Association between major depressive disorder and a functional polymorphism of the 5-hydroxytryptamine (serotonin) transporter gene : a meta-analysis

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Association between major depressive disorder and a functional polymorphism of the 5-hydroxytryptamine (serotonin) transporter gene: a meta-analysis

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Running head: Major depressive disorder and the *5-HTTLPR* polymorphism

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Abstract

Objectives: A functional polymorphism in the promoter region of the 5-hydroxytryptamine (serotonin) transporter (*5-HTT*) gene, termed *5-HTTLPR*, alters transcription of the *5-HTT* gene. The short variation (S allele) produces less transcriptional efficiency of serotonin, which can partly account for psychiatric disorders. Despite strong biologic plausibility, the relationship between *5-HTTLPR* and the risk of major depressive disorder (MDD) is unclear. To elucidate the relationship, we applied meta-analysis techniques to molecular studies of *5-HTTLPR* and MDD.

Methods: A total of 22 articles were identified from MEDLINE through March 2008, using the search keywords "depression," "5-HTTLPR" and "polymorphism." The authors assessed the evidence of genotypic association using STATA Version 8.2.

Results: Summary frequencies of the S allele of 5-*HTTLPR* among Caucasians and Asians based on the random effects model were 42.1% (95% confidence interval (CI) = 40.5 - 43.6) and 76.8% (95% CI = 73.9 - 79.7), respectively. The distribution of the S allele was significantly different between Asians and Caucasians ($P < 0.001$). The SS genotype was significantly associated with an increased risk of MDD among Caucasian populations (odds ratio = 1.41, 95% CI = 1.15 - 1.72), although there was no significant association among Asians.

Conclusions: Although the summary risk for developing MDD in individuals with the "at risk" SS genotype of *5-HTTLPR* may be small, MDD is such a common disease that even a small increase in risk translates to a large number of excess MDD cases in the population. Thus, *5-HTT* may be a candidate MDD susceptibility gene.

Introduction

The World Health Organization (WHO) has estimated that each year about 877,000 people die from suicide. Furthermore, mental health disorders, particularly depression and substance abuse, are associated with more than 90% of all cases of suicide (http://www.who.int/mental_health/prevention/suicide/suicideprevent/en/). The general term depression is often used to describe the disorder, but it is also used to describe a depressed mood. Major depressive disorder (MDD), also known as clinical depression, major depression, unipolar depression, or unipolar disorder, is defined by the Diagnostic and Statistical Manual of Mental Disorders, Fourth edition [(DSM-IV (1994)] as a disabling condition which adversely affects a person's family, work or school life, sleeping and eating habits, and general health. Although both genetic and environmental factors are involved through pathogenesis of any mental disorders as shown by vulnerability-stress model, genetic factors have been considered to play a more important role in the occurrence of mental disorders such as schizophrenia, mood disorders including MDD, than in neurotic disorders such as anxiety disorders or dissociative disorders due to psychogenic reaction, which are mainly caused by environmental stressors.

The heritability of MDD is much lower than that of bipolar disorder or schizophrenia. The heritability of MDD is likely to be in the range of 31%−42%. This is probably at the lower end of the range, and the level of heritability is likely to be substantially higher for reliably diagnosed MDD and subtypes, such as recurrent MDD. In comparison, the heritabilities of schizophrenia and bipolar disorder are estimated to be approximately 70% (Kendler, 1983). MDD is caused by multiple genes and does not follow Mendelian patterns of inheritance. MDD is a common disease that results

from a complex interplay of genes and environmental risk factors just like other common multifactorial diseases, such cancer, diabetes mellitus and cardiovascular disease. Several excellent reviews have been written on the topic of stress and depression in the past 15 years (Kessler, 1997; Mazure, 1998; Monroe and Hadjiyannakis, 2002; Tennant, 2002; Paykel, 2003; Hammen, 2005; Belmaker and Agam, 2008). Environmental factors, such as prenatal factors, loss, deprivation, grief, stress, natural disasters, war, social support systems, nutrition, exercise, drug effects, and medical illness, have all been linked to MDD (Wong and Licinio, 2001). As for genetic factors, only minor susceptibility genes have been reliably identified.

The serotonin system provides a logical source for susceptibility genes linked to depression, because this system is the target of selective serotonin reuptake–inhibitor drugs that are effective in the treatment of depression. The 5-hydroxytryptamine (serotonin) transporter (*5-HTT*) has received particular attention because it is involved in the reuptake of serotonin at brain synapses. A polymorphism in the promoter region of the *5-HTT* gene, termed *5-HTTLPR*, has two frequent alleles designated long (L) and short (S). A repeat of 20−23 base pairs has been observed as a motif within *5-HTTLPR*: one consisting of 14 repeats (S allele) and another of 16 repeats (L allele). The S allele leads to less transcriptional efficiency of serotonin (Heils *et al*., 1996; Heils *et al*., 1997), and it can partly account for anxiety-related personality traits (Lesch *et al*., 1996). Characteristics of individual studies on *5-HTTLPR* and MDD are summarized in Table 1. The first study suggests that variation at *5-HTTLPR* may influence susceptibility to MDD (Collier *et al.*, 1996). Three case-control studies were separately carried out in the British, German and Italian populations. A significant

association between the presence of the S allele and MDD, whether by allele or genotype, was observed in a combined European sample of three populations, although no significant association was found. However, Rees *et al*. (1997) found an increased frequency of the S allele in controls. The SS genotype or S allele was not significantly associated with a decreased risk of depression in the British population (Rees *et al.*, 1997). A nonsignificant protective effect of the S allele was observed among Spaniards (Arias *et al*., 2003) and Germans (Frodl *et al.*, 2004; Dannlowski *et al.*, 2008). Four studies of whites (Hoehe *et al.*, 1998; Geijer *et al*., 2000; Taylor *et al*., 2005; Grünblatt *et al*., 2006) found no substantial relationships between the *5-HTTLPR* genotypes and MDD. In contrast, seven Caucasian studies (Bellivier *et al*., 1998; Furlong *et al.*, 1998; Minov *et al.*, 2001; Serretti *et al.*, 2002; Cervilla *et al*., 2006; Dorado *et al.*, 2007; Hickie *et al.*, 2007) found that the SS genotype was associated with a modest increase in the risk of depression. Furthermore, one Polish study (Hauser *et al*., 2003) and one German study (Hoefgen *et al.*, 2005) found that the SS genotype was associated with a significant increase in the risk of depression. Frisch *et al*. found no association of this polymorphism with MDD in Ashkenazi and non-Ashkenazi Jews (Frisch *et al*., 1999). Likewise, Asian studies did not find any significant difference between patients with MDD and controls (Kunugi *et al*., 1997; Ohara *et al*., 1998; Kim *et al*., 2000). Despite strong biologic plausibility, the relationship between *5-HTTLPR* and MDD risk is unclear. The individual results of these studies have been inconsistent, and definite conclusions are difficult to establish. A reliable method for assessing individual studies and generating combined results is provided by systematic reviews using meta-analytical techniques.

In this paper, we carried out a systematic review by retrieving, assessing, and

combining individual studies investigating the association between MDD and a functional genetic polymorphism of *5-HTTLPR*.

Materials and methods

1. Identification and eligibility of relevant studies

We conducted MEDLINE, Current Contents and Web of Science searches using "depression", "5-HTTLPR" and "polymorphism" for papers published before March 2008. Additional articles were identified through the references cited in the first series of articles selected. Articles included in the meta-analysis were in any language, involved human subjects, were published in the primary literature and had no obvious overlap of subjects with other studies. We excluded studies with the same data or overlapping data by the same authors. Case-control studies were eligible if they had determined the distribution of the relevant genotypes in depression cases and in concurrent controls using a molecular method for genotyping. Using the MEDLINE database, we identified 22 genetic epidemiological studies that provided information on depression associated with *5-HTTLPR*. No additional articles through Current Contents or Web of Science were identified.

2. Data extraction and assessment of study quality

For each study, characteristics, such as authors, year of publication, ethnic group of the study population, source of control population, number of genotyped cases and controls, diagnostic criteria, diagnostic instrument, crude odds ratio (OR), the method of genotyping and the method for quality control of genotyping, were obtained.

For studies including subjects of different ethnic groups, data were extracted separately for each ethnic group whenever possible. Our meta-analysis did not include the large European collaborative study of Mendelwics *et al.* (2004) due to the absence of information on genotype frequency.

Methods for defining study quality in genetic studies are more clearly defined than those for observational studies. We assessed the Hardy-Weinberg equilibrium (HWE) via a goodness-of-fit χ^2 test (Pearson) to compare the observed and expected genotype frequencies among controls. When the P value for HWE exceeded 0.05, we estimated that the study population was under the Hardy-Weinberg equilibrium. We also assessed the homogeneity of the study population [ethnicity (Caucasians or Asians), diagnostic criteria for MDD (DMS-IV or any international criterion) and control source (general or healthy population)].

3. Meta-analysis

Data were combined using fixed effects (the inverse variance-weighted method) and random effects models. The Cochran's Q statistics test is used for the assessment of heterogeneity. The fixed effects model is used when the effects are assumed to be homogenous, while the random effects model is used when they are heterogenous. In the absence of between-study heterogeneity, the two methods provide identical results. The random effects model incorporates an estimate of the between-study variance and tends to provide wider CIs when the results of the constituent studies differ among themselves. As the random effects model is more appropriate when heterogeneity is present (DerSimonian and Laird, 1986), the summary

OR and prevalence were essentially based on the random effects model. The meta-analyses were performed on crude ORs, since the adjusted ORs were not comparable because of different covariates' included in the multivariate regression models. Using individuals homozygous for the long (L) allele (LL genotype) as the reference group, we calculated ORs for individuals with the SS genotype or for those with the LS genotype. The Q statistic was considered significant for $P<0.10$ because of the low power of the statistic (Cochran, 1954; Whitehead and Whitehead, 1991).

Publication bias is always a concern in meta-analysis. The presence of publication bias indicates that nonsignificant or negative findings remain unpublished. To test for publication bias, both Begg's (Begg and Mazumdar, 1994) and Egger's (Egger *et al.*, 1997) tests were used to assess whether smaller studies reported greater associations than larger studies. Publication bias was considered significant for $P<0.10$ because of the low power of the statistic.

All the calculations were performed with the computer program STATA Version 8.2 (Stata Corporation, College Station, TX).

Results

All studies analyzed in this paper were based on the polymerase chain reaction-restriction fragment length polymorphism method. Quality control of genotyping (replication of a random sample, direct sequencing, etc.) was not performed in all studies. As shown in Table 1, the 22 case-control studies in 25 different ethnic populations of MDD and *5-HTTLPR* included 7,919 subjects (2,934 depressed cases and 4,985 controls). As for the prevalence of the S allele in controls, we found strong

evidence of between-study heterogeneity among all studies (Table 1, $P < 0.0001$). To remove the heterogeneity, stratified analysis by ethnicity was carried out. Significant heterogeneity remained among Caucasian populations ($P = 0.002$). Heterogeneity can be taken into account by applying the random effects model. Based on the random effects model, summary frequencies of the S allele among Caucasians and Asians were 42.1% (95% CI =40.5 - 43.6%) and 76.8% (95% CI = 73.9 - 79.7%), respectively (Table 1). The distribution of the S allele was significantly different between Asians and Caucasians ($P < 0.001$). Studies included in the meta-analysis in ascending order of the S allele frequency by ethnic group are presented in Figure 1. As shown in Figure 1, the distribution of the S allele among controls was dramatically different, not only between Asians and Caucasians but also within Caucasian populations. The frequencies of the S allele were lowest (34.4%) in the study by Hickie *et al*. (2007) and highest (49.1%) in the study by Arias *et al.* (2003). As between-study heterogeneity may be due to differences in control sample selection among Caucasian populations, further stratified analysis was done. When studies were stratified by control source, frequencies of the S allele was 42.3% (95% CI = 38.7 - 45.9%) using data from general population-based studies and 41.6% (95% CI = 40.2 - 43.1%) using data from healthy population (blood donors, healthy volunteers, healthy staff and etc.)-based studies. The heterogeneity was removed after exclusion of the data from general population-based studies data set ($P = 0.86$). There was no statistical difference between the two groups with respect to frequency of the S allele ($P = 0.62$, data not shown), however. The Begg's and Egger's tests for publication bias were not statistically significant in any analyses.

As shown in Table 1, the summary OR for the SS genotype vs. the LL genotype was 1.34 (95% CI = 1.14 - 1.57) among all studies. A lack of equilibrium can indicate that the genotype distribution in the control group was not representative of the general population, from which the cases presumably arose, suggesting the possibility of selection bias. The distribution of the *5-HTTLPR* genotypes among controls was in agreement with HWE in all studies. The summary OR for the SS genotype vs. the LL genotype was 1.40 (95% CI = 1.19-1.65) among Caucasian studies. When Caucasian populations were restricted to the studies based on DSM-IV, the summary OR for the SS genotype was 1.41 (95% CI = 1.15-1.72). Our results were robust in sensitivity analyses that were restricted to studies of Caucasians or studies of Caucasians based on DSM-IV criteria. The Cochran's Q test for heterogeneity did not show a statistical significance in both sensitivity analyses (P = 0.33 for Caucasian studies and P =0.23 for Caucasian studies based on DSM-IV). The Begg's and Egger's tests for publication bias were not also statistically significant in both analyses. When Caucasian populations were stratified by control source, the summary OR for the SS genotype was 1.36 (95% CI= 1.07 - 1.74) using data from general population-based studies and 1.43 (95% CI = 1.12 - 1.84) using data from healthy population-based studies. There was a slight but nonsignificant difference between the two groups with respect to the summary OR for the SS genotype. Evidence for heterogeneity and publication bias was absent in the analyses.

In three Asian studies (all Japanese), the OR for MDD with the SS genotype was 1.04 (95% CI = 0.51 - 2.16). When Asian populations were restricted to the studies based on DSM-IV criteria, the summary OR for the SS genotype was 0.94 (95%

CI = 0.35 - 2.49). Heterogeneity and publication bias were absent in the analyses (P = 0.78 for Asian studies and P = 0.53 for Asian studies based on DSM-IV). The S allele may be a disease allele; thus, we calculated ORs for the LS genotype compared with the LL genotype. The LS genotype was not associated with an increased risk of MDD in any analysis. The S allele might act in a recessive fashion among Caucasian populations. When the genetic model is assumed to be recessive, the LS and LL genotypes can be combined. The summary OR for the SS genotype compared with the LS and LL genotypes combined among Caucasian studies based on DSM-IV was 1.33 (95% CI = 1.15 - 1.54, data not shown). When Asian populations (all Japanese) were restricted to the studies based on DSM-IV criteria, the summary OR for the SS compared with the LS and LL combined genotype was 1.40 (95% CI = 0.84-2.43, data not shown). Evidence for heterogeneity and publication bias was absent in the analyses. *5-HTTLPR* was not significantly associated with MDD risk among Asians, although there are only a small number of articles available regarding Asian populations.

As shown in Figure 2, studies included in the meta-analysis were sorted in ascending order of OR among Caucasian populations. The summary OR for MDD based on DSM-IV with the SS genotype was tolerably different among Caucasian populations. The ORs for the SS genotype among Caucasians were lowest (OR = 0.59) in the study by Rees *et al*. (1997) and highest (3.30) in the study by Hauser *et al*. (2003).

Discussion

When Caucasian populations were restricted to the studies based on DSM-IV (2,176 depressed cases and 3,580 controls), the SS genotype of *5-HTTLPR* was associated with a 41% increase in MDD risk (Table 1). There was no evidence of heterogeneity between the results of individual studies (the SS genotype vs. the LL genotype or the LS genotype vs. the LL genotype) within Caucasian populations. There was evidence of heterogeneity in the prevalence of S allele (P = 0.01) although in general there was very little heterogeneity among Caucasians (Garte *et al.*, 2001). Stratified by control source, significant heterogeneity remained in general population-based studies (P <0.0001) but not in healthy population-based studies (P = 0.86). The reason for the more heterogenous prevalence in general populations is not clear. General populations may be more heterogenous than healthy populations in relation to the frequencies of the "at risk" alleles including the S allele of *5-HTTLPR*. A "healthy volunteer effect" may occur in molecular epidemiological studies based on populations of volunteers, such as blood donors. Blood donors must be well on the day of donation and not currently under medical care for serious illnesses. Any group of workers or volunteers (blood donors) may be healthier, on average, than subjects of general population ("health worker effect"). Therefore, frequency of the "at risk" genotype is expected to be higher in general populations than in healthy populations. Although these biases will inflate prevalence of the S allele in general population and non-general population, there was no statistical difference in the risks between the two groups in this study. It may be unlikely to introduce biases in the comparison of estimated MDD risks between the two groups. Anyway, ORs for the SS genotype compared with the LL or LS genotype was

unaffected by the presence or absence of heterogeneity. Our meta-analysis did suggest a major role of *5-HTTLPR* in MDD among Caucasian populations. To date, two meta-analyses on the association between *5-HTTLPR* and MDD have been published in 2004 (Lotrich and Pollock, 2004) and 2005 (Lasky-Su *et al.*, 2005). In the first meta-analysis, based on 10 case-control studies, the number of depressed cases and controls was 910 and 2017, respectively. The first meta-analysis showed that individuals with the SS genotype had a 16% (95% CI = 1.03 - 1.31) increased risk of MDD compared with individuals with the LL genotype. The results supported the hypothesis that individuals with the SS genotype are at higher risk of developing MDD. Five individual studies (Collier *et al.*, 1996; Rees *et al.*, 1997; Bellivier *et al.*, 1998; Furlong *et al.*, 1998; Kunugi *et al.*, 1997) were included in both meta-analyses. The second meta-analysis was somewhat different from the first one, because ORs for the S allele compared with the L allele were summarized. The second meta-analysis, based on 10 case-control studies, was comprised of 1,961 cases and 3,402 controls and showed that the summary OR was close to unity (OR= 1.05, 95% CI = 0.96-1.14). However, this meta-analysis suggested that the S allele was significantly associated with an increased risk of bipolar disorder (OR= 1.13, 95% CI = 1.05 - 1.22). Our meta-analysis, on the other hand, showed that the S allele was significantly associated with an increased risk of MDD based on DSM-IV criteria among Caucasian populations (OR= 1.18, 95% CI = 1.06 - 1.31, data not shown). Therefore, the results of the present study are dissimilar to the results of the second meta-analysis. The reasons for this discrepancy are not clear, but the inconsistent results are probably due to differences in the number of studies included (statistical

power). Furthermore, there was a significant heterogeneity of the frequency of the S allele within Caucasian populations. The presence of significant heterogeneity suggests that the estimated frequency in each study is not homogeneous. Possible sources of heterogeneity are characteristics of control subjects (age, sex, ethnicity, source of population, population admixture and so on). Sensitivity analyses (e.g., stratified by sex or nationality) may produce a stronger association between *5-HTTLPR* and MDD. As stated earlier, the S allele of a functional *5-HTTLPR* may be recessive among Caucasian populations in our meta-analysis. An additional major concern is the grouping of genotypes for calculation of ORs; without functional data to dictate genotype groupings, it seems prudent to present two ORs per polymorphism (one for heterozygotes vs. common-allele homozygotes and one for rare-allele homozygotes vs. common-allele homozygotes) so that dominant, codominant, or recessive patterns may be elucidated.

Our meta-analysis did not support a major role for *5-HTTLPR* in MDD among Asians, while the polymorphism was significantly associated with MDD risk in Caucasian populations. The ethnic difference of the association between *5-HTTLPR* and MDD is not clear. Generally, the low frequency of the "at risk" genotype reduces the statistical power. As the prevalence of the S allele was significantly higher in Asians than in Caucasians ($p<0.001$), this is not the case. Given the higher frequency of the S allele of the *5-HTTLPR* in Japanese subjects, if this allele is associated with an increased risk of MDD, then the prevalence of MDD would be higher among Japanese than Caucasians. The 12-month prevalence rate (standard error) among Japanese was 2.1 (0.3) (Kawakami *et al*., 2008). On the other hand, the corresponding figures

among Caucasians (German, Latin or Slav) were 3.0 (0.3, Germany), 4.9 (0.5, the Netherlands), 3.0 (0.2, Italy) and 8.4 (0.6, Ukraine) (Alonso *et al*., 2008; de Graaf *et al*., 2008; de Girolamo *et al*., 2008; Bromet *et al*., 2008). The higher figure for Ukraine was due to a higher prevalence of Ukrainian women. Their prevalence of 11.3% was almost twice as many as the women's figure reported for the European Study of the Epidemiology of Mental Disorders (Bromet *et al*., 2008). In addition, high prevalence may be explained by several social factors such as loss of spouse, loss of income, or difficulty adjusting to the changing sociopolitical climate (Bromet *et al*., 2008). Thus, the 12-month prevalence rate among Japanese might be a bit low compared with that among different Caucasians. Stigma in response to psychiatric disorders may cause underestimate of MDD in Japan. Stigmatising attitudes towards psychiatric disorders are more common among Japanese than among Caucasians (Griffiths *et al.*, 2006).

MDD is a multifactorial disease that results from complex interactions between many genetic and environmental factors. Ethnic differences in roles of the polymorphism may be caused by gene-environment (social, relational cultural, lifestyle factors and so on) interactions. Each ethnic group may have its own set of environmental and genetic factors that contributes to the MDD risk. Despite the growing awareness of the relevance of gene-environment interactions in human disease, true progress in the identification of common genetic alterations that by themselves may not substantially impact risk, but in concert with environmental exposures may lead to disease development, has been limited. Some genetic variants may exert population-specific effects that are independent of the other genetic profile of the individual and environmental exposures, while other population-specific effects may be

generated under differential gene-environment interactions (Hunter, 2005).

Continued advances in SNP maps and in high-throughput genotyping methods will facilitate the analysis of multiple polymorphisms within genes and the analysis of multiple genes within the same pathways. The effects of polymorphisms are best represented by their haplotypes. Data from multiple polymorphisms within a gene can be combined to create haplotypes, the set of multiple alleles on a single chromosome. The analysis of haplotypes can increase the power to detect disease associations because of higher heterozygosity and tighter linkage disequilibrium with disease-causing mutations (Stephens *et al.*, 2001; Judson *et al.*, 2000; Fallin *et al.*, 2001). In addition, analysis of haplotypes offers the advantage of not assuming that any of the genotyped polymorphisms is functional; rather, it allows for the possibility of an ungenotyped functional variant to be in linkage disequilibrium with the genotyped polymorphisms (Khoury *et al.*, 1993). Another common polymorphism of the *5-HTT* gene is a variable number tandem repeat (VNTR) in intron 2 (STin2), which has three alleles consisting of either 9 (STin2.9), 10 (STin2.10) or 12 (STin2.12) repeats with the positive association between the STin2 allele 10 and the *5-HTTLPR* L allele (Collier *et al*., 1996). Variation at the VNTR can also influence expression of the transporter with the polymorphic VNTR regions acting as transcriptional regulators (McKenzie and Quinn, 1999), although this is unlikely to have a significant effect on function. Ogilvie *et al.* (1996) first demonstrated that there was an excess of the 9-repeat allele of another *5-HTT* STin2 polymorphism in MDD patients in comparison to controls. However, this finding was not replicated in other Caucasian (Rees *et al*., 1997; Hoehe *et al.*, 1998; Bellivier *et al.*, 1998; Furlong *et al*., 1998; Stöber *et al*., 1996; Gutiérrez *et al*., 1998; Mellerup *et al.*, 2001; Collier *et al*., 1996) or Asian studies (Kunugi *et al*., 1997) using

independent samples. The rarity of the STin2.9 allele (below 1%) means that the risk attributable to this allele is small. Liu *et al.* (1999) demonstrated that the 12-repeat allele was associated with MDD in a Chinese population. This polymorphism and *5-HTTLPR* were shown to be in modest or weak linkage disequilibrium (Collier *et al*., 1996; Kunugi *et al*., 1997; Rees *et al*., 1997; Bellivier *et al.*, 1998). Some of the studies reviewed here reported haplotype (*5-HTTLPR* and *5-HTT* STin2) analyses (Collier *et al*., 1996; .Rees *et al.*, 1997) but did not show significant evidence for MDD-haplotype association. Although 5-*HTTLPR* may play a pathogenic role, it seems more likely that it is in linkage disequilibrium with polymorphisms other than the STin2 polymorphisms within or close to the *5-HTT* gene. In addition, an analysis of data from multiple genes can provide more comprehensive insight into the studied associations. Identification of gene-gene interactions has become increasingly important in understanding psychiatric disorders. Such an analysis may shed light on the complexities of the many pathways involved with the monoaminergic pathway and MDD development and provide hypotheses for future functional studies. Because of concerns over inflated type I error rates in pathway-wide or genome-wide association studies, methods of statistical analysis seeking to obviate this problem are under development (Hoh *et al*., 2001). The ability to include haplotype information and data from multiple genes and to model their interactions will provide more powerful and comprehensive assessments of the monoaminergic pathways. Further investigations of the combined effects of polymorphisms between monoaminergic genes (tryptophan hydroxylase, catechol-*O*-methyltransferase, serotonin receptor, brain-derived neurotrophic factor, norepinephrine transporter, dopamine receptors, etc.) may help to clarify the influence of genetic variation in the process of developing the depressive

state. In addition to single locus analysis, haplotype analysis or analysis of combination of variations in multiple genes may be a future direction of research for *5-HTTLPR.*

Although the risk associated with *5-HTTLPR* may not be large, the public health implication may be large because of their high frequency in the general population. It is essential that epidemiological investigations of monoaminergic polymorphisms are adequately designed. Unfortunately a fairly large number of studies are limited by their sample size and, consequently, low power to detect effects that may truly exist. Also, given the borderline significance of some associations and multiple comparisons, there is a possibility that one or more of these findings are false-positives (Wacholder *et al.*, 2004). Large and combined analyses such as those by Healey *et al.* (2000) and Spurdle *et al.* (2002) are preferred to minimize the likelihood of both false-positive and false-negative results. In addition, a susceptibility factor in one population may not be a factor in another. There are differences in the prevalence of the *5-HTTLPR* polymorphisms across populations. In a population where the prevalence of an "at-risk genotype in a given polymorphism is very low, the "at-risk" allele or "at-risk" genotype may be too infrequent to assess its associated risk. Finally, the major burden of MDD in the population probably results from complex interactions between many genetic and environmental factors over time. Consortia and international collaborative studies, which may maximize study efficacy and overcome the limitations of individual studies, are needed to help further illuminate the complex landscape of MDD risk and genetic variations.

In conclusion, the SS genotype of *5-HTTLPR* was significantly associated with an increased risk of MDD among Caucasians (OR = 1.41, 95% CI = 1.15 - 1.72).

Although the summary risk for developing MDD in individuals with the SS ("at-risk") genotype may not be large, MDD is such a common disease that even a small increase in risk can translate to a large number of excess MDD cases. Therefore, polymorphisms, even those not strongly associated with MDD, should be considered as a potentially important public health issue.

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(Figure Legends)

Figure 1. The S allele frequency of 20 Caucasian populations and 3 Asian populations among controls.

The center of a box and the horizontal line (logarithm) indicate the prevalence and the 95% confidence interval (CI) in each study, with the areas of the boxes representing the weight of each study. The summary prevalence based on the random effects model is represented by the middle of a diamond whose width indicates the 95% CI. The summary prevalence is also shown by the dotted vertical line. The summary prevalence of Caucasians and Asians based on the random effects model are 42.1% (95% CI = 40.5-43.6) and 76.8% (95C% CI = 73.9-79.7), respectively. Statistical heterogeneity between studies among Caucasians and Asians were assessed as Q =34.9, P = 0.01 and Q =2.60, P = 0.27, respectively, by Cochran's Q test.

Figure 2. Meta-analysis of *5-HTTLPR* and major depressive disorder according to DSM-IV among 16 Caucasian populations.

The center of a box and the horizontal line (logarithm) indicate the odds ratio (OR) and the 95% confidence interval (CI) in each study, with the areas of the boxes representing the weight of each study. The summary OR based on the random effects model is represented by the middle of a diamond whose width indicates the 95% CI. The summary OR is also shown by the dotted vertical line. The summary OR (SS vs. LL) is 1.41 (95% CI =1.15- 1.72). Statistical heterogeneity between studies was assessed with Cochran's Q test (Q =18.71, P = 0.23).

Table 1 Studies of the *5-HTTLPR* polymorphism and risk of major depressive disorder

Researcher, published year, study location	Race/ ethnicity (residence of subjects)	Diagnostic criteria/ instrument	No. of Cases/ Controls	Frequency (%) of SS genotype		Source of controls	Crude OR (95% CI)		P_{HWE}†
				Cases	Controls		SS vs. LL	LS vs. LL	
Collier et al., 1996, Germany	Caucasian (UK)[a]	DSM-IV/ No information	81/174	24 (29.6)	36 (20.7)	Hospital controls	1.59 (0.78-3.24)	0.98 (0.52-1.84)	0.65
	Caucasian (Italy)[b]	DSM-IIIR/ No information	22/95	7 (31.8)	15 (15.8)	Blood donors	1.87 (0.46-6.33)	0.62 (0.20-1.87)	0.26
	Caucasian (Germany)[c]	DSM-IV/ No information	47/301	10 (21.2)	50 (16.9)	Blood donors	1.35 (0.57-3.18)	0.99 (0.49-1.99)	0.82
	Combined	−	150/570	41 (27.3)	101 (17.7)	−	1.69 (1.04-2.74)	0.94 (0.60-1.47)	0.99
Rees et al., 1997, UK	Caucasian (Europe)	DSM-IV/ SADS-L	80/118	13 (16.3)	24 (20.3)	Blood donors	0.59 (0.26-1.36)	0.76 (0.36-1.60)	0.92
Kunugi et al., 1997, Japan	Asian (Japan)	DSM-IV/ No information	49/207	36 (73.5)	132 (63.8)	Healthy volunteers	0.75 (0.23-2.50)	0.39 (0.10-1.48)	0.38
Bellivier et al., 1998, France	Caucasian (France)	DSM-IV/ No information	37/99	8 (21.6)	12 (12.1)	Healthy volunteers	1.49 (0.49-4.50)	0.92 (0.41-2.07)	0.37
Furlong et al., 1998, UK	Caucasian (UK)	DSM-IV /SADS-L	125/169	26 (20.8)	29 (17.2)	DNA Bank of Molecular Genetics Laboratory	1.51 (0.77-2.97)	1.33 (0.78-2.27)	0.89
Hoehe et al., 1998, France	Caucasian (West Europe)	DSM-IV /SADS-L	36/281	5 (13.9)	48 (17.1)	Healthy staff and students	1.10 (0.35-3.46)	1.68 (0.74-3.81)	0.86

Ohara et al., 1998, Japan	Asian (Japan)	DSM-IV/ SADS	41/92	26 (63.4)	54 (58.7)	Healthy volunteers	1.44 (0.27-7.65)	1.22 (0.22-6.84)	0.67
Frisch et al., 1999, Israel	Ashkenazi Jewish[a]	DSM-IV /SADS-L	63/112	15 (23.8)	28 (25.0)	Healthy volunteers	0.58 (0.25-1.37)	0.47 (0.23-0.99)	0.44
	non-Ashke-nazi Jewish[b]	DSM-IV /SADS-L	60/39	7 (17.9)	11 (18.3)	Healthy volunteers	1.03 (0.28-3.77)	1.08 (0.39-3.01)	0.12
Geijer et al., 2000, Sweden	Caucasian (Europe)	DSM-IV/ SCID-I	45/99	7 (15.6)	15 (10.1)	Healthy staff and students	1.04 (0.34-3.16)	1.01 (0.45-2.27)	0.18
Kim et al., 2000, Korea	Asian (Korea)	DSM-3R/ HDRS 17	120/252	68 (56.7)	137 (54.4)	Healthy volunteers	1.19 (0.40-3.52)	1.10 (0.36-3.28)	0.20
Minov et al., 2001, Germany	Caucasian (Germany)	DSM-IV/ HAM-D17, CGI	173/121	40 (23.1)	17 (14.0)	General population	1.78 (0.88-3.58)	0.93 (0.55-1.58)	0.28
Serretti et al., 2002, Italy	Caucasian (Italy)	DSM-IV /OPCRIT checklist	667/457	135 (20.2)	75 (16.4)	Healthy staff	1.33 (0.94-1.88)	1.05 (0.81-1.37)	0.80
Arias et al., 2003, Spain	Caucasian (Spain)	DSM-IV/ SCID-I	131/163	27 (20.6)	38 (23.3)	General population	0.71 (0.37-1.37)	0.75 (0.44-1.29)	0.69
Hauser et al., 2003, Poland	Caucasian (Poland)	DSM-IV, ICD10/ SCID-I	94/213	26 (27.7)	35 (16.4)	Blood donors	3.30 (1.61-6.79)	2.27 (1.23-4.19)	0.59
Frodl et al., 2004, Germany	Caucasian (Germany)	DSM-IV/ HDRS-21	40/40	8 (20.0)	10 (25.0)	General population	0.65 (0.20-2.12)	0.76 (0.28-2.08)	0.36
Mendlewicz et al., 2004, Belgium	Caucasian (Europe)	DSM-IV/ SADS-L	539/821	Not shown*	161 (19.6)	General population	Not calculable	Not calculable	0.26
Hoefgen et al., 2005, Germany	Caucasian (Germany)	DSM-IV/ SADS-L, SCID-I, CIDI	466/827	99 (21.4)	127 (15.4)	General population	1.63 (1.17-2.26)	1.14 (0.88-1.48)	0.50

Taylor et al., 2005, USA	Not specifed	No information /DDES	135/83	21 (15.6)	13 (15.7)	General population	1.00 (0.43-2.29)	1.01 (0.55-1.85)	0.80
Grünblatt et al., 2006 Austria	Caucasian (Austria)	DSM-IV/ SCID-I, HDRS	36/360	6 (16.7)	52 (14.4)	Healthy cohort member	1.51 (0.53-4.29)	1.51 (0.70-3.29)	0.63
Cervilla et al., 2006, Spain	Caucasian (Spain)	ICD10/ CIDI	261/476	77 (29.5)	101 (21.2)	Healthy cohort member	1.52 (0.99-2.32)	0.97 (0.67-1.41)	0.37
Dorado et al., 2007, Spain	Caucasian (Spain)	DSM-IV/ No information	70/142	17 (24.3)	26 (18.3)	Healthy volunteers	2.26 (0.95-5.40)	1.95 (0.94-4.04)	0.83
Hickie et al., 2007, Australia	Caucasian (Australia)	DSM-IV/ HDRS-21	45/16	12 (26.7)	3 (16.0)	General population	2.13 (0.46-9.84)	1.92 (0.52-7.12)	0.22
Dannlowski et al., 2008, Germany	Caucasian (Germany)	No information /SCID	28/28	5 (17.8)	7 (25.0)	Healthy volunteers	0.57 (0.13-2.50)	0.80 (0.24-2.67)	0.71

Summary

Ethnicity	No. of populations (total no. of subjects)	No. of cases	No. of controls	Frequency (%) among controls **		OR (95% CI)**			
				S allele	p‡	SS vs. LL	p‡	LS vs. LL	p‡
All	25 (7919)	2934	4985	47.2 (42.0-52.5)	<0.0001	1.34 (1.14-1.57)	0.34	1.05 (0.93-1.19)	0.39
Stratified by ethnicity									
Caucasian	20 (6884)	2622	4262	42.1 (40.5-43.6)	0.01	1.40 (1.19-1.65)	0.33	1.08 (0.96-1.22)	0.50
Asian	3 (761)	210	551	76.8 (73.9-79.7)	0.27	1.04 (0.51-2.16)	0.78	0.80 (0.37-1.71)	0.43

Stratified by diagnostic criteria (DSM-IV)									
All	20 (6419)	2368	4051			1.32 (1.07-1.61)	0.20	1.07 (0.91-1.25)	0.20
Caucasian	16 (5756)	2176	3580			1.41 (1.15-1.72)	0.23	1.12 (0.97-1.29)	0.34
Asian	2 (389)	90	299			0.94 (0.35-2.49)	0.53	0.60 (0.20-1.79)	0.30
Stratified by control source among Caucasian populations									
General population	8 (3368)	1282	2086	42.3 (38.7-45.9)	<0.0001	1.36 (1.07-1.74)	0.33	1.04 (0.88-1.23)	0.75
Healthy population	12 (3516)	1340	2176	41.6 (40.2-43.1)	0.86	1.43 (1.12-1.84)	0.29	1.14 (0.93-1.40)	0.25

* Distribution of genotypes in cases was not shown.
** Based on random effects model.
†Hardy-Weinberg equilibrium (p for Pearson χ^2)
‡Cochran's Q test for heterogeneity.

SADS-L, Schedules for Affective Disorders and Schizophrenia, Lifetime Version
SCID-I, Structured Clinical Interview for DSM-IV Axis I Disorders
HDRS, Hamilton Rating Scale for Depression
CGI, Clinical Global Impression scale
DDES, Duke Depression Evaluation Schedule
CIDI, Composite International Diagnostic Interview, version 1.1
DSM, Diagnostic and Statistical Manual of Mental Disorders

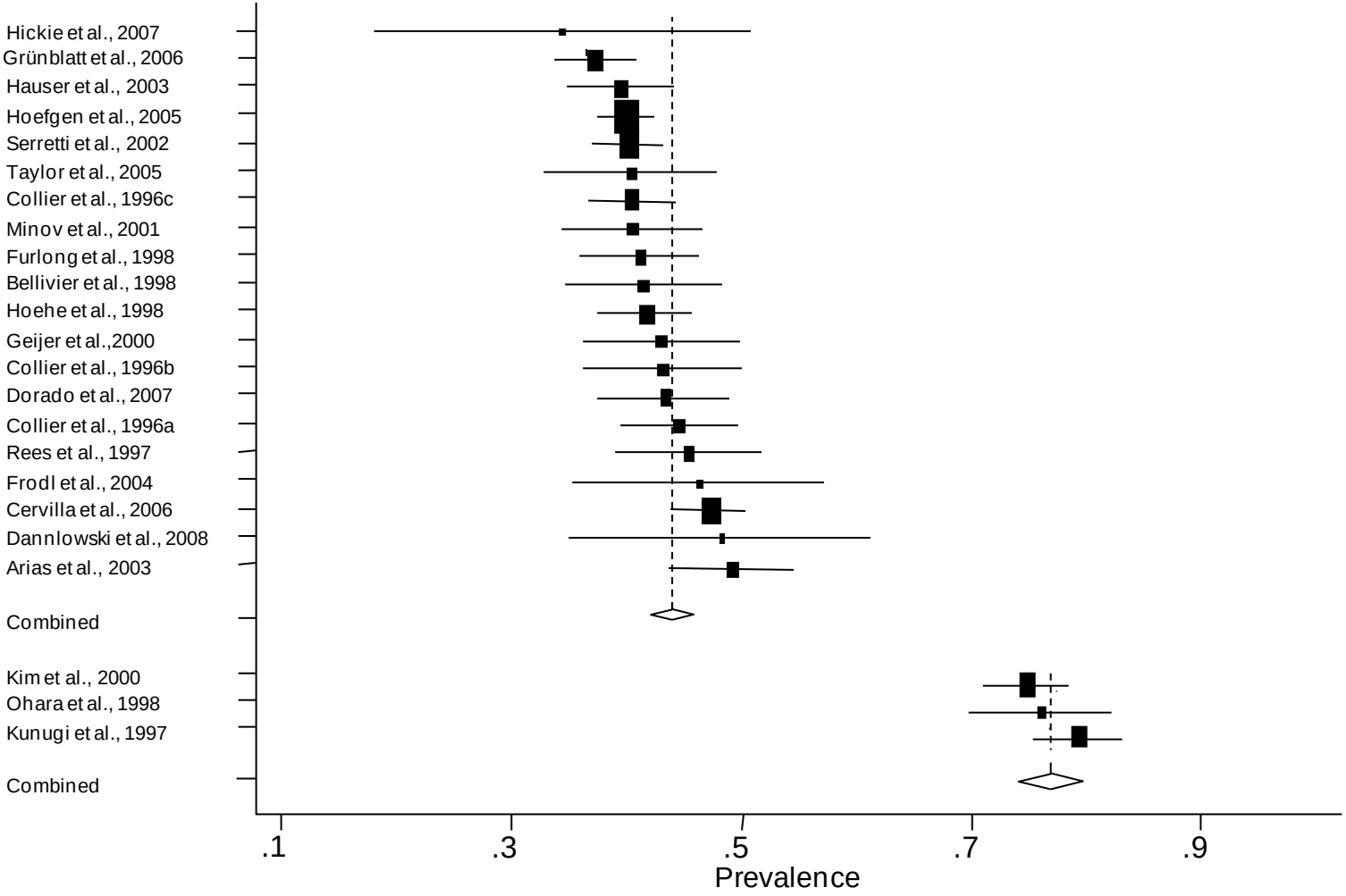

Fig. 1 The S allele frequency of 20 Caucasian populations and 3 Asian populations among controls

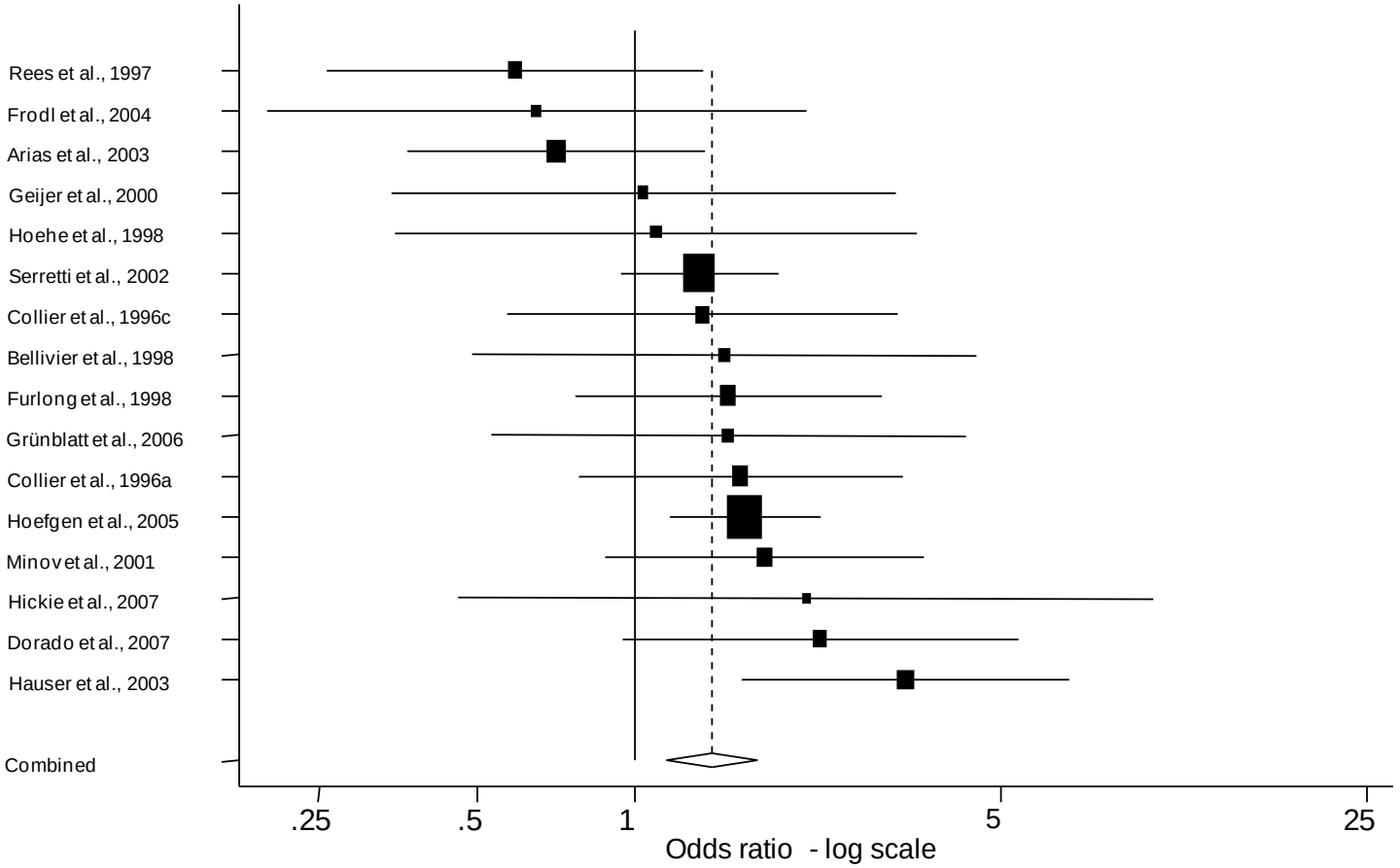

Fig. 2 Meta-analysis of *5-HTTLPR* and major depressive disorder according to DSM IV among 16 Caucasian populations. ;