Is there an association between NPY and neuroticism?

Arising from: Z. Zhou *et al. Nature* 452, 997–1001 (2008)

Psychiatric genetics has been hampered by the fact that initially exciting findings from underpowered studies are so often not replicated in larger, more powerful, data sets. Here we show that the claims of Zhou *et al.*[1] that neuropeptide Y (*NPY*) diplotype-predicted expression is correlated with trait anxiety (neuroticism) is not replicated in a data set consisting of phenotypically extreme individuals drawn from a large ($n = 88{,}142$) non-clinical population. We found no association between *NPY* diplotype or diplotype-predicted expression and neuroticism. Our reply to Zhou and colleagues forms part of a larger debate[2–5] (see, for example, http://www.nature.com/news/2008/080709/full/454154a.html) about the efficacy and replicability of candidate driven versus genome wide approaches to psychiatric genetics.

In their recent study, Zhou and colleagues[1] used a candidate gene driven approach to select NPY for investigation as a possible modulator of genetic susceptibility to anxiety and neuroticism. Zhou *et al.* concluded that "haplotype-driven *NPY* expression…inversely correlates with trait anxiety" and that their results "help to explain inter-individual variation in resiliency to stress, a risk factor for many diseases"[1].

To test their claims we genotyped all seven single nucleotide polymorphisms (SNPs) investigated by Zhou *et al.*[1] in 582 singletons from the extreme 5% tails of the Eysenck Personality Questionnaire neuroticism score distribution from a non-clinical population of 88,142 individuals from the south-west of England[2]. This sample has close to 100% power to detect a genetic effect accounting for 1.25% of phenotypic variance at an alpha level of 0.01. As Zhou *et al.* state that NPY explains between 3.3% and 3.4% of variance in trait anxiety[1], we have close to 100% power to test their claims.

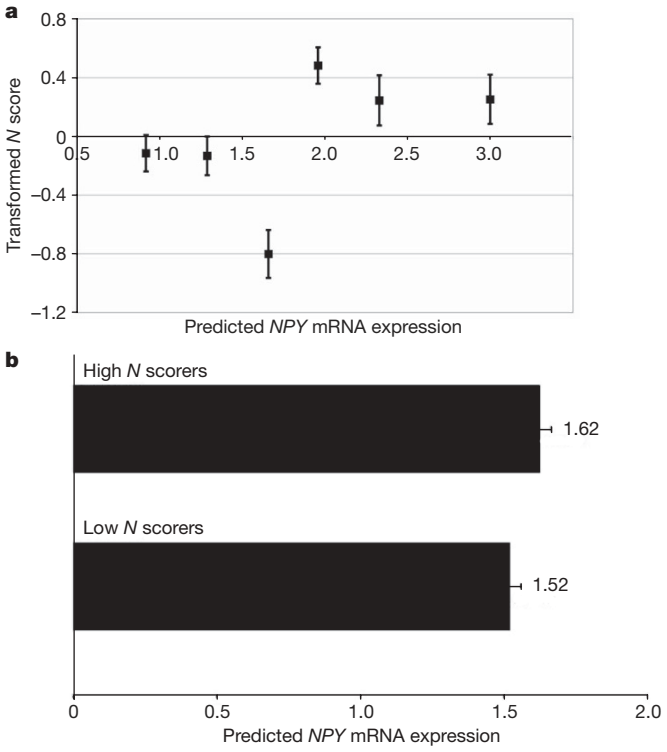

Figure 1 | Diplotype-predicted *NPY* expression and neuroticism. **a**, Regression of transformed age and sex-regressed *N* scores (mean ± s.e.m.) and diplotype-predicted expression values in 507 subjects (from left to right: H1/H1, $n = 151$; H1/H3, $n = 129$; H3/H3, $n = 16$; H1/H2, $n = 139$; H2/H3, $n = 33$; and H2/H2, $n = 39$). **b**, Diplotype-predicted *NPY* mRNA expression levels (mean and s.e.m.) of high neuroticism scorers ($n = 265$) and low neuroticism scorers ($n = 242$) compared with a two-tailed *t*-test ($P = 0.06$).

Diplotypes were assigned to each sample using the five haplotype definitions outlined by Zhou and colleagues[1]. The three most common haplotypes (H1, H2 and H3) formed six common diplotypes that had each been assigned an expression profile on the basis of lymphoblast *NPY* messenger RNA levels: low (LL:H1/H1), intermediate (LH:H1/H3, H3/H3 and H1/H2) and high (HH:H2/H3 and H2/H2). Subjects with minor diplotypes ($n = 75$) were not included in further analyses. Figure 1a shows the distribution of neuroticism scores by diplotype-predicted mRNA expression levels. Neuroticism was compared among diplotype groups by analysis of variance (ANOVA) and regression analysis. The diplotype-predicted values of mRNA expression were taken from Zhou *et al.*[1] as predicted by a co-dominant model. One-way ANOVA on all samples demonstrated no effect of *NPY* diplotype on neuroticism phenotype ($F(5) = 1.38$; $P = 0.14$) nor of *NPY*-diplotype-predicted expression ($F(2) = 1.01$; $P = 0.36$). Furthermore, *NPY*-diplotype-predicted expression was not correlated with transformed age and sex-regressed neuroticism scores (Fig. 1a). Furthermore, *NPY* diplotype-predicted mRNA levels did not differ significantly between subjects with high and low neuroticism scores ($P = 0.06$; Fig. 1b).

If *NPY* diplotype does in fact exert an effect on neuroticism, then the main effect size must be smaller than 1.25% and probably smaller than 0.5% (power = 87.6%). This lack of replication highlights the problems inherent in candidate gene driven approaches to psychiatric genetics.

METHODS

Oligonucleotide primers specific for seven different SNP markers (rs3037354, rs17149106, rs16147, rs16139, rs9785023, rs5574 and rs16475) were used to amplify the target *NPY* fragments by PCR. Sequencing was performed with Sequenom's MassARRAY technology[6].

Statistical power was calculated by simulation methods and implemented in Perl[2]. We ran 1,000 simulations of effect sizes ranging from 2.0% to 0.1% and using either 0.05 or 0.01 alpha levels, and calculated the proportion of times that a significant result was obtained.

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Zhou et al. *reply*

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The inability of Cotton *et al.*[1] to detect an effect of a functional haplotype (and locus) of neuropeptide Y (NPY), a stress regulatory neuropeptide, on neuroticism is interesting. Although it is important to measure effects of functional loci on complex behaviours, the strength of our study[2], and primary basis of its conclusions, was the larger and convergent effects of NPY on intermediate phenotypes, including regional brain responses to emotional stimuli and pain, and brain *NPY* messenger RNA and plasma NPY levels. Eysenck Neuroticism is a trait that we did not directly investigate. We reported modest association of NPY with two Harm Avoidance subscales from the Tridimensional Personality Questionnaire. Association of NPY with the complex trait of anxiety, especially when measured differently, is not the first place we would look to validate our results.

Concerning their advocacy of genome-wide approaches, if we follow the conclusions of their genome-wide association study with the same data set[3] then no loci contribute >1% of the variance in neuroticism. This is plausible, and could explain why they found no effect of NPY. However, Cotton *et al.*[1] genotyped the extremes of a large but relatively uncharacterized sample. Theoretically powerful, this approach may in practice be problematic. At the extremes of the distribution various confounds such as severe environmental stresses, rare functional alleles and measurement errors are more likely to be over-represented. Their study did not identify new functional loci for anxiety nor confirm functional loci for which there is independent evidence, as mentioned later. It is reasonable to request evidence that a tool works before using it to 'weed the garden'.

There is indeed debate as to how to proceed in gene discovery for behaviour. However, candidate gene and genome-wide approaches are not at war. The goal of genome-wide studies is to identify locations of functional polymorphisms. Studies using intermediate phenotypes, on which alleles exert larger effects than complex behaviours, may be better able to expand our understanding of mechanism. Consistent and convergent effects of several functional alleles on intermediate phenotypes have demonstrated the validity of this approach. Recent discoveries relating common alleles to behaviour have primarily relied on brain imaging tools. Examples include the serotonin-transporter-linked polymorphic region (5-HTTLPR) that has a weak effect on depression and anxiety—an association that was indeed obscured when only the extremes of the distribution were compared[4]—but strong effects on brain metabolic responses to emotional stimuli[5] and the uncoupling of limbic feedback circuitry (accounting for 30% of the variance in anxious temperament[6]). Brain imaging studies have also shown that a functional missense variant (Val158Met) of COMT alters brain activity during cognition[7], pain[8] and response to emotional stimuli (accounting for 38% of the variance in emotionality[9]), while having much more modest effects on complex behaviours, including anxiety. If allele effects on crudely measured behavioural phenotypes are undetectable in very large data sets, this may suggest that genome-wide genetic methods should be applied to data sets of more modest size, in which intermediate phenotypes have been measured that are more robust in detecting genetic influences on behaviour.

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