The Serotonin Transporter Gene Length Polymorphism (5-HTTLPR) and Life Events: No Evidence for an Interaction Effect on Neuroticism and Anxious Depressive Symptoms

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The finding of a significant gene by environment interaction effect on depression of the serotonin transporter length polymorphism (5-HTTLPR) and the Number of experienced Life Events (NLE) was not replicated in two large meta-analyses (Munafo et al., 2009; Risch et al., 2009). These meta-analyses have been criticized on the grounds that large studies that get most weight in meta-analyses have the poorest measurement quality of life events and, as a consequence, do not find an effect. Another issue is the time frame across which the NLE are measured. Proximal life events appear to be better predictors of depression than more distal events. We present the results of analyses of the 5-HTTLPR × NLE effect on anxious depression and neuroticism scores in a sample of 1,155 twins and their parents and siblings from 438 families. The interaction effect was tested separately for NLE experienced across the life span and NLE experienced in the past year. There was a significant main effect of NLE on anxious depression and neuroticism, especially when these were experienced in the past year. No interaction with 5-HTTLPR was found for NLE either experienced across the life span or across the past year. Our results support the two recent meta-analyses. Given recent insights from genome wide association studies, it seems more useful to focus on the joint effect of several genes, that are, for example, part of the same biological pathway, in interaction with the environment, than on one candidate gene.

Keywords: anxiety, depression, serotonin transporter gene, life events, gene environment interaction

There has been an ongoing debate regarding the role of gene-environment interaction in psychiatric disorders. One of the most investigated genes is the serotonin transporter gene (SLC6A4 also known as 5-HTT). The finding that the length polymorphism in this gene (5-HTTLPR) does not show a main effect on depression, but that the s-allele increases the risk on depression once an individual is exposed to one or more life events has received most attention (Caspi et al., 2003). Two meta-analyses, including 5 and 14 studies, respectively, yielded no evidence for an effect of 5-HTTLPR in interaction with life events on depression (Munafo et al., 2009; Risch et al., 2009). Especially the meta-analysis of Risch et al. (2009) has received a critical reception. An often mentioned argument to question the conclusions was that the quality of the measurement of life events was poorer in the negative, large studies, which are given most weight in a meta-analysis, than in the positive, often smaller studies (Caspi et al., 2010; Koenen & Galea, 2009; Lotrich & Lenze, 2009; Rieckmann et al., 2009; Risch et al., 2009; Rutter et al., 2009; Schwahn & Grabe, 2009; Uher & McGuffin, 2010).

Another issue is the timeframe across which the number of life events (NLE) is measured. More recent life events appear to be better predictors of depression than more distal events (Monroe & Reid, 2008). We tested the interaction of 5-HTTLPR with lifetime accumulated as well as recent life events in a large sample of twins and their parents and siblings. Part of this data set was presented in previous studies (Middeldorp et al., 2007; Middeldorp et al., 2008; Middeldorp & Boomsma, 2009) and in the meta-analysis of Risch et

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al. (2009). However, in the meta-analysis, the sample was restricted to one member from each family and only a measure of lifetime experienced life events was included (Risch et al., 2009). The current paper presents the results of the interaction analyses between 5-HTTLPR and number of life events (NLE) experienced in the previous year and NLE experienced across the lifetime with anxious depressive symptoms and neuroticism using the whole sample, while correcting for the dependency between measures of family members.

Materials and Methods

Subjects

The data for this study come from the longitudinal survey study of the Netherlands Twin Register (NTR). Sample selection and response rates are described in detail in Boomsma et al. (2002; 2006) and Vink et al. (2004; 2008). Data collected in twins and siblings (in 2000) and twins, siblings and parents (in 2002) were analyzed. Subjects aged between 18 years and 65 years and twins whose zygosity was known were included. For the majority of the twin pairs, zygosity was determined from questionnaire items. Information on zygosity was available from DNA polymorphisms for 726 same-sex twin pairs. The agreement between zygosity diagnoses from questionnaire and DNA data was 97% (Willemsen et al., 2005).

Neuroticism, anxious depression and exposure to life events were measured with self-report surveys. Mean age, scores on neuroticism, and anxious depression and frequencies of experienced life events were comparable in the two surveys. Therefore, the largest possible sample was created by combining the data collected in 2000 and 2002. This led to a sample with cross-sectional data on life events, anxious depression and neuroticism for 3,775 men and 6,303 women.

Genotyped Sample

As part of a project aiming to find the genes underlying the susceptibility to anxiety and depression, a subsample of twins and their family members were approached to provide DNA. Seven hundred and fifty-five men and 1,049 women were genotyped for 5-HTTLPR (Middeldorp et al., 2007).

There were 1,155 subjects with 5-HTTLPR genotypes, data on exposure to life events and either anxious depression (N = 1,154) or neuroticism scores (N = 1,147) including 126 fathers, 135 mothers, 87 monozygotic male twins, 161 monozygotic female twins, 238 DZ male twins/brothers and 408 DZ female twins/sisters from 438 families with a mean number of participants per family of 2.64. Mean age of the participants at the time of completing the survey was 37.5 years (SD = 13.1).

Instruments

Neuroticism was measured with the Amsterdamse Biografische Vragenlijst (ABV) (Wilde, 1970). The ABV neuroticism scale was modeled after the Eysenck Personality Questionnaire (Eysenck & Eysenck, 1964). Anxious depression was measured with the Adult Self Report (ASR) (Achenbach & Rescorla, 2003). Examples of items are: In the last six months 'I cry a lot', 'I am nervous or tense', 'I am unhappy, sad or depressed'. Cronbach's alpha was 0.89. The scores were log transformed following earlier analyses of these data (Boomsma et al., 2000).

An earlier NTR study showed that scores on the anxious depression scale are strongly related to DSM-IV major depression and anxiety disorders (Middeldorp et al., 2006). Subjects diagnosed with one or more of these disorders scored at least one standard deviation higher than subjects without any of these diagnoses. Another study also demonstrated excellent convergence between the anxious depression scale and major depression (Doyle et al., 2007).

In the 2000 and 2002 surveys, a Dutch life event scale (the Schokverwerkings Inventarisatie Lijst = SchIL) (Van der Velden et al., 1992) asked about the experience of the following life events: death of a spouse, father, mother, child, sibling or significant other, serious illness or injury of self or a significant other, divorce/break-up of a relationship, traffic accident, violent and sexual assault and robbery. Response categories were *Never experienced*, *0–6 months ago*, *6–12 months ago*, *1–5 years ago* and *More than five years ago*. The response categories *0–6 months ago* and *6–12 months ago* were combined to *Last year* as too few life events were reported 0–6 months ago to make this a useful variable for the analysis. We analyzed the sum score of life events experienced in the previous year in addition to the sum score of the ever experienced life events.

Genotyping

The 5-HTT regulatory gene region was amplified using a polymerase chain reaction (PCR) of oligonucleotide primers (Gelernter et al., 1997; Greenberg et al., 1999). PCR was performed in a 40 µl volume containing 10 ng of genomic DNA, 0.33 mM of each primer, 0.4 mM deoxynucleotide triphosphates, 2.5% dimethyl sulfoxide (DMSO), 1.6 units of rTaq DNA polymerase (Amersham Biosciences). Initial denaturation at 94 °C for 3 min. was followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 59 °C for 30 s, and extension at 72 °C for 1 min. 30 s. The PCR procedure was terminated by extension at 72 °C for 6 min. Amplified 469/513 bp fragments were electrophoresed on 2% agarose gel and were visualized by ultraviolet illumination upon ethidium bromide staining.

Statistical Analyses

Regression analyses were performed with anxious depression or neuroticism as the dependent variable. First, main effects of NLE in the previous year, NLE ever and 5-HTTLPR genotype (number of short alleles) were tested. Next, the interaction terms of genotype x NLE were added to the model. Sex was included as a covariate. To correct for the dependency of measures of

family members, we used the option 'robust cluster' in STATA 9.2 (StataCorp, College Station, Texas, USA).

Results

The allele frequencies of 57% for the long allele and 43% for the short allele were similar to the frequencies reported by Lesch et al. (1996). The genotype frequencies were 33% homozygotes for the long allele, 19% homozygotes for the short allele and 48% heterozygotes. The null hypothesis of Hardy Weinberg Equilibrium, tested in the total genotyped sample, was not rejected (p = .84).

Table 1 shows the anxious depression and neuroticism scores per genotype and per number of life events in the previous year and ever. The β coefficients (SE) and p values of the main and interaction effects can be found in Tables 2a and 2b. There was a marginally significant main effect of 5-HTTLPR on neuroticism before including the interaction term in the model. The effect became non-significant after including the interaction term. No significant main effect was found for 5-HTTLPR on anxious depression.

Life events experienced in the previous year were significantly related to both anxious depression and neuroticism, before and after including the interaction term. The effect of ever experienced life events was marginally significant before including the interaction term and non significant after including the interaction term.

The interaction terms did not show a significant effect on anxious depression or neuroticism.

Discussion

The current study does not provide evidence for a genotype by environment interaction effect for 5-HTTLPR and NLE on anxious depression or neuroticism scores. This is in line with the results of two meta-analyses (Munafo et al., 2009; Risch et al., 2009) with one including the anxious depression and ever experienced life event data of a subsample of unrelated individuals selected from the sample used in the current study (Risch et al., 2009).

As found in previous studies, there was a significant main effect of life events on anxious depression and neuroticism, especially when these were experienced in the previous year. This supports the view that recent life events have more influence on depression than more distal events (Monroe & Reid, 2008). A main effect of 5-HTTLPR on these traits does not seem likely from the results in our sample. The marginally significant effect on neuroticism is probably due to chance as our previous paper clearly showed that this effect is not consistent across several time points (Middeldorp et al., 2007).

A potential shortcoming of this study is the measurement of life events with a self-report instrument. In depression research, two of the important concerns are mood congruence recall bias and 'effort after meaning' which make depressed subjects more likely to report life events (Caspi et al., 2010; Monroe & Reid, 2008). As our data were originally collected in a longitudinal way, we were able to check whether inconsistencies in life event report; that is, the report of a life event at one time and not at another was related to higher anxious depression scores at the time of the report of the life event. This was not the case (Middeldorp et al., 2008). Thus, it seems that these measurement issues cannot explain the lack of positive results in our study. This does not withstand the fact that measurement and the way life events are analyzed is an important issue in research on life events or stress as carefully outlined by Monroe et al. (2008).

There are two other points, besides the issue of measurement error, which seem important in the whole debate. They have both already been raised by Munafo et al. (2009), but underexposed in the discussion following the two meta-analyses, First, the genetic effect of 5-HTTLPR differs across the positive

Table 1

Log Transformed Anxious Depression and Neuroticism Scores (SD) per 5-HTTLPR Genotype (ss, sl, and ll)

	Anxious depression				Neuroticism			
	N	ss	sl	ll	*N*	ss	sl	ll
N	1154	222	551	381	1147	220	548	379
0 life events previous year	722	18.5 (11.2)	20.1 (10.6)	19.1 (10.4)	715	18.1 (2.8)	18.3 (3.0)	17.7 (3.0)
1 life event previous year	295	21.6 (10.6)	21.4 (9.6)	20.1 (10.6)	295	19.0 (2.8)	18.7 (2.9)	18.3 (3.2)
2 or more life events previous year	137	23.9 (9.1)	23.2 (10.7)	21.1 (10.3)	137	19.4 (2.9)	18.9 (3.2)	18.2 (3.0)
0 life events ever	95	15.5 (12.3)	23.0 (10.2)	18.9 (11.2)	95	17.6 (2.6)	18.4 (3.2)	17.3 (3.3)
1 life event ever	207	19.1 (11.4)	18.7 (10.1)	19.4 (11.9)	206	18.2 (3.1)	17.9 (2.8)	18.4 (3.4)
2 life events ever	271	22.9 (10.6)	20.6 (10.5)	18.7 (10.4)	270	19.2 (3.0)	18.7 (2.9)	17.8 (3.0)
3 life events ever	223	18.3 (10.5)	20.1 (10.7)	18.4 (10.3)	223	18.7 (2.6)	18.4 (2.9)	17.4 (3.0)
4 life events ever	175	20.9 (11.4)	22.4 (9.5)	21.3 (9.6)	172	18.8 (3.0)	18.7 (3.1)	18.0 (2.7)
5 or more life events ever	183	21.6 (9.1)	21.6 (10.7)	21.6 (9.3)	181	18.4 (2.5)	18.6 (3.2)	18.8 (2.8)

Note: Scores are for individuals who were (1) not exposed to a negative life event, such as death of a significant other, serious illness, divorce; (2) exposed to one life event; or (3) exposed to two or more life events in the previous year (above bold line) and for individuals who were not exposed to a negative life events or to 1 to 5 or more life events ever.

Table 2a

Coefficients of Main Effects of NLE in the Previous Year, NLE Across the Lifetime and 5-HTTLPR: Main Effects from Model Without the Interaction Effects

	Anxious depression		Neuroticism	
	β (SE)	*p* value	β (SE)	*p* value
Main effects				
NLE previous year	1.68 (.41)	< .0001	.43 (.13)	.001
NLE lifetime	.52 (.22)	.02	.13 (.06)	.04
5-HTTLPR	.37 (.51)	.47	.35 (.15)	.02

Table 2b

Coefficients Main and Interaction Effects of NLE in the Previous Year, NLE Across the Lifetime and 5-HTTLPR

	Anxious depression		Neuroticism	
	β (SE)	*p* value	β (SE)	*p* value
Main effects				
NLE previous year	2.53 (.73)	.001	.59 (.21)	0.006
NLE lifetime	.56 (.43)	.19	.11 (.11)	.33
5-HTTLPR	.28 (1.02)	.79	.39 (.29)	.17
Interaction effects				
5-HTTLPR x NLE previous year	.81 (.59)	.17	. 15 (.17)	.37
5-HTTLPR x NLE ever	.04 (.30)	.90	.02 (.09)	.84

reports. Additive, recessive as well as dominant effects were all reported. We have also tested dominant and recessive models, but these tests did not yield any significant interaction effect either. Second, it is unlikely to find an interaction effect when no main genetic effect is found with genotype and exposure frequencies similar to 5-HTTLPR and life events. The frequency of subjects exposed to life events is that high that the mean depression score would be sufficiently increased in the group with the short allele to detect a genetic main effect even without taking an interaction effect into account (Munafo et al., 2009).

Another complicating fact is the recent insight following the genome wide association studies that the genetic architecture of psychiatric disorders (and other traits) is probably more complex than previously thought. Effect sizes seem to be smaller than previously expected and several authors have suggested that the heritability of psychiatric traits reflects the influence of a large number of genes, each with a very small effect (Demirkan et al., 2010; Purcell et al., 2009; Yang et al., 2010). In addition, rare variants (minor allele frequency < 0.01%) may also play a more important role (McClellan & King, 2010; Yang et al., 2010). It seems unlikely that genotype × environment interaction effects on depression will be more easily detected than these main effects.

Overall, we agree with the Psychiatric GWAS Consortium (PGC) that genotype by environment interaction research is only useful after a robust association with the genotype has been detected (Psychiatric GWAS Consortium, 2009). Morever, even then, in light of all the issues mentioned above, it seems more useful to focus on the joint effect of several genes, that are, for example, part of the same biological pathway, in interaction with the environment, than on one candidate gene.

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