ORIGINAL ARTICLE

Role of the Serotonin Transporter Promoter Polymorphism in Anxiety-Related Traits

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Background: The heritability of interindividual variation in anxiety and other aspects of personality establishes that variants of genes influence these traits. A functional polymorphism in the promoter of the human serotonin transporter gene (*SLC6A4*C*) was identified and found to be linked to an anxiety-related personality trait, Neuroticism. The polymorphism affects gene transcription and, ultimately, gene function. We have attempted to confirm the role of *SLC6A4*C* in anxiety-related personality traits by sibpair analysis and association studies.

Methods: Sibpair linkage analysis and association study were performed in 655 Finns. The index cases were 182 alcoholic criminal offenders, through which 258 relatives were ascertained to obtain 366 sibpairs. In addition, 215 unrelated population controls were collected. Each individual was psychiatrically interviewed, blind-rated for *DSM-III-R* diagnoses, and assessed with the Tridimensional Personality Questionnaire.

Results: The sibpair analysis revealed a positive linkage between *SLC6A4*C* and the 2 anxiety-related subdimensions of Harm Avoidance: HA1 (Anticipatory Worry) and HA2 (Fear of Uncertainty) ($P = .003$). However, there was no consistent association between *SLC6A4*C* and any Tridimensional Personality Questionnaire trait.

Conclusions: In the present study we replicated the relationship of *SLC6A4*C* to anxiety by sibpair linkage analysis but found no evidence of association, raising the question of whether *SLC6A4*C* locus is itself affecting anxiety or is linked to another still unknown functional variant.

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NEUROPHYSIOLOGICAL processes, which are genetically regulated, induce basic personality dimensions that determine inclinations to activate, preserve, or repress specific behaviors within the range available in a given society.[1] Variation in personality is one important component of human individuality and this variation is due to the interaction of interindividual differences in genes and environment.[2]

In the tridimensional theory of personality proposed by Cloninger,[3,4] each of 3 personality dimensions is postulated to be related to the primary action of 1 neurotransmitter system: Novelty Seeking (NS) to dopamine activity, Harm Avoidance (HA) to serotonin activity, and Reward Dependence (RD) to norepinephrine activity.[5] Among the 3 dimensions, HA is most closely akin to anxiety, being influenced by anxiety state, anxiety trait, and sex.[6] Individuals scoring higher than average in HA are characterized by anxiousness and shyness, and high HA scores are found in patients with generalized anxiety disorder.[6-9] Involvement of serotonin in HA and anxiety[10] is consistent with the positive therapeutic effects of selective serotonin reuptake inhibitors (SSRIs) in both anxiety and depression. The genetic epidemiology of anxiety and depression is consistent with a shared vulnerability for these disorders.[11] Although both SSRIs and benzodiazepines are commonly used in the treatment of anxiety, the SSRIs have a wider spectrum of action and are highly effective in depression.

Recently, a functional polymorphism in the human serotonin transporter (SLC6A4) promoter has been described[12] and associated by inference to 2 subdimensions of HA, Anticipatory Worry (HA1) and Fear of Uncertainty (HA2), and directly to

SUBJECTS AND METHODS

SUBJECTS

The sample (n = 655) consisted of alcoholic criminal offenders (ACOs), their relatives, and population controls. The index cases were 182 unrelated ACOs who because of the nature of their crimes were remanded to forensic psychiatric examination at the time of their incarceration. In addition, 258 relatives were collected through index cases. Therefore, 366 sibpairs were obtained and consisted of some of the index cases (ACOs) and their non-ACO sibs, so the sibpairs did not necessarily involve 1 ACO as in families with more than 2 children. The population control sample consisted of 215 nonrelated psychiatrically interviewed Finnish volunteers who were recruited by advertisement and paid for their participation. The Structured Clinical Interview for *DSM-IV* (SCID) was administered by 2 psychiatrists (H.N. and M.E.). Psychiatric diagnoses and TPQ scores were ascertained blind to subject identities and history. Alcoholism was defined by *DSM-III-R* criteria for alcohol dependence or alcohol abuse.[15]

All subjects provided an informed written consent before entering the study. This protocol was approved by the Institutional Review Boards of the National Institutes of Health and the National Institute of Mental Health, Rockville, Md, of the Department of Psychiatry, University of Helsinki and of the University of Helsinki Central Hospital, Helsinki, Finland, and by the Office for Protection from Research Risks, Bethesda, Md.

TPQ TRAITS

The TPQ measures 3 higher-order personality dimensions.[3,4] These 3 personality dimensions are Novelty Seeking (NS), Harm Avoidance (HA), and Reward Dependence (RD), each of which is divided into 4 subdimensions as given in **Table 1**. High NS is a heritable tendency toward frequent exploratory activity and excitement in response to novel stimuli. High HA refers to the tendency to avoid situations likely related to punishment. High RD reflects a need for constant reassurance and positive reinforcement.

POLYMERASE CHAIN REACTION (PCR)

The genomic DNA was isolated from Epstein-Barr virus—immortalized lymphoblastoma cell lines. DNA amplification was accomplished using the 2 flanking primers suggested in 1996 by Heils et al[12]:
5-HTTU:5′GGCGTTGCCGCTCTUAATGC3′, nt-1416,-1397
5-HTTL:5′GAGGGACTGAGCTGGACAACCAC, nt-910,-889.
This set of primers amplifies a 484/528 fragment corresponding to the *SLC6A4*C* short and long allele, respectively. The PCR conditions were slightly modified from Heils et al. The PCR reaction was carried out in a total volume of 20 µL consisting of 100 ng of genomic DNA, 0.1 µmol of primers per liter, 40-µmol/L deoxynucleotide triphosphates, 20-µmol/L 7-deaza-2′deoxyguanosine, and 1 unit of AmpliTaq with the appropriate buffer (Perkin-Elmer Biosystem, Foster City, Calif). To enhance the specificity and sensitivity of DNA amplification, TaqStart (Clontech, Palo Alto, Calif), a neutralizing monoclonal antibody directed against *Taq* DNA polymerase, was added so that HotStart-PCR could be performed. Cycling conditions were as follows: 1 denaturing cycle at 95°C for 5 minutes, 2 cycles with a touchdown annealing temperature of 63°C and 62°C, respectively for 30 seconds, and 38 cycles with an annealing temperature at 61°C. Final DNA elongation was at 72°C for 10 minutes. DNA bands were visualized in prestained (0.4-µg/mL ethidium bromide) 2% agarose gels that were run for 1 hour at 120 V.

STATISTICAL ANALYSIS

Linkage analysis was conducted using the Haseman-Elston sibpair method.[16] The Haseman-Elston approach evaluates allele sharing by sibpairs for a polymorphic marker (as in this case the *SLC6A4*C*). At the locus of interest, the sibpairs share 0, 1, or 2 alleles identical by descent (IBD) (direct inheritance of copies of the same parental allele). The *P* values for sibpair linkage analysis are obtained by regression analysis of the squared trait difference Δ^2 between each pair of siblings vs the number of alleles shared, IBD: 0, 1, or 2. For linkage, a negative slope is expected because siblings who resemble each other in the trait of interest tend to share alleles that are IBD.[16-18] Because the accuracy of sibpair analysis depends on large sample approximations, *P* values were verified by computer simulations. While holding the phenotype, family structures, and genotype distribution constant, the different *SLC6A4*C* alleles were randomly assigned to the founders of the pedigrees based on their population frequencies. These simulated genotypes were randomly transmitted to the offspring and analyzed for sibpair linkage using the SAGE (Statistical Analysis for Genetic Epidemiology) sibpal module.[19] By replicating this procedure 12 000 times, a new empirical distribution was created that was used to obtain simulation-derived *P* values.

Association studies are most powerful when applied to functionally significant variations in genes having a clear biological relation to the trait. Most often, a case-control comparison is performed in which genotype or allele frequencies are compared in groups that differ in affectation.[18] Here we compare different genotype groups for a difference in a continuous variable. *P* values were obtained using 1-way analysis of variance.

an equivalent anxiety-related personality trait on the NEO Personality Inventory–Revised: Neuroticism.[13] The polymorphism (*SLC6A4*C*) consists of a repetitive sequence (variable number of tandem repeats), located 1 kilobase upstream of the transcription site. The allele with a smaller number of repeats (*SLC6A4*C14*) (frequency = 0.40) has lower transcriptional activity leading to reductions in messenger RNA levels, serotonin binding, and serotonin reuptake compared with the longer allele (*SLC6A4*C16*).[13,14]

In this study, we examined the linkage of the *SLC6A4*C* locus to anxiety-related traits by performing sibpair analysis and association studies in a population of 655 Finns that included unrelated and related indi-

Table 1. Sibpair Linkage Analysis of *SLC6A4*C* and Tridimensional Personality Questionnaire Dimensions and Subdimensions*

Dimension and Subdimension	F Value	*P* Value
Novelty Seeking (NS)		
NS 1, exploratory excitability vs stoic rigidity	−0.63	.27
NS 2, impulsiveness vs reflection	−0.45	.33
NS 3, extravagance vs reserve	−0.45	.33
NS 4, disorderliness vs regimentation	−0.45	.33
NS, total	1.13	.87
Harm Avoidance (HA)		
HA 1, anticipatory worry vs uninhibited optimism	−2.81	.003
HA 2, fear of uncertainty vs confidence	−2.85	.003
HA 3, shyness with strangers vs gregariousness	−1.13	.13
HA 4, fatigability and asthenia vs vigor	0.69	.75
HA, total	−0.13	.45
Reward Dependence (RD)		
RD 1, sentimentality vs insensitiveness	−0.73	.23
RD 2, persistence vs irresoluteness	−1.00	.16
RD 3, attachment vs detachment	0.30	.62
RD 4, dependence vs independence	−1.20	.11
RD, total	−0.41	.34
df = 171		

**Sibpair linkage analysis was performed in 86 Finnish families with a total of 366 sibpairs to investigate the relationship between the* SLC6A4*C *locus and Tridimensional Personality Questionnaire dimensions and subdimensions.*

viduals, all of whom were psychiatrically interviewed and evaluated with the Tridimensional Personality Questionnaire (TPQ).

RESULTS

Linkage of *SLC6A4*C* to TPQ personality dimensions was evaluated in 366 sibpairs. As given in Table 1, the *SLC6A4*C* locus was strongly linked to the 2 anxiety-related subdimensions, HA1 (Anticipatory Worry, $P = .003$) and HA2 (Fear of Uncertainty, $P = .003$). These significant *P* values were verified by computer simulations but were not corrected for multiple analyses because the prior hypothesis was that the *SLC6A4*C* polymorphism contributes to anxiety-related traits, in particular HA1 and HA2.[13] Linkage to the other domains of personality assessed by the TPQ was also evaluated in exploratory analyses. None showed evidence of linkage (Table 1).

Association study of *SLC6A4*C* to TPQ personality dimensions was tested in 215 unrelated Finnish controls, in 182 unrelated Finnish ACOs, in the total unrelated sample (n = 397) (**Table 2** and **Table 3**). Neither HA1 nor HA2 showed a significant association. In exploratory analyses, NS1 (exploratory excitability vs stoic rigidity) and NS total did show *P* values of $P = .03$ and = .04, respectively, in unrelated controls (Table 2). Also, RD3 (attachment vs detachment) and RD total showed *P* values of $P = .01$ and = .03, respectively, in unrelated ACOs (Table 2) and $P = .009$ in controls + ACOs (Table 3). However, since there was no prior hypothesis that these traits were affected by *SLC6A4*C*, these results would not withstand correction for multiple testing.

COMMENT

Harm Avoidance reflects a tendency to respond more intensely to aversive stimuli. People with high HA scores are more fatiguable, shy and anxious with strangers, and tend to worry and become tense in unfamiliar situations.[5,7] It has been suggested that variations in serotonin function in the septohippocampal system are involved in increased HA and in anxiety.[4,10]

The serotonin transporter plays a crucial role in the regulation of serotonin neurotransmission by terminating the action of serotonin in the synapse and is the site of action of the SSRIs, used in the treatment of both depression and anxiety. The discovery that a functional serotonin transporter polymorphism was linked to anxiety and a possible association of another serotonin transporter marker to depression were therefore consonant with current knowledge of the origins and molecular targets for treatment of anxiety and depression.[13,20] In the present study, we were able to replicate the relationship of *SLC6A4*C* to anxiety (HA1 and HA2) by sibpair linkage analysis but found no evidence of significant association.

Sibpair analysis is an allele-sharing method that involves testing under random mendelian segregation whether affected relatives inherit a region IBD from their parents more often than expected. Siblings share an IBD linkage in either 0, 1, or 2 alleles at any autosomal locus, allowing a test of a locus' effect on any trait by regression analysis. On the other hand, valid associations are detected either when the allele is in linkage disequilibrium to a functional allele at a nearby locus or when the allele itself influences the trait. Association studies are therefore identity by state analyses and it can be seen that they are most powerful when applied to functionally significant variations in genes that have a clear biological relationship to the trait.[18]

In view of the functional importance of *SLC6A4*C*, we were therefore somewhat surprised to observe IBD linkage without identity by state association. Since the sibpairs also included individuals who were ACOs, we also conducted a sibpair linkage analysis to *DSM-III-R* alcoholism, but we did not detect linkage (data not shown). In this way, we can rule out the possibility that we have actually detected a linkage to alcoholism instead of to HA. However, there are other possible explanations why the IBD linkage analysis may have had more power to detect an effect of *SLC6A4*C* than did the association design. Sibpairs may offer an advantage in the detection of allele effects on phenotypes of complex genetic and environmental origins such as TPQ traits. In sibpairs, much of the environment is shared and 50% of the background genotype is held constant. Moreover it may also be that the number of individuals studied is still too small. Consistent with results from previous studies,[13] individuals homozygous or heterozygous for *SLC6A4*14* scored higher in HA1 and HA2 compared with *SLC6A4*16* homozygotes. Finally the serotonin transporter promoter polymorphism could be linked to another

Table 2. Population Association Study Between *SLC6A4*C* and Tridimensional Personality Questionnaire Dimensions and Subdimensions*

Dimension and Subdimension	Unrelated Controls, Genotype (No. of Individuals)							
	14/14 (41)		14/16 (106)		16/16 (68)			
	Mean	SD	Mean	SD	Mean	SD	F Value	*P* Value
Novelty Seeking (NS)								
NS 1	4.4	1.9	5.0	1.7	5.3	1.7	3.42	.03
NS 2	3.3	2.1	3.8	2.0	3.4	2.1	1.40	.25
NS 3	4.0	1.7	4.5	1.5	4.4	1.5	2.04	.13
NS 4	4.0	1.6	4.5	1.7	4.4	1.7	1.50	.22
NS, total	15.6	5.0	17.8	4.6	17.5	4.8	3.27	.04
Harm Avoidance (HA)								
HA 1	3.2	1.9	2.9	2.1	3.0	2.0	0.33	.72
HA 2	3.5	1.4	3.1	1.6	3.3	1.8	0.69	.50
HA 3	2.6	1.8	2.3	1.8	2.3	1.8	0.53	.59
HA 4	2.4	1.9	2.3	1.8	2.6	2.2	0.49	.61
HA, total	11.7	4.9	10.6	5.5	11.2	5.8	0.58	.56
Reward Dependence (RD)								
RD 1	2.2	1.3	2.4	1.3	2.8	1.4	2.90	.06
RD 2	4.0	1.7	3.7	1.8	3.8	1.9	0.49	.61
RD 3	6.7	2.6	7.2	2.4	7.7	2.5	1.85	.16
RD 4	2.9	1.2	3.0	1.4	3.1	1.4	0.36	.70
RD, total	15.8	4.2	16.4	3.4	17.3	4.3	1.74	.18
df = 212								

Dimension and Subdimension	Unrelated Alcoholic Criminal Offenders, Genotype (No. of Individuals)							
	14/14 (35)		14/16 (90)		16/16 (57)			
	Mean	SD	Mean	SD	Mean	SD	F Value	*P* Value
NS								
NS 1	4.6	1.6	4.4	1.8	4.3	1.7	0.27	.76
NS 2	4.6	2.0	4.9	2.2	4.5	2.3	0.67	.51
NS 3	5.2	1.4	4.6	1.7	4.9	1.7	1.59	.21
NS 4	5.1	2.0	5.6	2.0	5.1	2.0	1.65	.20
NS, total	19.5	5.0	19.5	5.0	18.7	4.8	0.52	.60
HA								
HA 1	5.1	2.5	4.9	2.5	4.8	2.0	0.24	.79
HA 2	4.6	2.1	4.2	2.0	4.4	1.8	0.60	.55
HA 3	4.4	2.3	4.4	2.1	4.1	2.0	0.61	.54
HA 4	3.9	2.7	4.3	2.6	3.7	2.5	1.01	.36
HA, total	18.1	7.3	17.9	6.8	17.0	6.2	0.42	.66
RD								
RD 1	2.8	1.3	2.7	1.5	2.8	1.4	0.17	.85
RD 2	4.1	2.0	3.9	1.8	4.4	2.0	1.23	.29
RD 3	5.7	2.6	5.3	2.5	6.7	2.7	4.51	.01
RD 4	2.0	1.5	2.6	1.3	2.6	1.3	2.02	.14
RD, total	15.8	4.2	14.5	4.5	16.5	4.8	3.46	.03
df = 179								

**Tridimensional Personality Questionnaire scores were assessed in a group of 215 unrelated Finnish controls consisting of 41 14/14 homozygous, 106 14/16 heterozygous, and 68 16/16 homozygous individuals, and in a group of 182 unrelated Finnish alcoholic criminal offenders consisting of 35 14/14 homozygous, 90 14/16 heterozygous, and 57 16/16 homozygous individuals. Genotypes and Tridimensional Personality Questionnaire scores were compared using 1-way analysis of variance. See footnote to Table 1 for explanation of each subdimension.*

nearby functional locus whose effect is detected by sibpair linkage analysis but not by the association design.

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Table 3. Population Association Study Between *SLC6A4 *C* and Tridimensional Personality Questionnaire Dimensions and Subdimensions***

	Unrelated Controls + Alcoholic Criminal Offenders, Genotype (No. of Individuals)							
	SS (76)		SL (196)		LL (125)			
Dimension and Subdimension	Mean	SD	Mean	SD	Mean	SD	F Value	*P* Value
Novelty Seeking (NS)								
NS 1	4.9	1.8	4.7	1.8	4.9	1.7	1.06	.35
NS 2	3.9	2.1	4.3	2.2	3.9	2.2	1.81	.16
NS 3	4.5	1.7	4.6	1.6	4.6	1.6	0.08	.93
NS 4	4.5	1.9	5.0	1.9	4.7	1.9	2.55	.08
NS, total	17.5	5.3	18.6	4.8	18.0	4.8	1.74	.18
Harm Avoidance (HA)								
HA 1	4.1	2.3	3.8	2.4	3.8	2.1	0.34	.71
HA 2	4.0	1.8	3.7	1.8	3.8	2.0	1.17	.31
HA 3	3.5	2.2	3.2	2.2	3.1	2.1	0.77	.47
HA 4	3.1	2.4	3.2	2.5	3.1	2.4	0.15	.86
HA, total	14.7	6.9	14.0	7.0	13.8	6.6	0.37	.69
Reward Dependence (RD)								
RD 1	2.5	1.3	2.5	1.4	2.8	1.4	1.92	.15
RD 2	4.1	1.8	3.8	1.8	4.0	2.0	1.02	.36
RD 3	6.3	2.6	6.4	2.6	7.2	2.6	4.76	.009†
RD 4	2.5	1.4	2.8	1.4	2.9	1.4	1.91	.15
RD, total	15.3	4.5	15.5	4.3	16.9	4.5	4.73	.009†
df = 394								

**Tridimensional Personality Questionnaire scores were assessed in a group of 397 unrelated Finnish controls + alcoholic criminal offenders consisting of 76 14/14 homozygous, 196 14/16 heterozygous, and 125 16/16 homozygous individuals. See footnote to Table 1 for explanation of each subdimension.*

†*These* P *values will not stand correction for multiple analyses.*

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