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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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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 β=0.044; P=0.0053 and β=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 β=0.0023; P=0.00005 and β=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* inluding Val158. The results also imply

gender-specific mechanisms of mood regulation and responses to environmental effectors.

ARTICLE SUMMARY

Article focus

- Gene-environment and gene-gender interaction in the etiology of depression
- Effect of early neurodevelopmental and social risk environments on depression
- Impact on depression of monoaminergic candidate genes with prior evidence of gene-environment interaction for affective disorders, and the dopamine receptor genes

Key messages

- Genes of monoamine neurotransmission play a role in the etiology of depression, especially in males
- Early developmental risk environment interacts with a *COMT* high risk haplotype inluding Val158
- Gender-specific mechanisms of mood regulation and responses to environmental effectors are evident

Strengths and limitations of this study Limitations of this study include that depression as defined does not necessary imply clinical diagnosis of major depression, but instead was defined based on self-report or on the score from

the HSCL measure, which has its limitations. Nevertheless, the prevalence of depressed mood was in the same range as in earlier reports. Furthermore, 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. 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. Furthermore, the subjects were representative, with all cohort members born in the same year and within a geographically defined area. Also, 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 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 from the effects of aging. Furthermore, a complete coverage of the major candidate genes that are relevant with the present focus is provided.

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).

According to the monoamine hypothesis, depression is caused by underactivity in brain monoamines, such as dopamine, serotonin, and norepinephrine.[10] Recent results of neuroimaging studies have provided further support for this theory.[11] The most solid evidence from candidate gene studies has perhaps been obtained for the interaction of serotonin transporter and stressful life events,[12] although a recent meta-analysis objects those findings.[13] Other robust genetic findings have been obtained on the *COMT* gene for cathecol-O-methyltransferase, an enzyme catabolising catecholamines such as dopamine and noradrenaline, that has been implicated f.ex. in cognition,[14] and on monoamine oxidase A, an enzyme oxidizing neurotransmitter and dietary monoamines such as serotonin, noradrenaline and dopamine, in which a mutation for an early stop codon was found to segregate in a family with antisocial behaviour,[15] and the gene was later related to antisocial behaviour after maltreatment in childhood.[16]

To advance our understanding of the etiology of depression, we aimed to investigate candidate genes of monoamine neurotransmission and their interaction with early developmental and social risk factors for depression in a sample of 5225 individuals from a large Finnish isolated population cohort. As gender is an important confounder for depression and at least some of the

genetic liability is gender-specific,[2] we also examined gene-gender interactions in this sample.

METHODS

Setting

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

Study subjects

The Northern Finland Birth Cohort 1966 (NFBC 1966) is a longitudinal one-year birth cohort from an unselected population (N=12058 live births) comprising inhabitants of the two northernmost provinces of Finland.[17] Data collection has started from the antenatal period, and follow-up studies have been performed at the ages of 1, 14 and 31 years. The cohort study has been approved by the Ethical Committee of Oulu University Faculty of Medicine, and written informed consent has been obtained from all participants.

In 1997 in the 31-year follow-up study[18] all cohort members alive with a known address (N=11540) were sent a postal questionnaire surveying lifestyle, social status and health (76% participated), including the Hopkins Symptom Check List–25 (HSCL).[19] and items on self-reported lifetime depression diagnosis ("Has your doctor ever diagnosed a depressive disorder?"). Additionally, cohort members who lived in Northern Finland or in the capital area (N=8465) were invited to a clinical examination (71% participated) with another questionnaire to be filled in later and sent to the research group (61% participated).[20] It included, among others, a validated Finnish translation of Cloninger's Temperament and Character Inventory (TCI) questionnaire.[21]

Current depressive symptoms were assessed by the HSCL-questionnaire,[22] a 25-item shortened version of an originally 90-item questionnaire. HSCL contains 13-item depression and 10-item anxiety subscales assessing presence and intensity of depressive and anxiety symptoms during the previous week. Answers

are scored on a scale from 1 (not bothered) to 4 (extremely bothered). HSCL total score is the sum of items divided by the number of items answered. We used mainly HSCL total score as symptoms of depression and anxiety are known to overlap significantly. In post hoc analyses in order to better understand the original association signals, the separate HSCL subscales for depressive and anxiety symptoms were also taken into consideration. In addition to current depressive symptoms (HSCL score) and lifetime (diagnosed) depression, we used the TCI temperament trait Harm avoidance[5-7] and its subcomponents as a measure of proneness to depression.

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 early neurodevelopmental and social risk environments (Table 1). *The early developmental risk environment* was defined by 1) low birth weight (<2500 g),[17] considered to reflect suboptimal growth environment during fetal life and to increase risk for somatic and psychiatric diseases such as depression in adulthood,[23] 2) late motor development as reflected by first standing later than at the age of 10 months,[24] and 3) late development of speech, defined by no words at the age of one year.[24] If two out of these risk indicators were present, one was classified as having experienced a high risk environment for early brain development. *The social risk environment* was defined by 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),[25] 2) low socio-economic status, linked with depression in the offspring in earlier studies,[26] as defined by father's social class 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 high risk groups as 43% and 41% of the individuals with high risk early developmental and social environments, and 47% and 46% of those with the respective low risk environments, were available for study.

[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 SNPs analysed 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 analysis and 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 multiple testing. *(i)* Primarily analyses were performed to identify genetic risk variants for current depressive symptoms (HSCL score) interacting with early developmental risk (GxE_{Dev}) and social risk (GxE_{Soc}) environments. For variants giving significant evidence of interaction, we also performed analyses separately in subgroups of high and low risk, respectively. As gender is an important confounder for depression and at least some of the genetic liability is gender-specific,[2] we also examined gene-gender interactions (GxSex). For variants showing significant evidence of gene-gender interaction, we also performed analyses separately in males and females. In order to form a complete view of the effects of the examined genes on depressive symptoms in the cohort, we also examined their influence on the HSCL score gender-adjusted in the complete sample regardless of environmental effectors. Finally, we tested for gene-environment correlations (rGE_{Dev} and rG_{Soc}) and associations of the risk environments with the HSCL score. *(ii)* Haplotype analyses were performed when two SNPs located at close vicinity physically had given association signals of $P<0.05$ when analyzed

separately. *(iii)* Genetic variants and haplotypes identified in the previous analyses were analyzed post hoc with respect to HSCL subscales (depressive and anxiety symptoms), depression diagnosis and TCI temperament Harm avoidance. We report point-wise empirical p-values generated by PLINK's max(T) permutation throughout the manuscript, and explicitly state where corrected empirical p-values are reported. SNPs with Hardy-Weinberg Equilibrium p-values <0.05 were excluded from all analyses.

RESULTS

Gene-environment and gene-gender interaction and association analyses on HSCL score

We examined the effects of nine candidate genes of monomine 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 early growth environments with indicators for potentially disturbed neurobehavioral development (early developmental risk environment) or 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, four showed some evidence of interaction (P<0.05) with high early developmental risk environment with respect to the HSCL score. Three of the variants were located in *COMT*, namely an intronic variant rs737866 at the 5' end of the gene (P=0.028), rs2239393 (P=0.005) and rs4680 (P=0.020), and one in the 3' end of *DRD3*, rs9825563 (P=0.045). All of them were associated with HSCL score in individuals of the high risk group (P=0.036, β=0.0414 for rs737866; P=0.008, β=0.0440 for rs2239393; P=0.042, β=0.0320 for rs4680; and P=0.022, β=-0.0396 for rs9825563, respectively). None of the variants gave evidence of interaction with the social risk environment in relation to the HSCL score, nor did they show evidence of gene-environment correlations (rGE). Despite a priori evidence for the role of the indicators of the risk environments in psychiatric health and wellbeing, the risk environments did not correlate with the HSCL score. Five of the genetic variants showed evidence of interaction with gender (P<0.05), including rs737866 and rs5993883 in *COMT* and rs4274224 in *DRD2*. Out of these, only rs4274224 associated at P<0.05 with one of the genders (P=0.0006, β=0.023 in males). Finally, we observed some evidence of association of four variants with the HSCL score in the complete sample: rs1487275 in *TPH2*, (P=0.049, β=0.008), rs4646316 in *COMT* (P=0.026, β=0.012), rs4274224 and rs4581480 in *DRD2* (P=0.022, β=0.011; and P=0.009, β=0.022, respectively), and rs13106539 in *DRD5* (P=0.044, β=-0.008).

None of the association findings of these primary analyses, namely interaction analysis or association analysis in the complete sample, survived correction for multiple testing. However, post hoc analysis of associations with interaction with gender lead 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 yielding the observed suggestive associations.

Haplotype analysis of *COMT* and *DRD2* variants on HSCL score

As described above, two adjacent variants in *COMT*, rs2239393 and rs4680, showed evidence of interaction with early developmental risk environment, increasing risk for depressive symptoms in individuals with the high risk environment (Table 2). We performed 2-SNP and 3-SNP haplotype analyses combining these SNPs as well as their neighbouring variants using the sliding window approach. Evidence of association was observed for rs5993883-rs2239393 haplotype CG spanning a region from the space in-between LD blocks 1 and 2 to block 2 of *COMT* (Supplementary figure 1) (P=0.0049, β=0.055), for rs2239393-rs4680 haplotype GG in block 2 (P=0.0072, β=0.044), and for rs5993883-rs2239393-rs4680 haplotype CGG (P=0.0046, β=0.055) in individuals with the high early developmental risk environment (Table 3).

Haplotype analysis of variants outside that region towards both ends of the gene gave no further evidence for association (P>0.01 for all allelic combinations). Thus, the results of these analyses are in synchrony with those of single variants, as both allele G of rs2239393 and allele G of rs4680 increase risk for depressive symptoms in individuals with the high early developmental risk environment.

As one of the variants in this *COMT* haplotype, rs5993883, also gave evidence of interaction with gender (Table 2), we further examined association of these haplotypes in males and females of the high risk group separately. We found that the haplotypes increased risk for depressive symptoms in males, but not in females (P=0.004, β=0.083 for rs5993883-rs2239393 haplotype CG; P=0.0037, β=0.072 for rs2239393-rs4680 haplotype GG; and P=0.0053, β=0.083 for rs5993883-rs2239393-rs4680 haplotype CGG) (Table 3).

Two adjacent variants in *DRD2* LD block 3, rs4274224 and rs4581480 (Supplementary figure 1), associated with HSCL score in the complete sample (regardless of environmental risk) (Table 2). 2-SNP haplotype analysis of these SNPs as well as their neighbouring variants gave evidence of association of rs4648318-rs4274224 haplotype GG spanning from block 2 to block 3 of *DRD2* (P=0.0007, β=0.041), rs4274224-rs4581480 haplotype GG in block 3 (P=0.0069, β=0.022), and rs4581480-rs7131056 haplotype GA spanning from block 3 to block 4 (P=0.0071, β=0.022) with HSCL score. 3-SNP haplotypes rs4648318-rs4274224-rs4581480 haplotype GGG (P=0.0027, β=0.032), and rs4274224-

rs4581480-rs7131056 haplotype GGA (P=0.0081, β=0.021), gave evidence of association in synchrony with the findings from 2-SNP haplotypes as well as the single variants (Table 4).

As one of the variants contained within these haplotypes, rs4274224, also gave evidence of interaction with gender as well as association with HSCL score in males, we also examined association in males alone. The association signal strengthened for all of the risk haplotypes, being strongest for rs4648318-rs4274224 haplotype GG (P=0.00005, β=0.069).

[Insert Tables 3, 4 and Supplementary figure 1 into the supplementary]

Haplotype analysis of *COMT* and *DRD2* variants on other neurobehavioral traits

Encouraged by the findings of the haplotype analyses, we tested associations of haplotypes rs5993883-rs2239393 in *COMT* and rs4648318-rs4274224 in *DRD2*, as well as the single variant rs737866 in *COMT* to other traits related to depression, including the HSCL depression and anxiety subscales, depression diagnosis and TCI temperament trait Harm avoidance (Table 5). In case of both of the genes, it is evident that the association with HSCL stems mainly from the subscale reflecting symptoms of depression and not that reflecting anxiety (with HSCL depression subscale, P=0.018, β=0.075 for *COMT* haplotype CG and

P=0.0015, β=0.060 for *DRD2* haplotype GG; with HSCL anxiety subscale, P=0.288, β=0.02 and P=0.02 and β=0.033, respectively). We did not detect any evidence of association with depression diagnosis or with Harm avoidance or its subcomponents.

[Insert Table 5]

DISCUSSION

We investigated potential genetic and environmental risk factors for depression in a genetically isolated Finnish birth cohort by assessing relative impacts of candidate gene variants from monoamine neurotransmission in environments of contrasting (high and low) early developmental and social risk. Our study sample provided evidence of association of allelic variants of *COMT* and *DRD2* with current symptoms of depression. In case of *COMT* we detected evidence for interaction with high early developmental risk environment particularly in males. The genetic risk from *DRD2* seemed, on the other hand, to arise from mechanisms not related to the environmental risks assessed here. Also here the associations were detected particularly in males.

Cathecol-O-methyltransferase degrades catecholamine transmitters including 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.[27] The enzyme encoded by the Val158 allele has 3-4 fold higher activity than that encoded by the Met158 allele. Here, we found association of the haplotype comprising rs5993883 between LD blocks 1 and 2 of *COMT,* as well as rs2239393 and rs4680, two variants in virtually complete linkage disequilbrium in block 2, to depressive symptoms in males with the high developmental risk environment (P=0.0053). The allele G of rs4680 from the high risk haplotype corresponds to the high activity variant Val158 of COMT. This allele has repeatedly been found to be associated with a poor response to pharmacological treatment of depression[28, 29] and a European multicenter study identified an association between that allele and early onset major depression.[30] The Val158 allele has also earlier been found to associate with cognitive deficits including poor performance in tasks related to higher-order components of processing[14] and perseverative errors, less efficient physiologic responses in the prefrontal cortex,[31] as well as with schizophrenia based on a meta-analysis,[32] although the effect was not significant when studies with allele frequencies deviating from Hardy-Weinberg equilibrium were excluded.

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

interaction could not be explained through gene-environment correlations. Low birth weight is considered to reflect suboptimal growth environment during fetal life and it has been found to increase risk for many somatic and psychiatric diseases, including depression.[23] The observed risk seemed to arise from an aggregation of these indicators, as none of the risk items separately showed evidence for GxE with risk variants from *COMT* or *DRD3* (data not shown). This could relate to the degree of developmental problems altogether, so that mild signs alone are not sufficient to comprise such an environment that would interact with *COMT* and affect the development of emotional regulation, at least not on the level that could be detected in this study. There is some prior evidence of interaction of *COMT* with a risk environment on psychosis, antisocial behaviour and dissociation. A study on children with ADHD showed a main gene-environment interaction of the Val/Val genotype and low birth weight on early-onset antisocial behaviour,[33] and the Val158 allele was also found to interact with cannabis use and psychotic symptoms[34] and with increasing levels of dissociation in those exposed to higher levels of childhood trauma.[35] Interestingly, a recent report[36] revealed an impact of that polymorphism on gender-related patterns of regulation of emotions (activation in limbic and paralimbic regions) in line with findings from the present study.

Another major 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,[37] nominal[38] and negative[39, 40] findings, and results on depression conditional on risk environment.[37, 39, 41]

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, 42] as well as temperament traits[42] have previously been reported in various populations, including the current one,[43] and the prevalence of depression is higher in women.[44] A true gender-specific effect of genetic variants on depressiveness would not be surprising, as there is evidence for example of gender differences in dopaminergic function[45] that may be estrogen-dependent.

It is noteworthy that despite previous reports of the 5-HTTLPR variant,[12] we did not detect association evidence for *SLC6A4*. However, a recent meta-analysis did not find any evidence of association with depression alone, or in interaction with stressful life-events.[13] The *SLC6A4* SNPs included in our study tag the 5-

HTTLPR well (D'>0.9), as determined using genotypes from a population-based Finnish Health 2000 study.[46] 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, represented by the Health 2000 study sample.[47]

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 the results from the primary analyses (Table 2) do not survive conservative correction, having a neurobiological a priori hypothesis for these genes' involvement in depressiveness supports their validity. Furthermore, additional analyses performed on the variants that had given any evidence of interaction in the primary analyses yielded a relatively strong association signal of *DRD2*'s rs4274224 with HSCL score in males (P=0.0006), which remains close to statistical significance even when taking into account the amount of multiple testing performed. The finding was further supported by results of analysis of haplotypes containing rs4274224, showing a statistically significant association with HSCL score in males (P=0.00005).

There are some limitations in the present study. First, it is notable that depression as defined here did not necessary signify a clinical diagnosis of major depression. Instead it was defined either based on self-report or on the score from HSCL, which as a measure has its limitations. However, the prevalence of

depressed mood was in the same range as in earlier reports.[1, 48] Secondly, there was a notable drop-out rate among the original material of all cohort members. About half of the original cohort members did not participate in this study. Finally, when the NFBC 1966 study was initiated it was not possible to predict that an investigation such as the present one would one day be conducted. Therefore, we are limited by the original choice of variables to be collected. It is also noteworthy that we did not detect association with our measure of current depression of either the early social or the developmental risk environment, despite them being formulated based on previous reports of their effects on psychiatric health and wellbeing.[23-26] However, the effect of genetic risk may be modulated by early life stress even though the direct link between early life environment and current status would be missing, and this modulating effect may be seen in the results of the GxE analysis.

The current study has several potential advantages, such as the availability of longitudinal follow-up data starting antenatally enabling us to include the environmental dimension without any risk of recall bias. Another advantage is the unique genetic structure of our study cohort, characterized by isolation, founder effect, multiple bottlenecks and more genetic homogeneity compared to many other isolates,[49] allowing us to identify genetic risk loci that may be missed in the screening of other more heterogeneous populations. Furthermore, the subjects were representative, with all cohort members born in the same year and within a geographically defined area.

Furthermore, the size of the sample is sufficient to identify genetic variants of moderate impact. We also have both genders represented in almost equal amounts (48% males, 52% females), which is notable since gender differences are evident both in depression[2, 42] and in temperament traits, for example Harm avoidance.[42] 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[50] of temperament, are age-dependent. We can therefore isolate genetic effects from the effects of aging. Furthermore, we provide a complete coverage of the major candidate genes that are relevant with the present focus.

Our results support the role of *COMT* and *DRD2*, two genes of monoamine neurotransmission, in the etiology of depression particularly in males, and they imply gender-specific mechanisms of mood regulation and responses to risk environments. The findings imply that the role of monoaminergic genes in depression should be examined further in future studies. However, these findings are pending replication in other, independent population samples.

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CONTRIBUTORS

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

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Table 1. Composition of the study sample from the NFBC 1966.

				Early developmental risk environment[1]			Social risk environment[2]		
	N	*HSCL score>1.75*	*Depression diagnosis*	*High-risk*[3]	*sLow-risk*	*nd*[4]	*High-risk*[3]	*Low-risk*	*nd*[4]
Males	2509	169 (7%)	79(3%)	229 (9%)	2094 (83%)	186 (7%)	912 (36%)	1574 (63%)	23 (0.9%)
Females	2716	269(10%)	136(5%)	193 (7%)	2328 (86%)	195 (7%)	1034 (38%)	1649 (61%)	33 (1.2%)
All	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	**0.037**[8]	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	**0.016**[8]	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	**0.049**[10]
		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	**0.028**[4]	0.853	**0.024**[8]	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	**0.025**[8]	0.920

		rs2239393	18330428	G	0.31	**0.005**[5]	0.765	0.256	0.930
		rs4680	18331271	G	0.45	**0.020**[6]	0.956	0.501	0.346
		rs4646316	18332132	A	0.18	0.205	0.165	0.933	**0.026**[11]
		rs165774	18332561	A	0.25	0.081	0.516	0.089	0.215
		rs165815	18339473	G	0.20	0.537	0.309	0.431	0.281
		rs887199	18341955	A	0.20	0.544	0.325	0.401	0.306
		rs2239395	18342203	C	0.02	0.144	0.655	0.153	0.390
MAOA	X	rs909525	43438146	G	0.45	0.559	0.871	0.554	0.165
		rs12843268	43458610	A	0.40	0.271	0.837	0.266	0.103
		rs6610845	43472954	G	0.41	0.232	0.795	0.263	0.170
		rs3027409	43491977	C	0.02	0.748	0.928	0.950	0.194
		rs6609257	43497652	G	0.50	0.848	0.320	0.470	0.077
		rs3027415	43499385	G	0.18	0.218	0.550	0.905	0.613
DRD1	5	rs265973	174793305	G	0.50	0.529	0.614	0.549	0.888
		rs265974	174793846	G	0.35	0.391	0.612	0.912	0.659
		rs265976	174795026	A	0.23	0.578	0.707	0.915	0.826
		rs5326	174802802	A	0.19	0.615	0.886	0.852	0.588
DRD2	11	rs1800497	112776038	A	0.17	0.079	0.825	0.691	0.467
		rs2242592	112784640	G	0.37	0.757	0.466	0.283	0.736
		rs1076563	112801119	C	0.50	0.053	0.813	0.897	0.662
		rs2471857	112803549	A	0.17	0.518	0.494	0.823	0.901
		rs4620755	112814829	A	0.22	0.383	0.997	0.951	0.176
		rs7125415	112815891	A	0.19	0.084	0.389	0.789	0.231
		rs4648318	112818599	G	0.34	0.711	0.885	0.631	0.684
		rs4274224	112824662	G	0.24	0.067	0.777	**0.017**[9]	**0.022**[12]
		rs4581480	112829684	G	0.07	0.184	0.521	0.210	**0.009**[13]
		rs7131056	112834984	C	0.49	0.564	0.795	0.413	0.964
		rs4938019	112846601	G	0.23	0.069	0.651	0.643	0.584

		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
DRD3	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
DRD4	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
DRD5	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.

				Males and females with high risk E_{Dev}		*Males with high risk E_{Dev}*		*Females with high risk E_{Dev}*	
Gene	*Variant*	*Haplotype*	*Frequency*	*Beta*	*P*	*Beta*	*P*	*Beta*	*P*
COMT	*2-SNP haplotype analysis*								
	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
	3-SNP haplotype analysis								
	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.

				Males and females		*Males*	
Gene	*Variant*	*Haplotype*	*Frequency*	*Beta*	*P*	*Beta*	*P*
DRD2	*2-SNP haplotype analysis*						
	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**
	3-SNP haplotype analysis						
	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.

				HSCL(total)		*HSCL(depression)*		*HSCL(anxiety)*		*Depression diagnosis*		*Harm avoidance*	
Gene	*Variant*	*Group*	*Gender*	*Beta*	*P*	*Beta*	*P*	*Beta*	*P*	*Odds ratio*	*P*	*Beta*	*P*
COMT	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
DRD2	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

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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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 β=0.056 for rs5993883-rs2239393-rs4680 risk haplotype CGG including Val158), particularly in males (P=0.0053 and β=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 β=0.0023; P=0.00005 and β=0.069 for rs4648318-rs4274224 haplotype GG). Our results support the role of genes of

monoamine neurotransmission in the etiology of depression conditional on environmental risk and sex, but not direct major effects of monoaminergic genes in this unselected population.

ARTICLE SUMMARY

Article focus

- Impact on depression of monoaminergic candidate genes with prior evidence of gene-environment interaction for affective disorders, and of dopamine receptor genes
- Gene-environment and gene-gender interaction in the etiology of depression
- Effect of early neurodevelopmental and social risk environments on depression

Key messages

- Genes of monoamine neurotransmission play a role in the etiology of depression conditional on environmental risk, especially in males and in individuals from an early developmental risk environment; in particular there is evidence of an Interaction with a *COMT* high risk haplotype including Val158
- Gender-specific mechanisms and responses to environmental effectors are evident in regulation of mood

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

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

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).

According to the monoamine hypothesis, depression is caused by underactivity in brain monoamines, such as dopamine, serotonin, and norepinephrine.[11] Recent results of neuroimaging studies have provided further support for this theory.[12] The most solid evidence from candidate gene studies has perhaps been obtained for the interaction of the *SLC6A4* gene for serotonin transporter and stressful early and current life events,[13] including positive results from a recent review[14] and meta-analysis of all studies to date[15], although there are also contradicting results.[16] Other robust genetic findings have been obtained on the *COMT* gene for catechol-O-methyltransferase, an enzyme catabolising catecholamines such as dopamine and noradrenaline, which has been implicated in depression in conjunction with stress,[17] and on the *MAOA* gene for monoamine oxidase A, an enzyme oxidizing neurotransmitter and dietary monoamines such as serotonin, noradrenaline and dopamine, which has been associated with depression in interaction with severity of maltreatment in childhood.[17] Furthermore, *TPH2* gene for tryptophan hydroxylase 2, which is the brain-specific form of the key enzyme in serotonin synthesis, has been implicated to interact with stress on disorders of cognitive control and emotional regulation, including depression.[18] Within the dopamine transmission the *DRD2* gene for dopamine receptor D2 has been associated with depressiveness and anxiety, combined with an effect of parenting in childhood,[19] and the *DRD4* gene for dopamine receptor D4 has been associated with increased risk for obesity in women with seasonal affective disorder.[20] Thus, genes from the

monoamine neurotransmission system are among the most thoroughly studied in psychiatric genetics and in particular in the etiology of mood disorders, and have provided perhaps the most robust evidence so far for interaction with various types of risk environments, including childhood environment.

We chose to include these candidate genes of monoamine neurotransmission, including *SLC6A4*, *TPH2*, *COMT*, *MAOA*, as well as the dopamine receptor genes *DRD1-DRD5*, in our study on the etiology of depression with a particular focus on their interaction with available markers reflecting early developmental and social risk environments. The study was performed in a sample of 5225 individuals from a large Finnish isolated population cohort. As gender is an important confounder for depression and at least some of the genetic liability is gender-specific,[2] we also examined gene-gender interactions in this sample.

METHODS

Setting

We utilized the genetically isolated Northern Finland Birth Cohort (NFBC 1966) to investigate effects of candidate genes and environmental risk factors during the development on depressiveness. We subdivided the study sample based on

developmental risk factors arising from the fetal growth environment and neurological development during the first year of life (early developmental risk environment) as well as from the family environment during pregnancy and early childhood (social risk environment). We examined interactions of these environments with candidate genes of the monoamine neurotransmitter systems, many of which have prior evidence of gene-environment interaction on affective disorders, namely *SLC6A4, TPH2, COMT, MAOA,* and the dopamine receptor genes *DRD1-DRD5*.

Study subjects

The Northern Finland Birth Cohort 1966 (NFBC 1966) is a longitudinal one-year birth cohort from an unselected population (N=12058 live births) comprising inhabitants of the two northernmost provinces of Finland.[21] Data collection was begun during the antenatal period, and follow-up studies have been performed at the ages of 1, 14 and 31 years. The cohort study has been approved by the Ethical Committee of Oulu University Faculty of Medicine, and written informed consent has been obtained from all participants.

In 1997 for the 31-year follow-up study[22] all alive cohort members with a known address (N=11540) were sent a postal questionnaire surveying lifestyle, social status and health (76% participated), including the Hopkins Symptom Check List–25 (HSCL)[23] and items on self-reported lifetime depression diagnosis (e.g.

"Has your doctor ever diagnosed a depressive disorder?"). Additionally, cohort members who lived in Northern Finland or had moved to the Helsinki area (N=8465) were invited to a clinical examination (71% participated) with another questionnaire to be filled in later and sent to the research group (61% participated).[24] It included, among others, a validated Finnish translation of Cloninger's Temperament and Character Inventory (TCI) questionnaire.[25]

Current depressive symptoms were assessed by the HSCL-questionnaire,[26] a 25-item shortened version of an originally 90-item questionnaire. HSCL contains 13-item depression and 10-item anxiety subscales assessing presence and intensity of depressive and anxiety symptoms during the previous week. Answers are scored on a scale from 1 (not bothered) to 4 (extremely bothered). The HSCL total score is the sum of items divided by the number of items answered. We used mainly HSCL total score as symptoms of depression and anxiety are known to overlap significantly. In the post hoc analyses, in order to better understand the original association signals, the separate HSCL subscales for depressive and anxiety symptoms were also taken into consideration. In addition to current depressive symptoms (HSCL score) and lifetime (diagnosed) depression, we used the TCI temperament trait Harm avoidance[5-7] and its subcomponents as a measure of proneness to depression.

The subjects (n=5225; 2509 males, 2716 females; 45 % of the 31 year follow-up study sample or 43% of the original study sample) were divided into high and low

risk groups based on the available information reflecting early neurodevelopmental and social risk environments (Table 1). The markers for the *early developmental risk environment* 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 *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 social class 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 the individuals with high risk early developmental and social environments, and 47% and 46% of those with the respective low risk environments, were available for study.

[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 multiple testing. *(i)* Primarily analyses were performed to identify genetic risk variants for current depressive symptoms (HSCL score) interacting with early

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

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

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

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

led to a finding close to statistical significance even when taking into account the amount of multiple testing performed (P=0.0006 for males with rs4274224 in *DRD2*). 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, β=0.055), for the rs2239393-rs4680 haplotype GG in block 2 (P=0.0072, β=0.044), and the rs5993883-rs2239393-rs4680 haplotype CGG (P=0.0046, β=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, β=0.083 for rs5993883-rs2239393 haplotype CG; P=0.0037,

β=0.072 for rs2239393-rs4680 haplotype GG; and P=0.0053, β=0.083 for rs5993883-rs2239393-rs4680 haplotype CGG) (Table 3).

Haplotype analysis of rs4274224 and rs4581480 from *DRD2*, which gave suggestive evidence of an association with the HSCL score in the complete sample, and of their neighbouring variants gave evidence of an association of rs4648318-rs4274224 haplotype GG spanning from block 2 to block 3 of *DRD2* (P=0.0007, β=0.041), rs4274224-rs4581480 haplotype GG in block 3 (P=0.0069, β=0.022), and rs4581480-rs7131056 haplotype GA spanning from block 3 to block 4 (P=0.0071, β=0.022) with the HSCL score. The 3-SNP haplotypes rs4648318-rs4274224-rs4581480 haplotype GGG (P=0.0027, β=0.032), and rs4274224-rs4581480-rs7131056 haplotype GGA (P=0.0081, β=0.021), gave evidence of an association in agreement with the findings from the 2-SNP haplotypes as well as the single variants (Table 4). As one of the variants contained within these haplotypes, namely rs4274224, also gave evidence of interaction with gender as well as association with the HSCL score in males, we also examined the association in males alone. The association signal became stronger for all of the risk haplotypes, being strongest for rs4648318-rs4274224 haplotype GG (P=0.00005, β=0.069).

[Insert Tables 3, 4 and Supplementary figure 1 into the supplementary]

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, β=0.075 for *COMT* haplotype CG and P=0.0015, β=0.060 for *DRD2* haplotype GG; with HSCL anxiety subscale, P=0.288, β=0.02 and P=0.02 and β=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

monoaminergic candidate genes for depression in environments 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 specific environmental factors were considered, some signals for association were observed, although none of them survive correction for multiple testing. Our study sample provided evidence of an interaction of *COMT* with the high early developmental risk environment particularly in males, and a contribution of an allelic variant of *DRD2* to genetic risk for depressiveness particularly in males (Table 2).

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 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 disequilbrium 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 an early developmental risk environment on depressive symptoms. This interaction could not be explained through gene-environment correlations. Nor were we able to detect a significant correlation of the developmental risk environment with depressive symptoms, despite the prior evidence for the role of its markers, which were low birth weight[21, 27] and late motor or verbal development[28], in decreased psychiatric health and wellbeing, including depression. This finding may reflect the presence of other environmental risk indicators which were not examined in our study. However, they may also reflect individual variability in response to the risk environment and presence of genetic factors (such as the *COMT* haplotype containing Met158) that may relate to resilience, adaptive changes in regulation of emotion reactivity and successful coping with stress.[38]

The observed risk also seemed to arise from an aggregation of the environmental indicators, as none of the risk items separately gave evidence of GxE with the risk variants from *COMT* or *DRD3* (data not shown). This could reflect a cumulative nature of these environmental influences, such that the effect of one marker may be weak, but the accumulated effect of multiple markers, together with genetic susceptibility, would be strong enough to increase the risk for a deviant development of emotional regulation and thus depressiveness.[39] There is some prior evidence of interaction of *COMT* with a risk environment on psychosis, antisocial behaviour and dissociation. A study on children with ADHD showed a gene-environment interaction between the Val/Val genotype and low birth weight on early-onset antisocial behaviour,[40] and the Val158 allele was also found to associate with cannabis use and psychotic symptoms[41] and with increasing levels of dissociation in those exposed to higher levels of childhood trauma.[42] Interestingly, a recent report[43] revealed an impact of that polymorphism on gender-related patterns of regulation of emotions (activation in limbic and paralimbic regions) in line with findings of the present study.

Another major 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.

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 environmental risk or gender. Still, the strongest association signal, obtained using *DRD2*'s rs4274224 with HSCL score in males (P=0.0006), remains close to statistical significance even when taking into account the amount of multiple testing performed. The finding was further supported by results of our haplotype analysis containing rs4274224, which showed a statistically significant association with the HSCL score in males (P=0.00005).

There are some limitations in the present study. First, it is notable that depression as defined here did not necessarily signify a clinical diagnosis of major depression. Instead it was defined either based on self-report or on the score from HSCL, which as a measure has its limitations. However, the

prevalence of depressed mood was in the same range as in earlier reports.[1, 55] Secondly, there was a notable drop-out rate among the original material of all cohort members. About half of the original cohort members did not participate in this study. Finally, when the NFBC 1966 study was initiated it was not possible to predict that an investigation such as the present one would one day be conducted. Therefore, we are limited by the original choice of variables to be collected, and the environmental risk factors may only be indicators or markers of risk rather than risk factors themselves.[39] It is also noteworthy that we did not detect any association with our measure of current depression and the developmental risk environment, despite it being formulated based on previous reports of their effects on psychiatric health and wellbeing.[27-30]. However, the effect of genetic risk may be modulated by early life stress even though the direct link between early life environment and current status would be too weak to be detected in our study sample, and this modulating effect may be seen in the results of the GxE analysis.

The current study has several potential advantages, such as the availability of longitudinal follow-up data starting antenatally, enabling us to include the environmental dimension without any risk of recall bias. Another advantage is the unique genetic structure of our study cohort, characterized by isolation, founder effect, multiple bottlenecks and more genetic homogeneity compared to many other isolates,[56] allowing us to identify genetic risk loci that may be missed in the screening of other more heterogeneous populations. Furthermore, the

subjects were representative, with all cohort members born in the same year and within a geographically defined area.

In addition, the size of the sample is sufficient to identify genetic variants of moderate impact. We also have both genders represented in almost equal amounts (48% males, 52% females), which is notable since gender differences are evident both in depression[2, 49] and in temperament traits, for example Harm avoidance.[49] 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[57] of temperament, are age-dependent. We can therefore isolate genetic effects from the effects of aging. Furthermore, we provide a complete coverage of the major candidate genes that are relevant with the present focus.

Our results support the role of *COMT* and *DRD2*, two genes of monoamine neurotransmission, in the etiology of depression conditional on environmental risk particularly in males, though not direct effects of monoaminergic genes in this unselected population. These findings imply that the nature of the role of monoaminergic genes in depression should be examined further in future studies, and are pending replication in other, independent population samples.

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CONTRIBUTORS

Tiina Paunio, Emma Nyman, Sonja Sulkava, Jouko Miettunen, Matti Joukamaa and Juha Veijola designed the study and wrote the protocol, with help also from Nelson Freimer, Pirjo Mäki, Leena Peltonen and Marjo-Riitta Järvelin. Emma Nyman and to some extent Tiina Paunio also managed the literature searches. Sonja Sulkava and Emma Nyman undertook the statistical analyses. Emma 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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Table 1. Composition of the study sample from the NFBC 1966.

		HSCL	*Depression*	*Early developmental risk environment*[2]			*Social risk environment*[3]		
	N	*score>1.75*[1]	*diagnosis*	*High-risk*[4]	*sLow-risk*	*nd*[5]	*High-risk*[4]	*Low-risk*	*nd*[4]
Males	2509	169 (7%)	79(3%)	229 (9%)	2094 (83%)	186 (7%)	912 (36%)	1574 (63%)	23 (0.9%)
Females	2716	269(10%)	136(5%)	193 (7%)	2328 (86%)	195 (7%)	1034 (38%)	1649 (61%)	33 (1.2%)
All	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	**0.037**[8]	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	**0.016**[8]	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	**0.049**[10]	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	**0.028**[4]	0.853	**0.024**[8]	0.755	0.623	0.489

			rs1544325	18311668	G	0.48	0.318	0.376	0.192	0.822	0.999	0.931
			rs174675	18314051	A	0.29	0.465	0.278	0.580	0.958	0.724	0.532
			rs5993883	18317638	C	0.36	0.230	0.495	**0.025** [8]	0.920	0.363	0.219
			rs2239393	18330428	G	0.31	**0.005** [5]	0.765	0.256	0.930	0.838	0.459
			rs4680	18331271	G	0.45	**0.020** [6]	0.956	0.501	0.346	0.412	0.498
			rs4646316	18332132	A	0.18	0.205	0.165	0.933	**0.026** [11]	0.521	0.392
			rs165774	18332561	A	0.25	0.081	0.516	0.089	0.215	0.538	0.239
			rs165815	18339473	G	0.20	0.537	0.309	0.431	0.281	0.338	0.158
			rs887199	18341955	A	0.20	0.544	0.325	0.401	0.306	0.314	0.168
			rs2239395	18342203	C	0.02	0.144	0.655	0.153	0.390	0.224	0.664
MAOA	*Monoamine oxidase A*	X	rs909525	43438146	G	0.45	0.559	0.871	0.554	0.165	0.255	0.932
			rs12843268	43458610	A	0.40	0.271	0.837	0.266	0.103	0.052	0.932
			rs6610845	43472954	G	0.41	0.232	0.795	0.263	0.170	0.060	0.524
			rs3027409	43491977	C	0.02	0.748	0.928	0.950	0.194	0.703	0.068
			rs6609257	43497652	G	0.50	0.848	0.320	0.470	0.077	0.075	0.898
			rs3027415	43499385	G	0.18	0.218	0.550	0.905	0.613	0.105	0.408
DRD1	*Dopamine receptor D1*	5	rs265973	174793305	G	0.50	0.529	0.614	0.549	0.888	**0.047**	0.831
			rs265974	174793846	G	0.35	0.391	0.612	0.912	0.659	0.066	0.859
			rs265976	174795026	A	0.23	0.578	0.707	0.915	0.826	0.077	0.933
			rs5326	174802802	A	0.19	0.615	0.886	0.852	0.588	0.273	0.197
DRD2	*Dopamine receptor D2*	11	rs1800497	112776038	A	0.17	0.079	0.825	0.691	0.467	0.921	0.264
			rs2242592	112784640	G	0.37	0.757	0.466	0.283	0.736	0.143	0.393
			rs1076563	112801119	C	0.50	0.053	0.813	0.897	0.662	0.234	0.856
			rs2471857	112803549	A	0.17	0.518	0.494	0.823	0.901	0.884	0.126
			rs4620755	112814829	A	0.22	0.383	0.997	0.951	0.176	0.065	0.992
			rs7125415	112815891	A	0.19	0.084	0.389	0.789	0.231	0.163	0.947

			rs4648318	112818599	G	0.34	0.711	0.885	0.631	0.684	0.214	0.466
			rs4274224	112824662	G	0.24	0.067	0.777	**0.017**[9]	**0.022**[12]	0.536	0.766
			rs4581480	112829684	G	0.07	0.184	0.521	0.210	**0.009**[13]	0.082	0.760
			rs7131056	112834984	C	0.49	0.564	0.795	0.413	0.964	0.622	0.138
			rs4938019	112846601	G	0.23	0.069	0.651	0.643	0.584	0.320	0.059
			rs12364283	112852165	G	0.08	0.280	0.504	0.441	0.861	0.633	0.804
			rs10891556	112857971	A	0.24	0.076	0.519	0.638	0.589	0.380	0.052
			rs6589377	112860946	G	0.17	0.286	0.617	0.061	0.552	0.502	0.915
DRD3	*Dopamine receptor D3*	3	rs2087017	115324703	G	0.43	0.937	0.921	0.606	0.743	0.835	0.828
			rs2134655	115340891	A	0.28	0.454	0.554	0.129	0.507	0.209	0.330
			rs963468	115345577	A	0.38	0.809	0.777	0.902	0.608	0.609	0.580
			rs3773678	115352768	A	0.06	0.780	0.855	0.487	0.770	0.972	0.556
			rs2630351	115357749	A	0.03	0.954	0.144	0.168	0.999	0.811	0.211
			rs167771	115358965	G	0.18	0.862	0.638	0.406	0.514	0.416	0.966
			rs167770	115362252	G	0.31	0.260	0.911	0.298	0.694	0.593	0.982
			rs226082	115363703	G	0.31	0.261	0.911	0.301	0.690	0.594	0.983
			rs324029	115364313	A	0.31	0.259	0.913	0.296	0.722	0.593	0.964
			rs10934256	115368342	A	0.17	0.229	0.898	0.478	0.246	0.147	0.669
			rs1486009	115371222	G	0.12	0.721	0.667	0.745	0.571	0.387	0.600
			rs6280	115373505	G	0.33	0.159	0.485	0.141	0.667	0.386	0.880
			rs9825563	115382910	G	0.23	**0.045**[7]	0.902	0.211	0.215	**0.028**	0.927
DRD4	*Dopamine receptor D4*	11	rs3758653	626399	G	0.23	0.300	0.752	0.249	0.980	0.322	0.905
			rs11246226	631191	A	0.49	0.749	0.748	0.166	0.925	0.908	0.097
DRD5	*Dopamine receptor D5*	4	rs1878943	9375986	A	0.21	0.586	0.686	0.482	0.386	0.988	0.605
			rs13106539	9406801	G	0.39	0.735	0.062	0.067	**0.044**[14]	0.532	0.384

[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.

				Males and females with high risk E_{Dev}		*Males with high risk E_{Dev}*		*Females with high risk E_{Dev}*	
Gene	*Variant*	*Haplotype*	*Frequency*	*Beta*	*P*	*Beta*	*P*	*Beta*	*P*
COMT	*2-SNP haplotype analysis*								
	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
	3-SNP haplotype analysis								
	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.

				Males and females		*Males*	
Gene	*Variant*	*Haplotype*	*Frequency*	*Beta*	*P*	*Beta*	*P*
DRD2	*2-SNP haplotype analysis*						
	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**
	3-SNP haplotype analysis						
	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.

				HSCL(total)		*HSCL(depression)*		*HSCL(anxiety)*		*Depression diagnosis*		*Harm avoidance*	
Gene	*Variant*	*Group*	*Gender*	*Beta*	*P*	*Beta*	*P*	*Beta*	*P*	*Odds ratio*	*P*	*Beta*	*P*
COMT	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
DRD2	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.

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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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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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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 β=0.056 for rs5993883-rs2239393-rs4680 risk haplotype CGG including Val158), particularly in males (P=0.0053 and β=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 β=0.0023;

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P=0.00005 and β=0.069 for rs4648318-rs4274224 haplotype GG). Our results support the role of genes of monoamine neurotransmission in the etiology of depression conditional on environmental risk and sex, but not direct major effects of monoaminergic genes in this unselected population.

ARTICLE SUMMARY

Article focus

- Impact on depression of monoaminergic candidate genes with prior evidence of gene-environment interaction for affective disorders, and of dopamine receptor genes
- Gene-environment and gene-gender interaction in the etiology of depression
- Effect of measures of early development and of social environment on depression

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Key messages

- Genes of monoamine neurotransmission play a role in the etiology of depression conditional on environmental risk, especially in males and in individuals of high early developmental risk group; in particular there is evidence of an Interaction with a *COMT* high risk haplotype including Val158
- Gender-specific mechanisms and responses to environmental effectors 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.

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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).

According to the monoamine hypothesis, depression is caused by underactivity in brain monoamines, such as dopamine, serotonin, and norepinephrine.[11] Recent results of neuroimaging studies have provided further support for this theory.[12] The most solid evidence from candidate gene studies has perhaps been obtained for the interaction of the *SLC6A4* gene for serotonin transporter and stressful early and current life events,[13] including positive results from a recent review[14] and meta-analysis of all studies to date[15], although there are also contradicting results.[16] Other robust genetic findings have been obtained on the *COMT* gene for catechol-O-methyltransferase, an enzyme catabolising catecholamines such as dopamine and noradrenaline, which has been implicated in depression in conjunction with stress,[17] and on the *MAOA* gene for monoamine oxidase A, an enzyme oxidizing neurotransmitter and dietary monoamines such as serotonin, noradrenaline and dopamine, which has been associated with depression in interaction with severity of maltreatment in childhood.[17] Furthermore, *TPH2* gene for tryptophan hydroxylase 2, which is the brain-specific form of the key enzyme in serotonin synthesis, has been implicated to interact with stress on disorders of cognitive control and emotional regulation, including depression.[18] Within the dopamine transmission the *DRD2* gene for dopamine receptor D2 has been associated with depressiveness and anxiety, combined with an effect of parenting in childhood,[19] and the *DRD4* gene for dopamine receptor D4 has been associated with increased risk for obesity in women with seasonal affective disorder.[20] Thus, genes from the

monoamine neurotransmission system are among the most thoroughly studied in psychiatric genetics and in particular in the etiology of mood disorders, and have provided perhaps the most robust evidence so far for interaction with various types of risk environments, including childhood environment.

We chose to include these candidate genes of monoamine neurotransmission showing prior evidence of gene-environment interaction, including *SLC6A4*, *TPH2*, *COMT*, *MAOA*, as well as the dopamine receptor genes *DRD1-DRD5*, in our study on the etiology of depression with a particular focus on their interaction with available markers reflecting measures of early development and of social environment. The study was performed in a sample of 5225 individuals from a large Finnish isolated population cohort. As gender is an important confounder for depression and at least some of the genetic liability is gender-specific,[2] we also examined gene-gender interactions in this sample.

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METHODS

Setting

We utilized the genetically isolated Northern Finland Birth Cohort (NFBC 1966) to investigate effects of candidate genes and environmental risk factors during the

development on depressiveness. We subdivided the study sample based on measures of early development arising from the fetal growth environment and neurological development during the first year of life (measure of early development) as well as from the family environment during pregnancy and early childhood (measure of social environment). We examined interactions of these measures with candidate genes of the monoamine neurotransmitter systems, which have prior evidence of gene-environment interaction on affective disorders, namely *SLC6A4, TPH2, COMT, MAOA,* and the dopamine receptor genes *DRD1-DRD5*.

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Study subjects

The Northern Finland Birth Cohort 1966 (NFBC 1966) is a longitudinal one-year birth cohort from an unselected population (N=12058 live births) comprising inhabitants of the two northernmost provinces of Finland.[21] Data collection was begun during the antenatal period, and follow-up studies have been performed at the ages of 1, 14 and 31 years. The cohort study has been approved by the Ethical Committee of Oulu University Faculty of Medicine, and written informed consent has been obtained from all participants.

In 1997 for the 31-year follow-up study[22] all alive cohort members with a known address (N=11540) were sent a postal questionnaire surveying lifestyle, social status and health (76% participated), including the Hopkins Symptom Check

List–25 (HSCL)[23] and items on self-reported lifetime depression diagnosis (e.g. "Has your doctor ever diagnosed a depressive disorder?"). Additionally, cohort members who lived in Northern Finland or had moved to the Helsinki area (N=8465) were invited to a clinical examination (71% participated) with another questionnaire to be filled in later and sent to the research group (61% participated).[24] It included, among others, a validated Finnish translation of Cloninger's Temperament and Character Inventory (TCI) questionnaire.[25]

Current depressive symptoms were assessed by the HSCL-questionnaire,[26] a 25-item shortened version of an originally 90-item questionnaire. HSCL contains 13-item depression and 10-item anxiety subscales assessing presence and intensity of depressive and anxiety symptoms during the previous week. Answers are scored on a scale from 1 (not bothered) to 4 (extremely bothered). The HSCL total score is the sum of items divided by the number of items answered. We used mainly HSCL total score as symptoms of depression and anxiety are known to overlap significantly. In the post hoc analyses, in order to better understand the original association signals, the separate HSCL subscales for depressive and anxiety symptoms were also taken into consideration. In addition to current depressive symptoms (HSCL score) and lifetime (diagnosed) depression, we used the TCI temperament trait Harm avoidance[5-7] and its subcomponents as a measure of proneness to depression.

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

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

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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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and rs13106539 in *DRD5* (P=0.044, β=-0.008). Three variants of *COMT* and one of *DRD3* showed some evidence of interaction (P<0.05) with high early developmental risk with respect to the HSCL score (P=0.028 for rs737866,; P=0.005 for rs2239393, and, P=0.020 from rs4680 from *COMT*, and rs9825563, P=0.045 from *DRD3*). All of them were associated with the HSCL score in individuals of the high risk group (P=0.036, β=0.0414 for rs737866; P=0.008, β=0.0440 for rs2239393; P=0.042, β=0.0320 for rs4680; and P=0.022, β=-0.0396 for rs9825563, respectively). None of the variants gave any evidence of interaction with the measure of social environment in relation to the HSCL score. Five of the genetic variants showed some evidence of interaction with gender (P<0.05), including rs737866 and rs5993883 in *COMT* and rs4274224 in *DRD2*. Out of these, only rs4274224 associated at P<0.05 with one of the genders (P=0.0006, β=0.023 in males). The evidence for gene-environment correlations (rGE) was observed only nominally about rs1906451 from *TPH2* (P=0.035), rs265973 from *DRD1* (P=0.047), and rs9825563 from *DRD3* (P=0.028). Despite a priori evidence for the role of the markers which indicate high developmental risk for psychiatric health and wellbeing, namely low birth weight[21, 27] and late motor or verbal development[28], there was no correlation between these markers and the HSCL score in the present sample (P=0.131), whereas the social high risk environment, correlated significantly with the score (P = 0.00001).

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

led to a finding close to statistical significance even when taking into account the amount of multiple testing performed (P=0.0006 for males with rs4274224 in *DRD2*). Furthermore, as there was an accumulation of association signals within two highly plausible candidate genes, *DRD2* and *COMT*, we proceeded to perform haplotype analyses on these genes in order to better characterize the allelic variants which yielded the observed suggestive associations, and to obtain a maximal amount of information on the nature of the associations observed.

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, β=0.055), for the rs2239393-rs4680 haplotype GG in block 2 (P=0.0072, β=0.044), and the rs5993883-rs2239393-rs4680 haplotype CGG (P=0.0046, β=0.055) in the high early developmental risk group, 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

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females (P=0.004, β=0.083 for rs5993883-rs2239393 haplotype CG; P=0.0037, β=0.072 for rs2239393-rs4680 haplotype GG; and P=0.0053, β=0.083 for rs5993883-rs2239393-rs4680 haplotype CGG) (Table 3). As is evident from the β-values, each of the haplotypes accounts for more variance in depression than any individual constituent SNP.

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Haplotype analysis of rs4274224 and rs4581480 from *DRD2*, which gave suggestive evidence of an association with the HSCL score in the complete sample, and of their neighbouring variants gave evidence of an association of rs4648318-rs4274224 haplotype GG spanning from block 2 to block 3 of *DRD2* (P=0.0007, β=0.041), rs4274224-rs4581480 haplotype GG in block 3 (P=0.0069, β=0.022), and rs4581480-rs7131056 haplotype GA spanning from block 3 to block 4 (P=0.0071, β=0.022) with the HSCL score. The 3-SNP haplotypes rs4648318-rs4274224-rs4581480 haplotype GGG (P=0.0027, β=0.032), and rs4274224-rs4581480-rs7131056 haplotype GGA (P=0.0081, β=0.021), gave evidence of an association in agreement with the findings from the 2-SNP haplotypes as well as the single variants (Table 4). As one of the variants contained within these haplotypes, namely rs4274224, also gave evidence of interaction with gender as well as association with the HSCL score in males, we also examined the association in males alone. The association signal became stronger for all of the risk haplotypes, being strongest for rs4648318-rs4274224 haplotype GG (P=0.00005, β=0.069). Similarly as for the *COMT* haplotypes, the

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β-values, imply that each of the *DRD2* haplotypes accounts for more variance in depression than any individual constituent SNP.

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[Insert Tables 3, 4 and Supplementary figure 1 into the supplementary]

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, β=0.075 for *COMT* haplotype CG and P=0.0015, β=0.060 for *DRD2* haplotype GG; with HSCL anxiety subscale, P=0.288, β=0.02 and P=0.02 and β=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 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

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 disequilbrium 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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may reflect the presence of other environmental risk indicators which were not examined in our study. However, they may also reflect individual variability in response to the risk environment and presence of genetic factors (such as the *COMT* haplotype containing Met158) that may relate to resilience, adaptive changes in regulation of emotion reactivity and successful coping with stress.[38] The observed risk also seemed to arise from an aggregation of the environmental indicators, as none of the risk items separately gave evidence of GxE with the risk variants from *COMT* or *DRD3* (data not shown). This could reflect a cumulative nature of these environmental influences, such that the effect of one marker may be weak, but the accumulated effect of multiple markers, together with genetic susceptibility, would be strong enough to increase the risk for a deviant development of emotional regulation and thus depressiveness.[39] There is some prior evidence of interaction of *COMT* with a risk environment on psychosis, antisocial behaviour and dissociation. A study on children with ADHD showed a gene-environment interaction between the Val/Val genotype and low birth weight on early-onset antisocial behaviour,[40] and the Val158 allele was also found to associate with cannabis use and psychotic symptoms[41] and with increasing levels of dissociation in those exposed to higher levels of childhood trauma.[42] Interestingly, a recent report[43] revealed an impact of that polymorphism on gender-related patterns of regulation of emotions (activation in limbic and paralimbic regions) in line with findings of the present study.

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]

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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.

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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showed a statistically significant association with the HSCL score in males (P=0.00005).

There are some limitations in the present study. First, it is notable that depression as defined here did not necessarily signify a clinical diagnosis of major depression. Instead it was defined either based on self-report or on the score from HSCL, which as a measure has its limitations. However, the prevalence of depressed mood was in the same range as in earlier reports.[1, 55] Secondly, there was a notable drop-out rate among the original material of all cohort members. About half of the original cohort members did not participate in this study. Finally, when the NFBC 1966 study was initiated it was not possible to predict that an investigation such as the present one would one day be conducted. Therefore, we are limited by the original choice of variables to be collected, and the measures of early development or of social environment may only be indicators or markers of risk rather than risk factors themselves.[39] It is also noteworthy that we did not detect any association of our measure of current depression with the measure of high early developmental risk, despite it being formulated based on previous reports of their effects on psychiatric health and wellbeing.[27-30]. However, the effect of genetic risk may be modulated by early life stress even though the direct link between early life environment and current status would be too weak to be detected in our study sample, and this modulating effect may be seen in the results of the GxE analysis.

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The current study has several potential advantages, such as the availability of longitudinal follow-up data starting antenatally, enabling us to include the environmental dimension without any risk of recall bias. Another advantage is the unique genetic structure of our study cohort, characterized by isolation, founder effect, multiple bottlenecks and more genetic homogeneity compared to many other isolates,[56] allowing us to identify genetic risk loci that may be missed in the screening of other more heterogeneous populations. Furthermore, the subjects were representative, with all cohort members born in the same year and within a geographically defined area.

In addition, the size of the sample is sufficient to identify genetic variants of moderate impact. We also have both genders represented in almost equal amounts (48% males, 52% females), which is notable since gender differences are evident both in depression[2, 49] and in temperament traits, for example Harm avoidance.[49] Furthermore, it is beneficial that the sample is a one-year birth cohort, as it is well established that some psychiatric traits, such as Harm avoidance[57] of temperament, are age-dependent. We can therefore isolate genetic effects from the effects of aging.

Our results support a modest role of *COMT* and *DRD2*, two genes of monoamine neurotransmission, in the etiology of depression conditional on environmental risk particularly in males, though not direct effects of monoaminergic genes in this unselected population. These findings imply that the nature of the role of

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monoaminergic genes in depression should be examined further in future studies, and are pending replication in other, independent population samples.

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CONTRIBUTORS

Tiina Paunio, Emma Nyman, Sonja Sulkava, Jouko Miettunen, Matti Joukamaa and Juha Veijola designed the study and wrote the protocol, with help also from Nelson Freimer, Pirjo Mäki, Leena Peltonen and Marjo-Riitta Järvelin. Emma Nyman and to some extent Tiina Paunio also managed the literature searches. Sonja Sulkava and Emma Nyman undertook the statistical analyses. Emma

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.