Universiteit Utrecht

Universitair Medisch Centrum Utrecht

The interactive effects of Catechol-O-methyltransferase (COMT), D-amino acid oxidase-activator (DAO-A) and stressful life events on neuroticism and depression in a healthy Caucasian population.

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	July 15th, 2012
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Summary

Extensive research identified several vulnerability factors for psychiatric disorders, including personality traits, environmental stress and genetic risk. Relatively little is known about the interaction between environmental stressors and multiple genes. The present study investigates the (interactive) effects of dopaminergic gene COMT and glutamatergic gene DAO-A, as well as the (interactive) effects of early life stress and recent life stress on neuroticism and depression. The personality trait neuroticism is considered a predictor for numerous psychiatric disorders, including depression. It is expected that the considered high-risk carriers of a gene have increased neuroticism levels due to altered neurotransmitter availability. Also, increased experienced environmental stress – either in early life or in recent life – is expected to raise neuroticism. Besides main effects, it is expected that genetic risk can combine with environmental stressors in elevating neuroticism. Also, both environmental stress and genetic risk are expected to accumulate their adverse effects to further increase neuroticism. These hypotheses are tested in a healthy population with Caucasian background. For genetic analyses, only carriers of homozygote gene-variants are included. Gene groups are matched on social economic status, age and sex. Indeed, the risk-variant of COMT leads to higher neuroticism levels. However, DAO-A does not influence neuroticism. As expected, increases in experienced stress increase neuroticism and depression. In line with expectations, although not consistent in all genes, genetic and environmental influences can combine since COMT interacts with early life stress and recent life stress in increasing neuroticism. Environmental stress and genetic risk seem to be able to accumulate their adverse effects, but this effect is moderate and inconsistent. The findings of this study address the influences of genetic variation in its ability (by itself and in combination with environmental factors) to produce adverse psychological effects. Also, the impact of early life stress and recent life stress on psychological wellbeing is made clearer, even more because of the seemingly accumulating properties of stress.

Introduction

Repeatedly, research has investigated predisposing factors that contribute to the development of psychiatric disorders. Genetic background as well as environmental factors have turned out to be of great importance. Genetics influence the capacity of an environmental risk to bring about psychiatric disorders (Caspi, 2006). Studies investigating this gene and environment interaction (G x E) have mainly focussed on the interaction between one or multiple environmental variables and one selected gene. Less is known about the interaction between environmental variables combined with *multiple genes*. Does the susceptibility to the development of psychiatric disorders increase when people have the risk-variant of more than one gene, because of the way these genes interact with each other?

Over the past years, several vulnerability genes for psychiatric conditions have been identified. D-amino acid oxidase-activator (DAO-A, formerly called G72) is one of these candidate genes. The gene DAO-A produces the enzyme DAO (also called DAAO), which degrades D-serine. D-serine acts as a co-agonist of glutamate at NMDA glutamatergic receptors (Boks *et al.*, 2007; Ohide, Miyoshi, Maruyama, Hamase & Konno, 2011). DAO-A is known for its strong associations with several psychiatric disorders, including schizophrenia, bipolar disorder (Detera-Wadleigh & McMahon, 2006) and major depressive disorder (Rietschel *et al.*, 2008). DAO-A will be included in the present study by looking at the homozygote alleles of rs2391191 (M15; gene-variants A/A and G/G) and rs3918342 (M23; C/C and T/T). Interestingly, despite its clear associations with various psychiatric conditions, there are no consistent results across studies when it comes to the assumption which allele is the high-risk variant of DAO-A (Detera-Wadleigh & McMahon, 2006). Considering M23, one study found that the supposed risk variant C/C increases the risk of schizophrenia and affective disorders, but at the same time leads to beneficial effects on cognitive performance (Jansen *et al.*, 2009). As for M15, many studies presenting significant results for this marker either do not mention which allele (A or G) produced these effects (Chumakov *et al.*, 2002), or show contradictory results considering which allele is the high-risk variant (Nicodemus *et al*, 2007; Schultz *et al,* 2011; Schumacher *et al.*, 2004; Wang *et al.*, 2004; Williams *et al.*, 2006). The current study identifies the homozygotic A/A variant as the high-risk allele of M15. This is in line with previous findings on associations of the A-allele with schizophrenia (Schultz *et al.*, 2011) non-psychotic bipolar disorder (Grigoroiu-Serbanescu *et al.,* 2010) and major mood disorder (Williams *et al.*, 2006). Based on found associations of the M23 C-allele with schizophrenia, affective disorder (Jansen *et al.*, 2009) and psychotic bipolar disorder (Schulze *et al.,* 2005), the homozygotic C/C variant is considered the high-risk variant of M23.

Besides research on glutamatergic functioning, many studies have also investigated genes that regulate dopaminergic functioning (Ellis *et al.*, 2011; Kang, Kim, Namkoong & An, 2010; Nemoda, Szekely & Sasvari-Szekely, 2011; Savitz, Solms & Ramesar, 2006). Dopamine belongs to the category of monoamines – along with norepinephrine and serotonin. Monoamines have a crucial role in the regulation of emotion processing and mood (Defrancesco *et al.,* 2011). Other studies focussed on genes regulating glutamatergic functioning (Detera-Wadleigh & McMahon, 2006; Rietschel *et al.,* 2008). Glutamatergic neurotransmission, especially through the NMDA-receptor, has gained much attention for its association with schizophrenia (Javitt, 2008). Both glutamate and dopamine are excitatory neurotransmitters, but they have a balancing effect on each other: dopamine modulates glutamate transmission. This modulation depends on which glutamate or dopamine receptors are activated (Tseng & O'Donnell, 2004). Unlike the many investigations addressing either dopamine or glutamate for their associations with psychiatric conditions, fewer studies included genes regulating dopaminergic functioning as well as glutamatergic functioning to examine their possible interaction. Besides investigating the effects of DAO-A, the present study also includes a gene that is crucially implicated in central dopamine function: Catechol-O-methyltransferase (Witte & Flöel, 2011).

The catechol-O-methyltransferase gene (COMT) is well-known as a functional candidate for a broad range of psychiatric disorders, personality traits and cognitive functioning (Craddock, Owen & O'Donovan, 2006, Hettema *et al.*, 2008; Wray *et al,* 2008). COMT produces the COMT-enzyme. This enzyme is involved in

the catabolism of monoamines. It breaks down catecholamines, including dopamine (Craddock *et al.*, 2006). The single nucleotide polymorphism (SNP) at codon 158 of the COMT gene replaces the production of the protein Valine with the protein Methionine. This SNP is called Val158Met (Harrison & Tunbridge, 2008). The Val-allele is associated with higher activity of the dopamine-catabolization, which results in a reduced availability of dopamine (Kang *et al*., 2010), whereas the Met-allele is associated with less dopamine-catabolization (Enoch, White, Waheed & Goldman, 2008), leading to increased dopamine availability. Despite a large quantity of studies having investigated the effect of Val158Met on psychiatric disorders, results have been contradictory. Initially, the Met-allele was associated with schizophrenia (Kotler *et al.*, 1999; Park, Yoon, Park, Hirvonen & Kang, 2002), but later studies showed associations between the Val-allele and schizophrenia (Glatt, Faraone & Tsuang, 2003; Wang, Fang, Shen & Xu, 2010; Wonodi, Stine, Mitchell, Buchanan & Thaker, 2003). In addition, the Val-allele was associated with increased depression severity (Domschke *et al.*, 2010), early onset major depressive disorder (Massat *et al.*, 2005), panic disorder (Domschke *et al.*, 2004; Domschke, Deckert, O'Donovan & Glatt, 2007), phobic anxiety (McGrath *et al.*, 2004) and neuroticism (Hettema *et al.*, 2008). These findings indicate that, for certain psychiatric disorders and associated personality traits , the high-risk allele of COMT is the Val-allele. COMT is dynamically regulated; its expression can be altered during normal brain development and in reaction to environmental influences (Witte & Flöel, 2011). Also, there is evidence that an epistasis might exist between DAO-A and COMT (Nicodemus *et al.*, 2007; Nixon *et al.,* 2011). This suggests that it is possible for these two genes to be modifying each other (Cordell, 2002).

However, not just genetic vulnerabilities determine which people will develop a psychiatric disorder or not. Stress and stress-causing events have proved to be risk factors for this development as well (Caspi & Moffitt, 2006). Stress in early life can have long-term effects on behavior and underlying neural function (Nelson, 2000; Post, 2007). Several ways have been identified through which childhood trauma influences the development of psychiatric disorders, including low self-esteem, a cognitive attribution style and family dysfunctions (Roy, 2002). Also, childhood trauma can predispose to psychiatric disorders by being a partial determinant of neuroticism (Roy, 2002).

Another way of measuring the occurrence of stressful life events is by focussing on recent stressors: stressful life events in adulthood can interact with early life stress by contributing to the process of stress sensitization (Nelson, 2000; Post, 2007). Stegenga and colleagues (2012) showed that recent life events, regardless the type of life event, pose the largest risk for the onset of major depressive disorder in mid-life.

The personality trait neuroticism (the proneness to experience negative affect) is considered a predictor for numerous psychiatric disorders (Rietschel *et al.,* 2008; Lahey, 2009; Taylor, Asmundson & Jang, 2011). This trait consists of multiple facets that are highly correlated but partially distinct, including anger, sadness, anxiety, worry, and hostility (Weiss & Costa, 2005). Neuroticism is a broad etiologic factor influencing different kinds of psychopathology (Lahey, 2009; Taylor, Asmundson & Jang, 2011). It is suggested that neuroticism is shaped by a constellation of genetic and environmental determinants. Evidence indicates that neuroticism interacts with psychosocial stress to generate psychopathology (Kendler, Gardner & Prescott, 2003; Kendler, Kuhn & Prescott, 2004). Previous studies showed evidence for an association between genes and neuroticism, including the DAO-A gene (Rietschel *et al.,* 2008) and the COMT-gene (Hettema *et al.*, 2008).

Rietschel and colleagues (2008) observed associations between several SNPs of the DAO-A gene (including M15 and M23) and multiple psychiatric disorders. The fact that they also found an association with the personality trait neuroticism suggests that DAO-A could contribute to the susceptibility to psychiatric disorders through neuroticism. Results on COMT effects are, as said above, inconsistent. Some studies suggest sex differences in the relation between the high activity Val-allele of COMT and neuroticism, but the association between COMT and neuroticism is observed nonetheless (Eley *et al.*, 2003; Pełka-Wysiecka *et al.*, 2012).

Narrowing down from a broad representation of subclinical psychopathology to a more specific psychiatric phenotype, this study will also investigate the effects of genes and environment on depression. This is in line with the dimensional approach to personality traits as applied in the coming DSM-V (Bachrach, Croon & Bekker, 2012; Wright *et al.*, 2012). Axis-I disorders like mood disorders and anxiety disorders are explained by the factor neuroticism (Bachrach *et al.*, 2012). Indeed, neuroticism has repeatedly been reported as a predictor for major depression (Kendler, Gardner & Prescott, 2003; Kendler, Kuhn & Prescott, 2004). For both DAO-A and COMT, studies gathered evidence that these genes show associations to depression (Massat *et al.,* 2004; Rietschel *et al.*, 2008, Williams *et al*, 2006).

Based on findings in previous literature, the following hypotheses considering COMT and DAO-A can be formed: it is expected that carriers of the high-risk variant of each SNP – COMT (Val/Val), M15 (A/A) or M23 (C/C) – score higher on neuroticism and have a higher incidence of depression compared to carriers of the low-risk variant (i.e. Met/Met, G/G or T/T). In addition, it is expected that carriers of the high-risk variants of two or three SNPs will score higher on neuroticism than carriers of just one high-risk variant due to interaction effects. Considering stressful life events, it is hypothesized that higher levels of experienced stress (either in childhood or in the past year) produce higher neuroticism scores and higher incidence of depression. Participants who experienced both early life stress and recent stress are expected to score higher on neuroticism than participants who experienced only one kind of stressful life event. Coming to the G x E interactions: it is expected that stressful life events (either in childhood or in the past year) show an interaction with the high-risk variants of each SNP, leading to increased neuroticism scores. Furthermore, it is known that women score higher on neuroticism (Costa & McCrae, 1992) and have higher depression rates than men (Sadock & Sadock, 2007). Hence it is investigated whether the main effects of genes and environment are influenced by sex differences.

Methods

Genotyping

The current study is based on a 'forward genetics' approach (Boks, Derks, Dolan, Kahn & Ophoff, 2010). This means all participants were selected based on genes known for their association with psychiatric disorders. Since all participants come from a healthy population, it is suggested that these candidate genes will present themselves in subclinical psychiatric phenotypes. 'Forward genetics' proved itself a cost-efficient method that increases statistical power (Boks *et al.,* 2010). For this reason, all heterozygous gene-variants are excluded

from analyses. COMT did not deviate from the Hardy-Weinberg equilibrium (X^2 (2) = .173, p = .932), nor did M15 (X^2 (2) = 3.052, p = .217) and M23 (X^2 (2) = .232, p = .891).

Genotyping was done using Illumina® technology operated by the 'Complex Genetic Groups' department in the University Medical Centre (UMC) Utrecht.

Participants

Participants were selected from a general population in Leidsche Rijn, a suburb near the city of Utrecht, the Netherlands. All inhabitants (N=10.000) of Leidsche Rijn were screened while participating in the Utrecht Health Program (UHP; Grobbee *et al.*, 2005). This is a large-scale health care monitoring study and epidemiologic research which is approved by the Medical Ethics Committee of the UMC Utrecht. Since genes can have various effects in different ethnicities, only Dutch participants were selected. Furthermore, people with major medical conditions were excluded. This selection of healthy participants (N=3200) had a blood sample taken for a quality control on genetic background and were genotyped for a number of candidate genes for psychiatric disorders. For COMT and DAO-A 2400 people were genotyped. This selection of participants completed several questionnaires, including the List of Threatening Event Questionnaire (LTE-Q; Brugha, Bebbington, Tennant & Hurry, 1985) and the neuroticism domain of the NEO-PI-R (Costa & McCrae, 1992).

For the current study, a second filter was applied to the selection of UHP-participants. Since this study focuses on gene x gene (x environment) interactions, it was decided to invite more homozygote gene variants than normally represented in the general population. Due to this enrichment in homozygote variants, there is increased power to make assumptions based on gene x gene interaction effects and gene x gene x environment interactions. By selecting only the participants carrying homozygote alleles, high-risk groups and low-risk groups could be formed for each candidate gene. An exception is the DAO-A gene, because so far it is uncertain which allele is the risk-variant. Between gene groups, people were matched on postal code (for social economic status), age and sex.

Procedure

After demographic matching between gene groups, the remaining selection of people received an invitation letter with details and information about the study. A few weeks after receiving the invitation, people were called to ask if they were willing to participate. It was explained that the study consisted of two parts: an appointment for some attention and memory tests and two interviews lasting 2.5 hours in total. The second part involved filling out online questionnaires – including the Childhood Trauma Questionnaire (CTQ; Bernstein et al., 1994), the LTE-Q, the PHQ-9 and the NEO-PI-R – taking about 1.5 hours to complete. If they agreed on participation, an appointment was made to conduct neuropsychological tests, a medical questionnaire, a psychiatric interview and a venepuncture to collect blood samples for a control check on genotype. This appointment was either in the UMC Utrecht, at their homes or at their general practitioner's building. In return for participation they received 40 euros and compensation for travel expenses. Participants who had completed both parts of the study (questionnaires and appointment) were included for analysis.

Demographics of the participants (N=397) are found in Table 1a to 1e, Addendum A. Also, this table shows demographics of the participants categorized by grouping variable (COMT, DAO-A M15, DAO-A M23, childhood trauma and recent life events). There were no significant inter-subgroup differences. An exception was childhood trauma, which showed a significant inter-subgroup difference in age and social economic status derived from education.

Instruments

Neuroticism.

Neuroticism will be measured using the neuroticism subscale of the NEO-PI-R (Costa & McCrae, 1992). This 48-item subscale consists of six facets measuring anxiety, hostility, depression, self-consciousness, impulsiveness, and vulnerability to stress (Costa & McCrae, 1992). The NEO-PI-R is a self-assessment questionnaire where participants fill in, on a five-point scale, how much they identify with each question ranging from "*strongly disagree*" to "*strongly agree*" (Costa & McCrae, 1992). To correct for sex differences, the total scores were transformed to a sex-specific standardized nine-point scale, i.e. stanines (Hoekstra, Ormel & Fruyt, 2007). These stanines correct for the fact that on average, women score higher on neuroticism than men (Costa & McRae, 1992; Sadock & Sadock,2007). A stanine of 1 is considered a very low neuroticism score. Stanines 4, 5 and 6 are regarded as average scores. Very high neuroticism is indicated by stanine 9.

Depression.

The 9-item depression module of the Patient Health Questionnaire (PHQ-9: Spitzer, Kroenke &Williams, 1999) is used to measure depression. The PHQ-9 is derived from the Primary Care Evaluation of Mental Disorders (Spitzer *et al.*, 1994), an instrument for detecting five common mental disorders (i.e. depression, anxiety, alcohol abuse, somatoform disorder and eating disorder). The PHQ-9 measures the level of depression in the past two weeks, scores range from 0 to 27 (Zuithoff *et al*, 2010). The presence and severity of depressive symptoms is based on the total PHQ-9 score. A total score lower than 10 is considered no or mild depressive symptoms. For the current study, participants scoring lower than 10 are regarded as having no depression. All participants scoring above or equal to 10 are regarded as having a probable depression, or moderate to severe depressive symptoms (Zuithoff *et al.,* 2010).

Early life stress.

To measure early life stress, a Dutch translation of the Childhood Trauma Questionnaire Short Form (CTQ; Bernstein *et al*., 1994) is used (Arntz & Wessel, 1996). This version of the CTQ consists of 25 questions spread out over five categories: Physical Abuse (e.g. "*I believe that I was physically abused*"), Emotional Abuse (e.g. "*People in my family called me 'stupid, lazy, or ugly' "*), Sexual Abuse (e.g. "*I believe I was sexually abused*"), Physical Neglect (e.g. "*I had to wear dirty clothes*") and Emotional Neglect (e.g. "*I felt that someone in my family hated me*"). Answering options range from 1 ("*Never true*") to 5 ("*Very often true*"). One item of the Sexual Abuse scale ("*I was molested*") was deleted due to ambiguity caused by a difference in meaning of the Dutch translation "*Ik ben door iemand gemolesteerd*". The English translation of the Dutch verb "*molesteren*"

can be interpreted as "*beat up"* (Martin, Tops, Broeders, Roos & Schrama, 1991, in: Thombs, Bernstein, Lobbestael & Arntz, 2009), which should refer to the Physical Abuse scale (Thombs *et al.*, 2009) This resulted in a 24-item questionnaire. According to the amount of experienced childhood trauma, a division is made to distinct people who have experienced stressful life events in childhood from those who have barely experienced these events or not at all. The cut-off score for the two groups was made based on the median of the total score on the CTQ. All participants having a total score under or equal to the median (< 35) are put in Group 1. Group 2 consists of participants having a total score above the median (> 34).

Recent life events.

Recent stressors are included in the present study by using the List of Threatening Events Questionnaire (LTE-Q; Brugha *et al*., 1985). This questionnaire indexes twelve categories of events reflecting the presence of life stressors during the past year (e.g. diagnosis of a serious illness or injury to the participant, a serious illness or injury to a close relative, death of a first-degree or second-degree relative, divorce, unemployment or financial crisis). Participants answer by registering how many times this event happened in the past year, ranging from 0 times to 99 times. Based on their score on the questionnaire, participants are either put in the group "No", meaning they have not experienced any stressors in the past twelve months, or in the group "Yes", indicating the occurrence of one or multiple stressful life events.

Statistical Analyses

All analyses will be done using SPSS 15.0 (2007). To check for normality, a Kolmogorov-Smirnov test will be conducted for each variable, and Levene's tests will be done to check the homogeneity of variance. Since all predictor variables (i.e. COMT, M15, M23, CTQ and RLE) are dichotomous, point-biserial correlations will be calculated to investigate the main effects of each predictor on neuroticism. To test the interactive influences of COMT, DAO-A (M15 and M23), childhood trauma and recent life events on neuroticism, independent factorial analyses of variance (ANOVA) will be conducted (Field, 2005). Considering the possible sex differences that could moderate the effects of genes and environment on neuroticism, the main effect of sex on neuroticism and depression is checked first. Secondly, an analysis of covariance (ANCOVA) will be performed for each predictor variable, with sex included as a covariate. Chi-square statistics are calculated to assess the main effects of genes and stressful life events on the incidence of depressive symptoms.

Results

Main effects on neuroticism

Three gene SNPs – one from the COMT gene and two SNPs from the DAO-A gene – and two different types of stressful life events were tested for their association with neuroticism. The main effects of COMT, DAO-A, childhood trauma and recent life stress on neuroticism are shown in Table 2. Also, the main effect of sex on neuroticism was tested to examine if sex differences influenced neuroticism.

Table 2: Main effects of gene SNPs (COMT Val158Met, DAO-A M15 and DAO-A M23), stressful life events (childhood trauma and recent life stress) and sex on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex-differences.

	Groups	Neuroticism stanines Mean (SD)	Statistical value	Significance
COMT (N=191)	Val/Val (N=81)	6.46 (2)	r_{pb} = .15	p = .037*
	Met/Met (N=110)	5.83 (2.1)		
DAO-A M15 (N=214)	A/A (N=77)	6.13 (2.1)	r_{pb} = .03	p = .714
	G/G (N=137)	6.02 (2)		
DAO-A M23 (N=204)	C/C (N=110)	6.19 (2)	r_{pb} = .27	p = .328
	T/T (N=94)	5.9 (2.1)		
Childhood trauma (N=397)	Below average (N=212)	5.56 (2.1)	r_{pb} = .1	p = .000***
	Above average (N=185)	6.71 (2.1)		
Recent life stress (N=397)	Yes (N=239)	6.36 (2.2)	r_{pb} = .15	p = .002**
	No (N=158)	5.69 (2.1)		
Sex (N=397)	Male (N=181)	6 (2.2)	r_{pb} = .04	p = .408
	Female (N=216)	6.18 (2.2)		

* Significant at a $p < .05$ level (two-tailed).
** Significant at a $p < .01$ level (two-tailed).
*** Significant at a $p < .001$ level (two-tailed).

The SNP of the COMT gene showed a significant relationship with neuroticism (r_{pb} = 15, p < .05), with the homozygote Val-variant – considered as the high-risk variant of the COMT gene – having a higher score on neuroticism. The DAO-A SNPs M15 and M23 did not show significant main effects. Sex did not have a significant effect either, as was expected since neuroticism scores were already made into standardized values to correct for naturally occurring sex differences. Considering stressful life events, both childhood trauma (r_{pb} = .1, $p < .001$) and recent life stress (r_{pb} = .15, $p < .01$) correlated significantly with neuroticism. For childhood trauma, the group that had experienced more trauma in their youth (above average group) showed higher neuroticism levels, with a mean difference of more than a whole stanine. For recent life stress, the group that had experienced one of multiple stressful life events in the past year had higher neuroticism scores.

Gene (x Gene) x Environment (x Environment) interaction-effects

After investigating the main effects and sex differences, several interaction models were made to assess the way these five factors contribute to levels of neuroticism. A complete list of result tables is found in Addendum B. An interesting significant interaction-effect on neuroticism was found in a gene x environment x environment model, combining COMT with both variants of stressful life events (F (7, 183) = 4.7, $p < .05$). As can be seen in Table 3 and Figure 1, the highest levels of neuroticism came from people who were carrying the Val-(high-risk) variant of COMT, had experienced above average childhood trauma and had also experienced recent life stress. The lowest neuroticism level is seen in the group of people who had experienced the least stress in their childhood and carried the Met-variant of COMT. Interestingly, these people had experienced a stressful life events in the past year. The mean difference between the highest and lowest neuroticism scores was more than 1.5 stanine.

Table 3: Interaction effects of homozygote COMT gene-variants, below or above average experienced childhood trauma and experienced recent life stress on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex-differences.

COMT gene variant	Childhood trauma	Recent life stress	Neuroticism stanines Mean (SD)	Statistical value	Significance
Val/Val	Below average (N=46)	Yes (N=25)	6.8 (2.1)		
		No (N=21)	5.24 (1.7)		
	Above average (N=35)	Yes (N=24)	7.12 (1.7)		
		No (N=11)	6.55 (2.1)		
	Total	N=81	6.46 (2)		
Met/Met	Below average (N=61)	Yes (N=26)	5.23 (2.1)	*F* = 4.7	*p* = .031*
		No (N=35)	5.63 (2)		
	Above average (N=49)	Yes (N=32)	6.72 (2.1)		
		No (N=17)	5.47 (2.1)		
	Total	N=110	5.83 (2.1)		
Total		N=191	6.09 (2.1)		

* Significant at a $p < .05$ level.

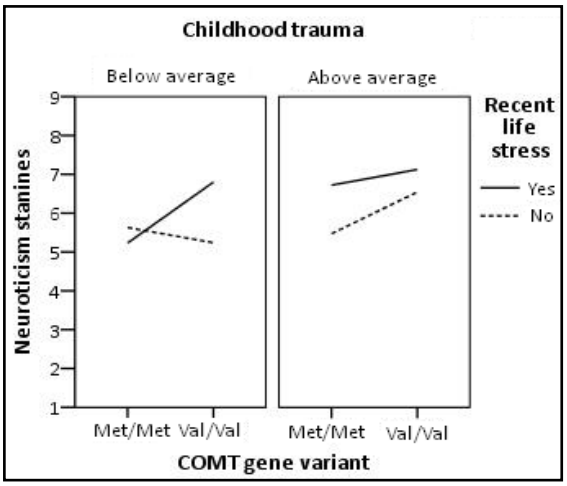


Figure 1: Interaction effects of COMT, childhood trauma and recent life stress on standardized mean neuroticism scores.

None of the remaining investigated gene x gene interactions and gene x gene x environment interactions with neuroticism showed significant results. However, there was a marginally significant gene x gene interaction between COMT and M15 of the DAO-A gene on neuroticism (F (3, 103) = 3.85, p = .053), as demonstrated in Table 4. Figure 2 below Table 4 displays the found interaction-effect graphically. This effect shows that homozygote carriers of the low-risk Met-variant of COMT had slightly higher neuroticism levels when also carrying the homozygote G-variant of DAO-A M15, which is considered the low-risk variant. Interestingly, the opposite is true for homozygote high-risk Val-carriers of the COMT gene. They scored considerably higher on neuroticism when they also carried the homozygote high-risk A-variant of DAO-A M15.

Table 4: Interaction effects of homozygote COMT gene-variants and homozygote DAO-A M15 gene-variants on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex differences.

COMT gene variant	DAO-A M15 gene variant	Neuroticism stanines Mean (SD)	Statistical value	Significance
Val/Val (N=44)	A/A (N=17)	6.76 (2.1)		
	G/G (N=27)	5.56 (2)		
Met/Met (N=63)	A/A (N=23)	5.7 (2.1)	*F* = 3.85	*p* = .053
	G/G (N=40)	6.1 (1.9)		
Total	N=107	5.98 (2)		

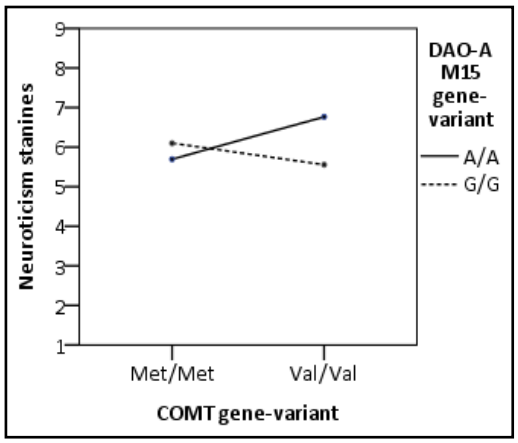
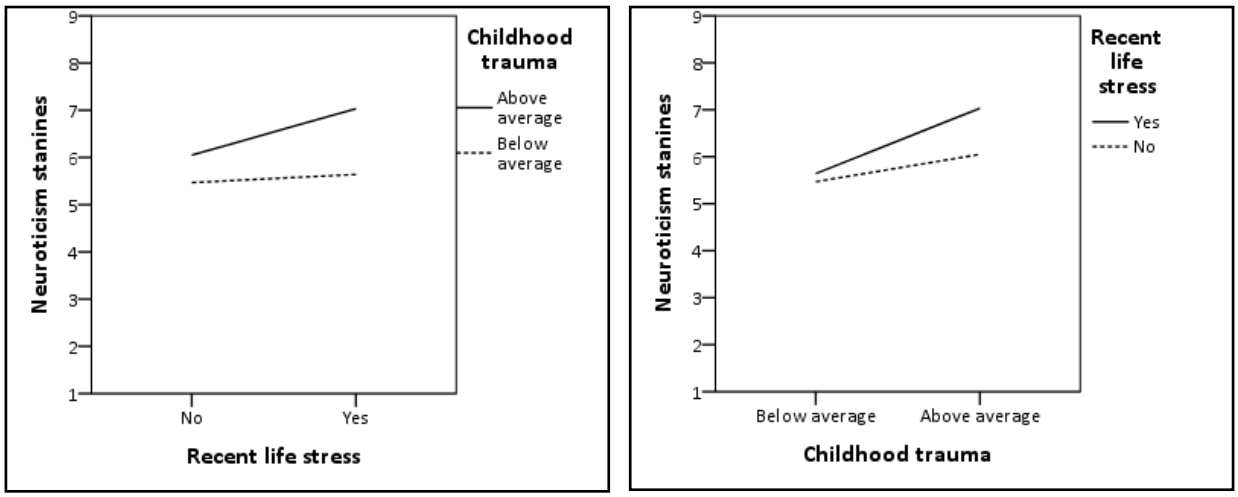


Figure 2: Interaction effect of COMT and DAO-A M15 on mean standardized neuroticism scores.

Considering the environmental contributions to neuroticism levels, another marginally significant result was found in the interaction between childhood trauma and recent life stress on neuroticism, as can be seen in Table 5 and Figures 3a and 3b (F (3, 393) = 3.53, p = .061). This finding points to a trend indicating that people who had experienced stressful life events in the past year scored higher on neuroticism if they had also experienced above average amounts of stress in their youth, compared to those who had experienced *less* than average amounts of stress in their youth. Furthermore, the differences in neuroticism levels between people who had experienced below average or above average stress in their childhood, are higher than the differences in neuroticism levels between those who had or had not experienced recent life stress. This environment-only model also shows that the found significant effect in Table 3 (COMT x childhood trauma x recent life stress) was caused by the additional genetic effects of the COMT-gene, not by environmental stressors alone.

Table 5: Interaction effects of the amount of experienced childhood trauma (below average or above average) and experienced recent life stress or no recent life stress on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex-differences.

	Chorus	Recent life stress	Neuroticism stanines Mean (SD)	Statistical value	Significance
Childhood trauma	Below average (N=212)	Yes (N=114)	5.64 (2.2)	*F* = 3.53	*p* = .061
		No (N=98)	5.47 (2)		
	Above average (N=185)	Yes (N=125)	7.03 (1.9)		
		No (N=60)	6.05 (2.2)		
	Total	N= 397	6.1 (2.2)		



Figures 3a and 3b: Interaction effects between childhood trauma and recent life stress on mean standardized neuroticism scores.

Influence of sex on neuroticism

As for the hypotheses about sex affecting the influences of genes and environment on neuroticism, there was no main effect of sex on neuroticism, as seen in Table 2. Sex was used as a covariate in the investigation of the effects of gene or environment on neuroticism. Results are displayed in Table 6. In a model combined with COMT, sex was significantly related to neuroticism (F (1, 189) = 7.86, p < .01). There was still a significant effect of COMT on neuroticism after controlling for sex (F (1, 189) = 4.83, p < .05). No significant association of sex on neuroticism was found in a model combined with DAO-A M15, DAO-A M23, childhood trauma or recent life stress. The effects of both types of stressful life events stayed significant after controlling for sex (childhood trauma: F (1, 395) = 30.52, p < .001); recent life events: F (1, 395) = 9.56, p,< 01).

Table 6: Sex as a covariate of gene SNPs effects (COMT, DAO-A M15 and DAO-A M23) and environmental effects (childhood trauma and recent life stress) on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex-differences.

	Chorus	Neuroticism stanines Mean (SD)	Statistical value	Significance
COMT (N=191)	Val/Val (N=81)	6.46 (2)	Sex: F = 7.86	p = .006**
	Met/Met (N=110)	5.83 (2.1)	COMT: F = 4.83	p = .029*
DAO-A M15 (N=214)	A/A (N=77)	6.13 (2.1)	Sex: F = 3.2	p = .075
	G/G (N=137)	6.02 (2)	M15: F = .34	p = .562
DAO-A M23 (N=204)	C/C (N=110)	6.19 (2)	Sex: F = 1.33	p = .251
	T/T (N=94)	5.9 (2.1)	M23: F = .89	p = .347
Childhood trauma (N=397)	Below average (N=212)	5.56 (2.1)	Sex: F = 1.13	p = .288
	Above average (N=185)	6.71 (2.1)	CT: F = 30.52	p = .000***
Recent life stress (N=397)	Yes (N=239)	6.37 (2.2)	Sex: F = .7	p = .402
	No (N=158)	5.69 (2.1)	RLE: F = 9.56	p = .002**

* Significant at a $p < .05$ level (two-tailed).
** Significant at a $p < .01$ level (two-tailed).
*** Significant at a $p < .001$ level (two-tailed).

Main effects on depression

Considering the investigated main effects of genes and environment on the depression prevalence in the used population, childhood trauma showed a significant correlation on depression rates (X^2 (1) = 11.21, $p < .01$), with more experienced childhood trauma leading to higher depression frequencies, as seen in Table 7. Also, there was a significant effect of experienced life stress in the past year on depression rates (X^2 (1) = 5.19, $p < .05$). People who had experienced recent life stress reported depression more often than people without stressful life events in the past year. No significant results were found for the effects of genes on depression. Finally, sex did not have a significant effect on depression, as was expected given the standardized neuroticism scores.

Table 7: Main effects of homozygote gene-variants (COMT, DAO-A M15 and DAO-A M23), environmental stress (childhood trauma and recent life stress) and sex on depression rates (and percentages).

	Groups	Depression frequencies	Statistical value	Significance
COMT (N=191)	Val/Val (N=81)	4 (4.9%)	X^2 = .2	p = .724
	Met/Met (N=110)	4 (3.6%)		
DAO-A M15 (N=214)	A/A (N=74)	3 (3.9%)	X^2 = .53	p = .669
	G/G (N=134)	3 (2.2%)		
DAO-A M23 (N=204)	C/C (N=110)	5 (4.5%)	X^2 = .89	p = .455
	T/T (N=94)	2 (2.1%)		
Childhood trauma (N=397)	Below average (N=212)	2 (0.9%)	X^2 = 11.21	p = .001**
	Above average (N=185)	14 (7.6%)		
Recent life stress (N=397)	Yes (N=239)	14 (5.9%)	X^2 = 5.19	p = .034*
	No (N=158)	2 (1.3%)		
Sex (N=397)	Male (N=181)	7 (3.9%)	X^2 = .02	p = .88
	Female (N=216)	9 (4.2%)		

* Significant at a $p < .05$ level (two-tailed).
** Significant at a $p < .01$ level (two-tailed).

Post Hoc analyses: influence of sex on neuroticism

Since the neuroticism scores were corrected for naturally occurring sex differences, the significant effect of sex on COMT (as seen in Table 6) was unexpected. Therefore, subsequent post hoc analyses were conducted to examine this finding. To further explore how sex affected genetic and environmental effects on neuroticism, sex was put in models together with each variable that was expected to contribute to neuroticism differences (COMT, DAO-A M15, DAO-A M23, childhood trauma and recent life events). The subgroup of people carrying a homozygote high-risk gene-variants was divided from people carrying the low-risk gene-variant in order to examine sex differences separately. Childhood trauma and recent life stress were also divided into their two pre-determined subgroups, based on amount of experienced stress. Differences in sample size per subgroup were already displayed in Table 1a-1e (Appendum A), showing no significant inter-subgroup sex differences in amount of involved people. Subsequently, each subgroup was investigated for their differences in male and female neuroticism scores. An overview of the results of these analyses is displayed in Table 8.

Table 8: Sex differences in standardized neuroticism scores (and standard deviations) in the homozygote gene-variant groups of COMT (Val or Met), DAO-A M15 (A or G) and DAO-A M23 (C or T), the level of experienced stress in childhood (below or above average) and the occurrence of stressful recent life events (yes or no).

	Groups	Sex	Neuroticism stanines Mean (SD)	Statistical Value	Significance
COMT gene-variant (N=181)	Val/Val (N=71)	Male (N=38)	6.05 (2)	r_{pb} = .21	p = .031*
		Female (N=43)	6.81 (2)		
	Met/Met (N=110)	Male (N=49)	5.35 (2.2)	r_{pb} = .19	p = .084
		Female (N=61)	6.21 (2)		
DAO-A M15 gene-variant (N=214)	A/A (N=77)	Male (N=41)	5.7 (2.3)	r_{pb} = .24	p = .038*
		Female (N=36)	6.7 (1.9)		
	G/G (N=137)	Male (N=56)	5.9 (2.1)	r_{pb} = .05	p = .536
		Female (N=81)	6.1 (2)		
DAO-A M23 gene variant (N=204)	C/C (N=110)	Male (N=49)	5.7 (2.2)	r_{pb} = .21	p = .028*
		Female (N=61)	6.6 (1.8)		
	T/T (N=94)	Male (N=45)	6 (2.2)	r_{pb} = -.06	p = .54
		Female (N=49)	5.8 (2)		
Childhood trauma (N=397)	Below average (N=212)	Male (N=93)	5.5 (2.1)	r_{pb} = .02	p = .734
		Female (N=119)	5.6 (2.1)		
	Above average (N=185)	Male (N=88)	6.5 (2.1)	r_{pb} = .09	p = .23
		Female (N=97)	6.9 (2)		
Recent life events (N=397)	Yes (N=239)	Male (N=109)	6.4 (2.1)	r_{pb} = .11	p = .16
		Female (N=130)	6.4 (2.2)		
	No (N=158)	Male (N=72)	5.4 (2.2)	r_{pb} = -.003	p = .958
		Female (N=86)	5.9 (2.1)		

* Significant at a p < .05 level (two-tailed).

As can be seen in Table 8, a significant sex difference in neuroticism only existed in the high-risk gene-variants of all three SNPs. For COMT, a significant sex difference appeared in the group of people carrying the homozygote Val-variant (r_{pb} = .21, p = < .05). Women had noticeably higher neuroticism levels than men in this specific high-risk gene group. There was no significant sex difference in the homozygote low-risk Met-variant carriers, although it showed a trend towards women scoring higher on neuroticism than men (r_{pb} = .19, p = .084). This

difference is displayed graphically in Figure 4a. Appliance of a division between DAO-A M15 gene-variants revealed that another significant sex difference was present in only one of the two gene-variants (Figure 1b). This result shows that, although neither DAO-A M15 nor sex had a main effect on neuroticism, a sex difference in neuroticism levels could be seen in the high-risk A-variant of DAO-A M15 (r_{pb} = .24, p < .05). Interestingly, Figure 4b shows that the high-risk A-allele seemed to have an opposite effect in men, who scored higher on neuroticism when carrying the low-risk G-allele. Again, there was no significant difference in the low-risk group (homozygote G-variant; r_{pb} = .05, p = ns). For the M23 marker of DAO-A, another significant difference in neuroticism levels appeared between male and female carriers of the homozygote C-variant of DAO-A M23 (r_{pb} = .21, p < .05). Women scored higher on neuroticism than men when carrying the homozygote C-variant, as displayed in Figure 4c. This found sex difference did not appear between carriers of the homozygote low-risk T-variant (r_{pb} = -.06, p = ns). Figure 4c shows that, just as for DAO-A M15, the high-risk gene-variant of DAO-A M23 has the opposite effect in men, who have a higher neuroticism level when carrying the low-risk T-allele. Considering childhood trauma and recent life events, no sex differences were found in either environmental stress category.

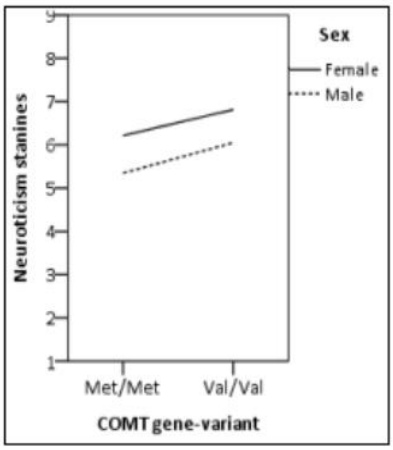


Figure 4a: Sex difference in the effects of COMT gene-variant on standardized neuroticism scores.

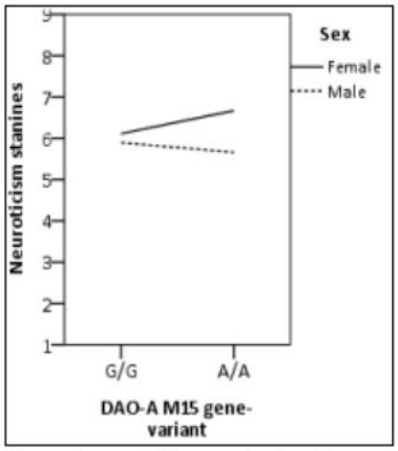


Figure 4b: Sex difference in the effects of DAO-A M15 gene-variant on standardized neuroticism scores.

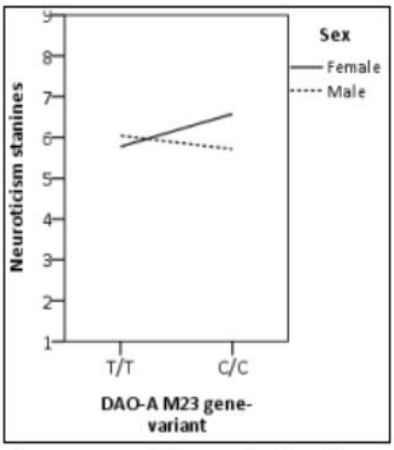


Figure 4c: Sex difference in the effects of A M23 gene-variant on standardized neuroticism scores.

Discussion

The aim of this study is to investigate how different dopaminergic and glutamatergic gene-variants affect neuroticism and depression directly, as well as in interaction with environmental stressors. The effects of environmental stress on neuroticism and depression is looked at from two perspectives: early life stress in the shape of childhood trauma, and recent life stress, formed by experienced stressful life events in the past year. This study also examines whether sex differences influence the effects of genes and environmental stress on neuroticism.

It is expected that homozygote carriers of the high-risk gene-variants of COMT (i.e. Val/Val), DAO-A M15 (i.e. A/A) and DAO-A M23 (i.e. C/C) will have higher scores on neuroticism and have higher depression prevalence than homozygote carriers of the low-risk gene-variants (i.e. Met/Met, G/G and T/T, respectively). Furthermore, it is hypothesized that people carrying more than one high-risk gene-variant will have an accumulated risk and will subsequently score even higher on neuroticism than people carrying just one high-risk gene-variant. Considering environmental stressors, it is expected that the more stress people have experienced, the higher their neuroticism and depression levels will be. This means that people who had experienced above average childhood trauma have higher neuroticism scores and higher depression prevalence than people who had experienced below average childhood trauma. This also means that people who had experienced stressful life events in the past year score higher on neuroticism and depression than people who had not experienced as such. Moreover, it is hypothesized that stress too has an accumulating effect, meaning that the highest levels of neuroticism come from people who had experienced recent life stress on top of above average experienced stress in their childhood. Concerning gene x environment interactions, it is expected that stressful life events (either in childhood or in the past year) show an interaction with the high-risk variants of each SNP, leading to increased neuroticism scores. On account of the finding that women score higher on neuroticism (Costa & McCrae, 1992) and have higher depression rates than men (Sadock & Sadock, 2007), it is investigated whether the main effects of genes and environment are influenced by sex differences, even after controlling for these differences by using standardized neuroticism scores.

Concerning the hypothesized effects of genes, results show that, in line with the expectations, homozygote carriers of COMT's high-risk gene-variant (Val/Val) have higher neuroticism scores than low-risk Met/Met-carriers. However, this is not the case for both DAO-A SNPs (M15 and M23) as there is no difference in neuroticism scores between DAO-A's high-risk and low-risk groups. Since COMT is involved in dopaminergic functioning, it seems that differences in amount of dopamine availability caused by gene-variation on SNP Val158Met do affect neuroticism, which confirms results found by Hettema and colleagues (2008). The glutamatergic DAO-A SNPs do not have main effects on neuroticism, which could indicate that the influences of glutamate are not (strongly enough) affecting neuroticism. Previous studies that found DAO-A results on psychiatric disorders and neuroticism (Detera-Wadleigh & McMahon, 2006; Rietschel et al., 2008) used a different design for their studies. Rietschel and colleagues (2008) did not specify high-risk and low-risk gene-variants, but merely based their findings on prevalence of each SNP in cases versus controls. The meta-analysis conducted by Detera-Wadleigh and McMahon (2006) confirmed the lack of consistent results across studies

when it comes to the associated alleles of DAO-A. They even suggest that there may not exist any distinctive haplotype that correlates with susceptibility to psychiatric conditions. Considering the expected accumulation of risk when carrying more than one high-risk gene-variant, DAO-A M15 does however show a trend towards higher neuroticism when combined with COMT, with the group carrying both homozygote high-risk alleles presenting the highest neuroticism scores. This trend may be explained by the finding that dopamine and glutamate modulate each other (Tseng & O'Donnell, 2004).

On the subject of hypothesized environmental influences of stress, a clear and strong influence of both childhood trauma and recent life stress is seen on elevated neuroticism levels, which was consistent with what was expected. People who, as a child, experienced above average trauma have considerably higher neuroticism levels, which increases their susceptibility to develop psychiatric disorders (Lahey, 2009; Taylor, Asmundson & Jang, 2011). The same is true for recent life stress: people who had experienced one or more stressful life events in the past year have increased neuroticism levels compared to people who had not experienced such an event. This also displays the dynamic properties of the personality trait neuroticism to rise after experiencing stress. The finding that the neuroticism difference between below or above experienced childhood trauma is bigger than the neuroticism difference between having or not having experienced recent life stress, suggests that early life stress has a more profound impact than recent life. This is supported by a biological explanation on the effects of stress. Environmental stress during early development can alter the functioning and structure of the brain (Nelson, 2000) because of the influence of released stress hormones, and thereby increase the vulnerability for later development of psychiatric conditions (Van der Kolk, McFarlane & Weisaeth, 1996; Teicher, 2002). For environmental stress, it is also expected that early life stress and recent stress can accumulate by further increasing neuroticism. This theory is supported by a trend indicating that people who had experienced recent stress scored higher on neuroticism if they had also experienced above average amounts of stress in their youth. This is in line with the conviction that neuroticism interacts with psychosocial stress (Kendler, Gardner & Prescott, 2003; Kendler, Kuhn & Prescott, 2004).

Considering gene x environment interactions, an interesting result is that the high-risk Val-allele of COMT interacts with childhood trauma and recent life stress to increase neuroticism significantly. This finding provides evidence for the hypothesis that genetic and environmental risks can combine, producing higher neuroticism. It supports the belief that genetics influence the capacity of an environmental risk to bring about psychiatric disorders (Caspi, 2006). However, a lack of similar findings on DAO-A M15 and M23 cannot further support this hypothesis.

In line with the expectations on depression, depression was more prevalent in the group of people who had experienced above average childhood trauma, compared to people who had experienced below average childhood trauma. As for recent life stress, a higher depression rate is seen in the group of people who had experienced stressful events in the past year, which is consistent with the expectations. This means that the factors increasing neuroticism (early life stress and recent life stress) also increase depression. These findings accord with studies reporting neuroticism as a predictor for major depression (Kendler, Gardner & Prescott, 2003; Kendler, Kuhn & Prescott, 2004). Neither gene-SNP had significant differences in depression rates. As for COMT, where the high-risk allele led to higher neuroticism but not to higher depression rates, a possible

explanation for this inconsistency is that environmental adversity has a larger effect on the development of psychiatric disorders than genetic risk. This interpretation is supported by Stegenga and colleagues (2012), who showed that recent life events, regardless the type of life event, pose the largest risk for the onset of major depressive disorder in mid-life. Also, multiple ways have been identified through which early life stress influences the development of psychiatric disorders, including low self-esteem, a cognitive attribution style and family dysfunctions (Roy, 2002).

An unexpected post hoc finding is that a significant sex difference in neuroticism is present in only the high-risk groups of all three included gene SNPs. Women have higher neuroticism levels than men when carrying the homozygote high-risk variant of either gene-variant. This corresponds to previous studies that found an association between COMT and neuroticism: they suggest sex-differences in the relation between the high activity Val-allele of COMT and neuroticism (Eley *et al.*, 2003; Pełka-Wysiecka *et al.*, 2012). Also, for both DAO-A SNPs M15 and M23, it seems that men score slightly higher when carrying the low-risk variant (i.e. G/G and T/T, respectively) compared to men carrying the high-risk variant (i.e. A/A and C/C). This small but interesting effect could clarify some of the inconsistency amongst studies as to which gene-variant is DAO-A's risk variant. However, given the focus of the present study, no extended investigation will be done to further examine these results.

The current study has several strengths and limitations. A strength of the study is its innovative combination of environment and multiple genes, involved in both dopaminergic and glutamatergic functioning. Also, all participants are selected from the large-scale research of the Utrecht Health Program (UHP; Grobbee *et al.*, 2005), which allows for replication of the analyses on a large sample size. Per participant, a lot of data is collected, which makes it a quantatively strong dataset. Another strength is that, between gene groups, people were matched on postal code (for social economic status), age and sex, which facilitates accurate between-group comparisons. Also, there are little data missing, which increases the accuracy of the between-group matching. Since genotyping is a precarious procedure, the participants were genotyped multiple times for quality-controls on which gene-variants each participant is carrying. A limitation of the current study is its inclusion of only healthy, Caucasian participants. This decreases its generalizing properties. Also, the fact that only healthy participants were included, could have lowered depression frequencies. A third limitation of the study is the relatively small sample size, which might have decreased genetic effects. Another limitation is that a selection bias could have occurred, since participants were invited but were not obligated to participate. This could have filtered away the people who have a busier daily life, who see no use in scientific research and/or who are experiencing adverse times and therefore do not want to participate.

Further research could include a broader range of people who are more evenly grouped on social economic status and education levels to increase generalizability. Also, a larger sample size could improve the results that are now marginally significant. A larger sample size could also lead to stronger genetic effects. It would be interesting for further research to investigate the interaction-effects of genetic risk and environmental stress on depression. Also, the unexpected result of women having higher neuroticism when carrying the high-risk gene-variant of COMT, DAO-A M15 or DAO-A M23 requires additional examination. Considering gene-

variants, uncertainty and inconsistency still exists on which gene-variant can be considered high-risk, especially for the DAO-A gene.

Concluding, the current study made meaningful contributions to the investigation on how genetic and environmental factors contribute to the susceptibility of developing psychiatric disorders. Decreased dopamine availability caused by the Val-allele of the COMT gene leads to significant increases in neuroticism, a personality trait that is considered a predictor for numerous psychiatric disorders (Rietschel *et al.,* 2008; Lahey, 2009; Taylor, Asmundson & Jang, 2011). No effect of the glutamatergic DAO-A SNPs M15 and M23 is found. The amount of experienced environmental stress, either in childhood or in recent life, produces strong raises in neuroticism as well as in depression frequency. Early life stress has more profound effects on neuroticism than recent life stress, possibly caused by alterations in the functioning and structure of the brain by the influence of released stress hormones (Nelson, 2000; Van der Kolk, McFarlane & Weisaeth, 1996; Teicher, 2002). A trend indicates that environmental stress can accumulate, since people who have experienced recent life stress have increased neuroticism if they also have experienced above average childhood trauma. Support is found for the belief that genes and environment interact, considering that COMT, childhood trauma and recent life stress combine in elevating neuroticism. A trend is found on gene x gene interaction, as a combination of COMT and DAO-A M15 slightly increases neuroticism. An unexpected but interesting sex difference occurs in the high-risk gene-variants of COMT and DAO-A, asking for further analysis.

The findings of this study address the important influences of genetic variation in its ability (by itself and in combination with environmental factors) to produce measurable adverse psychological effects. Also, the impact of early life stress and recent life stress on psychological wellbeing is considerable, even more because of the seemingly accumulating properties of stress. This emphasizes the importance of good parenting and the availability of suiting psychological treatment for people experiencing life stress in order to offset their increased susceptibility to develop psychiatric disorders.

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Addendum A – Demographics

Table 1a: Descriptive statistics (Age, Sex, Social Economic Status derived from education, Social Economic Status derived from income) sorted by variants of the COMT-gene (Homozygous Val, Heterozygous Val/Met, Homozygous Met).

	Total Group (N=397)	**COMT Val/Val (N=81)**	**COMT Val/Met (N=172)**	**COMT Met/Met (N=110)**	**Statistical value**	**Significance**
Age						
Mean (SD)	45 (12.4)	46 (12)	45 (12)	46 (13)	F = .026	*p* = .974
Range	20-86	23-85	20-83	22-86		
Sex						
Male	181 (45.6%)	38 (46.9%)	81 (47.1%)	49 (44.5%)	F = .095	*p* = .909
Female	216 (54.4%)	43 (53.1%)	91 (52.9%)	61 (55.5%)		
SES from education						
Low	57 (14.4%)	10 (12.3%)	27 (15.7%)	16 (14.5%)		
Medium	137 (34.5%)	29 (35.8%)	58 (33.7%)	42 (38.2%)	F = .456	*p* = .634
High	177 (44.6%)	39 (48.1%)	79 (45.9%)	43 (39.1%)		
Missing	*26 (6.5%)*	*3 (3.7%)*	*8 (4.7%)*	*9 (8.2%)*		
SES from income						
Low	28 (7.1%)	7 (8.6%)	14 (8.1%)	6 (5.5%)		
Medium	35 (8.8%)	4 (4.9%)	18 (10.5%)	11 (10%)	F = .325	*p* = .723
High	308 (77.6%)	67 (82.7 %)	132 (76.7%)	84 (76.4%)		
Missing	*26 (6.5%)*	*3 (3.7%)*	*8 (4.7%)*	*9 (8.2%)*		

Table 1b: Descriptive statistics (Age, Sex, Social Economic Status derived from education, Social Economic Status derived from income) sorted by rs2391191 variants of the DAO-A gene (Homozygous A, Heterozygous A/G, Homozygous G).

	Total Group (N=397)	**DAO-A M15 A/A (N=77)**	**DAO-A M15 A/G (N=122)**	**DAO-A M15 G/G (N=137)**	**Statistical value**	**Significance**
Age						
Mean (SD)	45 (12.4)	46 (10)	48 (12)	45 (12)	F = 1.393	*p* = .25
Range	20-86	25-74	26-83	29-86		
Sex						
Male	181 (45.6%)	41 (53.2%)	56 (45.9%)	56 (40.9%)	F = 1.526	*p* = .219
Female	216 (54.4%)	36 (46.8%)	66 (54.1%)	81 (59.1%)		
SES from education						
Low	57 (14.4%)	11 (14.3%)	18 (14.8%)	21 (15.3%)		
Medium	137 (34.5%)	36 (46.8%)	42 (34.4%)	45 (32.8%)	F = .767	*p* = .465
High	177 (44.6%)	30 (39%)	62 (50.8%)	71 (51.8%)		
Missing	*26 (6.5%)*					
SES from income						
Low	28 (7.1%)	4 (5.2%)	7 (5.7%)	11 (8%)		
Medium	35 (8.8%)	13 (16.9%)	11 (9%)	7 (5.1%)	F = .405	*p* = .668
High	308 (77.6%)	60 (77.9%)	104 (85.2%)	119 (86.9%)		
Missing	*26 (6.5%)*					

Table 1c: Descriptive statistics (Age, Sex, Social Economic Status derived from education, Social Economic Status derived from income) sorted by rs3918342 variants of the DAO-A gene (Homozygous C, Heterozygous C/T, Homozygous T).

	Total Group (N=397)	**DAO-A M23 C/C (N=110)**	**DAO-A M23 C/T (N=138)**	**DAO-A M23 T/T (N=94)**	**Statistical value**	**Significance**
Age						
Mean (SD)	45 (12.4)	46 (11)	48 (12)	46 (11)	F = .882	*p* = .415
Range	20-86	25-82	29-86	29-83		
Sex						
Male	181 (45.6%)	49 (44.5%)	63 (45.7%)	45 (47.9%)	F = .115	*p* = .891
Female	216 (54.4%)	61 (55.5%)	75 (54.3%)	49 (52.1%)		
SES from education						
Low	57 (14.4%)	21 (19.1%)	22 (15.9%)	10 (10.6%)		
Medium	137 (34.5%)	39 (35.5%)	44 (31.9%)	41 (43.6%)	F = .627	*p* = .535
High	177 (44.6%)	50 (45.5%)	72 (52.2%)	43 (45.7%)		
Missing	*26 (6.5%)*					
SES from income						
Low	28 (7.1%)	9 (8.2%)	8 (5.8%)	7 (7.4%)		
Medium	35 (8.8%)	13 (11.8%)	11 (8%)	7 (7.4%)	F = .723	*p* = .486
High	308 (77.6%)	88 (80%)	119 (86.2%)	80 (85.1%)		
Missing	*26 (6.5%)*					

Table 1d: Descriptive statistics (Age, Sex, Social Economic Status derived from education, Social Economic Status derived from income) sorted by amount of experienced childhood trauma (below average or above average)

	Total Group (N=397)	**Childhood trauma: below average N=212)**	**Childhood trauma: above average (N=185)**	**Statistical value**	**Significance**
Age					
Mean (SD)	45 (12.4)	44 (12.3)	47 (12.3)	F = 4.673	*p* = .031*
Range	20-86	20-86	21-82		
Sex					
Male	181 (45.6%)	93 (43.9%)	88 (47.6%)	F = .543	*p* = .462
Female	216 (54.4%)	119 (56.1%)	97 (52.4%)		
SES from education					
Low	57 (14.4%)	24 (11.3%)	33 (17.8%)		
Medium	137 (34.5%)	68 (32.1%)	69 (37.3%)	F = 6.213	*p* = .013*
High	177 (44.6%)	105 (49.5%)	72 (38.9%)		
Missing	*26 (6.5%)*	15 (7.1%)	11 (5.9%)		
SES from income					
Low	28 (7.1%)	15 (7.1%)	13 (7%)		
Medium	35 (8.8%)	15 (7.1%)	20 (10.8%)	F = .354	*p* = .552
High	308 (77.6%)	167 (78.8%)	141 (76.2%)		
Missing	*26 (6.5%)*	15 (7.1%)	11 (5.9%)		

* Significant at a $p < .05$ level (two-tailed).

Table 1e: Descriptive statistics (Age, Sex, Social Economic Status derived from education, Social Economic Status derived from income) sorted by experienced recent life events (yes or no).

	Total Group (N=397)	**Recent life events: yes (N=239)**	**Recent life events: no (N=158)**	**Statistical value**	**Significance**
Age					
Mean (SD)	45 (12.4)	45 (12.6)	45 (12.1)	F = .026	*p* = .974
Range	20-86	21-85	20-86		
Sex					
Male	181 (45.6%)	109 (45.6%)	72 (45.6%)	F = .079	*p* = .778
Female	216 (54.4%)	130 (54.4%)	86 (54.4%)		
SES from education					
Low	57 (14.4%)	33 (13.8%)	24 (15.2%)		
Medium	137 (34.5%)	88 (36.8%)	49 (31%)	F < .001	*p* = .994
High	177 (44.6%)	102 (42.7%)	75 (47.5%)		
Missing	*26 (6.5%)*	16 (6.7%)	10 (6.3%)		
SES from income					
Low	28 (7.1%)	14 (5.9%)	14 (8.9%)		
Medium	35 (8.8%)	20 (8.4%)	15 (9.5%)	F = 1.499	*p* = .222
High	308 (77.6%)	189 (79.1%)	119 (75.3%)		
Missing	*26 (6.5%)*	16 (6.7%)	10 (6.3%)		

Addendum B – Statistical Analyses

Main effects

Table 2: Main effects of gene SNPs (COMT Val158Met, DAO-A M15 and DAO-A M23), stressful life events (childhood trauma and recent life stress) and sex on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex-differences.

	Groups	Neuroticism stanines Mean (SD)	Statistical value	Significance
COMT (N=191)	Val/Val (N=81)	6.46 (2)	r_{pb} = .15	p = .037*
	Met/Met (N=110)	5.83 (2.1)		
DAO-A M15 (N=214)	A/A (N=77)	6.13 (2.1)	r_{pb} = .03	p = .714
	G/G (N=137)	6.02 (2)		
DAO-A M23 (N=204)	C/C (N=110)	6.19 (2)	r_{pb} = .27	p = .328
	T/T (N=94)	5.9 (2.1)		
Childhood trauma (N=397)	Below average (N=212)	5.56 (2.1)	r_{pb} = .1	p = .000***
	Above average (N=185)	6.71 (2.1)		
Recent life stress (N=397)	Yes (N=239)	6.36 (2.2)	r_{pb} = .15	p = .002**
	No (N=158)	5.69 (2.1)		
Sex (N=397)	Male (N=181)	6 (2.2)	r_{pb} = .04	p = .408
	Female (N=216)	6.18 (2.2)		

* Significant at a $p < .05$ level (two-tailed).
** Significant at a $p < .01$ level (two-tailed).
*** Significant at a $p < .001$ level (two-tailed).

Gene x Gene (x Gene) interaction-effects

Table 4a: Interaction effects of homozygote COMT gene-variants and homozygote DAO-A M15 gene-variants on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex differences.

COMT gene variant	DAO-A M15 gene variant	Neuroticism stanines Mean (SD)	Statistical value	Significance
Val/Val	A/A (N=17)	6.76 (2.1)	*F* = 3.85	*p* = .053
	G/G (N=27)	5.56 (2)		
Met/Met	A/A (N=23)	5.7 (2.1)		
	G/G (N=40)	6.1 (1.9)		
Total	N=107	5.98 (2)		

Table 4b: Interaction effects of homozygote COMT gene-variants and homozygote DAO-A M23 gene-variants on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex differences.

COMT gene variant	DAO-A M23 gene variant	Neuroticism stanines Mean (SD)	Statistical value	Significance
Val/Val	C/C (N=20)	6.7 (1.8)	*F* = 1.63	*p* = .205
	T/T (N=23)	5.87 (2.1)		
Met/Met	C/C (N=35)	5.83 (1.9)		
	T/T (N=26)	6.03 (2.3)		
Total	N=104	6.06 (2)		

Table 4c: Interaction effects of homozygote DAO-A M15 gene-variants and homozygote DAO-A M23 gene-variants on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex-differences.

DAO-A M15 gene variant	DAO-A M23 gene variant	Neuroticism stanines Mean (SD)	Statistical value	Significance
A/A	C/C (N=29)	6.17 (2.2)	*F* = .8	*p* = .374
	T/T (N=33)	6.27 (1.9)		
G/G	C/C (N=50)	6.18 (1.9)		
	T/T (N=29)	5.66 (2.1)		
Total	N=141	6.09 (2)		

Table 4d: Interaction effects of homozygote COMT gene-variants, homozygote DAO-A M15 gene-variants and homozygote DAO-A M23 gene-variants on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex differences.

COMT gene variant	DAO-A M15 gene variant	DAO-A M23 gene variant	Neuroticism stanines Mean (SD)	Statistical value	Significance
Val/Val (N=31)	A/A (N=14)	C/C (N=5)	6.2 (2.2)	*F* = .24	*p* = .626
		T/T (N=9)	6.44 (2.2)		
	G/G (N=17)	C/C (N=7)	6.9 (1.7)		
		T/T (N=10)	5.1 (2.3)		
Met/Met (N=45)	A/A (N=19)	C/C (N=8)	5.38 (2.4)		
		T/T (N=11)	6.27 (2)		
	G/G (N=26)	C/C (N=19)	6.11 (1.9)		
		T/T (N=7)	6 (2)		
Total		N=76	6.03 (2)		

Gene x Environment interaction-effects

Table 5a: Interaction effects of below or above average experienced childhood trauma and COMT gene-variants on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex differences.

	Groups	COMT gene variant	Neuroticism stanines Mean (SD)	Statistical value	Significance
Childhood trauma	Below average (N=107)	Val/Val (N=46)	6.09 (2.1)	*F* = .002	*p* = .961
		Met/Met (N=61)	5.46 (2)		
	Above average (N=84)	Val/Val (N=35)	6.94 (1.8)		
		Met/Met (N=49)	6.29 (2.1)		
	Total	N=191	6.09 (2.1)		

Table 5b: Interaction effects of below or above average experienced childhood trauma and the DAO-A M15 gene-variants on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex differences.

	Groups	DAO-A M15 gene variant	Neuroticism stanines Mean (SD)	Statistical value	Significance
Childhood trauma	Below average (N=109)	A/A (N=39)	5.77 (2)	*F* = .23	*p* = .63
		G/G (N=70)	5.53 (1.9)		
	Above average (N=105)	A/A (N=38)	6.5 (2.2)		
		G/G (N=67)	6.54 (2.1)		
	Total	N=214	6.06 (2.1)		

Table 5c: Interaction effects of below or above average experienced childhood trauma and the DAO-A M23 gene-variants on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex differences.

	Groups	DAO-A M23 gene variant	Neuroticism stanines Mean (SD)	Statistical value	Significance
Childhood trauma	Below average (N=108)	C/C (N=54)	6.02 (1.8)	*F* = .81	*p* = .371
		T/T (N=54)	5.54 (2.1)		
	Above average (N=96)	C/C (N=56)	6.36 (2.3)		
		T/T (N=40)	6.4 (2.1)		
	Total	N=204	6.06 (2.1)		

Table 6a: Interaction effects of experienced recent life stress and COMT gene-variants on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex differences.

	Groups	COMT Gene variant	Neuroticism stanines Mean (SD)	Statistical value	Significance
Recent life stress	Yes (N=107)	Val/Val (N=49)	6.96 (1.9)	*F* = 1.78	*p* = .184
		Met/Met (N=58)	6.05 (2.2)		
	No (N=84)	Val/Val (N=32)	5.69 (1.9)		
		Met/Met (N=52)	5.58 (2)		
	Total	N=191	6.09 (2.1)		

Table 6b: Interaction effects of experienced recent life stress and the DAO-A M15 gene-variants on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex differences.

	Groups	DAO-A M15 Gene variant	Neuroticism stanines Mean (SD)	Statistical value	Significance
Recent life stress	Yes (N=126)	A/A (N=42)	6.71 (2)	*F* = .69	*p* = .408
		G/G (N=84)	6.33 (2)		
	No (N=88)	A/A (N=35)	5.43 (2.1)		
		G/G (N=53)	5.53 (1.9)		
	Total	N=214	6.06 (2.1)		

Table 6c: Interaction effects of experienced recent life stress and the DAO-A M23 gene-variants on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex differences.

	Groups	DAO-A M23 Gene variant	Neuroticism stanines Mean (SD)	Statistical value	Significance
Recent life stress	Yes (N=118)	C/C (N=72)	6.47 (2)	*F* = .21	*p* = .649
		T/T (N=46)	6.46 (2.2)		
	No (N=86)	C/C (N=38)	5.66 (2.1)		
		T/T (N=48)	5.38 (1.9)		
	Total	N=204	6.06 (2.1)		

Environment x Environment interaction-effect

Table 7: Interaction effects of below or above average experienced childhood trauma and experienced recent life stress on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex differences.

	Groups	Recent life stress	Neuroticism stanines Mean (SD)	Statistical value	Significance
Childhood trauma	Below average (N=212)	Yes (N=114)	5.64 (2.2)	*F* = 3.53	*p* = .061
		No (N=98)	5.47 (2)		
	Above average (N=185)	Yes (N=125)	7.03 (1.9)		
		No (N=60)	6.05 (2.2)		
	Total	N= 397	6.1 (2.2)		

Gene x Gene x Environment interaction-effects

Table 8a: Interaction effects of below or above average experienced childhood trauma, homozygote COMT gene-variants and homozygote DAO-A M15 gene-variants on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex differences.

Childhood trauma	COMT	M15	Neuroticism stanines Mean (SD)	Statistical value	Significance
Below average (N=58)	Val/Val	A/A (N=12)	6.5 (2)	*F* = .15	*p* = .697
		G/G (N=16)	5.06 (1.9)		
	Met/Met	A/A (N=10)	5.1 (2)		
		G/G (N=20)	5.75 (1.8)		
Above average (N=49)	Val/Val	A/A (N=5)	7.4 (2.5)		
		G/G (N=11)	6.27 (2.1)		
	Met/Met	A/A (N=13)	6.15 (2.1)		
		G/G (N=20)	6.45 (2.1)		
Total		N=107	5.98 (2)		

Table 8b: Interaction effects of below or above average experienced childhood trauma, homozygote COMT gene-variants and homozygote DAO-A M23 gene-variants on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex differences.

Childhood trauma	COMT	M23	Neuroticism stanines Mean (SD)	Statistical value	Significance
Below average (N=58)	Val/Val	C/C (N=13)	6.62 (1.4)	*F* = .03	*p* = .87
		T/T (N=16)	5.6 (2.2)		
	Met/Met	C/C (N=16)	5.69 (1.6)		
		T/T (N=13)	5.77 (2.4)		
Above average (N=46)	Val/Val	C/C (N=7)	6.86 (2.5)		
		T/T (N=7)	6.43 (2)		
	Met/Met	C/C (N=19)	5.95 (2.2)		
		T/T (N=13)	6.31 (2.3)		
Total		N=104	6.06 (2)		

Table 8c: Interaction effects of below or above average experienced childhood trauma, homozygote DAO-A M15 gene-variants and homozygote DAO-A M23 gene-variants on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex differences.

Childhood trauma	M15	M23	Neuroticism stanines Mean (SD)	Statistical value	Significance
Below average (N=72)	A/A	C/C (N=15)	6 (2.1)	*F* = .08	*p* = .781
		T/T (N=19)	6.16 (1.9)		
	G/G	C/C (N=24)	6.04 (1.5)		
		T/T (N=14)	5.36 (2)		
Above average (N=69)	A/A	C/C (N=14)	6.36 (2.5)		
		T/T (N=14)	6.43 (1.9)		
	G/G	C/C (N=26)	6.31 (2.3)		
		T/T (N=15)	5.82 (1.9)		
Total		N=141	6.09 (2)		

Gene x Environment x Environment interaction-effects

Table 9a: Interaction effects of homozygote COMT gene-variants, below or above average experienced childhood trauma and experienced recent life stress on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex differences.

COMT gene variant	Childhood trauma	Recent life stress	Neuroticism stanines Mean (SD)	Statistical value	Significance
Val/Val	Below average (N=46)	Yes (N=25)	6.8 (2.1)		
		No (N=21)	5.24 (1.7)		
	Above average (N=35)	Yes (N=24)	7.12 (1.7)		
		No (N=11)	6.55 (2.1)		
	Total	N=81	6.46 (2)		
Met/Met	Below average (N=61)	Yes (N=26)	5.23 (2.1)	*F* = 4.7	*p* = .031*
		No (N=35)	5.63 (2)		
	Above average (N=49)	Yes (N=32)	6.72 (2.1)		
		No (N=17)	5.47 (2.1)		
	Total	N=110	5.83 (2.1)		
Total		N=191	6.09 (2.1)		

* Significant at a $p < .05$ level.

Table 9b: Interaction effects of homozygote DAO-A M15 gene-variants, below or above average experienced childhood trauma and experienced recent life stress on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex differences.

DAO-A M15 gene variant	Childhood trauma	Recent life stress	Neuroticism stanines Mean (SD)	Statistical value	Significance
A/A	Below average (N=39)	Yes (N=19)	6 (2.1)		
		No (N=20)	5.56 (2)		
	Above average (N=38)	Yes (N=23)	7.3 (1.7)		
		No (N=15)	5.27 (2.4)		
	Total	N=77	6.13 (2.1)		
G/G	Below average (N=70)	Yes (N=45)	5.71 (2)	*F* = .59	*p* = .445
		No (N=25)	5.2 (1.6)		
	Above average (N=67)	Yes (N=39)	7.1 (1.9)		
		No (N=28)	5.83 (1.9)		
	Total	N=137	6.02 (2)		
Total		N=214	6.06 (2.1)		

Table 9c: Interaction effects of homozygote DAO-A M23 gene-variants, below or above average experienced childhood trauma and experienced recent life stress on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex differences.

DAO-A M23 gene variant	Childhood trauma	Recent life stress	Neuroticism stanines Mean (SD)	Statistical value	Significance
C/C	Below average (N=54)	Yes (N=32)	6.19 (1.9)		
		No (N=22)	5.77 (1.7)		
	Above average (N=56)	Yes (N=40)	6.7 (2)		
		No (N=16)	5.5 (2.6)		
	Total	N=110	6.19 (2)		
T/T	Below average (N=54)	Yes (N=24)	5.54 (2.2)	*F* = 1.77	*p* = .185
		No (N=30)	5.53 (2)		
	Above average (N=40)	Yes (N=22)	7.45(1.7)		
		No (N=18)	5.11 (1.78)		
	Total	N=94	5.9 (2.1)		
Total		N=204	6.06 (2.1)		

Influence of sex differences on main effects

Table 10: Sex as a covariate of gene SNPs effects (COMT, DAO-A M15 and DAO-A M23) and environmental effects (childhood trauma and recent life stress) on mean neuroticism scores (and standard deviations), standardized into stanines to correct for sex-differences.

	Groups	Neuroticism stanines Mean (SD)	Statistical value	Significance
COMT (N=191)	Val/Val (N=81)	6.46 (2)	Sex: F = 7.86 COMT: F = 4.83	p = .006** p = .029*
	Met/Met (N=110)	5.83 (2.1)		
DAO-A M15 (N=214)	A/A (N=77)	6.13 (2.1)	Sex: F = 3.2 M15: F = .34	p = .075 p = .562
	G/G (N=137)	6.02 (2)		
DAO-A M23 (N=204)	C/C (N=110)	6.19 (2)	Sex: F = 1.33 M23: F = .89	p = .251 p = .347
	T/T (N=94)	5.9 (2.1)		
Childhood trauma (N=397)	Below average (N=212)	5.56 (2.1)	Sex: F = 1.13 CT: F = 30.52	p = .288 p = .000***
	Above average (N=185)	6.71 (2.1)		
Recent life stress (N=397)	Yes (N=239)	6.37 (2.2)	Sex: F = .7 RLE: F = 9.56	p = .402 p = .002**
	No (N=158)	5.69 (2.1)		

* Significant at a p < .05 level (two-tailed).
** Significant at a p < .01 level (two-tailed).
*** Significant at a p < .001 level (two-tailed).

Table 11: Sex differences in standardized neuroticism scores (and standard deviations) in the homozygote gene-variant groups of COMT (Val or Met), DAO-A M15 (A or G) and DAO-A M23 (C or T), the level of experienced stress in childhood and the occurrence of stressful recent life events.

	Chorus	Sex	Neuroticism stanines Mean (SD)	Statistical Value	Significance
COMT gene-variant (N=181)	Val/Val (N=71)	Male (N=38)	6.05 (2)	r_{pb} = .21	p = .031*
		Female (N=43)	6.81 (2)		
	Met/Met (N=110)	Male (N=49)	5.35 (2.2)	r_{pb} = .19	p = .084
		Female (N=61)	6.21 (2)		
DAO-A M15 gene-variant (N=214)	A/A (N=77)	Male (N=41)	5.7 (2.3)	r_{pb} = .24	p = .038*
		Female (N=36)	6.7 (1.9)		
	G/G (N=137)	Male (N=56)	5.9 (2.1)	r_{pb} = .05	p = .536
		Female (N=81)	6.1 (2)		
DAO-A M23 gene variant (N=204)	C/C (N=110)	Male (N=49)	5.7 (2.2)	r_{pb} = .21	p = .028*
		Female (N=61)	6.6 (1.8)		
	T/T (N=94)	Male (N=45)	6 (2.2)	r_{pb} = -.06	p = .54
		Female (N=49)	5.8 (2)		
Childhood trauma (N=397)	Below average (N=212)	Male (N=93)	5.5 (2.1)	r_{pb} = .02	p = .734
		Female (N=119)	5.6 (2.1)		
	Above average (N=185)	Male (N=88)	6.5 (2.1)	r_{pb} = .09	p = .23
		Female (N=97)	6.9 (2)		
Recent life events (N=397)	Yes (N=239)	Male (N=109)	6.4 (2.1)	r_{pb} = .11	p = .16
		Female (N=130)	6.4 (2.2)		
	No (N=158)	Male (N=72)	5.4 (2.2)	r_{pb} = -.003	p = .958
		Female (N=86)	5.9 (2.1)		

* Significant at a p < .05 level (two-tailed).

Main effects on depression

Table 12: Main effects of gene-variants (COMT, DAO-A M15 and DAO-A M23) and environment (below or above average experienced childhood trauma and experienced recent life stress) on depression rates (and percentage).

	Groups	Depression frequencies	Statistical value	Significance
COMT (N=191)	Val/Val (N=81)	4 (4.9%)	X^2 = .2	p = .724
	Met/Met (N=110)	4 (3.6%)		
DAO-A M15 (N=214)	A/A (N=74)	3 (3.9%)	X^2 = .53	p = .669
	G/G (N=134)	3 (2.2%)		
DAO-A M23 (N=204)	C/C (N=110)	5 (4.5%)	X^2 = .89	p = .455
	T/T (N=94)	2 (2.1%)		
Childhood trauma (N=397)	Below average (N=212)	2 (0.9%)	X^2 = 11.21	p = .001**
	Above average (N=185)	14 (7.6%)		
Recent life stress (N=397)	Yes (N=239)	14 (5.9%)	X^2 = 5.19	p = .034*
	No (N=158)	2 (1.3%)		
Sex (N=397)	Male (N=181)	7 (3.9%)	X^2 = .023	p = .88
	Female (N=216)	9 (4.2%)		

* Significant at a $p < .05$ level.

** Significant at a $p < .01$ level.