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NCAM1, *TACR1* and *NOS* Genes and Temperament: A Study on Suicide Attempters and Controls

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Temperament · *NCAM1* · *TACR1* · *NOS*

Abstract
Suicide, one of the leading causes of death among young adults, seems to be plausibly modulated by both genetic and personality factors. The aim of this study was to dissect the potential association between genetics and temperament in a sample of 111 suicide attempters and 289 healthy controls. We focused on 4 genes previously investigated in association with suicide on the same sample: the nitric oxide synthase 1 and 3 *(NOS1* and *NOS3)*, the neuronal cell adhesion molecule 1 *(NCAM1)*, and the tachykinin receptor 1 *(TACR1)* genes. In particular, we investigated whether a set of genetic variants in these genes (*NOS1*: rs2682826, rs1353939, rs693534; *NOS3*: rs2070744, rs1799983, rs891512; *NCAM1*: rs2301228, rs1884, rs1245113, rs1369816, rs2196456, rs584427; *TACR1*: rs3771810, rs3771825, rs726506, rs1477157) were associated with temperamental traits at the Temperament and Character Inventory (TCI). No strong evidence was found for the association between TCI personality traits and the polymorphisms considered in the 4 genes, with the exception of an association between reward dependence trait and the rs2682826 SNP in *NOS1* in the healthy sample. However, this result could be plausibly interpreted as a false-positive finding. In conclusion, our study did not support the thesis of a direct modulation of these genes on temperament; however, further studies on larger samples are clearly required in order to confirm our preliminary findings and to exclude any possible minor influence.

Introduction

Recent studies have extensively focused on both the dissection and prevention of suicidal behavior, the most frequent cause of death among young adults and psychiatric patients [1]. In particular, temperamental features that could lead to a predisposition towards suicidal behavior have been considered. In particular, 2 traits have been repeatedly associated with suicidal behaviors: Harm Avoidance/Neuroticism and Novelty Seeking/

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Extraversion [2]. Recent studies confirmed the association for Harm Avoidance [3–7], while some discrepancies have been found regarding Novelty Seeking [2, 3, 5, 8].

Furthermore, temperament has repeatedly been associated with neurochemical substrates that have a genetic basis [9–14]. In particular, consistent evidence has suggested that the serotonin system – especially serotonin transporter and serotonin receptor variants – plays a key role in the modulation of temperamental traits, particularly the Harm Avoidance/Neuroticism trait.

Consequently, considering the complex and multifaceted nature of suicidal behavior, focusing on the potential link between genetics and temperament, rather than on the direct modulation of genes on suicidal behavior, could be a useful approach. Accordingly, we focused on the genetic modulation of temperamental traits in suicide. Considering that genetic variants of the serotonin system alone could not completely explain the genetic loading of temperament and character traits [13, 15], and that other systems still need evaluation, we focused on 4 genes previously investigated in the same sample in relation to both suicide and anger/aggression traits [16–18]: the nitric oxide synthase 1 and 3 genes *(NOS1* and *NOS3)*, the neuronal cell adhesion molecule 1 gene *(NCAM1)*, and the tachykinin receptor 1 gene *(TACR1)*.

Specifically, in this study we investigated whether a panel of markers in *NOS1* (rs2682826, rs1353939, rs693534), *NOS3* (rs2070744, rs1799983, rs891512), *NCAM1* (rs2301228, rs1884, rs1245113, rs1369816, rs2196456 and rs584427) and *TACR1* (rs3771810, rs3771825, rs726506 and rs1477157) genes was associated with temperament traits measured with the Temperament and Character Inventory (TCI) in a German sample of 111 patients with a history of suicide attempts and 289 healthy subjects. To the best of our knowledge, this is the first study investigating the relationship between these genes and temperament measured with the TCI.

Materials and Methods

The sample features as well as the recruitment methodology of patients and healthy controls have been extensively described elsewhere [7, 19].

In summary, the patient group consisted of 111 suicide attempters (38.7% males, mean age 39.2 ± 13.6 years) that were consecutively referred to general psychiatric wards of the Department of Psychiatry, Ludwig Maximilians University of Munich, Germany. Current and lifetime diagnoses of mental disorders were assessed close to discharge by applying the Structured Clinical Interview for DSM-IV (SCID-I and II) [20, 21]. Patients with mental disorders due to a general medical condition or with dementia were excluded. DSM-IV lifetime diagnoses of mental disorders among the patients were affective spectrum (n = 76; 68.5%), schizophrenia spectrum (n = 17; 15.3%) and borderline personality disorder (n = 18; 16.2%).

Healthy volunteers were randomly selected from Munich, and contacted by mail. To exclude subjects with neuropsychiatric disorders, we conducted screenings before the volunteers were enrolled in the study. Firstly, subjects who responded were initially screened by phone. Detailed medical and psychiatric histories were assessed by using systematic forms. Secondly, SCID-I and II were administered. Subjects with relevant somatic diseases or a lifetime history of any axis I or II disorders or suicidal behavior were excluded. Finally, 289 healthy subjects (42.6% males, mean age 45.2 ± 14.9 years) were included.

The study was approved by the local ethics committee and carried out in accordance to the ethical standards laid down in the 1964 Declaration of Helsinki.

The TCI – a 240-item tool to assess individual differences in the basic dimensions of the Cloninger biosocial model of personality [9] – was administered to the final sample.

DNA analysis of the *NOS1* and *NOS3* polymorphisms [16], *TACR1* gene [17] and *NCAM1* gene [18] has been described elsewhere.

Table 1. SNPs considered in this study

SNP ID	Position[1]	Distance	Alleles	Location
NCAM1 SNPs				
rs2301228	112336669 (–875)		A/G	promoter
rs1884	112385807 (48264)	49139	C/G	intron 1
rs1245113	112456235 (118692)	70428	C/G	intron 1
rs1369816	112497911 (160368)	41676	G/T	intron 1
rs2196456	112561921 (224378)	64285	T/C	intron 1
rs584427	112609206 (271663)	47285	C/A	exon 12
NOS1 SNPs				
rs2682826	116137221 (116036)		G/A	exon 29, 3′ UTR
rs1353939	116159736 (93521)		C/T	intron 20
rs693534	116269101 (–15845)		G/A	intron 1
NOS3 SNPs				
rs2070744	150321012 (–814)		T/C	intron 1
rs1799983	150327044 (5219)		G/T	exon 8
rs891512	150339022 (17197)		G/A	intron 23
TACR1 SNPs				
rs3771810	75161161 (118407)		T/C	intron 2
rs3771825	75208988 (70580)		C/T	intron 1
rs726506	75262526 (17042)		C/G	intron 1
rs1477157	75282736 (–3169)		G/A	promoter

[1] Absolute chromosomal position. The relative position to the start codon is given in parentheses. All data from snpper.chip.org.

Table 2. Temperament and character traits stratified for *NOS1* genotypes in healthy controls

TCI traits	rs1353939			F	d.f.		p
	C/C (n = 149)	C/T (n = 102)	T/T (n = 38)				
HA	46.52 ± 5.20	46.59 ± 5.38	46.95 ± 5.41	0.02	2	281	0.98
NS	60.55 ± 5.46	59.52 ± 4.89	60.71 ± 5.50	2.02	2	281	0.13
RD	39.48 ± 3.24	38.77 ± 3.75	39.13 ± 3.43	2.70	2	281	0.07
P	11.26 ± 2.01	11.42 ± 1.90	11.24 ± 2.23	0.12	2	281	0.89
SD	80.17 ± 4.93	80.57 ± 4.71	79.60 ± 5.80	0.64	2	281	0.53
C	75.58 ± 4.03	74.84 ± 4.19	74.29 ± 4.46	2.52	2	281	0.08
ST	43.97 ± 5.83	42.40 ± 6.48	43.45 ± 6.64	6.74	2	281	0.13
TCI traits	rs2682826			F	d.f.		p
	A/A (n = 18)	A/G (n = 94)	G/G (n = 177)				
HA	48.39 ± 3.57	46.00 ± 5.60	46.74 ± 5.22	2.18	2	281	0.11
NS	59.67 ± 6.01	60.08 ± 4.91	60.33 ± 5.42	2.46	2	281	0.34
RD	40.61 ± 3.07	38.08 ± 3.74	39.62 ± 3.19	11.42	2	281	<0.001
P	11.61 ± 2.45	11.38 ± 1.98	11.25 ± 1.97	0.31	2	281	0.73
SD	80.33 ± 4.28	80.68 ± 5.04	79.99 ± 5.01	0.99	2	281	0.37
C	74.39 ± 4.61	74.57 ± 4.19	75.53 ± 4.07	2.40	2	281	0.09
ST	44.72 ± 6.78	41.9 ± 6.29	43.94 ± 5.99	3.06	2	281	0.05
TCI traits	rs693534			F	d.f.		p
	A/A (n = 51)	A/G (n = 141)	G/G (n = 97)				
HA	46.01 ± 5.70	46.38 ± 5.37	47.23 ± 4.88	1.34	2	281	0.26
NS	59.45 ± 5.36	60.74 ± 5.23	59.82 ± 5.28	1.17	2	281	0.31
RD	39.16 ± 3.42	39.32 ± 3.08	39.00 ± 3.98	0.80	2	281	0.45
P	11.67 ± 2.22	11.30 ± 1.97	11.14 ± 1.92	1.94	2	281	0.14
SD	80.88 ± 4.70	80.12 ± 5.19	80.07 ± 4.79	0.96	2	281	0.38
C	75.02 ± 4.36	75.33 ± 4.23	74.96 ± 3.98	0.39	2	281	0.68
ST	43.76 ± 6.38	43.35 ± 6.27	43.11 ± 6.04	0.79	2	281	0.45

Data presented as means ± SD, where indicated. HA = Harm avoidance; NS = novelty seeking; RD = reward dependence; P = persistence; SD = self-directedness; C = cooperativeness; ST = self-transcendence.

Multivariate analysis of covariance (MANCOVA) was used to test the possible influence of polymorphisms on TCI scores. With the aim of reducing possible sources of variance, we included genotype and sex as main factors in all analyses and education and age as covariates in the MANCOVA model. This was performed because of the influence of such variables on the TCI [9] and an unequal distribution in our study sample [22, 23]. Haploview 3.2 was used to generate a linkage disequilibrium map and to test for Hardy-Weinberg equilibrium [24]. Tests for associations using multi-marker haplotypes were performed using the statistics environment 'R' (http://www.R-project.org), package 'haplo.score', to compare TCI scores between haplotypes. Sex, age and education were added as covariates. Permutations (n = 10,000) were performed to estimate the global significance of the results for all haplotype analyses and to validate the expectation-maximization values.

All p values were 2-tailed, and statistical significance was conservatively set at the 0.0017 level (0.05 divided by 30 TCI factors). Traditional statistical analyses were performed using the Statistica package for Windows (StatSoft).

Results

Sociodemographic features as well as temperamental features of this sample have been extensively described elsewhere [7, 16–19].

All polymorphisms examined in this study (table 1) were in Hardy-Weinberg equilibrium in both control and suicide attempter samples (data not shown). In both sui-

Table 3. Temperament and character traits stratified for *NOS1* genotypes in suicide attempters

TCI traits	rs1353939			F	d.f.		p
	C/C (n = 58)	C/T (n = 38)	T/T (n = 15)				
HA	57.91 ± 7.56	56.03 ± 8.75	60.40 ± 6.02	1.16	2	103	0.32
NS	57.91 ± 5.52	58.53 ± 6.45	60.73 ± 5.55	1.60	2	103	0.21
RD	39.24 ± 3.71	39.97 ± 3.88	39.47 ± 3.44	0.32	2	103	0.72
P	11.81 ± 2.27	11.81 ± 1.69	11.00 ± 1.87	0.69	2	103	0.50
SD	69.60 ± 8.69	70.54 ± 8.71	63.17 ± 8.43	0.23	2	99	0.79
C	72.62 ± 5.46	73.29 ± 4.69	67.60 ± 9.95	3.35	2	103	0.04
ST	45.02 ± 6.60	46.10 ± 7.63	50.00 ± 7.84	2.99	2	103	0.05
TCI traits	rs2682826			F	d.f.		p
	A/A (n = 9)	A/G (n = 33)	G/G (n = 69)				
HA	59.78 ± 3.99	58.03 ± 8.64	57.11 ± 7.89	0.51	2	103	0.60
NS	60.67 ± 4.21	59.54 ± 6.70	57.72 ± 5.56	2.67	2	103	0.07
RD	39.89 ± 3.62	39.73 ± 3.73	39.38 ± 3.77	0.36	2	103	0.70
P	10.28 ± 1.60	11.82 ± 1.63	11.81 ± 2.22	1.08	2	101	0.34
SD	64.83 ± 6.49	68.34 ± 9.47	69.98 ± 8.71	0.14	2	99	0.87
C	70.00 ± 6.16	71.73 ± 7.91	72.67 ± 5.28	0.97	2	103	0.38
ST	51.00 ± 8.89	46.85 ± 7.53	45.04 ± 6.68	2.91	2	103	0.06
TCI traits	rs693534			F	d.f.		p
	A/A (n = 10)	A/G (n = 51)	G/G (n = 50)				
HA	57.80 ± 8.71	57.70 ± 7.37	57.46 ± 8.35	0.14	2	103	0.87
NS	59.10 ± 5.90	58.82 ± 6.30	58.06 ± 5.51	0.43	2	103	0.65
RD	40.40 ± 3.47	39.69 ± 3.49	39.18 ± 4.01	0.57	2	103	0.56
P	11.50 ± 1.78	12.02 ± 2.15	11.45 ± 1.97	0.36	2	101	0.36
SD	69.90 ± 9.28	69.75 ± 8.72	68.50 ± 9.07	0.17	2	99	0.85
C	74.10 ± 3.98	72.47 ± 5.67	71.48 ± 7.08	0.59	2	103	0.56
ST	44.30 ± 6.36	47.16 ± 7.61	45.30 ± 7.01	1.08	2	103	0.34

Data presented as means ± SD, where indicated. HA = Harm avoidance; NS = novelty seeking; RD = reward dependence; P = persistence; SD = self-directedness; C = cooperativeness; ST = self-transcendence.

cide and healthy samples, the linkage disequilibrium was similar to the one previously reported for a similar sample [for *NCAM1*, *TACR1*, *NOS1* and *NOS3* genes see respectively: 16–18].

We also performed haplotype analyses on polymorphisms showing a medium-low linkage disequilibrium in order to identify any possible association.

Concerning temperamental traits, we only found a significant association in the healthy sample between the rs2682826 SNP in *NOS1* and 'reward dependence' (RD) ($p = 0.000017$) as well as the subscale 'sentimentality' (RD1) ($p = 0.0009$). See tables 2 and 3 for results regarding *NOS1*. In particular, subjects with the A/A genotype showed higher scores in both RD and RD1.

No other association was detected analyzing genotypes of the other genes, allele frequencies as well as haplotypes, both in controls and suicide attempters (data not shown).

Discussion

The aim of this study was to investigate whether a panel of markers in *NOS1* (rs2682826, rs1353939, rs693534), *NOS3* (rs2070744, rs1799983, rs891512), *NCAM1* (rs2301228, rs1884, rs1245113, rs1369816, rs2196456, rs584427) and *TACR1* (rs3771810, rs3771825, rs726506, rs1477157) genes was associated with temperamental

traits measured with the TCI in a German sample of 111 suicide attempters and 289 healthy subjects. In the healthy sample we found an association between RD and the rs2682826 SNP in *NOS1*. However, this result could be due to the small sample size of A/A subjects (only 18 vs. 177 subjects with G/G genotype), and, therefore, this could plausibly be considered a false-positive finding. Consequently, overall our results did not support the hypothesis of a direct modulation of these genetic variants on temperamental traits in the considered sample.

To the best of our knowledge, this is the first study investigating the association between these genes and TCI traits; therefore, our results should be considered as preliminary findings.

However, in the same sample, we previously detected an association between haplotypes in *NOS1* and *NOS3* genes and both suicidal behavior and aggressiveness traits measured using the Questionnaire for Measuring Factors of Aggression [16]. Moreover, recently 3 independent studies of non-clinical samples reported an association between the *NOS1* ex1f variable number tandem repeat (VNTR) – functional promoter polymorphism – and impulsivity traits measured with different questionnaires [25–27]. Hence, it is plausible that *NOS1* and *NOS3* genes could be implicated in the modulation of impulsive and/or aggressive traits. Consequently, further studies should be performed on larger samples of psychiatric patients with and without previous suicide attempts and healthy volunteers. In addition, further polymorphisms should be considered, such as the ex1f VNTR. Clearly, the possibility of the lack of modulation of these genes on temperament measured by TCI should also be considered. Moreover, further studies on the association between *NCAM1* and *TACR1* and temperamental traits should be performed.

Our study is not without limitations: (1) the considered SNPs are mostly not known to alter functional proprieties of the respective genes or proteins and the functional relevance mostly remains to be demonstrated or, alternatively, the causative genetic variations remain to be identified; (2) only a few SNPs from each gene were selected; (3) the power of our sample was not high, considering the complexity of the personality phenotype, and it could have raised false-negative findings.

In conclusion, this study reported the lack of a direct modulation of *NOS1*, *NOS3*, *NCAM1* and *TACR1* genes on temperamental traits in both suicide patients and controls; however, further studies should be performed to deeply dissect this issue, considering in particular a number of recent findings reporting associations between *NOS1* gene and impulsivity traits measured with different scales.

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