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No Human Tryptophan Hydroxylase-2 Gene R441H Mutation in a Large Cohort of Psychiatric Patients and Control subjects

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Background: It was recently reported that a rare functional variant, R441H, in the human tryptophan hydroxylase-2 gene (*hTPH2*) could represent an important risk factor for unipolar major depression (UP) since it was originally found in 10% of UP patients (vs. 1.4% in controls).

Methods: We explored the occurrence of this variation in patients with affective disorders (n=646), autism spectrum disorders (n=224) and OCD (n=201), in healthy volunteers with no psychiatric disorders (n=246), and in an ethnic panel of control individuals from North Africa, Sub-Saharan Africa, India, China and Sweden (n=277).

Results: Surprisingly, we did not observe the R441H variant in any of the individuals screened (3188 independent chromosomes).

Conclusions: Our results do not confirm the role of the R441H mutation of the *TPH2* gene in the susceptibility to UP. The absence of the variant from a large cohort of psychiatric patients and controls suggests that the findings reported in the original study could be due to a genotyping error or to stratification of the initial population reported. Additional data by other groups should contribute to the clarification of the discrepancy between our results and those previously published.

In the human brain, serotonin (5-HT) is synthesized from tryptophan by the enzyme tryptophan hydroxylase-2 (TPH2). Recently a rare variant (R441H) due to a single nucleotide change (G1463A) in the human tryptophan hydroxylase-2 gene (*hTPH2*) encoding the rate-limiting enzyme of neuronal serotonin synthesis, human tryptophan hydroxylase-2 (*hTPH2*), was demonstrated to dramatically reduce enzymatic activity. *In vitro* studies indicate that the ability of the mutant enzyme to synthesize serotonin (5-HT) is decreased 80% compared to the wild-type enzyme (Zhang et al 2005). Interestingly, this rare genetic variant was found in 9/87 patients with unipolar major depression (UP) and only in 3/219 controls (all the affected individuals were Caucasians except for one control who was African American). Therefore, the R441H mutation could represent an important risk factor for UP. Given the predominant role of *hTPH2* in brain serotonergic neurons (Walther et al 2003; Patel et al 2004), and the large number of studies suggesting a dysfunction of the 5-HT system in many psychiatric conditions, we determined the incidence (or allelic frequency) of this variant in independent cohorts of patients with UP and other neuropsychiatric disorders.

Methods and Materials

Subjects

We studied the G1463A polymorphism of the gene in a large sample of 1071 individuals with psychiatric disorders including UP (n=265), major depressive disorder (MDD) (n=297), bipolar disorder (BP) (n=84), obsessive compulsive disorder (OCD) (n=201), autism spectrum disorders (ASD) (n=224), in 246 healthy volunteers controlled for the absence of personal and familial history of psychiatric disorders, and in an ethnic panel of individuals recruited among the general population

in North Africa, Sub-Saharan Africa, India, China and Sweden (n=277). Control DNAs comprising both 1463A homozygous and heterozygous individuals were kindly provided by Dr. Marc Caron.

The patients were recruited at departments of child and adult psychiatry in four European countries (France, Germany, Sweden, and Switzerland). Among those with affective disorders (UP, BP and MDD), 542 were of West European Caucasian origin and 104 were of mixed ethnic origin (Central European Caucasians, North Africans, Asians and sub-Saharan Africans). All patients met DSM-IV diagnostic criteria; diagnoses were based on the Diagnostic Interview for Genetic Studies, the Schedule of Affective Disorders and Schizophrenia, the Mini International Neuropsychiatric Interview, and the Autism Diagnostic Interview-Revised. The local Research Ethics Boards approved the study protocol. Written informed consent was obtained from all participating subjects and/or their parents.

Genotyping procedures

The G1463A SNP was analyzed using three different methods: with amplification refractory mutation system (ARMS)-PCR as described by Zhang et al (2005), with PCR-RFLP or by sequencing. For ARMS-PCR and PCR-RFLP, the PCR was done with the same external primers with Eurobio Taq polymerase, the PCR product was digested with 5 U of Fok I (Biolabs, Bioconcept, Basel- CH) during 5 hours at 37°C and the final product was run on a clearose gel (Elchrom). For sequencing, primers for the PCR were located in intron 9 (5'-CTGTAGGATTACCGAGCTATC-3') and intron 11 (5'-CGTCGTCAAGTCAGTTACAGT-3').

Results

We did not observe the 1463A allele in any of the individuals tested (Table 1). All the individuals were 1463G homozygous. Genotyping procedures were robust, since we were able to detect the mutation in genomic DNA from 1463A homozygous and heterozygous individuals obtained from Dr. Caron. The frequency of the 1463A allele in UP reported by Zhang et al. (2005) is 6.89%; therefore we expected to observe about 36 1463A alleles in our sample of 265 UP patients (530 chromosomes). For bipolar patients we confirmed the absence of the 1463A allele (Zhang et al 2005). In the original paper, eleven individuals with the 1463A allele were of Caucasian origin and one was an African American. To explore the possibility that this rare variation is more frequent in other genetic backgrounds, we screened an ethnic panel of 277 control subjects from North Africa (n=62), Sub-Saharan Africa (n=48), India (n=42), China (n=54) and Sweden (n=71). We could not detect the 1463A allele in any of these individuals. In total, we failed to detect the 1463A polymorphism in 3188 chromosomes.

Discussion

Compared to the original report (Zhang et al 2005), the absence of the *hTPH2* mutation in our study could not be solely explained by a difference in ethnicity since both study groups consisted predominantly of Caucasian individuals. It is also unlikely that diagnosis and sex-ratio could explain the apparent contradictory findings. One obvious difference between the individuals carrying the *hTPH2* 1463A allele in the study by Zhang et al (2005) and those described here is the age of the patients. In the report of Zhang et al UP patients were 60 years of age or older, whereas those in our study had a mean age at interview of 39.2 ± 13.9 years. However, this difference remains difficult to interpret, since no information on the age at onset of UP in the Zhang et al cohort is given.

In conclusion, we were unable to replicate the findings of Zhang et al (2005). In addition, a recent study that systematically sequenced *hTPH2* exons in subjects with autism and controls did not observe the G1463A variation (Coon et al 2005). One reason for the discrepancy could be a sub-stratification of the population presented in the original report. Indeed, when compared to the expected values at the Hardy Weinberg equilibrium, Zhang et al observed an excess of homozygotes for the 1463A allele, both in UP (3 vs. 0.41 expected) and controls (1 vs. 0.02 expected). There was also a significant difference between the frequency observed in their control population and ours (Fischer exact test, $\chi^2 = 43.71$, $p = 0.0003$). Alternatively, a genotyping error in the study by Zhang et al (2005) could explain the discrepancy. Indeed, their PCR-based genotyping method (ARMS-PCR) was not validated by sequencing analysis. Thus, a possible contamination of the subject samples by the cloning vector can not be excluded. Nevertheless, it remains possible that the G1463A *hTPH2* variation is associated with a yet unknown UP-associated trait and/or an older age at onset. Further screening is warranted to address this open question.

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Table 1 Population screened for the TPH2 R441H mutation

	Male:Female ratio	Age at Interview	Age at Onset	Personal History of Suicide Attempt
Unipolar major disorder (n=265)	108 : 157	39.2 ± 13.9	23.3 ± 10.9	136
Other affective disorders				
Bipolar disorder (n=84)	18 : 66	39.1 ± 12.2	25.2 ± 11.9	68
Major depressive disorder (n=297)	64 : 233	40.3 ± 13.3	31.1 ± 12.2	272
Obsessive compulsive disorder (n=201)	99 : 102	30.0 ± 16.0	17.1 ± 10.4	0
Autism spectrum disorders (n=224)	174 : 50	— ^a	< 3 years	0
Healthy volunteers with no psychiatric disorders (n=246)				
French (n=187)	108 : 79	41.9 ± 11.3	— ^b	0
German (n=59)	24 : 35	38.0 ± 16.6	— ^b	0

TPH2, tryptophan hydroxylase-2.

^aParents were interviewed.

^bInformation not relevant.