Response to Zhang et al. (2005) Loss-of-Function Mutation in Tryptophan Hydroxylase-2 Identified in Unipolar Major Depression. Neuron *45*, 11–16

Zhang and colleagues reported the G1463A variant (c.1322G>A) that predicted an amino acid substitution at a highly conserved position in tryptophan hydroxylase-2 (TPH2) (p.Arg441His). Functional analysis of 441His showed an ~80% loss in serotonin production when expressed in PC12 cells. The authors' genetic data showed the presence of the 1463 A-allele in 9 of 87 elderly unipolar affective disorder (UP) patients (>60 years) and in 3 of 219 healthy control individuals.

Based on these finding, we genotyped the G1463A variant in two large patient-control samples obtained in northern-Sweden and Belgium. The northern-Swedish sample was comprised of 135 UP patients (87 females, 48 males, mean age at inclusion was 56.6 years), 182 bipolar (BP) patients (96 females, 86 males, mean age at inclusion was 56.3 years), and 364 healthy age-, gender-, and ethnicity-matched control individuals. The Belgian sample consisted of 182 UP patients (121 females, 61 males, mean age at inclusion was 46.6 years), 182 BP patients (99 females, 83 males, mean age at inclusion was 45.3 years) and 364 healthy age-, gender-, and ethnicity-matched control individuals. Genotyping was performed by pyrosequencing on a PSQ^{TM}HS96 pyrosequencer (http://www.pyrosequencing.com/) with the use of 5′-bio-GTTTATTCTGCAGGGACTTTGC-3′ and 5′-CGAAGGTCCTGCACCACA-3′ as PCR primers and 5′-GAAGTATACTGAGAAGG-3′ as reverse sequencing primer. We did not observe the A-allele in any of the patients nor in the control individuals. To confirm the validity of our pyrosequencing assay, we subsequently sequenced genomic DNA of all patients and control individuals by using PCR-based direct sequencing with primers 5′-AGCCTTTGACCCAAAGACAA-3′ (forward) and 5′-AGATCATGCTGGCAACAACA-3′ (reverse). Sequence trace files were analyzed for the occurrence of the G1463A variant with novoSNP (Weckx et al., 2005) and visual inspection. The sequencing data confirmed the absence of the 1463A-allele in all subjects. This unexpected absence of the *TPH2* 1463A-allele in 681 patients with affective disorders and 728 control individuals from two independent patient-control cohorts obtained from northern-Sweden and Belgium raises doubts about the true nature of this polymorphism. Even if the A-allele was rare in our Caucasian populations, it would have a frequency of <1/2818 chromosomes, or <0.035%. This contradicts the high prevalence of this polymorphism in 15/662 chromosomes (2.3%) in Caucasians reported by Zhang et al. (2005). Because the authors selected older patients (>60 years) for their study, we cannot exclude that the A-allele is enriched due to an associated protective survival effect. However, because the frequency of affective disorders is very similar worldwide and no studies have reported increased survival due to reduced serotonin levels, this assumption is unlikely to explain their genetic data. Furthermore, 7 of the 9 depressed patients with the 1463A-allele failed to respond to SSRI treatment, while 2 patients required high concentration. Therefore, it seems more plausible that the observed high frequency of the 1463A-allele reported by Zhang et al. (2005) is the result of severe patient population stratification based on age of inclusion and/or SSRI treatment failure. Anyway, even with the strong functional data of which we do not doubt the quality, the report of a functional relevant genetic *THP2* polymorphism associated with affective disorders is at this stage uncertain and needs to be replicated independently. Even more, recent guidelines for genetic association studies in complex diseases are firm when it comes to sample sizes and replication, even for functionally relevant variants in important candidate genes, and therefore warrant genotyping the G1463A variant in large patient-control populations (Smyth et al., 2005; Qu et al., 2005).

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Selected Reading

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We genotyped 1023 samples from human subjects with a diagnosis of unipolar depression for the neuronal tryptophan hydroxylase-2 (TPH2) mutation reported by Zhang et al. (2005). With the use of a Taqman 5′ nuclease assay (ABI, Assay by Design) we genotyped the entire Genetics of Response to Antidepressant Drugs (GRAD) sample at the G1463A TPH2 mutation and did not