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Risk-Taking Behavior in a Gambling Task Associated with Variations in the *Tryptophan Hydroxylase 2* Gene: Relevance to Psychiatric Disorders

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Decision making, choosing the best option from the possible outcomes, is impaired in many psychiatric conditions including affective disorders. We tested the hypothesis that variations in serotonergic genes (*TPH2*, *TPH1*, *SLC6A4*, *HTR1A*), which influence serotonin availability, affect choice behavior in a probabilistic gambling task. A population cohort ($N = 1035$) completed a paper-and-pencil gambling task, filled out personality and symptom questionnaires and gave consent for the use of their DNA in a genetic association study. A subgroup of subjects ($N = 69$) also completed a computer version of the task. The gambling task was designed to estimate an individual's tendency to take a risk when choosing between a smaller but more certain 'win' and a larger, less probable one. We genotyped seven haplotype tagging SNPs in the *TPH2* gene, and previously reported functional polymorphisms from the other genes (*rs1800532, 5HTTLPR*, and *rs6295*). Carriers of the more prevalent TPH2 haplotype, which was previously associated with less active enzyme variant, showed reduced risk taking on both tasks compared with subjects not carrying the common haplotype. The effect of TPH2 haplotypes on risk-taking was independent of current depression and anxiety symptoms, neuroticism and impulsiveness scores. We did not find an association between functional polymorphisms in the *TPH1, SLC6A4, HTR1A* genes and risk-taking behavior. In conclusion, our study demonstrates the role of the *TPH2* gene and the serotonin system in risk taking and suggests that *TPH2* gene may contribute to the expression of psychiatric phenotypes through altered decision making.
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INTRODUCTION

Decision making depends on a complex set of processes that are orchestrated in various brain systems to find an optimal outcome (Elliott and Deakin, 2005; Paulus, 2007). Risk-taking refers to the willingness to accept a possible negative outcome in order to potentially achieve a desirable outcome, and typically involves assessing the relative probability of winning or losing against the values of the outcomes (Anderson *et al*, 2003; Cardinal, 2006). Subjects with depression or anxiety are less likely than controls to take risks to gain reward (Elliott *et al*, 1997; Forbes *et al*, 2006; Smoski *et al*, 2008; Paulus, 2007). In contrast, pathological gamblers are more risk-seeking (Holt *et al*, 2003). Another aspect of decision making is self-control, or the ability to delay gratification, which is more related to impulsiveness and is impaired in patients with substance-use disorder (Rogers *et al*, 1999; Bechara *et al*, 2002; Paulus, 2007).

Manipulation of serotonin (5-HT) appears to influence decision making. Acute tryptophan depletion (ATD) leads to impulsive choice and impaired decision making related to different magnitudes of gains, in both animals and humans (Rogers *et al*, 1999, 2003; Mobini *et al*, 2000; Walderhaug *et al*, 2007). Furthermore, a recent study showed that tryptophan supplementation significantly decreased loss-aversion (Murphy *et al*, 2008). Although

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5-HT appears to influence choice involving delayed reinforcement or different magnitudes of gains, it remains unclear whether 5-HT affects choice related to the assessment of probability (Mobini *et al*, 2000; Anderson *et al*, 2003; Rogers *et al*, 2003; Cardinal, 2006), especially at a genetic level.

It has been reported that possession of the less active S allele of the 5-HT transporter *5HTTLPR* polymorphism (*SLC6A4* gene short/S–long/L promoter variant) increased sensitivity to the probability of winning in a 'risky-choice task' (Roiser *et al*, 2006) and promoted disadvantageous choices in the Iowa Gambling Task (IGT) probably due to lack of persistence (Must *et al*, 2007; Homberg *et al*, 2008) or to slower learning of advantageous decision making (Jollant *et al*, 2007). It has also been suggested that *5HTTLPR* variations modulate sensitivity to punishment rather than reward, as the LL homozygous individuals are less sensitive to punishment-related information (Blair *et al*, 2008; Roiser *et al*, 2009). Jollant *et al* (2007) showed that genetic variations in the *TPH1, TPH2* (tryptophan hydroxylase 1 and 2, respectively), and *MAOA* (monoamine oxydase A) genes that have been associated with suicide were also associated with poorer performance on the IGT in suicide attempters. However, all of the above-mentioned studies were carried out in small samples (less than 200 subjects) and require confirmation in larger samples.

In this study, the main candidate gene was the *TPH2* that codes for the majority of TPH in the human brain (Walther *et al*, 2003; Zill *et al*, 2007a). Variations in the *TPH2* gene have been repeatedly implicated in major depression disorder (MDD), and in suicidal behavior in MDD patients (Zill *et al*, 2004a, b; Zhang *et al*, 2005; Zhou *et al*, 2005; Lopez *et al*, 2007a; Haghighi *et al*, 2008). To cover this gene, we used haplotype tagging SNPs (htSNPs) similar to the previous studies.

As the availability of synaptic 5-HT depends on other serotonergic genes, we also investigated the well-known *5HTTLPR* polymorphism and a functional *TPH1* gene variant *(A218C, rs1800532)*. *A218C* has been shown to alter TPH immunoreactivity in postmortem brain samples of both suicide victims and controls (Ono *et al*, 2002) and has been associated with suicidal behavior (Li and He, 2006). It has been demonstrated that *TPH1* is expressed in several human brain areas, although at lower levels than *TPH2* in the raphe (Zill *et al*, 2007a). Finally, we genotyped for a functional variant in the *5-HTR1A* receptor gene *(C(-1019)G, rs6295)* that modulates the expression of 5-HT1A autoreceptors in raphe cells and that controls 5-HT neuronal firing, and this influence synaptic 5-HT release (Lemonde *et al*, 2003). This functional polymorphism has previously been associated with depression-, suicide-, and anxiety-related traits (Lemonde *et al*, 2003; Strobel *et al*, 2003).

In order to investigate risky decision-making behavior in a large population, we developed a pencil-and-paper probabilistic gambling task administered remotely with questionnaires assessing personality data, current mood, and anxiety symptoms. On the basis of previous literature, we hypothesized that subjects with a self-reported history of depression or with a higher neuroticism, depression and/or anxiety scores would make safer (less risky) choices. Furthermore, we hypothesized that variants in the serotonergic genes and especially in the *TPH2* gene that regulates the availability of synaptic 5-HT would influence risky decision making.

MATERIALS AND METHODS

Participants

This study was part of the EU funded NewMood (New Molecules for Mood Disorders) research program. We recruited participants aged 18–60 years from Greater Manchester, United Kingdom, through general practices, and a website (http://www.newmood.co.uk); details of our recruitment strategy and responses were published previously (Juhasz *et al*, 2009). The included participants were of Caucasian European origin and could have a self-reported history of depression or anxiety disorders; we excluded those reporting manic or hypomanic episodes, psychotic symptoms, or obsessive–compulsive disorder (all information based on background self-report questionnaire data). Thus, the population sample in this study consisted of 1188 participants who fulfilled the inclusion criteria, sent back the questionnaire with a completed gambling task and provided DNA. Population details are shown in Table 1. The study was approved by the local Ethics Committees and was carried out in accordance with the Declaration of Helsinki.

Phenotype Assessments

The NewMood booklet consisted of brief or adapted versions of standard questionnaires that have been validated at a second level of the study in a subset of subjects ($n = 142$) by full versions of the questionnaires and face-to-face interviews. The background questionnaire was adapted from a structured self-rating questionnaire (Hunt *et al*, 1999) that consists of 22 items and collects information about medical history, including personal psychiatric history (particularly depression), family psychiatric history, and socioeconomic background. Impulsivity was measured by the Impulsiveness, Venturesome and Empathy Questionnaire (IVE) Impulsiveness subscale (Eysenck and Eysenck, 1978). For the analysis, a continuous weighted dimension score (sum of item scores divided by the number of items completed) was calculated. The IVE Impulsivity score showed significant correlation with the NEO PI-R (Costa and McCrae, 1992) Impulsiveness (N5) facet score (Pearson's correlation: $R = 0.47$, $p < 0.001$, $n = 142$) in our validation sample. To assess personality we used the Big Five Inventory (BFI-44) (John *et al*, 1991), and a continuous weighted dimension score was calculated for Neuroticism. The 53-item Brief Symptom Inventory (BSI) (Derogatis, 1993) was used to measure anxiety symptoms (using the anxiety subscale) and depressive symptoms (using the depression subscale plus additional items); a continuous weighted dimension score was calculated for Depression and Anxiety. Validation data of neuroticism and symptom scores were published previously (Juhasz *et al*, 2009).

The Recent Negative Life Events questionnaire was based on the validated List of Life Threatening Experiences (LTE) (Brugha *et al*, 1985; Rijsdijk *et al*, 2001) and the sum of life event items was used in the analysis. Questions based on the Childhood Trauma Questionnaire (CTQ) (Bernstein *et al*,

Table 1 Population Details for Those Who Fulfilled the Inclusion Criteria for the Study ($n = 1188$)

Demographics	
Sex	
Female	816 (69%)
Male	372 (31%)
Age (mean ± SEM)	33.79 ± 0.29
Education (A levels or higher)	771 (65%)
Financial situation	
Living comfortably	672 (57%)
Just getting by	376 (32%)
Finding it difficult to make ends meet	140 (11%)
Personal psychiatric history	
Reported depression	630 (53%)
Single episode	171 (14%)
Recurrent episodes	459 (39%)
Reported suicide attempt	181 (15%)
Reported anxiety	338 (28%)
Reported substance use disorder	80 (7%)
Family psychiatric history	
Reported depression in immediate blood relatives	421 (35%)
Personality scores	
BFI Neuroticism (mean ± SEM; range 1–5)	3.30 ± 0.03
IVE Impulsiveness (mean ± SEM; range 0–1)	0.36 ± 0.01
Symptom scores	
BSI Depression (mean ± SEM; range 0–4)	0.98 ± 0.03
BSI Anxiety (mean ± SEM; range 0–4)	0.92 ± 0.03
Adversities	
Recent negative life events (mean ± SEM)	1.27 ± 0.04
Childhood Adversity (mean ± SEM)	3.54 ± 0.10

Abbreviations: BFI, Big Five Inventory; BSI, Brief Symptom Inventory; IVE, Impulsiveness, Venturesome and Empathy Questionnaire.

1994) were used to determine Childhood Adversity related to emotional and physical abuse, and emotional and physical neglect. An additional question asked about parental loss during childhood. The sum of item scores was used in the analysis. Summary scores from the full version of CTQ significantly correlated with scores derived from our short questionnaire (Pearson's correlation: $R = 0.75$, $p < 0.001$, $n = 142$).

Gambling Task

This task was derived from that of Rachlin *et al* (1986). Subjects were presented with a picture of two 'wheel of fortune'-like spinners each with a pointer at the top. Each spinner was divided into a white and a gray segment, and

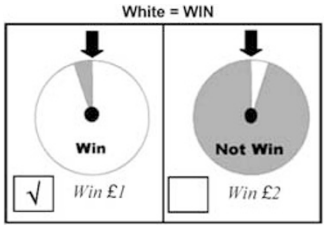

Figure 1 Decision-making task sample trial.

the subject was told to imagine they would 'win' if the spinner stopped with the white segment under the pointer, but not with the gray (Figure 1). Subjects were instructed to indicate which spinner they would choose to maximize their winnings. Dial 'A' provided a smaller (£1) but more likely 'win,' whereas dial 'B' gave a 'win' twice the amount but with a lower probability that was systematically varied. As our reward task was imaginary, subjects did not gain real monetary reward.

The probability of winning on 'A' (pA) was set to $p = 0.95$, 0.5, 0.33, and 0.25, whereas probability of winning on 'B' was varied from $p = 0.80$ to 0.025 but was always lower than pA. As the probability of winning on 'B' increased, an indifference point is reached where the participant values both option equally, and may select 'B' as it offers the potential for a larger 'win.' Odds against winning (θ) were calculated as [1/p]−1, giving four θA values (0.05, 1, 2, 3). The odds against winning on 'B' (θB) at which the participant switched from dial 'A' to dial 'B' (the indifference point) was calculated for each value of 'A' by linear interpolation. To assess an individual's overall tendency to take a safer *vs* more risky choice, we plotted θB against θA and calculated a global area under the curve measure (AUCθ) using the trapezoid method requiring all four indifference points; a smaller value indicating safer choice. Using AUCθ gives weight to choices, in which wins are unlikely and is sensitive to how much the individual is willing to take a risk for a greater reward under conditions where both outcomes are uncertain (Ho *et al*, 1999).

Given the study's constraints, we were not able to determine directly whether participants understood the task and so we included two trials, in which one of the choices was so disadvantageous that its selection was irrational and if chosen the individual was excluded. We also required choices on 'B' to follow the same rank order as their corresponding value on 'A' (allowing one occurrence of the same value for contiguous choices on 'B'). This excluded participants who, for example, chose a smaller indifference probability on 'B' for $p = 0.33$ than for $p = 0.25$ on 'A.' Using these rules, 87.1% ($n = 1035$) of participants were included in the final analysis.

From these subjects, 72 completed the computer version of a similar decision-making task and 69 provided calculable data. During this task, the participants were shown two 'wheel of fortune' spinners—A and B—that had a green (win) and a red (lose) segment. Alternative A had a reward of 10p; alternative B had a reward of 20p but a lower probability chance of winning compared to A (2 blocks of 41 trials; θA: $\theta = 0.00$, 0.33, 1, and 3; θB: from $\theta = 0.00$ to ∞). Participants pressed A or B on the keyboard depending on which alternative they preferred. The outcome (which had been decided in a pseudorandom manner) was then

shown to the subject. The reward was imaginary again, so the subjects did not get real monetary reward. A global AUCθ was calculated from these data similarly to the paper version of the task. Results from the two tasks showed significant correlation (Pearson's $R = 0.30$, $p = 0.012$) in this population.

Genotyping

Buccal mucosa cells were collected from the participants and genomic DNA was extracted according to a previously described method (Freeman *et al*, 2003).

HaploView software (http://www.broad.mit.edu/personal/jcbarret/haploview/) was used to identify htSNPs using the confidence interval method (Barrett *et al*, 2005; Gabriel *et al*, 2002). The tagging was based on the CEPH (Utah residents with ancestry from northern and western Europe) data that were available at the International HapMap Project Phase I. June 2005 release (http://www.hapmap.org), thus SNPs (functional or tagging) that were identified later were not included in this study. We also genotyped a further three functionally relevant serotonergic polymorphisms: *5HTTLPR*, *rs6295*, and *rs1800532*.

The chosen SNPs were genotyped using the Sequenom® MassARRAY technology (Sequenom, San Diego, http://www.sequenom.com) using 25 ng of DNA. Determination of the *5HTTLPR* genotype was performed in a Thermo-Fast® 384 PCR plate (ABgene Ltd, UK) on a PTC-220 Dyad TM thermocycler (Bio-Rad, Hercules, CA). Polymerase chain reaction products were analyzed using an ABI3100 Genetic Analyzer and GeneScan analysis software (Applied Biosystems, Netherlands). Genotyping was performed blinded with regard to phenotype. All laboratory work was performed under the ISO 9001:2000 quality management requirements. The genotyping methods in more details can be seen in Supplementary Document 1.

Statistical Analysis

Statistical analyses were performed using SPSS for Windows Statistical Analysis Software, Version 15.0. A p-value of <0.05 was adopted for all statistical testing, and all reported p-values were two-tailed.

We used the HaploView program to explore the haplotype structure of *TPH2* gene (Gabriel *et al*, 2002; Barrett *et al*, 2005); Quanto 1.2 version (http://hydra.usc.edu/gxe) to calculate the power of the recruited populations; and PLINK v1.06 (http://pngu.mgh.harvard.edu/purcell/plink/) for testing association of different genetic models (dominant, recessive, and additive; linear regression model covariation with age and sex), interactions between SNPs (epistasis) and with sex, and for calculating Hardy–Weinberg analysis.

Haplotype analyses were performed with HelixTree™ 6.4.1 (Golden Helix, USA) using haplotype trend regression. Only haplotypes with a frequency greater than 5% were used in the linear regression analysis, similar to the method described previously (Juhasz *et al*, 2009). In all cases, data were adjusted for age and sex. To reduce the influence of multiple testing, we used a permutation test, randomly grouping the sample 10 000 times.

For both single-marker association and haplotypic association analyses, we used false discovery rate calculation at a level of 5% (FDR; Qvalue: http://genomics.princeton.edu/storeylab/qvalue/) to adjust p-values according to the number of hypothesis tested (Storey and Tibshirani, 2003). We report q–value, which is a measure of significance of each test of many tests performed simultaneously.

RESULTS

Decision-Making Task

Women were less likely to make risky decisions than men, represented by lower AUCθ values ($F = 9.38$, $df = 1,1032$, $p = 0.002$; AUCθ mean ± SEM after covariation for age: males—10.42 ± 0.36, females—9.09 ± 0.24; explained variance 0.9%). Subjects with reported depression were also less likely to make risky decisions ($F = 4.83$, $df = 1,1031$, $p = 0.028$; AUCθ mean ± SEM after covariation for age and gender: no depression—9.99 ± 0.30, depression—9.09 ± 0.28). Higher neuroticism (Pearson's correlation $R = -0.13$, $p < 0.001$), depression (Pearson correlation $R = -0.13$, $p < 0.001$), and anxiety (Pearson's correlation $R = -0.13$, $p < 0.001$) scores were also associated with risk aversion. These factors explained a further 1.6% variance in risk-taking behavior. However, impulsivity measured by IVE and risk taking measured by our task were independent of each other (Pearson's correlation $R = -0.02$, $p = 0.233$). Both Childhood Adversity (Pearson's $R = -0.06$, $p = 0.039$) and Recent Negative Life Events (Pearson's $R = -0.06$, $p = 0.042$) significantly correlated with less risk taking, but after correction for depressive and anxiety symptoms this effect became nonsignificant (Childhood Adversity: Pearson's $R = -0.02$, $p = 0.629$; Recent Negative Life Events: Pearson's $R = -0.03$, $p = 0.344$).

After covariation for age and gender, there were no significant difference in neuroticism ($p = 0.45$), impulsivity ($p = 0.20$), symptom scores (depression, $p = 0.80$; anxiety, $p = 0.82$) or education level ($p = 0.24$) between those who did not provide consistent task results ($n = 153$) and who did ($n = 1035$).

Using the computer version of the decision-making task, females were less likely to make risky decisions ($F = 3.78$, $df = 1,66$, $p = 0.056$; AUCθ mean ± SEM after covariation for age: males ($n = 30$)—11.17 ± 0.67, females ($n = 39$)—9.44 ± 0.59; explained variance 5.4%), although this effect missed the significance threshold. We did not find a significant difference between those with and without lifetime major depressive disorder according to SCID ($F = 0.91$, $df = 1,64$, $p = 0.344$; AUCθ mean ± SEM after covariation for age and gender: no psychiatric disorder ($n = 28$)—9.66 ± 0.71, lifetime MDD ($n = 40$)—10.56 ± 0.59). However, subjects with low symptom scores (MADRS $\leqslant 7$; $n = 53$) tended to make more risky decisions than those who had high symptom scores (MADRS > 7, $n = 16$; $F = 3.42$, $df = 1,65$, $p = 0.069$; AUCθ mean ± SEM after covariation for age and gender: low symptom score—10.65 ± 0.50, high symptom score—8.68 ± 0.93; explained variance 2.8%).

Genotyping Results

HaploView analysis of the *TPH2* gene—based on the HapMap Project Phase I. (June 2005) release CEPH population data—resulted in seven htSNPs that were

Table 2 Summary of the Genotyped SNPs

SNP no.	DbSNP[a] no.	Position[b]	Location	Allele (minor/major)	Manchester		CEPH[c]
					MAF (%)	HWE *p*	MAF (%)
TPH2 (Chr12q21.1)							
1	rs1843809	70634965	Intron5	G/T	16	0.118	12
2	rs1386493	70641446	Intron5	T/C	18	0.420	15
3	rs6582078	70661158	Intron7	G/T	43	0.305	34
4	rs10506645	70671767	Intron7	T/C	24	0.125	22
5	Rs1352250	70684051	Intron8	A/G	43	0.856	38
6	rs1487275	70696559	Intron8	G/T	27	0.759	25
7	rs1386485	70698634	Intron8	C/A	34	0.690	30
SLC6A4 (Chr17q11.1–q12)							
8	5HTTLPR	25588244-25588593	Promoter	S/L	44	0.676	43[d]
HTR1A (Chr5q11.2–q13)							
9	rs6295	63294321	Promoter	G/C	48	0.125	54[e]
TPH1 (Chr11p15.3–p14)							
10	rs1800532	18004392	Intron5	A/C	40	0.195	40

HWE, Hardy–Weinberg equilibrium; MAF, minor allele frequency.
[a]Genotyping success rate was above 93% for all SNPs.
[b]Position according to National Center for Biotechnology Information (NCBI, March 2007).
[c]CEPH: collection consists of 30 mother–father–child trios with Northern and Western Europe ancestry, data based on the HapMap Project Phase II. (November 2008) release.
[d]No CEPH data, based on Lesch *et al* (1996).
[e]No CEPH data, based on Strobel *et al* (2003).

genotyped in our study. Details about the *TPH2* htSNPs and the genotyped three functional serotonergic polymorphisms can be seen in Table 2. Although no genomic control by random marker genotyping was performed in the study population, we attempted to reduce ethnic variation and stratification effects by including only independent Caucasian subjects of European origin in the analysis. Indeed, all of the genotyped polymorphisms were in Hardy–Weinberg equilibrium both in the total population and separately for those who reported depression and who did not (data not shown). *TPH2* linkage disequilibrium (LD) block structure is similar in our population and in the CEPH population (data from the HapMap Project Phase II. (November 2008) release; see Figure 2). Although in this study we did not genotype functional SNPs in the *TPH2* gene, we do show the LD data between our htSNPs and *rs4570625 (−703G/A)* (Chen *et al*, 2008) and *rs7305115* (Lim *et al*, 2007) in the CEPH population in order to interpret our results. There are no available data in the CEPH population about other functional SNPs *(rs11178997: −473T/A; rs11178998: 90A/G)* (Chen *et al*, 2008; Lin *et al*, 2007; Scheuch *et al*, 2007).

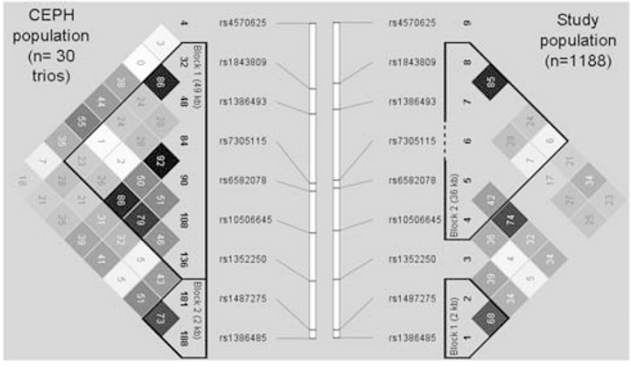

Figure 2 Linkage disequilibrium (LD) R^2 data of the *TPH2* gene in the CEPH population (HapMap Project Phase II. November 2008 release) and in our study. Blocks of LD have been identified on the basis of the method published by Gabriel *et al* (2002) using Haploview program.

Single-Marker Associations

We found significant association of the AUCθ and *TPH2* htSNPs, and after FDR correction the association remained significant for *rs6582078* and *rs1352250* (Table 3). The minor alleles were positively associated with increased risk taking in all htSNPs. There was no significant marker-specific association between *5HTTLPR, rs6295* or *rs1800532* and risk taking. None of the SNPs showed association with symptom or personality scores after correction for multiple testing (Supplementary Table S1).

Because AUCθ showed significant difference between males and females, we tested the interaction of SNPs and sex. None of the SNPs showed significant interaction with sex on risk-taking AUCθ after correction for multiple testing (Supplementary Table S2a). Furthermore, *TPH2*

Table 3 Single-Marker Association Data for Risk Taking (AUCθ) Using the Paper-and-Pencil Task ($n = 1035$)

No.	SNP	A1	Test	Beta	SE	95% CI Lower	95% CI Upper	t	Cohen's d	p-value	FDR q
1	rs1843809	G	ADD	0.883	0.411	0.077	1.690	2.148	0.14	**0.032**	0.239
		G	DOM	0.987	0.454	0.097	1.877	2.173	0.14	**0.030**	0.239
		G	REC	1.004	1.520	−1.974	3.982	0.661	0.04	0.509	0.839
2	rs1386493	T	ADD	0.850	0.388	0.090	1.610	2.193	0.14	**0.029**	0.239
		T	DOM	0.867	0.440	0.004	1.729	1.970	0.12	**0.049**	0.318
		T	REC	1.911	1.277	−0.592	4.413	1.496	0.09	0.135	0.589
3	rs6582078	G	ADD	1.173	0.300	0.584	1.761	3.904	0.25	**0.0001**	**0.005**
		G	DOM	1.272	0.445	0.399	2.145	2.855	0.18	**0.004**	0.119
		G	REC	1.926	0.543	0.861	2.991	3.545	0.22	**0.0004**	**0.014**
4	rs10506645	T	ADD	0.747	0.352	0.056	1.438	2.119	0.13	**0.034**	0.239
		T	DOM	0.618	0.422	−0.209	1.446	1.465	0.09	0.143	0.589
		T	REC	2.267	0.946	0.414	4.120	2.398	0.15	**0.017**	0.239
5	rs1352250	A	ADD	1.223	0.300	0.636	1.810	4.082	0.26	**0.00005**	**0.005**
		A	DOM	1.243	0.446	0.370	2.117	2.789	0.18	**0.005**	0.122
		A	REC	2.139	0.541	1.079	3.200	3.954	0.25	**0.00008**	**0.005**
6	rs1487275	G	ADD	0.804	0.342	0.134	1.474	2.352	0.15	**0.019**	0.239
		G	DOM	0.947	0.426	0.112	1.781	2.224	0.14	**0.026**	0.239
		G	REC	1.169	0.844	−0.484	2.822	1.386	0.09	0.166	0.627
7	rs1386485	C	ADD	0.837	0.316	0.217	1.456	2.648	0.17	**0.008**	0.160
		C	DOM	0.904	0.427	0.068	1.741	2.118	0.14	**0.034**	0.239
		C	REC	1.496	0.663	0.196	2.796	2.255	0.14	**0.024**	0.239
8	5HTTLPR	A	ADD	−0.014	0.293	−0.588	0.560	−0.047	0.00	0.963	0.841
		A	DOM	0.297	0.442	−0.569	1.163	0.672	0.04	0.502	0.772
		A	REC	−0.459	0.522	−1.483	0.565	−0.879	−0.06	0.380	0.688
9	rs6295	G	ADD	−0.037	0.283	−0.592	0.517	−0.132	−0.01	0.895	0.833
		G	DOM	0.174	0.457	−0.723	1.070	0.379	0.02	0.705	0.826
		G	REC	−0.295	0.477	−1.229	0.639	−0.619	−0.04	0.536	0.784
10	rs1800532	A	ADD	−0.163	0.304	−0.758	0.432	−0.537	−0.03	0.592	0.824
		A	DOM	0.167	0.433	−0.681	1.014	0.385	0.02	0.700	0.826
		A	REC	−0.899	0.581	−2.037	0.240	−1.547	−0.10	0.122	0.438

In all calculations, age and sex were covariates. *t*, *t*-test statistics; Cohen's *d*, standardized effect sizes; *p*, uncorrected significance value; FDR *q*, false discovery rate significance value.
The significance values <0.05 are indicated in bold.

htSNPs did not show significant interaction (epistasis) with *5HTTLPR, rs6295,* or *rs1800532* on AUCθ (Supplementary Table S2b).

Assuming a continuous trait such as AUCθ data, using an independent-individual study design and assuming an additive linear model relating the phenotype to genotype, we had a 90–93% power in our population ($N = 1035$ and $N = 1188$) to detect a polymorphism (with a minor allele frequency $\geqslant 10\%$) that could explain 1% of the variance of the trait at the 5% two-tailed significance level. To detect gene × sex interaction in the same model as above and assuming that sex explains 5% variance, we had a 91–95% power in our population. Furthermore, for epistasis we had a 90–94% power in our population if the other gene also explains 1% variance and the gene × gene interaction explains further 1% variance.

Using the computer task data, single-marker associations showed similar results with higher effect sizes to the pencil-and-paper task results (see Table 4).

TPH2 Haplotype Association With AUCθ

The overall genetic effect of *TPH2* haplotypes for the AUCθ data was significant and remained significant after permutation tests and correction for multiple testing (Table 5). The *TPH2* haplotypes explained 1.9% variance in risk taking. The haplotypic association did not change after adding BSI Depression and Anxiety scores (permutated FDR corrected $q = 0.018$) or BFI neuroticism scores (permutated FDR corrected $p = 0.018$) as covariates in the analysis. These results suggest that there is a genetic effect on this measure of decision making, which is independent of current mood and anxiety state or neuroticism. Indeed, the *TPH2* gene showed no significant haplotypic association with BSI depression (permutated FDR corrected $q = 0.337$), BSI anxiety (permutated FDR corrected $q = 0.172$), BFI neuroticism (permutated FDR corrected $q = 0.314$), or IVE impulsivity (permutated FDR corrected $q = 0.174$) scores.

Table 4 Single-Marker Association Data For Risk Taking (AUCθ) Using the Computer Task ($n = 69$)

No.	SNP	A1	Test	Beta	SE	95% CI Lower	95% CI Upper	t	Cohen's *d*	*p*-value
1	rs1843809	G	ADD	1.297	0.801	−0.273	2.867	1.619	0.41	0.111
		G	DOM	1.341	0.863	−0.350	3.032	1.555	0.39	0.125
		G	REC	2.372	3.395	−4.283	9.027	0.699	0.18	0.488
2	rs1386493	T	ADD	1.138	0.705	−0.243	2.520	1.616	0.41	0.111
		T	DOM	1.033	0.847	−0.627	2.693	1.220	0.31	0.227
		T	REC	3.162	1.956	−0.672	6.995	1.617	0.41	0.111
3	rs6582078	G	ADD	1.045	0.700	−0.327	2.417	1.493	0.37	0.141
		G	DOM	2.012	1.111	−0.166	4.191	1.811	0.45	0.075
		G	REC	0.649	1.123	−1.553	2.850	0.577	0.14	0.566
4	rs10506645	T	ADD	−0.107	0.762	−1.601	1.386	−0.141	−0.04	0.889
		T	DOM	0.011	0.972	−1.894	1.917	0.012	0.00	0.991
		T	REC	−0.603	1.750	−4.032	2.827	−0.345	−0.09	0.732
5	rs1352250	A	ADD	1.498	0.598	0.326	2.670	2.505	0.63	**0.015**
		A	DOM	2.507	0.911	0.721	4.293	2.750	0.69	**0.008**
		A	REC	1.231	1.039	−0.805	3.268	1.185	0.30	0.241
6	rs1487275	G	ADD	1.392	0.721	−0.020	2.805	1.932	0.49	0.058
		G	DOM	1.913	0.893	0.163	3.662	2.143	0.54	**0.036**
		G	REC	0.939	1.784	−2.558	4.436	0.526	0.13	0.601
7	rs1386485	C	ADD	1.642	0.565	0.535	2.750	2.906	0.73	**0.005**
		C	DOM	2.109	0.819	0.503	3.715	2.574	0.65	**0.013**
		C	REC	2.275	1.125	0.070	4.479	2.022	0.51	**0.048**
8	5HTTLPR	A	ADD	−0.040	0.685	−1.383	1.302	−0.059	−0.01	0.953
		A	DOM	−0.379	1.071	−2.477	1.720	−0.354	−0.09	0.725
		A	REC	0.333	1.168	−1.957	2.623	0.285	0.07	0.777
9	rs6295	C	ADD	−0.909	0.766	−2.411	0.593	−1.186	−0.30	0.240
		C	DOM	−2.256	1.191	−4.590	0.079	−1.894	−0.48	0.063
		C	REC	−0.013	1.126	−2.221	2.194	−0.012	0.00	0.991
10	rs1800532	A	ADD	0.728	0.615	−0.477	1.933	1.184	0.30	0.241
		A	DOM	0.007	0.897	−1.752	1.766	0.008	0.00	0.994
		A	REC	2.745	1.158	0.475	5.014	2.370	0.60	**0.021**

In all calculations, age and sex were covariates. *t*, *t*-test statistics; Cohen's *d*, standardized effect sizes; *p*, uncorrected significance value. The significance values <0.05 are indicated in bold.

Table 5 TPH2 Haplotypic Regression Results For Risk Taking Measured by AUCθ

No.	Haplotypes	Frequencies (%)	Beta	SE	t	*p*-value	FDR q
1	T,C,T,C,G,T,A	49	−1.239	0.551	−2.248	0.025	0.022
2	G,T,G,C,A,G,C	14	3.194	1.855	1.722	0.085	0.058
3	T,C,G,T,A,T,A	11	4.790	1.699	2.819	0.005	0.008
4	T,C,G,T,A,G,C	10	0.228	1.913	0.119	0.905	0.348
5	Rare haplotypes	16			**Left out regressor**		
	Full *vs* reduced model *p*-value	—	—	—	—	0.0004	0.003
	Permuted *p*-value	—	—	—	—	0.014	0.016

Age and sex were covariate in the calculations, and the order of the htSNPs in the haplotypes corresponds to the SNP order in Table 2. *t*, *t*-test statistics; *p*, uncorrected significance value; FDR *q*, false discovery rate significance value.

The most common haplotype T,C,T,C,G,T,A was found in 49% of study participants. Beta values suggest that carriers were relatively risk avoidant and made significantly less risky decisions than the other haplotype carriers (permutated FDR corrected $q = 0.022$). This effect was also significant for the risk-taking AUCθ calculated from the computer task ($\beta = -2.619$, SE = 1.271, $t = -2.06$, permutated $p = 0.044$, explained variance 5.9%, $n = 69$, frequency of the T,C,T,C,G,T,A haplotype = 45%).

DISCUSSION

Using haplotypic association, we found significant differences in risk-taking behavior on a performance gambling task between the most prevalent haplotype and the other haplotypes of the *TPH2* gene. Subjects carrying the most prevalent T,C,T,C,G,T,A haplotype had lower AUCθ parameters suggesting that they are less likely to make risky decisions. Thus, our finding implies involvement of the serotonergic system, namely, the *TPH2* gene, in decision making through altering willingness to take risks for reward. In addition, we tested whether differences in risk-taking behavior measured by our task were influenced by other serotonergic genes. We genotyped a further three serotonergic functional polymorphisms (*5HTTLPR* in serotonin transporter, *rs6295* in *5HTR1A* receptor, and *rs1800532* in *TPH1* genes) but could not demonstrate significant main effects of these polymorphisms or interaction with the *TPH2* htSNPs on risk taking. However, we could not exclude that these genes modulate other aspects of decision making that were not measured by our task, such as punishment- or feedback-related processing, as previous studies reported (Jollant *et al*, 2007; Must *et al*, 2007; Blair *et al*, 2008; Homberg *et al*, 2008).

Accumulating evidence suggest that the more common allele/haplotype variants in the *TPH2* gene are associated with lower cerebrospinal fluid 5-hydroxyindoleacetic acid concentrations (Zhou *et al*, 2005) and with decreased *TPH2* mRNA expression (Chen *et al*, 2008; Chen and Miller, 2008; Haghighi *et al*, 2008; Lim *et al*, 2007). Thus, a considerable proportion of the population, who carry the wild-type (most ancient) variation of the *TPH2* gene, express TPH2 at low level that results in decreased serotonin availability at the raphe nucleus and its projections, and also show risk aversion on the basis of our results. This situation is somewhat parallel to the ATD, which has been shown to reduce 5-HT levels to a functionally significant degree. However, human studies have not demonstrated a consistent effect of reducing 5-HT function using dietary ATD on probabilistic choice behavior: Rogers *et al* (1999) reported that after ATD subjects chose the most likely option less often, while Talbot *et al* (2006) demonstrated the opposite and our group could not find any significant difference (Anderson *et al*, 2003). The most plausible explanation for these discrepancies is that these studies used different tasks to measure risk taking and probably were not adequately powered to detect the effect of ATD on probabilistic choice. Indeed, on the basis of our results, variability in serotonin synthesis caused by the *TPH2* gene only explained 1.9% (paper-and-pencil task)—5.9% (computer task) of the variance in risk taking. Another possible explanation is that the genetic make-up of the participants interfered with the effect of the ATD, as has been suggested in relation of the *5HTTLPR* variant (Blair *et al*, 2008).

Our results suggest that there is a biological distinction to be made between impulsive and safe/risky decision making as measured with our task. Although impulsivity has usually been associated with an increased tendency to take risks, such as in DSM-IV-defined impulse control disorders (American Psychiatric Association, 2000), it only appears to predict some risky behaviors, whereas others are more linked to neuroticism and extraversion (Cooper *et al*, 2000). Impulsivity may be more related to inability to delay gratification and Crean *et al* (2000) demonstrated that delay discounting has a closer relationship to self-reported impulsivity measurements than probability discounting. Low 5-HT function has been associated with increased impulsivity (eg Dolan *et al*, 2001) in contrast to our finding that the common *TPH2* variant, likely to be associated with reduced 5-HT function, is associated with risk aversion rather than risk taking when faced with a probabilistic choice. This highlights the complex role of 5-HT in behavior and mood and the need to carefully specify neuropsychological function when investigating the neurobiology of behavior.

Our task has face validity when considered in relation to mood in our subjects. Depression is characterized by reduced motivation to obtain reward and reduced enjoyment of it (Elliott *et al*, 1997; Forbes *et al*, 2006), whereas anxiety is associated with intolerance of uncertainty (Paulus *et al*, 2003; Paulus, 2007). Hence, subjects with depression and anxiety, which are frequently comorbid disorders, would be expected to be less motivated, indeed averse, to take more risks for a greater uncertain reward compared to controls (Smoski *et al*, 2008). This is consistent with the findings in our sample, in which higher depression, anxiety, and neuroticism ratings were associated with a lower tendency to take risks (AUCθ) and explained 1.6%–2.8% variance in this phenotype. However, our study also demonstrated that the effect of TPH2 on risk taking is independent of depression and anxiety symptom scores and also independent of neuroticism, thus indicating a separate mode of action. Furthermore, we could not demonstrate any association between haplotype variants of the *TPH2* gene and BSI depression, BSI anxiety, BFI neuroticism, or IVE impulsiveness scores. This is in agreement with recent studies which failed to find any association between the *TPH2* gene and depression/suicidal behavior (Mann *et al*, 2008; Must *et al*, 2007; Zill *et al*, 2007b; De Luca *et al*, 2006; Lopez *et al*, 2007b), but in contrast to earlier findings reporting such an involvement (Zill *et al*, 2004a, b; Zhou *et al*, 2005; Van Den Bogaert *et al*, 2006; Haghighi *et al*, 2008; Lopez *et al*, 2007a; Ke *et al*, 2006). Therefore, the interpretation we favor is that the variations in the *TPH2* gene might be linked to an endophenotype influencing risk-taking behavior that could be an additive or in some cases a vulnerability factor, for psychiatric disorders, for example, affective disorders or disturbed mood in impulse control or substance misuse disorders. As previous studies have investigated severe clinical populations (hospitalized major depressive patients, completed suicides with MDD), it is likely they represented extreme cases in which *TPH2* haplotypes may have contributed to the phenotypic picture through altered risk-taking behavior, potentially leading to patient selection bias and an apparent association between depression/suicide and *TPH2* haplotypes. A modulatory rather than causative role of serotonin and *TPH2* in psychiatric disorders was suggested by *TPH2* and *TPH1*/*TPH2* double knockout (KO) animal data. *TPH2* KO animals did not show apparent neuronal developmental abnormalities (Gutknecht *et al*, 2008), and even double KOs showed only mildly increased anxiety-like behavior (Savelieva *et al*, 2008).

The main strength of our study is that this is the first attempt to investigate the role of serotonergic genes in decision-making behavior in a large sample using a performance measure plus personality and symptom questionnaires. Furthermore, we were able to validate the questionnaire findings in a subgroup using a more extensive assessment including questionnaire and observer ratings and a computerized task. We have also recently reported that there is no difference in risky choice behavior between receiving real and hypothetical rewards when using this computerized task (Hinvest and Anderson, 2009). The findings in the subgroup largely replicated the findings in the main sample. The computerized task showed only a modest, although significant, correlation with the pencil-and-paper task, which is likely to be explained by methodological differences (amount to be won and different probabilities) and sampling error, as each indifference point was only assessed on one occasion in the pencil-and-paper task but on two occasions in the computerized version. Differences in genetic effect sizes (small effect sizes for the paper-and-pencil task and medium-large effect sizes for the computerized task, see Tables 3 and 4) probably are related to these methodological differences as the computerized task is likely to be a more sensitive tool to measure decision-making differences.

Our *TPH2* finding has limitations, as we did not genotype recently identified functional variants in this gene. Therefore, we compared the LD pattern in our population and in the CEPH population, using our htSNPs (based on the International HapMap Project Phase I. June 2005 release) and recently genotyped functional SNPs (CEPH population, HapMap Project Phase II. November 2008 release), to interpret our results (Figure 2). We can conclude that our study satisfactorily captured the possible influence of functional variants that resides in the region around introns 5–8 and previously have been implicated in regulation of splicing, and therefore might influence the level of the active enzyme (Haghighi *et al*, 2008; Lim *et al*, 2007). Nonetheless, we have limited information about the promoter region of the *TPH2* gene, which exhibits only moderate LD with our two most significant SNPs (*rs6582078* and *rs1352250*) (Figure 2b) (Chen *et al*, 2008; Lin *et al*, 2007; Scheuch *et al*, 2007). Associations have been demonstrated with the *TPH2* promoter region and traits related to emotional dysregulation, such as harm avoidance (Gutknecht *et al*, 2007; Reuter *et al*, 2007; Strobel *et al*, 2007) and amygdala activation during emotional processing (Brown *et al*, 2005; Canli *et al*, 2005). However, we did not find any association between haplotype variants of the *TPH2* gene and depression, anxiety, neuroticism, or impulsiveness scores that might be influenced by functional variations in the TPH2 promoter region. Using denser htSNP cover on the full length of the *TPH2* gene and including independent replication populations into the study would help us to answer these questions and support our new findings. The lack of independent replication population in this study is a major limitation and warrants further research in this field.

We genotyped functional polymorphisms in other serotonergic genes but could not replicate previous positive findings (Ono *et al*, 2002; Lesch *et al*, 1996; Lemonde *et al*, 2003), despite our study being sufficiently powered to identify genetic effect that explain 1% variance in personality and symptom scores (Juhasz *et al*, 2009). Our findings are however consistent with the recent scientific literature, as a large meta-analysis showed that several studies have failed to show association between anxiety-related traits and *5HTTLPR*, except neuroticism measured by NEO (Munafo *et al*, 2009): it has been argued that genetic effects on neuroticism might be complex and smaller than 1% per genetic variants (Shifman *et al*, 2008).

In conclusion, our study is the first to support a role for the *TPH2* gene as a trait factor in determining risky choice behavior as tested in a probabilistic choice task. We also demonstrated that risk-taking behavior is modified by state-dependent elements, such depression and anxiety, independently of the *TPH2* gene effect. Thus, this biological marker may contribute to the expression of psychiatric phenotypes and possibly to a vulnerability to develop specific disorders. Our results support the importance of identifying quantitative traits, biological markers, or endophenotypes that might be more informative for genetic studies than heterogenic, complex disorders such as depression or complex traits such as impulsivity.

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DISCLOSURE

The authors declare that, except for income received from their primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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