



Interaction of Early Environment, Gender and Genes of Monoamine Neurotransmission in the Etiology of Depression in a Large Population-Based Finnish Birth Cohort

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Interaction of Early Environment, Gender and Genes of Monoamine Neurotransmission in the Etiology of Depression in a Large Population-Based Finnish Birth Cohort

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44 Key words: Environment, Monoamine, Gene, Depression, Cohort Study

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ABSTRACT

Objectives Depression is a worldwide leading cause of morbidity and disability. Genetic studies have recently begun to elucidate its molecular etiology. We investigated candidate genes of monoamine neurotransmission and early environmental risk factors for depressiveness in the genetically isolated population-based Northern Finland Birth Cohort 1966 (12058 live births).

Design We ascertained and subdivided the study sample (n=5225) based on early developmental and social risk environments and examined candidate genes of monoamine neurotransmission, many of which have shown prior evidence of gene-environment interaction (GxE) for affective disorders, namely *SLC6A4*, *TPH2*, *COMT*, *MAOA*, and the dopamine receptor genes *DRD1-DRD5*.

Results and Conclusion Four variants (three in *COMT* and one in *DRD3*) interacted with high early developmental risk environment, the strongest evidence being for rs2239393 in *COMT* ($P=0.005$) that showed evidence of association in the high risk group ($P=0.008$ and $\beta=0.044$; $P=0.0053$ and $\beta=0.083$ for rs5993883-rs2239393-rs4680 risk haplotype CGG including Val158 in high risk males). Only one variant, rs4274224 from *DRD2*, interacted with gender showing significant association in males ($P=0.0006$ and $\beta=0.0023$; $P=0.00005$ and $\beta=0.069$ for rs4648318-rs4274224 haplotype GG). Our results support the role of genes of monoamine neurotransmission in the etiology of depression, particularly in males, and interaction of the early developmental risk environment with a high risk haplotype of *COMT* including Val158. The results also imply

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3 gender-specific mechanisms of mood regulation and responses to environmental
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5 effectors.
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11 **ARTICLE SUMMARY**

14 **Article focus**

- 17 - Gene-environment and gene-gender interaction in the etiology of
18 depression
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- 20 - Effect of early neurodevelopmental and social risk environments on
21 depression
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- 23 - Impact on depression of monoaminergic candidate genes with prior
24 evidence of gene-environment interaction for affective disorders, and the
25 dopamine receptor genes
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35 **Key messages**

- 36 - Genes of monoamine neurotransmission play a role in the etiology of
37 depression, especially in males
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- 39 - Early developmental risk environment interacts with a *COMT* high risk
40 haplotype including Val158
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- 43 - Gender-specific mechanisms of mood regulation and responses to
44 environmental effectors are evident
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52 **Strengths and limitations of this study** Limitations of this study include that
53 depression as defined does not necessary imply clinical diagnosis of major
54 depression, but instead was defined based on self-report or on the score from
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3 the HSCL measure, which has its limitations. Nevertheless, the prevalence of
4 depressed mood was in the same range as in earlier reports. Furthermore, there
5 was a notable drop-out rate among the original material of all cohort members,
6 and about half of the original cohort members did not participate in this study.
7
8 Advantages of this study include the availability of longitudinal follow-up data
9 starting antenatally, enabling inclusion of the environmental dimension without
10 risk of recall bias. Another advantage is the unique genetic structure of the study
11 cohort, characterized by isolation, founder effect, multiple bottlenecks and more
12 genetic homogeneity compared to many other isolates, permitting identification of
13 genetic risk loci that may be missed when using more heterogeneous
14 populations. Furthermore, the subjects were representative, with all cohort
15 members born in the same year and within a geographically defined area. Also,
16 the size of the study sample is sufficient for identifying genetic variants of
17 moderate impact. Both genders are also represented in almost equal amounts,
18 which is notable since gender differences are evident in both depression and
19 temperament traits, such as Harm avoidance. It is also beneficial that the sample
20 is a one-year birth cohort, as it is well established that some psychiatric
21 traits, such as Harm avoidance are age-dependent. The genetic effects may
22 therefore be isolated from the effects of aging. Furthermore, a complete
23 coverage of the major candidate genes that are relevant with the present focus is
24 provided.
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INTRODUCTION

Depression is a major cause of morbidity worldwide, major depression affecting 5-7% of the population annually and 16% over the lifetime.[1] Although a genetic component in the etiology of major depression is evident with a 40-50% heritability,[2] the predisposing genetic background has so far remained largely undefined, and recent findings from genome-wide association studies also point to a complex underlying architecture.[3] Depressed patients frequently exhibit comorbidities such as anxiety and alcohol abuse,[4] and certain personality types[5-7] have been associated with depression proneness.

Environmental risk factors, in particular stressors influencing during development,[8] are considered to have a significant impact on the development and course of depression. It is likely that many of the genetic risk factors for depression interact with the early developmental environment, but recapture of these interactions has remained a challenge for etiological studies of depression. Although the interplay between genes and environment has been investigated with respect to several psychiatric disorders[9] including depression, this vast subject remains still to a large extent unexplored. On the other hand, addressing the effects of genes and environment on psychiatric morbidity enables us to examine the two main constituents in their etiology. Therefore, we wanted to include the environmental dimension in our study in order to also explore gene-environment interactions (GxE).

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7 According to the monoamine hypothesis, depression is caused by underactivity
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9 in brain monoamines, such as dopamine, serotonin, and norepinephrine.[10]
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11 Recent results of neuroimaging studies have provided further support for this
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13 theory.[11] The most solid evidence from candidate gene studies has perhaps
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15 been obtained for the interaction of serotonin transporter and stressful life
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17 events,[12] although a recent meta-analysis objects those findings.[13] Other
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19 robust genetic findings have been obtained on the *COMT* gene for catechol-O-
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21 methyltransferase, an enzyme catabolising catecholamines such as dopamine
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23 and noradrenaline, that has been implicated f.ex. in cognition,[14] and on
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25 monoamine oxidase A, an enzyme oxidizing neurotransmitter and dietary
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27 monoamines such as serotonin, noradrenaline and dopamine, in which a
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29 mutation for an early stop codon was found to segregate in a family with
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31 antisocial behaviour,[15] and the gene was later related to antisocial behaviour
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33 after maltreatment in childhood.[16]
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45 To advance our understanding of the etiology of depression, we aimed to
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47 investigate candidate genes of monoamine neurotransmission and their
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49 interaction with early developmental and social risk factors for depression in a
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51 sample of 5225 individuals from a large Finnish isolated population cohort. As
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53 gender is an important confounder for depression and at least some of the
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3 genetic liability is gender-specific,[2] we also examined gene-gender interactions
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6 in this sample.
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10 11 12 **METHODS**

13 14 15 16 17 **Setting**

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22 We utilized the genetically isolated Northern Finland Birth Cohort (NFBC 1966) to
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24 investigate effects of candidate genes and environmental risk factors during
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26 development on depressiveness. We subdivided the study sample based on
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28 developmental risk factors arising from the fetal growth environment and
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30 neurological development during the first year of life (early developmental risk
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32 environment) as well as from the family environment during pregnancy and early
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34 childhood (social risk environment). We examined interactions of these
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36 environments with candidate genes of the monoamine neurotransmitter systems,
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38 many of which have prior evidence of gene-environment interaction on affective
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40 disorders, namely *SLC6A4*, *TPH2*, *COMT*, *MAOA*, and the dopamine receptor
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42 genes *DRD1-DRD5*.
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50 51 **Study subjects**

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3 The Northern Finland Birth Cohort 1966 (NFBC 1966) is a longitudinal one-year
4 birth cohort from an unselected population (N=12058 live births) comprising
5 inhabitants of the two northernmost provinces of Finland.[17] Data collection has
6 started from the antenatal period, and follow-up studies have been performed at
7 the ages of 1, 14 and 31 years. The cohort study has been approved by the
8 Ethical Committee of Oulu University Faculty of Medicine, and written informed
9 consent has been obtained from all participants.
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23 In 1997 in the 31-year follow-up study[18] all cohort members alive with a known
24 address (N=11540) were sent a postal questionnaire surveying lifestyle, social
25 status and health (76% participated), including the Hopkins Symptom Check
26 List-25 (HSCL).[19] and items on self-reported lifetime depression diagnosis
27 (“Has your doctor ever diagnosed a depressive disorder?”). Additionally, cohort
28 members who lived in Northern Finland or in the capital area (N=8465) were
29 invited to a clinical examination (71% participated) with another questionnaire to
30 be filled in later and sent to the research group (61% participated).[20] It
31 included, among others, a validated Finnish translation of Cloninger’s
32 Temperament and Character Inventory (TCI) questionnaire.[21]
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49 Current depressive symptoms were assessed by the HSCL-questionnaire,[22] a
50 25-item shortened version of an originally 90-item questionnaire. HSCL contains
51 13-item depression and 10-item anxiety subscales assessing presence and
52 intensity of depressive and anxiety symptoms during the previous week. Answers
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3 are scored on a scale from 1 (not bothered) to 4 (extremely bothered). HSCL
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5 total score is the sum of items divided by the number of items answered. We
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8 used mainly HSCL total score as symptoms of depression and anxiety are known
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10 to overlap significantly. In post hoc analyses in order to better understand the
11
12 original association signals, the separate HSCL subscales for depressive and
13
14 anxiety symptoms were also taken into consideration. In addition to current
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16 depressive symptoms (HSCL score) and lifetime (diagnosed) depression, we
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18 used the TCI temperament trait Harm avoidance[5-7] and its subcomponents as
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20 a measure of proneness to depression.
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27 The subjects (n=5225; 2509 males, 2716 females; 45 % of the 31 year follow up
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29 study sample or 43% of the original study sample) were divided into high and low
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31 risk groups based on early neurodevelopmental and social risk environments
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33 (Table 1). *The early developmental risk environment* was defined by 1) low birth
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35 weight (<2500 g),[17] considered to reflect suboptimal growth environment during
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37 fetal life and to increase risk for somatic and psychiatric diseases such as
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39 depression in adulthood,[23] 2) late motor development as reflected by first
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41 standing later than at the age of 10 months,[24] and 3) late development of
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43 speech, defined by no words at the age of one year.[24] If two out of these risk
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45 indicators were present, one was classified as having experienced a high risk
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47 environment for early brain development. *The social risk environment* was
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49 defined by the occurrence of two or more of the following five indicators for high
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51 risk social environment during pregnancy and early childhood: 1) unwantedness
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3 of pregnancy (rated by mothers of the cohort members at the sixth or seventh
4 month of pregnancy),[25] 2) low socio-economic status, linked with depression in
5 the offspring in earlier studies,[26] as defined by father's social class at birth (no
6 occupation, unskilled worker, or farmer with area under cultivation under 8
7 hectares), 3) single parenthood at birth, 4) low level of education of mother (less
8 than nine years of primary school), and 5) low level of information retrieval by the
9 mother related to pregnancy and child care. There was no significant drop-out in
10 either high risk groups as 43% and 41% of the individuals with high risk early
11 developmental and social environments, and 47% and 46% of those with the
12 respective low risk environments, were available for study.
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[Insert Table 1]

34 **Genotyping methods**

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38 We investigated genes relevant within the context of the monoamine hypothesis
39 of depression: *SLC6A4*, *TPH2*, *COMT*, *MAOA*, and the dopamine receptor genes
40 *DRD1-DRD5* (Table 2). The genotyping was performed at the Broad Institute
41 (Cambridge, MA, USA) on the HumanCNV370-duo chip (Illumina, San Diego, CA
42 USA) platform according to the manufacturer's instructions. The SNPs analysed
43 included HapMap tag SNPs (<http://www.hapmap.org/index.html.en>) and were
44 relatively evenly spaced to cover the genes and flanking regions.
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8 **Statistical analysis**

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12 LD structures were determined using HAPLOVIEW. Interaction analysis and
13 linear and logistic regression with permutation was performed using PLINK
14 Software Package Version 1.04, in a step-wise manner to maximize our ability to
15 detect associations and to minimize multiple testing. (i) Primarily analyses were
16 performed to identify genetic risk variants for current depressive symptoms
17 (HSCL score) interacting with early developmental risk ($G \times E_{Dev}$) and social risk
18 ($G \times E_{Soc}$) environments. For variants giving significant evidence of interaction, we
19 also performed analyses separately in subgroups of high and low risk,
20 respectively. As gender is an important confounder for depression and at least
21 some of the genetic liability is gender-specific,[2] we also examined gene-gender
22 interactions ($G \times Sex$). For variants showing significant evidence of gene-gender
23 interaction, we also performed analyses separately in males and females. In
24 order to form a complete view of the effects of the examined genes on
25 depressive symptoms in the cohort, we also examined their influence on the
26 HSCL score gender-adjusted in the complete sample regardless of
27 environmental effectors. Finally, we tested for gene-environment correlations
28 ($r_{GE_{Dev}}$ and r_{GSoc}) and associations of the risk environments with the HSCL
29 score. (ii) Haplotype analyses were performed when two SNPs located at close
30 vicinity physically had given association signals of $P < 0.05$ when analyzed
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3 separately. (iii) Genetic variants and haplotypes identified in the previous
4 analyses were analyzed post hoc with respect to HSCL subscales (depressive
5 and anxiety symptoms), depression diagnosis and TCI temperament Harm
6 avoidance. We report point-wise empirical p-values generated by PLINK's
7 max(T) permutation throughout the manuscript, and explicitly state where
8 corrected empirical p-values are reported. SNPs with Hardy-Weinberg
9 Equilibrium p-values <0.05 were excluded from all analyses.
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24 **RESULTS**

25 **Gene-environment and gene-gender interaction and association analyses** 26 **on HSCL score** 27 28 29 30 31 32

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35 We examined the effects of nine candidate genes of monomine
36 neurotransmission on current depressive symptoms (HSCL score) in a
37 longitudinal population-based NFBC 1966 cohort. In particular, we searched for
38 evidence of interaction of variants in these genes with early growth environments
39 with indicators for potentially disturbed neurobehavioral development (early
40 developmental risk environment) or with risk factors from social environment for
41 normal emotional development (social risk environment). The results are
42 presented in Table 2 in which nominal P-values are reported.
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3 Out of the 69 genetic variants examined, four showed some evidence of
4 interaction ($P < 0.05$) with high early developmental risk environment with respect
5 to the HSCL score. Three of the variants were located in *COMT*, namely an
6 intronic variant rs737866 at the 5' end of the gene ($P = 0.028$), rs2239393
7 ($P = 0.005$) and rs4680 ($P = 0.020$), and one in the 3' end of *DRD3*, rs9825563
8 ($P = 0.045$). All of them were associated with HSCL score in individuals of the high
9 risk group ($P = 0.036$, $\beta = 0.0414$ for rs737866; $P = 0.008$, $\beta = 0.0440$ for rs2239393;
10 $P = 0.042$, $\beta = 0.0320$ for rs4680; and $P = 0.022$, $\beta = -0.0396$ for rs9825563,
11 respectively). None of the variants gave evidence of interaction with the social
12 risk environment in relation to the HSCL score, nor did they show evidence of
13 gene-environment correlations (rGE). Despite a priori evidence for the role of the
14 indicators of the risk environments in psychiatric health and wellbeing, the risk
15 environments did not correlate with the HSCL score. Five of the genetic variants
16 showed evidence of interaction with gender ($P < 0.05$), including rs737866 and
17 rs5993883 in *COMT* and rs4274224 in *DRD2*. Out of these, only rs4274224
18 associated at $P < 0.05$ with one of the genders ($P = 0.0006$, $\beta = 0.023$ in males).
19 Finally, we observed some evidence of association of four variants with the
20 HSCL score in the complete sample: rs1487275 in *TPH2*, ($P = 0.049$, $\beta = 0.008$),
21 rs4646316 in *COMT* ($P = 0.026$, $\beta = 0.012$), rs4274224 and rs4581480 in *DRD2*
22 ($P = 0.022$, $\beta = 0.011$; and $P = 0.009$, $\beta = 0.022$, respectively), and rs13106539 in
23 *DRD5* ($P = 0.044$, $\beta = -0.008$).
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3 None of the association findings of these primary analyses, namely interaction
4 analysis or association analysis in the complete sample, survived correction for
5 multiple testing. However, post hoc analysis of associations with interaction with
6 gender lead to a finding close to statistical significance even when taking into
7 account the amount of multiple testing performed ($P=0.0006$ for males with
8 rs4274224 in *DRD2*). Furthermore, as there was an accumulation of association
9 signals within two highly plausible candidate genes, *DRD2* and *COMT*, we
10 proceeded to perform haplotype analyses on these genes in order to better
11 characterize the allelic variants yielding the observed suggestive associations.
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27 **Haplotype analysis of *COMT* and *DRD2* variants on HSCL score**

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31 As described above, two adjacent variants in *COMT*, rs2239393 and rs4680,
32 showed evidence of interaction with early developmental risk environment,
33 increasing risk for depressive symptoms in individuals with the high risk
34 environment (Table 2). We performed 2-SNP and 3-SNP haplotype analyses
35 combining these SNPs as well as their neighbouring variants using the sliding
36 window approach. Evidence of association was observed for rs5993883-
37 rs2239393 haplotype CG spanning a region from the space in-between LD
38 blocks 1 and 2 to block 2 of *COMT* (Supplementary figure 1) ($P=0.0049$,
39 $\beta=0.055$), for rs2239393-rs4680 haplotype GG in block 2 ($P=0.0072$, $\beta=0.044$),
40 and for rs5993883-rs2239393-rs4680 haplotype CGG ($P=0.0046$, $\beta=0.055$) in
41 individuals with the high early developmental risk environment (Table 3).
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3 Haplotype analysis of variants outside that region towards both ends of the gene
4 gave no further evidence for association ($P > 0.01$ for all allelic combinations).
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6 Thus, the results of these analyses are in synchrony with those of single variants,
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8 as both allele G of rs2239393 and allele G of rs4680 increase risk for depressive
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10 symptoms in individuals with the high early developmental risk environment.
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17 As one of the variants in this *COMT* haplotype, rs5993883, also gave evidence of
18 interaction with gender (Table 2), we further examined association of these
19 haplotypes in males and females of the high risk group separately. We found that
20 the haplotypes increased risk for depressive symptoms in males, but not in
21 females ($P = 0.004$, $\beta = 0.083$ for rs5993883-rs2239393 haplotype CG; $P = 0.0037$,
22 $\beta = 0.072$ for rs2239393-rs4680 haplotype GG; and $P = 0.0053$, $\beta = 0.083$ for
23 rs5993883-rs2239393-rs4680 haplotype CGG) (Table 3).
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36 Two adjacent variants in *DRD2* LD block 3, rs4274224 and rs4581480
37 (Supplementary figure 1), associated with HSCL score in the complete sample
38 (regardless of environmental risk) (Table 2). 2-SNP haplotype analysis of these
39 SNPs as well as their neighbouring variants gave evidence of association of
40 rs4648318-rs4274224 haplotype GG spanning from block 2 to block 3 of *DRD2*
41 ($P = 0.0007$, $\beta = 0.041$), rs4274224-rs4581480 haplotype GG in block 3 ($P = 0.0069$,
42 $\beta = 0.022$), and rs4581480-rs7131056 haplotype GA spanning from block 3 to
43 block 4 ($P = 0.0071$, $\beta = 0.022$) with HSCL score. 3-SNP haplotypes rs4648318-
44 rs4274224-rs4581480 haplotype GGG ($P = 0.0027$, $\beta = 0.032$), and rs4274224-
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3 rs4581480-rs7131056 haplotype GGA ($P=0.0081$, $\beta=0.021$), gave evidence of
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6 association in synchrony with the findings from 2-SNP haplotypes as well as the
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8 single variants (Table 4).
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12 As one of the variants contained within these haplotypes, rs4274224, also gave
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14 evidence of interaction with gender as well as association with HSCL score in
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16 males, we also examined association in males alone. The association signal
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18 strengthened for all of the risk haplotypes, being strongest for rs4648318-
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20 rs4274224 haplotype GG ($P=0.00005$, $\beta=0.069$).
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27 [Insert Tables 3, 4 and Supplementary figure 1 into the supplementary]
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31 32 **Haplotype analysis of *COMT* and *DRD2* variants on other neurobehavioral** 33 34 **traits** 35

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38 Encouraged by the findings of the haplotype analyses, we tested associations of
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40 haplotypes rs5993883-rs2239393 in *COMT* and rs4648318-rs4274224 in *DRD2*,
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42 as well as the single variant rs737866 in *COMT* to other traits related to
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44 depression, including the HSCL depression and anxiety subscales, depression
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46 diagnosis and TCI temperament trait Harm avoidance (Table 5). In case of both
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48 of the genes, it is evident that the association with HSCL stems mainly from the
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50 subscale reflecting symptoms of depression and not that reflecting anxiety (with
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52 HSCL depression subscale, $P=0.018$, $\beta=0.075$ for *COMT* haplotype CG and
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3 P=0.0015, β =0.060 for *DRD2* haplotype GG; with HSCL anxiety subscale,
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5 P=0.288, β =0.02 and P=0.02 and β =0.033, respectively). We did not detect any
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8 evidence of association with depression diagnosis or with Harm avoidance or its
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10 subcomponents.
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22 DISCUSSION

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27 We investigated potential genetic and environmental risk factors for depression in
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29 a genetically isolated Finnish birth cohort by assessing relative impacts of
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31 candidate gene variants from monoamine neurotransmission in environments of
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33 contrasting (high and low) early developmental and social risk. Our study sample
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35 provided evidence of association of allelic variants of *COMT* and *DRD2* with
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37 current symptoms of depression. In case of *COMT* we detected evidence for
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39 interaction with high early developmental risk environment particularly in males.
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42 The genetic risk from *DRD2* seemed, on the other hand, to arise from
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44 mechanisms not related to the environmental risks assessed here. Also here the
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46 associations were detected particularly in males.
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3 group from S-adenosylmethionine to the catecholamines. Its enzymatic activity
4 varies according to a G-to-A transition at codon 158 in the *COMT* gene resulting
5 in a valine-to-methionine substitution (Val158Met) on the protein level.[27] The
6 enzyme encoded by the Val158 allele has 3-4 fold higher activity than that
7 encoded by the Met158 allele. Here, we found association of the haplotype
8 comprising rs5993883 between LD blocks 1 and 2 of *COMT*, as well as
9 rs2239393 and rs4680, two variants in virtually complete linkage disequilibrium in
10 block 2, to depressive symptoms in males with the high developmental risk
11 environment ($P=0.0053$). The allele G of rs4680 from the high risk haplotype
12 corresponds to the high activity variant Val158 of *COMT*. This allele has
13 repeatedly been found to be associated with a poor response to pharmacological
14 treatment of depression[28, 29] and a European multicenter study identified an
15 association between that allele and early onset major depression.[30] The
16 Val158 allele has also earlier been found to associate with cognitive deficits
17 including poor performance in tasks related to higher-order components of
18 processing[14] and perseverative errors, less efficient physiologic responses in
19 the prefrontal cortex,[31] as well as with schizophrenia based on a meta-
20 analysis,[32] although the effect was not significant when studies with allele
21 frequencies deviating from Hardy-Weinberg equilibrium were excluded.
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Here we observed evidence for interaction of *COMT* with an early risk environment comprised of indicators related to poor neurodevelopment, namely low birth weight, as well as late development of motor system and speech. This

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3 interaction could not be explained through gene-environment correlations. Low
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5 birth weight is considered to reflect suboptimal growth environment during fetal
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7 life and it has been found to increase risk for many somatic and psychiatric
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9 diseases, including depression.[23] The observed risk seemed to arise from an
10
11 aggregation of these indicators, as none of the risk items separately showed
12
13 evidence for GxE with risk variants from *COMT* or *DRD3* (data not shown). This
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15 could relate to the degree of developmental problems altogether, so that mild
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17 signs alone are not sufficient to comprise such an environment that would
18
19 interact with *COMT* and affect the development of emotional regulation, at least
20
21 not on the level that could be detected in this study. There is some prior evidence
22
23 of interaction of *COMT* with a risk environment on psychosis, antisocial
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25 behaviour and dissociation. A study on children with ADHD showed a main gene-
26
27 environment interaction of the Val/Val genotype and low birth weight on early-
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29 onset antisocial behaviour,[33] and the Val158 allele was also found to interact
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31 with cannabis use and psychotic symptoms[34] and with increasing levels of
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33 dissociation in those exposed to higher levels of childhood trauma.[35]
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35 Interestingly, a recent report[36] revealed an impact of that polymorphism on
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37 gender-related patterns of regulation of emotions (activation in limbic and
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39 paralimbic regions) in line with findings from the present study.
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50 Another major finding of the present study, and statistically the strongest one,
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52 was observed in the dopamine receptor D2 gene *DRD2*, where a haplotype
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54 comprising the intronic variants rs4648318 in LD block 2 and rs4274224 in block
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3 was found to associate with depressive symptoms particularly in males,
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5 regardless of their early environment ($P=0.00005$). Dopamine receptors have key
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7 roles in a variety of processes in the vertebrate central nervous system, and
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9 dysfunction in dopaminergic neurotransmission may therefore predispose to a
10
11 variety of neuropsychiatric disorders. Among the receptor genes, *DRD2* has
12
13 attracted the most attention and has been implied to have a role in the etiology of
14
15 several psychiatric disorders. However, there are only a few previous reports on
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17 unipolar depression, including positive,[37] nominal[38] and negative[39, 40]
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19 findings, and results on depression conditional on risk environment.[37, 39, 41]
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27 Our varying results for males and females in general imply different mechanisms
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29 of mood regulation and possible gender-specific responses to environmental
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31 effectors. Gender differences in depression[2, 42] as well as temperament
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33 traits[42] have previously been reported in various populations, including the
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35 current one,[43] and the prevalence of depression is higher in women.[44] A true
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37 gender-specific effect of genetic variants on depressiveness would not be
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39 surprising, as there is evidence for example of gender differences in
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41 dopaminergic function[45] that may be estrogen-dependent.
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48 It is noteworthy that despite previous reports of the 5-HTTLPR variant,[12] we did
49
50 not detect association evidence for *SLC6A4*. However, a recent meta-analysis
51
52 did not find any evidence of association with depression alone, or in interaction
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54 with stressful life-events.[13] The *SLC6A4* SNPs included in our study tag the 5-
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3 HTTLPR well ($D' > 0.9$), as determined using genotypes from a population-based
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5 Finnish Health 2000 study.[46] Moreover, the LD measure thus obtained is
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7 conservative, since in the population under current study LD has been shown to
8
9 be stronger than in the general Finnish population, represented by the Health
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11 2000 study sample.[47]
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17 We did not use the Bonferroni correction for multiple testing due to limitations of
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19 sample size and expected magnitude of gene effects in complex traits. Although
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21 the results from the primary analyses (Table 2) do not survive conservative
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23 correction, having a neurobiological a priori hypothesis for these genes'
24
25 involvement in depressiveness supports their validity. Furthermore, additional
26
27 analyses performed on the variants that had given any evidence of interaction in
28
29 the primary analyses yielded a relatively strong association signal of *DRD2*'s
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31 rs4274224 with HSCL score in males ($P=0.0006$), which remains close to
32
33 statistical significance even when taking into account the amount of multiple
34
35 testing performed. The finding was further supported by results of analysis of
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37 haplotypes containing rs4274224, showing a statistically significant association
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39 with HSCL score in males ($P=0.00005$).
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49 There are some limitations in the present study. First, it is notable that
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51 depression as defined here did not necessary signify a clinical diagnosis of major
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53 depression. Instead it was defined either based on self-report or on the score
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55 from HSCL, which as a measure has its limitations. However, the prevalence of
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3 depressed mood was in the same range as in earlier reports.[1, 48] Secondly,
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5 there was a notable drop-out rate among the original material of all cohort
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7 members. About half of the original cohort members did not participate in this
8
9 study. Finally, when the NFBC 1966 study was initiated it was not possible to
10
11 predict that an investigation such as the present one would one day be
12
13 conducted. Therefore, we are limited by the original choice of variables to be
14
15 collected. It is also noteworthy that we did not detect association with our
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17 measure of current depression of either the early social or the developmental risk
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19 environment, despite them being formulated based on previous reports of their
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21 effects on psychiatric health and wellbeing.[23-26] However, the effect of genetic
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23 risk may be modulated by early life stress even though the direct link between
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25 early life environment and current status would be missing, and this modulating
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27 effect may be seen in the results of the GxE analysis.
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36 The current study has several potential advantages, such as the availability of
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38 longitudinal follow-up data starting antenatally enabling us to include the
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40 environmental dimension without any risk of recall bias. Another advantage is the
41
42 unique genetic structure of our study cohort, characterized by isolation, founder
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44 effect, multiple bottlenecks and more genetic homogeneity compared to many
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46 other isolates,[49] allowing us to identify genetic risk loci that may be missed in
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48 the screening of other more heterogeneous populations. Furthermore, the
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50 subjects were representative, with all cohort members born in the same year and
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52 within a geographically defined area.
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6 Furthermore, the size of the sample is sufficient to identify genetic variants of
7 moderate impact. We also have both genders represented in almost equal
8 amounts (48% males, 52% females), which is notable since gender differences
9 are evident both in depression[2, 42] and in temperament traits, for example
10 Harm avoidance.[42] It is also beneficial that the sample is a one-year birth
11 cohort, as it is well established that some psychiatric traits, such as Harm
12 avoidance[50] of temperament, are age-dependent. We can therefore isolate
13 genetic effects from the effects of aging. Furthermore, we provide a complete
14 coverage of the major candidate genes that are relevant with the present focus.
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29 Our results support the role of *COMT* and *DRD2*, two genes of monoamine
30 neurotransmission, in the etiology of depression particularly in males, and they
31 imply gender-specific mechanisms of mood regulation and responses to risk
32 environments. The findings imply that the role of monoaminergic genes in
33 depression should be examined further in future studies. However, these findings
34 are pending replication in other, independent population samples.
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22 **CONTRIBUTORS**

23
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26
27 Emma Nyman, Tiina Paunio, Jouko Miettunen, Matti Joukamaa and Juha Veijola
28 designed the study and wrote the protocol, with help also from Nelson Freimer,
29 Pirjo Mäki, Leena Peltonen and Marjo-Riitta Järvelin. Emma Nyman and to some
30 extent Tiina Paunio also managed the literature searches and analyses. Emma
31 Nyman and Sonja Sulkava undertook the statistical analysis. Emma Nyman
32 wrote the first draft of the manuscript, and Tiina Paunio and Sonja Sulkava also
33 contributed to its later versions. All authors contributed to and have approved the
34 final manuscript.
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Table 1. Composition of the study sample from the NFBC 1966.

	<i>N</i>	<i>HSCL score > 1.75</i>	<i>Depression diagnosis</i>	<i>Early developmental risk environment¹</i>			<i>Social risk environment²</i>		
				<i>High-risk³</i>	<i>sLow-risk</i>	<i>nd⁴</i>	<i>High-risk³</i>	<i>Low-risk</i>	<i>nd⁴</i>
<i>Males</i>	2509	169 (7%)	79(3%)	229 (9%)	2094 (83%)	186 (7%)	912 (36%)	1574 (63%)	23 (0.9%)
<i>Females</i>	2716	269(10%)	136(5%)	193 (7%)	2328 (86%)	195 (7%)	1034 (38%)	1649 (61%)	33 (1.2%)
<i>All</i>	5225	438(8%)	215(4%)	422 (8%)	4422 (85%)	381 (7%)	1946 (37%)	3223 (62%)	56 (1.1%)

¹ Defined by the presence of two out of three possible indicators for the early developmental risk environment: low birth weight, late motor development, and late development of speech.

² Defined by the presence of two out of five possible indicators for the social risk environment: unwantedness of pregnancy, low socio-economic status, single parenthood, low level of education of mother, and low activity for information retrieval by the mother. For further details, see text.

³ Both early developmental and environmental risk present in 92 males (3,6%) and 67 females (2,4%).

⁴ Not defined.

Table 2. Interaction between genetic variants of genes of monoamine neurotransmission and early developmental risk environment (GxE_{Dev}),¹ social risk environment (GxE_{Soc}),² and gender (GxSex) on current depressive symptoms (HSCL score), and genetic association to HSCL score in the complete study sample from the NFBC 1966 (All). The analyses were performed using PLINK's linear regression model and interaction analysis. Empirical P-values based on max(T) permutation are reported, with P-values <0.05 shown in bold.

Gene	Chromosome	SNP	Position/bp	Minor allele	MAF ³	P(GxE _{Dev})	P(GxE _{Soc})	P(GxSex)	P(All)
SLC6A4	17	rs1906451	25539605	G	0.44	0.608	0.363	0.784	0.268
		rs3794808	25555919	A	0.41	0.365	0.263	0.799	0.320
		rs140700	25567515	A	0.09	0.133	0.460	0.037⁸	0.876
		rs2066713	25575791	A	0.46	0.253	0.499	0.505	0.550
		rs8071667	25576899	A	0.15	0.473	0.682	0.122	0.606
TPH2	12	rs4131348	70610746	G	0.12	0.844	0.937	0.400	0.497
		rs2129575	70626340	A	0.22	0.787	0.682	0.432	0.423
		rs1386496	70637057	G	0.16	0.983	0.404	0.293	0.792
		rs2171363	70646531	A	0.43	0.762	0.983	0.016⁸	0.814
		rs10506645	70671767	A	0.23	0.996	0.756	0.102	0.789
		rs1386497	70678557	C	0.17	0.816	0.131	0.452	0.797
		rs1487276	70691326	A	0.21	0.888	0.088	0.838	0.908
		rs9325202	70693744	A	0.48	0.805	0.074	0.488	0.473
		rs1487275	70696559	C	0.37	0.972	0.054	0.625	0.049¹⁰
rs1386483	70698761	A	0.47	0.574	0.090	0.326	0.437		
rs1872824	70716581	A	0.35	0.652	0.121	0.211	0.494		
COMT	22	rs6518591	18304021	G	0.16	0.688	0.255	0.919	0.303
		rs737866	18310109	G	0.18	0.028⁴	0.853	0.024⁸	0.755
		rs1544325	18311668	G	0.48	0.318	0.376	0.192	0.822
		rs174675	18314051	A	0.29	0.465	0.278	0.580	0.958
		rs5993883	18317638	C	0.36	0.230	0.495	0.025⁸	0.920

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13	MAOA	X	rs909525	43438146	G	0.45	0.559	0.871	0.554	0.165
14			rs12843268	43458610	A	0.40	0.271	0.837	0.266	0.103
15			rs6610845	43472954	G	0.41	0.232	0.795	0.263	0.170
16			rs3027409	43491977	C	0.02	0.748	0.928	0.950	0.194
17			rs6609257	43497652	G	0.50	0.848	0.320	0.470	0.077
18			rs3027415	43499385	G	0.18	0.218	0.550	0.905	0.613
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21	DRD1	5	rs265973	174793305	G	0.50	0.529	0.614	0.549	0.888
22			rs265974	174793846	G	0.35	0.391	0.612	0.912	0.659
23			rs265976	174795026	A	0.23	0.578	0.707	0.915	0.826
24			rs5326	174802802	A	0.19	0.615	0.886	0.852	0.588
25										
26										
27	DRD2	11	rs1800497	112776038	A	0.17	0.079	0.825	0.691	0.467
28			rs2242592	112784640	G	0.37	0.757	0.466	0.283	0.736
29			rs1076563	112801119	C	0.50	0.053	0.813	0.897	0.662
30			rs2471857	112803549	A	0.17	0.518	0.494	0.823	0.901
31			rs4620755	112814829	A	0.22	0.383	0.997	0.951	0.176
32			rs7125415	112815891	A	0.19	0.084	0.389	0.789	0.231
33			rs4648318	112818599	G	0.34	0.711	0.885	0.631	0.684
34			rs4274224	112824662	G	0.24	0.067	0.777	0.017 ⁹	0.022 ¹²
35			rs4581480	112829684	G	0.07	0.184	0.521	0.210	0.009 ¹³
36			rs7131056	112834984	C	0.49	0.564	0.795	0.413	0.964
37			rs4938019	112846601	G	0.23	0.069	0.651	0.643	0.584
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			rs12364283	112852165	G	0.08	0.280	0.504	0.441	0.861
			rs10891556	112857971	A	0.24	0.076	0.519	0.638	0.589
			rs6589377	112860946	G	0.17	0.286	0.617	0.061	0.552
<i>DRD3</i>	3		rs2087017	115324703	G	0.43	0.937	0.921	0.606	0.743
			rs2134655	115340891	A	0.28	0.454	0.554	0.129	0.507
			rs963468	115345577	A	0.38	0.809	0.777	0.902	0.608
			rs3773678	115352768	A	0.06	0.780	0.855	0.487	0.770
			rs2630351	115357749	A	0.03	0.954	0.144	0.168	0.999
			rs167771	115358965	G	0.18	0.862	0.638	0.406	0.514
			rs167770	115362252	G	0.31	0.260	0.911	0.298	0.694
			rs226082	115363703	G	0.31	0.261	0.911	0.301	0.690
			rs324029	115364313	A	0.31	0.259	0.913	0.296	0.722
			rs10934256	115368342	A	0.17	0.229	0.898	0.478	0.246
			rs1486009	115371222	G	0.12	0.721	0.667	0.745	0.571
			rs6280	115373505	G	0.33	0.159	0.485	0.141	0.667
			rs9825563	115382910	G	0.23	0.045 ⁷	0.902	0.211	0.215
<i>DRD4</i>	11		rs3758653	626399	G	0.23	0.300	0.752	0.249	0.980
			rs11246226	631191	A	0.49	0.749	0.748	0.166	0.925
<i>DRD5</i>	4		rs1878943	9375986	A	0.21	0.586	0.686	0.482	0.386
			rs13106539	9406801	G	0.39	0.735	0.062	0.067	0.044 ¹⁴

¹ Defined by the presence of two out of three possible indicators for the early developmental risk environment: low birth weight, late motor development, and late development of speech.

² Defined by the presence of two out of five possible indicators for the social risk environment: unwantedness of pregnancy, low socio-economic status, single parenthood, low level of education of mother, and low activity for information retrieval by the mother.

³ Minor allele frequency.

⁴ P=0.0364 (β=0.0414), ⁵ P=0.008 (β =0.0440), ⁶ P=0.042 (β =0.0320) and ⁷ P=0.022 (β =-0.0396) in individuals with high risk environment; ⁸ P>0.05 in both genders; ⁹ P=0.0006 (β=0.023) in males; ¹⁰ β=0.008, ¹¹ β=0.012, ¹² β=0.011, ¹³ β=0.022, ¹⁴ β=-0.

Table 3. Haplotype analysis of *COMT* variants on current depressive symptoms (HSCL score) in individuals with high early developmental risk (E_{Dev})¹ from the NFBC 1966. Empirical P-values based on permutation are reported, with P-values <0.05 shown in bold.

Gene	Variant	Haplotype	Frequency	Males and females with high risk E_{Dev}		Males with high risk E_{Dev}		Females with high risk E_{Dev}	
				Beta	P	Beta	P	Beta	P
<i>COMT</i>	<i>2-SNP haplotype analysis</i>								
	rs5993883-rs2239393	CG	0.21	0.0552	0.0049	0.0828	0.0040	0.0216	0.4420
	rs2239393-rs4680	GG	0.32	0.0440	0.0072	0.0720	0.0037	0.0119	0.4914
		AA	0.55	-0.0320	0.0428	-0.0411	0.0827	-0.0207	0.3370
	rs4680-rs4646316	GA	0.17	0.0434	0.0331	0.0624	0.0226	0.0206	0.3950
	<i>3-SNP haplotype analysis</i>								
	rs5993883-rs2239393-rs4680	CGG	0.21	0.0548	0.0046	0.0826	0.0053	0.0211	0.4569
rs2239393-rs4680-rs4646316	GGA	0.17	0.0433	0.0344	0.0614	0.0258	0.0213	0.4311	

¹ Defined by the presence of two out of three possible indicators for the early developmental risk environment: low birth weight, late motor development, and late development of speech.

Table 4. Haplotype analysis of *DRD2* variants on current depressive symptoms (HSCL score) in the complete sample from the NFBC 1966. Empirical P-values based on permutation are reported, with P-values <0.05 shown in bold.

Gene	Variant	Haplotype	Frequency	Males and females		Males	
				Beta	P	Beta	P
<i>DRD2</i>	<i>2-SNP haplotype analysis</i>						
	rs4648318-rs4274224	GG	0.05	0.0409	0.0007	0.0694	0.00005
	rs4274224-rs4581480	GG	0.07	0.0220	0.0069	0.0321	0.0023
		AA	0.48	0.0116	0.0161	-0.0237	0.0004
	rs4581480-rs7131056	GA	0.07	0.0220	0.0071	0.0322	0.0026
	<i>3-SNP haplotype analysis</i>						
	rs4648318-rs4274224-rs4581480	GGG	0.05	0.0326	0.0027	0.0437	0.0019
rs4274224-rs4581480-rs7131056	GGA	0.07	0.0215	0.0081	0.0317	0.0033	

Table 5. Haplotype analysis of *COMT* and *DRD2* variants on other neurobehavioral traits in the NFBC1966. Empirical P-values based on permutation are reported, with P-values <0.05 shown in bold.

Gene	Variant	Group	Gender	HSCL(total)		HSCL(depression)		HSCL(anxiety)		Depression diagnosis		Harm avoidance	
				Beta	P	Beta	P	Beta	P	Odds ratio	P	Beta	P
<i>COMT</i>	rs737866	High risk	Males	0.0640	0.0254	0.0440	0.2239	0.0150	0.6157	0.7130	0.5004	0.7820	0.3799
	rs5993883- rs2239393 (CG)	High risk	Males	0.0830	0.0040	0.0750	0.0176	0.0200	0.2877	0.2100	0.1506	1.2040	0.1433
<i>DRD2</i>	rs4648318- rs4274224 (GG)	All	Males	0.0694	0.00005	0.0600	0.0015	0.0326	0.0212	0.8280	0.6798	1.0430	0.07009

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cohort studies

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3-4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6-7
Objectives	3	State specific objectives, including any prespecified hypotheses	7-8
Methods			
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8-11
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	8-11
		(b) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8-11
Bias	9	Describe any efforts to address potential sources of bias	8-11
Study size	10	Explain how the study size was arrived at	8-11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	12-13
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	12-13
		(b) Describe any methods used to examine subgroups and interactions	12-13
		(c) Explain how missing data were addressed	
		(d) If applicable, explain how loss to follow-up was addressed	
		(e) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8-11
		(b) Give reasons for non-participation at each stage	8-11
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-11
		(b) Indicate number of participants with missing data for each variable of interest	8-11
		(c) Summarise follow-up time (eg, average and total amount)	8-11
Outcome data	15*	Report numbers of outcome events or summary measures over time	8-13
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13-18
		(b) Report category boundaries when continuous variables were categorized	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	13-18
Discussion			
Key results	18	Summarise key results with reference to study objectives	18-22, 24
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	22-24
Generalisability	21	Discuss the generalisability (external validity) of the study results	24
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	24-25

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.



Interaction of Early Environment, Gender and Genes of Monoamine Neurotransmission in the Etiology of Depression in a Large Population-Based Finnish Birth Cohort

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Keywords:	PSYCHIATRY, Depression & mood disorders < PSYCHIATRY, GENETICS
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Interaction of Early Environment, Gender and Genes of Monoamine Neurotransmission in the Etiology of Depression in a Large Population-Based Finnish Birth Cohort

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44 Key words: Environment, Monoamine, Gene, Depression, Cohort Study

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48 Word count text: 4333

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50 Word count abstract: 236

ABSTRACT

Objectives Depression is a worldwide leading cause of morbidity and disability. Genetic studies have recently begun to elucidate its molecular etiology. We investigated candidate genes of monoamine neurotransmission and early environmental risk factors for depressiveness in the genetically isolated population-based Northern Finland Birth Cohort 1966 (12058 live births).

Design We ascertained and subdivided the study sample (n=5225) based on early developmental and social risk environments and examined candidate genes of monoamine neurotransmission, many of which have shown prior evidence of gene-environment interaction (GxE) for affective disorders, namely *SLC6A4*, *TPH2*, *COMT*, *MAOA*, and the dopamine receptor genes *DRD1-DRD5*.

Results and Conclusion We observed no major genetic effects of the analyzed variants on depressiveness. However, when specific environmental factors were considered, some evidence of interaction was observed. Allelic variants of *COMT* interacted with high early developmental risk environment (P=0.005 for rs2239393 and P=0.02 for rs4680) so that the association with depression was detected only in individuals of high developmental risk group (P=0.0046 and $\beta=0.056$ for rs5993883-rs2239393-rs4680 risk haplotype CGG including Val158), particularly in males (P=0.0053 and $\beta=0.083$ for the haplotype CGG). Rs4274224 from *DRD2* interacted with gender (P=0.017) showing significant association with depressiveness in males (P=0.0006 and $\beta=0.0023$; P=0.00005 and $\beta=0.069$ for rs4648318-rs4274224 haplotype GG). Our results support the role of genes of

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3 monoamine neurotransmission in the etiology of depression conditional on
4 environmental risk and sex, but not direct major effects of monoaminergic genes
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8 in this unselected population.
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10 11 12 13 14 **ARTICLE SUMMARY**

15 16 17 18 **Article focus**

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20
21 - Impact on depression of monoaminergic candidate genes with prior
22 evidence of gene-environment interaction for affective disorders, and of
23 dopamine receptor genes
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29 - Gene-environment and gene-gender interaction in the etiology of
30 depression
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34 - Effect of early neurodevelopmental and social risk environments on
35 depression
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38 39 **Key messages**

- 40
41 - Genes of monoamine neurotransmission play a role in the etiology of
42 depression conditional on environmental risk, especially in males and in
43 individuals from an early developmental risk environment; in particular
44 there is evidence of an Interaction with a *COMT* high risk haplotype
45 including Val158
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50 - Gender-specific mechanisms and responses to environmental effectors
51 are evident in regulation of mood
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Strengths and limitations of this study Depression as defined does not necessary imply clinical diagnosis of major depression, but instead is based on self-report or on the score from the HSCL measure. Despite this limitation, the prevalence of depressed mood was in the same range as in earlier reports. There was a notable drop-out rate among the original material of all cohort members, and about half of the original cohort members did not participate in this study. 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. Advantages of this study include the availability of longitudinal follow-up data starting antenatally, enabling inclusion of the environmental dimension without risk of recall bias. Another advantage is the unique genetic structure of the study cohort, characterized by isolation, founder effect, multiple bottlenecks and more genetic homogeneity compared to many other isolates, permitting identification of genetic risk loci that may be missed when using more heterogeneous populations. The subjects were also representative of the population, with all cohort members born in the same year and within a geographically defined area. The size of the study sample is sufficient for identifying genetic variants of moderate impact. Both genders are also represented in almost equal amounts, which is notable since gender differences are evident in both depression and related temperament traits, such as Harm avoidance. It is also beneficial that the sample is a one-year birth cohort, as it is well established that some psychiatric traits, such as Harm avoidance are age-dependent. The genetic effects may therefore be isolated

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from the effects of aging. Finally, a complete coverage of the major candidate genes that are relevant with the present focus is provided.

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INTRODUCTION

Depression is a major cause of morbidity worldwide, major depression affecting 5-7% of the population annually and 16% over the lifetime.[1] Although a genetic component in the etiology of major depression is evident with a 40-50% heritability,[2] the predisposing genetic background has so far remained largely undefined, and recent findings from genome-wide association studies also point to a complex underlying architecture.[3] Depressed patients frequently exhibit comorbidities such as anxiety and alcohol abuse,[4] and certain personality types[5-7] have been associated with depression proneness.

Environmental risk factors, in particular stressors influencing during development,[8] are considered to have a significant impact on the development and course of depression. It is likely that many of the genetic risk factors for depression interact with the early developmental environment, but recapture of these interactions has remained a challenge for etiological studies of depression. Although the interplay between genes and environment has been investigated with respect to several psychiatric disorders[9, 10] including depression, this vast subject remains still to a large extent unexplored. On the other hand, addressing the effects of genes and environment on psychiatric morbidity enables us to examine the two main constituents in their etiology. Therefore, we wanted to include the environmental dimension in our study in order to also explore gene-environment interactions (GxE).

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7 According to the monoamine hypothesis, depression is caused by underactivity
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9 in brain monoamines, such as dopamine, serotonin, and norepinephrine.[11]
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11 Recent results of neuroimaging studies have provided further support for this
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13 theory.[12] The most solid evidence from candidate gene studies has perhaps
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15 been obtained for the interaction of the *SLC6A4* gene for serotonin transporter
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17 and stressful early and current life events,[13] including positive results from a
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19 recent review[14] and meta-analysis of all studies to date[15], although there are
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21 also contradicting results.[16] Other robust genetic findings have been obtained
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23 on the *COMT* gene for catechol-O-methyltransferase, an enzyme catabolising
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25 catecholamines such as dopamine and noradrenaline, which has been implicated
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27 in depression in conjunction with stress,[17] and on the *MAOA* gene for
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29 monoamine oxidase A, an enzyme oxidizing neurotransmitter and dietary
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31 monoamines such as serotonin, noradrenaline and dopamine, which has been
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33 associated with depression in interaction with severity of maltreatment in
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35 childhood.[17] Furthermore, *TPH2* gene for tryptophan hydroxylase 2, which is
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37 the brain-specific form of the key enzyme in serotonin synthesis, has been
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39 implicated to interact with stress on disorders of cognitive control and emotional
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41 regulation, including depression.[18] Within the dopamine transmission the *DRD2*
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43 gene for dopamine receptor D2 has been associated with depressiveness and
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45 anxiety, combined with an effect of parenting in childhood,[19] and the *DRD4*
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47 gene for dopamine receptor D4 has been associated with increased risk for
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49 obesity in women with seasonal affective disorder.[20] Thus, genes from the
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3 monoamine neurotransmission system are among the most thoroughly studied in
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5 psychiatric genetics and in particular in the etiology of mood disorders, and have
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7 provided perhaps the most robust evidence so far for interaction with various
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9 types of risk environments, including childhood environment.
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17 We chose to include these candidate genes of monoamine neurotransmission,
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19 including *SLC6A4*, *TPH2*, *COMT*, *MAOA*, as well as the dopamine receptor
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21 genes *DRD1-DRD5*, in our study on the etiology of depression with a particular
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23 focus on their interaction with available markers reflecting early developmental
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25 and social risk environments. The study was performed in a sample of 5225
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27 individuals from a large Finnish isolated population cohort. As gender is an
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29 important confounder for depression and at least some of the genetic liability is
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31 gender-specific,[2] we also examined gene-gender interactions in this sample.
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41 **METHODS**

42 43 44 45 **Setting**

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50 We utilized the genetically isolated Northern Finland Birth Cohort (NFBC 1966) to
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52 investigate effects of candidate genes and environmental risk factors during the
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54 development on depressiveness. We subdivided the study sample based on
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3 developmental risk factors arising from the fetal growth environment and
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5 neurological development during the first year of life (early developmental risk
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7 environment) as well as from the family environment during pregnancy and early
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9 childhood (social risk environment). We examined interactions of these
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11 environments with candidate genes of the monoamine neurotransmitter systems,
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13 many of which have prior evidence of gene-environment interaction on affective
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15 disorders, namely *SLC6A4*, *TPH2*, *COMT*, *MAOA*, and the dopamine receptor
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17 genes *DRD1-DRD5*.
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24 **Study subjects**

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30 The Northern Finland Birth Cohort 1966 (NFBC 1966) is a longitudinal one-year
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32 birth cohort from an unselected population (N=12058 live births) comprising
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34 inhabitants of the two northernmost provinces of Finland.[21] Data collection was
35
36 begun during the antenatal period, and follow-up studies have been performed at
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38 the ages of 1, 14 and 31 years. The cohort study has been approved by the
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40 Ethical Committee of Oulu University Faculty of Medicine, and written informed
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42 consent has been obtained from all participants.
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50 In 1997 for the 31-year follow-up study[22] all alive cohort members with a known
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52 address (N=11540) were sent a postal questionnaire surveying lifestyle, social
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54 status and health (76% participated), including the Hopkins Symptom Check
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56 List-25 (HSCL)[23] and items on self-reported lifetime depression diagnosis (e.g.
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3 “Has your doctor ever diagnosed a depressive disorder?”). Additionally, cohort
4 members who lived in Northern Finland or had moved to the Helsinki area
5 (N=8465) were invited to a clinical examination (71% participated) with another
6 questionnaire to be filled in later and sent to the research group (61%
7 participated).[24] It included, among others, a validated Finnish translation of
8 Cloninger’s Temperament and Character Inventory (TCI) questionnaire.[25]
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20 Current depressive symptoms were assessed by the HSCL-questionnaire,[26] a
21 25-item shortened version of an originally 90-item questionnaire. HSCL contains
22 13-item depression and 10-item anxiety subscales assessing presence and
23 intensity of depressive and anxiety symptoms during the previous week. Answers
24 are scored on a scale from 1 (not bothered) to 4 (extremely bothered). The HSCL
25 total score is the sum of items divided by the number of items answered. We
26 used mainly HSCL total score as symptoms of depression and anxiety are known
27 to overlap significantly. In the post hoc analyses, in order to better understand
28 the original association signals, the separate HSCL subscales for depressive and
29 anxiety symptoms were also taken into consideration. In addition to current
30 depressive symptoms (HSCL score) and lifetime (diagnosed) depression, we
31 used the TCI temperament trait Harm avoidance[5-7] and its subcomponents as
32 a measure of proneness to depression.
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53 The subjects (n=5225; 2509 males, 2716 females; 45 % of the 31 year follow-up
54 study sample or 43% of the original study sample) were divided into high and low
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3 risk groups based on the available information reflecting early
4 neurodevelopmental and social risk environments (Table 1). The markers for the
5 *early developmental risk environment* included 1) low birth weight (<2500 g),[21]
6 considered to reflect suboptimal growth environment during fetal life and to
7 increase risk for somatic and psychiatric diseases such as depression in
8 adulthood,[27] 2) late motor development as reflected by first standing later than
9 at the age of 10 months,[28] and 3) late development of speech, defined by no
10 words at the age of one year.[28] If two out of these risk indicators were present,
11 the subject was classified as having experienced a high risk environment for
12 early brain development. The markers for the *social risk environment* included
13 the occurrence of two or more of the following five indicators for high risk social
14 environment during pregnancy and early childhood: 1) unwantedness of
15 pregnancy (rated by mothers of the cohort members at the sixth or seventh
16 month of pregnancy),[29] 2) low socio-economic status, shown to be linked with
17 depression in the offspring in earlier studies,[30] as defined by father's social
18 class at birth (no occupation, unskilled worker, or farmer with area under
19 cultivation under 8 hectares), 3) single parenthood at birth, 4) low level of
20 education of mother (less than nine years of primary school), and 5) low level of
21 information retrieval by the mother related to pregnancy and child care. There
22 was no significant drop-out in either of the high risk groups, as 43% and 41% of
23 the individuals with high risk early developmental and social environments, and
24 47% and 46% of those with the respective low risk environments, were available
25 for study.
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10 **Genotyping methods**

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15 We investigated genes relevant within the context of the monoamine hypothesis
16 of depression: *SLC6A4*, *TPH2*, *COMT*, *MAOA*, and the dopamine receptor genes
17 *DRD1-DRD5* (Table 2). The genotyping was performed at the Broad Institute
18 (Cambridge, MA, USA) on the HumanCNV370-duo chip (Illumina, San Diego, CA
19 USA) platform according to the manufacturer's instructions. The analysed SNPs
20 included HapMap tag SNPs (<http://www.hapmap.org/index.html.en>) and were
21 relatively evenly spaced to cover the genes and flanking regions.
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39 **Statistical analysis**

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43 LD structures were determined using HAPLOVIEW. Interaction and
44 association/correlation analyses using linear and logistic regression with
45 permutation was performed using PLINK Software Package Version 1.04, in a
46 step-wise manner to maximize our ability to detect associations and to minimize
47 multiple testing. (i) Primarily analyses were performed to identify genetic risk
48 variants for current depressive symptoms (HSCL score) interacting with early
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3 developmental risk ($G \times E_{Dev}$) and social risk ($G \times E_{Soc}$) environments. For variants
4 giving significant evidence of interaction, we also performed analyses separately
5 in subgroups of high and low risk, respectively. As gender is an important
6 confounder for depression and at least some of the genetic liability is gender-
7 specific,[2] we also examined gene-gender interactions ($G \times Sex$). For variants
8 showing significant evidence of gene-gender interaction, we also performed
9 analyses separately in males and females. In order to achieve a more complete
10 view of the effects of the examined genes on depressive symptoms in the cohort,
11 we also examined their influence on the gender-adjusted HSCL score in the
12 complete sample regardless of environmental effectors. Finally, we tested for
13 gene-environment correlations ($r_{GE_{Dev}}$ and $r_{G_{Soc}}$) and associations of the risk
14 environments with the HSCL score (PASW Statistics 18, linear regression
15 model). (ii) Haplotype analyses were performed when two SNPs located at
16 physically close vicinity had given association signals of $P < 0.05$ when analyzed
17 separately. (iii) Genetic variants and haplotypes which had been identified in the
18 previous analyses were analyzed post hoc with respect to HSCL subscales
19 (depressive and anxiety symptoms), depression diagnosis and TCI temperament
20 Harm avoidance. We report point-wise empirical p-values generated by PLINK's
21 max(T) permutation (10 000 permutations) throughout the manuscript, and
22 explicitly state where corrected empirical p-values are reported. SNPs with
23 Hardy-Weinberg Equilibrium p-values < 0.05 were excluded from all analyses.
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RESULTS

Gene-environment and gene-gender interaction and association analyses in relation to the HSCL score

We examined the effects of nine candidate genes of monoamine neurotransmission on current depressive symptoms (HSCL score) in a longitudinal population-based NFBC 1966 cohort. In particular, we searched for evidence of interaction of variants in these genes with two early growth environments, one with indicators for potentially disturbed neurobehavioral development (early developmental risk environment) and the other with risk factors from social environment for normal emotional development (social risk environment). The results are presented in Table 2 in which nominal P-values are reported.

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. We observed nominal evidence for association with the HSCL score ($P < 0.05$) in the complete sample in the cases of rs1487275 in *TPH2*, ($P = 0.049$, $\beta = 0.008$), rs4646316 in *COMT* ($P = 0.026$, $\beta = 0.012$), rs4274224 and rs4581480 in *DRD2* ($P = 0.022$, $\beta = 0.011$; and $P = 0.009$, $\beta = 0.022$, respectively), and rs13106539 in *DRD5* ($P = 0.044$, $\beta = -0.008$). Three variants of *COMT* and one

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3 of *DRD3* showed some evidence of interaction ($P < 0.05$) with high early
4 developmental risk environments with respect to the HSCL score ($P = 0.028$ for
5 *rs737866*; $P = 0.005$ for *rs2239393*, and, $P = 0.020$ from *rs4680* from *COMT*, and
6 *rs9825563*, $P = 0.045$ from *DRD3*). All of them were associated with the HSCL
7 score in individuals of the high risk group ($P = 0.036$, $\beta = 0.0414$ for *rs737866*;
8 $P = 0.008$, $\beta = 0.0440$ for *rs2239393*; $P = 0.042$, $\beta = 0.0320$ for *rs4680*; and $P = 0.022$,
9 $\beta = -0.0396$ for *rs9825563*, respectively). None of the variants gave any evidence
10 of interaction with the social risk environment in relation to the HSCL score. Five
11 of the genetic variants showed some evidence of interaction with gender
12 ($P < 0.05$), including *rs737866* and *rs5993883* in *COMT* and *rs4274224* in *DRD2*.
13 Out of these, only *rs4274224* associated at $P < 0.05$ with one of the genders
14 ($P = 0.0006$, $\beta = 0.023$ in males). The evidence for gene-environment correlations
15 (rGE) was observed only nominally about *rs1906451* from *TPH2* ($P = 0.035$),
16 *rs265973* from *DRD1* ($P = 0.047$), and *rs9825563* from *DRD3* ($P = 0.028$). Despite
17 a priori evidence for the role of the markers which indicate a developmental high
18 risk environment for psychiatric health and wellbeing, namely low birth weight[21,
19 27] and late motor or verbal development[28], there was no correlation between
20 these markers and the HSCL score in the present sample ($P = 0.131$), whereas
21 the social high risk environment, correlated significantly with the score ($P =$
22 0.00001).

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53 Although none of the association findings of these primary analyses survived
54 correction for multiple testing, post hoc association analyses in gender groups
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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*). 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.

Haplotype analysis of *COMT* and *DRD2* variants in relation to the HSCL score

We performed 2-SNP and 3-SNP haplotype analyses combining rs2239393 and rs4680 from *COMT* and their neighbouring variants using the sliding window approach. Evidence of association was observed for the rs5993883-rs2239393 haplotype CG spanning a region from the space between LD blocks 1 and 2 to block 2 of *COMT* (Supplementary figure 1) ($P=0.0049$, $\beta=0.055$), for the rs2239393-rs4680 haplotype GG in block 2 ($P=0.0072$, $\beta=0.044$), and the rs5993883-rs2239393-rs4680 haplotype CGG ($P=0.0046$, $\beta=0.055$) in high early developmental risk individuals, in agreement with analyses using single variants (Table 3). As rs5993883 from the haplotype had also given evidence of interaction with gender (Table 2), we further examined haplotype association in males and females of the high risk group separately. We found that the haplotypes increased the risk for depressive symptoms in males, but not in females ($P=0.004$, $\beta=0.083$ for rs5993883-rs2239393 haplotype CG; $P=0.0037$,

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3 $\beta=0.072$ for rs2239393-rs4680 haplotype GG; and $P=0.0053$, $\beta=0.083$ for
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6 rs5993883-rs2239393-rs4680 haplotype CGG) (Table 3).
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10 Haplotype analysis of rs4274224 and rs4581480 from *DRD2*, which gave
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12 suggestive evidence of an association with the HSCCL score in the complete
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14 sample, and of their neighbouring variants gave evidence of an association of
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16 rs4648318-rs4274224 haplotype GG spanning from block 2 to block 3 of *DRD2*
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18 ($P=0.0007$, $\beta=0.041$), rs4274224-rs4581480 haplotype GG in block 3 ($P=0.0069$,
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20 $\beta=0.022$), and rs4581480-rs7131056 haplotype GA spanning from block 3 to
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22 block 4 ($P=0.0071$, $\beta=0.022$) with the HSCCL score. The 3-SNP haplotypes
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24 rs4648318-rs4274224-rs4581480 haplotype GGG ($P=0.0027$, $\beta=0.032$), and
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26 rs4274224-rs4581480-rs7131056 haplotype GGA ($P=0.0081$, $\beta=0.021$), gave
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28 evidence of an association in agreement with the findings from the 2-SNP
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30 haplotypes as well as the single variants (Table 4). As one of the variants
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32 contained within these haplotypes, namely rs4274224, also gave evidence of
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34 interaction with gender as well as association with the HSCCL score in males, we
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36 also examined the association in males alone. The association signal became
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38 stronger for all of the risk haplotypes, being strongest for rs4648318-rs4274224
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40 haplotype GG ($P=0.00005$, $\beta=0.069$).
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51 [Insert Tables 3, 4 and Supplementary figure 1 into the supplementary]
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Haplotype analysis of *COMT* and *DRD2* variants in relation to other neurobehavioral traits

Encouraged by the findings of the haplotype analyses, we tested for associations of haplotypes rs5993883-rs2239393 in *COMT* and rs4648318-rs4274224 in *DRD2*, as well as the single variant rs737866 in *COMT* with other traits related to depression, including the HSCL depression and anxiety subscales, depression diagnosis and TCI temperament trait Harm avoidance (Table 5). In both of the genes, it is evident that the association with HSCL stems mainly from the subscale which reflects symptoms of depression and not that reflecting anxiety (with HSCL depression subscale, $P=0.018$, $\beta=0.075$ for *COMT* haplotype CG and $P=0.0015$, $\beta=0.060$ for *DRD2* haplotype GG; with HSCL anxiety subscale, $P=0.288$, $\beta=0.02$ and $P=0.02$ and $\beta=0.033$, respectively). We did not detect any evidence of an association with depression diagnosis or with Harm avoidance or its subcomponents.

[Insert Table 5]

DISCUSSION

We investigated genetic and environmental risk factors for depression in a genetically isolated Finnish birth cohort by assessing the relative impacts of

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3 monoaminergic candidate genes for depression in environments of contrasting
4 (high and low) early developmental and social risk. We did not observe any
5 robust genetic effects of the analyzed variants on depressiveness. However,
6 when specific environmental factors were considered, some signals for
7 association were observed, although none of them survive correction for multiple
8 testing. Our study sample provided evidence of an interaction of *COMT* with the
9 high early developmental risk environment particularly in males, and a
10 contribution of an allelic variant of *DRD2* to genetic risk for depressiveness
11 particularly in males (Table 2).
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27 The *COMT* gene encoding for catechol-O-methyltransferase enzyme is among
28 the most investigated genes in psychiatric genetics. The enzyme degrades
29 catecholamine neurotransmitters such as dopamine, noradrenaline and
30 adrenaline by catalyzing the transfer of a methyl group from S-
31 adenosylmethionine to the catecholamines. Its enzymatic activity varies
32 according to a G-to-A transition at codon 158 in the *COMT* gene, resulting in a
33 valine-to-methionine substitution (Val158Met) on the protein level.[31] The
34 enzyme encoded by the Val158 allele has a 3-4 fold higher activity than that
35 encoded by the Met158 allele. Here, we found an association of the haplotype
36 comprising rs5993883 between LD blocks 1 and 2 of *COMT*, as well as
37 rs2239393 and rs4680, which are two variants in virtually complete linkage
38 disequilibrium in block 2, with depressive symptoms in high developmental risk
39 males (P=0.0053). The high risk haplotype included the high activity variant
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3 Val158 of *COMT*, the allele G of rs4680. This allele has repeatedly been found to
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5 be associated with a poor response to pharmacological treatment of
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7 depression,[32, 33] and a European multicenter study identified an association
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9 between that allele and early onset major depression.[34] The Val158 allele has
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11 already earlier been found to associate with cognitive deficits including poor
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13 performance in tasks related to higher-order components of processing[35] and
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15 perseverative errors, less efficient physiologic responses in the prefrontal
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17 cortex,[36] and even schizophrenia based on a meta-analysis,[37] although the
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19 effect was not significant when studies with allele frequencies deviating from the
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21 Hardy-Weinberg equilibrium were excluded.
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29 In our study we observed evidence for interaction between *COMT* and an early
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31 developmental risk environment on depressive symptoms. This interaction could
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33 not be explained through gene-environment correlations. Nor were we able to
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35 detect a significant correlation of the developmental risk environment with
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37 depressive symptoms, despite the prior evidence for the role of its markers,
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39 which were low birth weight[21, 27] and late motor or verbal development[28], in
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41 decreased psychiatric health and wellbeing, including depression. This finding
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43 may reflect the presence of other environmental risk indicators which were not
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45 examined in our study. However, they may also reflect individual variability in
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47 response to the risk environment and presence of genetic factors (such as the
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49 *COMT* haplotype containing Met158) that may relate to resilience, adaptive
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51 changes in regulation of emotion reactivity and successful coping with stress.[38]
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3 The observed risk also seemed to arise from an aggregation of the
4 environmental indicators, as none of the risk items separately gave evidence of
5 GxE with the risk variants from *COMT* or *DRD3* (data not shown). This could
6 reflect a cumulative nature of these environmental influences, such that the effect
7 of one marker may be weak, but the accumulated effect of multiple markers,
8 together with genetic susceptibility, would be strong enough to increase the risk
9 for a deviant development of emotional regulation and thus depressiveness.[39]
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11 There is some prior evidence of interaction of *COMT* with a risk environment on
12 psychosis, antisocial behaviour and dissociation. A study on children with ADHD
13 showed a gene-environment interaction between the Val/Val genotype and low
14 birth weight on early-onset antisocial behaviour,[40] and the Val158 allele was
15 also found to associate with cannabis use and psychotic symptoms[41] and with
16 increasing levels of dissociation in those exposed to higher levels of childhood
17 trauma.[42] Interestingly, a recent report[43] revealed an impact of that
18 polymorphism on gender-related patterns of regulation of emotions (activation in
19 limbic and paralimbic regions) in line with findings of the present study.
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43 Another major finding of the present study, and statistically the strongest one,
44 was observed in the dopamine receptor D2 gene *DRD2*, where a haplotype
45 comprising the intronic variants rs4648318 in LD block 2 and rs4274224 in block
46 3 was found to associate with depressive symptoms particularly in males,
47 regardless of their early environment ($P=0.00005$). Dopamine receptors have key
48 roles in a variety of processes in the vertebrate central nervous system, and
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3 dysfunction in dopaminergic neurotransmission may therefore predispose to a
4 variety of neuropsychiatric disorders. Among the receptor genes, *DRD2* has
5 attracted the most attention and has been implied to have a role in the etiology of
6 several psychiatric disorders. However, there are only a few previous reports on
7 unipolar depression, including positive,[44] nominal[45] and negative[46, 47]
8 findings, and for results on depression conditional on risk environment.[44, 46,
9 48]

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22 Our varying results for males and females in general imply different mechanisms
23 of mood regulation and possible gender-specific responses to environmental
24 effectors. Gender differences in depression[2, 49] as well as in temperament
25 traits[49] have previously been reported in various populations, including the
26 current one,[50] and the prevalence of depression is higher in women.[51] A true
27 gender-specific effect of genetic variants on depressiveness would not be
28 surprising, as there is evidence of gender differences in dopaminergic
29 function[52] that may be estrogen-dependent.

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43 It is noteworthy that despite previous reports of the 5-HTTLPR variant,[13] we did
44 not detect association evidence for *SLC6A4*. Similarly, a recent meta-analysis did
45 not find any evidence of an association with depression alone, or in interaction
46 with stressful life-events,[16] although a current review[14] and a meta-analysis
47 of all studies to date[15] support the positive association findings and the role of
48 5-HTTLPR and stress in depression. The *SLC6A4* SNPs included in our study
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3 tag the 5-HTTLPR well ($D' > 0.9$), as determined by using genotypes from a
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5 population-based Finnish Health 2000 study.[53] Moreover, the LD measure thus
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7 obtained is conservative, since in the population under current study LD has
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9 been shown to be stronger than in the general Finnish population, which was
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11 represented by the Health 2000 study sample.[54]
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17 We did not use the Bonferroni correction for multiple testing due to limitations of
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19 sample size and expected magnitude of gene effects in complex traits. Although
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21 none of the results from the primary analyses (Table 2) survive conservative
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23 correction, a neurobiological a priori hypothesis based on previously published
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25 studies supports the validity of our most robust findings. It is, however,
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27 noteworthy that they were observed only when the sample was conditioned on
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29 environmental risk or gender. Still, the strongest association signal, obtained
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31 using *DRD2*'s rs4274224 with HSCL score in males ($P = 0.0006$), remains close to
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33 statistical significance even when taking into account the amount of multiple
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35 testing performed. The finding was further supported by results of our haplotype
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37 analysis containing rs4274224, which showed a statistically significant
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39 association with the HSCL score in males ($P = 0.00005$).
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48 There are some limitations in the present study. First, it is notable that
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50 depression as defined here did not necessarily signify a clinical diagnosis of
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52 major depression. Instead it was defined either based on self-report or on the
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54 score from HSCL, which as a measure has its limitations. However, the
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3 prevalence of depressed mood was in the same range as in earlier reports.[1, 55]
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5 Secondly, there was a notable drop-out rate among the original material of all
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7 cohort members. About half of the original cohort members did not participate in
8
9 this study. Finally, when the NFBC 1966 study was initiated it was not possible to
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11 predict that an investigation such as the present one would one day be
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13 conducted. Therefore, we are limited by the original choice of variables to be
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15 collected, and the environmental risk factors may only be indicators or markers of
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17 risk rather than risk factors themselves.[39] It is also noteworthy that we did not
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19 detect any association with our measure of current depression and the
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21 developmental risk environment, despite it being formulated based on previous
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23 reports of their effects on psychiatric health and wellbeing.[27-30]. However, the
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25 effect of genetic risk may be modulated by early life stress even though the direct
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27 link between early life environment and current status would be too weak to be
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29 detected in our study sample, and this modulating effect may be seen in the
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31 results of the GxE analysis.
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41 The current study has several potential advantages, such as the availability of
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43 longitudinal follow-up data starting antenatally, enabling us to include the
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45 environmental dimension without any risk of recall bias. Another advantage is the
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47 unique genetic structure of our study cohort, characterized by isolation, founder
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49 effect, multiple bottlenecks and more genetic homogeneity compared to many
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51 other isolates,[56] allowing us to identify genetic risk loci that may be missed in
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53 the screening of other more heterogeneous populations. Furthermore, the
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3 subjects were representative, with all cohort members born in the same year and
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5 within a geographically defined area.
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10 In addition, the size of the sample is sufficient to identify genetic variants of
11 moderate impact. We also have both genders represented in almost equal
12 amounts (48% males, 52% females), which is notable since gender differences
13 are evident both in depression[2, 49] and in temperament traits, for example
14 Harm avoidance.[49] It is also beneficial that the sample is a one-year birth
15 cohort, as it is well established that some psychiatric traits, such as Harm
16 avoidance[57] of temperament, are age-dependent. We can therefore isolate
17 genetic effects from the effects of aging. Furthermore, we provide a complete
18 coverage of the major candidate genes that are relevant with the present focus.
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34 Our results support the role of *COMT* and *DRD2*, two genes of monoamine
35 neurotransmission, in the etiology of depression conditional on environmental
36 risk particularly in males, though not direct effects of monoaminergic genes in
37 this unselected population. These findings imply that the nature of the role of
38 monoaminergic genes in depression should be examined further in future
39 studies, and are pending replication in other, independent population samples.
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27 **CONTRIBUTORS**

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31 Tiina Paunio, Emma Nyman, Sonja Sulkava, Jouko Miettunen, Matti Joukamaa
32
33 and Juha Veijola designed the study and wrote the protocol, with help also from
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36
37 Nyman and to some extent Tiina Paunio also managed the literature searches.
38
39 Sonja Sulkava and Emma Nyman undertook the statistical analyses. Emma
40
41 Nyman and Tiina Paunio wrote the first draft of the manuscript, and other authors
42
43 contributed to its later versions. All authors contributed to and have approved the
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45 final manuscript.
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Table 1. Composition of the study sample from the NFBC 1966.

	<i>N</i>	<i>HSCL score > 1.75</i> ¹	<i>Depression diagnosis</i>	<i>Early developmental risk environment</i> ²			<i>Social risk environment</i> ³		
				<i>High-risk</i> ⁴	<i>sLow-risk</i>	<i>nd</i> ⁵	<i>High-risk</i> ⁴	<i>Low-risk</i>	<i>nd</i> ⁴
<i>Males</i>	2509	169 (7%)	79(3%)	229 (9%)	2094 (83%)	186 (7%)	912 (36%)	1574 (63%)	23 (0.9%)
<i>Females</i>	2716	269(10%)	136(5%)	193 (7%)	2328 (86%)	195 (7%)	1034 (38%)	1649 (61%)	33 (1.2%)
<i>All</i>	5225	438(8%)	215(4%)	422 (8%)	4422 (85%)	381 (7%)	1946 (37%)	3223 (62%)	56 (1.1%)

¹ There is prior support for using the HSCL score 1.75 as a cut-off when aiming to identify clinical depression.

² Defined by the presence of two out of three possible indicators for the early developmental risk environment: low birth weight, late motor development, and late development of speech.

³ Defined by the presence of two out of five possible indicators for the social risk environment: unwantedness of pregnancy, low socio-economic status, single parenthood, low level of education of mother, and low activity for information retrieval by the mother. For further details, see text.

⁴ Both early developmental and environmental risk present in 92 males (3,6%) and 67 females (2,4%).

⁵ Not defined.

Table 2. Interaction (GxE) and correlation (rGE) between genetic variants of genes of monoamine neurotransmission and early developmental risk environment (GxE_{Dev}, rGE_{Dev}),¹ social risk environment (GxE_{Soc}, rG_{Soc}),² and gender (GxSex) on current depressive symptoms (HSCL score), and genetic association to HSCL score in the complete study sample from the NFBC 1966 (All). The analyses were performed using PLINK's linear and logistic regression models and interaction analysis. Empirical P-values based on max(T) permutation are reported, with P-values <0.05 shown in bold.

Gene	Gene name	Chromosome	SNP	Position/bp	Minor allele	MAF ³	P(GxE _{Dev})	P(GxE _{Soc})	P(GxSex)	P(All)	P(rGE _{Dev})	P(rG _{Soc})
SLC6A4	Serotonin transporter	17	rs1906451	25539605	G	0.44	0.608	0.363	0.784	0.268	0.747	0.035
			rs3794808	25555919	A	0.41	0.365	0.263	0.799	0.320	0.402	0.064
			rs140700	25567515	A	0.09	0.133	0.460	0.037⁸	0.876	0.209	0.614
			rs2066713	25575791	A	0.46	0.253	0.499	0.505	0.550	0.778	0.092
			rs8071667	25576899	A	0.15	0.473	0.682	0.122	0.606	0.961	0.827
TPH2	Tryptophan hydroxylase 2	12	rs4131348	70610746	G	0.12	0.844	0.937	0.400	0.497	0.705	0.241
			rs2129575	70626340	A	0.22	0.787	0.682	0.432	0.423	0.173	0.298
			rs1386496	70637057	G	0.16	0.983	0.404	0.293	0.792	0.837	0.226
			rs2171363	70646531	A	0.43	0.762	0.983	0.016⁸	0.814	0.692	0.940
			rs10506645	70671767	A	0.23	0.996	0.756	0.102	0.789	0.888	0.721
			rs1386497	70678557	C	0.17	0.816	0.131	0.452	0.797	0.640	0.172
			rs1487276	70691326	A	0.21	0.888	0.088	0.838	0.908	0.591	0.219
			rs9325202	70693744	A	0.48	0.805	0.074	0.488	0.473	0.913	0.675
			rs1487275	70696559	C	0.37	0.972	0.054	0.625	0.049¹⁰	0.861	0.638
			rs1386483	70698761	A	0.47	0.574	0.090	0.326	0.437	0.294	0.625
rs1872824	70716581	A	0.35	0.652	0.121	0.211	0.494	0.772	0.331			
COMT	Catechol-O-methyltransferase	22	rs6518591	18304021	G	0.16	0.688	0.255	0.919	0.303	0.150	0.385
			rs737866	18310109	G	0.18	0.028⁴	0.853	0.024⁸	0.755	0.623	0.489

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3				rs1544325	18311668	G	0.48	0.318	0.376	0.192	0.822	0.999	0.931
4				rs174675	18314051	A	0.29	0.465	0.278	0.580	0.958	0.724	0.532
5				rs5993883	18317638	C	0.36	0.230	0.495	0.025⁸	0.920	0.363	0.219
6				rs2239393	18330428	G	0.31	0.005⁵	0.765	0.256	0.930	0.838	0.459
7				rs4680	18331271	G	0.45	0.020⁶	0.956	0.501	0.346	0.412	0.498
8				rs4646316	18332132	A	0.18	0.205	0.165	0.933	0.026¹¹	0.521	0.392
9				rs165774	18332561	A	0.25	0.081	0.516	0.089	0.215	0.538	0.239
10				rs165815	18339473	G	0.20	0.537	0.309	0.431	0.281	0.338	0.158
11				rs887199	18341955	A	0.20	0.544	0.325	0.401	0.306	0.314	0.168
12				rs2239395	18342203	C	0.02	0.144	0.655	0.153	0.390	0.224	0.664
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18	MAOA	Monoamine oxidase A	X	rs909525	43438146	G	0.45	0.559	0.871	0.554	0.165	0.255	0.932
19				rs12843268	43458610	A	0.40	0.271	0.837	0.266	0.103	0.052	0.932
20				rs6610845	43472954	G	0.41	0.232	0.795	0.263	0.170	0.060	0.524
21				rs3027409	43491977	C	0.02	0.748	0.928	0.950	0.194	0.703	0.068
22				rs6609257	43497652	G	0.50	0.848	0.320	0.470	0.077	0.075	0.898
23				rs3027415	43499385	G	0.18	0.218	0.550	0.905	0.613	0.105	0.408
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27	DRD1	Dopamine receptor D1	5	rs265973	174793305	G	0.50	0.529	0.614	0.549	0.888	0.047	0.831
28				rs265974	174793846	G	0.35	0.391	0.612	0.912	0.659	0.066	0.859
29				rs265976	174795026	A	0.23	0.578	0.707	0.915	0.826	0.077	0.933
30				rs5326	174802802	A	0.19	0.615	0.886	0.852	0.588	0.273	0.197
31													
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34	DRD2	Dopamine receptor D2	11	rs1800497	112776038	A	0.17	0.079	0.825	0.691	0.467	0.921	0.264
35				rs2242592	112784640	G	0.37	0.757	0.466	0.283	0.736	0.143	0.393
36				rs1076563	112801119	C	0.50	0.053	0.813	0.897	0.662	0.234	0.856
37				rs2471857	112803549	A	0.17	0.518	0.494	0.823	0.901	0.884	0.126
38				rs4620755	112814829	A	0.22	0.383	0.997	0.951	0.176	0.065	0.992
39				rs7125415	112815891	A	0.19	0.084	0.389	0.789	0.231	0.163	0.947
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3			rs4648318	112818599	G	0.34	0.711	0.885	0.631	0.684	0.214	0.466	
4			rs4274224	112824662	G	0.24	0.067	0.777	0.017 ⁹	0.022 ¹²	0.536	0.766	
5			rs4581480	112829684	G	0.07	0.184	0.521	0.210	0.009 ¹³	0.082	0.760	
6			rs7131056	112834984	C	0.49	0.564	0.795	0.413	0.964	0.622	0.138	
7			rs4938019	112846601	G	0.23	0.069	0.651	0.643	0.584	0.320	0.059	
8			rs12364283	112852165	G	0.08	0.280	0.504	0.441	0.861	0.633	0.804	
9			rs10891556	112857971	A	0.24	0.076	0.519	0.638	0.589	0.380	0.052	
10			rs6589377	112860946	G	0.17	0.286	0.617	0.061	0.552	0.502	0.915	
11													
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15	<i>DRD3</i>	<i>Dopamine receptor D3</i>	3	rs2087017	115324703	G	0.43	0.937	0.921	0.606	0.743	0.835	0.828
16				rs2134655	115340891	A	0.28	0.454	0.554	0.129	0.507	0.209	0.330
17				rs963468	115345577	A	0.38	0.809	0.777	0.902	0.608	0.609	0.580
18				rs3773678	115352768	A	0.06	0.780	0.855	0.487	0.770	0.972	0.556
19				rs2630351	115357749	A	0.03	0.954	0.144	0.168	0.999	0.811	0.211
20				rs167771	115358965	G	0.18	0.862	0.638	0.406	0.514	0.416	0.966
21				rs167770	115362252	G	0.31	0.260	0.911	0.298	0.694	0.593	0.982
22				rs226082	115363703	G	0.31	0.261	0.911	0.301	0.690	0.594	0.983
23				rs324029	115364313	A	0.31	0.259	0.913	0.296	0.722	0.593	0.964
24				rs10934256	115368342	A	0.17	0.229	0.898	0.478	0.246	0.147	0.669
25				rs1486009	115371222	G	0.12	0.721	0.667	0.745	0.571	0.387	0.600
26				rs6280	115373505	G	0.33	0.159	0.485	0.141	0.667	0.386	0.880
27				rs9825563	115382910	G	0.23	0.045 ⁷	0.902	0.211	0.215	0.028	0.927
28													
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33	<i>DRD4</i>	<i>Dopamine receptor D4</i>	11	rs3758653	626399	G	0.23	0.300	0.752	0.249	0.980	0.322	0.905
34				rs11246226	631191	A	0.49	0.749	0.748	0.166	0.925	0.908	0.097
35													
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38	<i>DRD5</i>	<i>Dopamine receptor D5</i>	4	rs1878943	9375986	A	0.21	0.586	0.686	0.482	0.386	0.988	0.605
39				rs13106539	9406801	G	0.39	0.735	0.062	0.067	0.044 ¹⁴	0.532	0.384
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4 ¹ Defined by the presence of two out of three possible indicators for the early developmental risk environment: low birth weight, late motor
5 development, and late development of speech.

6 ² Defined by the presence of two out of five possible indicators for the social risk environment: unwantedness of pregnancy, low socio-economic
7 status, single parenthood, low level of education of mother, and low activity for information retrieval by the mother.

8 ³ Minor allele frequency.

9 ⁴ $P=0.0364$ ($\beta=0.0414$), ⁵ $P=0.008$ ($\beta =0.0440$), ⁶ $P=0.042$ ($\beta =0.0320$) and ⁷ $P=0.022$ ($\beta =-0.0396$) in individuals with high risk environment; ⁸ $P>0.05$
10 in both genders; ⁹ $P=0.0006$ ($\beta=0.023$) in males; ¹⁰ $\beta=0.008$, ¹¹ $\beta=0.012$, ¹² $\beta=0.011$, ¹³ $\beta=0.022$, ¹⁴ $\beta=-0$.

Table 3. Haplotype analysis of *COMT* variants on current depressive symptoms (HSCL score) in individuals with high early developmental risk (E_{Dev})¹ from the NFBC 1966. Empirical P-values based on permutation are reported, with P-values <0.05 shown in bold.

Gene	Variant	Haplotype	Frequency	Males and females with high risk E_{Dev}		Males with high risk E_{Dev}		Females with high risk E_{Dev}			
				Beta	P	Beta	P	Beta	P		
<i>COMT</i>	<i>2-SNP haplotype analysis</i>										
		rs5993883-rs2239393	CG	0.21	0.0552	0.0049	0.0828	0.0040	0.0216	0.4420	
		rs2239393-rs4680	GG	0.32	0.0440	0.0072	0.0720	0.0037	0.0119	0.4914	
			AA	0.55	-0.0320	0.0428	-0.0411	0.0827	-0.0207	0.3370	
		rs4680-rs4646316	GA	0.17	0.0434	0.0331	0.0624	0.0226	0.0206	0.3950	
		<i>3-SNP haplotype analysis</i>									
		rs5993883-rs2239393-rs4680	CGG	0.21	0.0548	0.0046	0.0826	0.0053	0.0211	0.4569	
	rs2239393-rs4680-rs4646316	GGA	0.17	0.0433	0.0344	0.0614	0.0258	0.0213	0.4311		

¹ Defined by the presence of two out of three possible indicators for the early developmental risk environment: low birth weight, late motor development, and late development of speech.

Table 4. Haplotype analysis of *DRD2* variants on current depressive symptoms (HSCL score) in the complete sample from the NFBC 1966. Empirical P-values based on permutation are reported, with P-values <0.05 shown in bold.

Gene	Variant	Haplotype	Frequency	Males and females		Males	
				Beta	P	Beta	P
<i>DRD2</i>	<i>2-SNP haplotype analysis</i>						
	rs4648318-rs4274224	GG	0.05	0.0409	0.0007	0.0694	0.00005
	rs4274224-rs4581480	GG	0.07	0.0220	0.0069	0.0321	0.0023
		AA	0.48	0.0116	0.0161	-0.0237	0.0004
	rs4581480-rs7131056	GA	0.07	0.0220	0.0071	0.0322	0.0026
	<i>3-SNP haplotype analysis</i>						
	rs4648318-rs4274224-rs4581480	GGG	0.05	0.0326	0.0027	0.0437	0.0019
rs4274224-rs4581480-rs7131056	GGA	0.07	0.0215	0.0081	0.0317	0.0033	

Table 5. Haplotype analysis of *COMT* and *DRD2* variants on other neurobehavioral traits in the NFBC1966. Empirical P-values based on permutation are reported, with P-values <0.05 shown in bold.

Gene	Variant	Group	Gender	HSCL(total)		HSCL(depression)		HSCL(anxiety)		Depression diagnosis		Harm avoidance	
				Beta	P	Beta	P	Beta	P	Odds ratio	P	Beta	P
<i>COMT</i>	rs737866	High risk	Males	0.0640	0.0254	0.0440	0.2239	0.0150	0.6157	0.7130	0.5004	0.7820	0.3799
	rs5993883- rs2239393 (CG)	High risk	Males	0.0830	0.0040	0.0750	0.0176	0.0200	0.2877	0.2100	0.1506	1.2040	0.1433
<i>DRD2</i>	rs4648318- rs4274224 (GG)	All	Males	0.0694	0.00005	0.0600	0.0015	0.0326	0.0212	0.8280	0.6798	1.0430	0.07009

STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cohort studies

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3-4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6-7
Objectives	3	State specific objectives, including any prespecified hypotheses	7-8
Methods			
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8-11
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	8-11
		(b) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8-11
Bias	9	Describe any efforts to address potential sources of bias	8-11
Study size	10	Explain how the study size was arrived at	8-11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	12-13
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	12-13
		(b) Describe any methods used to examine subgroups and interactions	12-13
		(c) Explain how missing data were addressed	
		(d) If applicable, explain how loss to follow-up was addressed	
		(e) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8-11
		(b) Give reasons for non-participation at each stage	8-11
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-11
		(b) Indicate number of participants with missing data for each variable of interest	8-11
		(c) Summarise follow-up time (eg, average and total amount)	8-11
Outcome data	15*	Report numbers of outcome events or summary measures over time	8-13
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13-18
		(b) Report category boundaries when continuous variables were categorized	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	13-18
Discussion			
Key results	18	Summarise key results with reference to study objectives	18-22, 24
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	22-24
Generalisability	21	Discuss the generalisability (external validity) of the study results	24
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	24-25

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.



Interaction of Early Environment, Gender and Genes of Monoamine Neurotransmission in the Etiology of Depression in a Large Population-Based Finnish Birth Cohort

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Keywords:	PSYCHIATRY, Depression & mood disorders < PSYCHIATRY, GENETICS

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Interaction of Early Environment, Gender and Genes of Monoamine Neurotransmission in the Etiology of Depression in a Large Population-Based Finnish Birth Cohort

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Key words: Environment, Monoamine, Gene, Depression, Cohort Study

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ABSTRACT

Objectives Depression is a worldwide leading cause of morbidity and disability. Genetic studies have recently begun to elucidate its molecular etiology. We investigated candidate genes of monoamine neurotransmission and early environmental risk factors for depressiveness in the genetically isolated population-based Northern Finland Birth Cohort 1966 (12058 live births).

Design We ascertained and subdivided the study sample (n=5225) based on measures of early development, and of social environment, and examined candidate genes of monoamine neurotransmission, many of which have shown prior evidence of gene-environment interaction (GxE) for affective disorders, namely *SLC6A4*, *TPH2*, *COMT*, *MAOA*, and the dopamine receptor genes *DRD1-DRD5*.

Results and Conclusion We observed no major genetic effects of the analyzed variants on depressiveness. However, when measures of early development and of social environment were considered, some evidence of interaction was observed. Allelic variants of *COMT* interacted with high early developmental risk (P=0.005 for rs2239393 and P=0.02 for rs4680) so that the association with depression was detected only in individuals of high developmental risk group (P=0.0046 and $\beta=0.056$ for rs5993883-rs2239393-rs4680 risk haplotype CGG including Val158), particularly in males (P=0.0053 and $\beta=0.083$ for the haplotype CGG). Rs4274224 from *DRD2* interacted with gender (P=0.017) showing significant association with depressiveness in males (P=0.0006 and $\beta=0.0023$;

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2 P=0.00005 and $\beta=0.069$ for rs4648318-rs4274224 haplotype GG). Our results
3
4 support the role of genes of monoamine neurotransmission in the etiology of
5
6 depression conditional on environmental risk and sex, but not direct major effects
7
8 of monoaminergic genes in this unselected population.
9

10 11 12 13 14 ARTICLE SUMMARY

15 16 17 18 Article focus

- 19 - Impact on depression of monoaminergic candidate genes with prior
20 evidence of gene-environment interaction for affective disorders, and of
21 dopamine receptor genes
22
- 23 - Gene-environment and gene-gender interaction in the etiology of
24 depression
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- 26 - Effect of measures of early development, and of social environment on
27 depression
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30 31 32 33 34 Key messages

- 35 - Genes of monoamine neurotransmission play a role in the etiology of
36 depression conditional on environmental risk, especially in males and in
37 individuals of high early developmental risk group; in particular there is
38 evidence of an Interaction with a *COMT* high risk haplotype including
39 Val158
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- 42 - Gender-specific mechanisms and responses to environmental effectors
43 are evident in regulation of mood
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Strengths and limitations of this study Depression as defined does not necessary imply clinical diagnosis of major depression, but instead is based on self-report or on the score from the HSCL measure. Despite this limitation, the prevalence of depressed mood was in the same range as in earlier reports. There was a notable drop-out rate among the original material of all cohort members, and about half of the original cohort members did not participate in this study. Measures of early development and of social environment were chosen to reflect risk as precisely as possible, however, the choice was limited by the availability of variables collected. Advantages of this study include the availability of longitudinal follow-up data starting antenatally, enabling inclusion of the environmental dimension without risk of recall bias. Another advantage is the unique genetic structure of the study cohort, characterized by isolation, founder effect, multiple bottlenecks and more genetic homogeneity compared to many other isolates, permitting identification of genetic risk loci that may be missed when using more heterogeneous populations. The subjects were also representative of the population, with all cohort members born in the same year and within a geographically defined area. The size of the study sample is sufficient for identifying genetic variants of moderate impact. Both genders are also represented in almost equal amounts, which is notable since gender differences are evident in both depression and related temperament traits, such as Harm avoidance. Finally, it is also beneficial that the sample is a one-year birth cohort, as it is well established that some psychiatric traits, such as Harm

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avoidance are age-dependent. The genetic effects may therefore be isolated
from the effects of aging.

Deleted: Finally, a complete coverage of the major candidate genes that are relevant with the present focus is provided.

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INTRODUCTION

Depression is a major cause of morbidity worldwide, major depression affecting 5-7% of the population annually and 16% over the lifetime.[1] Although a genetic component in the etiology of major depression is evident with a 40-50% heritability,[2] the predisposing genetic background has so far remained largely undefined, and recent findings from genome-wide association studies also point to a complex underlying architecture.[3] Depressed patients frequently exhibit comorbidities such as anxiety and alcohol abuse,[4] and certain personality types[5-7] have been associated with depression proneness.

Environmental risk factors, in particular stressors influencing during development,[8] are considered to have a significant impact on the development and course of depression. It is likely that many of the genetic risk factors for depression interact with the early developmental environment, but recapture of these interactions has remained a challenge for etiological studies of depression. Although the interplay between genes and environment has been investigated with respect to several psychiatric disorders[9, 10] including depression, this vast subject remains still to a large extent unexplored. On the other hand, addressing the effects of genes and environment on psychiatric morbidity enables us to examine the two main constituents in their etiology. Therefore, we wanted to include the environmental dimension in our study in order to also explore gene-environment interactions (GxE).

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5 According to the monoamine hypothesis, depression is caused by underactivity
6 in brain monoamines, such as dopamine, serotonin, and norepinephrine.[11]
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8 Recent results of neuroimaging studies have provided further support for this
9 theory.[12] The most solid evidence from candidate gene studies has perhaps
10 been obtained for the interaction of the *SLC6A4* gene for serotonin transporter
11 and stressful early and current life events,[13] including positive results from a
12 recent review[14] and meta-analysis of all studies to date[15], although there are
13 also contradicting results.[16] Other robust genetic findings have been obtained
14 on the *COMT* gene for catechol-O-methyltransferase, an enzyme catabolising
15 catecholamines such as dopamine and noradrenaline, which has been implicated
16 in depression in conjunction with stress,[17] and on the *MAOA* gene for
17 monoamine oxidase A, an enzyme oxidizing neurotransmitter and dietary
18 monoamines such as serotonin, noradrenaline and dopamine, which has been
19 associated with depression in interaction with severity of maltreatment in
20 childhood.[17] Furthermore, *TPH2* gene for tryptophan hydroxylase 2, which is
21 the brain-specific form of the key enzyme in serotonin synthesis, has been
22 implicated to interact with stress on disorders of cognitive control and emotional
23 regulation, including depression.[18] Within the dopamine transmission the *DRD2*
24 gene for dopamine receptor D2 has been associated with depressiveness and
25 anxiety, combined with an effect of parenting in childhood,[19] and the *DRD4*
26 gene for dopamine receptor D4 has been associated with increased risk for
27 obesity in women with seasonal affective disorder.[20] Thus, genes from the
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2 monoamine neurotransmission system are among the most thoroughly studied in
3 psychiatric genetics and in particular in the etiology of mood disorders, and have
4 provided perhaps the most robust evidence so far for interaction with various
5 types of risk environments, including childhood environment.
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14 We chose to include these candidate genes of monoamine neurotransmission
15 [showing prior evidence of gene-environment interaction](#), including *SLC6A4*,
16 *TPH2*, *COMT*, *MAOA*, as well as the dopamine receptor genes *DRD1-DRD5*, in
17 our study on the etiology of depression with a particular focus on their interaction
18 with available markers reflecting [measures of](#) early development, [and of](#) social
19 environment. The study was performed in a sample of 5225 individuals from a
20 large Finnish isolated population cohort. As gender is an important confounder
21 for depression and at least some of the genetic liability is gender-specific,[2] we
22 also examined gene-gender interactions in this sample.
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37 METHODS

40 Setting

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42 We utilized the genetically isolated Northern Finland Birth Cohort (NFBC 1966) to
43 investigate effects of candidate genes and environmental risk factors during the
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2 development on depressiveness. We subdivided the study sample based on
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4 measures of early development arising from the fetal growth environment and
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6 neurological development during the first year of life (measure of early
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8 development) as well as from the family environment during pregnancy and early
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10 childhood (measure of social environment). We examined interactions of these
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12 measures with candidate genes of the monoamine neurotransmitter systems,
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14 which have prior evidence of gene-environment interaction on affective disorders,
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16 namely *SLC6A4*, *TPH2*, *COMT*, *MAOA*, and the dopamine receptor genes
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18 *DRD1-DRD5*.
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Study subjects

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27 The Northern Finland Birth Cohort 1966 (NFBC 1966) is a longitudinal one-year
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29 birth cohort from an unselected population (N=12058 live births) comprising
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31 inhabitants of the two northernmost provinces of Finland.[21] Data collection was
32
33 begun during the antenatal period, and follow-up studies have been performed at
34
35 the ages of 1, 14 and 31 years. The cohort study has been approved by the
36
37 Ethical Committee of Oulu University Faculty of Medicine, and written informed
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39 consent has been obtained from all participants.
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44 In 1997 for the 31-year follow-up study[22] all alive cohort members with a known
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46 address (N=11540) were sent a postal questionnaire surveying lifestyle, social
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48 status and health (76% participated), including the Hopkins Symptom Check
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2 List-25 (HSCL)[23] and items on self-reported lifetime depression diagnosis (e.g.
3 “Has your doctor ever diagnosed a depressive disorder?”). Additionally, cohort
4 members who lived in Northern Finland or had moved to the Helsinki area
5 (N=8465) were invited to a clinical examination (71% participated) with another
6 questionnaire to be filled in later and sent to the research group (61%
7 participated).[24] It included, among others, a validated Finnish translation of
8 Cloninger’s Temperament and Character Inventory (TCI) questionnaire.[25]
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19 Current depressive symptoms were assessed by the HSCL-questionnaire,[26] a
20 25-item shortened version of an originally 90-item questionnaire. HSCL contains
21 13-item depression and 10-item anxiety subscales assessing presence and
22 intensity of depressive and anxiety symptoms during the previous week. Answers
23 are scored on a scale from 1 (not bothered) to 4 (extremely bothered). The HSCL
24 total score is the sum of items divided by the number of items answered. We
25 used mainly HSCL total score as symptoms of depression and anxiety are known
26 to overlap significantly. In the post hoc analyses, in order to better understand
27 the original association signals, the separate HSCL subscales for depressive and
28 anxiety symptoms were also taken into consideration. In addition to current
29 depressive symptoms (HSCL score) and lifetime (diagnosed) depression, we
30 used the TCI temperament trait Harm avoidance[5-7] and its subcomponents as
31 a measure of proneness to depression.
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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 [measures of early neurodevelopment](#), and [of social environment](#) (Table 1). The markers for the [measure of high early developmental risk](#) included 1) low birth weight (<2500 g),[21] considered to reflect suboptimal growth environment during fetal life and to increase risk for somatic and psychiatric diseases such as depression in adulthood,[27] 2) late motor development as reflected by first standing later than at the age of 10 months,[28] and 3) late development of speech, defined by no words at the age of one year.[28] If two out of these risk indicators were present, the subject was classified as having experienced a high risk environment for early brain development. The markers for the [measure of high social risk environment](#) included the occurrence of two or more of the following five indicators for high risk social environment during pregnancy and early childhood: 1) unwantedness of pregnancy (rated by mothers of the cohort members at the sixth or seventh month of pregnancy),[29] 2) low socio-economic status, shown to be linked with depression in the offspring in earlier studies,[30] as defined by father's [occupation](#) at birth (no occupation, unskilled worker, or farmer with area under cultivation under 8 hectares), 3) single parenthood at birth, 4) low level of education of mother (less than nine years of primary school), and 5) low level of information retrieval by the mother related to pregnancy and child care. There was no significant drop-out in either of the high risk groups, as 43% and 41% of

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the individuals of high early developmental and social risk groups, and 47% and 46% of those of the respective low risk groups, were available for study.

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[Insert Table 1]

Genotyping methods

We investigated genes relevant within the context of the monoamine hypothesis of depression: *SLC6A4*, *TPH2*, *COMT*, *MAOA*, and the dopamine receptor genes *DRD1-DRD5* (Table 2). The genotyping was performed at the Broad Institute (Cambridge, MA, USA) on the HumanCNV370-duo chip (Illumina, San Diego, CA USA) platform according to the manufacturer's instructions. The analysed SNPs included HapMap tag SNPs (<http://www.hapmap.org/index.html.en>) and were relatively evenly spaced to cover the genes and flanking regions.

[Insert Table 2]

Statistical analysis

LD structures were determined using HAPLOVIEW. Interaction and association/correlation analyses using linear and logistic regression with permutation was performed using PLINK Software Package Version 1.04, in a step-wise manner to maximize our ability to detect associations and to minimize

1
2 multiple testing. (i) Primarily analyses were performed to identify genetic risk
3 variants for current depressive symptoms (HSCL score) interacting with
4 measures of early development (GxE_{Dev}) and of social environment (GxE_{Soc}). For
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8 variants giving significant evidence of interaction, we also performed analyses
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10 separately in subgroups of high and low risk, respectively. As gender is an
11 important confounder for depression and at least some of the genetic liability is
12 gender-specific,[2] we also examined gene-gender interactions (GxSex). For
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14 variants showing significant evidence of gene-gender interaction, we also
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16 performed analyses separately in males and females. In order to achieve a more
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18 complete view of the effects of the examined genes on depressive symptoms in
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20 the cohort, we also examined their influence on the gender-adjusted HSCL score
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22 in the complete sample regardless of environmental effectors. Finally, we tested
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24 for gene-environment correlations (rGE_{Dev} and rG_{Soc}) and associations of the risk
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26 environments with the HSCL score (PASW Statistics 18, linear regression
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28 model). (ii) Haplotype analyses were performed when two SNPs located at
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30 physically close vicinity had given association signals of P<0.05 when analyzed
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32 separately. (iii) Genetic variants and haplotypes which had been identified in the
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34 previous analyses were analyzed post hoc with respect to HSCL subscales
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36 (depressive and anxiety symptoms), depression diagnosis and TCI temperament
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38 Harm avoidance. We report point-wise empirical p-values generated by PLINK's
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40 max(T) permutation (10 000 permutations) throughout the manuscript, and
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42 explicitly state where corrected empirical p-values are reported. SNPs with
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44 Hardy-Weinberg Equilibrium p-values <0.05 were excluded from all analyses.
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RESULTS

Gene-environment and gene-gender interaction and association analyses in relation to the HSCL score

We examined the effects of nine candidate genes of monoamine neurotransmission on current depressive symptoms (HSCL score) in a longitudinal population-based NFBC 1966 cohort. In particular, we searched for evidence of interaction of variants in these genes with two [measures of early growth](#), one with indicators for potentially disturbed neurobehavioral development ([measure of early development](#)) and the other with risk factors from social environment for normal emotional development ([measure of social environment](#)).

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The results are presented in Table 2 in which nominal P-values are reported.

Out of the 69 genetic variants examined, none gave a statistically significant association signal with depressiveness or for an interaction with [measures of early development](#), or [of social environment](#), which would survive correction for multiple testing. We observed nominal evidence for association with the HSCL score ($P < 0.05$) in the complete sample in the cases of rs1487275 in *TPH2*, ($P = 0.049$, $\beta = 0.008$), rs4646316 in *COMT* ($P = 0.026$, $\beta = 0.012$), rs4274224 and rs4581480 in *DRD2* ($P = 0.022$, $\beta = 0.011$; and $P = 0.009$, $\beta = 0.022$, respectively),

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2 and rs13106539 in *DRD5* ($P=0.044$, $\beta=-0.008$). Three variants of *COMT* and one
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4 of *DRD3* showed some evidence of interaction ($P<0.05$) with high early
5
6 developmental risk with respect to the HSCL score ($P=0.028$ for rs737866;
7
8 $P=0.005$ for rs2239393, and, $P=0.020$ from rs4680 from *COMT*, and rs9825563,
9
10 $P=0.045$ from *DRD3*). All of them were associated with the HSCL score in
11
12 individuals of the high risk group ($P=0.036$, $\beta=0.0414$ for rs737866; $P=0.008$,
13
14 $\beta=0.0440$ for rs2239393; $P=0.042$, $\beta=0.0320$ for rs4680; and $P=0.022$, $\beta=-0.0396$
15
16 for rs9825563, respectively). None of the variants gave any evidence of
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18 interaction with the [measure of social environment in relation to the HSCL score](#).
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21 Five of the genetic variants showed some evidence of interaction with gender
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23 ($P<0.05$), including rs737866 and rs5993883 in *COMT* and rs4274224 in *DRD2*.
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25 Out of these, only rs4274224 associated at $P<0.05$ with one of the genders
26
27 ($P=0.0006$, $\beta=0.023$ in males). The evidence for gene-environment correlations
28
29 (rGE) was observed only nominally about rs1906451 from *TPH2* ($P=0.035$),
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31 rs265973 from *DRD1* ($P=0.047$), and rs9825563 from *DRD3* ($P=0.028$). Despite
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33 a priori evidence for the role of the markers which indicate [high developmental](#)
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35 [risk for psychiatric health and wellbeing, namely low birth weight\[21, 27\] and late](#)
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37 motor or verbal development[28], there was no correlation between these
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39 markers and the HSCL score in the present sample ($P=0.131$), whereas the
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41 social high risk environment, correlated significantly with the score ($P = 0.00001$).
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45 Although none of the association findings of these primary analyses survived
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47 correction for multiple testing, post hoc association analyses in gender groups
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2 led to a finding close to statistical significance even when taking into account the
3 amount of multiple testing performed ($P=0.0006$ for males with rs4274224 in
4 *DRD2*). Furthermore, as there was an accumulation of association signals within
5
6 *DRD2*). Furthermore, as there was an accumulation of association signals within
7
8 two highly plausible candidate genes, *DRD2* and *COMT*, we proceeded to
9
10 perform haplotype analyses on these genes in order to better characterize the
11
12 allelic variants which yielded the observed suggestive associations, [and to obtain](#)
13
14 [a maximal amount of information on the nature of the associations observed.](#)
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17 18 19 **Haplotype analysis of *COMT* and *DRD2* variants in relation to the HSCL** 20 21 **score**

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24 We performed 2-SNP and 3-SNP haplotype analyses combining rs2239393 and
25
26 rs4680 from *COMT* and their neighbouring variants using the sliding window
27
28 approach. Evidence of association was observed for the rs5993883-rs2239393
29
30 haplotype CG spanning a region from the space between LD blocks 1 and 2 to
31
32 block 2 of *COMT* (Supplementary figure 1) ($P=0.0049$, $\beta=0.055$), for the
33
34 rs2239393-rs4680 haplotype GG in block 2 ($P=0.0072$, $\beta=0.044$), and the
35
36 rs5993883-rs2239393-rs4680 haplotype CGG ($P=0.0046$, $\beta=0.055$) in [the](#) high
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38 early developmental risk [group](#), in agreement with analyses using single variants
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40 (Table 3). As rs5993883 from the haplotype had also given evidence of
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42 interaction with gender (Table 2), we further examined haplotype association in
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44 males and females of the high risk group separately. We found that the
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46 haplotypes increased the risk for depressive symptoms in males, but not in
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2 females ($P=0.004$, $\beta=0.083$ for rs5993883-rs2239393 haplotype CG; $P=0.0037$,
3 $\beta=0.072$ for rs2239393-rs4680 haplotype GG; and $P=0.0053$, $\beta=0.083$ for
4 rs5993883-rs2239393-rs4680 haplotype CGG) (Table 3). [As is evident from the](#)
5 [β-values, each of the haplotypes accounts for more variance in depression than](#)
6 [any individual constituent SNP.](#)
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14 Haplotype analysis of rs4274224 and rs4581480 from *DRD2*, which gave
15 suggestive evidence of an association with the HSCL score in the complete
16 sample, and of their neighbouring variants gave evidence of an association of
17 rs4648318-rs4274224 haplotype GG spanning from block 2 to block 3 of *DRD2*
18 ($P=0.0007$, $\beta=0.041$), rs4274224-rs4581480 haplotype GG in block 3 ($P=0.0069$,
19 $\beta=0.022$), and rs4581480-rs7131056 haplotype GA spanning from block 3 to
20 block 4 ($P=0.0071$, $\beta=0.022$) with the HSCL score. The 3-SNP haplotypes
21 rs4648318-rs4274224-rs4581480 haplotype GGG ($P=0.0027$, $\beta=0.032$), and
22 rs4274224-rs4581480-rs7131056 haplotype GGA ($P=0.0081$, $\beta=0.021$), gave
23 evidence of an association in agreement with the findings from the 2-SNP
24 haplotypes as well as the single variants (Table 4). As one of the variants
25 contained within these haplotypes, namely rs4274224, also gave evidence of
26 interaction with gender as well as association with the HSCL score in males, we
27 also examined the association in males alone. The association signal became
28 stronger for all of the risk haplotypes, being strongest for rs4648318-rs4274224
29 haplotype GG ($P=0.00005$, $\beta=0.069$). [Similarly as for the *COMT* haplotypes, the](#)
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3 β -values, imply that each of the *DRD2* haplotypes accounts for more variance in
4 depression than any individual constituent SNP.
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9 [Insert Tables 3, 4 and Supplementary figure 1 into the supplementary]
10

11 12 **Haplotype analysis of *COMT* and *DRD2* variants in relation to other** 13 **neurobehavioral traits** 14

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18 Encouraged by the findings of the haplotype analyses, we tested for associations
19 of haplotypes rs5993883-rs2239393 in *COMT* and rs4648318-rs4274224 in
20 *DRD2*, as well as the single variant rs737866 in *COMT* with other traits related to
21 depression, including the HSCL depression and anxiety subscales, depression
22 diagnosis and TCI temperament trait Harm avoidance (Table 5). In both of the
23 genes, it is evident that the association with HSCL stems mainly from the
24 subscale which reflects symptoms of depression and not that reflecting anxiety
25 (with HSCL depression subscale, $P=0.018$, $\beta=0.075$ for *COMT* haplotype CG and
26 $P=0.0015$, $\beta=0.060$ for *DRD2* haplotype GG; with HSCL anxiety subscale,
27 $P=0.288$, $\beta=0.02$ and $P=0.02$ and $\beta=0.033$, respectively). We did not detect any
28 evidence of an association with depression diagnosis or with Harm avoidance or
29 its subcomponents.
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DISCUSSION

We investigated genetic and environmental risk factors for depression in a genetically isolated Finnish birth cohort by assessing the relative impacts of monoaminergic candidate genes for depression in groups of contrasting (high and low) early developmental and social risk. We did not observe any robust genetic effects of the analyzed variants on depressiveness. However, when measures of early development and social environment were considered, some signals for association were observed, although none of them survive correction for multiple testing. Our study sample provided modest evidence of an interaction of *COMT* with the measure of high early developmental risk, particularly in males, and a contribution of an allelic variant of *DRD2* to genetic risk for depressiveness particularly in males (Table 2).

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The *COMT* gene encoding for catechol-O-methyltransferase enzyme is among the most investigated genes in psychiatric genetics. The enzyme degrades catecholamine neurotransmitters such as dopamine, noradrenaline and adrenaline by catalyzing the transfer of a methyl group from S-adenosylmethionine to the catecholamines. Its enzymatic activity varies according to a G-to-A transition at codon 158 in the *COMT* gene, resulting in a valine-to-methionine substitution (Val158Met) on the protein level.[31] The enzyme encoded by the Val158 allele has a 3-4 fold higher activity than that

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encoded by the Met158 allele. Here, we found an association of the haplotype comprising rs5993883 between LD blocks 1 and 2 of *COMT*, as well as rs2239393 and rs4680, which are two variants in virtually complete linkage disequilibrium in block 2, with depressive symptoms in high developmental risk males ($P=0.0053$). The high risk haplotype included the high activity variant Val158 of *COMT*, the allele G of rs4680. This allele has repeatedly been found to be associated with a poor response to pharmacological treatment of depression,[32, 33] and a European multicenter study identified an association between that allele and early onset major depression.[34] The Val158 allele has already earlier been found to associate with cognitive deficits including poor performance in tasks related to higher-order components of processing[35] and perseverative errors, less efficient physiologic responses in the prefrontal cortex,[36] and even schizophrenia based on a meta-analysis,[37] although the effect was not significant when studies with allele frequencies deviating from the Hardy-Weinberg equilibrium were excluded.

In our study we observed evidence for interaction between *COMT* and a [measure of](#) early developmental risk on depressive symptoms. This interaction could not be explained through gene-environment correlations. Nor were we able to detect a significant correlation of the [measure of early](#) developmental risk 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

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3 may reflect the presence of other environmental risk indicators which were not
4 examined in our study. However, they may also reflect individual variability in
5 response to the risk environment and presence of genetic factors (such as the
6 *COMT* haplotype containing Met158) that may relate to resilience, adaptive
7 changes in regulation of emotion reactivity and successful coping with stress.[38]
8
9 The observed risk also seemed to arise from an aggregation of the
10 environmental indicators, as none of the risk items separately gave evidence of
11 GxE with the risk variants from *COMT* or *DRD3* (data not shown). This could
12 reflect a cumulative nature of these environmental influences, such that the effect
13 of one marker may be weak, but the accumulated effect of multiple markers,
14 together with genetic susceptibility, would be strong enough to increase the risk
15 for a deviant development of emotional regulation and thus depressiveness.[39]
16
17 There is some prior evidence of interaction of *COMT* with a risk environment on
18 psychosis, antisocial behaviour and dissociation. A study on children with ADHD
19 showed a gene-environment interaction between the Val/Val genotype and low
20 birth weight on early-onset antisocial behaviour,[40] and the Val158 allele was
21 also found to associate with cannabis use and psychotic symptoms[41] and with
22 increasing levels of dissociation in those exposed to higher levels of childhood
23 trauma.[42] Interestingly, a recent report[43] revealed an impact of that
24 polymorphism on gender-related patterns of regulation of emotions (activation in
25 limbic and paralimbic regions) in line with findings of the present study.
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Another main finding of the present study, and statistically the strongest one, was observed in the dopamine receptor D2 gene *DRD2*, where a haplotype comprising the intronic variants rs4648318 in LD block 2 and rs4274224 in block 3 was found to associate with depressive symptoms particularly in males, regardless of their early environment ($P=0.00005$). Dopamine receptors have key roles in a variety of processes in the vertebrate central nervous system, and dysfunction in dopaminergic neurotransmission may therefore predispose to a variety of neuropsychiatric disorders. Among the receptor genes, *DRD2* has attracted the most attention and has been implied to have a role in the etiology of several psychiatric disorders. However, there are only a few previous reports on unipolar depression, including positive,[44] nominal[45] and negative[46, 47] findings, and for results on depression conditional on risk environment.[44, 46, 48]

Our varying results for males and females in general imply different mechanisms of mood regulation and possible gender-specific responses to environmental effectors. Gender differences in depression[2, 49] as well as in temperament traits[49] have previously been reported in various populations, including the current one,[50] and the prevalence of depression is higher in women.[51] A true gender-specific effect of genetic variants on depressiveness would not be surprising, as there is evidence of gender differences in dopaminergic function[52] that may be estrogen-dependent.

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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. The *SLC6A4* SNPs included in our study tag the 5-HTTLPR well ($D' > 0.9$), as determined by using genotypes from a population-based Finnish Health 2000 study.[53] Moreover, the LD measure thus obtained is conservative, since in the population under current study LD has been shown to be stronger than in the general Finnish population, which was represented by the Health 2000 study sample.[54]

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 [measures of early development or of social environment](#), 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

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2 showed a statistically significant association with the HSCL score in males
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4 (P=0.00005).
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9 There are some limitations in the present study. First, it is notable that
10 depression as defined here did not necessarily signify a clinical diagnosis of
11 major depression. Instead it was defined either based on self-report or on the
12 score from HSCL, which as a measure has its limitations. However, the
13 prevalence of depressed mood was in the same range as in earlier reports.[1, 55]
14
15 Secondly, there was a notable drop-out rate among the original material of all
16 cohort members. About half of the original cohort members did not participate in
17 this study. Finally, when the NFBC 1966 study was initiated it was not possible to
18 predict that an investigation such as the present one would one day be
19 conducted. Therefore, we are limited by the original choice of variables to be
20 collected, and the [measures of early development or of social environment](#), may
21 only be indicators or markers of risk rather than risk factors themselves.[39] It is
22 also noteworthy that we did not detect any association [of our measure of current](#)
23 depression [with the measure of high early developmental risk](#), despite it being
24 formulated based on previous reports of their effects on psychiatric health and
25 wellbeing.[27-30]. However, the effect of genetic risk may be modulated by early
26 life stress even though the direct link between early life environment and current
27 status would be too weak to be detected in our study sample, and this
28 modulating effect may be seen in the results of the GxE analysis.
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2 The current study has several potential advantages, such as the availability of
3 longitudinal follow-up data starting antenatally, enabling us to include the
4 environmental dimension without any risk of recall bias. Another advantage is the
5 unique genetic structure of our study cohort, characterized by isolation, founder
6 effect, multiple bottlenecks and more genetic homogeneity compared to many
7 other isolates,[56] allowing us to identify genetic risk loci that may be missed in
8 the screening of other more heterogeneous populations. Furthermore, the
9 subjects were representative, with all cohort members born in the same year and
10 within a geographically defined area.
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22 In addition, the size of the sample is sufficient to identify genetic variants of
23 moderate impact. We also have both genders represented in almost equal
24 amounts (48% males, 52% females), which is notable since gender differences
25 are evident both in depression[2, 49] and in temperament traits, for example
26 Harm avoidance.[49] ~~Furthermore, it is beneficial that the sample is a one-year~~
27 birth cohort, as it is well established that some psychiatric traits, such as Harm
28 avoidance[57] of temperament, are age-dependent. We can therefore isolate
29 genetic effects from the effects of aging.
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41 Our results support a modest role of *COMT* and *DRD2*, two genes of monoamine
42 neurotransmission, in the etiology of depression conditional on environmental
43 risk particularly in males, though not direct effects of monoaminergic genes in
44 this unselected population. These findings imply that the nature of the role of
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2 monoaminergic genes in depression should be examined further in future
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4 studies, and are pending replication in other, independent population samples.
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10 **ACKNOWLEDGEMENTS, COMPETING INTERESTS, FUNDING**

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22 that could have influenced the performance or presentation of the current study.
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35 **CONTRIBUTORS**

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37
38 Tiina Paunio, Emma Nyman, Sonja Sulkava, Jouko Miettunen, Matti Joukamaa
39 and Juha Veijola designed the study and wrote the protocol, with help also from
40 Nelson Freimer, Pirjo Mäki, Leena Peltonen and Marjo-Riitta Järvelin. Emma
41 Nyman and to some extent Tiina Paunio also managed the literature searches.
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47 Sonja Sulkava and Emma Nyman undertook the statistical analyses. Emma
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Nyman and Tiina Paunio wrote the first draft of the manuscript, and other authors contributed to its later versions. All authors contributed to and have approved the final manuscript.

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STROBE 2007 (v4) Statement—Checklist of items that should be included in reports of cohort studies

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(a) Indicate the study’s design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	3-4
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6-7
Objectives	3	State specific objectives, including any prespecified hypotheses	7-8
Methods			
Study design	4	Present key elements of study design early in the paper	8
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	8-11
Participants	6	(a) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	8-11
		(b) For matched studies, give matching criteria and number of exposed and unexposed	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	8-11
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8-11
Bias	9	Describe any efforts to address potential sources of bias	8-11
Study size	10	Explain how the study size was arrived at	8-11
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	12-13
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	12-13
		(b) Describe any methods used to examine subgroups and interactions	12-13
		(c) Explain how missing data were addressed	
		(d) If applicable, explain how loss to follow-up was addressed	
		(e) Describe any sensitivity analyses	
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	8-11
		(b) Give reasons for non-participation at each stage	8-11
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	8-11
		(b) Indicate number of participants with missing data for each variable of interest	8-11
		(c) Summarise follow-up time (eg, average and total amount)	8-11
Outcome data	15*	Report numbers of outcome events or summary measures over time	8-13
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	13-18
		(b) Report category boundaries when continuous variables were categorized	
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	13-18
Discussion			
Key results	18	Summarise key results with reference to study objectives	18-22, 24
Limitations			
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	22-24
Generalisability	21	Discuss the generalisability (external validity) of the study results	24
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	24-25

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at www.strobe-statement.org.