

Association between DRD2/DRD4 Interaction and Conduct Disorder: A Potential Developmental Pathway to Alcohol Dependence

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In a recent article we reported the association of alcohol dependence with interacting genotypes of the dopamine D2 (DRD2) and D4 (DRD4) receptor genes [Mota et al., 2013]. The interaction between the two dopamine receptor genes is thought to reflect different DRD2–DRD4 heteromerization patterns, previously shown by experimental studies [Borroto-Escuela et al., 2011; Gonzalez et al., 2012]. The present study extends the investigation of the DRD2–DRD4 interaction to behavioral phenotypes during childhood and adolescence known to contribute to the development of drug and alcohol dependence in adulthood.

Childhood attention deficit hyperactivity disorder (ADHD) predicts a higher prevalence of substance use disorder (SUD) in adulthood [Charach et al., 2011] and comorbid conduct disorder (CD) plays a mediating role in the association of ADHD with both SUD in general [Brook et al., 2010] and alcohol dependence specifically [Tuithof et al., 2012]. It is therefore possible that the reported DRD2–DRD4 association with adult alcohol dependence could be mediated by CD, suggesting a developmental role for the DRD2–DRD4 interaction effect on early behavioral precursors of alcohol dependence.

To investigate this hypothesis we used a subset of the International Multicenter ADHD Genetics (IMAGE) project, for which the same DRD2 and DRD4 polymorphisms (rs2283265 and 48bp-VNTR, respectively) that had been used in the previous report were available [Mota et al., 2013]. The sample comprised 559 unrelated children and adolescents with a research diagnosis of DSM-IV combined subtype ADHD, of which 142 had comorbid CD; with a mean age of 10.86 years (SD = 2.692, range 7–17). Details of the sample, ascertainment, diagnostic procedures and genotyping have been reported previously [Brookes et al., 2006].

The DRD2 and DRD4 polymorphisms investigated in the present study are both thought to have functional effects and to interact with each other. In brief, the expression ratio of the short-(D2S) and long-(D2L) DRD2 isoforms (mainly presynaptic and postsynaptic, respectively) are affected by the DRD2 rs2283265 SNP, where the minor allele (T) favours the expression of the D2L form. This allele has been associated with cocaine abuse [Moyer et al., 2011] and reduced working memory and attention control performances [Zhang et al., 2007]. For the DRD4 48-bp variable number tandem repeat (VNTR) polymorphism in exon 3, the most common variants are the 2-, 4- and 7-repeat alleles (2R, 4R, and 7R, respectively), with 7R being implicated in ADHD [Li et al., 2006].

Subjects were classified according to the presence of the DRD2 and DRD4 risk alleles (T and 7R, respectively) and, as in the previous study, subjects with rare DRD4 alleles (not 2R, 4R, or 7R) were excluded from all analyses; all P-values are two-tailed. Chi-squared tests were first used to test the association of each SNP separately with CD. The presence of the T allele of the DRD2 rs2283265 SNP was associated with higher prevalence of CD ($\chi^2 = 6.628$, $P = 0.01$), but there was no effect of the DRD4 7R allele ($\chi^2 = 0.073$, $P = 0.79$). Logistic regression revealed a significant DRD2-DRD4 interaction ($P = 0.041$) associated with CD (Table 1a). Estimation of the odds ratio (OR) in the presence of interaction was performed as previously described [Mota et al., 2013].

The findings indicated that the presence of the DRD2 T allele conferred an increased risk of CD ($OR_{CI,95} = 2.33$ (1.41–3.86)) for subjects that did not have the DRD4 7R allele, whereas there was no risk associated with the DRD2 T allele for DRD4 7R carriers ($OR_{CI,95} = 0.96$ (0.48–1.91)) (Table 1b). This DRD2–DRD4 interaction pattern is similar to

the previously reported association with alcohol dependence in adults with and without ADHD [Mota et al., 2013]. In the earlier study, the concomitant presence of both DRD2 T and DRD4 7R alleles was associated with a protective effect, compared to the presence of either one of them alone (i.e., in the absence of the other). Although in the present study no significant protective effect by the presence of both alleles was observed, the current results are in line with the previous study as they suggest that the significantly increased risk conferred by the DRD2 T allele is suppressed by the concomitant presence of the DRD4 7R

risk allele.

It has been shown that DRD2 and DRD4 receptors can be colocalized in the prefrontal cortex and striatal regions, where they may be pre- and postsynaptically located [Svingos et al., 2000; Rivera et al., 2002; De Mei et al., 2009; De Almeida Mengod, 2010]. Experimental studies on DRD2–DRD4 heteromerization show that the D2L variant is able to heteromerize with the three main DRD4 variants, although less effectively with the DRD4 7R [Borroto-Escuela et al., 2011]. These authors suggest an enhancing allosteric

TABLE I.

Interaction Effects Between DRD4 48bp VNTR and DRD2 rs2283265 Polymorphisms in Conduct Disorder

	B	SE	Wald	df	OR (95% CI)	P-value
(a) Logistic Regression						
Model Main Effects						
rs2283265 (T+ vs. GG)	0.526	0.205	6.555	1	1.692 (1.13–2.53)	0.010
DRD4 7R (DRD4 7R+ vs. 7R-)	-0.054	0.201	0.073	1	0.947 (0.64–1.41)	0.787
Interaction						
rs2283265 ^a DRD4 7R	-0.889	0.436	4.163	1	0.411 (0.18–0.97)	0.041
					OR (95% CI)	P-value
(b) Odds ratio estimation^a						
rs2283265 (T+ vs. GG) in DRD4 7R+					0.96 (0.48–1.91)	0.902
rs2283265 (T+ vs. GG) in DRD4 7R-					2.33 (1.41–3.86)	0.001

^a Odds ratio estimation and corresponding confidence intervals (95% CI) for the presence of DRD2 rs2283265 T allele (T+ vs. GG) in DRD4 7R carriers (DRD4 7+) and non-carriers (DRD4 7R-).

receptor–receptor interaction exists with the D2L-DRD4 2R and 4R heteromers, but not between D2L and DRD4 7R and that these different processes may play a role in the forebrain. Meanwhile, the D2S isoform is able to form heteromers only with DRD4 2R and 4R receptor types, but not with the DRD4 7R variant [Gonzalez et al., 2012]. Based on in vivo and in vitro results, the authors strongly suggest that these D2S-DRD4 heteromers may have a

key role in dopamine-mediated modulation of striatal glutamate release.

Although the present study does not test heteromerization patterns directly, it is possible that our results may be reflecting the expected different DRD2–DRD4 heteromerization outcomes according to the concomitant presence (or absence) of the DRD4 7R, which is not able to heteromerize (or does it less efficiently) with D2S and D2L isoforms [Borroto-Escuela et al., 2011; Gonzalez

et al., 2012]. Further interpretation of these results requires additional studies to clarify how the different DRD2-DRD4 heteromerization processes modulate dopaminergic and/or glutamatergic neurotransmission and whether any such functional changes explain the DRD2-DRD4 genetic associations reported in these studies.

A limitation of our study is the reliance on the candidate gene approach and the use of an interaction term, increasing the chances of a type I error. However within this context there is no substitute for replication and it is therefore of some interest that we see similar genetic effects on related externalizing phenotypes. Our previous study [Mota et al., 2013] implicated a significant DRD2-DRD4 interaction effect in alcohol dependence in two independent samples. In the present study we were able to demonstrate that such interaction is also associated with CD in a sample of children/adolescents with ADHD. Despite the above stated limitations and differences between the two studies, the findings of the DRD2-DRD4 interaction association with childhood/adolescent CD, a frequent precursor of later SUD, suggest a potential mechanism for one possible causal pathway that links childhood CD to the later development of alcohol dependence.

In summary, this is the third independent sample where disorders

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related to externalizing behavior are found to be associated with a DRD2-DRD4 interaction term. While these results look highly promising, further replication is still required to clarify the role of the DRD2-DRD4 interaction on the course and outcome of childhood behavioral disorders on adult alcohol dependence, and to establish the processes involved throughout development.

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