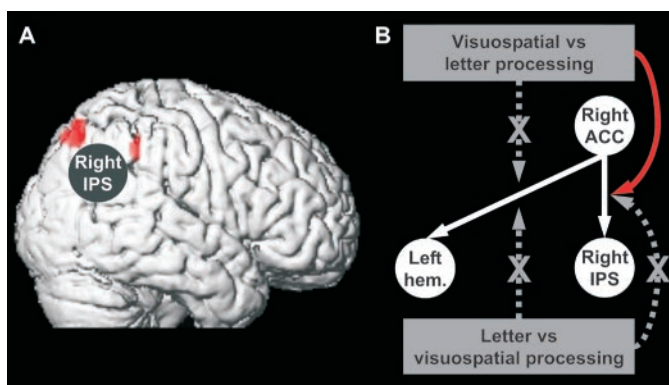Fig. 4. PPI of the right ACC. (**A**) All areas are shown that receive a significant context-dependent contribution from right ACC during visuospatial decisions, projected on the same rendered brain as in Fig. 1. Right ACC significantly increased its influence on posterior (28/–72/48, $t_{max} = 4.49$, $P < 0.015$, corrected) and anterior parts of the right IPS (42/–42/44, $t_{max} = 4.63$, $P < 0.034$, correct-

ed) during visuospatial decisions. Note the specificity of this result: Even when the threshold was reduced to $P < 0.05$, uncorrected, no other significant clusters were found throughout the brain. (**B**) This schema summarizes the negative findings for right ACC: As indicated by the gray dashed lines, right ACC shows no context-dependent contributions to any left-hemispheric area during visuospatial decisions and none to any left- or right-hemispheric area at all during letter decisions.

tivity (*25*) that investigated two tasks with right hemisphere dominance demonstrated top-down effects that were specific for the right hemisphere, i.e., from the right middle frontal gyrus (area 46) on right extrastriate areas. It is thus likely that our findings generalize to other lateralized tasks. Although we cannot exclude lateralization contingent on stimulus type in some situations, our results are consistent with previous findings from split-brain patient studies (*7*) and positron emission tomography (*11*) showing hemispheric specialization based on task demands. Research on hemispheric specialization should move beyond analyses of asymmetric regional activations and focus more strongly on functional interactions within and between hemispheres.

References and Notes

1. J. L. Bradshaw, N. C. Nettleton, *Behav. Brain Sci.* **4**, 51 (1981).
2. J. B. Hellige, *Annu. Rev. Psychol.* **41**, 55 (1990).
3. J. C. Marshall, *Behav. Brain Sci.* **4**, 72 (1981).
4. K. Hugdahl, R. J. Davidson, *The Asymmetrical Brain* (MIT Press, Cambridge, MA, 2003).
5. J. Sergent, *Psychol. Bull.* **93**, 481 (1983).
6. C. Chiarello, J. Senehi, M. Soulier, *Neuropsychologia* **4**, 521 (1986).
7. P. M. Corballis, M. G. Funnell, M. S. Gazzaniga, *NeuroReport* **10**, 2183 (1999).
8. C. J. Price, R. J. S. Wise, R. S. J. Frackowiak, *Cereb. Cortex* **6**, 62 (1996).
9. G. R. Fink *et al.*, *Nature* **382**, 626 (1996).
10. L. E. Nystrom *et al.*, *Neuroimage* **11**, 424 (2000).
11. S. M. Courtney, L. G. Ungerleider, K. Keil, J. V. Haxby, *Cereb. Cortex* **6**, 39 (1996).
12. J. Levy, C. Trevarthen, *J. Exp. Psychol. Hum. Percept. Perform.* **2**, 299 (1976).
13. M. S. Gazzaniga, *Brain* **123**, 1293 (2000).
14. M. I. Posner, S. E. Petersen, *Annu. Rev. Neurosci.* **13**, 25 (1990).
15. T. Shallice, *From Neuropsychology to Mental Structure* (Cambridge Univ. Press, Cambridge, 1988).
16. R. Desimone, J. Duncan, *Annu. Rev. Neurosci.* **18**, 193 (1995).
17. Materials and methods are available as supporting material on *Science* Online.
18. Using a 2 × 2 × 2 factorial design, with letter vs. visuospatial decisions, left vs. right visual fields of presentation, and left vs. right response hand as the three experimental factors, the eight conditions occurred equally often and were varied systematically as blocked conditions in a pseudorandom fashion.
19. A. W. MacDonald, J. D. Cohen, V. A. Stenger, C. S. Carter, *Science* **288**, 1835 (2000).
20. B. J. Casey *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 8728 (2000).
21. C. S. Carter *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **97**, 1944 (2000).
22. G. R. Fink *et al.*, *Brain* **122**, 497 (1999).
23. C. Büchel, K. J. Friston, *Cereb. Cortex* **7**, 768 (1997).
24. A. K. Engel, P. Fries, W. Singer, *Nature Rev. Neurosci.* **2**, 704 (2001).
25. A. R. McIntosh *et al.*, *J. Neurosci.* **14**, 655 (1994).
26. G. Bush, P. Luu, M. I. Posner, *Trends Cogn. Sci.* **4**, 215 (2000).
27. K. J. Friston *et al.*, *NeuroImage* **6**, 218 (1997).
28. C. Cavada, P. S. Goldman-Rakic, *J. Comp. Neurol.* **287**, 393 (1989).
29. D. N. Pandya, G. W. Van Hoesen, M. M. Mesulam, *Exp. Brain Res.* **42**, 319 (1981).
30. This work was supported by the Deutsche Forschungsgemeinschaft (K.Z., G.R.F), the U.K. Medical Research Council (J.C.M), and the Wellcome Trust (K.E.S., K.J.F). Additional support for this Human Brain Project/Neuroinformatics research was provided jointly by the National Institute of Mental Health, National Institute of Neurological Disorders and Stroke, National Institute on Drug Abuse, and the National Cancer Institute. We thank our volunteers, K. Amunts for helpful anatomical discussions, and the radiographers at the Research Center Jülich for technical assistance.

Influence of Life Stress on Depression: Moderation by a Polymorphism in the 5-HTT Gene

Avshalom Caspi,[1,2] Karen Sugden,[1] Terrie E. Moffitt,[1,2]* Alan Taylor,[1] Ian W. Craig,[1] HonaLee Harrington,[2] Joseph McClay,[1] Jonathan Mill,[1] Judy Martin,[3] Antony Braithwaite,[4] Richie Poulton[3]

In a prospective-longitudinal study of a representative birth cohort, we tested why stressful experiences lead to depression in some people but not in others. A functional polymorphism in the promoter region of the serotonin transporter (5-HTT) gene was found to moderate the influence of stressful life events on depression. Individuals with one or two copies of the short allele of the 5-HTT promoter polymorphism exhibited more depressive symptoms, diagnosable depression, and suicidality in relation to stressful life events than individuals homozygous for the long allele. This epidemiological study thus provides evidence of a gene-by-environment interaction, in which an individual's response to environmental insults is moderated by his or her genetic makeup.

Depression is among the top five leading causes of disability and disease burden throughout the world (*1*). Across the life span, stressful life events that involve threat, loss, humiliation, or defeat influence the onset and course of depres-

sion (*2–5*). However, not all people who encounter a stressful life experience succumb to its depressogenic effect. Diathesis-stress theories of depression predict that individuals' sensitivity to stressful events depends on their genetic makeup (*6*, *7*). Behavioral genetics research supports this prediction, documenting that the risk of depression after a stressful event is elevated among people who are at high genetic risk and diminished among those at low genetic risk (*8*). However, whether specific genes exacerbate or buffer the effect of stressful life events on depression is unknown. In this study, a functional polymorphism in the pro-

[1]Medical Research Council Social, Genetic, and Developmental Psychiatry Research Centre, Institute of Psychiatry, King's College London, PO80 De Crespigny Park, London, SE5 8AF, UK. [2]Department of Psychology, University of Wisconsin, Madison, WI 53706, USA. [3]Dunedin School of Medicine, [4]Department of Pathology, University of Otago, Dunedin, New Zealand.

*To whom correspondence should be addressed. E-mail: t.moffitt@iop.kcl.ac.uk

moter region of the serotonin transporter gene (*SLC6A4*) was used to characterize genetic vulnerability to depression and to test whether 5-HTT gene variation moderates the influence of life stress on depression.

The serotonin system provides a logical source of candidate genes for depression, because this system is the target of selective serotonin reuptake–inhibitor drugs that are effective in treating depression (*9*). The serotonin transporter has received particular attention because it is involved in the reuptake of serotonin at brain synapses (*10*). The promoter activity of the 5-HTT gene, located on 17q11.2, is modified by sequence elements within the proximal 5′ regulatory region, designated the 5-HTT gene-linked polymorphic region (5-HTTLPR). The short ("s") allele in the 5-HTTLPR is associated with lower transcriptional efficiency of the promoter compared with the long ("l") allele (*11*).

Evidence for an association between the short promoter variant and depression is inconclusive (*12*). Although the 5-HTT gene may not be directly associated with depression, it could moderate the serotonergic response to stress. Three lines of experimental research suggest this hypothesis of a gene-by-environment (G × E) interaction. First, in mice with disrupted 5-HTT, homozygous and heterozygous (5-HTT –/– and +/–) strains exhibited more fearful behavior and greater increases in the stress hormone (plasma) adrenocorticotropin in response to stress compared to homozygous (5-HTT +/+) controls, but in the absence of stress no differences related to genotype were observed (*13*). Second, in rhesus macaques, whose length variation of the 5-HTTLPR is analogous to that of humans, the short allele is associated with decreased serotonergic function [lower cerebrospinal fluid (CSF) 5-hydroxyindoleacetic acid concentrations] among monkeys reared in stressful conditions but not among normally reared monkeys (*14*). Third, human neuroimaging research suggests that the stress response is mediated by variations in the 5-HTTLPR. Humans with one or two copies of the s allele exhibit greater amygdala neuronal activity to fearful stimuli compared to individuals homozygous for the l allele (*15*). Taken together, these findings suggest the hypothesis that variations in the 5-HTT gene moderate psychopathological reactions to stressful experiences.

We tested this G × E hypothesis among members of the Dunedin Multidisciplinary Health and Development Study (*16*). This representative birth cohort of 1037 children (52% male) has been assessed at ages 3, 5, 7, 9, 11, 13, 15, 18, and 21 and was virtually intact (96%) at the age of 26 years. A total of 847 Caucasian non-Maori study members, without stratification confounds, were divided into three groups on the basis of their 5-HTTLPR genotype (*11*): those with two copies of the s allele (s/s homozygotes; n =

147; 17%), those with one copy of the s allele (s/l heterozygotes; n = 435; 51%), and those with two copies of the l allele (l/l homozygotes; n = 265; 31%). There was no difference in genotype frequencies between the sexes [$\chi^2(2) = 0.02$, $P = 0.99$]. Stressful life events occurring after the 21st birthday and before the 26th birthday were assessed with the aid of a life-history calendar (*17*), a highly reliable method for ascertaining life-event histories (*18*). The 14 events included employment, financial, housing, health, and relationship stressors. Thirty percent of the study members experienced no stressful life events; 25% experienced one event; 20%, two events; 11%, three events; and 15%, four or more events. There were no significant differences between the three genotype groups in the number of life events they experienced, $F(2,846) = 0.56$, $P = 0.59$, suggesting that 5-HTTLPR genotype did not influence exposure to stressful life events.

Study members were assessed for past-year depression at age 26 with the use of the Diagnostic Interview Schedule (*19*), which yields a quantitative measure of depressive symptoms and a categorical diagnosis of a major depressive episode according to *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV) criteria (*20*). 17% of study members (58% female versus 42% male; odds ratio = 1.6; 95% confidence interval from 1.1 to 2.2) met criteria for a past-year major depressive episode, which is comparable to age and sex prevalence rates observed in U.S. epidemiological studies (*21*). In addition, 3% of the study members reported past-year suicide attempts or recurrent thoughts about suicide in the context of a depressive episode. We also collected informant reports about symptoms of depression for 96% of study members at age 26 by mailing a brief questionnaire to persons nominated by each study member as "someone who knows you well."

We used a moderated regression framework (*22*), with sex as a covariate, to test the association between depression and (i) 5-HTTLPR genotype, (ii) stressful life events, and (iii) their interaction (table S1). The interaction between 5-HTTLPR and life events showed that the effect of life events on self-reports of depression symptoms at age 26 was significantly stronger ($P = 0.02$) among individuals carrying an s allele than among l/l homozygotes (Fig. 1A). We further tested whether life events could predict within-individual increases in depression symptoms over time among individuals with an s allele by statistically controlling for the baseline number of depressive symptoms they had before the life events occurred (table S1). The significant interaction ($P = 0.05$) showed that individuals carrying an s allele whose life events occurred after their 21st birthday experienced increases in depressive symptoms from the age of 21 to 26 years ($b = 1.55$,

SE = 0.66, $t = 2.35$, $P = 0.02$ among s/s homozygotes and $b = 1.25$, SE = 0.34, $t = 3.66$, $P < 0.001$ among s/l heterozygotes) whereas l/l homozygotes did not ($b = 0.17$, SE = 0.41, $t = 0.41$, $P = 0.68$).

The G × E interaction also showed that stressful life events predicted a diagnosis of major depression among carriers of an s allele but not among l/l homozygotes ($P = 0.056$, Fig. 1B). We further tested whether life events could predict the onset of new diagnosed depression among carriers of an s allele (table S1). We excluded from analysis study members who were diagnosed with depression before age 21. The significant interaction ($P = 0.02$) showed that life events occurring after their 21st birthdays predicted depression at age 26 among carriers of an s allele who did not have a prior history of depression ($b = 0.79$, SE = 0.25, $z = 3.16$, $P = 0.002$ among s/s homozygotes and $b = 0.41$, SE = 0.12, $z = 3.29$, $P = 0.001$ among s/l heterozygotes) but did not predict onset of new depression among l/l homozygotes ($b = 0.08$, SE = 0.20, $z = 0.42$, $P = 0.67$). Further analyses showed that stressful life events predicted suicide ideation or attempt among individuals carrying an s allele but not among l/l homozygotes ($P = 0.05$, Fig. 1C). The hypothesized G × E interaction was also significant when we predicted informant reports of age-26 depression ($P < 0.01$), an analysis that ruled out the possibility of self-report bias (Fig. 1D). The interaction showed that the effect of life events on informant reports of depression was stronger among individuals carrying an s allele than among l/l homozygotes. These analyses attest that the 5-HTT gene interacts with life events to predict depression symptoms, an increase in symptoms, depression diagnoses, new-onset diagnoses, suicidality, and an informant's report of depressed behavior.

This evidence that 5-HTTLPR variation moderates the effect of life events on depression does not constitute unambiguous evidence of a G × E interaction, because exposure to life events may be influenced by genetic factors; if individuals have a heritable tendency to enter situations where they encounter stressful life events, these events may simply be a genetically saturated marker (*23*, *24*). Thus, what we have identified as a gene × environment interaction predicting depression could actually reflect a gene × "gene" interaction between the 5-HTTLPR and other genes we did not measure. We reasoned that, if our measure of life events represents merely genetic risk, then life events would interact with 5-HTTLPR even if they occurred after the depression episode. However, if our measure of life events represents environmental stress, then the timing of life events relative to depression must follow cause-effect order and life events that
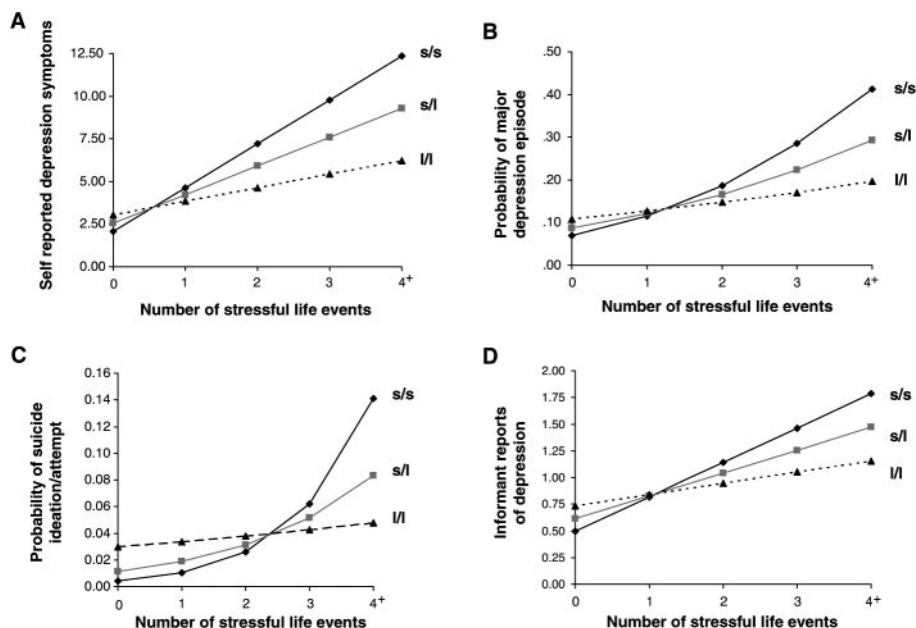
Fig. 1. Results of multiple regression analyses estimating the association between number of stressful life events (between ages 21 and 26 years) and depression outcomes at age 26 as a function of 5-HTT genotype. Among the 146 s/s homozygotes, 43 (29%), 37 (25%), 28 (19%), 15 (10%), and 23 (16%) study members experienced zero, one, two, three, and four or more stressful events, respectively. Among the 435 s/l heterozygotes, 141 (32%), 101 (23%), 76 (17%), 49 (11%), and 68 (16%) experienced zero, one, two, three, and four or more stressful events. Among the 264 l/l homozygotes, 79 (29%), 73 (28%), 57 (21%), 26 (10%), and 29 (11%) experienced zero, one, two, three, and four or more stressful events. (**A**) Self-reports of depression symptoms. The main effect of 5-HTTLPR (i.e., an effect not conditional on other variables) was marginally significant ($b = -0.96$, SE $= 0.52$, $t = 1.86$, $P = 0.06$), the main effect of stressful life events was significant ($b = 1.75$, SE $= 0.23$, $t = 7.45$, $P < 0.001$), and the interaction between 5-HTTLPR and life events was in the predicted direction ($b = -0.89$, SE $= 0.37$, $t = 2.39$, $P = 0.02$). The interaction showed that the effect of life events on self-reports of depression symptoms was stronger among individuals carrying an s allele ($b = 2.52$, SE $= 0.66$, $t = 3.82$, $P < 0.001$ among s/s homozygotes, and $b = 1.71$, SE $= 0.34$, $t = 5.02$, $P < 0.001$ among s/l heterozygotes) than among l/l homozygotes ($b = 0.77$, SE $= 0.43$, $t = 1.79$, $P = 0.08$). (**B**) Probability of major depressive episode. The main effect of 5-HTTLPR was not significant ($b = -0.15$, SE $= 0.14$, $z = 1.07$, $P = 0.29$), the main effect of life events was significant ($b = 0.37$, SE $= 0.06$, $z = 5.99$, $P < 0.001$), and the G × E was in the predicted direction ($b = -0.19$, SE $= 0.10$, $z = 1.91$, $P = 0.056$). Life events predicted a diagnosis of major depression among s carriers ($b = 0.52$, SE $= 0.16$, $z = 3.28$, $P = 0.001$ among s/s homozygotes, and $b = 0.39$, SE $= 0.09$, $z = 4.24$, $P < 0.001$ among s/l heterozygotes) but not among l/l homozygotes ($b = 0.16$, SE $= 0.13$, $z = 1.18$, $P = 0.24$). (**C**) Probability of suicide ideation or attempt. The main effect of 5-HTTLPR was not significant ($b = -0.01$, SE $= 0.28$, $z = 0.01$, $P = 0.99$), the main effect of life events was significant ($b = 0.51$, SE $= 0.13$, $z = 3.96$, $P < 0.001$), and the G × E interaction was in the predicted direction ($b = -0.39$, SE $= 0.20$, $t = 1.95$, $P = 0.051$). Life events predicted suicide ideation or attempt among s carriers ($b = 0.48$, SE $= 0.29$, $z = 1.67$, $P = 0.09$ among s/s homozygotes, and $b = 0.91$, SE $= 0.25$, $z = 3.58$, $P < 0.001$ among s/l heterozygotes) but not among l/l homozygotes ($b = 0.13$, SE $= 0.26$, $z = 0.49$, $P = 0.62$). (**D**) Informant reports of depression. The main effect of 5-HTTLPR was not significant (b $= -0.06$, SE $= 0.06$, t $= 0.98$, $P = 0.33$), the main effect of life events was significant ($b = 0.23$, SE $= 0.03$, $t = 8.47$, $P < 0.001$), and the G × E was in the predicted direction ($b = -0.11$, SE $= 0.04$, $t = 2.54$, $P < 0.01$). The effect of life events on depression was stronger among s carriers ($b = 0.39$, SE $= 0.07$, $t = 5.23$, $P < 0.001$ among s/s homozygotes, and $b = 0.17$, SE $= 0.04$, $t = 4.51$, $P < 0.001$ among s/l heterozygotes) than among l/l homozygotes ($b = 0.14$, SE $= 0.05$, $t = 2.69$, $P < 0.01$).

Fig. 2. Results of regression analysis estimating the association between childhood maltreatment (between the ages of 3 and 11 years) and adult depression (ages 18 to 26), as a function of 5-HTT genotype. Among the 147 s/s homozygotes, 92 (63%), 39 (27%), and 16 (11%) study members were in the no maltreatment, probable maltreatment, and severe maltreatment groups, respectively. Among the 435 s/l heterozygotes, 286 (66%), 116 (27%), and 33 (8%) were in the no, probable, and severe maltreatment groups. Among the 265 l/l homozygotes, 172 (65%), 69 (26%), and 24 (9%) were in the no, probable, and severe maltreatment groups. The main effect of 5-HTTLPR was not significant ($b = -0.14$, SE $= 0.11$, $z = 1.33$, $P = 0.19$), the main effect of childhood maltreatment was significant ($b = 0.30$, SE $= 0.10$, $z = 3.04$, $P = 0.002$), and the G × E interaction was in the predicted direction ($b = -0.33$, SE $= 0.16$, $z = 2.01$, $P = 0.05$). The interaction showed that childhood stress predicted adult depression only among individuals carrying an s allele ($b = 0.60$, SE $= 0.26$, $z = 2.31$, $P = 0.02$ among s/s homozygotes, and $b = 0.45$, SE $= 0.16$, $z = 2.83$, $P = 0.01$ among s/l heterozyotes) and not among l/l homozygotes ($b = -0.01$, SE $= 0.21$, $z = 0.01$, $P = 0.99$).

occur after depression should not interact with 5-HTTLPR to postdict depression. We tested this hypothesis by substituting the age-26 measure of depression with depression assessed in this longitudinal study when study members were 21 and 18 years old, before the occurrence of the measured life events between the ages of 21 and 26 years. Whereas the 5-HTTLPR × life events interaction predicted depression at the age of 26 years, this same interaction did not postdict

depression reported at age 21 nor at the age of 18 years (table S2), indicating our finding is a true G × E interaction.

If 5-HTT genotype moderates the depressogenic influence of stressful life events, it should moderate the effect of life events that occurred not just in adulthood but also of stressful experiences that occurred in earlier developmental periods. Based on this hypothesis, we tested whether adult depression was predicted by the interaction between 5-
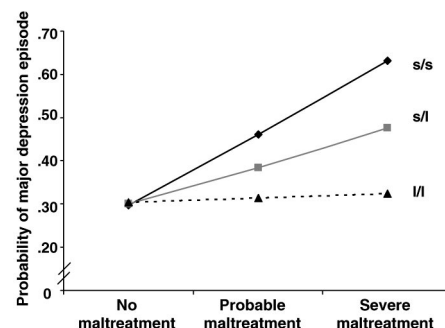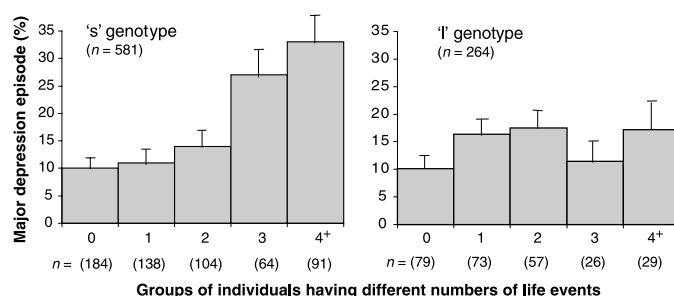
HTTLPR and childhood maltreatment that occurred during the first decade of life (*16, 25*). Consistent with the G × E hypothesis, the longitudinal prediction from childhood maltreatment to adult depression was significantly moderated by 5-HTTLPR (table S3). The interaction showed ($P = 0.05$) that childhood maltreatment predicted adult depression only among individuals carrying an s allele but not among l/l homozygotes (Fig. 2).

We previously showed that variations in the gene encoding the neurotransmitter-metabolizing enzyme monoamine oxidase A (MAOA) moderate children's sensitivity to maltreatment (*25*). MAOA has high affinity for 5-HTT, raising the possibility that the protective effect of the l/l allele on psychiatric morbidity is further augmented by the presence of a genotype conferring high MAOA activity (*13, 26*). However, we found that the moderation of life stress on depression was specific to a polymorphism in the 5-HTT gene, because this effect was observed regardless of the individual's MAOA gene status (tables S4 and S5).

Until this study's findings are replicated, speculation about clinical implications is pre-

Fig. 3. The percentage of individuals meeting diagnostic criteria for depression at age 26, as a function of 5-HTT genotype and number of stressful life events between the ages of 21 and 26. The figure shows individuals with either one or two copies of the short allele (**left**) and individuals homozygous for the long allele (**right**). In a hierarchical logistic regression model, the main effect of genotype (coded as s group = 0 and l group = 1) was not significant, $b = -0.15$, SE = 0.21, $z = 0.72$, $P = 0.47$; the main effect of number of life events was significant, $b = 0.34$, SE = 0.06, $z = 5.70$, $P < 0.001$; and the interaction between genotype and number of life events was significant, $b = -0.30$, SE = 0.15, $z = 1.97$, $P = 0.05$.

mature. Nonetheless, although carriers of an s 5-HTTLPR allele who experienced four or more life events constituted only 10% of the birth cohort, they accounted for almost one-quarter (23%) of the 133 cases of diagnosed depression. Moreover, among cohort members suffering four or more stressful life events, 33% of individuals with an s allele became depressed, whereas only 17% of the l/l homozygotes developed depression (Fig. 3). Thus, the G × E's attributable risk and predictive sensitivity indicate that more knowledge about the functional properties of the 5-HTT gene may lead to better pharmacological treatments for those already depressed. Although the short 5-HTTLPR variant is too prevalent for discriminatory screening (over half of the Caucasian population has an s allele), a microarray of genes might eventually identify those needing prophylaxis against life's stressful events (27).

Evidence of a direct relation between the 5-HTTLPR and depression has been inconsistent (12), perhaps because prior studies have not considered participants' stress histories. In this study, no direct association between the 5-HTT gene and depression was observed. Previous experimental paradigms, including 5-HTT knockout mice (13), stress-reared rhesus macaques (14), and human functional neuroimaging (15), have shown that the 5-HTT gene can interact with environmental conditions, although these experiments did not address depression. Our study demonstrates that this G × E interaction extends to the natural development of depression in a representative sample of humans. However, we could not test hypotheses about brain endophenotypes (28) intermediate between the 5-HTT gene and depression because of the difficulty of taking CSF or functional magnetic resonance imaging measurements in an epidemiological cohort.

Much genetic research has been guided by the assumption that genes cause diseases, but the expectation that direct paths will be found from gene to disease has not proven fruitful for complex psychiatric disorders (29). Our findings of G × E interaction for the 5-HTT gene and another candidate gene, MAOA (25), point to a different, evolutionary model. This model assumes that genetic variants maintained at high prevalence in the population probably act to promote organisms' resistance to environmental pathogens (30). We extend the concept of environmental pathogens to include traumatic, stressful life experiences and propose that the effects of genes may be uncovered when such pathogens are measured (in naturalistic studies) or manipulated (in experimental studies). To date, few linkage studies detect genes, many candidate gene studies fail consistent replication, and genes that replicate account for little variation in the phenotype (29). If replicated, our G × E findings will have implications for improving research in psychiatric genetics. Incomplete gene penetrance, a major source of error in linkage pedigrees, can be explained if a gene's effects are expressed only among family members exposed to environmental risk. If risk exposure differs between samples, candidate genes may fail replication. If risk exposure differs among participants within a sample, genes may account for little variation in the phenotype. We speculate that some multifactorial disorders, instead of resulting from variations in many genes of small effect, may result from variations in fewer genes whose effects are conditional on exposure to environmental risks.

References and Notes

1. C. J. Tang, A. D. Lopez, *Lancet* **349**, 1498 (1997).
2. G. W. Brown, *Soc. Psychiatry Psychiatr. Epidemiol.* **33**, 363 (1998).
3. K. S. Kendler, L. M. Karkowski, C. A. Prescott, *Am. J. Psychiatry* **156**, 837 (1999).
4. R. C. Kessler, *Annu. Rev. Psychol.* **48**, 191 (1997).
5. D. S. Pine, P. Cohen, J. G. Johnson, J. S. Brook, *J. Affect. Disorders* **68**, 49 (2002).
6. E. J. Costello *et al.*, *Biol. Psychiatry* **52**, 529 (2002).
7. S. M. Monroe, A. D. Simons, *Psychol. Bull.* **110**, 406 (1991).
8. K. S. Kendler *et al.*, *Am. J. Psychiatry* **152**, 833 (1995).
9. C. A. Tamminga *et al.*, *Biol. Psychiatry* **52**, 589 (2002).
10. K. P. Lesch, M. D. Greenberg, J. D. Higley, A. Bennett, D. L. Murphy, in *Molecular Genetics and the Human Personality*, J. Benjamin, R. P. Ebstein, R. H. Belmaker, Eds. [American Psychiatric Association (APA), Washington, DC, 2002], pp. 109–136.
11. K. P. Lesch *et al.*, *Science* **274**, 1527 (1996).
12. K. P. Lesch, in *Behavioral Genetics in the Postgenomics Era*, R. Plomin, J. C. DeFries, I. W. Craig, P. McGuffin, Eds. (APA, Washington, DC, 2003), pp. 389–424.
13. D. L. Murphy *et al.*, *Brain Res. Bull.* **56**, 487 (2001).
14. A. J. Bennett *et al.*, *Mol. Psychiatry* **7**, 188 (2002).
15. A. R. Hariri *et al.*, *Science* **297**, 400 (2002).
16. Materials and methods are available as supporting material on *Science* Online.
17. A. Caspi *et al.*, *Int. J. Methods Psychiatr. Res.* **6**, 101 (1996).
18. R. F. Belli, W. L. Shay, F. P. Stafford, *Public Opin. Q.* **65**, 45 (2001).
19. L. N. Robins, L. Cottler, K. Bucholtz, W. Compton, *Diagnostic Interview Schedule for DSM-IV* (Washington University, St. Louis, MO, 1995).
20. APA, *Diagnostic and Statistical Manual of Mental Disorders* (APA, Washington, DC, ed. 4, 1994).
21. R. C. Kessler, K. A. McGonagle, M. Swartz, D. G. Blazer, C. B. Nelson, *J. Affect. Disorders* **29**, 85 (1993).
22. L. S. Aiken, S. G. West, *Multiple Regression: Testing and Interpreting Interactions* (Sage, Thousand Oaks, CA, 1991).
23. K. S. Kendler, L. Karkowski-Shuman, *Psychol. Med.* **27**, 539 (1997).
24. R. Plomin, C. S. Bergeman, *Behav. Brain Sci.* **14**, 373 (1991).
25. A. Caspi *et al.*, *Science* **297**, 851 (2002).
26. N. Salichon *et al.*, *J. Neurosci.* **21**, 884 (2001).
27. W. E. Evans, M. V. Relling, *Science* **286**, 487 (1999).
28. I. I. Gottesman, T. D. Gould, *Am. J. Psychiatry* **160**, 636 (2003).
29. D. Hamer, *Science* **298**, 71 (2002).
30. A. V. S. Hill, *Br. Med. Bull.* **55**, 401 (1999).
31. We thank P. Silva, founder of the Dunedin Multidisciplinary Health and Development Study, Air New Zealand, and the study members, their families, and their friends. Supported by the Health Research Council of New Zealand and the University of Wisconsin Graduate School and by grants from the U.K. Medical Research Council, the William T. Grant Foundation, and the U.S. National Institute of Mental Health (MH49414 and MH45070). T.E.M. is a Royal Society–Wolfson Research Merit Award holder. The study protocol was approved by the institutional review boards of the participating universities.

SUPPLEMENTARY MATERIAL (Online)

Influence of life stress on depression: Moderation by a polymorphism in the 5-HTT gene, by

A. Caspi, K. Sugden, T. E. Moffitt, A. Taylor, I. W. Craig, HL Harrington, J. McClay, J. Mill,

J. Martin, A. Braithwaite, R. Poulton.

Materials and Methods

Research sample. Participants were members of the Dunedin Multidisciplinary Health

and Development Study. The birth cohort of 1,037 children (52% male) was established at age

3 when the investigators enrolled 91% of the consecutive births between April 1972 and March

1973 in Dunedin, New Zealand. Cohort families represent the full range of socioeconomic

status in the general population of New Zealand's South Island. Follow-ups have been carried

out at ages 3, 5, 7, 9, 11, 13, 15, 18, 21, and most recently at age 26, when we assessed 96% of

the living cohort members. The sample and its history are described in detail elsewhere (S1).

Serotonin transporter genetic variation. We selected to study the 5-HTT gene based on

two criteria. (a) Evidence of functionality and (b) evidence that it may moderate response to

stress. The promoter activity of the 5-HTT gene, located on 17q11.2, is modified by sequence

elements within the proximal 5' regulatory region, designated the serotonin transporter gene-

linked polymorphic region (5-HTTLPR). A 20-23 base pair repeat motif within this region

occurs as 2 prevalent alleles: One consisting of 14 repeats (the short allele 's') and another of

16 repeats (the long allele 'l'). This polymorphic region has functional significance; 'l/l'

homozygote lymphoblast cells produce 1.4-1.7 times the concentration of 5-HTT mRNA than

's/l' and 's/s' cells, uptake of labeled serotonin in 'l/l' homozygote lymphoblast cells is 2

times greater than in 's/l' or 's/s' cells, and the protein produced from 'l/l' cells binds 30-40%

more serotonin than cells with the short variant (S2). Although the short promoter variant has

not been conclusively linked to depression, experimental paradigms, including studies of 5-HTT knockout mice (S3), stress-reared macaques (S4), and human functional neuroimaging (S5) have shown that the 5-HTT gene can interact with environmental conditions to shape reactions to stressful experiences, suggesting to us the hypothesis that variations in the 5-HTT gene may explain why stress leads to depression in some people but not in others.

DNA extraction and genotyping. When the Study members were age 26 years, we obtained DNA from 953 participants (97% of those assessed at that age; 51% male); 93% of the DNA samples were obtained via blood and 7% via buccal swabs for those not wishing to undergo phlebotomy. DNA was extracted from blood samples using standard procedures (S6, S7). A modified procedure was used to extract DNA from buccal cells (S8). Primer sequences for 5-HTTLPR are described by Gelernter *et al.* (S9), the forward primer having the sequence (5'- ATGCCAGCACCTAACCCCTAATGT-3') and the reverse (5'-GGACCGCAAGGTGGGCGGGA-3'). This amplifies a 419 base pair product for the 16 repeat ('l') allele and a 375 base pair product for the 14 repeat ('s') allele. PCR was carried out on a PTC-225 DNA engine (MJ Research), using the following cycling conditions: initial 15-min denaturing step at 95°C, followed by 35 cycles of 94°C for 30 sec, 66°C for 30 sec and 72°C for 40 sec, and a final extension phase of 72°C for 15 min. Reactions were performed in 10X reaction Buffer IV (ABgene), 1.5mM MgCl$_2$, 50ng of genomic DNA, 5pmols of each primer, 0.3mM dNTPs and 1 unit of Native *Taq* (Promega). PCR products were separated on a 2.5% agarose gel (MultiABgarose, ABgene) supplemented with Ethidium bromide (0.03%, BDH) and visualised by ultraviolet transillumination.

Population stratification can probably be ruled out as a confounding factor in this study. First, cohort members reporting Maori ethnicity (7%) were not included in our analysis. Second, a genomic control approach based on latent class analysis was adopted, which suggested that the Caucasian sample was genetically homogeneous (S10). Third, allele

frequencies among the non-Maori members of our study were consistent with previously

reported allele frequencies in Caucasian populations (S11): 57% for the 16 repeat ('l') allele

and 43% for the 14 repeat ('s') allele. No other alleles were detected.

We followed the well-documented functional classification described by Lesch et al.

(S2). The sample was split into three groups on the basis of genotype, s/s (N=147, 17% of

sample, 51% male), s/l (N=435, 51% of sample, 51% male) and l/l (N=265, 31% of sample,

51% male). The three groups were in Hardy-Weinberg equilibrium ($\chi^2(2)$=1.91, p=0.41), and

there was no significant difference in genotype frequencies between the sexes ($\chi^2(2)$=0.02,

p=0.99).

Stressful life events were assessed at age 26 with the aid of a life history calendar

(S12), a highly-reliable method for ascertaining life-event histories (S13). The 5-year reporting

period covered events occurring after the 21st birthday and before the 26th birthday. Events

included employment problems (long-term unemployment; being made redundant; losing a job

because the company moved; being fired); financial problems (problems with debt, such as

having items repossessed; not having enough money to pay for food or household expenses;

lacking money for medical expenses; difficulty paying bills); housing problems (homelessness;

multiple residential changes); health problems (a disabling physical illness lasting a month or

more; a disabling injury); and relationship problems (being involved in a physically violent

relationship; a break-up of a cohabiting, intimate relationship). To ensure that the collection of

information on life events was not influenced by knowledge of psychiatric outcomes, this

information was gathered from Study members by a different interviewer in a separate session.

30% of the Study members experienced no stressful life events, 25% experienced 1 event, 20%

2 events, 11% 3 events, and 15% 4 or more events. Males experienced more stressful life

events than females, $X^2(4) = 10.6$, p = .03. There were no significant differences between the

three genotype groups in the number of life events they experienced, $F (2,846) = .56$, $P = .59$,

suggesting that 5-HTTLPR genotype did not influence exposure to stressful life events in adulthood.

Childhood maltreatment. To assess children's experience of stressful life events, we measured their experience of maltreatment between ages 3 to 11 years, as previously described by Caspi et al. (S14). Evidence of childhood maltreatment was ascertained using behavioral observations, parental reports, and retrospective reports by the Study members. First, mother-child interactions were observed during the child's age-3 assessment. The mother was rated by an observer on eight categories: mother's affect toward the child was consistently negative; harshness toward the child; rough, awkward handling of the child; no effort to help child; unaware or unresponsive to child's needs; indifferent to child's performance; demanding of child's attention; soiled, unkempt appearance of child. Mothers engaging in 2 or more such behaviors were classified as rejecting. Second, harsh discipline was measured at ages 7 and 9 using a checklist on which parents indicated if they engaged in ten disciplinary behaviors such as "smack him or hit him with something." Parents scoring in the top decile of the sample-wide distribution were classified as unusually harsh, relative to the culture in which this cohort grew up. Third, changes in the person occupying the role of the child's primary caregiver were ascertained at each assessment. Children who experienced 2 or more such changes during the first decade of life were classified as having suffered disruptive caregiver changes. Fourth, exposure to child physical abuse was assessed retrospectively at age 26 as part of an interview about victimization. Study members were classified as physically abused if they reported multiple episodes of severe physical punishment (e.g., strapping leaving welts; whipping with electric cords) resulting in lasting bruising or injury before age 11. Fifth, unwanted sexual contact was assessed retrospectively at age 26 as part of an interview about reproductive health. Study members were classified as sexually abused if they reported having their genitals touched, touching another's genitals, or attempted/ completed sexual intercourse before age 11.

We derived a cumulative exposure index for each child by counting the number of maltreatment experiences during the first decade of life; in the full sample, 64% of the children experienced no maltreatment, 27% experienced 1 indicator of maltreatment, and 9% experienced 2 or more indicators of maltreatment. There was no significant association between the three genotype groups and maltreatment ($X^2(4) = 1.67$, p = .80), suggesting that 5-HTTLPR genotype did not influence exposure to maltreatment in childhood.

Depression outcomes at age 26. Depression was assessed at age 26 using the Diagnostic Interview Schedule (S15), administered by clinicians with a medical or clinical psychology degree. The reporting period was 12 months prior to interview, which occurred within 60 days of the 26th birthday. This structured interview yields a continuous measure of depressive symptoms (M = 5.2, SD = 10.5; Cronbach's alpha = .95) as well as a diagnosis of a major depressive episode according to DSM-IV criteria (S16). The essential feature of a major depressive episode is a period of at least two weeks during which there is either depressed mood or the loss of interest or pleasure in all activities. One must also experience four of the following additional symptoms: changes in weight or appetite, sleep, or psychomotor activity; decreased energy; feelings of worthlessness or guilt; difficulty thinking or concentrating; or recurrent thoughts of death or suicidal ideation. Lastly, the episode must be accompanied by clinically significant distress or impairment in social, occupational or other important areas of functioning. 17% of Study members (58% female vs. 42% male; OR = 1.6, 95% CI: 1.1-2.2) met criteria for a past-year major depressive episode, which is comparable to age and sex prevalence rates observed in U.S. epidemiological studies (S17). In addition to analyzing the diagnostic outcome of depression, we also examined specific evidence of suicide ideation/attempt; 3% of the Study members reported suicide attempts or recurrent thoughts about suicide in the context of a depressive episode. We also collected informant reports about

symptoms of depression for 96% of Study members at age 26, by mailing a brief questionnaire

to persons nominated by each Study member as "someone who knows you well." Informants

were best friends, partners, or other family members. Using a 3-point scale (0 = no, doesn't

apply; 1 = applies somewhat; 2 = certainly applies), informants rated the Study member on 4

different symptoms: "feels depressed, miserable, sad, or unhappy," "feels that no one loves

them," "seems lonely," and "talks about suicide" (M = 1.0; SD = 1.2; Cronbach's alpha = .80).

Measures of depression at ages 18 and 21. Depression symptoms and diagnoses were

derived in the same way at ages 18 and 21 as at age 26 (described above). Study members

were interviewed with the Diagnostic Interview Schedule at ages 18 and 21 years (S18). At

those assessments, the interviews covered the 12-month periods prior to the 18th (age 17 years)

and 21st (age 20 years) birthdays.

Statistical analysis. We used a moderated regression framework (S19) to estimate the

association between depression and (a) 5-HTTLPR genotype, (b) stressful life events, and (c)

their interaction. Sex was entered into the regressions as a covariate. The equation for the

models is as follows

Depression = b0 + b1(Sex) + b2(5-HTTLPR) + b3(Stress) + b4(5-HTTLPR * Stress),

where,

b0 is the intercept,

b1 is the regression coefficient associated with the effect of sex, which is coded as:

0 = female; 1 = male,

b2 is the regression coefficient associated with the effect of variations in the serotonin

transporter gene promoter, which is here coded to reflect the number of long ('l') alleles, such

that:

0 = ss; 1 = sl; 2 = ll,

b3 is the coefficient associated with the effect of stressful life events, coded to reflect the

number of life events, such that:

$$0 = \text{no stressful events;}$$
$$1 = 1 \text{ stressful event;}$$
$$2 = 2 \text{ stressful events;}$$
$$3 = 3 \text{ stressful events;}$$
$$4 = 4+ \text{ stressful events,}$$

b4 is the coefficient associated with the interaction effect, which is the product of the two

variables (5-HTTLPR and Stressful Life Events). For continuous measures (self-reports and

informant reports of depression symptoms), we used ordinary least squares (OLS) regression;

for categorical measures (diagnosis of major depression and suicide ideation/attempt), we used

logistic regression.

The full results of these regression analyses are provided in Supporting Tables S1

through S3. The coefficients (labeled as b) in all the Supporting Tables are the model

parameters for each type of model (e.g., OLS, logistic) before any transformation (e.g.,

exponentiation to obtains odds ratios). Predicted values can be plotted using variable values.

In additional analyses we examined the moderating effect of 5-HTTLPR on the

association between stress and depression, as a function of MAOA genotype. Genotyping

details about MAOA are provided in Caspi et al. (S14). Study members were grouped as

"low" MAOA activity (carrying the 2, 3 or 5 repeat variants; 61% male) and "high" MAOA

activity (carrying the 3.5 or 4 repeat variants; 75% male). As the gene is situated on the X

chromosome, only females are heterozygous (23% of the sample). We observed that the

influence of life stress on depression was moderated by variation in the 5-HTT gene, regardless

of individuals' MAOA genotype. Among carriers of an 's' allele, the effect of stressful life

events on depression was consistently significant, whether they had low- or high-MAOA

activity status (Supporting Table S4). In contrast, among l/l homozygotes, the effect of

stressful life events on depression was nonsignificant, regardless of MAOA status (Supporting

Table S5).

Assessing the robustness of the G x E effect. We incorporated five analytic features into this

study to test the robustness of the G x E effect. First, we tested that the G x E interaction on

depression obtained whether stress occurred in childhood or in adulthood. Second, we tested that

the G x E interaction predicted within-individual increases in depression from a baseline

measured before life events occurred. Third, we tested that the G x E interaction was not an

artefact of genetic vulnerability evoking life events. Fourth, we used informant reports of

depression to rule out the possibility of self-report biases. Fifth, we examined multiple outcome

measures, which is of particular importance in the behavioral sciences because different

measurements have different sources of error associated with them (S20). Conducting multiple

tests is problematic in the following situation: when (a) several tests are conducted, (b) only a

small subset of the tests attain significance, and (c) the small number that attain significance can

be explained by chance. This situation is even more problematic if (d) no hypothesis was stated

in advance, or (e) researchers selectively report only the test that attained significance. In

contrast, as in the present study, multiple statistical tests can provide evidence that a finding is

robust in the following situation: (a) several tests are conducted using different methods of

measurement and analysis, (b) all findings are in the same direction and all of the tests attain

significance (or very near-significance), and (c) this number of significant tests exceeds the

proportion that could be explained by chance. This situation provides even better evidence of a

sturdy finding if, as in the present study, (d) a clear hypothesis was stated in advance, and (e) the

researchers collect multiple outcome measures and report all of them to document that the

finding is not an artefact of one measurement approach.

SUPPORTING REFERENCES AND NOTES

S1. T. E. Moffitt, A. Caspi, M. Rutter, P. A. Silva, *Sex Differences in Antisocial Behavior: Conduct Disorder, Delinquency, and Violence in the Dunedin Longitudinal Study* (Cambridge Univ. Press, Cambridge, 2001).

S2. K. P. Lesch *et al., Science* **274**, 1527 (1996).

S3. D. L. Murphy *et al., Brain Res. Bull*. **56**, 487 (2001).

S4. A. J. Bennett *et al., Mol. Psychiatry* **7**, 188 (2002).

S5. A. R. Hariri et al., *Science* **297**, 400 (2002).

S6. D. Bowtell, *Anal. Biochem.* **162**, 463 (1987).

S7. M. Jeanpierre, *Nucleic. Acids. Res*. **15**, 9611 (1987).

S8. B. Freeman *et al*., *Behav. Genet.* **33,** 67 (2002).

S9. J. Gelernter, H. Kranzler, J. F. Cubells, *Hum. Genet*. **101**, 243 (1997).

S10. G. A. Satten, D. Flanders, Q. Yang, *Am. J. Hum. Genet.* **68** 466 (2001).

S11. K. P. Lesch, in *Behavioral Genetics in the Postgenomics Era*, R. Plomin, J. C. DeFries, I. W. Craig, P. McGuffin, Eds. (APA, Washington, DC, 2003), pp. 389-424.

S12. A. Caspi *et al., Int. J. Methods Psychiatric Res*. **6**, 101 (1996).

S13. R. F. Belli, W. L. Shay, F. P. Stafford, *Public Opin. Quart.* **65**, 45 (2001).

S14. A. Caspi *et al., Science* **297**, 851 (2002).

S15. L. N. Robins, L. Cottler, K. Bucholtz, W. Compton, *Diagnostic Interview Schedule for DSM-IV* (Washington University, St. Louis, MO, 1995).

S16. *Diagnostic and Statistical Manual of Mental Disorders* (American Psychiatric Association, Washington, DC, ed. 4, 1994).

S17. R. C. Kessler, K. A., McGonagle, M. Swartz, D. G. Blazer, C. B. Nelson, *J. Affect. Disorders* **29**, 85 (1993).

S18. S. R. Jaffee *et al*. *Arch. Gen. Psychiatry* **58** 215 (2002).

S19. L. S. Aiken, S. G. West, *Multiple Regression: Testing and Interpreting Interactions* (Sage, Thousand Oaks, CA, 1991).

S20. Institute of Medicine, *Reducing risks for mental disorders* (Washingon, DC, National Academy Press, 1994).

Table S 1. Results of final regression analyses testing G x E interaction effects on indices of depression at age 26. *The Table presents final models with main effects and interactions entered simultaneously*. For continuous measures (self-reports and informant reports of depression symptoms), we used ordinary least squares (OLS) regression; for categorical measures (diagnosis of major depressive episode [MDE] and suicide ideation/attempt), we used logistic regression (see Statistical Analysis section for details).

Depression outcomes at age 26	Predictor Variables																
	Intercept	Sex				5-HTTLPR				Life events, ages 21-26				5-HTTLPR x Life events			
		b	se	t/z	p	b	se	t/z	p	b	se	t/z	p	b	se	t/z	p
Self-report of depressive symptoms	3.32	-2.44	0.69	3.53	0.001	0.49	0.75	0.65	0.52	2.57	0.48	5.39	0.001	-0.89	0.37	2.39	0.02
Increase in self-report of depressive symptoms*	3.69	-1.12	0.67	1.67	0.100	0.44	0.72	0.62	0.54	1.80	0.47	3.84	0.001	-0.71	0.36	1.97	0.05
Diagnosis of MDE	-2.29	-0.62	0.20	3.11	0.002	0.25	0.24	1.03	0.30	0.56	0.13	4.35	0.001	-0.19	0.10	1.91	0.056
First diagnosis of MDE[†]	-2.93	-0.84	0.27	3.08	0.002	0.53	0.33	1.61	0.11	0.77	0.19	4.11	0.001	-0.34	0.15	2.37	0.02
Suicide ideation/attempt	-5.42	-0.07	0.38	0.17	0.870	0.98	0.58	1.71	0.09	0.91	0.28	3.22	0.001	-0.39	0.20	1.95	0.05
Informant report of depressive symptoms	0.61	-0.22	0.08	2.73	0.006	0.12	0.09	1.38	0.17	0.32	0.06	5.84	0.001	-0.11	0.04	2.54	0.01

[*] This regression equation contains an additional covariate which controls for self-reports of depression symptoms collected during diagnostic interviews with the Study members at ages 18 and 21 years. The model thus tests whether the 5-HTTLPR x Life events interaction predicts within-individual increases in depression symptoms over time.

[†] This regression equation excludes from analysis Study members who met diagnostic criteria for depression prior to age 21 (27%). The model thus tests whether the 5-HTTLPR x Life events interaction predicts new cases of depression at age 26 years.

Table S2. Results of final regression analyses testing G x E interaction effects on depressive symptoms at age 26 years, and on depressive symptoms at the age-21 and age-18 assessments. *The Table presents final models with main effects and interactions entered simultaneously.* The G x E interaction predicts depression occurring after life events (row 1), but not depression that occurred before life events (rows 2 and 3).

Self-reports of depression symptoms	Predictor Variables																
	Intercept	Sex				5-HTTLPR				Life events, ages 21-26				5HTTLPR x Life events			
		b	se	t	p	b	se	t	p	b	se	t	p	b	se	t	p
Depression symptoms, age 26	3.32	-2.44	0.69	3.53	0.001	0.49	0.75	0.65	0.52	2.57	0.48	5.39	0.001	-0.89	0.37	2.39	0.02
Depression symptoms, age 21	6.39	-2.69	0.76	3.53	0.001	-0.07	0.83	-0.09	0.93	2.18	0.53	4.09	0.001	-0.53	0.41	1.29	0.20
Depression symptoms, age 18	5.56	-4.20	0.70	6.01	0.001	0.30	0.76	0.40	0.69	1.67	0.49	3.40	0.001	-0.17	0.38	0.44	0.66

Table S3. Results of final regression analyses testing G x E interaction effects on indices of depression. *The Table presents final models with main effects and interactions entered simultaneously.* The first row shows the analysis predicting diagnosis of major depressive episode (MDE) at age 26 years; for this analysis, we used logistic regression. The second row shows a supplementary analysis, predicting the number of depression episodes experienced by Study members (range 0-3, as assessed according to independent psychiatric interviews carried out when the Study members were aged 18, 21, and 26 years old); for this analysis, we used a negative binomial regression. Childhood stressful events was treated as a single quantitative variable in the regression analyses, ranging from no maltreatment (= 0), to probable maltreatment (= 1), to severe maltreatment (=2).

Depression outcomes	Predictor Variables																	
	Intercept	Sex				5HTT				Childhood stressful events, ages 3-11				5HTT x Childhood events			
		b	se	z	p	b	se	z	p	b	se	z	p	b	se	z	p
Any MDE, (ages 18-26)	-0.39	-0.91	0.15	6.02	0.001	0.02	0.13	0.12	0.90	0.70	0.21	3.27	0.001	-0.33	0.16	2.01	0.05
Number of age periods with MDE diagnosis (ages 18, 21, 26)	-0.72	-0.54	0.11	5.01	0.001	0.05	0.10	0.51	0.61	0.51	0.13	3.87	0.001	-0.22	0.10	2.10	0.04

Table S4. The association between stressful life experiences and depression among individuals with either one or two copies of the 5-HTTLPR 's' allele, as a function of MAOA genotype. We used logistic regression analyses to examine the association between young-adult stress and major depression episode at age 26 years, and negative binomial regression analyses to examine the association between childhood stress and number of adult depression episodes between ages 18-26. Sex was a covariate in analyses carried out among the low- and high-MAOA activity groups, but not among the intermediate-MAOA activity group, as the MAOA gene is situated on the X chromosome and only females are heterozygous.

Individuals with a 5-HTTLPR 's' allele												
	MAOA genotype											
	Low-MAOA activity genotype (n = 141)				**Intermediate-MAOA activity genotype (n = 134)**				**High-MAOA activity genotype (n = 300)**			
	b	SE	z	p	b	SE	z	p	b	SE	z	p
Young-adult stress —> depression at age 26	.67	.17	3.98	.001	.43	.17	2.56	.01	.33	.11	3.00	.003
Childhood stress —> adult depression	.40	.16	2.57	.01	.33	.18	1.84	.07	.40	.12	3.25	.001

Table S5. The association between stressful life experiences and depression among individuals homozygous for the 5-HTTLPR 'l' allele, as a function of MAOA genotype. See Table S4 for details.

Individuals homozygous for the 5-HTTLPR 'l' allele												
	MAOA genotype											
	Low-MAOA activity genotype (n = 62)				Intermediate-MAOA activity genotype (n = 57)				High-MAOA activity genotype (n = 140)			
	b	SE	z	p	b	SE	z	p	b	SE	z	p
Young-adult stress —> depression at age 26	-.03	.32	.10	.92	.21	.24	.87	.39	.17	.21	.84	.40
Childhood stress —> adult depression	.06	.30	.21	.84	.02	.27	.07	.94	-.08	.27	.32	.75