Association of a functional variant of the nitric oxide synthase 1 gene with personality, anxiety, and depressiveness

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Abstract
A functional promoter polymorphism of the nitric oxide synthase 1 gene first exon 1f variable tandem repeat (NOS1 ex1f-VNTR) is associated with impulsivity and related psychopathology. Facets of impulsivity are strongly associated with personality traits; maladaptive impulsivity with neuroticism, and adaptive impulsivity with extraversion. Both high neuroticism and low extraversion predict anxiety and depressive symptoms. The aim of the present study was to evaluate the effect of the NOS1 ex1f-VNTR genotype and possible interaction with environmental factors on personality, anxiety, and depressiveness in a population-representative sample. Short allele carriers had higher neuroticism and anxiety than individuals with the long/long (l/l) genotype. Male short/short homozygotes also had higher extraversion. In the face of environmental adversity, females with a short allele had higher scores of neuroticism, anxiety, and depressiveness compared to the l/l genotype. Males were more sensitive to environmental conditions when they had the l/l genotype and low extraversion. In conclusion, the NOS1 ex1f-VNTR influences personality and emotional regulation dependent on gender and environment. Together with previous findings on the effect of the NOS1 genotype on impulse control, these data suggest that NOS1 should be considered another plasticity gene, because its variants are associated with different coping strategies.

Individuals with high neuroticism and impulsivity are predisposed to psychiatric disorders (Congdon & Canli, 2008; Lönnquist, Verkasalo, Mäkinen, & Henriksson, 2009; ten Have, Oldehinkel, Vollerbergh, & Ormel, 2005). Expression of these traits is moderated by environmental and genetic factors. The impact of genetic variation and environmental effects is known to converge at the level of regulation of neurochemical transmission. For example, emotional behavior and/or personality traits are related to serotonergic (Kriegebaum, Gütkencht, Schmitt, Lesch, & Reif, 2010; Oreland, Nordquist, Hallmann, Harro, & Nilsson, 2010) and dopaminergic systems (Kestler, Malhotra, Finch, Adler, & Breier, 2000; Reiner & Spangler, 2011; Smillie, Cooper, Proitsi, Powell, & Pickering, 2010). Both systems are regulated by nitric oxide (NO), a gaseous messenger molecule. NO is produced from the amino acid arginine catalyzed by the enzyme NO synthase (NOS). One form of this enzyme is the neuronal form NOS1 (Kiss & Vici, 2001). Recently, a dinucleotide variable number tandem repeat (VNTR) polymorphism in the promoter region of the alternative first exon 1f (ex1f) of the human neuronal nitric oxide synthase (NOS1 ex1f-VNTR) has been found to affect impulsivity and related psychopathology in different populations (Laas et al., 2010; Reif et al., 2009, 2011). The human NOS1 gene comprises 28 coding exons, as well as at least 9 different and alternative first exons (exons 1a–1j), which are spliced to the same second exon. Each of the first exons is driven by its own promoter; however, none of them is translated into protein. Exons 1c and 1f are most abundant in the brain. NOS1 ex1f-VNTR is highly polymorphic and thus has been dichotomized into long and short alleles. Dichotomization originally was done according to a median split of the repeat number (Reif et al., 2006), with up to 19 repeats being designated as “short.” Using a luciferase reporter assay, it was demonstrated that the long variant of NOS1 ex1f-VNTR results in significantly increased reporter gene activity as compared with intermediate and short variants (Reif et al., 2009; Rife et al., 2009). In healthy controls, 21% of subjects are homozygous for short alleles whereas 28% are homozygous for long alleles (n = 8,719 subjects; Reif et al., unpublished data). There is no significant difference between samples of different origin, as far as they have been tested (samples from Germany, Italy, Sweden, Estonia, Austria, Norway, and The Netherlands).

Studies in mice have revealed that knocking out the NOS1 results in behavioral changes involving the anxiolytic-like phenotype (Zhang et al., 2010) and increased aggressiveness (Nelson, Trainor, Chiavegatto, & Demas, 2006; Trainor,
Workman, Jessen, & Nelson, 2007). The low-expression NOS1 ex1f-VNTR short/short (s/s) genotype parallels this behavior: it is found more frequently among adults with impulsive behavior and impulsivity-related disorders, for example, attention-deficit/hyperactivity disorder and Cluster B personality disorder (Reif et al., 2009). In the general population, participants with the s/s genotype missed significantly more hits on the Stop signal task ($\eta_p^2 = 0.02$; Reif et al., 2011), which reflects deficits in vigilance (Malloy-Diniz, Fuentes, Borges Leite, Correa, & Bechara, 2007), and had higher maladaptive impulsivity if they had experienced inferior family relationships ($\eta_p^2 = 0.02–0.04$; Reif et al., 2011). In conclusion, neuronal NOS function is pertinent to impulsivity, and the people with the lower expressing NOS1 s/s genotype may be more vulnerable to impulsivity-related psychiatric disorders.

Impulsivity is considered to be a heterogeneous construct (Evenden, 1999). While there are quite a number of classifications of and approaches to impulsivity, few can be used to explain why such an apparently inferior trait is so prevalent. One attempt to explain the benefits of impulsivity is to divide it into functional impulsivity, which incorporates willingness to take risks in situations where this behavior is appropriate, and dysfunctional impulsivity, described as a tendency to act with less forethought, when this tendency is a source of difficulty (Dickman, 1990). Dysfunctional impulsivity is associated with increased neuroticism (Zadreve, Buicik, & Sočan, 2005), while functional impulsivity and sensation seeking rather correlate with extraversion (Aluja, García, & García, 2003; Smillie & Jackson, 2006; Zadreve, Buicik, & Sočan, 2005). Both high neuroticism and low extraversion predict anxiety and depressive symptoms (Chioqueta & Stiles, 2005; Kerscher, Rapee, & Schniering, 2009; Muris et al., 2009). Neuronal NOS has been associated with anxiety and depression in both animals and humans (Reif et al., 2006; Workman, Trainor, Finly, & Nelson, 2008; Wultsch et al., 2007; Zhang et al., 2010). Hence, investigation of the NOS1 genotype effect on basic personality traits, anxiety, and depressiveness is a rational consequence. Although neuroticism and depression share to a large extent their genetic underpinnings (Kendler, Gatz, Gardner, & Pedersen, 2006), extraversion does not have a similar association with depression. Nevertheless, high extraversion is associated with fewer symptoms of depression and lower anxiety (Jylhä & Isometsa, 2006). Because extraversion has been considered as a proxy measure for positive affect (Lucas, Le, & Dyrenforth, 2008), and positive affect has an independent, protective role in the pathogenesis of depression (Geschwind et al., 2011), the association between NOS1 and negative emotionality needs to be considered in the context of extraversion. Because there are gender differences in personality and depressiveness (Schmitt, Realo, Voracek, & Allik, 2008; Van de Velde, Bracke, & Leveque, 2010), gender was included in the analysis.

Starting from adolescence, measures of extraversion increase and measures of neuroticism decrease (Roberts, Walton, & Viechtbauer, 2006). Both of these personality traits are associated with the development of the prefrontal cortex: extraversion has been found positively related to orbitofrontal volume (Cremers et al., 2011; DeYoung et al., 2010) and neuroticism negatively associated with the volume of the right dorsomedial prefrontal cortex (DeYoung et al., 2010). The prefrontal cortex is still developing in adolescent years (Insel, 2010), and prefrontal functioning is suggested to be impaired in NOS1 ex1f-VNTR short allele carriers in a gene–dose dependent fashion (Reif et al., 2009). The development of the prefrontal cortex is associated also with environment (McLaughlin, Fox, Zeanah, & Nelson, 2011), and environmental conditions have been found to play an important role in gene–phenotype association studies (Uher & McGuffin, 2010).

Therefore, to clarify the potential impact of the NOS1 ex1f-VNTR on personality development and its possible interaction with environment, we performed a longitudinal analysis of a database on a population-representative sample of adolescents to examine the effect of NOS1 ex1f-VNTR on basic personality traits, anxiety, and depressiveness dependent on environmental conditions.

Materials and Methods

Participants

The sample of the present study was based on the younger cohort of the population-representative European Youth Heart Study, originally conducted in Estonia in 1998–1999, which was incorporated into the longitudinal Estonian Children Personality, Behaviour, and Health Study. The rationale and procedure of sample formation have been described elsewhere (Harro et al., 2001, 2009). In brief, all schools of Tartu County, Estonia, that agreed to participate (54 of the total of 56) were included in the sampling, using the probability proportional to the number of students of the respective age groups in the school, and 25 schools were selected. In 1998–1999, all children from Grades 3 and 9 were invited to participate, and written informed consent was received from 79% of the invited subjects and their parents. The total number of subjects in this sampling was 1,176, including 593 in the younger cohort. The data for the present study were collected during the follow-ups of the younger cohorts in 2004 and 2007, where we were able to recruit, respectively, 83% ($n = 483$; 222 males, 261 females) and 78% ($n = 453$; 201 males, 252 females) of the original sample. The mean age of the participants studied in 2004 and 2007 was $15.3 \pm 0.3$ and $18.0 \pm 0.3$ years, respectively. All participants were Caucasians. Adolescents and their parents gave their informed consent in all study waves, and the study procedure was approved by the Committee of Ethics of the University of Tartu, Estonia.

Measures

Measures of personality. The five-factor personality (neuroticism, extraversion, openness to experience, agreeableness,
and conscientiousness) assessment was carried out at age 15 with a 240-item questionnaire Estonian Personality Item Pool NEO (Möttus, Pullmann, & Allik, 2006) and at age 18 with a 60-item inventory “short five.” Both questionnaires measure the five-factor construct with adequate reliability and are highly intercorrelated (Konstabel, Lönnqvist, Walkowitz, Konstabel, & Verkasalo, 2012). On the basis of median value and both genders separately, the participants were divided to form the groups of less or more extrovert for $NOS1 \times \text{Environment} \times \text{Extraversion}$ interaction analysis. Data about personality at ages 15 and 18 were available for 468 and 452 participants, respectively.

**Measures of impulsivity.** We have previously reported on the effect of the $NOS1$ ex1f-VNTR genotype on impulsivity in this sample (Reif et al., 2011). In the current analysis, impulsivity measures were therefore used as covariates. Self-reported adaptive and maladaptive impulsivity scales (see, e.g., Laas et al., 2010) at ages 15 and 18 were used. Data were available for 481 and 445 participants at ages 15 and 18, respectively.

**Measurement of anxiety.** The Spielberger State Anxiety Inventory (Spielberger, 1983) was used at ages 15 and 18, and the Spielberger Trait Anxiety Inventory at age 18. Data were available for 450, 447, and 441 participants, respectively.

**Measurement of depressive symptoms.** Depressiveness was measured by the Montgomery–Åsberg Depression Rating Scale self-assessment version (Montgomery & Åsberg, 1979). Data were available for 440 participants.

**Measures of family relations.** Relationships in the family were measured using the Tartu Family Relationships Scale (Paaver, Kurrikoff, Nordquist, Oreland, & Harro, 2008). It has four subscales: closeness, support, misprize, and abuse. Based on the similarity in results in the presented analyses, the subscales of closeness and support were added together under a common name, warmth in the family, and the subscales of abuse and misprize were added together under a common name, maltreatment. On the basis of the median value of the warmth or maltreatment score, the participants were divided to form the groups with less and more maltreatment or warmth. Data were available for 388 and 411 participants, respectively.

**Stressful life events.** The history of stressful life events was self-reported at age 18. The list of stressful life events consisted of 15 adverse experiences, including parental death and divorce/separation, unemployed parent, parental alcoholism, poverty, poor living conditions, poor health, accidents and traumas, physical abuse, emotional abuse, severe burden/serious concerns, suicidal attempts, leaving home for several days without telling anyone, depression of a close relative, and committed suicide or suicide attempt of a close relative. Data on stressful life events were available for 452 adolescents. The events were recorded as dichotomous variables (present or not present) and were then counted to form the number of experienced adverse life events. On the basis of the median value (1), the participants were divided to form the groups with less (0–1; 57% of participants) or more stressful life events (2–12; 43% of participants).

**Genotyping**

$NOS1$ ex1f-VNTR has been analyzed as published previously (Reif et al., 2009). One of the primers was labeled with a fluorescent dye (cy-5; TIB MolBiol, Berlin) enabling detection of the polymerase chain reaction product. Electrophoretic separation of the polymerase chain reaction products was performed using a CEQ8000 DNA sequencer (Beckman–Coulter, Krefeld, Germany). $NOS1$ ex1f-VNTR alleles were grouped as short (180–196 repeats) and long (198–210 repeats). For the mixed-effect analysis of variance, which incorporated data from two follow-up studies, 2004 and 2007, data of the $NOS1$ ex1f-VNTR genotype were available for 524 participants. In interaction analysis, data from the 2007 follow-up were used: data of the $NOS1$ ex1f-VNTR genotype were available for 435 participants (Table 1). Quality control comprises genotyping two constant external controls every 90 samples, as well as genotyping 2% of all samples (randomly) twice. Concordance rates were 100%. The sample has been genotyped in one batch, and the call rate was 91%. Dropouts were due to missing amplicons, and repetition of genotyping dropouts did not result in recovery of genotypes, so we assume that the dropout most likely is due to DNA degradation. Genotypes were in Hardy–Weinberg equilibrium.

**Statistical analysis**

Participants were divided into groups with long/long (l/l), l/s, and s/s genotypes. A mixed-effects analysis of variance was used for testing $NOS1$ genotype, gender, and age effects on personality traits and anxiety. Contrasts were computed for statistically significant effects, using reduced models where nonsignificant independent variables were omitted (with the exception of lower-order terms included in significant interactions). Personality measures were standardized into $z$ scores, indicating how far and in what direction the group

<table>
<thead>
<tr>
<th></th>
<th>l/l</th>
<th>s/l</th>
<th>s/s</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>52 (26%)</td>
<td>113 (57%)</td>
<td>34 (17%)</td>
<td>199 (100%)</td>
</tr>
<tr>
<td>Females</td>
<td>63 (27%)</td>
<td>111 (47%)</td>
<td>62 (26%)</td>
<td>236 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>115 (26%)</td>
<td>224 (52%)</td>
<td>96 (22%)</td>
<td>435 (100%)</td>
</tr>
</tbody>
</table>

*Note:* l, long allele; s, short allele.
deviates from the whole sample’s mean expressed in units of its distribution’s standard deviation, according to the formula $Z_X = X - M_X / SD_X$, where $Z_X$ accounts for the $z$ score, $X$ for the raw score, $M$ for the sample’s mean, and $SD$ for the standard deviation of the mean.

For detecting Gene × Environment (G × E) interaction effects, participants were divided into two groups according to their median score on the family relations subscales. The median score was included in the groups with less aversive family relations and with less stressful life events. To detect the impact of extraversion on the G × E interaction, the participants were split into groups with high versus low extraversion using the median score. Multiple analysis of variance was carried out on data from the 18-year-olds to detect two-way or three-way interaction effects between genotype–family relations and among genotype–family relations–gender on personality, anxiety, and depressiveness. Fisher post hocs are given for a comparison of the groups. The raw scores of personality, anxiety, and depressiveness. Fisher post hocs are presented in Table 3.

Results

The effect of NOS1 genotype on neuroticism and extraversion

The $F$ statistics for mixed models, including NOS1 genotype, gender, and age as independent variables and personality traits as dependent variables, are presented in Table 2. Contrasts computed for gender, age, and genotype group comparisons are presented in Table 3.

Table 2. Nitric oxide synthase 1 (NOS1) exon 1f variable number tandem repeat genotype effects on aspects of personality and state–anxiety

<table>
<thead>
<tr>
<th>NOS1 Genotype (1)</th>
<th>NOS1 Genotype (1)</th>
<th>Sex (2)</th>
<th>Age (3)</th>
<th>$1 \times 2$</th>
<th>$1 \times 3$</th>
<th>$2 \times 3$</th>
<th>$1 \times 2 \times 3$</th>
<th>Impulsivity</th>
<th>Neuroticism</th>
<th>Adapative</th>
<th>Maladapative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extraversion</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>13.2**</td>
<td>0</td>
<td>2.9</td>
<td>0.3</td>
<td>3</td>
<td>4.7**</td>
<td></td>
<td>452.4***</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extraversion (AI)</td>
<td>1.0</td>
<td>25.2***</td>
<td>0</td>
<td>0.2</td>
<td>3</td>
<td>5.8**</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Openness</td>
<td>0.2</td>
<td>88.5***</td>
<td>0.2</td>
<td>1.6</td>
<td>0.1</td>
<td>2</td>
<td>0.4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agreeableness</td>
<td>0.8</td>
<td>27.5***</td>
<td>0.2</td>
<td>1.3</td>
<td>0</td>
<td>0.6</td>
<td>1.4</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Conscientiousness</td>
<td>1.5</td>
<td>1.4</td>
<td></td>
<td>0.4</td>
<td>0</td>
<td>0.1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroticism</td>
<td>3.2**</td>
<td>24.9***</td>
<td>0.1</td>
<td>0.2</td>
<td>0</td>
<td>0</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroticism (MI, AI)</td>
<td>4.9**</td>
<td>36.6***</td>
<td>0.1</td>
<td>1.2</td>
<td>0.3</td>
<td>0</td>
<td>1.6</td>
<td>104.4***</td>
<td>163.8***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>State anxiety</td>
<td>4.1*</td>
<td>4.7*</td>
<td>20.6***</td>
<td>0.9</td>
<td>1.1</td>
<td>2.3</td>
<td>0</td>
<td>48.8***</td>
<td>23.6***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>State anxiety (MI, AI)</td>
<td>5.4**</td>
<td>5.8*</td>
<td>22.7***</td>
<td>1.3</td>
<td>1.2</td>
<td>2.1</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>State anxiety (N)</td>
<td>5.9**</td>
<td>7.3**</td>
<td>19.9***</td>
<td>0.8</td>
<td>0.7</td>
<td>3.2</td>
<td>0.2</td>
<td>227.6***</td>
<td></td>
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</tr>
</tbody>
</table>

Note: Because of missing data, the error degrees of freedom vary across analyses: 330–346 for age and interactions involving age and 463–475 for other effects. The $F$ statistics are for terms in the mixed effects models. AI, adaptive impulsivity; MI, maladaptive impulsivity; N, neuroticism. Extraversion (AI), Neuroticism (MI, AI), State anxiety (MI, AI), and State anxiety (N) are $F$ statistics if AI, MI, and N are controlled for in the respective aspects or states. *$p < .05$. **$p < .01$. ***$p < .0001$. 

Age had no independent effect on any personality trait. Females scored significantly higher on neuroticism, $F (1, 472) = 25.10$, $p < .0001$, $\eta^2_p = 0.096$, and extraversion, $F (1, 471) = 13.22$, $p = .003$, $\eta^2_p = 0.053$, than males.

The analysis revealed a significant main effect of the NOS1 genotype on neuroticism, $F (2, 472) = 3.26$, $p = .04$, $\eta^2_p = 0.014$, indicating that short allele carriers scored higher on neuroticism than adolescents with the l/l genotype ($p = .02$; Figure 1a). The genotype effect (effect of being an short allele carrier) for neuroticism was independent of adaptive and maladaptive impulsivity, $F (2, 464) = 4.89$, $p = .008$, $\eta^2_p = 0.021$.

A three-way interaction effect of NOS1 genotype, gender, and age on the score of extraversion was detected, $F (2, 345) = 4.75$, $p = .009$, $\eta^2_p = 0.027$. Males carrying a NOS1 short allele had significantly higher scores than males with the l/l genotype ($p = .009$), while this effect was not observed among females (Figure 1b). At age 15, males with the s/s genotype had lower extraversion than the 15-year-old males with the long allele ($p < .03$), whereas this effect was not significant among 18-year-old boys. We found that the genotype effect on extraversion in males was partly due to the association between extraversion and adaptive impulsivity: when controlling for adaptive impulsivity, the two-way interaction between genotype and gender did not exist ($p = .50$; however, the three-way interaction remained statistically significant ($p = .004$). In females, extraversion did not depend upon genotype.

Because we had recorded personality with scales based on the five-factor model, data were available for other personality traits such as agreeableness, openness to experience, and conscientiousness. Nevertheless, the NOS1 genotype had no effect on these other personality traits of the five-factor model, thus suggesting more specific associations with neuroticism and extraversion.
The effect of NOS1 genotype on anxiety and depressiveness

Females had higher scores of state anxiety than males, $F(1, 465) = 4.69, p = .03, \eta_p^2 = 0.010$. The score of anxiety declined with age, $F(1, 335) = 20.70, p < .0001, \eta_p^2 = 0.058$. A significant main effect of the NOS1 genotype on state anxiety was found, $F(2, 465) = 4.10, p = .02, \eta_p^2 = 0.017$, indicating that short allele carriers had higher state anxiety than adolescents with the l/l genotype ($p = .007$; Figure 2a). The genotype effect (effect of being a short carrier) on state anxiety was independent of maladaptive and adaptive impulsivity, $F(2, 455) = 5.38, p = .005, \eta_p^2 = 0.023$, and neuroticism, $F(2, 460) = 5.86, p = .003, \eta_p^2 = 0.025$. No main effect of the genotype on trait anxiety or depressiveness was found.

NOS1 × Environment interactions on personality, anxiety, and depression

The NOS1 genotype had no interaction effect with family relationships or stressful life events on personality, trait anxiety, and depressiveness. However, stressful life events and the NOS1 genotype interacted in shaping state anxiety. The highest levels of state anxiety were observed in short allele carriers who had experienced more adverse life events, interaction effect $F(2, 393) = 3.38, p = .04, \eta_p^2 = 0.026$ (Figure 2b). The interaction effect of the NOS1 genotype and the number of stressful life events on state anxiety remained significant when the score of neuroticism, trait anxiety, maladaptive impulsivity, or adaptive impulsivity was added to the analysis as a covariate, $F(2, 392) = 3.94, p = .02, \eta_p^2 = 0.031; F(2, 385) = 3.77, p = .02, \eta_p^2 = 0.019; F(2, 375) = 2.99, p = .052, \eta_p^2$.

Note: Contrasts are in the mixed effects models. The numbers refer to regression coefficients for the respective contrasts. s, short allele; Short Carriers, contrasts s/s + s/long (1) with l/l; s/s, s/s homozygocity contrasts s/s with s/l + l/l. Extraversion (AI), Neuroticism (MI, AI), State anxiety (MI, AI), and State anxiety (N) are contrasts if AI, MI, and N are controlled for in the respective aspects or states. Contrasts for nonsignificant interactions were not computed.

*p < .05. **p < .01. ***p < .0001.

Table 3. Nitric oxide synthase 1 exon If variable number tandem repeat genotype effects on aspects of personality and state–anxiety

<table>
<thead>
<tr>
<th>Term</th>
<th>Genotype</th>
<th>Genotype × Sex</th>
<th>Sex</th>
<th>Age</th>
<th>Genotype × Age</th>
<th>Genotype × Sex × Age</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Short Carriers</td>
<td>s/s</td>
<td>Male</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extraversion</td>
<td>-0.07</td>
<td>-0.005</td>
<td>-0.22***</td>
<td>0.07</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Extraversion (AI)</td>
<td>-0.05</td>
<td>0.03</td>
<td>-0.44***</td>
<td>-0.01</td>
<td></td>
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</tr>
<tr>
<td>Neuroticism</td>
<td>0.07***</td>
<td>0.007</td>
<td>-0.41***</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neuroticism (MI, AI)</td>
<td>0.07***</td>
<td>-0.007</td>
<td>-0.21***</td>
<td>0.03</td>
<td></td>
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<tr>
<td>Openness</td>
<td>0.01</td>
<td>-0.05</td>
<td>-0.72***</td>
<td>0.02</td>
<td></td>
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</tr>
<tr>
<td>Agreeableness</td>
<td>-0.03</td>
<td>-0.03</td>
<td>-0.41</td>
<td>-0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Conscientiousness</td>
<td>-0.04</td>
<td>-0.03</td>
<td>-0.10</td>
<td>-0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>State anxiety (l/l)</td>
<td>0.09***</td>
<td>0.0003</td>
<td>-0.18**</td>
<td>0.26***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>State anxiety (s/l)</td>
<td>0.09***</td>
<td>-0.002</td>
<td>-0.07</td>
<td>0.30***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>State anxiety (s/s)</td>
<td>0.05</td>
<td>0.003</td>
<td>0.02</td>
<td>0.25***</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: Contrasts are in the mixed effects models. The numbers refer to regression coefficients for the respective contrasts. s, short allele; Short Carriers, contrasts s/s + s/long (1) with l/l; s/s, s/s homozygocity contrasts s/s with s/l + l/l. Extraversion (AI), Neuroticism (MI, AI), State anxiety (MI, AI), and State anxiety (N) are contrasts if AI, MI, and N are controlled for in the respective aspects or states. Contrasts for nonsignificant interactions were not computed.

*p < .05. **p < .01. ***p < .0001.

Figure 1. The standardized scores of (a) neuroticism and (b) extraversion in adolescents with nitric oxide synthase 1 long/long (l/l), short (s/l), and s/s genotypes. The limit bars above and below the symbols indicate 95% confidence intervals.
We found a significant three-way interaction effect on neuroticism, dependent on maltreatment in the family, \( F(2, 357) = 5.06, p = .007, \eta^2_p = 0.028 \). There was also a significant three-way interaction effect on state anxiety, dependent on warmth in the family, \( F(2, 335) = 3.00, p = .05, \eta^2_p = 0.018 \), which remained significant after adding the scores of neuroticism, trait anxiety, maladaptive impulsivity, or adaptive impulsivity as a covariate, \( F(2, 334) = 6.00, p = .003, \eta^2_p = 0.035 \); \( F(2, 328) = 6.99, p = .001, \eta^2_p = 0.041 \); \( F(2, 318) = 3.37, p = .04, \eta^2_p = 0.021 \); or \( F(2, 317) = 2.96, p = .05, \eta^2_p = 0.018 \), respectively. There were no three-way interaction effects on extraversion or trait anxiety.

\[ NOS1 \times \text{Environment} \times \text{Gender interactions} \]

\[ \text{in personality, anxiety, and depressiveness} \]

We found a significant three-way interaction effect on neuroticism, dependent on adverse life events, \( F(2, 382) = 4.08, p = .02; \eta^2_p = 0.021 \). The interaction remained significant when each score of neuroticism, state anxiety, maladaptive impulsivity, or adaptive impulsivity was added as a covariate, \( F(2, 381) = 3.54, p = .03, \eta^2_p = 0.018 \); \( F(2, 378) = 4.48, p = .01, \eta^2_p = 0.023 \); \( F(2, 365) = 3.15, p = .04, \eta^2_p = 0.017 \); or \( F(2, 363) = 2.96, p = .05, \eta^2_p = 0.016 \), respectively. When trait anxiety was added as a covariate, the interaction effect remained almost significant, \( F(2, 374) = 2.91, p = .056, \eta^2_p = 0.015 \).

\[ \text{Males.} \]

Males with the l/l genotype and higher exposure to environmental adversity had higher neuroticism and depressiveness than men with the same genotype from an advantageous environment. Higher neuroticism was found in l/l males who had experienced more maltreatment in family, and higher depressiveness was found in males with the l/l genotype who had had more adverse life events (Figure 3a, c, e).

\[ \text{Females.} \]

Female short allele carriers had higher neuroticism, state anxiety, and depressiveness in cases of adverse environment. Female short allele carriers had higher scores of neuroticism with higher experience of maltreatment, higher scores of state anxiety if having experienced less warmth in family, and higher scores of depressiveness in cases of more adverse life events (Figure 3a, c, e).

\[ NOS1 \times \text{Environment} \times \text{Gender interactions} \]

\[ \text{on psychological and somatic symptoms of depressiveness} \]

Based on factor analysis (data not shown), items of the Montgomery–Åsberg Depression Rating Scale were divided into two groups: psychological symptoms of depression (reported sadness, inner tension, lassitude, inability to feel, pessimistic thoughts, suicidal thoughts) and somatic symptoms of depression (reduced sleep, reduced appetite, concentration difficulties).

We found a significant three-way interaction effect on psychological symptoms of depressiveness, dependent on adverse life events, \( F(2, 378) = 3.78, p = .02, \eta^2_p = 0.020 \). Females with one or two short alleles and more adverse life events had significantly more psychological symptoms of depressiveness than other female groups. No similar effect was found in males (Figure 4a).

There was no significant three-way interaction effect on somatic symptoms of depressiveness, \( F(2, 381) = 1.75, p = .18, \eta^2_p = 0.009 \) (Figure 4b), but when analyzing males separately and comparing short allele carriers with the l/l genotype, there appeared an interaction effect, \( F(1, 170) = 3.94, p = .05, \eta^2_p = 0.023 \): Males with the l/l genotype and more adverse life events had more somatic symptoms of depression than male.
l/l participants who had fewer adverse life events ($p < 0.05$). Females showed no significant $\text{NOS1} \times \text{Adverse Life Events}$ interaction on somatic symptoms of depression.

**Figure 3.** Gene × Environment interaction effects on (a, b) neuroticism, (c, d) anxiety, and (e, f) depressiveness. The numbers in parentheses are the number of participants in each group. The limit bars above and below the symbols indicate 95% confidence intervals. (a, c, e) The effect of genotype and environment on males and females. (b, d, f) The effect of genotype and environment dependent upon the level of extroversion. *$p < 0.05$, different from adolescents with the same genotype, same sex, and better environmental conditions; **$p < 0.05$, different from adolescents with similar environmental conditions, same sex, and short/long (s/l) genotype; ***$p < 0.05$, different from adolescents with similar environmental conditions, same sex, and s/s genotype.

$NOS1 \times \text{Environment} \times \text{Extraversion interactions on neuroticism, anxiety, and depressiveness}$

$NOS1$ and extraversion had a significant interaction effect on neuroticism, dependent on maltreatment, $F (2, 150) = 3.06, p = 0.039$, a significant interaction effect on state anxiety dependent on warmth, $F (2, 140) = 3.57, p = 0.03$, $\eta^2_p = 0.048$, and a significant interaction effect on depressiveness, dependent on adverse life events, $F (2, 162) = 3.04, p = 0.05$, $\eta^2_p = 0.036$, in males.

Less extroverted males with the l/l genotype who had lived in a more adverse environment had higher neuroticism, state anxiety, and depressiveness scores than less extroverted males who had lived in a favorable environment. There was
no difference between males with different NOS1 genotypes and different environmental conditions among more extroverted males (Figure 3b, d, f).

NOS1, extraversion, and environment had a significant interaction effect on female depressiveness, $F(2, 208) = 3.48$, $p = .03$, $\eta^2_p = 0.032$. In contrast to what was found in males, females with low extraversion had significantly higher scores of depressiveness if they had been experiencing more adverse life events recently and had the s/s genotype (data not shown).

**Discussion**

The results of the present study indicate that the functional NOS1 ex1f-VNTR polymorphism is associated not only with impulsivity (Laas et al., 2010; Reif et al., 2011) but also with basic personality traits and state anxiety in the general population. While the effect of the genotype on neuroticism and state anxiety was independent of gender, the effect on extraversion was present only in males. NOS1 genotype interaction with life events appears to be gender specific.

Among psychiatric patients, subjects with the s/s genotype present a more severe course of the disorder, higher depression ratings, and lower scores in global assessment of functioning (Reif et al., 2006). In the general population, male s/s homozygotes have higher adaptive impulsivity (Laas et al., 2010; Reif et al., 2011). In the present analysis, we have found that adolescents carrying the short allele have higher neuroticism and state anxiety than participants with the l/l genotype. Although aspects of impulsivity are included in neuroticism in the five-factor model of personality, impulsivity did not explain the genotype effect on neuroticism. It appears that the environment-independent effects of the NOS1 ex1f-VNTR genotype on neuroticism and the adaptive side of impulsivity are at least in part separate.

Higher neuroticism and state anxiety leads to higher sensitivity toward life events (Homberg & Lesch, 2011). Therefore, people with NOS1 short alleles should be more sensitive to adversities in the environment. Accordingly, participants with the s/s genotype were found to describe themselves as more thoughtless and disinhibited persons, if having unfavorable relations in their family (Reif et al., 2011). The result of the short allele carriers being more vulnerable to adverse environment was found again in the present analysis, as adolescents carrying the short allele had high state anxiety if having been subjected to an adverse environment. In addition, female participants with the short allele and experience of adverse environment had higher neuroticism and depressiveness. This supports the notion that adverse life events interact with the risk allele to increase impulsivity in males and neuroticism/depressiveness in females, that is, the preponderant sex-specific sequelae of life adversity: While males may be imprisoned, females seek treatment. Thus, adverse environment leads to different psychological outcomes in males and females dependent on the NOS1 genotype. More difficult to explain, however, is the finding that in males this association was reversed in a way that, after experiencing an adverse environment, males with the l/l genotype had higher neuroticism and depressiveness. One can only speculate how such a balanced selection effect might make sense: Under positive conditions, male short allele carriers feature the advantageous genotype in that they display increased adaptive impulsivity (Reif et al., 2011) and extraversion. In the presence of life adversity, s/s carriers have to pay the price in featuring higher maladaptive impulsivity and an accordingly increased risk for related psychopathology. On the contrary, male l/l participants have lower impulsivity and extraversion, and could be led by an adverse environment to anxiety and affective disorders. If that is true, we would expect a G × E interaction in a way that male short allele carriers compared to the l/l participants are less prone to clinical depression, especially in the absence of an adverse life event, which is a hypothesis that seems worthy of testing in further studies.
Thus, our results support the notion that “vulnerability genes” or “risk alleles” could sometimes be more appropriately conceptualized as “plasticity genes” because they seem to make individuals more susceptible to environmental influences for better and for worse (Belsky et al., 2009). Genetically driven deficits would not have been maintained throughout evolution if they only exerted negative effects without conveying any gain of function. This plasticity gene effect on functional outcomes has been described for other genes. For example, most of serotonin transporter linked polymorphic region gene studies associate short allele carriers with maladaptive phenotypes like anxiety-related traits and increased risk for depression in interaction with psychosocial adversity across the life span. However, humans and nonhuman primates carry the short variant of the serotonin transporter linked polymorphic region gene outperform subjects carrying the long allele in an array of cognitive tasks and show increased social conformity (Homberg & Lesch, 2010). In addition, children with the s/s genotype were more likely to respond to cognitive behavior therapy than those carrying a long allele (Eley et al., 2012) and on average their higher neuroticism wanes during the transition from childhood to adolescence and adulthood, suggestive of a complex adaptive capacity (Harro et al., 2009). Thus, depending on environment and task, short allele carriers who appear to be characterized by general hypervigilance may react in either a less adaptive manner, with increased negative emotionality, or with more successful adaptation, based on cognitive regulation (Homberg & Lesch, 2010). The plasticity gene effect has also been described for the monoamine oxidase A gene (MAOA). Individuals with the less active version of the MAOA gene usually display higher levels of antisocial and risky behavior when they have experienced maltreatment in childhood, but they score lowest in antisocial behavior if they have not been exposed to child maltreatment (Belsky et al., 2009). In an experimental gambling task, participants with the low MAOA activity genotype engaged in more risky behavior only when it was advantageous to do so (Frydman, Camerer, Bossaerts, & Rangel, 2011).

Thus, subjects carrying the gene variants that can make them more vulnerable may not only passively benefit from the more advantageous environments but also might acquire active successful strategies to prevent devastating effects from their inherent vulnerability and turn vulnerability into success (Harro, 2010).

We found that the NOS1 ex1f-VNTR genotype affects extraversion in males. Male short allele carriers also have higher adaptive impulsivity (Reif et al., 2011). These two constructs are not dissimilar: Both include components that reflect dominance or even overt aggressiveness, such as activity and assertiveness. Analysis of covariance suggested that association of the NOS1 genotype with extraversion is related to its effect on adaptive impulsivity. Comparable results have been found in mouse studies: male mice lacking the Nos1 gene display more aggression-like behavior, which includes high locomotor activity, similar to extraversion and impulsivity in humans, whereas there was no such difference among female mice (Nelson, Trainor, Chiavegatto & Demas, 2006). Furthermore, in male mice, administration of a nNOS inhibitor prevented the chronic mild stress-induced behavioral despair (Zhou et al., 2007). Extraversion is negatively associated with depressiveness (Chioqueta & Stiles, 2005), and, therefore, the short allele leading males toward being more active and extroverted may prevent them from being increasingly depressed in adversity. That might also be an explanation as to why adverse environmental conditions affected males with the l/l genotype. Sensitivity to stressful conditions appeared only in males with the l/l genotype if they also had low extraversion (Figure 3f).

Males with the l/l genotype had more somatic symptoms of depression after experiencing multiple stressful life events, but there was no significant difference in psychological symptoms of depression. In contrast, women carrying the short allele had more psychological symptoms of depressiveness. Although the vast majority of people make initially exclusively somatic presentations to physicians in cases of depression (Kirmayer, Robbins, Dworkind, & Yaffe, 1993), women might be more prone to psychologization of their distress, which is the tendency to express distress in cognitive or affective terms.

Personality scores did not differ between 15- and 18-year-old participants, but state anxiety was lower in 18-year-old participants. While emotional stability starts to increase already from adolescence (Roberts, Walton, & Viechtbauer, 2006), this might be a result of maturing revealed in changes at state and not at trait level. Although we did not find age (15 to 18 years) being associated with personality traits, we found that extraversion was lower in 15-year-old males with the s/s genotype but higher in short allele carriers in males. This difference appeared mainly due to the increasing score of extraversion in s/s homozygotic males during the 3 years. As the prefrontal cortex is still developing in adolescents (Insel, 2010) and its functioning is most impaired in the short allele homozygotes (Reif et al., 2009), we might hypothesize that development of the prefrontal cortex and functions associated with it might in adolescent years be most detectable in male short allele homozygotes.

Conclusion

In addition to impulsivity as previously demonstrated, NOS1 ex1f-VNTR associates with neuroticism, extraversion, anxiety, and depressiveness in the general population. These associations are dependent on environmental adversity and are gender dependent. Clearly, because of the exploratory nature of some of the analyses, issues of multiple testing have to be considered in the interpretation of the data. However, the true number of independent tests is difficult to estimate, owing to high correlations between neuroticism, state anxiety, and depressiveness. A possibility exists that discovered interaction effects are found by chance and replications in independent data sets are awaited with further studies. However, although
in three-way interaction analysis the groups have remained quite small, the effect sizes in our study that on average explain 1% of the variance can be considered large for G × E studies and power adequate (Duncan & Keller, 2011).

Further studies that also incorporate functional imaging data are warranted to disentangle the effect of the NOSI and other putative plasticity genes on personality and psychopathology.

References


