PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	**Interaction of Early Environment, Gender and Genes of Monoamine Neurotransmission in the Etiology of Depression in a Large Population-Based Finnish Birth Cohort**
AUTHORS	Nyman, Emma; Sulkava, Sonja; Soronen, Pia; Miettunen, Jouko; Loukola, Anu; Leppä, Virpi; Joukamaa, Matti; Mäki, Pirjo; Järvelin, Marjo-Riitta; Freimer, Nelson; Peltonen, Leena; Veijola, Juha; Paunio, Tiina

VERSION 1 - REVIEW

REVIEWER	***Helge Frieling*** Professor for Molecular Psychiatry Department of Psychiatry, Socialpsychiatry and Psychotherapy Hannover Medical School (MHH) Germany Potential competing interest: none to declare
REVIEW RETURNED	15-Feb-2011

GENERAL COMMENTS	Reviewer completed checklist only. No further comments were made

REVIEWER	***Frances Rice*** Reader University College London UK
REVIEW RETURNED	03-Mar-2011

THE STUDY	This is potentially an interesting paper in a large cohort. However, as it stands there are a number of problems with the paper. The testing for the particular gene-environment interactions appears in the paper to be primarily exploratory (as opposed to based on a prior neurobiological hypotheses as stated on page 22). Thus, while some genes are selected for prior association or GxE with depression, others are selected of the basis of GxE with other phenotypes e.g. antisocial behaviour - MAOA or on association with other phenotypes e.g. cognition - COMT. Selecting genes on the basis of being involved in dopamine, serotonin and norepinephrine functioning appears to be rather broad. A clearer rationale for selecting these genes (in the context of the particular environmental factors) should be made in the introduction. The discussion of environmental factors in the introduction is very cursory. The particular environmental factors are not robustly associated with depression - why were birth weight, socio-economic status, unwanted pregnancy etc chosen? Presumably this is because that is what information was available rather than a strong a priori

	hypothesis for their involvement in the aetiology of depression and for putative interaction with monoamine function. These are environmental factors that are likely to be indicators or markers of risk rather than risk factors (see e.g. Moffitt et al 2005 who clearly outline reasons for focusing on environmental pathogens i.e. proximal environmental risk factors that are likely causal in GxE studies). I also note that there is no environmental association (reported in text on page 14) with depression. This appears to be problematic – there ought to be a main environmental effect if there is modification of the environmental effect size or direction by genotype i.e. GxE Important information is not given: We first need to see the univariate environmental and genetic associations separately before interaction is tested. I.e. is there a main G or E effect and what is the magnitude of the effect? If there is no main G and no main E effect (the lack of an environmental effect is reported in text) – how can the GxE results reported be interpreted? There is no correction for multiple testing. The authors mention this but it does seem a problem. More than 69 tests are run (69 tests also run separately by gender, by the two types of distal environmental factor and for the whole sample n=69x4). The authors still take an alpha value of p.05 as informative. 69 tests would be expected to yield between 3 and 4 p values<.05 purely by chance. Yet, then additional haplotype analyses are tested. Other comments: P14 How was gene environment correlation tested for? The paper is difficult to follow in places and the written English needs to be examined throughout. Some terms and tables are not defined or mentioned in text e.g. TPH2 is not mentioned in the introduction or defined in full. Table 1 is not mentioned in text. In Table 1 a cutpoint of 1.75 on the depression questionnaire is used – why was that cutpoint chosen?

REVIEWER	***Thalia Eley*** Reader in Developmental Behavioural Genetics SGDP Centre Institute of Psychiatry Kings College London
REVIEW RETURNED	31-Mar-2011

THE STUDY	As per my review they have missed out two key papers inteh area, and the English is of slightly poor quality throughout. ust needs minor editing basically.
RESULTS & CONCLUSIONS	As noted in my main review, the sample is really too small for the number of unrelated hypotheses tested here.
GENERAL COMMENTS	In this paper the authors examine interactions between measures of early environment, gender and SNPs in various neurotransmitter genes on the etiology of depression. Although they offer some positive findings these do not withstand correction for multiple testing, thus are indicative rather than definitive. The main strengths of the paper are the use of a relatively large founded population sample, and a relatively targeted approach to genotyping. The GxE approach is one that is gathering quite a bit of momentum at

	present, so these results are of interest, but they are somewhat limited by the following aspects. 1. The authors only minimally reflect on the current literature on GxE, and particularly the controversy over the SERT results. Indeed they do not mention the two most recent relevant papers, one a review (Uher & McGuffin, Molecular Psychiatry, 2010) the other a meta-analysis (Karg, Archives General Psychiatry, 2011, online early) both of which support a more positive take on the attempts at replication of the original findings as compared to the Risch paper. 2. This study is almost certainly one of convenience, making us of data already collected in an ongoing study. Had the authors set out to test the role of early aspects of the environment on subsequent adult depression, I doubt they would not have assessed the early environment as done here. Indeed it is notable that no main effect on depression is found which speaks for itself really. Do the authors have a measure of "age of mother at birth of first child"? This would perhaps function better as a global measure of social environmental risk. 3. The early developmental risk variables are also not entirely appropriate, particularly given the possibility that similar genes influence these factors AND depression, a possibility which is not addressed in the paper as far as I could see. 4. As the authors themselves acknowledge the sample is rather small for the number of tests calculated. This is particularly notable when they reveal that of 69 tests, just 4 are $p < .05$, which is exactly what would be expected by chance. 5. I found it surprising that given interest in the COMT gene, the authors did not specifically examine the val158met polymorphism. Until page 19 they did not even mention this marker which is extraordinary given that the bulk of positive findings for COMT relate specifically to that marker. They state on page 19 that the allele G of rs4680 "corresponds" to the val allele, but give no reference to this assertion and no further details. I found myself very doubtful about this as a result. If the val158 is in full LD with the G allele of rs4680 then this should be stated, with appropriate references, when the use of this SNP in the current study is first described. 6. Over-all I felt this paper would have benefited from being rather tighter. At present it reads rather like a wide net being cast with only modest catch. I would have preferred to see, for example, wide coverage of all SNPs in all serotonin related genes or something along those lines. The current paper included rather a surprising mixture if genes, and in particular the dopamine genes seem a strange choice here. 7. Finally, the paper could do with reading by a native English speaker as there are numerous points where the English is not as clear as it could be.

VERSION 1 – AUTHOR RESPONSE

Responses to Reviewer #1:

Reviewer: Frances Rice
Reader
University College London
UK

This is potentially an interesting paper in a large cohort. However, as it stands there are a number of

problems with the paper.

Reviewer's comment 1: The testing for the particular gene-environment interactions appears in the paper to be primarily exploratory (as opposed to based on a prior neurobiological hypotheses as stated on page 22). Thus, while some genes are selected for prior association or GxE with depression, others are selected of the basis of GxE with other phenotypes e.g. antisocial behaviour - MAOA or on association with other phenotypes e.g. cognition - COMT. Selecting genes on the basis of being involved in dopamine, serotonin and norepinephrine functioning appears to be rather broad. A clearer rationale for selecting these genes (in the context of the particular environmental factors) should be made in the introduction.

Author's reply: Thank you for the comments. We have now added more detailed explanation of the rationale for gene selection, including additional references to literature on the genes selected into the Introduction, in particular to literature on gene-environment interaction studies. In the article Introduction from line 4 on p. 8 to line 8 on p. 9: Instead of saying "According to the monoamine hypothesis, depression is caused by underactivity in brain monoamines, such as dopamine, serotonin, and norepinephrine.[10] Recent results of neuroimaging studies have provided further support for this theory.[11] The most solid evidence from candidate gene studies has perhaps been obtained for the interaction of serotonin transporter and stressful life events,[12] although a recent meta-analysis objects those findings.[13] Other robust genetic findings have been obtained on the COMT gene for cathecol-O-methyltransferase, an enzyme catabolising catecholamines such as dopamine and noradrenaline, that has been implicated f.ex. in cognition,[14] and on monoamine oxidase A, an enzyme oxidizing neurotransmitter and dietary monoamines such as serotonin, noradrenaline and dopamine, in which a mutation for an early stop codon was found to segregate in a family with antisocial behaviour,[15] and the gene was later related to antisocial behaviour after maltreatment in childhood.[16] To advance our understanding of the etiology of depression, we aimed to investigate candidate genes of monoamine neurotransmission and their interaction with early developmental and social risk factors for depression in a sample of 5225 individuals from a large Finnish isolated population cohort.", the text now reads "According to the monoamine hypothesis, depression is caused by underactivity in brain monoamines, such as dopamine, serotonin, and norepinephrine.[11] Recent results of neuroimaging studies have provided further support for this theory.[12] The most solid evidence from candidate gene studies has perhaps been obtained for the interaction of the SLC6A4 gene for serotonin transporter and stressful early and current life events,[13] including positive results from a recent review[14] and meta-analysis of all studies to date[15], although there are also contradicting results.[16] Other robust genetic findings have been obtained on the COMT gene for catechol-O-methyltransferase, an enzyme catabolising catecholamines such as dopamine and noradrenaline, which has been implicated in depression in conjunction with stress,[17] and on the MAOA gene for monoamine oxidase A, an enzyme oxidizing neurotransmitter and dietary monoamines such as serotonin, noradrenaline and dopamine, which has been associated with depression in interaction with severity of maltreatment in childhood.[17] Furthermore, TPH2 gene for tryptophan hydroxylase 2, which is the brain-specific form of the key enzyme in serotonin synthesis, has been implicated to interact with stress on disorders of cognitive control and emotional regulation, including depression.[18] Within the dopamine transmission the DRD2 gene for dopamine receptor D2 has been associated with depressiveness and anxiety, combined with an effect of parenting in childhood,[19] and the DRD4 gene for dopamine receptor D4 has been associated with increased risk for obesity in women with seasonal affective disorder.[20] Thus, genes from the monoamine neurotransmission system are among the most thoroughly studied in psychiatric genetics and in particular in the etiology of mood disorders, and have provided perhaps the most robust evidence so far for interaction with various types of risk environments, including childhood environment. We chose to include these candidate genes of monoamine neurotransmission, including SLC6A4, TPH2, COMT, MAOA, as well as the dopamine receptor genes DRD1-DRD5, in our study on the etiology of depression with a particular focus on their interaction with

markers reflecting early developmental and social risk environments. The study was performed in a sample of 5225 individuals from a large Finnish isolated population cohort.". We feel that this addition has made the article more complete in its discussion of the rationale for choosing genes for the study.

Reviewer's comment 2: The discussion of environmental factors in the introduction is very cursory. The particular environmental factors are not robustly associated with depression - why were birth weight, socio-economic status, unwanted pregnancy etc chosen? Presumably this is because that is what information was available rather than a strong a priori hypothesis for their involvement in the aetiology of depression and for putative interaction with monoamine function. These are environmental factors that are likely to be indicators or markers of risk rather than risk factors (see e.g. Moffitt et al 2005 who clearly outline reasons for focusing on environmental pathogens i.e. proximal environmental risk factors that are likely causal in GxE studies).

Author's reply: Thank you for this comment. We have now emphasized the nature of the analyzed environmental variables more clearly in the text, i.e. they present available markers which reflect the risk environments, and have added these details on lines 5-9 of Introduction on p. 9: The text now reads "We chose to include these candidate genes of monoamine neurotransmission, including SLC6A4, TPH2, COMT, MAOA, as well as the dopamine receptor genes DRD1-DRD5, in our study on the etiology of depression with a particular focus on their interaction with available markers reflecting early developmental and social risk environments. ", and on line 20 of Methods on p. 11 the text now reads "The subjects (n=5225; 2509 males, 2716 females; 45 % of the 31 year follow-up study sample or 43% of the original study sample) were divided into high and low risk groups based on the available information reflecting early neurodevelopmental and social risk environments (Table 1).". We also added to Strengths and limitations of this study on lines 7-9 on p. 5: "Markers of early developmental and social environments were chosen to reflect risk as precisely as possible, however, the choice was limited by the availability of variables collected." We feel that these additions have made the article more precise in its description of the environmental factors used.

Reviewer's comment 3: I also note that there is no environmental association (reported in text on page 14) with depression. This appears to be problematic – there ought to be a main environmental effect if there is modification of the environmental effect size or direction by genotype i.e. GxE

Author's reply: Thank you also for this valuable comment, based on the suggestion we have added on lines 12-16 of Results on p. 16 the following: "Despite a priori evidence for the role of the markers which indicate a developmental high risk environment for in psychiatric health and wellbeing, there was no correlation with the HSCL score (P=0.131), whereas the social high risk environment, correlated significantly with the score (P = 0.00001).". We also added the following to Discussion on lines 10-18 on p. 21: "Nor were we able to detect a significant correlation of the developmental risk environment with depressive symptoms, despite the prior evidence for the role of its markers, which were low birth weight[21, 27] and late motor or verbal development[28], in decreased psychiatric health and wellbeing, including depression. This finding may reflect the presence of other environmental risk indicators which were not examined in our study. However, they may also reflect individual variability in response to the risk environment and presence of genetic factors (such as the COMT haplotype containing Met158) that may relate to resilience, adaptive changes in regulation of emotion reactivity and successful coping with stress.[38]". Furthermore, we also added to Discussion on lines 5-12 on p. 25 the following: "It is also noteworthy that we did not detect any association with our measure of current depression and the developmental risk environment, despite it being formulated based on previous reports of their effects on psychiatric health and wellbeing.[27-30]. However, the effect of genetic risk may be modulated by early life stress even though the direct link between early life environment and current status would be too weak to be detected in our study sample, and this modulating effect may be seen in the results of the GxE analysis.". We feel that

these additions have made the article more precise in its description of the environmental effects.

Reviewer's comment 4: Important information is not given: We first need to see the univariate environmental and genetic associations separately before interaction is tested. I.e. is there a main G or E effect and what is the magnitude of the effect? If there is no main G and no main E effect (the lack of an environmental effect is reported in text) – how can the GxE results reported be interpreted?

Author's reply: Thank you for this comment, we clarify that in Table 2 column 11, P(All), the univariate genetic associations are given, and we have now also added information on the magnitudes of the environmental effects to the manuscript, into Results on lines 12-16 on p. 16: "Despite a priori evidence for the role of the markers which indicate a developmental high risk environment for psychiatric health and wellbeing, namely low birth weight[21, 27] and late motor or verbal development[28], there was no correlation between these markers and the HSCL score in the present sample (P=0.131), whereas the social high risk environment, correlated significantly with the score (P = 0.00001).". The lack of univariate effects in the presence of interaction, and possible reasons and interpretations therefore were already discussed in our answer to the previous comment (number 3) on main E effects. Similar reasons and interpretations for the lack of main G effects in the presence of observed interactions apply here as well.

Reviewer's comment 5: There is no correction for multiple testing. The authors mention this but it does seem a problem. More than 69 tests are run (69 tests also run separately by gender, by the two types of distal environmental factor and for the whole sample n=69x4). The authors still take an alpha value of p.05 as informative. 69 tests would be expected to yield between 3 and 4 p values<.05 purely by chance. Yet, then additional haplotype analyses are tested.

Author's reply: We are very well aware that multiple testing is a real problem in our study, as in all genetic studies, and has to be acknowledged fully. First of all, we planned our statistical analyses to be performed in a step-wise manner to maximize our ability to detect associations and to minimize multiple testing. Secondly, we now acknowledge the limitations imposed on our findings by multiple testing in the revised manuscript in several occasions, first in Results on lines 12-15 on p. 15 "Out of the 69 genetic variants examined, none gave a statistically significant association signal with depressiveness or for an interaction with early developmental or social risk environments, which would survive correction for multiple testing." and more later in the manuscript, in Results on lines 21-22 on p. 16 and lines 1-3 on p. 17: "Although none of the association findings of these primary analyses survived correction for multiple testing, post hoc association analyses in gender groups led to a finding close to statistical significance even when taking into account the amount of multiple testing performed (P=0.0006 for males with rs4274224 in DRD2).", and in Discussion on lines 3-6 on p. 20: "However, when specific environmental factors were considered, some signals for association were observed, although none of them survive correction for multiple testing.". Third, we also discuss multiple testing in the Descussion on lines 3-14 on p. 24: "We did not use the Bonferroni correction for multiple testing due to limitations of sample size and expected magnitude of gene effects in complex traits. Although none of the results from the primary analyses (Table 2) survive conservative correction, a neurobiological a priori hypothesis based on previously published studies supports the validity of our most robust findings. It is, however, noteworthy that they were observed only when the sample was conditioned on environmental risk or gender. Still, the strongest association signal, obtained using DRD2's rs4274224 with HSCL score in males (P=0.0006), remains close to statistical significance even when taking into account the amount of multiple testing performed. The finding was further supported by results of our haplotype analysis containing rs4274224, which showed a statistically significant association with the HSCL score in males (P=0.00005).".

Reviewer's comment 6: Other comments:
P14 How was gene environment correlation tested for?

Author's reply: This is now clarified in the revised version of the manuscript by stating in Statistical analysis on lines 8-11 p. 14: "we tested for gene-environment correlations (rGEDev and rGSoc) and associations of the risk environments with the HSCL score (PASW Statistics 18, linear regression model).". Furthermore, the numeric values for the gene-environment correlation results were added to Table 2, columns 12-13, P(rGEDev) and P(rGSoc).

Reviewer's comment 7: The paper is difficult to follow in places and the written English needs to be examined throughout.

Author's reply: We have corrected the language of the manuscript and aimed to make it more concise and easy to read.

Reviewer's comment 8: Some terms and tables are not defined or mentioned in text e.g. TPH2 is not mentioned in the introduction or defined in full. Table 1 is not mentioned in text. In Table 1 a cutpoint of 1.75 on the depression questionnaire is used – why was that cutpoint chosen?

Author's reply: We have now added the missing definitions and clarifications to the manuscript. The full names of the genes are added to Table 2, column 2, Gene name, and TPH2 is mentioned in Introduction on lines 14-17 p. 8: "Furthermore, TPH2 gene for tryptophan hydroxylase 2, which is the brain-specific form of the key enzyme in serotonin synthesis, has been implicated to interact with stress on disorders of cognitive control and emotional regulation, including depression.[18]". Table 1 is mentioned in the text in Methods lines 18-21 p. 11: "The subjects (n=5225; 2509 males, 2716 females; 45 % of the 31 year follow-up study sample or 43% of the original study sample) were divided into high and low risk groups based on the available information reflecting early neurodevelopmental and social risk environments (Table 1).". Furthermore, we have added as footnote to Table 1 the following clarification: "1 There is prior support for using the HSCL score 1.75 as a cut-off when aiming to identify clinical depression.". Such support is given for example in (Lehtinen et al., 1995): LEHTINEN, V., JOUKAMAA, M., KARLSSON, H. & ROUHE, E. (1995) Agreement on diagnoses of mental disorder in the primary health care of Turku, Finland. Eur Psychiatry, 10, 11-6.

Responses to Reviewer #2:

Reviewer: Thalia Eley
Reader in Developmental Behavioural Genetics
SGDP Centre
Institute of Psychiatry
Kings College London

As per my review they have missed out two key papers inteh area, and the English is of slightly poor quality throughout. Just needs minor editing basically.

As noted in my main review, the sample is really too small for the number of unrelated hypotheses tested here.

In this paper the authors examine interactions between measures of early environment, gender and SNPs in various neurotransmitter genes on the etiology of depression. Although they offer some positive findings these do not withstand correction for multiple testing, thus are indicative rather than definitive. The main strengths of the paper are the use of a relatively large founded population sample, and a relatively targeted approach to genotyping. The GxE approach is one that is gathering

quite a bit of momentum at present, so these results are of interest, but they are somewhat limited by the following aspects.

Reviewer's comment 1: The authors only minimally reflect on the current literature on GxE, and particularly the controversy over the SERT results. Indeed they do not mention the two most recent relevant papers, one a review (Uher & McGuffin, Molecular Psychiatry, 2010) the other a meta-analysis (Karg, Archives General Psychiatry, 2011, online early) both of which support a more positive take on the attempts at replication of the original findings as compared to the Risch paper.

Author's reply: We have now added discussion on the SERT results and the two references to Introduction on lines 4-8 p. 8: "The most solid evidence from candidate gene studies has perhaps been obtained for the interaction of the SLC6A4 gene for serotonin transporter and stressful early and current life events,[13] including positive results from a recent review[14] and meta-analysis of all studies to date[15], although there are also contradicting results.[16]". We also added to Discussion on lines 12-17 p. 24 the following: "It is noteworthy that despite previous reports of the 5-HTTLPR variant,[13] we did not detect association evidence for SLC6A4. Similarly, a recent meta-analysis did not find any evidence of an association with depression alone, or in interaction with stressful life-events,[16] although a current review[14] and a meta-analysis of all studies to date[15] support the positive association findings and the role of 5-HTTLPR and stress in depression.". We also added further reflection on the current literature on GxE into Introduction from line 8 p. 8 to line 7 p. 9 : "Other robust genetic findings have been obtained on the COMT gene for catechol-O-methyltransferase, an enzyme catabolising catecholamines such as dopamine and noradrenaline, which has been implicated in depression in conjunction with stress,[17] and on the MAOA gene for monoamine oxidase A, an enzyme oxidizing neurotransmitter and dietary monoamines such as serotonin, noradrenaline and dopamine, which has been associated with depression in interaction with severity of maltreatment in childhood.[17] Furthermore, TPH2 gene for tryptophan hydroxylase 2, which is the brain-specific form of the key enzyme in serotonin synthesis, has been implicated to interact with stress on disorders of cognitive control and emotional regulation, including depression.[18] Within the dopamine transmission the DRD2 gene for dopamine receptor D2 has been associated with depressiveness and anxiety, combined with an effect of parenting in childhood,[19] and the DRD4 gene for dopamine receptor D4 has been associated with increased risk for obesity in women with seasonal affective disorder.[20] Thus, genes from the monoamine neurotransmission system are among the most thoroughly studied in psychiatric genetics and in particular in the etiology of mood disorders, and have provided perhaps the most robust evidence so far for interaction with various types of risk environments, including childhood environment. We chose to include these candidate genes of monoamine neurotransmission, including SLC6A4, TPH2, COMT, MAOA, as well as the dopamine receptor genes DRD1-DRD5, in our study on the etiology of depression with a particular focus on their interaction with available markers reflecting early developmental and social risk environments.".

Reviewer's comment 2: This study is almost certainly one of convenience, making us of data already collected in an ongoing study. Had the authors set out to test the role of early aspects of the environment on subsequent adult depression, I doubt they would not have assessed the early environment as done here. Indeed it is notable that no main effect on depression is found which speaks for itself really. Do the authors have a measure of "age of mother at birth of first child"? This would perhaps function better as a global measure of social environmental risk.

Author's reply: We have addressed these questions in our answers to Reviewer #1's questions 2 and 4.

Reviewer's comment 3: The early developmental risk variables are also not entirely appropriate, particularly given the possibility that similar genes influence these factors AND depression, a

possibility which is not addressed in the paper as far as I could see.

Author's reply: We have clarified this issue in the manuscript, and our results indicate that the genetic factors with evidence of gene-environment interaction (GxE) do not correlate with the risk environments (rGE). This is now more clearly stated in Table 2 where also gene-environment correlation results are presented in columns 12 and 13 (P(rGEDev) and P(rGSoc)). We also write in Results on lines 12-14 on p. 16: "The evidence for gene-environment correlations (rGE) was observed only nominally about rs1906451 from TPH2 (P=0.035), rs265973 from DRD1 (P=0.047), and rs9825563 from DRD3 (P=0.028).", and in Discussion on lines 11-13 on p. 21: "In our study we observed evidence for interaction between COMT and an early developmental risk environment on depressive symptoms. This interaction could not be explained through gene-environment correlations.".

Reviewer's comment 4: As the authors themselves acknowledge the sample is rather small for the number of tests calculated. This is particularly notable when they reveal that of 69 tests, just 4 are $p < .05$, which is exactly what would be expected by chance.

Author's reply: Thank you for this valuable comment that we have addressed in our answer to Reviewer #1's question 5.

Reviewer's comment 5: I found it surprising that given interest in the COMT gene, the authors did not specifically examine the val158met polymorphism. Until page 19 they did not even mention this marker which is extraordinary given that the bulk of positive findings for COMT relate specifically to that marker. They state on page 19 that the allele G of rs4680 "corresponds" to the val allele, but give no reference to this assertion and no further details. I found myself very doubtful about this as a result. If the val158 is in full LD with the G allele of rs4680 then this should be stated, with appropriate references, when the use of this SNP in the current study is first described.

Author's reply: Thank you for this comment. It appears that we were not clear enough in expressing that the COMT Val158Met polymorphism is on DNA level the same exact polymorphism SNP rs4680, the G allele of which codes for the Val allele on the protein level. We therefore added the following clarification to lines 19-20 on p. 20 in Discussion: "The high risk haplotype included the high activity variant Val158 of COMT, the allele G of rs4680.".

Reviewer's comment 6: Over-all I felt this paper would have benefited from being rather tighter. At present it reads rather like a wide net being cast with only modest catch. I would have preferred to see, for example, wide coverage of all SNPs in all serotonin related genes or something along those lines. The current paper included rather a surprising mixture if genes, and in particular the dopamine genes seem a strange choice here.

Author's reply: We have discussed and further clarified our rationale for gene selection in our answer to Reviewer #1's question 1.

Reviewer's comment 7: Finally, the paper could do with reading by a native English speaker as there are numerous points where the English is not as clear as it could be.

Author's reply: We have corrected the language of the manuscript and aimed to make it more concise and easy to read.

VERSION 2 - REVIEW

REVIEWER	***Thalia Eley***
REVIEW RETURNED	07-Jun-2011

GENERAL COMMENTS	Nyman et al. Interaction of early environment, gender and genes on depression Please note that I am a behavioural geneticist, not a statistical geneticist, so I assume someone more familiar with haplotype analysis will also have reviewed this work. This paper tests the interesting hypothesis that the impact of monoamine genes on depression is moderated by early environmental influences and/or gender. The strengths of the study include a genetically homogenous and large sample, broad SNP coverage in each gene considered and clear writing style. They find modest evidence for SNPs (particularly when examined as haplotypes) in COMT and DRD2. I have the following suggestions for revision. 1. In the "strengths and limitations" section, you end with "Finally, a complete coverage of the major candidate genes that are relevant with the present focus is provided". I'm not sure I agree with this. You do cover each gene well with multiple SNPs, but were I to select monoamine genes for a paper of this kind I would also consider the serotonin receptors. You need to say why you did not include these genes, and you need to re-word this final sentence. 2. You describe two measures that are considered aspects of the early environment. I agree that for the "social environment" measure this is a reasonably appropriate label. However, your other variable is definitely not an environmental variable, but rather an index of early development. I would simply state that you have a measure of early development, a measure of social environment, and gender as your three covariates in these analyses. 3. On page 12 where you say "as defined by father's social class at birth" you then list occupations. Thus this would be more accurate, i.e. "as defined by father's occupation at birth". 4. With respect to your haplotype analyses, which are undoubtedly where the interest lies in this paper, it would be good to know that each of these accounts for more variance in depression than any single individual constituent SNP. 5. The discussion is too long particularly given the somewhat marginal nature of the findings. 6. Although in places the authors are appropriately circumspect in their conclusions, in others they make their results sound more significant/substantial than they are. For example, on page 20, 6th line of text, an adjective such as "modest" should be inserted, i.e. "Our study sample provided modest evidence of…" Similarly on the 2nd paragraph of page 22, which currently begins with "Another major finding…" None of these are major findings. A more appropriate phrase would be "Another main finding of the present study…" Similarly in the final paragraph, again it should be "Our results support A MODEST role of COMT and DRD2…" My over-all view remains similar – this could be published as is if you want to publish it. BUT having just gone back to my earlier review I do not feel they have really addressed many of the points I made. Most importantly, they still over-state the importance of their findings and the paper is over-long (particularly the discussion).

VERSION 2 – AUTHOR RESPONSE

Responses to Reviewer #1:

Reviewer: Thalia Eley
Reader in Developmental Behavioural Genetics
SGDP Centre
Institute of Psychiatry
Kings College London

This paper tests the interesting hypothesis that the impact of monoamine genes on depression is moderated by early environmental influences and/or gender. The strengths of the study include a genetically homogenous and large sample, broad SNP coverage in each gene considered and clear writing style. They find modest evidence for SNPs (particularly when examined as haplotypes) in COMT and DRD2. I have the following suggestions for revision.

Reviewer's comment 1: In the "strengths and limitations" section, you end with "Finally, a complete coverage of the major candidate genes that are relevant with the present focus is provided". I'm not sure I agree with this. You do cover each gene well with multiple SNPs, but were I to select monoamine genes for a paper of this kind I would also consider the serotonin receptors. You need to say why you did not include these genes, and you need to re-word this final sentence.

Author's reply: Thank you for the comments. We have now modified the manuscript text to reflect this comment by removing the final sentence of the Strengths and limitations of this study section from p. 6 line 2, "Finally, a complete coverage of the major candidate genes that are relevant with the present focus is provided." Similarly, we have also removed the last sentence of the second-to the-last paragraph of Discussion from p. 26 line 10, "Furthermore, we provide a complete coverage of the major candidate genes that are relevant with the present focus." Moreover, we have added further clarification of our rationale of selecting genes for study. Namely, in the last paragraph of Introduction on p. 9 line 6 we have added the phrase "showing prior evidence of gene-environment interaction", so that the sentence now reads "We chose to include these candidate genes of monoamine neurotransmission showing prior evidence of gene-environment interaction, including SLC6A4, TPH2, COMT, MAOA, as well as the dopamine receptor genes DRD1-DRD5, in our study on the etiology of depression with a particular focus on their interaction with available markers reflecting measures of early development and of social environment." Likewise, we have removed the words "many of" from the end of the first paragraph of Methods on p. 10 line 7, so that the sentence now reads "We examined interactions of these measures with candidate genes of the monoamine neurotransmitter systems, which have prior evidence of gene-environment interaction on affective disorders, namely SLC6A4, TPH2, COMT, MAOA, and the dopamine receptor genes DRD1-DRD5."

Reviewer's comment 2: You describe two measures that are considered aspects of the early environment. I agree that for the "social environment" measure this is a reasonably appropriate label. However, your other variable is definitely not an environmental variable, but rather an index of early development. I would simply state that you have a measure of early development, a measure of social environment, and gender as your three covariates in these analyses.

Author's reply: We agree with the reviewer and have changed the wording in the manuscript accordingly. We now use the phrases "measure of early development" and "measure of social

environment" throughout the manuscript to describe these environmental indices, namely in the Abstract on p. 3 lines 8 and 14, in Article focus section on p. 4 line 12, in Strengths and limitations of this study on p. 5 line 7, in Introduction on p. 9 line 9, in Methods on p. 10 lines 2, 3, 5 and 6, and on p. 12 lines 3-5 and line 12, and on p. 14 line 3, as well as in Results on p. 15 lines 7-10 and 13-14, and on p.16 line 9, as well as in Discussion on p. 20 lines 5 and 9, and on p. 21 lines 13-15, and on p. 24 line 10, and on p. 25 lines 5 and 9. We also changed the wording similarly in Table 1 column headings and table footnotes, in Table 2 label and footnotes, and in Table 3 label, columns headings and table footnotes.

Reviewer's comment 3: On page 12 where you say "as defined by father's social class at birth" you then list occupations. Thus this would be more accurate, i.e. "as defined by father's occupation at birth".

Author's reply: We agree with the reviewer on this and have now changed the wording in Methods on p. 12 line 18 to "…as defined by father's occupation at birth…"

Reviewer's comment 4: With respect to your haplotype analyses, which are undoubtedly where the interest lies in this paper, it would be good to know that each of these accounts for more variance in depression than any single individual constituent SNP.

Author's reply: We have now taken this comment into account by adding the following to the manuscript text: In Results in the first paragraph on p. 18 lines 3-5 we have added the sentence "As is evident from the β coefficient values, the effect of each of the haplotypes on depression is greater than that of any individual constituent SNP.". Similarly, we have added to the end of the next paragraph, starting from the last line of p. 18 to the second line of p. 19 "Similarly as for the COMT haplotypes, higher β coefficient values imply that the effects of DRD2 haplotypes on depression are greater than those of individual constituent SNPs." Furthermore, we have also added to Results to the first paragraph on p. 17 lines 6-7 the phrase "and to obtain a maximal amount of information on the nature of the associations observed" so that the full sentence now reads "Furthermore, as there was an accumulation of association signals within two highly plausible candidate genes, DRD2 and COMT, we proceeded to perform haplotype analyses on these genes in order to better characterize the allelic variants which yielded the observed suggestive associations, and to obtain a maximal amount of information on the nature of the associations observed."

Reviewer's comment 5: The discussion is too long particularly given the somewhat marginal nature of the findings.

Author's reply: We have attempted to trim the length of the Discussion throughout by making the wording as concise as possible to accommodate this comment.

Reviewer's comment 6: Although in places the authors are appropriately circumspect in their conclusions, in others they make their results sound more significant/substantial than they are. For example, on page 20, 6th line of text, an adjective such as "modest" should be inserted, i.e. "Our study sample provided modest evidence of…" Similarly on the 2nd paragraph of page 22, which currently begins with "Another major finding…" None of these are major findings. A more appropriate phrase would be "Another main finding of the present study…" Similarly in the final paragraph, again it should be "Our results support A MODEST role of COMT and DRD2…"

Author's reply: We have made changes to the manuscript text to take this comment fully into account, not only in the two instances mentioned in the comment above, but also elsewhere in the manuscript. Namely, in the Abstract on the last line of p. 3 we have removed the word "significant", and on p. 4 lines 1-2 added the word "a modest", as well as in the Key messages section on p. 4 line 15 have added the word "modest", as well as in Discussion on p. 20 line 8 have added the word "modest", and on p. 22 line 15 have changed the word "major" to the word "main", and on p. 26 line 11 added the word "a modest".

My over-all view remains similar – this could be published as is if you want to publish it. BUT having just gone back to my earlier review I do not feel they have really addressed many of the points I made.
Most importantly, they still over-state the importance of their findings and the paper is over-long (particularly the discussion).