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Research paper

Nice guys: Homozygocity for the *TPH2* -703G/T (rs4570625) minor allele promotes low aggressiveness and low anxiety

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ABSTRACT

Background: Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in the synthesis of serotonin. We examined whether the *TPH2* polymorphism -703G/T (rs4570625) is associated with aggressiveness and impulsivity, and the prevalence of psychiatric disorders, in a population-representative sample.
Methods: We used self and proxy reports on aggressive behaviour in the younger birth cohort of the longitudinal Estonian Children Personality, Behaviour and Health Study collected at age 25, and earlier collected impulsivity and related data of both ECPBHS cohorts.
Results: The TT homozygous males reported less aggressive behaviour in the Life History of Aggression interview at age 25. They also had significantly lower scores in Illinois Bully Scale peer reports, and less ADHD symptoms rated by teachers both at ages 9 and 15. The TT homozygotes of both sexes had the lowest Maladaptive Impulsivity at ages 18 and 25, and the highest Adaptive Impulsivity at age 25. The TT homozygotes also had low depressiveness and trait anxiety by age 25, and the odds ratio for the prevalence of anxiety disorders was 9.38 for the G-allele carriers.
Limitations: The main limitation of the study is the naturally occurring low number of subjects with the TT genotype.
Conclusions: Subjects with the *TPH2* rs4570625 TT genotype, especially males, exhibit less aggression and a favourable impulsivity profile, and develop anxiety disorders by young adulthood less often.

1. Background

Tryptophan hydroxylase (TPH) is the rate-limiting enzyme in the synthesis of serotonin. The role of the serotonergic system in regulating emotions, including aggressiveness is relatively well established (e.g., Bortolato et al., 2013; Lesch et al., 2012; Miczek et al., 2015). However, there is no simple explanation how alterations in serotonergic homeostasis lead to different types of psychopathology, or other- or self-directed aggressive behaviour. Therefore, additional information about the role of naturally existing variations in the serotonin system, including genetic variation, in emotion regulation and aggressive traits is crucial for understanding the underlying neurobiology.

There are two isoenzymes of TPH in humans, TPH1 and TPH2, the latter being predominantly expressed in the brain (Carkaci-Salli et al., 2011; Walther et al., 2003). Variations in genes encoding both TPH-s have been linked to emotion regulation and aggression-related traits (Bortolato et al., 2013; Waider et al., 2011), including the potentially functional rs4570625 SNP in the TPH2 gene that leads to G to T base substitution in the promoter region at position -703. The functionality of this polymorphism still requires establishment: Two studies have found differences in gene expression in haplotypes containing the -703G/T (Chen et al., 2008; Lin et al., 2007), but Scheuch et al. (2007) found no G/T difference in promoter activity. Nevertheless, in studies on brain and behaviour, the T-allele of the -703G/T polymorphism has been described as a psychiatric risk allele as it is linked to biased amygdala responsiveness (Brown et al., 2005; Canli et al., 2005) and is overrepresented in cluster B and C personality disorders, as well as in both affective and anxiety disorders with a sample of patients with personality disorders (Gutknecht et al., 2007). The meta-analysis by Gao et al. (2012) found T-allele carriers at greater risk of affective

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disorders, and one report (Mandelli et al., 2012) has suggested higher sensitivity to stressful life events in this genotype. In some contrast, Gutknecht et al. (2007) linked the haplotype containing rs4570625 G-allele to higher TPQ Harm Avoidance and NEO Neuroticism in healthy adults, and Reuter et al. (2007a) found lower harm avoidance in TT homozygotes. The G-allele has been found transmitted more often to the children affected by ADHD (Walitza et al., 2005) and OCD (Mössner et al., 2006); GG homozygotes, both controls and ADHD patients, had altered prefrontal function during response inhibition task (Baehne et al., 2009). Furthermore, increased frequency of the G-allele was associated with suicide attempts in patients with major depression (Yoon and Kim, 2009). Perez-Rodriguez et al. (2010) found evidence of the involvement of *TPH2* in aggressiveness but they tested haplotypes that did not contain rs4570625. TPH2 -703G/T SNP may have important effect on susceptibility to suicidal behaviour in major depression.

In case of the *TPH2* G/T polymorphism, for practical reasons, carriers of the low frequency T-allele have mostly been compared with the GG genotype. However, in our recent study (Lehto et al., 2015) the population-derived sample was large enough to allow the comparison of all genotypes separately. We found that the TT homozygotes differed significantly from other genotype groups: Specifically, *TPH2* G-allele homozygotes and GT heterozygotes had similar personality profiles while the TT homozygotes had significantly lower Neuroticism, and higher Extraversion and Conscientiousness.

Given the important role of serotonergic function in emotion regulation and aggressiveness, and the findings on *TPH2* described above, we sought to test how the variation in *TPH2* rs4570625 affects aggressive behaviour and related traits, and prevalence of mood, anxiety and alcohol use disorders in a population-representative sample of young adults.

2. Method

2.1. Sample

This study was carried out on the Estonian sample of the European Youth Heart Study (1998/99), which was subsequently incorporated into the longitudinal Estonian Children Personality Behaviour and Health Study (ECPBHS). The EYHS sample of the ECPBHS consists of two birth cohorts. Data on aggressive and antisocial behaviour, bullying and victimisation are currently available for the younger cohort only. Hence, most of the present analysis is focused on data of this cohort, but relevant measures of both cohorts were subject to analysis if available. The rationale and procedure of sample formation have been described elsewhere in detail (Harro et al., 2001; Tomson et al., 2011). The total number of subjects in the first wave in 1998/99 was 1176; 583 in the younger cohort (M_{Age}=9.6 ± 0.5) and 593 in the older cohort (M_{Age}=15.6 ± 0.6). The follow-up studies for the younger cohort took place in 2004 (n=483, M_{Age}=15.3 ± 0.5), 2007 (n=454, M_{Age}=18.3 ± 0.5) and 2014 (n=440, M_{Age}=25.3 ± 0.5); for the older cohort, the follow-ups were in 2001 (n=449, including 62 additional subjects, M_{Age}=18.4 ± 0.9) and 2008 (n=541, M_{Age}=24.7 ± 0.7). The number of subjects with valid genotype and psychometric data is given in Table 1. All the subjects were of Caucasian origin. The study was approved by the Tartu University Ethics Review Committee on Human Research.

2.2. Measures

2.2.1. Illinois Bully Scale (IBS)

Illinois Bully Scale is an 18-item scale with three subscales, Bully, Fight, and Victim, assessing the frequency of bullying behaviour, fighting, and victimization by peers (Espelage and Holt, 2001). We used the conventional self-report and a version of the scale adopted to be filled in by classmates where we asked subjects to rate their peers (peer reports, mostly two raters per person; each rater also reported on two classmates as a rule). In both cases, we asked subjects to recall the times in primary school assessing the frequency of listed behaviours in 5-point scale ranging from “never” to “very often”. In case of peer ratings, subjects were shown the list of classmates and were asked to select the first one or two peers they remembered well. For this reason, the number of subjects for whom proxy reports were obtained is larger than the number of participants in this particular study wave. The list was narrowed gradually as reports were obtained, but when a subject could not make a decision, he/she was permitted to pick someone from the list of already rated peers. Averaged scores were used in case of more than one rating per subject.

2.2.2. ADHD symptoms by teacher reports

ADHD symptoms were assessed by teachers as described in Kiive et al. (2010) using the Hyperactivity Scale (Af Klinteberg and Oreland, 1995). The data of both birth cohorts for ages 15 and 18 were combined, while for age 9, data were available for the younger birth cohort only (Table 1).

2.2.3. Impulsivity self-reports

Participants filled in the Adaptive and Maladaptive Impulsivity Scale (Laas et al., 2010; Paaver et al., 2008;) with subscales measuring Fast decision making and Excitement seeking (functional or adaptive impulsivity), and Disinhibition and Thoughtlessness (dysfunctional or maladaptive impulsivity). The data of both birth cohorts collected at ages 18 and 25 were combined (Table 1).

2.2.4. Psychiatric interview at age 25

Psychiatric assessment based on DSM-IV was carried out in both birth cohorts at age 25 (Table 1) by experienced clinical psychologists using the Mini-International Neuropsychiatric Interview (M.I.N.I.5.0.0; Sheehan et al., 1998; Estonian version: Shlik et al., 1999). We used lifetime prevalence of disorders in the analysis.

2.2.5. Life History of Aggression interview

The Life History of Aggression interview (LHA, Coccaro et al., 1997) was carried out in the younger birth cohort immediately after the M.I.N.I. interview to assess dimensions of aggression (Table 1). Items were scored only for the history of actual behaviour. LHA has three subscales: Aggression (temper tantrums, verbal aggression, indirect aggression, non-specific fighting, and physical assault against people); Consequences/Antisocial Behaviour (school disciplinary problems, problems with supervisors, antisocial behaviour not resulting in police involvement, and antisocial behaviour involving the police); and Self-Directed Aggression (self-injurious behaviour, and suicide attempts). Each item was rated on a 5-point scale, ranging from 0=No events to 5=More events than can be counted.

2.2.6. TPH2 rs4570625 genotyping

Genomic DNA was extracted from whole blood samples using Qiagen QIAamp® DNA Blood Midi Kit. Genotyping for *TPH2* G-703T (rs4570625) was performed as described in Lehto et al. (2015) with the Applied Biosystems ViiA™ 7 Real-Time PCR System using the TaqMan® Pre-Designed SNP Genotyping Assay with Solis BioDyne 5× HOT FIREPol® Probe qPCR Mix Plus (ROX). All DNA samples were successfully genotyped. The genotype frequencies were in Hardy–Weinberg equilibrium. The distribution of *TPH2* genotype (Table 2) was in Hardy-Weinberg equilibrium and did not differ between birth cohorts (Fisher's Exact Test p=0.546) and. The frequency of the minor T-allele was 21.8%.

2.3. Statistical analysis

Subjects were analyzed by the *TPH2* genotype groups. Due to genotype distribution (see Table 2), the group sizes were too unequal to rely on parametric statistical tests like analysis of variance, so we have