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PERSPECTIVE

The moderation by the serotonin transporter gene of environmental adversity in the etiology of depression: 2009 update

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An updated review of 34 human observational studies indicates that the length polymorphism of the serotonin transporter gene moderates the effect of environmental adversity in the development of depression. This finding depends on the use of contextual or objective methods to assess environmental adversity and is attenuated when self-report instruments are used. Inconsistent findings in male adolescents suggest a developmental stage and sex-specific protective mechanism. These systematic relationships between method and results should be followed up to specify causal mechanisms leading to depression.
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Introduction

Over the past 6 years, a series of studies demonstrating interactions between specific genes and environmental factors brought a new paradigm into research on the causation of mental illness.[1] The fact that these gene–environment interactions are often not accompanied by direct gene-illness associations suggests a reason why genome-wide association studies may not detect genes involved in the causation of mental disorders.[2] As researchers are contemplating the need for and feasibility of including environmental factors in genome-wide searches for causative mechanisms, the replicability of gene–environment interactions has become topical. A primary focus has been on the interaction of a length polymorphism in the serotonin transporter gene (5-HTTLPR) and environmental adversity in the causation of depression.[3] Although the report of this gene–environment interaction was followed by a number of replications enhancing confidence in this gene–environment interaction as a causal mechanism in depression, several large studies have shown results that were inconsistent with the original findings. In 2007, we reviewed 18 studies published on this topic, including the original study, 11 replications, 3 partial-replications and 3 non-replications.[4] We found that the method used to assess environmental adversity could explain most discrepancies in results. Studies using objective evidence or detailed interviews to assess environmental adversity in context consistently found an interaction in the expected direction, but studies relying on brief self-report measures of adversity often showed negative findings. Age also appeared to be a significant factor, with studies in adolescents (especially male adolescents) and elderly often giving negative results. We concluded that the substantial heterogeneity in the methodology of these studies was systematically related to their outcome and precluded a meta-analytic synthesis of all published studies.

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Motivation for update

In 2009, two meta-analyses have been published, based on a selection of 5 and 14 studies, respectively, and concluding that there is insufficient evidence for the existence of an interaction between 5-HTTLPR and stressful life events in depression.[5,6] As these meta-analyses did not take into account methodological heterogeneity and only included a selection of published studies, we have undertaken to update our systematic review to include all relevant studies published up to the end of March 2009 (the same date as in the larger of the two meta-analyses[6]). The aims were to find out whether the relationship between methodology and results holds for studies published since our 2007 review and explore whether methodological heterogeneity could have confounded the published meta-analyses.

Approach to review

We performed PubMed and Web of Knowledge database searches for papers with a combination of

serotonin transporter (or its abbreviations) and stress, life events, environment or gene–environment interaction in the abstract or title. In addition, we performed cited reference searches on relevant recent articles and have contacted colleagues to help us identify omissions. We sought to include all relevant published studies that tested the interaction between environmental adversity and serotonin transporter with either continuous measure of depressive symptoms or diagnosis of depression as the outcome. Consistently with our previous review,[4] we excluded studies with fewer than 100 subjects, as such small studies could not be expected to provide a test of a gene-environment interaction and would have been unlikely to be published if they had given negative results (all studies excluded on this ground reported positive gene–environment interaction results). As in our previous review,[4] we also excluded studies with a neurological or multisystemic illness as stressor, as a different pathogenic mechanism is likely to be involved (this also led to the exclusion of positive studies). We classified the assessment of adversity according to the level of objectivity: self-report questionnaires are classified as the most subjective method, as they rely entirely on the memory and judgment of study participants; interviews are still based on subjective verbal report of participants, but the interviewer is trained to introduce a level of objectivity by systematically probing and making a judgment about the presence and severity of reported stressors; measures of adversity were classified as 'objective' if they were collected independently of participants' report and of researchers (for example, social services record of child abuse, natural disaster or physical illness established by objective examination) or if they were facts very unlikely to be influenced by any reporting bias (for example, growing up in a single parent family). Studies where a self-report questionnaire was administered by an interviewer who was not probing or making judgment were conservatively classified as 'self-report'. The test of gene–environment interaction was taken as reported in the original peer-reviewed articles, with studies reporting findings in the direction consistent with the original report[3] with a type I error probability less than 5% classified as 'replications'. Studies where multiple tests of gene–environment interaction (for example, separate analyses of males and females or for multiple measures of adversity) provided a mix of positive and negative findings were classified as 'part-replications'. Studies with nonsignificant results and studies with results in the direction opposite to the original report[3] were considered 'non-replications'.

Review of published literature

We identified 34 published human observational studies that reported a test of interaction between 5-HTTLPR and environmental adversity (Table 1). There were 17 replications of the original gene–environment interaction in the expected direction, 8 partial replications (finding an interaction only in females or only with one of several types of adversity) and 9 non-replications (finding no interaction or an interaction in the opposite direction). The relationship between the method of adversity assessment and results held almost perfectly: all studies using objective indicators or structured interviews to assess stress replicated the gene–environment interaction fully or partially, whereas all non-replications relied on brief self-report measures of adversity (Table 1). This relationship between the method used to assess environmental adversity and results was statistically significant ($\chi^2_{(1)}$ test for trend = 8.51, $P = 0.004$). This result held across a range of sensitivity analyses probing the influence of review methodology: inclusion of studies with small sample size and with neurological illness or reclassification of studies with interviewer-aided administration of a questionnaire as interviews strengthened this result. The preponderance of non-replications and partial replications in adolescent samples also held for the updated list of 34 studies ($\chi^2_{(1)}$ test for trend = 5.30, $P = 0.02$; Table 1). Most part-replications in adolescent samples found the gene–environment interaction in female but not male adolescents. The two studies that reported findings in the opposite direction were both in adolescent samples and used self-report to assess adversity.[7,8]

Self-report, interviews and subjectivity of adversity assessment

The method used to assess environmental adversity has emerged as an important determinant of heterogeneity among studies of gene–environment interactions. The strong relationship between methodology and results is not surprising in the light of previous demonstrations of major differences between self-report and interview methods of assessing stress and life events,[9] including different predictive value for depression treatment outcomes.[10] A number of studies have shown that semistructured interviews with contextual ratings are preferable to self-report questionnaires.[9,11] Self-report questionnaires both over-report adverse events by including trivial occurrences,[11,12] and under-report severe events important for the onset of depression.[9,13] The problem with self-report is not a simple inability to recall, as important events can be reliably recalled over long periods in semistructured interview setting.[14] Self-report is prone to biases that make it less reliable and more influenced by subjective states, including current mood and desire to please the investigator.[9] Such biases can systematically influence results across studies. Self-report instruments may tap into emotional memory, which may have different genetic and neuroendocrine underpinnings.[15] Although interviews are preferable to unaided self-report, they are still based on verbal report and memory of research participants, and therefore are unlikely to be immune to subjective influences. Therefore, we separately

Table 1 Published studies on gene–environment interactions involving the 5-HTTLPR, environmental adversity and depression, ordered by method of assessment of environmental adversity

		Replications	*Adol*	*Meta*	*Part-replications*	*Adol*	*Meta*	*Non-replications*	*Adol*	*Meta*
Method used to assess environmental adversity	Objective	Kaufman *et al.* (2004) Nakatani *et al.* (2005) Kilpatrick *et al.*[16] Otte *et al.*[18] Kohen *et al.*[17] Nobile *et al.* (2009)	A		Cicchetti *et al.* (2007)	A				
	Interview	Caspi *et al.*[3] Kendler *et al.* (2005) Mandelli *et al.* (2007) Zalsman *et al.* (2006) Drachmann Bukh (2009)		R, M	Grabe *et al.* (2005) Wilhelm *et al.* (2006) Sjoberg *et al.* (2006)	 A	R R			
	Self-report	Taylor *et al.* (2006) Jacobs *et al.* (2006) Cervilla *et al.* (2007) Kim *et al.* (2007) Dick *et al.* (2007) Lazary *et al.* (2008)		R R R, M	Eley *et al.* (2004) Scheid *et al.* (2007) Brummett *et al.* (2008) Aguilera *et al.* (2009)	A	R, M M	Gillespie *et al.* (2005) Surtees *et al.* (2006) Covault *et al.* (2007) Chipman *et al.*[7a] Chorbov *et al.* (2007) Wichers *et al.* (2008) Power *et al.* (2008) Laucht *et al.*[9a] Zhang *et al.* (2009) Middeldorp *et al.*[b]	 A A A A 	R R, M R R R R R

Studies are divided into 'replications', showing a significant effect in the same direction as the original Caspi *et al.* (report, 'part-replications' showing a significant effect in the same direction only in some analyses (for example, only one gender or only for one type of adversity), and 'non-replications' including nonsignificant findings and effects in the opposite direction. 'Objective' measures of adversity include records that are collected independently of participants' report and of researchers (for example, social services record of child abuse, natural disaster or physical illness established by objective examination), 'Interview' measures involve an interviewer making a judgment on the significance of life stress in context, whereas 'Self-report' leaves the measurement of stress to the direct report of participants. Studies in adolescent samples are marked by 'A'. Studies included in the Risch *et al.*[6] (meta-analysis are marked with an 'R' and those included by Munafo *et al.*[5] (are marked by an 'M'.

[a]These studies reported findings in a direction opposite to the original report.

[b]A study by Middledorp *et al.* was included in the meta-analysis by Risch *et al.*[6] although no test of gene–environment interaction was reported in the available publications. Full references to all studies are included in Supplementary Information.

consider studies that used an 'objective' record of adversity assessment, which is completely independent of subjects' memory. Even though such reported adversity is often limited to a single specific condition, data provided in Table 1 suggests that such objectively reported adversities provide a reliable 'E' component for gene–environment interaction studies.

Contribution of objective and experimental studies

A major advance in the literature over the last 5 years has been a proliferation of studies where environmental adversity is objectively ascertained, including a natural catastrophe[16] or objectively diagnosed physical illness.[17,18] These studies assess a narrow range of exposures compared with questionnaires and interviews, but are valuable as subjective factors in the report of adversity are excluded and gene–environment correlation is unlikely to be involved. It is encouraging that these studies consistently support the serotonin transporter gene–environment interaction in the causation of depression (Table 1). Further evidence is coming from high-quality experimental studies, where exposure to stressful stimuli is manipulated and gene–environment correlation is definitely excluded. These experimental studies provide additional evidence in support of the gene–environment interaction, by finding that the 5-HTTLPR short allele is associated with attentional bias toward threatening stimuli[19,20] and stronger reactivity of cortisol to experimental stressors.[21,22]

Examination of published meta-analyses

We further explored whether the systematic relationship between methodology and results could have confounded the published meta-analyses. We found that studies using self-report measures of adversity were preferentially included in the published meta-analyses. As a result, the inclusion of studies in the meta-analyses was significantly biased toward negative studies. The meta-analysis by Risch *et al*.[6] included 7 out of 10 negative studies, 3 out of 8 part replications and only 4 out of 17 positive studies ($\chi^2_{(1)}$ test for trend = 5.48, $P < 0.02$). In addition, one unpublished negative study was included in the meta-analysis (neither of the two referenced papers by Middeldorp *et al*.[23,24] reported a test of gene–environment interaction). We conclude that the published meta-analyses are confounded by methodological heterogeneity and do not provide an unbiased synthesis of the literature.

Limits of synthesis in heterogeneous literature

Any synthesis of the current literature is complicated by a systematic relationship between the study size and methodology, with larger studies being more likely to use brief self-report measures of adversity. As a result, the effects of study size and assessment method cannot be reliably separated. A meta-analysis weighs studies by sample size and assigns more importance to larger studies using self-report questionnaires to assess adversity. Such approach would be suitable for synthesis across a homogeneous group of studies, but does not do justice to important differences in method. In contrast, the present review considers each study with a sample size of at least one hundred an equivalent unit of analysis. This approach is suited for testing relationships between method and outcome but is relatively inefficient in making conclusions about overall replicability of findings. With an increasing number and quality of studies, a method-specific meta-analysis may become a possibility that will give a more definite answer to this conundrum.

Another limitation of this review is that it relies on the published version of findings and cannot place different studies on a single scale of measurement. This may be important with respect to the genetic model used, as most studies included in Table 1 used either an additive or a dominant genetic model. A pessimistic reviewer may suggest that each study might have had two goes at achieving significant findings. However, as we have required replication in the same direction and two-tailed *P*-values have been reported, the rate of false positives due to chance remains at 1 out of 20. Repeated testing would also not explain the preponderance of positive findings in the expected direction (17 studies) compared with significant findings in the opposite direction (2 studies) and the systematic relationship between methods and results. Thus, given the heterogeneity of studies in this field, we believe that reliance on published peer-reviewed findings is preferable to the Procrustean error of stretching very different studies onto a single metric.

Conclusions

An updated review confirms that there is a systematic relationship between the method used to assess environmental adversity and the results of studies of interaction with the length variant of the promoter of the serotonin transporter gene, suggesting that the genetic variant affects the impact of objectively occurring adversity rather than its representations in memory. This interaction is revealed by objective indicators of adversity and may be attenuated by inaccuracies of retrospective self-report. Several authors have expressed concerns about the standards of stress assessment in these studies and have recommended the use of contextual or objective methods of assessment.[25,26] The lack of interaction between 5-HTTLPR and measured adversity in male adolescents may be pointing to a developmentally specific protective mechanism, but may also reflect the fact that commonly measured aspects of adversity are less relevant to this age group or existence of alterative adverse outcomes. The large number of good quality studies that replicate the original finding and especially the coherent set of findings in studies

using objective methodology to assess environmental adversity militate against the conclusion that the gene–environment interaction is a chance finding. Rather than reaching a premature yes-or-no verdict in a selective meta-analysis, appreciation of methodological heterogeneity and developmental context is needed to further our understanding of the etiology of mental illness. A follow-up of the systematic relationships between method and results is likely to provide specific conclusions about the causal mechanisms involved.

Conflict of interest

Uher declare no conflict of interest. McGuffin has received consultancy fees and honoraria for participating in expert panels for pharmaceutical companies, including Lundbeck, Astra-Zeneca, GlaxoSmithKline and Bristol Myers Squib.

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