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5-HTTLPR Genotype and Anxiety-Related Personality Traits: A meta-analysis and new data

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Abstract

We investigated the strength of evidence for association of the 5-HTTLPR polymorphism and the personality trait of Harm Avoidance. We used new primary data from a large sample of adults drawn from the Finnish population. We also applied meta-analytic techniques to synthesize existing published data. The large number studies of the 5-HTTLPR polymorphism allowed us to apply a formal test of publication bias, as well as formally investigate the impact of potential moderating factors such as measurement instrument.

Univariate ANOVA of primary data (n = 3,872), with 5-HTTLPR genotype as a between-groups factor, indicated no evidence of association with Harm Avoidance (p = 0.99). Meta-analysis indicated no evidence of significant association of 5-HTTLPR with Harm Avoidance (d = 0.02, p = 0.37), or EPQ Neuroticism (d = 0.01, p = 0.71), although there was evidence of association with NEO Neuroticism (d = 0.18, $p < 0.001$).

Our analyses indicate that the 5-HTTLPR variant is not associated with Harm Avoidance. Together with our previous analyses of a large sample of participants with extreme Neuroticism scores (defined by the EPQ), we have data that excludes a meaningful genetic effect of the 5-HTTLPR on two measures of anxiety-related personality traits. There remains the possibility that the variant influences the NEO personality questionnaire measure of Neuroticism. However, a large, well-powered primary study is required to test this hypothesis directly and adequately.

Keywords

5-HTTLPR; Genotype; Meta-Analysis; Neuroticism; Harm Avoidance

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Introduction

While twin studies have shown that individual differences in personality traits are substantially influenced by genetic factors (Loehlin 1993), identifying the specific genetic variants that are responsible for the variation in personality has proved to be difficult (Munafo et al. 2003). Theories of personality propose that individual differences in personality traits are associated with individual differences in activity in specific neurotransmitter pathways (Depue and Collins 1999), and as a result various candidate genes with known functional effects on these pathways have been investigated (Munafo et al. 2003). Amongst these, the serotonin transporter gene is one of the most widely investigated, particularly in relation to anxiety-related personality traits (Munafo et al. 2003). However, despite a very large number of published studies, there remains uncertainty regarding the strength and nature of any association (Munafo et al. 2005a).

Anxiety-related traits are continuously distributed dimensions of normal human personality, and are of particular interest in psychiatric genetics as they are predictive of risk of psychiatric disease (Ormel et al. 2004), in particular major depression (Willis-Owen et al. 2005), but may be assessed in the healthy population. These traits can be measured using a variety of questionnaires, of which the most frequently used are derived from Costa and McCrae's (Costa and McCrae 1997) fivefactor model (FFM) of personality (NEO-PI, NEO-PI-R, NEO-FFI), Eysenck's (Eysenck and Eysenck 1991) model of personality (EPQ, EPQ-R) and Cloninger's (Cloninger 1986) tri-dimensional theory of personality (TPQ, TCI). The construct within each of these that corresponds most closely to an anxiety-related trait differs, with Costa and McCrae's FFM and Eysenck's model reflecting a Neuroticism (N) scale, and Cloninger's tri-dimensional theory reflecting a Harm Avoidance (HA) scale. The N construct reflects a tendency to experience negative emotions and interpret situations as threatening (Costa and McCrae 1997), whereas the HA construct is defined as avoidance of aversive stimuli, anxiety proneness and an aversion to risk-taking (Cloninger 1986; Zohar et al. 2003).

There is some evidence that N and HA may not be equivalent. Previous studies have shown that the variance shared by N and HA is between 26% and 55% (De Fruyt et al. 2000). While Costa and McCrae's N correlates highly with Eysenck's N (0.77) (Aluja et al. 2002), it correlates only modestly with Cloninger's HA (0.30) (Curtin et al. 1995), suggesting that measures of N may differ substantially from those of HA. Despite this, studies of the molecular genetic basis of anxiety-related traits have used both measures of N and HA almost equally.

The serotoninergic system has been the principal focus in molecular genetic investigations of emotional behaviour in general, and anxiety-related traits in particular (Lesch et al. 2003). The serotonin transporter (5-HTT) regulates the reuptake of 5-HT at synapses (Heils et al. 1996; Heinz et al. 2000; Lesch et al. 1996), and molecular genetic investigations of this pathway have focused in large part on the role of the serotonin transporter gene. The 5-HTTLPR is a polymorphism in the promoter region of the serotonin transporter gene (Heils et al. 1996), with two common alleles: a long allele with 16 repeats (L) and a short allele with 14 repeats (S). The dominant S allele (Lesch et al. 1996) has been reported to result in lower transcriptional activity than the L allele (Heils et al. 1996), leading to a relative reduction in mRNA levels, serotonin binding, and re-uptake (Little et al. 1998).

Following the first published report of association between the S allele of 5-HTTLPR and increased anxiety-related personality trait score (Lesch et al. 1996), which suggested that this polymorphism may account for up to 4% of phenotypic variance in anxiety-related personality (using both the NEO measure of N and the TPQ / TCI measure of HA), a large

number of studies have attempted to replicate this association. As this literature has grown, and in particular following several failures to replicate, a number of meta-analytic studies have attempted to determine with greater certainty the strength of the evidence for this association. Meta-analysis is an increasingly popular tool for assessing the strength of evidence for gene-phenotype associations, given the likely small effect size of single gene effects, and the resulting probability than most individual studies are likely to lack statistical power (Munafo and Flint 2004). In addition, meta-analytic techniques have the potential to identify unexpected sources of heterogeneity and test for possible publication bias.

We initially reported little evidence of an association between the 5-HTTLPR polymorphism and anxiety-related personality (Munafo et al. 2003). However, two subsequent meta-analyses suggested an association with N (as measured by NEO instruments) but not with HA (Schinka et al. 2004; Sen et al. 2004a). These meta-analyses did not include measures of N derived using EPQ instruments. We subsequently concluded that while the magnitude of the effect appeared to differ by measurement instrument, there was no formal evidence that measurement instrument moderated any association (Munafo et al. 2005a), although various re-analyses indicated that caution is necessary in the use of meta-analytic techniques as methods used to create standardised scores and weight individual studies may bias results (Munafo et al. 2005b; Schinka 2005; Sen et al. 2005).

Given that the genetic effect of the 5-HTTLPR on N is likely to be small, we analysed extreme scoring individuals from two large English populations (n = 88,142, and 20,921) (Willis-Owen et al. 2005). Although the combined sample had sufficient power to detect an effect contributing 0.5% of phenotypic variance at a significance threshold of $p = 0.01$, we were unable to find statistically significant evidence of genetic association. In that study N was measured using the EPQ, leaving open the possibility that there is an effect of 5-HTTLPR on anxiety-related personality traits defined by a different instrument.

In the present study, we investigated the strength of evidence for association of the 5-HTTLPR polymorphism and the TCI measure of HA. We used a large sample of adults drawn from the Finnish population. We also applied established meta-analytic techniques to synthesize existing published data. The large number studies of the 5-HTTLPR polymorphism allowed us to apply a formal test of publication bias, as well as investigate the impact of potential moderating factors such as measurement instrument.

Method

Participants

Participants in this study were part of the Northern Finland Birth Cohort (NFBC 1966), which consists of n = 12,058 live births to mothers in the two northern-most provinces of Finland in 1966 (Rantakallio 1988). DNA samples were collected from n = 5,923 participants within the NFBC 1966 birth cohort. Of these, data were available on a total of n = 3,872 participants who provided a DNA sample, were successfully genotyped for the 5-HTTLPR polymorphism, and completed the TCI measure of HA.

Measures

Participants completed a Finnish version of the TCI (Miettunen et al. 2004). Briefly, the original English version was translated into Finnish, and back-translated blindly to the original English scale, by a professional English translator. The original version and the back-translation were compared and corrections were made accordingly. Full details, including psychometric properties, are reported elsewhere (Miettunen et al. 2004). The TCI defines four personality dimensions: harm avoidance, novelty seeking, reward dependence

and persistence. Harm avoidance is defined as the relative balance of anxiety proneness versus risk taking (Cloninger 1986).

Genotyping

Participants were genotyped for the 5-HTTLPR polymorphism as follows: Amplification of 5-HTTLPR was carried out in a 20 ul reaction containing 40–80 ng genomic DNA, 0.2 mM dATP, 0.2 mM dCTP, 0.2 mM dTTP, 0.1 mM dGTP, 0.1 mM 7-deaza-dGTP (Roche), 0.25 mM of each primer (Heils et al. 1996), 1.5 mM $MgCl_2$, 1X Buffer II (Applied Biosystems), 2% DMSO, and 3.5 U AmpliTaq Gold Polymerase (Applied Biosystems). Thermal cycling conditions included an initial activation at 94 °C for 5 minutes, followed by two cycles of 94 (30s), 66 (30s) and 72°C (1 min), subsequent cycles with decreasing annealing temperature (three cycles of 65 °C [30 s], three cycles of 64 °C [30 s], 35 cycles of 63 °C [30 s]), and a final extension of 72 °C for 10 minutes. Data was collected on an ABI Prism 3700 DNA Analyzer, and analyzed using GeneScan v3.7 and Genotyper v3.6 NT. Scoring the LPR required an exceptionally rigorous procedure. Efforts to score this polymorphism using procedures similar to those employed for other length polymorphisms resulted in genotypes that were not in HWE. Each 5-HTTLPR genotype was independently scored by 3 readers. A fourth reader rescored all data using two filters to resolve the observed HWE discrepancies. The first filter removed scores with peak heights < 200. The second filter removed erroneous scores based on a background peak that was always present at a certain level but not always visible with the eye because of variable peak height of the second peak (allele 2); this filter compared: (peak height1 - peak height2) / peak height 1. Fidelity checks on a sub-set of samples genotyped in duplicate runs indicated a genotyping error rate of < 1%.

Selection of Studies for Inclusion in Meta-Analysis

Association studies of the serotonin transporter gene 5-HTTLPR polymorphism and anxiety-related traits in healthy adults (aged 16 and over) were included in the meta-analysis. Studies included those reporting data on male and female participants of any ethnic origin. Only studies that used validated, standardized, self-report questionnaire measures of HA or N were included. Data collected from clinical populations (including chronic headache, anorexia, alcoholism and organic disease) were excluded. In the case of studies that reported data previously reported elsewhere, the largest sample available was included only.

Search Strategy

The search was performed on two bibliographic databases: PubMed and PsychInfo. The search strategy included keywords of “personality”, “serotonin transporter gene”, “harm avoidance”, “anxiety”, “neuroticism” and “5-HTTLPR”. These databases were searched for appropriate studies from the earliest available data to 31st October 2007. Bibliographies of studies identified by the search strategy were also hand-searched for further relevant studies.

Data Extraction

The primary outcome measure was the mean score of the anxiety-related personality measure grouped by 5-HTTLPR genotype, with genotype grouped as presence (SS or SL) or absence (LL) of the 5-HTTLPR S allele. The data that were extracted included: number of participants in each genotype group, mean anxiety-related trait score and standard deviation by genotype, personality instrument, ancestry, and statement of Hardy-Weinberg equilibrium (HWE). Data from healthy controls were used in studies that assessed both control and clinical populations. If multiple questionnaires were administered to the same participants, results were extracted from all questionnaires, and data were identified as having been extracted from a duplicate sample. In cases where data were not reported in an

appropriate format for inclusion, authors were contacted directly and appropriate summary statistics requested.

Data Analysis

Primary data from the NFBC 1966 were analysed using linear regression models, for both the effect of number of S alleles, and genotype grouped as presence (SS or SL) or absence (LL) of the 5-HTTLPR S allele. This grouping assumes a dominant model of genetic action of the S allele, consistent with existing functional data and the majority of studies to date. We repeated all analyses adjusting for the effects of sex. Unadjusted and adjusted results are reported in the text. Data from all participants (i.e., unselected) were included in these analyses. Power analysis indicated 95% power to detect an effect size of $d = 0.12$, and 80% power to detect an effect size of $d = 0.09$ (in both cases equivalent to less than one point difference on the HA scale of the TCI).

Secondary data from the published literature were analyzed within both a fixed effects and a random effects framework, using Cohen's *d* for each individual study sample, with genotype grouped as presence (SS or SL) or absence (LL) of the 5-HTTLPR S allele. Data from both selected (i.e., extreme-scoring) and unselected study samples were included in these analyses. First we tested for an effect of construct, grouping individual study samples according to whether they measured HA or N. In the case of studies which reported measures of N using *both* EPQ and NEO instruments, data from the NEO instrument only were used. Where there was evidence of a significant difference between the sub-group estimates, we conducted further analyses stratified by construct and, in the case of measures of N, grouped individual study samples according to whether they measured N using questionnaires derived from the EPQ or NEO family of instruments.

Fixed effects analysis was used to combine individual study effect sizes within sub-groups, using inverse variance methods to generate a summary effect size and 95% confidence interval (*CI*). A fixed effects framework assumes that the effect of genotype is constant across studies, and between-study variation is considered to be due to chance or random variation. For the stratified analyses, random effects analyses were used in the presence of significant between-study heterogeneity, with effects sizes pooled using DerSimonian and Laird methods. A random effects framework assumes that between-study variation is due to both chance or random variation and an individual study effect. Random effects models are more conservative than fixed effects models and generate a wider confidence interval. The significance of the pooled effect sizes was determined using a *Z* test. We used a χ^2 test and the I^2 statistic to assess between-study heterogeneity, with the latter providing a measure of the proportion of variation that is explained by between-study variation (Higgins et al. 2003).

For the stratified analyses, where there was evidence of significant association, the effect size of the first published study was compared to the pooled effect size of the remaining studies using a *Z* test, as there is evidence for a substantially greater estimate of effect size in the first published study (Trikalinos et al. 2004). Meta-regression of individual study effect size against year of publication was also conducted, and potential ascertainment bias (e.g., due to publication bias) assessed using the Egger test (Egger et al. 1997).

Statistical power for the primary analysis of NFBC 1966 data was derived by simulation and implemented though a Perl (http://www.perl.com) interface designed for quantitative traits (i.e., HA). This derivation included several basic parameters including the size of sampling population, proportion selected for genotyping, alpha threshold, effect size, and allele frequency. In order to reduce complexity, power was only calculated for those samples lacking any family structure.

Data were analysed using SPSS (v.12) and Comprehensive Meta-Analysis (v.2) statistical software packages. Exact *p*-values are given throughout.

Results

Association Analysis of NFBC 1966 Data

We obtained genotypes for $n = 3,872$ individuals (44% male) born in 1966, from whom questionnaire data were collected in 1997. The mean HA score was 14 (range 0 – 34, $SD = 6$). Genotype frequencies did not deviate significantly from Hardy-Weinberg Equilibrium (SS: 17%, SL: 48%, LL: 35%; $p = 0.61$).

Univariate ANOVA of HA score across all participants, with 5-HTTLPR genotype as a between-groups factor (SS, SL, LL), indicated no evidence of association (F [2, 3869] = 0.01, $p = 0.99$). Grouping individuals with one or more S alleles and controlling for the effect of sex did not alter these results substantially. HA scores grouped by genotype are presented in Table 1.

We estimated power by simulation and found that at significance thresholds of $p = 0.05$ and 0.01 we had 100% power to detect effects of 1% and 0.5% of phenotypic variance. The sample has 63% power to detect an effect of just 0.1% of phenotypic variance at a significance threshold of $p = 0.05$.

Characteristics of Studies Included in Meta-Analysis

A total of 51 studies, including the primary data from the present study, consisting of $k = 64$ study samples, were eligible for inclusion in the meta-analysis. Of these, data were unavailable on $k = 9$ study samples (Bachner-Melman et al. 2005; Cohen et al. 2002; Kim et al. 2005; Kim et al. 2006a; Kremer et al. 2005; Lerman et al. 2000; Park et al. 2004; Schmidt et al. 2000; Thierry et al. 2004), so that a total of 42 studies, comprising $k = 55$ study samples, contributed to the meta-analysis (Ball et al. 1997; Benjamin et al. 2000a; Brummett et al. 2003; Comings et al. 2000; Cruz et al. 1995; Deary et al. 1999; Dragan and Oniszczenko 2006; Du et al. 2000; Ebstein et al. 1997; Flory et al. 1999; Greenberg et al. 2000; Ham et al. 2004; Hamer et al. 1999; Hariri et al. 2005; Herbst et al. 2000; Hu et al. 2000; Jacob et al. 2004; Joo et al. 2007; Jorm et al. 1998; Katsuragi et al. 1999; Kumakiri et al. 1999; Lang et al. 2004; Lanzagorta et al. 2006; Lesch et al. 1996; Mazzanti et al. 1998; Middeldorp et al. 2007; Monteleone et al. 2006; Munafo et al. 2006; Nakamura et al. 1997; Nilsson et al. 2007; Osher et al. 2000; Ricketts et al. 1998; Samochowiec et al. 2001; Samochowiec et al. 2004; Schmitz et al. 2007; Sen et al. 2004b; Stein et al. 2007; Strobel et al. 2000; Szekely et al. 2004; Tsai et al. 2002; Umekage et al. 2003; Vormfelde et al. 2006; Willis-Owen et al. 2005). This included 7 studies, comprising $k = 15$ study samples, that reported data on multiple personality instruments from the same sample (Jacob et al. 2004; Kumakiri et al. 1999; Nakamura et al. 1997; Osher et al. 2000; Samochowiec et al. 2004; Vormfelde et al. 2006; Willis-Owen et al. 2005), and 3 studies, comprising $k = 5$ study samples, that recruited participants based on a selected personality score (Ball et al. 1997; Deary et al. 1999; Willis-Owen et al. 2005). In addition, 1 study comprised unpublished data (Cruz et al. 1995), and 2 studies recruited from both patient and healthy control populations, so that only data from healthy controls were included (Brummett et al. 2003; Monteleone et al. 2006). The characteristics of these studies are described in Table 2.

A further 17 studies were identified by the search strategy but were ineligible for inclusion in the meta-analysis, either because they reported data that were reported elsewhere, or because they reported data on measures of personality other than HA or N (Benjamin et al. 2000b; Golimbet et al. 2006; Gonda and Bagdy 2006; Gonda et al. 2007; Gonda et al. 2006; Gursoy 2002; Gustavsson et al. 1999; Jang et al. 2001; Kim et al. 2006b; Melke et al. 2001;

Mizuno et al. 2006; Munafo et al. 2005c; Murakami et al. 1999; Park et al. 2006; Park et al. 2005; Sher et al. 2000; Sirota et al. 1999).

Meta-Analysis

The initial moderator analysis, excluding one study sample which reported EPQ N data where NEO N data were available for the same sample (Schmitz et al. 2007), did not indicate a moderating effect of construct on the association between 5-HTTLPR genotype and anxiety-related personality. For HA, there was no evidence of significant association (d = 0.02, *95% CI* −0.03, 0.07, Z = 0.90, p = 0.37), and no evidence of between-study heterogeneity (χ^2 [27] = 25.41, p = 0.55, I^2 = 0.00). For N, there was evidence of significant association (d = 0.07, *95% CI* 0.03, 0.11, Z = 3.44, p = 0.001), indicating higher N score in individuals with one or more copies of the S allele, and evidence of significant between-study heterogeneity (χ^2 [27] = 65.30, p < 0.001, I^2 = 58.65). However, these two sub-group effect size estimates did not differ significantly (χ^2 [1] = 2.24, p = 0.14). When the analysis was conducted within a mixed-effects framework, using random effects methods to combine individual study sample effect sizes within sub-groups, these results were not altered substantially, although the evidence for a moderating effect of construct constituted a trend (χ^2 [1] = 3.18, p = 0.08).

Given the trend for a moderating effect of construct in the mixed effects analysis, and the substantial between-study heterogeneity among the study samples which measured N, further exploratory analyses were conducted on study samples that used measures of N. For EPQ instruments, there was no evidence of significant association (d = 0.01, *95% CI* −0.04, 0.06, Z = 0.37, p = 0.71), and no evidence of between-study heterogeneity (χ^2 [7] = 10.57, p = 0.16, I^2 = 0.00). For NEO instruments, there was evidence of significant association (d = 0.18, *95% CI* 0.12, 0.24, Z = 5.57, p < 0.001), indicating higher N score in individuals with one or more copies of the S allele, and evidence of modest between-study heterogeneity (χ^2 [20] = 40.50, p = 0.004, I^2 = 50.62). These two sub-group effect size estimates differed significantly (χ^2 [1] = 17.14, p < 0.001). When the analysis was conducted within a mixed-effects framework, using random effects methods to combine individual study sample effect sizes within sub-groups, the evidence for a moderating effect of instrument was non-significant (χ^2 [1] = 2.60, p = 0.11).

On the basis of evidence of a significant moderating effect of instrument in the fixed effects analysis, and the reduction in between-study heterogeneity observed when study samples that used NEO instruments were considered alone, we conducted further analyses on study samples that used measures of NEO N. When the effect size estimate from the first published study (d = 0.29) (Lesch et al. 1996) was compared to the pooled effect size estimate from the remaining study samples (d = 0.16) there was no evidence that these differed (p = 0.20), and meta-regression did not indicate any evidence of association between year of publication and effect size estimate (p = 0.68). When the pooled effect size estimate from European study samples (d = 0.18) was compared to the pooled estimate from East Asian study samples (d = 0.11) there was no evidence that these differed (p = 0.74). Egger's test indicated evidence of possible ascertainment bias (t [19] = 1.85, p = 0.08), but this was in the *opposite* direction to that which would be predicted if publication bias were operating.

Discussion

Our analyses, both of new primary data drawn from a large, population-based sample and of secondary data analyzed using meta-analytic techniques, indicate that the 5-HTTLPR variant is not associated with Harm Avoidance. These results are broadly consistent with the results of previous meta-analyses (Schinka et al. 2004; Sen et al. 2004a), but extend on these by

including studies that used EPQ instruments, and those employing extreme-score designs, as well as incorporating data from approximately twenty additional studies compared with previous meta-analyses. Together with our previous report (Willis-Owen et al. 2005) of a large sample of participants with extreme Neuroticism scores (measured using the EPQ), we have data that excludes a genetic effect of the 5-HTTLPR on two measures of anxiety-related personality traits. There remains the possibility that the variant influences the NEO personality questionnaire measure of Neuroticism, and our meta-analysis provides support for this.

To address this question we currently are reliant upon relatively small sample sizes in the primary studies that used NEO instruments (albeit a relatively large number of individual studies). The 19 such studies included in our meta-analysis had a median sample size of n = 196, with a maximum of n = 759 subjects (Hu et al. 2000). Our meta-analysis found evidence of association with the 5-HTTLPR, modest between-study heterogeneity, no evidence of publication bias, and no association between individual study effect size and year of publication. This result leaves open the possibility that variation in an anxiety-related personality trait defined by the NEO instrument is influenced by the 5-HTTLPR, although we suggest that a large, well-powered primary study is needed to confirm or refute the observation. Based on the effect size estimate derived from our meta-analysis, this would require a sample size in excess of n = 1,000 to have 80% power at a significance level of p = 0.05. However, although a measurement-specific genetic effect is possible (and indeed plausible, given the imperfect correlation between even the two measures of Neuroticism), it is not clear how useful a genetic effect that is specific to one questionnaire will prove to be.

There are certain limitations to our primary sample which should be considered. First, we did not explicitly control for potential population stratification (for example, by using genomic controls). However, our sample was drawn from a relatively homogeneous population within a geographically-limited region. We therefore regard the risk of population stratification as low. Second, a functional SNP within the 5-HTTLPR variant has been identified which appears to modify the functional consequences of carrying the L allele (Hu et al. 2006). It would have been valuable to have genotyped this variant, although unfortunately resources were not available to allow this. Nevertheless, given the marked absence of an effect of the 5-HTTLPR, we regard it as unlikely that genotyping this additional SNP would have altered our results substantially.

It is worth commenting here on other possible confounds that may have hidden the genetic effect and those that may have contributed to false positive results. Interactions may be to blame here – if the effect attributable to the S and L alleles at the 5-HTTLPR susceptibility depend on the co-occurrence of alleles at other loci in the gene (Parsey et al. 2006) (or elsewhere in the genome), and if those alleles occur at different frequencies in the populations sampled, then inconsistent findings of positive and negative associations may be reported. Similarly, if the effect is only apparent when those carrying the susceptibility allele are exposed to the relevant environment, differences in the prevalence of environmental stressors between study populations is another cause of inconsistent findings. However even if interactions occur, they do not exclude the simplest hypothesis, that there is no main effect attributable to the 5-HTTLPR locus.

A final issue, rarely considered in the literature, is technical. The DNA in which the 5-HTTLPR is embedded has a high GC content (about 70%), and PCR-based assays require modifications to obtain reliable results. We have often observed that, under these conditions, additional, artifactual bands may be produced. We therefore routinely carry out the PCR in duplicate, with case and control participants randomly ordered on the same PCR analysis plate, and have the genotypes called blind to the status of the participant. We have found

that these controls are essential to avoid bias in the genotyping, but we do not know to what extent similar controls are used in other laboratories. It is possible that technical artifacts may explain some of the inconsistencies in the literature.

In conclusion, our results indicate that the 5-HTTLPR variant is not associated with Harm Avoidance, or Neuroticism as measured by the EPQ. There remains the possibility that the variant influences the NEO personality questionnaire measure of Neuroticism. Meta-analysis, while valuable and offering the potential to generate new hypotheses, is not a replacement for adequately-powered primary studies. Therefore, a large, well-powered primary study is required to test this hypothesis directly and adequately.

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Table 1

Harm Avoidance Score by 5-HTTLPR Genotype.

		SS (*n* = 661)	SL (*n* = 1859)	LL (*n* = 1352)
Male	*M*	13	13	13
	SD	(6)	(6)	(6)
Female	*M*	14	15	15
	SD	(6)	(6)	(6)
Total	*M*	14	14	14
	SD	(6)	(6)	(6)

Table 2

Characteristics of Included Studies.

Author	Year	*5-HTTLPR*									Measure	Phenotype	Ancestry	Remarks
		SS			SL			LL						
		(mean)	(SD)	(n)	(mean)	(SD)	(n)	(mean)	(SD)	(n)				
Cruz 1995	1995	13.50	5.70	20	10.30	4.60	26	12.00	5.40	26	EPQ	Neuroticism	European	Unpublished data
Lesch 1996	1996	56.30	11.20	95	56.60	12.00	247	53.10	11.50	163	NEO	Neuroticism	European	
Ebstein 1997	1997	13.15	4.58	32	12.15	6.17	66	12.86	5.90	23	TPQ/TCI	Harm Avoidance	European	
Nakamura 1997 (NEO)	1997	142.99	9.55	128	143.29	10.88	55	137.33	9.45	3	NEO	Neuroticism	East Asian	Duplicate sample
Nakamura 1997 (TPQ/TCI)	1997	18.14	5.98	118	17.21	7.20	52	11.67	3.06	3	TPQ/TCI	Harm Avoidance	East Asian	Duplicate sample
Jorm 1998	1998	4.36	3.50	155	4.49	3.27	350	4.42	3.33	254	EPQ	Neuroticism	European	
Mazzanti 1998	1998	11.70	4.90	41	10.60	5.50	106	11.20	5.80	68	TPQ/TCI	Harm Avoidance	European	
Ricketts 1998	1998	16.80	6.36	10	12.71	6.96	14	10.85	5.05	13	TPQ/TCI	Harm Avoidance	European	
Flory 1999	1999	47.50	9.40	37	51.40	10.10	112	50.50	10.80	76	NEO	Neuroticism	European	
Hamer 1999	1999	13.44	7.07	108	13.75	7.30	336	12.25	7.48	190	TPQ/TCI	Harm Avoidance	European	
Katsuragi 1999	1999	17.53	5.77	66	13.42	6.29	31	17.00	2.94	4	TPQ/TCI	Harm Avoidance	East Asian	
Kumakiri 1999 (NEO)	1999	104.00	22.00	85	104.50	22.00	48	103.60	25.90	11	NEO	Neuroticism	East Asian	Duplicate sample
Kumakiri 1999 (TPQ/TCI)	1999	18.90	6.30	85	18.60	7.20	48	18.40	6.20	11	TPQ/TCI	Harm Avoidance	East Asian	Duplicate sample
Benjamin 2000	2000	13.27	6.04	346	Combined with s/s			12.51	6.01	108	TPQ/TCI	Harm Avoidance	European	
Comings 2000	2000	48.61	11.89	23	48.63	11.97	39	47.03	11.34	19	TPQ/TCI	Harm Avoidance	European	
Du 2000	2000	41.98	10.32	40	39.93	8.49	86	40.42	9.24	60	NEO	Neuroticism	European	
Greenberg 2000	2000	59.02	12.25	66	60.17	11.21	217	56.29	11.65	114	NEO	Neuroticism	European	
Herbst 2000	2000	10.20	6.30	79	10.40	6.10	198	11.20	6.50	148	TPQ/TCI	Harm Avoidance	European	
Hu 2000	2000	57.67	11.46	135	58.55	11.95	390	54.65	11.73	234	NEO	Neuroticism	European	
Lerman 2000	2000	Data Not Available									EPQ	Neuroticism	European	Not included
Osher 2000 (NEO)	2000	91.50	25.60	39	92.80	23.80	70	92.30	24.30	35	NEO	Neuroticism	European	Duplicate sample
Osher 2000 (TPQ/TCI)	2000	14.62	6.93	39	14.37	6.37	73	11.81	6.80	36	TPQ/TCI	Harm Avoidance	European	Duplicate sample
Schmidt 2000	2000	Data Not Available									NEO	Neuroticism	European	Not included
Strobel 2000	2000	15.58	7.34	26	13.55	6.61	69	13.54	6.65	39	TPQ/TCI	Harm Avoidance	European	
Samochowiec 2001	2001	16.10	5.90	18	15.40	6.40	67	17.50	6.10	41	TPQ/TCI	Harm Avoidance	European	
Cohen 2002	2002	Data Not Available									TPQ/TCI	Harm Avoidance	European	Not included

Author	Year	*5-HTTLPR*									Measure	Phenotype	Ancestry	Remarks
		SS			SL			LL						
		(mean)	(SD)	(n)	(mean)	(SD)	(n)	(mean)	(SD)	(n)				
Tsai 2002	2002	14.90	6.40	100	15.00	7.50	71	14.70	7.10	21	TPQ/TCI	Harm Avoidance	East Asian	
Brummett 2003	2003	39.80	9.20	13	40.20	8.80	51	44.80	9.00	35	NEO	Neuroticism	European	Control sample
Umekage 2003	2003	103.90	21.10	103	99.10	15.30	46	101.50	17.10	10	NEO	Neuroticism	East Asian	
Ham 2004	2004	17.94	6.15	93	16.91	6.67	47	16.40	9.10	6	TPQ/TCI	Harm Avoidance	East Asian	
Jacob 2004 (NEO)	2004	95.89	31.49	40	94.18	25.65	140	98.18	23.17	101	NEO	Neuroticism	European	Duplicate sample
Jacob 2004 (TPQ/TCI)	2004	14.80	6.97	40	15.54	6.50	140	15.07	6.38	101	TPQ/TCI	Harm Avoidance	European	Duplicate sample
Lang 2004	2004	31.60	7.20	41	29.90	7.60	102	28.80	5.74	85	NEO	Neuroticism	European	Duplicate sample
Park 2004	2004	Data Not Available									TPQ/TCI	Harm Avoidance	East Asian	Not included
Samochowiec 2004 (NEO)	2004	5.00	2.34	56	Combined with s/s			5.11	20.60	44	NEO	Neuroticism	European	Duplicate sample
Samochowiec 2004 (TPQ/TCI)	2004	14.18	4.93	56	Combined with s/s			15.23	5.67	44	TPQ/TCI	Harm Avoidance	European	Duplicate sample
Sen 2004	2004	87.80	18.86	83	84.50	20.83	183	79.90	19.41	149	NEO	Neuroticism	European	
Szekely 2004	2004	14.55	5.80	29	14.52	6.22	69	14.62	6.66	53	TPQ/TCI	Harm Avoidance	European	
Thierry 2004	2004	Data Not Available									TPQ/TCI	Harm Avoidance	European	Not included
Bachner-Melman 2005	2005	Data Not Available									TPQ/TCI	Harm Avoidance	Other	Not included
Hariri 2005	2005	10.22	5.48	65	Combined with s/s			9.60	5.92	27	TPQ/TCI	Harm Avoidance	European	
Kim 2005	2005	Data Not Available									TPQ/TCI	Harm Avoidance	East Asian	Not included
Kremer 2005	2005	Data Not Available									TPQ/TCI	Harm Avoidance	Other	Not included
Willis-Owen 2005 (singletons)	2005	14.15	9.98	129	13.65	10.09	327	13.22	10.19	274	EPQ	Neuroticism	European	Selected sample
Willis-Owen 2005 (siblings)	2005	12.39	9.03	97	13.98	8.74	254	11.90	9.27	177	EPQ	Neuroticism	European	Selected sample
Willis-Owen 2005 (EPIC)	2005	4.73	4.38	755	4.74	3.84	2029	4.88	4.37	1391	EPQ	Neuroticism	European	Selected sample
Dragan 2006	2006	24.23	7.14	22	24.85	9.30	74	19.73	9.00	100	NEO	Neuroticism	European	
Kim 2006a	2006	16.15	6.58	128	17.82	8.18	81	Combined with s/l			TPQ/TCI	Harm Avoidance	East Asian	Not included
Lanzagorta 2006	2006	13.60	5.60	21	12.70	5.60	21	13.30	5.00	15	TPQ/TCI	Harm Avoidance	European	
Monteleone 2006	2006	95.50	13.20	33	90.50	11.50	39	91.00	9.60	22	TPQ/TCI	Harm Avoidance	European	Control sample
Munafo 2006	2006	14.59	5.94	46	11.71	5.77	129	12.59	5.78	76	EPQ	Neuroticism	European	
Nilsson 2006 (females)	2006	14.58	7.91	26	12.50	7.36	60	14.61	7.06	31	TPQ/TCI	Harm Avoidance	European	
Nilsson 2006 (males)	2006	11.44	6.45	18	10.73	6.59	30	11.45	5.67	31	TPQ/TCI	Harm Avoidance	European	
Joo 2007	2007	17.14	7.11	95	16.59	7.71	54	18.78	4.02	9	TPQ/TCI	Harm Avoidance	East Asian	

Author	Year	*5-HTTLPR*									Measure	Phenotype	Ancestry	Remarks
		SS			SL			LL						
		(mean)	(SD)	(n)	(mean)	(SD)	(n)	(mean)	(SD)	(n)				
Middeldorp 2007	2007	32.62	10.13	203	32.30	10.05	283	32.64	10.77	86	EPQ	Neuroticism	European	
Schmitz 2007 (NEO)	2007	2.96	0.82	143	2.89	0.75	196	2.75	0.68	71	NEO	Neuroticism	European	Duplicate sample
Schmitz 2007 (TPQ/TCI)	2007	16.28	7.58	143	15.49	7.35	196	15.01	6.98	71	TPQ/TCI	Harm Avoidance	European	Duplicate sample
Schmitz 2007 (EPQ)	2007	13.49	5.99	143	12.55	5.81	196	11.23	5.53	71	EPQ	Neuroticism	European	Duplicate sample
Stein 2007	2007	48.62	9.87	68	47.97	9.98	108	47.30	10.38	71	NEO	Neuroticism	European	
Vormfelde 2007 (females) (NEO)	2007	92.00	22.00	38	98.00	21.00	40	92.00	26.00	20	NEO	Neuroticism	European	Duplicate sample
Vormfelde 2007 (females) (TPQ/TCI)	2007	14.50	5.80	38	14.90	5.90	40	15.10	5.50	20	TPQ/TCI	Harm Avoidance	European	Duplicate sample
Vormfelde 2007 (males) (NEO)	2007	91.00	21.00	31	80.00	23.00	50	81.00	26.00	16	NEO	Neuroticism	European	Duplicate sample
Vormfelde 2007 (males) (TPQ/TCI)	2007	13.60	6.20	31	12.00	7.00	50	11.10	7.50	16	TPQ/TCI	Harm Avoidance	European	Duplicate sample
NFBC	2007	13.99	6.18	2520	Combined with s/s			13.99	6.21	1352	TPQ/TCI	Harm Avoidance	European	

Author	Year	*5-HTTLPR*									Measure	Phenotype	Ancestry	Remarks
		SS			SL			LL						
		High	Low	% High	High	Low	% High	High	Low	% High				
Ball 1997	1997	4	10	7.7	30	21	57.7	18	23	34.6	NEO	Neuroticism	European	Selected sample
Deary 1999	1999	24	19	23.5	44	57	44.1	32	28	32.4	NEO	Neuroticism	European	Selected sample