Functional serotonin transporter gene polymorphisms and anxiety personality traits: new study and meta-analysis on a psychiatrically healthy population.

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Abstract

**Background**: Genetic liability for anxiety personality traits in healthy subjects has been associated with the functional serotonin transporter promoter polymorphism (5-HTTLPR) with conflicting results. Moreover, only one study has investigated the functional significance of the 5-HTTLPR/rs25531 haplotypes in relation to anxiety traits in healthy subjects. We tested whether the 5-HTTLPR and the haplotypes are linked to Harm Avoidance (HA) by an association study and a meta-analytic approach.

**Methods**: Two-hundred-eighty-seven unrelated Italian volunteers were screened for DSM-IV Axis I disorders diagnoses and they were genotyped for the 5-HTTLPR and rs25531.

**Results**: Using criteria described in the majority of studies, we found that S allele in homozygosity is associated with higher scores in HA. However, when we carried out the analyses only including subjects without any DSM-IV axis I disorders, we lost the association. Moreover the results on 5-HTTLPR meta-analyses and anxiety-related traits in the whole sample confirmed the association of SS genotype with higher anxiety-related traits scores in Caucasoid, but whatever we executed the analysis including only the studies using a structured psychiatric screening, no association was found.

**Conclusions**: This study does not find an association between functional serotonin transporter gene polymorphisms and anxiety traits in healthy subjects screened through a structured psychiatric interview. Furthermore, it demonstrates the relevance to perform analysis only in DSM-IV axis I disorder-free subjects.

**Keywords**: Neuroticism, Harm Avoidance, 5-HTTLPR, rs25531, meta-analysis
INTRODUCTION

Personality traits can be defined as individual qualities or characteristics that influence cognitions, emotions, and behaviors and lead to adaptive or maladaptive responses. Human personality is a multidimensional structure that is affected by both environmental and genetic factors. According to the literature, individual variation of the heritable component is estimated to account for 30-40% of the variance in personality traits [1]. To date, the most frequently studied candidate gene for personality traits is the functional polymorphism 5-HTTLPR in the promoter region of the gene SLC6A4, which encodes the serotonin transporter and results in a short (S) and a long (L) variant [2, 3].

Functional studies of the activity of the SLC6A4 promoter in transfected cell lines, human post-mortem brain, and lymphoblasts confirmed that the L allele is associated with higher levels of transcriptional activity and influences the rate of serotonin uptake more than the S variant [4]. According to recent findings, the S allele could be a risk factor for Major Depression Disorder (MDD) [5] and shows a less favorable response to SSRIs treatment [6, 7].

Recently it has been critically discussed that the analysis of 5-HTTLPR is incomplete, because other polymorphisms have been found in the proximity of the Ins/Del locus, such as rs25531, rs25532, rs2020933, and a 17-bp variable tandem repeat in the second intron (STin2) [4, 8, 9]. In particular, rs25531, the polymorphism nearest 5-HTTLPR, results in an A to G substitution and has been shown to modulate the effect of 5-HTTLPR on transcriptional efficacy. Our recent work [10] evidenced that the location of this polymorphism is immediately outside of the 5-HTTLPR segment resulting 5-HTTLPR and rs25531 as two independent polymorphisms. It has been reported that the G allele of
rs25531 is in phase with the 5-HTTLPR long allele and attenuates the transcriptional efficacy compared to the 5-HTTLPR short allele. Therefore, the modulation of 5-HTTLPR by rs25531 results in haplotypes with a high (L_A) or low (L_G, S_A or S_G) transcriptional efficacy [4, 11].

The inventories most used in biological studies of personality are the NEO-Personality Inventory [12] and the Temperament and Character Inventory (TCI) [13]. Although NEO and TCI have relevant differences, they seem to be similar when evaluating anxiety traits such as Neuroticism (N) and Harm Avoidance (HA), respectively. Several studies have shown that N is highly related to HA [14, 15], but contrarily, there is evidence that N and HA may not be equivalent [16].

Since the first paper of Lesch [3] was published, a large number of studies have been conducted in order to find evidence of association between the 5-HTTLPR polymorphism and anxiety-related personality traits. Despite all of these investigations, the strength and nature of any association is still uncertain. Controvert results were obtained using both TCI and NEO scales. In addition, five meta-analyses [17-21] provided conflicting results. In particular, while Munafò [17] reported little evidence of an association between the 5-HTTLPR polymorphism and avoidance traits, the two successive meta-analyses [20, 21] found an association with N and no link was observed with HA. In 2005, another meta-analysis [18] was conducted and the results indicated an association between the 5-HTTLPR polymorphism and HA and not with N, showing opposite data respect to those reported in the previous works [20, 21]. The authors concluded that the obtained results could depend on the measurement instruments used and they hypothesized that identification of significant sources of heterogeneity (instruments, ethnicity, gender) is
relevant for avoiding important biases in the results. Furthermore, the conclusion of the Schinka and Sen’s meta-analyses was biased by the inclusion of data from both healthy and psychiatric populations; personality traits of pathological people could have been a confounding factor. Finally, the most recent meta-analysis [19] provided results that were broadly consistent with those of previous studies [20, 21], although it included only healthy subjects in computations. Importantly all these meta-analyses do not take in consideration the psychiatric screening of the subjects involved in the studies. Indeed, it has been known that depression and anxiety disorders are associated with higher anxiety-related traits scores [22-25]. In addiction, a higher frequency of the S allele was observed in depressed and anxiety disorders patients [5].

Thus, we carried out the following analyses: 1) association study between the 5-HTTLPR and rs25531 and the relative estimated/phased haplotypes with anxiety personality traits measured using the self-rated TCI scale in whole sample of controls as well as in subjects without any DSM-IV axis I disorders screened by structured interviews; 2) meta-analyses of 5-HTTLPR and HA or N in controls and in screened samples.

**METHODS AND MATERIAL**

**New study**

**Participants**

Two-hundred-eighty-seven unrelated volunteers (age: 50.05 ± 15.94 years (mean ± SD) range: 22-87 years; 117 males, 170 females) were recruited for the study through different sources (university, newspaper advertisement, elderly association). The study protocol was approved by an Ethics Committee (Fatebenefratelli hospital “San Giovanni
di Dio” - Brescia, Italy) and written informed consent was obtained from all subjects. They were screened for DSM-IV Axis I disorders diagnoses through the Mini-International Neuropsychiatric Interview (M.I.N.I.) [26] by expert psychologists. Only healthy volunteers without history of drug or alcohol abuse or dependence and without a personal or first-degree family history of psychiatric disorders were enrolled in the study. The personality traits were assessed by the Italian version of M.I.N.I., a 240-item true-false self-report questionnaire [27]. Only subjects who obtained a score of at least 27/30 on the Mini Mental State Examination (M.M.S.E.) [28] were included in the study in order to avoid biases in the filling of the TCI.

5-HTTLPR and rs25531 analyses

DNA isolation, 5-HTTLPR and rs25531 genotyping and estimated/phased haplotypes classification (S'S', S'S', L'S', L'S', as S'S', L'S', S'S', and L'L' as L'L' ) were described in Bonvicini’ study [10]. In the genotyping analyses, we did not detect any L'L' and S'S'.

Statistical analysis

The association between TCI scores and 5-HTTLPR, and 5-HTTLPR/rs25531 was analyzed by an analysis of variance, using the HA score as the dependent variable, genotypes and sex as independent variables, and age as a covariate (ANCOVA). All analyses were conducted using the SPSS statistical software version 12.0 (SPSS Inc., Chicago, IL).

Meta-analysis

Literature search
To identify eligible studies for meta-analysis, we performed a search for all available association researches between the serotonin transporter and anxiety personality traits conducted in healthy adults, through PubMed at the National Library of Medicine using the following search terms: serotonin transporter polymorphism, serotonin transporter gene, 5-HTTLPR, Neuroticism, Harm Avoidance, anxiety, and personality. Once articles had been collected, bibliographies were then manually searched for additional references.

**Inclusion criteria**

All association studies that measured anxiety traits using all versions of NEO (NEO-PI, NEO-PI-R, NEO-FFI) or TCI (and TPQ version) on male and/or female participants of any ethnic origin were included. Studies in which psychiatric patients and control data were compared, only data from controls were included. Data that appeared in more than one published study were included only once in the analyses. Furthermore, papers not written in English [29] were excluded.

**Data extraction**

We recorded the number of participants, mean of N and/or HA trait scores, and standard deviation for each of the three genotype groups (LL, LS, SS) in each study included in our analysis. Furthermore, we extracted data regarding the male/female ratio, mean age, the ethnic compositions of the sample, and the structured clinical interview used for screening. Genotype frequencies were used to calculate the Hardy-Weinberg equilibrium (HWE; program http://www.genemapping.cn). In cases where all or part of this information was not available in the publication, the authors were contacted by email.

**Statistical analysis**
The Review Manager was used to analyze data (RevMan Version 5.0.16; Copenhagen, The Nordic Cochrane Centre, The Cochrane Collaboration, 2008).

Firstly, data were analyzed with the fixed effects model in order to combine individual study effect sizes (Cohen’s $d$s) using inverse variance methods to generate a summary $d$ and 95% confidence interval (CI). We analyzed possible association by both comparing LL genotype versus carriers of the S allele and SS genotype versus carriers of the L allele. The significance of the pooled effect sizes was determined by the $Z$-test and the between-study heterogeneity was assessed using a $\chi^2$ test of goodness of fit and $I^2$ statistic [30]. The significant $p$ value was set at 0.05. In a fixed effects model, the fundamental assumption is that a single true effect size underlies all study results and that observed estimates vary only as a function of chance. The error term in a fixed effects model represents only within-study variation, and between–study variation is ignored.

Where the results showed a significant effect in the presence of significant between-study heterogeneity, a random effects model was utilized, with $d$s pooled using the DerSimonian and Laird methods [31]. In contrast, a random effects model assumes that each study estimates different, yet related, true effects and that the distribution of the various effects is normally distributed around a mean effect size value. This model takes both within- and between-study variation into account. When there is little heterogeneity, both models yield essentially identical results. When heterogeneity is extensive, however, the analyses will yield different estimates of the mean effect size, and the confidence intervals around the estimates are different sizes. When there is heterogeneity across studies, the random effects model yields wider confidence intervals than the fixed effects model and is thus usually more conservative.
RESULTS

New study on 5-HTTLPR and 5-HTTLPR/rs25531

In the whole sample of 287 healthy volunteers, the minor S allele frequency of the 5-HTTLPR polymorphism was 0.39; the genotype frequencies and HA (mean score +/- SD) of LL, LS, and SS were 0.37 (43.42 +/- 17.14), 0.48 (42.55 +/- 17.96) and 0.15 (48.57 +/- 20.18), respectively (Table 1a). The genotype distributions were in the HWE ($\chi^2 = 0.05; p = 0.82$). The results indicated an association trend between 5-HTTLPR and anxiety-related scale for genotypes ($p = 0.06$), and a significant effect was found when we considered the L allele as dominant ($p = 0.02$). Concerning the analysis of the 5-HTTLPR/rs25531 the ANCOVA results showed an effect using a dominant L model (L’L’ + L’S’ vs. S’S’ $p = 0.05$, Table 1b). Based on the assessment performed by means of M.I.N.I., the sample consisted of 229 (80%) subjects without and 58 subjects (20%) with lifetime DSM-IV Axis I disorders. In the disordered group, 38 subjects had MDD, 2 subjects had Panic Disorder, 22 had Generalized Anxiety Disorder, and 6 had Dysthymia (the total number exceeds the number of subjects due to the presence of comorbidity), 1 was affected by Bipolar Disorder, 1 subject had alcohol abuse and 1 substance abuse. Because the literature has largely shown that people affected by unipolar major depression and anxiety disorders present homogeneous patterns of personality traits compared to other subjects [22-25, 32-35], the 55 participants with depression and/or anxiety lifetime diagnosis were regrouped.

Thus, in order to evaluate whether the results from participants excluded by M.I.N.I. influenced the previous analyses, we carried out a ANCOVA using HA score as the
dependent variable, groups (healthy and disordered), genotypes, and sex as independent variables, and age as a covariate for both the 5-HTTLPR and the estimated/phased haplotypes. The results indicated that, the disordered group showed significantly higher HA scores than healthy subjects (F = 46.72, p < 0.0001). No association between the polymorphism and anxiety traits was found (F = 1.34, p = 0.26), while a significant interaction was observed between the 5-HTTLPR genotype and groups (F = 4.52, p = 0.03). The same pattern was obtained when the SS genotype was compared to allele L carriers (F = 4.41, p = 0.04; Fig. 1). Concerning the 5-HTTLPR/rs25531, a significant interaction was detected with the dominant L model (L’L’ + L’S’ vs. S’S’; p = 0.02).

Consequently, in the sample of subjects without any DSM-IV axis I disorders (health subjects), we carried out a ANCOVA analysis for testing the possible association between polymorphisms and HA showing no significant results (Tab 1a and 1b).

**Meta-analysis of 5-HTTLPR polymorphism**

A total of 50 studies [3, 19, 36-84] met our inclusion criteria and their features are shown in Table 2. Six studies were excluded from our analysis for significant deviation from HWE (p ≤ 0.05) [42, 48, 56, 61, 70, 77], while one was excluded for excessive ethnic heterogeneity [52]. Furthermore, nine other studies [46, 58, 59, 63, 64, 67, 72, 74, 82] were not included because data regarding anxiety traits for each genotype and/or data to test HWE were insufficient, and we were unable to obtain these information from the authors.

The meta-analysis was therefore computed using the results of 35 studies; including seven studies [37, 45, 51, 55, 65, 80, 81] that reported data for both inventories, another
that generated data regarding NEO on two different independent samples, and the data of present work, a total of 44 samples were available for analysis.

Because of the ethnic differences in 5-HTTLPR genotype distribution, the studies on Asian and Caucasoid populations were analyzed independently. In the Caucasoid population, when we conducted a comparison analysis between the LL genotype and S allele carriers (Fig. 2), no association was observed between 5-HTTLPR and HA (p = 0.94) and no evidence of between-study heterogeneity was apparent. A significant association with N (p < 0.01) indicating higher anxiety-trait score in the S allele carriers group and evidence of strong significant between-study heterogeneity (p < 0.0001, I² = 74%) were found. When the analysis was rerun using the random effects method, the significant effect just described was not significant anymore. No evidence for an association between the 5-HTTLPR genotype and N (p = 0.09) as well as no overall effect (p = 0.11) was shown.

When we tested the L allele carriers versus SS genotype in the same ethnic population (Fig. 3), no association was found between 5-HTTLPR and neither HA nor N, and no evidence of between-study heterogeneity existed. Instead, a significant overall association effect was obtained (p = 0.03), and the two subgroups did not show significant differences ($\chi^2 [1] = 0.01$, p = 0.95, I² = 0%).

Concerning the analyses in the Asian populations, no association was observed between LL genotype versus carriers of the S allele both using TCI (d = -0.01, 95% CI = -0.24, 0.22, Z = 0.10, p = 0.92) and NEO (d = -0.15, 95% CI = -0.54, 0.24, Z = 0.75, p = 0.46). In clustering of the L allele carriers versus SS genotype, no significant evidence of an association between 5-HTTLPR with either HA or N (d = -0.06, 95% CI = -0.16, 0.04, Z
= 1.13, p = 0.26; d = -0.12, 95% CI = -0.29, 0.05, Z = 1.38, p = 0.17; respectively) was observed. We did not find between-study heterogeneity in any groups.

Because of the inherent bias of a mix of healthy subjects with depressed or anxiety symptomatic people, we carried out a meta-analysis including only the studies with structured psychiatric interview screening [38, 39, 55, 62, 69, 71, 84]. No significant result was found when we considered an L dominant model (TCI: d = 0.00, 95% CI -0.12, 0.12, Z = 0.01, p = 1.00; NEO: d = -0.02, 95% CI -0.22, 0.18, Z = 0.19, p = 0.85; Overall effect p = 0.92) as well as taking into account the recessive model (TCI: d = -0.10, 95% CI = -0.25, 0.04, Z = 1.40, p = 0.16; NEO: d = -0.12, 95% CI = -0.39, 0.14, Z = 0.93, p = 0.35; Overall effect p = 0.09).

DISCUSSION

The present study demonstrates the relevance of employing more stringent inclusion/exclusion criteria in association studies on healthy subjects. Our results indicates the influence of a mistaken selection, underscoring the importance of use of a structured psychiatric interview when people are enrolled as control samples for this class of study. Indeed, when we performed the analyses on the whole sample of 287 healthy volunteers, an effect on the susceptibility to HA were found for the SS genotype and S’S’ haplotypes. However, when people that suffered from depression or anxiety disorders, assessed by M.I.N.I., were excluded from the analysis, these associations were lost. These data are largely consistent with previous studies that used structured psychiatric screening for enrolling subjects [38, 55, 62, 71, 84], supporting a lack of involvement of 5-
HTTLPR in anxiety-related traits. Also with respect to the 5-HTTLPR/rs25531, our data are congruent with those reported in Kazantseva study [44].

In addition, we have conducted a meta-analysis involving about eighteen thousands healthy subjects, among Caucasoid and Asian populations, considering anxiety traits measured by TPQ/TCI or NEO. The results confirmed an association of S allele in homozygosity with higher scores of anxiety-related traits, measured by NEO and TCI in Caucasoid, but whatever we executed the analysis including only the studies using a structured psychiatric screening, no association was found.

Moreover, when we tested the dominant L model, our results indicated an association between the 5-HTTLPR and anxiety-related traits, showing a significant overall effect and no differences between the two subgroups (NEO and TCI). Although the authors of a previous meta-analysis [18] hypothesized that the type of inventory used could play a crucial role in detecting an association, our data from meta-analysis did not support this hypothesis, instead showing that both TCI and NEO are useful tools for measuring anxiety traits in genetic studies.

Because it is noteworthy that the S allele is much more prevalent in Asians than in Caucasians [37, 45, 54], suggesting that major ethnic differences may be a potential confounding factor in association studies of the 5-HTTLPR genotype, we have conducted separate analyses for both populations to avoid biased conclusions. No significant association was found between 5-HTTLPR and both N or HA.

In general, the majority of studies enrolled volunteers without information about psychiatric life-time history. Several lines of evidence support that several psychiatric disorders are associated with higher anxiety-trait scores [22-25, 32-35, 85, 86]. In our
sample in which subjects have been excluded for depressive and anxiety symptomatology by means of a structured psychiatric interview, an association between SS genotype and HA was found, despite the small size of the group. Similarly, we observed a significant effect of S’S’ haplotypes in the 5-HTTLPR/rs25531 analyses. These results are in agreement with those obtained in the whole sample where no screening was conducted and this supports the hypothesis that the absence of a psychiatric screening interview might represent an important confounding variable in studies regarding the biological basis of personality traits in healthy populations. Moreover, the results on subjects with depressive and anxiety symptomatology and homozygotes for the S allele support the current literature showing that this allele is a risk factor for Major Depression/anxiety spectrum disorders [5, 6].

Concerning the meta-analysis, when we carried out further analyses including studies with structured psychiatric screening [38, 39, 55, 62, 69, 71, 84], no association of the 5-HTTLPR with anxiety traits was observed. However, these analyses included few studies and further evidence is needed to clarify this issue.

In summary, this study supports the following conclusions: 1. the lack of structured psychiatric screening of the subjects might represent an important bias in genetic association studies with personality traits in healthy subjects. The symptomatology of depressive and anxiety disorders could interfere with anxiety-related traits in the possible associations with the serotonin transporter and also the higher frequency of the S allele observed in depressed and anxiety disorder patients; 2. the SLC6A4 gene is not involvement in anxiety-related traits measured by TCI and NEO in psychiatrically
healthy subjects; 3. the S allele was confirmed to be risk factor for major depression/anxiety spectrum disorders.

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**Conflict of Interest:** The authors declare no conflict of interest.

**References**


Figures legend:

Figure 1: Harm Avoidance scores in healthy and disordered groups for the carriers L allele and SS homozygotes. The disordered subjects with SS genotype show higher HA scores (p = 0.04).

Figure 2: Meta-analysis of association studies of serotonin transporter gene and anxiety-related personality traits measured by NEO and TCI in Caucasoid population. It was used fixed effects model testing the comparison between LL genotype versus carriers S allele. Bars represent individual study 95% CI, with a central block proportional to study effect size, while summary diamond bar represents the pooled effect size estimate and 95% CI.

Figure 3: Meta-analysis of association studies of serotonin transporter gene and anxiety-related personality traits measured by NEO and TCI in Caucasoid population. It was used fixed effects model testing the comparison between carriers L allele
versus SS genotype. Bars represent individual study 95% CI, with a central block proportional to study effect size, while summary diamond bar represents the pooled effect size estimate and 95% CI.
1.3.1 TCI

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>LL genotypes</th>
<th>Carriers S allele</th>
<th>Std. Mean Difference IV, Fixed, 95% CI</th>
</tr>
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<tbody>
<tr>
<td>Comings 2000</td>
<td>47.03</td>
<td>11.34</td>
<td>62 0.4% -0.09 [-0.61, 0.42]</td>
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<tr>
<td>Ebstein 1997</td>
<td>12.86</td>
<td>5.85</td>
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<td>Gonda 2009</td>
<td>15.69</td>
<td>5.59</td>
<td>62 1.2% -0.17 [-0.48, 0.14]</td>
</tr>
<tr>
<td>Herbst 2000</td>
<td>11.2</td>
<td>6.5</td>
<td>148 3.0% 0.14 [-0.06, 0.34]</td>
</tr>
<tr>
<td>Jacob 2004</td>
<td>15.07</td>
<td>6.38</td>
<td>101 2.0% -0.01 [-0.25, 0.23]</td>
</tr>
<tr>
<td>Katsantera 2008</td>
<td>8.97</td>
<td>4.73</td>
<td>69 1.6% 0.00 [-0.27, 0.27]</td>
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<tr>
<td>Mazzanti 1998</td>
<td>11.2</td>
<td>5.8</td>
<td>68 1.4% 0.01 [-0.28, 0.30]</td>
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<tr>
<td>Montealeone 2006</td>
<td>91</td>
<td>9.6</td>
<td>22 0.5% -0.17 [-0.65, 0.31]</td>
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<tr>
<td>Munafò 2008</td>
<td>13.99</td>
<td>6.21</td>
<td>1352 27.0% 0.00 [-0.07, 0.07]</td>
</tr>
<tr>
<td>Nilsson 2007</td>
<td>13.03</td>
<td>6.37</td>
<td>62 1.3% 0.10 [-0.20, 0.41]</td>
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<tr>
<td>Osher 2000</td>
<td>11.81</td>
<td>6.8</td>
<td>36 0.8% -0.40 [-0.78, 0.02]</td>
</tr>
<tr>
<td>Present Study 2010</td>
<td>41.63</td>
<td>15.66</td>
<td>93 1.7% 0.18 [-0.08, 0.45]</td>
</tr>
<tr>
<td>Ricketts 1998</td>
<td>10.85</td>
<td>5.05</td>
<td>13 0.2% -0.62 [-1.31, 0.07]</td>
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<td>Saiz 2010</td>
<td>16</td>
<td>6.4</td>
<td>122 2.6% -0.07 [-0.28, 0.14]</td>
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<td>Samochowiec 2001</td>
<td>17.5</td>
<td>6.1</td>
<td>41 0.8% 0.28 [-0.09, 0.66]</td>
</tr>
<tr>
<td>Samochowiec 2004</td>
<td>15.23</td>
<td>5.67</td>
<td>44 0.8% 0.20 [-0.20, 0.59]</td>
</tr>
<tr>
<td>Schmitz 2007</td>
<td>15.01</td>
<td>6.98</td>
<td>143 3.1% -0.12 [-0.32, 0.08]</td>
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<td>Serretti 2006</td>
<td>20.7</td>
<td>7.1</td>
<td>38 0.8% 0.10 [-0.28, 0.47]</td>
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<td>Szekely 2004</td>
<td>14.62</td>
<td>6.66</td>
<td>53 1.1% 0.01 [-0.32, 0.35]</td>
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<td>Vorfelde 2006</td>
<td>13.1</td>
<td>6.5</td>
<td>36 0.9% -0.10 [-0.46, 0.26]</td>
</tr>
</tbody>
</table>

Subtotal (95% CI) 2545 5214 51.9% -0.00 [-0.05, 0.05]

Heterogeneity: Chi² = 19.05, df = 19 (P = 0.45); I² = 0%
Test for overall effect: Z = 0.08 (P = 0.94)

1.3.2 NEO

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>LL genotypes</th>
<th>Carriers S allele</th>
<th>Std. Mean Difference IV, Fixed, 95% CI</th>
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<td>Brummett 2003</td>
<td>44.8</td>
<td>9</td>
<td>35 0.7% 0.53 [0.11, 0.95]</td>
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<td>Dragan 2006</td>
<td>19.73</td>
<td>9</td>
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<td>Du 2000</td>
<td>40.42</td>
<td>9.24</td>
<td>60 1.2% -0.06 [-0.36, 0.25]</td>
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<td>Flory 1999</td>
<td>50.5</td>
<td>10.8</td>
<td>76 1.5% 0.10 [-0.17, 0.38]</td>
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<td>Hu 2000</td>
<td>54.65</td>
<td>11.73</td>
<td>234 4.9% -0.30 [-0.45, -0.14]</td>
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<td>98.18</td>
<td>23.17</td>
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<td>Lang 2004</td>
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<td>5.74</td>
<td>85 1.6% -0.27 [-0.54, -0.00]</td>
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<td>11.5</td>
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<td>Osher 2000</td>
<td>85.1</td>
<td>24.6</td>
<td>35 0.8% -0.28 [-0.67, 0.10]</td>
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<tr>
<td>Samochowiec 2004</td>
<td>5.11</td>
<td>2.06</td>
<td>44 0.8% 0.04 [-0.36, 0.43]</td>
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<td>Schmitz 2007</td>
<td>2.75</td>
<td>0.68</td>
<td>71 1.8% -0.24 [-0.49, 0.02]</td>
</tr>
<tr>
<td>Terracciano A 2009</td>
<td>56.4</td>
<td>8.65</td>
<td>1026 23.2% -0.00 [-0.08, 0.07]</td>
</tr>
<tr>
<td>Terracciano B 2009</td>
<td>48.4</td>
<td>8.71</td>
<td>191 3.8% 0.13 [-0.05, 0.30]</td>
</tr>
<tr>
<td>Vorfelde 2006</td>
<td>86.5</td>
<td>26</td>
<td>36 0.9% -0.15 [-0.51, 0.22]</td>
</tr>
</tbody>
</table>

Subtotal (95% CI) 2257 5532 48.1% -0.07 [-0.12, -0.02]

Heterogeneity: Chi² = 49.41, df = 13 (P < 0.00001); I² = 74%
Test for overall effect: Z = 2.76 (P = 0.006)

Total (95% CI)

<table>
<thead>
<tr>
<th>LL Genotypes</th>
<th>Carriers S allele</th>
<th>Std. Mean Difference IV, Fixed, 95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>4802</td>
<td>10746</td>
<td>100.0% -0.03 [-0.07, -0.00]</td>
</tr>
</tbody>
</table>

Heterogeneity: Chi² = 72.19, df = 33 (P < 0.0001); I² = 54%
Test for overall effect: Z = 1.97 (P = 0.05)

Test for subgroup differences: Chi² = 3.73, df = 1 (P = 0.05), I² = 73.2%
2.2.2 TCI

Study or Subgroup                  | Carriers L allele Mean | Carriers L allele SD | Carriers L allele Total | SS genotypes Mean | SS genotypes SD | SS genotypes Total | Weight | Std. Mean Difference IV, Fixed, 95% CI
---|---|---|---|---|---|---|---|---|---
Comings 2000                     | 47.83 | 11.66 | 58 | 48.61 | 11.89 | 23 | 0.6% | -0.07 [-0.55, 0.42] |
Ebstein 1997                      | 12.51 | 9.34  | 89 | 13.15 | 4.58  | 32 | 0.9% | -0.08 [-0.48, 0.33] |
Gonda 2009                        | 16.2  | 6.12  | 145| 16.79 | 6.47  | 24 | 0.8% | -0.10 [-0.53, 0.34] |
Herbst 2000                       | 10.8  | 6.3   | 346| 10.2  | 6.3   | 79 | 2.5% | 0.10 [-0.15, 0.34]  |
Jacob 2004                        | 15.3  | 6.44  | 24 | 14.8  | 6.97  | 40 | 1.4% | 0.08 [-0.26, 0.41]  |
Katsantera 2008                   | 8.9   | 4.62  | 209| 9.09  | 4.74  | 92 | 2.5% | -0.04 [-0.29, 0.20] |
Mazzanti 1998                     | 10.9  | 5.65  | 174| 11.7  | 4.9   | 41 | 1.3% | -0.14 [-0.49, 0.20] |
Monteleone 2006                   | 90.75 | 10.55 | 61 | 95.5  | 13.2  | 33 | 0.8% | -0.41 [-0.84, 0.02] |
Munafò 2008                       | 14    | 6     | 6  | 321   | 6     | 661| 21.6%| 0.00 [-0.08, 0.08] |
Nilsson 2007                      | 12.33 | 6.68  | 152| 13    | 7.18  | 44 | 1.3% | -0.10 [-0.43, 0.24] |
Osher 2000                        | 13.09 | 6.59  | 109| 14.62 | 6.93  | 39 | 1.1% | -0.23 [-0.58, 0.14] |
Present Study 2010                 | 40.25 | 16.25 | 202| 37.24 | 12.11 | 27 | 0.9% | 0.19 [-0.21, 0.59] |
Ricketts 1998                     | 11.78 | 6.01  | 27 | 16.8  | 6.36  | 10 | 0.3% | -0.80 [-1.56, -0.05]|
Saiz 2010                         | 16.05 | 6.25  | 314| 16.8  | 6.6   | 90 | 2.8% | -0.12 [-0.35, 0.12] |
Samochowiec 2001                  | 16.45 | 6.25  | 108| 16.1  | 5.9   | 18 | 0.6% | 0.06 [-0.44, 0.56] |
Samochowiec 2004                  | 14.5  | 6.12  | 88 | 14.3  | 5.34  | 12 | 0.4% | 0.03 [-0.57, 0.64] |
Schmitz 2007                      | 15.25 | 7.17  | 267| 16.28 | 7.58  | 143| 3.7% | -0.14 [-0.34, 0.06] |
Serretti 2006                     | 20.05 | 6.9   | 103| 20.6  | 7.5   | 29 | 0.9% | -0.08 [-0.49, 0.33] |
Szekely 2004                      | 14.57 | 6.44  | 122| 14.55 | 5.8   | 29 | 0.9% | -0.12 [-0.41, 0.17] |
Vorfelder 2006                    | 13.28 | 6.5   | 6  | 126   | 14.05 | 6  | 1.8% | 0.00 [-0.40, 0.41] |

Subtotal (95% CI)                | 6152  | 1535 | 47.3%| -0.04 [-0.10, 0.01] |

Heterogeneity: $\chi^2 = 14.12$, df = 19 ($P = 0.78$); $I^2 = 0$
Test for overall effect: $Z = 1.45$ ($P = 0.15$)

2.2.3 NEO

Study or Subgroup                  | Carriers L allele Mean | Carriers L allele SD | Carriers L allele Total | SS genotypes Mean | SS genotypes SD | SS genotypes Total | Weight | Std. Mean Difference IV, Fixed, 95% CI
---|---|---|---|---|---|---|---|---|---
Brummell 2003                     | 42.5  | 8.9   | 86 | 39.8  | 9.2   | 13 | 0.4% | 0.30 [-0.29, 0.88] |
Dragan 2006                       | 22.29 | 9.15  | 174| 24.23 | 7.14  | 22 | 0.8% | -0.22 [-0.66, 0.23] |
Du 2000                           | 40.18 | 8.87  | 146| 41.98 | 10.32 | 40 | 1.2% | -0.19 [-0.55, 0.18] |
Flory 1999                        | 50.95 | 10.45 | 188| 47.5  | 9.4   | 37 | 1.2% | -0.33 [-0.02, 0.69] |
Hu 2000                           | 56.6  | 11.84 | 624| 57.67 | 11.46 | 135| 4.4% | -0.09 [-0.28, 0.10] |
Jacob 2004                        | 96.18 | 24.41 | 241| 95.89 | 31.49 | 40 | 1.4% | 0.01 [-0.32, 0.35] |
Lang 2004                         | 29.39 | 6.67  | 187| 31.6  | 7.2   | 41 | 1.3% | -0.33 [-0.66, 0.01] |
Lesch 1996                        | 54.8  | 11.75 | 410| 56.3  | 11.2  | 95 | 3.0% | -0.13 [-0.35, 0.09] |
Osher 2000                        | 88.95 | 24.2  | 105| 91.5  | 25.6  | 39 | 1.1% | -0.10 [-0.47, 0.26] |
Samochowiec 2004                  | 5.1   | 2.28  | 88 | 4.97  | 2.01  | 12 | 0.4% | 0.06 [-0.55, 0.66] |
Schmitz 2007                      | 2.82  | 0.72  | 267| 2.96  | 0.82  | 143| 3.7% | -0.18 [-0.39, 0.02] |
Terracciano A 2009                | 56.4  | 12.76 | 2946| 56.5  | 8.71  | 967| 28.7%| -0.01 [-0.08, 0.06] |
Terracciano B 2009                | 47.35 | 12.37 | 447| 47.5  | 8.64  | 101| 3.3% | -0.01 [-0.23, 0.20] |
Vorfelder 2006                    | 87.75 | 24    | 126| 90.5  | 20.5  | 69 | 1.8% | -0.12 [-0.41, 0.17] |

Subtotal (95% CI)                | 6035  | 1754 | 52.7%| -0.04 [-0.10, 0.01] |

Heterogeneity: $\chi^2 = 13.85$, df = 13 ($P = 0.38$); $I^2 = 6$
Test for overall effect: $Z = 1.62$ ($P = 0.11$)

Total (95% CI)                    | 12187 | 3289 | 100.0%| -0.04 [-0.08, -0.00] |

Heterogeneity: $\chi^2 = 27.98$, df = 33 ($P = 0.72$); $I^2 = 0$
Test for overall effect: $Z = 2.17$ ($P = 0.03$)
Test for subgroup differences: $\chi^2 = 0.00$, df = 1 ($P = 0.95$); $I^2 = 0$
Additional files provided with this submission:

Additional file 1: Table 1a and 1b.doc, 60K
http://www.biomedcentral.com/imedia/1301620041431030/supp1.doc

Additional file 2: Table 2.doc, 114K
http://www.biomedcentral.com/imedia/5567702944310302/supp2.doc