levels and clinical outcome in nortriptyline-treated, depressed patients. This curvilinear relationship has also been clearly demonstrated using nortriptyline (but not desipramine) in the tail suspension test, an animal model for screening antidepressant drugs, in which a self-inhibiting effect of nortriptyline is evident when high doses are used.[3] Thus, our findings show that the observed clinical therapeutic window found for nortriptyline is mimicked in certain brain areas at molecular level, as measured by c-Fos expression.

The mechanisms explaining the inverted U-shaped dose-response effect of nortriptyline in clinical practice require further elucidation. The occurrence of a similar phenomenon regarding c-Fos expression in the group of animals receiving nortriptyline demonstrated here also needs clarification. There is some evidence that hydroxy-metabolites may be involved in the self-inhibiting effect of nortriptyline.[7] Indeed, nortriptyline-treated, depressed patients with high plasma levels of E-10-OH-nortriptyline have been shown to respond poorly or not at all to the treatment. In this situation, the self-inhibitory effect of nortriptyline may be explained by the fact that, at high doses, increased plasma levels of nortriptyline hydroxy-metabolites with lower therapeutic efficacy exert a pharmacological competition with the parent compound, reducing its effect. Additionally, some of the brain areas in which the pattern of c-Fos expression appears as an inverted U-shaped dose–response curve are related to depression. Indeed, altered hippocampal volume detected by neuroimaging studies has been reported in humans.[8] Also, the hypothalamic areas studied here are involved in the regulation of hypothalamic–pituitary–adrenal function, a neuroendocrine axis that is closely related to the mechanisms triggering and maintaining depression.[9,10]

The data presented here suggest that the MPOL, MnPO, PVN, SCh, CA1 and AHiPM may be involved in depression and that functional image-based protocols should be used to scrutinize their function in depressed patients.

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## References

1 Asberg M, Chronholm B, Sjoqvist F, Tuck D. *BMJ* 1971; **3**: 331–334.
2 Ribeiro MG, Pereira ELA, Santos-Jesus R, de-Sena EP, Petribú K, de-Oliveira IR. *Rev Br Psiq* 2000; **22**: 51–56.
3 De-Oliveira IR, Diquet B, Van der Meersch V, Dardennes R, Gonidec J, Prado-Lima PA. *Psychopharmacology* 1990; **102**: 553–556.
4 Beck CH. *J Psychiatry Neurosci* 1995; **20**: 25–32.
5 Dahmen N, Fehr C, Reuss S, Hiemke C. *Biol Psychiatry* 1997; **42**: 317–323.
6 Fraga IC, Fregoneze JB, Carvalho FLQ, Dantas KB, Azevedo CS, Pinho CB *et al. Neuroscience* 2005; **134**: 327–334.
7 Young RC, Alexopoulos GS, Shindledecker R, Dhar AK, Kutt H. *Neuropsychopharmacology* 1988; **1**: 213–215.
8 Sheline YI, Gado MH, Kraemer HC. *Am J Psychiatry* 2003; **160**: 1516–1518.
9 Gillespie CF, Nemeroff CB. *Psychosom Med* 2005; **67**(Suppl 1): S26–S28.
10 Plotsky PM, Owens MJ, Nemeroff CB. *Psychiatr Clin N Am* 1998; **21**: 293–307.

# CREB1 haplotypes and the relative reinforcing value of nicotine

*Molecular Psychiatry* (2007) **12,** 615–617;
doi:10.1038/sj.mp.4002002

Genetic and pharmacological studies in mice have demonstrated an important role of the $\mu$-opioid receptor (MOR) and cAMP responsive element-binding protein (CREB) in nicotine reward/dependence. We tested for an interaction between these two genes (*OPRM1* and *CREB1*) in a human pharmacogenetic laboratory study.

Preclinical studies support the importance of both CREB and MOR in nicotine reward.[1] Nicotine increases phosphorylation of CREB (P-CREB) in the ventral tegmental area,[1] while nicotine withdrawal decreases P-CREB.[2] Nicotine-induced P-CREB increase is absent in $MOR^{-/-}$ knockout (KO) mice and is also blocked by the MOR antagonist naloxone in wild-type mice.[1] The MOR promoter has a cAMP-responsive element that binds P-CREB, which is thought to drive expression of MOR.[1,3] Finally, the $CREB^{\alpha\Delta-/-}$ KO and $MOR^{-/-}$ KO mice do not exhibit nicotine-conditioned place preference.[1,4]

We translated these findings to a human behavioral pharmacology investigation testing the role of MOR in the relative reinforcing value of nicotine.[5] We focused the genetic analysis on the functional *OPRM1* A118G variant in exon1, for which the G allele is associated with reduced mRNA and protein levels.[6] Here, we report new data on the interacting effects of *CREB1* and *OPRM1* in nicotine reward. As described previously,[5] 60 smokers (30 homozygous for the wild-type *OPRM1* A118G A/A and 30 with at least one *OPRM1* G allele) participated in a within-subject, double-blind investigation of effects of the MOR antagonist naltrexone (NTX) vs placebo on the relative reinforcing value of nicotine. We used a

validated nicotine cigarette choice paradigm in which number of nicotine cigarette puffs (vs denicotinized cigarette puffs) over a 3-h period reflects the relative reinforcing value of nicotine.

There is very high linkage disequilibrium (LD) across markers on the 69 kb *CREB1* gene forming a single haplotype block.[7] Owing to the lack of known functional variants in *CREB1*, we selected four single nucleotide polymorphisms (SNPs) close to the multi-marker haplotype-tagging SNPs with minor allele frequency $>0.1$. Because of the small sample size in the current study, LD was estimated[8] in a population of 367 Caucasian smokers described previously.[9] Pairwise LD was high for all of the SNPs (see Figure 1). *CREB1* SNP rs2254137 was perfectly correlated with rs2551640 for all cases and was therefore omitted from analysis.

Haplotype analyses utilized Haplo.Score and Haplo.GLM (generalized linear model),[10] which use the EM algorithm to estimate haplotype frequencies and a GLM of haplotype associations with binary or continuous traits. Rare haplotypes ($<5\%$) were omitted from the analysis. For the *CREB1* SNPs, rs2551640, rs13029936 and rs6740584 respectively, the three common haplotypes were A_C_C (49.1%), A_T_T (19.1%) and G_C_T (30.9%) with omitted haplotypes totaling under 1%. The outcome was the continuous trait of the effects of treatment on nicotine preference (nicotine puffs on placebo minus nicotine puffs on NTX). The overall regression model (Table 1) was significant (LR $\chi^2(7)=14.33$, $P=0.046$) and the presence of the *OPRM1* G allele modified the effects of the *CREB1* haplotypes on nicotine reward (LR $\chi^2(2)=7.54$, $P=0.02$).

A linear regression model of the effect of medication on the relative reinforcing value of nicotine was estimated using individual SNP data. As in the haplotype analysis, the outcome was the difference in nicotine puffs between medication and placebo phases. Predictors included cigarettes per day at baseline, treatment order, *OPRM1* genotype and genotype at *CREB1* rs2551640, rs13029936 and rs6740584. The model also included terms representing the interaction of *OPRM1* genotype by each of the *CREB1* SNPs. Including these interaction terms in the model resulted in a significant improvement in the

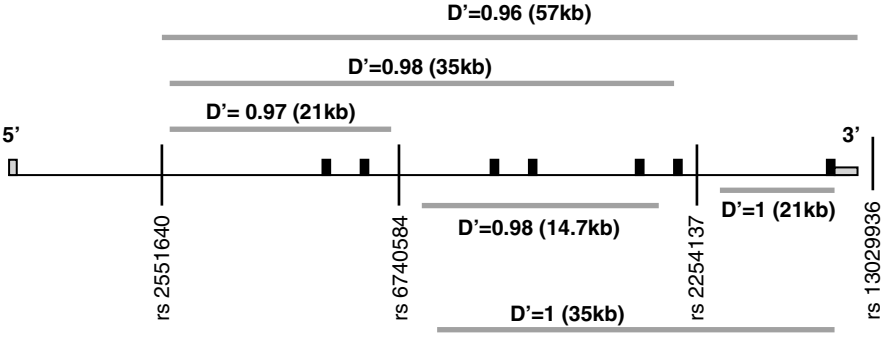


**Figure 1** SNPs genotyped in CREB1. The darker shaded boxes represent the exons and the lighter shaded boxes represent the untranslated regions (kb, kilobase; *D'*, linkage disequilibium estimate). Figure not drawn to scale.

**Table 1** Haplotype analysis of *CREB1* SNPs, *OPRM1* genotype and treatment condition

| *Variable* | *CREB1 haplotype* | *Nicotine preference (placebo−naltrexone)* | *s.e.* | t | P-*value* |
|---|---|---|---|---|---|
| OPRM1 A/A | A_C_C[a] | 2.36 | 1.76 | 1.34 | 0.19 |
| | A_T_T | 1.46 | 1.27 | 1.15 | 0.25 |
| | G_C_T | −2.44 | 1.03 | −2.35 | 0.02 |
| OPRM1 */G | A_C_C | −2.37 | 1.93 | −1.23 | 0.23 |
| | A_T_T | 1.08 | 1.94 | 0.56 | 0.58 |
| | G_C_T | 4.51 | 1.76 | 2.56 | 0.01 |
| Sex = female | | −0.21 | 1.04 | −0.2 | 0.84 |
| Cigarettes/day | | −0.06 | 0.06 | −1.1 | 0.27 |

Abbreviation: SNP, single nucleotide polymorphism.
Haplotype position 1 = rs2551640, haplotype position 2 = rs13029936, haplotype position 3 = rs6740584. Likelihood ratio for *CREB1* × *OPRM1* interaction: (LR) $\chi^2(2)=7.54$, $P=0.02$. Likelihood ratio for overall regression model: (LR) $\chi^2(7)=14.33$, $P=0.046$.
[a]Reference group.

predictive value (LR $\chi^2(3) = 12.75$, $P = 0.005$), which seemed to be due, in large part, to the interaction of *OPRM1* and *CREB1* rs2551640 ($P = 0.05$). Among cases with an *OPRM1* G allele, *CREB1* rs2551640 genotype had little effect on the difference in nicotine reinforcement between NTX and placebo. Among cases homozygous for the *OPRM1* A allele, those with *CREB1* rs2551640 A/A genotypes took 74% of puffs from the nicotine cigarette in the NTX session and 82% in the placebo session. For those with *CREB1* A/G or G/G genotypes, the direction of the treatment effect was reversed, with 83% nicotine puffs taken during NTX vs 73% during placebo.

Consistent with preclinical data,[1] these preliminary findings suggest that interaction between *CREB1* and *OPRM1* may be important in nicotine reward. Because of the small sample size and the fact that we did not adjust our $\alpha$ level for multiple comparisons, these results should be considered hypothesis-generating. Sequencing the *CREB1* exons or promoter region to identify novel functional SNPs may help elucidate more precisely the role of *CREB1* variation in nicotine reward. Studies exploring polymorphisms in other members of intracellular signal transduction pathways may also elucidate novel targets for therapeutic development for nicotine dependence.

## Acknowledgments

This research was supported by grants from the National Institute on Drug Abuse (RO1 DA017555-03) and National Cancer Institute (P50 CA/DA84718) (CL).

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## References

1 Walters CL, Cleck JN, Kuo YC, Blendy JA. *Neuron* 2005; **46**: 933–943.
2 Pluzarev O, Pandey SC. *J Neurosci Res* 2004; **77**: 884–891.
3 Lee PW, Lee YM. *Mol Pharmacol* 2003; **64**: 1410–1418.
4 Berrendero F, Kieffer BL, Maldonado R. *J Neurosci* 2002; **22**: 10935–10940.
5 Ray R, Jepson C, Patterson F, Strasser A, Rukstalis M, Perkins K *et al. Psychopharmacology (Berl)* 2006; **188**: 355–363.
6 Zhang Y, Wang D, Johnson AD, Papp AC, Sadee W. *J Biol Chem* 2005; **280**: 32618–32624.
7 The International Hapmap Project. http://www.hapmap.org.
8 Carter KW MPAPLJ. http://genepicomau/projects/jlin. 2004.
9 Lerman C, Wileyto EP, Patterson F, Rukstalis M, Audrian-McGovern J, Restine S *et al. Pharmacogenomics J* 2004; **4**: 184–192.
10 Sinwell JPS, Schaid DJ. *Haplo Stats (Version 1.2.1)* 2005.

# Role of the novel tryptophan hydroxylase-2 gene in Tourette syndrome

*Molecular Psychiatry* (2007) **12,** 617–619;
doi:10.1038/sj.mp.4002004

Tourette syndrome is thought to be of polygenic aetiology. Decreased concentrations of serotonin (5-HT) and its metabolite, 5-hydroxyindole acetic acid (5-HIAA), in brain and cerebrospinal fluid of Tourette patients led us to speculate that 5-HT synthesis might be implicated in the pathogenesis of the disorder. We therefore focussed on the novel enzyme responsible for 5-HT synthesis in the brain, tryptophan hydroxylase 2 (TPH2). Our findings indicate an important role of genetic variation of *TPH2* in the pathogenesis of Tourette syndrome.

Tourette syndrome is a complex neuropsychiatric disorder, which is characterized by chronic motor tics and at least one phonic tic. An increasing body of evidence points to an involvement of the serotonergic system in the pathophysiology of Tourette syndrome. Thus, serotonin (5-hydroxytryptamine; 5-HT) concentrations are decreased in subcortical brain regions in this disorder.[1] Similarly, levels of the 5-HT metabolite 5-HIAA are reduced in these brain regions[1] and in cerebrospinal fluid of Tourette syndrome patients.[2]

This allowed us to speculate that 5-HT synthesis might be implicated in the pathogenesis of Tourette syndrome. We therefore investigated the enzyme responsible for 5-HT synthesis in the brain, TPH2. We assessed two variants of *TPH2*, including a functional variant, in patients with Tourette syndrome. Our study sample consisted of 98 unrelated patients with Tourette syndrome. All patients were recruited from the Outpatient TS Clinic of Hannover Medical School (MHH) and examined personally by one of the authors (KRMV). All procedures were performed in accordance with the guidelines of the MHH Institutional Review Board. Control DNA samples were obtained from 178 healthy controls. All patients and controls were of German descent, according to their names and phenotypic appearance. Genomic DNA was extracted from EDTA–anticoagulated whole blood using QIAamp DNA Mini kits.

We assessed two single nucleotide polymorphisms (SNPs) of *TPH2* located in and close to the transcriptional control region of the gene. SNP rs4570625 is located in the transcriptional control region of *TPH2*, at position −703 with respect to the transcription start site (+1). SNP rs4565946 is located in intron 2 of *TPH2*. Genotyping of the SNPs was performed as described previously.[3,4] Statistical evaluations on allelic and genotypic distributions were performed using the SPSS package version 13.0 (SPSS Inc.,