Molecular Psychiatry (2004) 9, 908–915

www.nature.com/mp

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Gene–environment interaction analysis of serotonin system markers with adolescent depression

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We report analyses from a study of gene–environment interaction in adolescent depression. The sample was selected from 1990 adolescents aged 10–20 years: those with depression symptoms in the top or bottom 15% were identified and divided into high or low environmental risk groups. DNA was obtained from 377 adolescents, representing the four quadrants of high or low depression and high or low environmental risk. Markers within, or close to, each of the serotonergic genes *5HTT, HTR2A*, *HTR2C*, *MAOA* (monoamine oxidase type A) and tryptophan hydroxylase (*TPH*) were genotyped. Environmental risk group was a nonsignificant predictor and sex was a significant predictor of the depression group. *HTR2A* and *TPH* significantly predicted the depression group, independent of the effects of sex, environmental risk group and their interaction. In addition, there was a trend for an effect of *5HTTLPR*, which was significant in female subjects. Furthermore, there was a significant genotype–environmental risk interaction for *5HTTLPR* in female subjects only, with the effect being in the same direction as another recent study, reaffirming that an important source of genetic heterogeneity is exposure to environmental risk.
Molecular Psychiatry (2004) **9**, 908–915. doi:10.1038/sj.mp.4001546
Published online 6 July 2004

Keywords: serotonin receptors; serotonin transporter; tryptophan hydroxylase; monoamine oxidase A; depression; gene–environment interaction; adolescence

The past decade of research has revealed just how difficult it is to find genes that contribute to variance in complex traits.[1] Progress in the area as a whole has been slow, reflecting the complexity of the aetiology of these traits. It is now widely recognized not only that there are both genetic and environmental influences on almost every psychological trait and associated disorder, but that this genetic risk results from a large number of individually small effect sizes. Furthermore, genetic risks are likely to interact with environmental risks. As such, there is considerable heterogeneity in the effect of each genetic variant across different levels of environmental risk, with a small overall average effect size in the entire population.[1] As a result, the analysis of the main effects will fail to identify genetic risks to the extent that they operate only in conjunction with environmental risk.

Depression is an excellent example of a complex trait for which gene–environment interactions are likely to be important. In addition to the well-reported evidence for both genetic[2] and environmental[3] influences on depressive symptoms in adolescence, behavioural genetic research has begun to provide evidence for interactions between individual (biological, familial, or genetic) vulnerability and environmental stress in adolescent[4,5] samples (described in more detail below). In this study, we therefore examined gene–environment interactions associated with risk for high levels of depression symptoms in adolescence, concentrating on loci implicated in the serotonin (5HT) system.

Molecular genetic work has begun to identify targets and investigate their role as genetic risks for depression. The predominant theory for explaining the biological basis of depression has been the monoamine hypothesis, reflected in the development of antidepressants, that work on this system, most recently the important introduction of the serotonin reuptake inhibitors (SSRIs).[6] Other evidence implicating the serotonin system includes the finding that there is increased density of the 5HT receptor 2A (*5HT2A*) receptor-binding sites in brain regions of depressed individuals who committed suicide[7] and in platelets of depressed suicidal patients.[8] Candidate gene studies in this area have examined markers from genes covering many steps at different stages within the 5HT system. First, there are results relating the functional polymorphism in the 5HT transporter gene promotor region (*5HTTLPR*) and neuroticism and other personality traits associated with anxiety and depression,[9] which have been widely re-examined leading to both replications and nonreplications.[10] Research in this area has culminated recently in the demonstration that the variant associated with lower activity (short allele) confers risk for the development

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Received 18 November 2003; accepted 25 May 2004

of depression in the presence of adverse life events.[11] Second, several receptor genes have been examined. For example, *5HT2A* has been repeatedly investigated for association with depression, with both positive[12] and negative[13] results, and has also been found to be associated with learned helplessness in rats, an animal model of depression.[14] The C allele at the T102C SNP of the 5HT receptor 2A has also been associated with suicide[15] and suicidal ideation,[12] although again there have also been negative findings.[16] The 5HT receptor 2C (*5HT2C*) has also been explored, with some studies finding association with depression[17] and bipolar affective disorder,[18] while others do not.[19] Third, monoamine oxidase type A (*MAOA*) acts as catalyst in the degradation of neurotransmitters, including 5HT, and has been examined with regard to a high-activity allele within a VNTR in the promotor region of *MAOA*, which is associated with a number of related phenotypes including major depression,[20] suicide attempts,[21] panic disorder[22] and neuroticism.[20,23] Finally, tryptophan hydroxylase (*TPH1*) is a rate-limiting enzyme in the synthesis of 5HT. It has been associated with somatic anxiety in unipolar depression,[24] with paroxetine response in depression[25] and also with suicide,[26] while results from other studies are equivocal.[27]

Environmental influences associated with depression in adolescence can be broadly divided into two groups: child-specific risks and family-general risks. Child-specific risks include acute sources of stress such as life events and more chronic ongoing difficulties such as peer relationship problems. Family-general risks include parental psychopathology, which in the developmental psychology literature is often examined in the context of environmental risk,[28] social adversity factors such as poverty or low SES[29] and family-based stressful life events.[4] Recent reports indicate that shared environmental influences, which are likely to be family-general risks, are particularly important for high levels of depression, while being relatively unimportant for variation in the full range.[30] For this reason in the present study, we chose to use a measure of family-based environmental risk and classified adolescents as high or low on this risk variable.

Gene–environment interactions have recently begun to be identified. There are two main methods that have been used. The first is a rather indirect approach—showing that heritability of a phenotype varies as a function of environmental risk. For example, one study found that genetic risk for depression in adolescent girls was greater in the presence of life events.[4] Another example comes from research on the sample used in the present study regarding the prediction of high levels of adolescent depression, which revealed an interaction between a variable reflecting familial risk for anxiety, depression and neuroticism in parents and lack of parental educational qualifications.[5] However, a second and more direct approach is to assess specific aspects of both the genetic and environmental risks. For example, an interaction has been demonstrated between adverse life events and the short allele of the 5HT transporter promoter on depression and suicidality in a study of young adults.[11] To our knowledge, this current study is the first to examine gene–environment interaction *directly* for adolescent depression. Adolescence is a particularly good time to conduct a study of this kind, as it is when many individuals will be experiencing their first onset of depression. We assessed interactions between five markers in the 5HT system (*5HTTLPR, 5HT2A, 5HT2C, MAOA* and *TPH*) and a composite measure of family-based environmental risk (parental education, social adversity and family life events) in the prediction of high levels of adolescent depression. We selected as markers simple sequence repeat motifs within or close to the genes. For some, there was evidence that they influenced gene activity and for others that their high information content conferred advantage through their potential linkage disequilibrium with putative functional variants. An additional advantage was that some of the markers chosen could be combined in a single tube multiplex reaction.

Materials and methods

Procedure

Adults screened for a study of the genetics of depression and anxiety (GENESiS[31]), who had reported children living at home, were sent a letter asking them to pass on a booklet containing the short form of the Mood and Feelings Questionnaire (SMFQ)[32] to any offspring aged 12–19 years. This measure consists of 13 statements such as 'I did everything wrong', which are rated on a three-point scale for frequency over the past 2 weeks. In this study we used a 4-point scale in order to gain more differentiation at the extremes, then converted the scale back to have a range of 0–26, with higher scores reflecting higher levels of depressive symptoms. Completed questionnaire booklets were returned by 1900 adolescents (more details on this sample are given elsewhere[5]). The mean SMFQ score in this sample was 7.7 (SD = 5.5), and those with scores greater than 12 or less than 3 on the SMFQ (roughly the top and bottom 15%, N = 560) were selected for follow-up and sent buccal swab kits.[33] In all, 377 adolescents provided DNA (67%). Of this sample, 295 adolescents were unrelated and the remaining 82 were from sibling pairs.

Family environmental risk was assessed using three variables. First, the level of family social adversity was assessed using the Social Problems Questionnaire (SPQ),[34] which has a four-point rating scale. The scale assesses problems relating to finances, housing, work, relationships and social difficulties. Second, parental educational level was assessed using an eight-point scale ranging from 'No qualifications' to 'Postgraduate degree (eg Masters, PhD)', which was recoded such that higher scores reflected a poorer

level of education. Third, adverse life events were assessed with the 12-item 'List of Threatening Events' (LTE),[35] which relates to the previous 6 months. These events related to the parent or family as a whole and included items relating to serious illness, bereavement, relationship breakdowns, unemployment and financial crisis. A composite environmental measure was created by standardizing and combining the individual environmental measures, and this was dichotomized into above and below the entire sample mean.

The sample consisted of 93 (73 female), 117 (73 female), 57 (25 female) and 110 (49 female) in the high depression high environmental risk, high depression low environmental risk, low depression high environmental risk and low depression low environmental risk groups, respectively. The sex differences between these groups were highly significant, so we included sex in all analyses. There were no significant age differences between the four groups.

Genotyping

Markers from five genes within the serotonergic system were genotyped: *5HTT*, *5-HT2A*, *5-HT2C*, *MAOA* and *TPH*. Genotyping was carried out following previously published protocols, and for multiallelic markers the alleles were binned into two groups. For the 5HT transporter, we analysed the promoter polymorphic region (*5HTTLPR*) following the method described by Gelernter *et al*.[36] We categorized the alleles as either 'long' (L) or 'short' (S) as described by Lesch *et al*.[9] For *5HT2A*, we analysed the well-documented T102C SNP.[37] For the *5-HT2C* promoter repeat polymorphism,[38] the allele designated 'allele 1', which is associated with increased transcriptional activity in a haplotype construct, was analysed *vs* the remainder of the alleles. For MAOA, genotyping conditions and the classification of alleles into high (H) and low (L) functionality were as described by Deckert *et al*.[22] For the 3′ microsatellite of *TPH*,[39] we examined the 198 bp allele (*vs* all other alleles), which we designated as allele 5, being the fifth from shortest fragment length found within this sample. We chose this allele on the basis of work from the adult GENESiS sample in which it was shown to be a significant predictor of a psychometrically derived measure of familial vulnerability to depression and related traits. Although TPH2 could also be considered to be of interest as it is expressed in the brain stem of mice, rats and humans,[40] we did not genotype this marker as the expression data on TPH1 and TPH2 from within our own laboratories, suggesting that both forms are expressed at approximately equal levels in the brain (K Sugden personal communication), thus indicating that data from either can stand alone on their own merits. Further work on SNP analysis of relevant loci is in progress, but beyond the scope and time scale for this investigation. All genotyping was conducted blind to depression and environmental group membership.

Analyses

For the first stage of the analyses χ^2's were calculated to examine the main effects of genotype on the depression groups. Second, we used STATA[41] to compute multivariate logistic regressions to examine the joint effects of several variables in predicting the depression group. Furthermore, this analysis can take into account clustering within a data set due to nonindependent observations. As there were 41 sibling pairs within the data set, there were nonindependent observations, which would lead to an underestimation of confidence intervals in standard analyses. The 'robust, cluster' option was therefore used to take these related observations into account in order to utilize the entire data set. The variables entered into each analysis were sex, environmental risk group, the interaction between sex and environmental risk group, genotype and gene–environment interaction. No complications from ethnic stratification were anticipated as a latent class analysis of genotype data from a whole genome scan on the parents of the selected individuals found no evidence for population heterogeneity (Matthew Nash and Pak Sham 2003, personal communication).

Results

The genotype frequencies within the two depression groups are given in Table 1, separately for the two sexes, for *MAOA* and *5HT2C*, given their location on the X-chromosome. All markers were in Hardy–Weinberg equilibrium. As can be seen, a significant association with depression was observed for *HTR2A*, with the T allele more common in the high depression group. There was also a trend in the males for *MAOA*. However, these simplistic analyses do not take into account either the sex of the individual (apart from markers on the X-chromosome), their environmental risk group or interactions between these effects. In order to model these effects concurrently, we conducted a series of multiple logistic regressions. Initial analyses indicated a significant main effect of sex on depression group, with 70% of the high depression group being female as compared to 43% of the low depression group, an odds ratio (OR) of 2.84. Environmental risk group did not significantly predict depression group, but there was a nonsignificant trend indicating an interaction between sex and environmental group, such that the excess of females with high depression symptom scores was more extreme in the high environmental risk group (80%).

In the multiple logistic regression analyses, the OR for sex, environmental risk group and their interaction were 2.13 ($P=0.006$), 0.89 ($P=0.75$) and 2.20 ($P=0.09$), respectively. In addition to these three parameters, we then incorporated each genotype and the interaction with the environmental stress group resulting in five separate multiple logistic regressions, one for each marker. The results for the effects of each genotype and the interaction between this and environmental risk group are given in Table 2. For

Table 1 Genotypic frequencies for the 5HT system markers in the high and low depression groups

Marker (sex)	*Genotype*	*Depression groups*			χ^2
		Low N (%)	*High N (%)*	*Total N (%)*	
5HTTLPR	L,L	26 (16.0)	37 (18.0)	63 (17.1)	
	L,S	83 (50.9)	115 (55.8)	198 (53.7)	
	S,S	54 (33.1)	54 (26.2)	108 (29.3)	
	Total	163	206	369	2.11
HTR2A	C,C	65 (39.2)	65 (31.3)	130 (34.8)	
	C,T	82 (49.4)	101 (48.6)	183 (48.9)	
	T,T	19 (11.5)	42 (20.2)	61 (16.3)	
	Total	166	208	374	6.00*
HT2C (male)	0	65 (70.6)	38 (61.3)	103 (66.9)	
	1	27 (29.4)	24 (38.7)	51 (33.1)	
	Total	92	62	154	1.47
HT2C (female)	0,0	36 (48.7)	72 (49.3)	108 (49.1)	
	1,0	33 (44.6)	54 (37.0)	87 (39.6)	
	1,1	5 (6.8)	20 (13.7)	25 (11.4)	
	Total	74	146	220	2.81
MAOA (male)	L	36 (40.0)	17 (26.6)	53 (34.4)	
	H	54 (60.0)	47 (73.4)	101 (65.6)	
	Total	90	64	154	2.99+
MAOA (female)	L,L	6 (8.2)	12 (8.3)	18 (8.3)	
	L,H	27 (37.0)	62 (42.8)	89 (40.8)	
	H,H	40 (54.8)	71 (49.0)	111 (50.9)	
	Total	73	145	218	0.72
TPH	0,0	118 (71.1)	158 (76.0)	276 (73.8)	
	5,0	43 (25.9)	44 (21.2)	87 (23.3)	
	5,5	5 (3.0)	6 (2.9)	11 (2.9)	
	Total	166	208	374	1.20

Note: Alleles denoted '0' are any other than the allele of interest for that marker.
Significance levels given as $^*P<0.05$ or $^+P<0.10$.

Table 2 ORs and significance levels for main effects of genotype, and genotype by environment interaction from the logistic regression analyses

Marker	*Genotype*	*P-value*	*Genotype by E-risk group*	*P-value*
5HTTLPR	0.69	0.07	1.85	0.09
5HT2A	1.61	0.02	0.59	0.11
5HT2C	1.12	0.65	1.19	0.64
MAOA	1.12	0.63	1.01	1.0
TPH	0.49	0.02	2.39	0.07

5HTTLPR, there was a nonsignificant trend indicating an overall decrease in odds of depression for an increasing number of 'short' alleles (OR = 0.69) and a nonsignificant interaction between this genotype and environmental risk. However, in female subjects, the main effect of 'short' alleles was significant, with an OR of 0.56 (CI = 0.32–0.96, $P = 0.03$). Similarly, in female subjects the interaction with the environmental group was significant, with an OR of 2.82 (CI = 1.12–7.12, $P = 0.03$). For *5HT2A*, the T allele confers significantly increased risk of depression (OR = 1.61), indicating that the odds of severe depressive symptoms in the adolescent rise by a factor of 1.61 for each additional T allele. The interaction between *5HT2A* and environmental risk group was not significant. For both *5HT2C* and *MAOA*, the main effects and interactions with environmental risk group were all nonsignificant. For *TPH1*, allele 5 (198 bp fragment length) had a significant protective effect, such that each additional

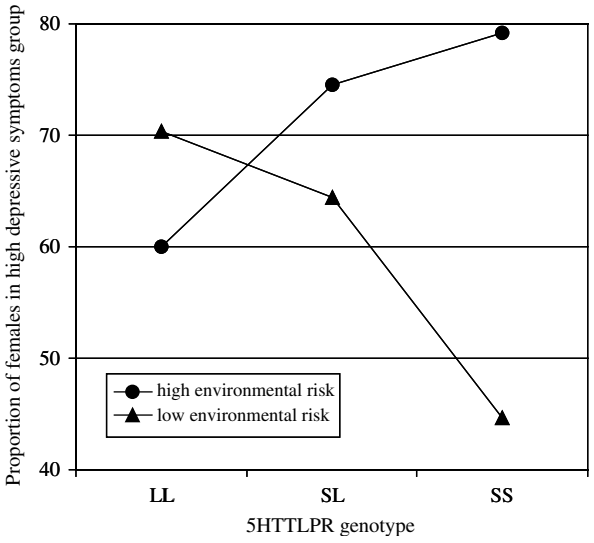

Figure 1 Proportion of female subjects with a high level of depression by environmental risk group and genotype.

allele 5 roughly halves the odds of being in the high depression group once sex and environmental risk are taken into account. There was also a nonsignificant interaction between *TPH1* and environmental risk group. This was found to be significant in the male subjects with an OR of 4.99 (CI = 1.03–24.26, $P<0.05$), although the effect was largely due to a small group of male subjects in the high environmental risk group with the 'TT' genotype, none of whom were in the high depression group. As a result of this small group leading to this finding, this result is not interpreted further.

It is of note that the interaction between *5HTTLPR* and the environmental risk group is significant in the larger group of female subjects (total $N = 216$), and is in the same direction as the only other published data examining gene–environment interactions for this marker in depression. In order to illustrate this interaction, the proportion of female subjects in the high depression group by *5HTTLPR* genotype for the two environmental risk groups is given in Figure 1. As can be seen from the figure, an increase in the number of 'short' alleles increases the likelihood of being in the high depression group for females within the high environmental risk group, such that those with two 'short' alleles are at nearly twice the risk of being in the high depression group as compared to those with two 'short' alleles but low environmental risk.

Discussion

This study is the first to report an analysis of the main effects and interactions between markers in the 5HT system and environmental risk on adolescent depression. We found evidence for the main effects of *5HT2A*, *TPH* and of *5HTTLPR* in female subjects only. In addition, we found evidence, albeit only in one of the sexes each, for interactions between both *5HT2A* and *5HTTLPR* and environmental risk group in prediction of depression group membership.

Results indicated that for *5HT2A*, there was a protective effect of the T allele, consistent with previous reports that the C allele is a risk factor for suicide ideation within a major depressive disorder.[12] These data are also in line with findings that the density of 5HT2A receptors is increased in the brain regions of depressed suicide victims[7] and in the platelets of suicidal individuals with a major depression.[8] These data indicate that *5HT2A* remains an important target for molecular genetic work in depression and related phenotypes.

Results for *TPH* revealed a protective effect for allele 5 (fragment length 198 bp). This allele was considered as it was found to be *putative* in the adult study, GENESiS.[42] However, as was noted in the original univariate analyses, there was no robust main effect in this sample independent of sex and environmental group, so further replication is required to examine this finding. Recently, a novel TPH gene (*TPH2)* has been described, which appears to be mainly responsible for TPH activity in the murine brain.[40] Nevertheless, given the currently available evidence, this situation may not be the case in humans as the reported sequence of cDNA derived from the human brain is from the *TPH1* gene rather than the *TPH2* gene.[43] However, it will be of interest to study the expression pattern of *TPH2* and any potential association of it with anxiety and/or depression.

For *5HTTPLR*, there was a significant risk from the 'short–short' genotype, but only for girls in the high environmental risk group. Indeed, the overall (nonsignificant) trend suggests an association of the short allele with low depressive symptoms groups. This is particularly interesting as the observations replicate in part recent findings from another study, which found that the short allele of the *5HTTPLR* interacts with life events to increase risk for depression and suicidality.[11] The main difference between the two sets of results is that in our study the increased risk of depression for those with both the short–short genotype and high environmental risk was only present for girls, whereas in the study of adults the finding applied to both sexes (K Sugden, personal communication). However, it should also be noted that our sample consisted of individuals in the adolescent age range, rather than young adulthood. Hormonal fluctuations in female subjects, starting in adolescence, may be especially relevant to mood disorders, which are twice as common in females as they are in males.[44] These findings are also in line with another recent study that revealed an interaction between the short allele of the *5HTTLPR* and fearful stimuli on increased amygdala activity in adults.[45] Furthermore, these results are consistent with research in animals on the topic, including an interaction between the *5HTTLPR*, stress and increased fearful behaviour in mice,[46] and an interaction between *5HTTLPR*, stressful-rearing conditions and

decreased serotonergic function in rhesus monkeys.[47] What clearly emerges from these studies is that the effects of *5HTTLPR* are dependent on environmental stress. This may in part explain the difficulties seen in trying to replicate the original association between the *5HTTLPR* and neuroticism and other anxiety- and depression-related traits.[48]

At face value, the recent observations suggest that the short (low-activity) allele of *5HTTPR* is a risk factor for depression may appear to be unexpected, given the efficacious prescription of SSRIs. However, much remains to be understood concerning the activity of 5HT at the synapse and its significance with respect to affective symptoms and disorders. The time course of the effects of SSRI drugs suggests that neurogenesis may be required for their activity.[49] The possibility of changes in concentrations of pre- and postsynaptic *5HT* receptors makes simplistic interpretations untenable and it is possible that drug effects may interact with other neurotransmission pathways. The hope must be that accumulated data from studies that examine the polymorphic variants at most, or all of the significant loci implicated in 5HT system and their interaction with environmental measures, will eventually lead to a clearer understanding of the important parameters in understanding and treating depression.

This study benefits from a number of strengths including a large sample size and unusual but important age-range (mid to late adolescence), when there is a high rate of first onsets of depression. The sample also gains power from the selected extremes design. However, there are also a number of limitations. First, the number of individuals in the four 'depression by environmental risk' groups differed, with the group in high environmental risk but low depression being only around half the size of the other three groups. While this may be expected, it does reduce the power of this specific group. Furthermore, the sex distribution across the four groups was not consistent, with a high preponderance of female subjects in the high relative to low depression groups. Again, this would be expected, given the frequency differences in depression between girls and boys from adolescence onwards. Replication of these analyses is clearly paramount, but as a result of these distributions, it would be particularly beneficial to examine these findings in a sample with a high number of those at high environmental risk but low depression, and of male subjects with high levels of depression. Second, many of our results were significant only at the 0.05 level, and this also adds to the need for replication. We have therefore presented confidence intervals for all our ORs. Our use of a selected extremes design increased power relative to an unselected sample, but nonetheless a larger sample would be of benefit. However, it should be noted that in order to halve the confidence interval, we would need to quadruple the sample size. Third, the measure of depression used was a self-report questionnaire rather than a diagnostic interview. Thus, the individuals with high levels of depression would not necessarily approach the criteria for a major depressive disorder. However, we selected the top 15% on our depression measure, so the sample is likely to reflect those with symptom levels approaching clinical significance. Finally, our measure of environmental stress was parent reported and related to family-wide aspects of environmental risk rather than child-specific aspects of the environment. Although this has the important benefit of providing a different source of information for the measure of depression (adolescent self-report) and the measure of environmental risk (parental report), future studies would also benefit from assessing individual-specific environment directly from the adolescents themselves.

In summary, this study considered the role of markers in the serotonergic system for adolescent depression. Furthermore, we examined the combined influence of genotype, sex and environmental risk and their interactions on depression symptoms. Both *HTR2A* and *TPH* significantly predicted high levels of depression symptoms, with a significant effect for *5HTTLPR* in female subjects only. There were also significant interactions with environmental risk for both 5*HTLPR* (in females) and *TPH* (in males) consistent with the hypothesis that there is heterogeneity in the effects of genetic risks, which may only be an impact on subgroups of the population. One of these findings (*5HTTLPR*) partially replicates recent data in the adult literature, indicating that this is likely to be more than a chance effect. While these results are clearly preliminary and in need of further replication, they indicate that further work is needed both with adolescent samples and using interaction approaches.

Acknowledgements

This research was supported by a project grant from the WT Grant Foundation and by a Career Development Award from the British Medical Research Council to the first author. The GENESiS project was supported by Grant G9901258 from the UK Medical Research Council. We thank the families of the G1219 study for their time, and Matthew Nash, Abram Sterne, Richard Williamson and Maria Napolitano for their contributions to the project.

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