Social Adversity, the Serotonin Transporter (5-HTTLPR) Polymorphism and Major Depressive Disorder

Paul G. Surtees, Nicholas W.J. Wainwright, Saffron A.G. Willis-Owen, Robert Luben, Nicholas E. Day, and Jonathan Flint

Background: *Recent evidence has suggested that the short allele of the serotonin transporter (5-HTT) gene-linked polymorphic region (5-HTTLPR of the human serotonin gene [SLC6A4]) is associated with increased risk of depressive disorder but only among individuals exposed to social adversity. We report an investigation designed to replicate this finding.*

Methods: *Data were available from a non-clinical sample of 4175 adult men and women, ages 41–80 years, selected from participants in the European Prospective Investigation into Cancer and Nutrition in Norfolk (EPIC-Norfolk, United Kingdom) study. Evidence of past-year prevalent episodic major depressive disorder (MDD), defined by restricted DSM-IV diagnostic criteria, was assessed through questionnaire. Adverse experiences in childhood and in adulthood (during the five years preceding assessment) were also assessed through self-report. The 5-HTTLPR variant was genotyped according to published protocols.*

Results: *One-year prevalent MDD criteria were met by 298 study participants. The experience of social adversity (both in childhood and adulthood) was strongly associated with increased rates of past-year prevalent MDD. No gene by environment (GxE) interactions between the 5-HTTLPR genotype, social adversity, and MDD were observed.*

Conclusions: *This study has not replicated a previous finding of a GxE interaction between the 5-HTTLPR genotype, social adversity, and depression.*

Key Words: Depression, stress, serotonin transporter, 5-HTTLPR, *SLC6A4*, gene-environment interaction

Interactions between genetic loci and the environment are known for a wide variety of biological phenotypes, such as the thalassaemias (Weatherall 2001) and reactive spondyloarthropathies (Scofield et al 1995). Few such interactions, however, have been successfully characterized in relation to human psychiatric phenotypes, despite clear indications from nonhuman mammal research that they exist. For example, investigations of the genetic contribution of the serotonin transporter (5-HTT) to behavioral measures of anxiety in both rodents and nonhuman primates have indicated a moderating role of social adversity, most specifically during early stages of development (Ansorge et al 2004; Bennett et al 2002; Champoux et al 2002).

Recently, Caspi et al (2003) reported that a length polymorphism (*SLC6A4*) in the promoter region of 5-HTT (5-HTTLPR) mediates the influence of stressful life events on human depression. They showed that individuals carrying one or more short (S) allele who were exposed to stressful life events were more likely to develop depression than those homozygous for the long (L) allele. They also reported that childhood maltreatment predicted adult depression only among individuals carrying a copy of the S allele.

This finding is intriguing for a number of reasons. First, as one of the few examples of a gene by environment (GxE) interaction, it provides an opportunity to investigate the phenomenon at a molecular level. Because of its potential importance, Caspi et al have emphasized the need for its replication. Second, the interaction effects reported seem to be present in the absence of a main effect of the *SLC6A4* promoter length polymorphism on depression. Although, historically, studies that have addressed this question in relation to (unipolar) depression have reached inconsistent conclusions, a recent large multicenter case-control study (involving 539 unipolar patients and 821 control subjects; Mendlewicz et al 2004) and a recent meta-analysis (Lasky-Su et al 2005) have both reported no main effect, indicating that the main effect, if present, must be very small. Third, the reported effect was observed on depression occurring in the year before assessment (at age 26) for individuals experiencing stressful life events in the preceding five years. In contrast, the reported impact of life events is typically limited to the 1–3 months preceding the onset of a depressive episode (Brown et al 1973; Kendler et al 1998; Surtees et al 1986).

We now report an attempt to replicate the GxE interaction between *SLC6A4* (5-HTTLPR) genotype, the experience of social adversity, and DSM-IV–defined (American Psychiatric Association 1994) major depressive disorder (MDD) among a large non-clinical sample of men and women participating in the European Prospective Investigation into Cancer and Nutrition in Norfolk (EPIC-Norfolk), United Kingdom study (Day et al 1999).

Methods and Materials

Participants

During 1993–1997, EPIC-Norfolk recruited, through general practice age-gender registers, a total of 30,414 men and women (then) ages 40–74 years and resident in Norfolk, England (Day et al 1999). The study was approved by the Norwich District Health Authority Ethics Committee and all participants gave signed informed consent. During 1996–2000, an assessment of social and psychological circumstances, on the basis of the Health and Life Experiences Questionnaire (HLEQ) (Surtees et al 2003b), was completed by a total of 20,921 participants, representing a response rate of 73.2% of the total eligible EPIC-Norfolk sample (28,582). A sample of 5000 participants was selected from the

From Strangeways Research Laboratory and University of Cambridge Department of Public Health and Primary Care (PGS, NWJW, RL, NED), Worts Causeway, Cambridge; and the Wellcome Trust Centre for Human Genetics (SAGW-O, JF), Headington, Oxford, United Kingdom.

Address reprint requests to Paul Surtees, Strangeways Research Laboratory, Worts Causeway, Cambridge, CB1 8RN, United Kingdom; E-mail: paul.surtees@srl.cam.ac.uk.

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EPIC-Norfolk HLEQ cohort. This sample was originally designed for a study of neuroticism (Willis-Owen, in press) and included 2500 men and 2500 women with DNA available, selected according to extremes of high and low neuroticism scores (assessed through completion of a 12-item scale; Eysenck et al 1985). The boundaries of high and low neuroticism varied by gender (high: scale score ranges 6–12 and 8–12 for men and women, respectively; and low: scale score ranges 0–1 and 0–2; see Willis-Owen, in press for further details). The final study sample consisted of 4175 study participants for whom the *SLC6A4* variant was successfully genotyped.

Mood State Assessment

The HLEQ includes a structured self-assessment approach to psychiatric symptoms embodying restricted DSM-IV (American Psychiatric Association 1994) criteria for MDD. The assessment was designed to identify those EPIC-Norfolk HLEQ participants thought likely to have met a putative diagnosis of MDD at any time in their lives. Where any episode was reported, participants were asked to estimate onset and (if appropriate) offset timings (see Surtees et al 2000 for full details of this assessment). The dependent variable was taken as 1-year prevalence of MDD (i.e., any episode that was either current at the time of or ended within 1 year of HLEQ completion). In addition, this measure was refined according to neuroticism score, with cases defined as those with both 1-year prevalent MDD and high neuroticism, and with control subjects defined as those free of 1-year prevalent MDD and with low neuroticism.

Social Adversity Assessment

The HLEQ included an assessment of social adversity, defined by adverse experience in childhood (0–16 years) and in adulthood (represented by stressful life events and enduring personal difficulties).

Adverse experience in childhood was assessed by the following eight circumstances: separation from mother for more than 1 year; hospital stay for two or more weeks; parental divorce; parental unemployment for several years when they wanted to be working; an experience that was so frightening as to be thought about for years following its occurrence; being sent away from home because of doing something wrong; parental alcohol or drug use sufficient to cause family problems; and experience of physical abuse by someone close. An overall measure of childhood adverse experience was constructed as the total number of circumstances reported (Surtees et al 2003a; Wainwright and Surtees 2002a).

Retrospective assessment of adverse event experience in adulthood was restricted to 16 specific events and a further undefined event of personal significance. Specific events involved serious illnesses (injuries or assaults) experienced by the participant (or a first degree relative), relationship events (concerning separation, divorce, termination of pregnancy), work events (retirement, redundancy, or being fired) and loss experiences (through death of first degree relatives). The undefined event provided an opportunity for the participant to describe (and rate) the lifetime experience of any other particularly stressful experience. Participants were asked to provide details of event timing to within a year of occurrence and a rating of their degree of upset (rated on a four-point scale) associated with each event experienced. These events were selected on the basis of the questionnaire version of the List of Threatening Experiences (LTE-Q) (Brugha et al 1985). Concurrent validity of the LTE-Q assessed against the Life Events and Difficulties Schedule (LEDS; Brown and Harris 1978) has shown both high specificity and sensitivity (Brugha and Cragg 1990) (see Surtees and Wainwright 1998, 2000 for further details). A calendar-based Personal Life Chart (PLC) was designed to allow each participant to record the date, description, and onset/offset times of (up to six) prolonged difficulties in their lives. The design format provided a brief way of representing periods of personal difficulty and stems from the methodological framework of the LEDS (Brown and Harris 1978) approach to the assessment of long-term difficulties adapted for use within a questionnaire format and to record experiences across a lifetime. Only those long-term difficulties reported to have been experienced by the study participant or close family (restricted to spouse/partner, parent, sibling, or child) were included. Adverse experience in adulthood was represented by the total number of moderately or extremely upsetting life events and/or a period of long-term difficulty reported to have been experienced during the past 5 years. In addition, these measures were refined to include only those adverse events and difficulties reported to have been experienced in the year preceding MDD episode onset (or time of HLEQ assessment for those with no 1-year prevalent MDD).

Genotyping

Deoxyribonucleic acid (DNA) was extracted from fresh 9-mL EDTA whole blood samples (Whatman International, Ely, United Kingdom) and arrayed onto 96 well plates. Two oligonucleotide primers (5′-GGCGTTGCCGCTCTGAATGC-3′, 5′-GAGGGACTGAGCTGGACAACCCAC-3′) were used to generate *SLC6A4* allele-specific fragments (484-base pair [bp], 528-bp) (Lerman et al 1998) by polymerase chain reaction (PCR). Genotype was then ascertained by agarose gel electrophoresis of PCR products, and visualized by transillumination. The PCR was initially performed in a 10-μL reaction including 5 μL of DNA template (10 ng), and this was increased to a final volume of 15 μL (16.8 ng DNA) where samples failed to produce amplification. Each 10-μL reaction included 1 μL NH_4 buffer, .25 μL dNTP (40 mmol/L), 2.35 μL 2xPolymate (Bio-Line, London, United Kingdom), 0.3 μL $MgCl_2$ (50 mmol/L), 1 μL primers, and .1 μL BioTaq DNA Polymerase (BioLine). The PCR consisted of a 5-min denature step at 95°C (1 cycle), 95°C for 30 seconds, 62°C for 45 seconds, and 72°C for 1 min (35 cycles), and 72°C for 4 min (1 cycle).

Statistical Analysis

Logistic regression was used to investigate the presence of GxE interaction for past-year prevalent MDD. Models were fit for the main effect of *SLC6A4* (5-HTTLPR) genotype (included as L/L; L/S and S/S, assuming a multiplicative effect for the S allele and, correspondingly, a 1 degree of freedom test of trend [allele test]) and main effect of environment (with childhood adverse experience classified as 0, 1, and 2 or more and adult life events or long-term difficulty experience as 0, 1, 2, 3, and 4 or more included as continuous variables, again with a 1 degree of freedom test of trend), and with subsequent addition of GxE interaction (through inclusion of the cross-product term for a 1 degree of freedom test of multiplicative interaction). All models included gender as a covariate. Secondary analyses were performed to confirm the absence of significant GxE interaction in these data. Analyses were repeated 1) for the refined measure of adverse experience in adulthood (in the year before MDD onset), 2) for lifetime MDD, 3) for the combined measure of past-year prevalent MDD and neuroticism, and 4) for past-year prevalent MDD by gender.

Table 1. Past-year Prevalent Major Depressive Disorder (MDD) According to *SLC6A4* (5-HTTLPR) Genotype and the Number of Adverse Experiences in Childhood

		Adverse Experience in Childhood		
Genotype	MDD[a]	0 (n = 2,103)	1 (n = 1,265)	≥2 (n = 807)
L/L	no	648	372	226
	yes	41	31	31
	%	6.0	7.7	12.1
L/S	no	944	559	331
	yes	51	55	35
	%	5.1	9.0	9.6
S/S	no	348	193	141
	yes	24	18	12
	%	6.5	8.5	7.8

MDD, major depressive disorder.
[a]115 missing values.

Table 3. Odds Ratios (OR) and 95% Confidence Intervals (CI) for the Main Effects of *SLC6A4* (5-HTTLPR) Genotype (G) and Social Adversity (E) and Their Interaction (G×E) (Adjusted for Gender) on Past-year Prevalent Major Depressive Disorder, Where Social Adversity Represented by A) Childhood Adverse Experience and B) Adult Events or Difficulties in the Past Five Years

	A		B	
	OR	95% CI	OR	95% CI
Main Effects				
G (per S allele)	.97	(.82–1.15)	1.00	(.84–1.19)
E (per adverse exposure)	1.37	(1.19–1.59)	1.81	(1.66–1.98)
Interactions				
G×E	.90	(.73–1.11)	.96	(.84–1.09)

Results

A total of 4175 genotype calls were made from a total of 4416 individuals genotyped (a 94.5% genotype call rate). Of the 4175 study participants, 2225 were men and 1950 were women. The age range of the sample was 41–80 years (mean 60.3 years, SD 9.1). Genotype frequencies were: L/L homozygotes, n = 1391 (33.3%); L/S heterozygotes, n = 2029 (48.6%); and S/S homozygotes, n = 755 (18.1%). Allele frequencies were in Hardy-Weinberg equilibrium [$\chi^2(1)$ = .10, p = .75]. The 1-year prevalence of MDD (in this neuroticism-enriched sample) was 7.1% (n = 298) and lifetime MDD prevalence was 18.3% (n = 767).

Past-year prevalence of MDD was 5.6%, 8.5%, and 10.1%, respectively, for those who reported 0, 1, and ≥ 2 adverse experiences in childhood, and was 2.9%, 5.5%, 11.2%, 14.5%, and 26.8%, respectively, for those who reported 0, 1, 2, 3, and ≥ 4 life events or difficulties. No differences were observed by genotype, either in the mean number of reported adverse experiences in childhood (.78 for L/L, .77 for L/S, and .80 for S/S, p = .75) or adverse experiences during adulthood (1.14 for L/L, 1.07 for L/S, and 1.05 for S/S, p = .19).

Table 1 shows the number (and percentage) of study participants with past-year prevalent MDD by *SLC6A4* (5-HTTLPR) genotype and according to the number of adverse experiences in childhood. Table 2 provides the same data according to the number of life events or long-term difficulties experienced during the five years before assessment. Table 3 shows the odds ratios (OR) for the main effect of *SLC6A4* genotype and social adversity and their interaction on past-year prevalent MDD. The table confirms the absence of any main effect for genotype and the presence of strong main effects for environment (as represented by either adverse experience in childhood or adult life events or difficulties) and reveals that no GxE interactions were observed in these data.

Adverse adult events or difficulties were reported in the year before MDD onset/HLEQ completion by 930 participants (732 [17.5%] reported one and 198 [4.7%] reported more than one event and/or difficulty in this 1-year period). This refined measure of adult adverse events showed a stronger association with past-year MDD (OR = 2.11, 95% confidence interval [CI]1.78–2.51 per event) but, again, no GxE interaction with *SLC6A4* (5-HTTLPR) genotype was observed (OR = .91, 95% CI .71–1.17). Nor was there a significant interaction between genotype and adverse events experienced in childhood or between genotype and adult adverse experience (in the past 5 years) on the basis of a lifetime MDD phenotype. Finally, we found no evidence for a significant interaction between genotype and adverse experience in childhood or adult adverse experience for the combined measure of past-year prevalent MDD and neuroticism (n = 276 cases and n = 2018 control subjects).

A significant interaction was observed for past-year prevalent MDD and adverse experience in childhood among men (p = .040) such that adverse experiences in childhood were associated with increased rates of past-year prevalent MDD for L/L homozygotes (OR = 1.69, 95% CI 1.17–2.44) but not for L/S heterozygotes (OR = 1.26, 95% CI .91–1.75) or S/S homozygotes (OR = .82, 95% CI .46–1.48). This finding is in the opposite direction of that previously reported for a GxE interaction (Caspi

Table 2. Past-year Prevalent Major Depressive Disorder (MDD) According to *SLC6A4* (5-HTTLPR) Genotype and the Number of Adult Events and Difficulties Experienced in the Past Five Years

		Adverse Life Events or Long-Term Difficulties				
Genotype	MDD[a]	0 (n = 1,766)	1 (n = 1,127)	2 (n = 680)	3 (n = 373)	≥4 (n = 229)
L/L	no	528	361	191	109	57
	yes	15	19	26	21	22
	%	2.8	5.0	12.0	16.2	27.8
L/S	no	815	503	286	152	78
	yes	21	36	35	21	28
	%	2.5	6.7	10.9	12.1	26.4
S/S	no	315	178	109	51	29
	yes	14	6	13	11	10
	%	4.3	3.3	10.7	17.7	25.6

MDD, major depressive disorder.
[a]115 missing values

et al 2003; Figure 2). No GxE interactions were observed for adverse experience in childhood and past-year prevalent MDD among women or for adult adverse experience and past-year prevalent MDD in either men or women. With Bonferroni correction, according to the number of subgroup analyses performed, the interaction for adverse experience in childhood and past-year prevalent MDD in men was not significant (required p value for nine tests, $p = .006$).

Discussion

This investigation was designed to attempt replication of a recent report of interaction between the experience of social adversity, the *SLC6A4* (5-HTTLPR) genotype, and past-year prevalent MDD (Caspi et al 2003). Our results, on the basis of a sample of 4175 study participants, did not support such a GxE interaction.

To our knowledge, five other human studies, to date, have focused on the relationship between the *SLC6A4* genotype, social adversity, and mood-related phenotypes (Eley et al 2004; Gillespie et al 2005; Grabe et al 2005; Kaufman et al 2004; Kendler et al 2005). Of these studies, one (Gillespie et al 2005) reported no evidence of replication, and four studies provided evidence for replication, though on the basis of different categorizations of genotype (full [Kaufman et al 2004], additive [Eley et al 2004], recessive [Kendler et al 2005], and dominant [Grabe et al 2005]). In addition, two of these studies found a result only when on the basis of a data subset (in both cases in women and not in men [Eley et al 2004; Grabe et al 2005]). Care is required in interpreting studies that only partially replicate an original study finding, because multiple testing can distort the type I error rate. Marginal evidence followed by partial replication does not provide strong evidence of association (Colhoun et al 2003). In addition, all of these replications were on the basis of small samples, of more limited statistical power than the population presented here. Our study carries approximately twice the number of MDD cases as in the original report by Caspi et al (2003); however, sample size requirements for interactions are approximately four times as great as those required for the detection of main effects of the same magnitude (Cooper 2003). Therefore, the current study, as in the other studies cited, remains underpowered to detect interactions other than those of large magnitude (Hwang et al 1994; Smith and Day 1984).

Associations between genotype(s) and complex phenotypes are likely to be of modest effect size and large sample sizes with, perhaps, thousands of cases required to detect and confirm associations at appropriate levels of statistical significance (Keavney et al 2004; Pharoah et al 2004; Zondervan and Cardon 2004). Where large sample sizes have been unavailable, many genetic association studies have employed liberal type I error rates (e.g., 5%) on the basis that, as few variants are studied, the need to correct for multiple comparisons is reduced (Freimer and Sabatti 2004). These studies should be followed by definitive replication (Thomas and Clayton 2004).

This study differs markedly both in design and methodology from the report by Caspi et al (2003). These differences could explain our non-replication of study findings. In particular, participants in the original Dunedin Multidisciplinary Health and Development Study were a representative birth cohort with repeated follow-up assessments with the GxE analyses on the basis of the cohort at age 26 years. In marked contrast, this study was on the basis of a single retrospective questionnaire assessment of a population cohort with age range 41–80. Therefore, consideration needs to be given to the possibility that either the GxE interaction is restricted to younger age groups (for example, one recent partial replication was on the basis of a younger cohort [Kendler et al 2005]) or that the strength of the interaction varies with age and that the large and older age range in this study might have obscured any potentially positive result.

In addition, the assessment of phenotype differed between the two studies. Participants in the birth cohort study were assessed with the Diagnostic Interview Schedule (Robins and Regier 1991) at ages 18, 21, and 26 years. An important consequence of these assessments is that most of the depressive episodes identified in the study were likely to be the participants' first such experience. In contrast, in the present study, a single comprehensive structured self-assessment was employed to provide evidence of each participant's history of MDD episodes (Surtees et al 2000). These data suggest that, of those 298 participants who met one-year prevalent DSM-IV MDD criteria, for only 9.4% was this their first episode, giving further emphasis to the difference between this and the original birth cohort study. In addition, clinical observations and epidemiological evidence have grown to support a "kindling" hypothesis (Post 1992; Post and Weiss 1998) that the strength of the association between social adversity and the onset of MDD decays with progressively increasing number of previous episodes (Kendler et al 2000). Therefore, these observations combine to compound the differences between this study and the original report by Caspi et al (2003). Our sample was enriched by participants meeting MDD criteria through implementation of an extreme-phenotype design to permit evaluation of an associated hypothesis (Willis-Owen, in press). As expected, the 1-year prevalence of MDD was much greater in the extreme high neuroticism group (13.2%) than in the low neuroticism group (1.1%). It remains possible that the absence of individuals with intermediate neuroticism scores might have acted to obscure any GxE interaction.

Our measures of adverse exposure history, both in childhood and adulthood, were reported at rates that closely matched those reported in the original study; however, in the context of a research program designed to investigate the association between diet and chronic disease incidence, the assessment of sexual abuse was considered inappropriate. Availability of the relative timings of measures of MDD onset, offset, and adverse event exposure in adulthood also permitted inclusion of only those events that occurred in the year before episode onset, and importantly, permitted exclusion of event exposures that followed episode onset. This refined measure of adverse exposure, again, provided no evidence for GxE interaction in these data. While population stratification could, theoretically, have impacted on our findings, this is unlikely to be an important consideration (Cardon and Palmer 2003), given the design characteristics of the EPIC-Norfolk HLEQ study.

We acknowledge that study limitations might follow from the collection of episodic MDD state and social adversity exposure history data through self-report, through the retrospective recall of early adverse experience and through the potential confounding of current emotional state with recall of adverse experience. In the EPIC-Norfolk HLEQ cohort ($n = 20{,}921$; age range 41–80; SD 9.3), the lifetime prevalence of MDD is 15.4% and annual MDD prevalence is 5.2%. These MDD prevalence estimates (and age-gender distributions) are comparable to those from interview-based assessments from United Kingdom and international psychiatric epidemiology studies (Surtees et al 2000). In addition, analysis of EPIC-Norfolk HLEQ episodic mood state data has revealed only a small amount of compression of reported MDD

episodes (clustering of episodes in the immediate pre-assessment period) (Surtees et al 2000) and has aided elucidation of the association between adverse event exposure history (including those experienced in childhood) and the onset (and recurrence) of depression (Wainwright and Surtees 2002a, 2002b).

The GxE evidence reported by Caspi et al (2003) represents an exciting development in psychiatric genetic research. Therefore, the finding has provoked considerable interest. The small sample sizes of the original study and of the replication studies so far reported could account for differences between findings. In addition, the failure of the current study to replicate the finding of Caspi et al could be due to differences in research design and measures employed; however, the original finding was of modest statistical significance, and the authors emphasized the requirement for replication. Subsequent evidence has been, largely, on the basis of studies that only partially replicate the original finding. In addition, large multicenter case-control studies and meta-analyses suggest that if there is any direct association between *SLC6A4* genotype and (unipolar) depression, it must be very small. Collectively, we believe that these considerations suggest that evidence, to date, is not supportive of a GxE interaction between the *SLC6A4* genotype, social adversity, and depression.

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