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Association of a functional variant of neuronal nitric oxide synthase gene with self-reported impulsiveness, venturesomeness and empathy in male offenders

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Abstract It has been shown that a functional promoter dinucleotide repeat length variation of the neuronal nitric oxide synthase gene (*NOS1* Ex1f-VNTR) is associated with impulsivity-related behavioral phenotypes. In this study, the Eysenck Impulsivity Questionnaire (IVE-7) was administered to 182 male offenders to prove the hypothesis that *NOS1* Ex1f-VNTR is associated with self-reported impulsiveness, venturesomeness and empathy. Multivariate analysis of variance (MANCOVA) revealed a significant multivariate effect of *NOS1* Ex1f-VNTR genotype on IVE-7 measures ($P = 0.0006$). The effect was more pronounced regarding impulsiveness and empathy ($P = 0.0052$ and $P = 0.0036$, respectively) as compared with venturesomeness, which was only of borderline significance. The findings give additional evidence that NOS-I is involved in the regulation of impulsive personality traits and support the notion that the *NOS1* gene takes part in the regulation of social behavior.

Keywords Impulsivity · Empathy · Nitric oxide synthase · Genetic · Offender

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Introduction

Recent behavioral genetic research includes the search for functional gene variants that might provide insight into the neurobiological mechanisms involved in antisocial behavioral phenotypes. For example, the role of functional variants in the serotonin transporter gene (*5-HTT*LPR; Heils et al. 1996, Retz et al. 2004, Reif et al. 2007) and the MAOA gene (*MAOA* uVNTR; Deckert et al. 1999, Kim-Cohen et al. 2006; Reif et al. 2007) in the development of antisocial behavior has been consistently replicated.

The *NOS1* gene is a complex gene located at 12q24.3 consisting of a ~125-kb region encoding for 28 exons and another ~125-kb region, termed the "variable region" that codes for at least 11 distinct alternative first exons of the enzyme. In a previous study, we have shown that a polymorphic promoter dinucleotide repeat length variation of the *NOS1* gene (*NOS1* Ex1f-VNTR) is of functional relevance. Using a luciferase assay it was demonstrated that long alleles of *NOS1* Ex1f-VNTR (204 repeats) result in significantly increased reporter gene activity as compared with intermediate (192 repeats) and even more so short (182 repeats) alleles (Reif et al. 2006, 2009). On a clinical level, associations of this polymorphism with traits related to impulsivity were found. Specifically, we demonstrated associations with cluster B personality disorders, adult attention-deficit/hyperactivity disorder (aADHD), as well as suicidal and violent behavior. On a neurophysiological level, *NOS1* Ex1f-VNTR variants were shown to modulate prefrontal brain activity, including the anterior cingulate cortex, a structure known to be involved in the processing of emotion and reward in behavioral control.

Impulsivity and empathy are personality traits which are closely related to disruptive behavior phenotypes. Empathy can be understood as vicarious responding with an

emphasis on the congruent response to the others' emotional state. High empathy is considered to be involved in moral reasoning, prosocial behavior and control of aggression (Eisenberg 2000). Further, it has been suggested by Eysenck et al. (1985) that impulsiveness and venturesomeness are two closely related dimensional traits that can be viewed as two facets of impulsivity. While impulsiveness has been conceptualized as spontaneous behaving without realizing the risk in the behavior, venturesomeness is defined as being conscious of the risk but acting anyway.

In this study, we report the results of an association study of self-rated impulsiveness, venturesomeness and empathy in order to test the hypothesis that *NOS1* Ex1f-VNTR is involved in impulsive and empathic personality traits.

Materials and methods

Subjects

A sample of 182 adult male, Caucasian offenders, who were referred to the Forensic-Psychiatric Institute of the University of Saarland Homburg/Saar, Germany for a forensic examination, entered the study. Only subjects who gave written informed consent after explanation about scope and aim of the investigation were enrolled. The study was approved by the Ethics Committee of the General Medical Council of the Saarland. Mean age was 34.1 years (SD 11.7 years). Subjects with a diagnosis of current substance dependence, acute schizophrenia, major depression/bipolar disorder, or any other severe Axis-I diagnosis according to DSM-IV as well as mentally retarded subjects (IQ < 70) were not included. Psychiatric lifetime diagnoses according to DSM-IV criteria comprised substance use disorders (46.2%), psychotic disorders (8.2%), paraphilias (4.5%), affective disorders (2.2%), neurotic disorders and disorders of impulse control (3.8%). Cluster B personality disorders were present in 27.2%, personality disorders of clusters A and C in 7.6% of the sample. All subjects underwent a semi-structured psychiatric interview by well-trained psychiatrists and a neurological examination. Criminal offences of the participants of the study comprised homicide, physical injury and robbery ($n = 75$), property offenses and fraud ($n = 41$), sexual offenses ($n = 33$), drug offenses ($n = 12$) and others ($n = 67$).

Psychometric assessments

Impulsiveness, venturesomeness and empathy were assessed using the German version of the Eysenck Impulsivity Questionnaire (IVE-7). High scores indicate high levels of the respective personality characteristics. Gender-specific T-values can be calculated from German norm groups (Eysenck et al. 1990). Childhood ADHD symptomatology was assessed by the German version of the Wender Utah Rating Scale (WURS-k; Retz et al. 2002, 2003). Subjects were assigned as "violent" or "nonviolent" according to their history of criminal charges, based on the careful evaluation of all available documents. Violence was defined as any recurrent and overt physical injury against persons or things according to Volavka (1999).

Genotyping

NOS1 Ex1f-VNTR has been determined as described previously by PCR amplification and product size determination (Reif et al. 2006). *NOS1* Ex1f-VNTR was found to be in Hardy–Weinberg equilibrium.

Statistics

Due to their proposed functionality and analogous to previous studies, *NOS1* Ex1f-VNTR alleles were dichotomized in short (i.e., 180–196 repeats) and long (i.e., 198–210 repeats) alleles. Genotypes were "homozygous" when both alleles belonged to the short or long group and "heterozygous" if one allele contained 180–196 repeats and the other 198–201 repeats. To analyze the impact of genotype on IVE-7 measures, a multivariate analysis of covariance (MANCOVA) was calculated with genotype as independent variables of interest, adjusted for the confounding variables age, diagnosis of personality disorder, childhood ADHD symptoms and history of violent offences, as the aim of the study was to evaluate *NOS1* ex1f-VNTR as a risk factor for personality traits beyond these possible confounding variables. Complete measures on these confounding variables were obtained for $N = 176$ individuals who were included in the analysis. Lifetime axis-I disorders were not included into the multivariate model as covariates as they were not related to the personality measures (ANOVA, impulsiveness: $F = 0.897$, df1, $P = 0.345$; venturesomeness: $F = 0.141$, df1, $P = 0.708$; empathy: $F = 0.055$, df1, $P = 0.814$). Post-hoc analysis was performed by separate ANCOVA models on the three IVE-7 measures. No adjustment for multiple testing had to be performed, as the results of the MANCOVA analysis were used as criterion for an association of genotypes with IVE-7 measures. Power was >0.80 to detect an R^2 of 0.05 explained by the effect of the genotypes.

Results

MANCOVA revealed a significant multivariate effect of *NOS1* Ex1f-VNTR genotype on IVE-7 impulsiveness,

Table 1 Effect of *NOS1* Ex1f-VNTR and confounding variables on IEV-7 personality domains analyzed by ANOVA

	DF	*F* value	*P*
Impulsiveness			
NOS1 Ex1f-VNTR	2	5.4	0.0052
Age	1	7.5	0.0069
Personality disorder	1	5.0	0.0261
Childhood ADHD symptoms (WURS-k)	1	51.6	<0.0001
Violent behavior	1	11.0	0.0011
Venturesomeness			
NOS1 Ex1f-VNTR	2	2.9	0.0566
Age	1	11.8	0.0007
Personality disorder	1	0.5	0.4803
Childhood ADHD symptoms (WURS-k)	1	5.7	0.0183
Violent behavior	1	3.8	0.0542
Empathy			
NOS1 Ex1f-VNTR	2	5.82	0.0036
Age	1	0.79	0.3744
Personality disorder	1	0.45	0.5043
Childhood ADHD symptoms (WURS-k)	1	0.26	0.6115
Violent behavior	1	1.74	0.1889

venturesomeness and empathy (Wilks' Lambda 0.868, *F* 4.09, Num DF 6, Den DF 334, $P = 0.0006$). As shown in Table 1, univariate analyses of variance (ANCOVAs) revealed a more pronounced effect of genotype regarding impulsiveness and empathy as compared with venturesomeness, which was only of borderline significance. Also age (Wilks' Lambda 0.914, *F* 5.26, Num DF 3, Den DF 167, $P = 0.0017$), childhood ADHD symptoms (Wilks; Lambda 0.763, *F* 17.25, Num DF 3, Den DF 167, $P < 0.0001$) and history of violent criminal behavior (Wilks' Lambda 0.922, *F* 4.72, Num DF 3, Den DF 167, $P = 0.0034$) but not diagnosis of personality disorder showed a significant multivariate effect on IVE-7 measures.

Using univariate analyses of variance, age showed a significant negative correlation with the mean subscores of impulsiveness and venturesomeness but no effect on empathy measures. Personality disorders were associated with increased impulsiveness scores, but did not affect those of venturesomeness and empathy. Further, ANOVA revealed significant effects of childhood ADHD symptoms as measured by WURS-k and history of violent behavior on impulsiveness and venturesomeness but not on empathy. High WURS-k scores were associated with higher ratings of impulsiveness and venturesomeness (Table 1). The highest impulsivity and venturesomeness mean scores were present in the heterozygous group, whereas homozygous individuals showed lower mean scores. In contrast, higher empathy mean scores were found in heterozygous as compared to homozygous individuals (Table 2).

Discussion

There are several lines of evidence that NOS-I is involved in disruptive behavior. Knockdown of the *NOS1* gene in mice results in behavioral changes involving learning deficits, decreased anxiety, impulsivity and increased aggressiveness (Nelson et al. 1995). Moreover, a functional polymorphism was associated with disruptive behavioral phenotypes and prefrontal brain dysfunction in humans (Reif et al. 2006, 2009). In this study, we extended the current knowledge by demonstrating that standardized measures of impulsiveness and empathy, as assessed by self-rating, were associated with *NOS1* Ex1f-VNTR. This result remained significant when analyses were controlled for age, diagnosis of personality disorder, childhood ADHD symptoms and violent criminal behavior.

Specifically, the findings indicate a heterosis effect, which refers to situations where subjects heterozygous for a genetic polymorphism show a stronger effect for a quantitative phenotype than subjects homozygous for either allele (Comings and MacMurray 2000). Interestingly, this finding is in line with previous reports of frontal cortical dysfunction in heterozygous carriers of the *NOS1* ex1f-VNTR polymorphism (Reif et al. 2006). Likewise, in our previous study we reported a significant association of this polymorphism with *Conscientiousness* in healthy females, of whom also heterozygous individuals nominally displayed lowest scores (Reif et al. 2009) indicating higher levels of impulsive behavior paralleling the findings of the present study. However, the lowest nominal scores of impulsivity and venturesomeness and the highest empathy scores were found in carriers homozygous for the short allele. Although this finding appears contradictory to prior findings, suggesting that the short allele increases the risk for cluster B personality disorder, ADHD and aggressive behaviors (Reif et al. 2009), it should be mentioned that such diverging results of association studies on related but not identical phenotypes are not unusual. For example, the *5-HTT*LPR long allele has been shown to be associated with ADHD (Faraone and Khan 2006; Retz et al. 2002, 2008), whereas the short allele of this gene was associated with other disruptive phenotypes like aggression and violence (Beitchman et al. 2006; Retz et al. 2004), although all these phenotypes are related. Further research has revealed a modulating effect of environmental factors on the impact of this genetic polymorphism, which might explain this differential gene effect (Retz and Rösler 2009). Moreover, it has to be emphasized that results of association studies highly depend on the sample investigated, which in the present case was extremely selected. Therefore, one should be cautious to generalize the findings reported here.

Table 2 Adjusted mean scores and 95% confidence intervals (CI) of IVE-7 domains

IVE-7 Mean *T* values (95% CI)	*NOS1* Ex1f-VNTR genotype		
	Short/short ($N = 31$)	Short/long ($N = 102$)	Long/long ($N = 49$)
Impulsiveness	56.5 (52.4–60.6)	63.8 (61.5–66.2)	60.3 (57.0–63.5)
Venturesomeness	47.4 (43.8–51.0)	49.9 (47.8–52.0)	45.8 (43.0–48.6)
Empathy	53.3 (50.7–55.9)	48.9 (47.4–50.4)	52.1 (50.0–54.1)

The discrepancy that the short–short genotype predisposed to aggressive behavior in the present sample (Reif et al. 2009), yet went along with decreased impulsiveness and increased empathy points to a more complex role of *NOS1* Ex1f VNTR than a mere association with impulsive behaviors. This resembles findings on the role of *MAOA* uVNTR and aggression, where it was shown that the variant predisposing to violent behavior most likely is operative by increasing the sensitivity to negative socioemotional experiences (Eisenberger et al. 2006). Therefore, the psychogenomic consequences of *NOS1* Ex1f-VNTR are far from being understood and require further research efforts.

Some limitations of this study have to be pointed out. As already mentioned, the association of *NOS1* Ex1f-VNTR with personality traits reported here refers to a sample of offenders with an unavoidable high load of antisocial behavior and psychiatric comorbidity. Therefore, the results cannot be easily extrapolated to general population. Moreover, they are restricted to males, since female subjects were not included in the study. Since no control group was implemented in this study not only replication but also verification of the findings in general population is required.

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