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Authors
Smith, Moyra
Filipek, Pauline A
Wu, Charles
et al.

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Dopamine genes and ADHD☆

J.M. Swanson[a,c,*], Pamela Flodman[a], James Kennedy[b], M. Anne Spence[a], Robert Moyzis[a], Sabrina Schuck[a], Michael Murias[a], Joan Moriarity[a], Cathy Barr[b], Moyra Smith[a], Michael Posner[c]

[a]*Child Development Center, University of California at Irvine, 19262 Jamboore, Irvine, CA 92715, USA*
[b]*University of Toronto, Toronto, Canada*
[c]*Sackler Institute, Cornell University, New York, NY, USA*

Abstract

Family, twin, and adoption studies have documented a strong genetic basis for ADHD/HKD, but these studies do not identify specific genes linked to the disorder. Molecular genetic studies can identify allelic variations of specific genes that are functionally associated with ADHD/HKD, and dopamine genes have been the initial candidates based on the site of action of the stimulants drugs, which for a half century have provided the primary pharmacological treatment for ADHD/HKD. Two candidate dopamine genes have been investigated and reported to be associated with ADHD/HKD: the dopamine transporter (DAT1) gene [Cook et al., American Journal of Human Genetics 1995;56:993–998, Gill et al., Molecular Psychiatry 1997;2:311–313] and the dopamine receptor D4 (DRD4) gene [LaHoste et al., Molecular Psychiatry 1996;1:121–124; Smalley et al., 1998;3:427–430; Swanson et al., Molecular Psychiatry 1998;3:38–41]. Speculative hypotheses [Swanson and Castellanos, NIH Consensus Development Conference: Diagnosis and Treatment of Attention Deficit Hyperactivity Disorder, November 1998. p. 37–42] have suggested that specific alleles of these dopamine genes may alter dopamine transmission in the neural networks implicated in ADHD/HKD (e.g. that the 10-repeat allele of the DAT1 gene may be associated with hyperactive re-uptake of dopamine or that the 7-repeat allele of the DRD4 gene may be associated with a subsensitive postsynaptic receptor). These and other variants of the dopamine hypothesis of ADHD will be discussed. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Dopamine genes; ADHD; Alleles

1. Introduction

Over the past decade, several large twin studies established that the heritability of ADHD is about 80 [1–5]. The next step is to perform molecular genetic studies to search for allelic variations of specific genes that are functionally associated with ADHD/HKD. Dopamine genes have been the initial candidates for application of advances in molecular biology, based on the dopamine theory of ADHD [6,7] and the site of action of the stimulants drugs [8], the primary pharmacological treatment for ADHD/HKD.

Two candidate dopamine genes have been investigated: (1) Refs. [9,10] provided the initial reports on the dopamine transporter (DAT1) gene, and (2) Refs. [5,11] provided the initial reports on the dopamine receptor D4 (DRD4) gene. Polymorphisms of these genes are defined by variable numbers of tandem repeats (VNTR), which for the DAT1 gene is a 40-bp repeat sequence on chromosome 5p15.3 and for the DRD4 gene is a 48-bp repeat sequence on chromosome 11p15.5. The most common variants of the DAT1 gene are specified by nine or 10 repeats (copies) of the 40-bp sequence, and the most common variants of the DRD4 gene are specified by two, four or seven repeats (copies) of the 48-bp sequence.

The literature on these candidate genes and ADHD is increasing. Eight molecular genetic studies (see Table 1) have been published, so far, about investigations of a hypothesized association of ADHD with the DAT1 gene and the DRD4 gene. Six of these initial studies used the preferred family-based association (FBA) designs, in which DNA from clinical cases and their parents is used to investigation association of a gene with a disorder, rather than population-based association (PBA) designs, in which DNA from clinical cases and non-affected controls is used. The results from PBA designs are often discounted, since

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* Corresponding author.
E-mail address: jmswanso@uci.edu (J.M. Swanson)

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Table 1
Research teams investigating dopamine genes and ADHD

Location	Reference	Dopamine gene: DAT1	Dopamine gene: DRD4	N for probands	Statis
Family-based association (FBA)					
Chicago, USA	Cook et al. [9]	+		57	HRR
Irvine, USA	Swanson et al. [2]		+	52	HRR
Dublin, Ireland	Gill et al. [10]	+		40	HRR
Atlanta, USA	Rowe et al. [17]		+	116	HRR
	Waldman et al. [15]	+		122	TDT
Los Angeles, USA	Smalley et al. [18]		+	133	TDT
Population-based association (PBA)					
Irvine, USA	LaHoste et al. [11]		+	39	
Washington, USA	Castellanos et al. [19]		−	41	

presence or absence of effects may be due to population stratification effects—i.e. the clinical and control groups may differ in the percentage of subjects from different ethnic groups, which are known to have different allele proportions, and has ethnic differences in the groups rather than the presence or absence of the disorder may account (or contribute) to the group differences.

As shown in Table 1, all three published studies of the DAT1 gene reported an association with ADHD, while four of the five published data on both the DAT1 and DRD4 genes and their association with ADHD in the same sample of ADHD subjects. It is likely that for a common psychiatric (such as ADHD), complexity will be derived from the interaction of multiple genes, each with small effects. This will create significant problems for locating these genes [12].

A primary purpose of this study is to present data on the DAT1 gene and its association with ADHD, as defined by a refined phenotype of AADHD [4] and from the clinical samples used from investigations of the DRD4 gene and ADHD [5,11]. Another purpose of this study is to estimate allele frequencies in the reported studies, which are known to vary across ethnic groups [13]. This may help evaluate the population stratification hypothesis in the published studies that used PBA designs.

2. Materials and methods

Allele proportions: The eight published studies were examined, and when possible the allele proportions were calculated. For the FBA designs, the "Haplotype Relative Risk" (HRR) tables [14] were used to specify the allele proportions for the Parents (based on "2*n*" alleles), Probands (based on the "*n*" transmitted alleles), and theoretical controls (based on the "*n*" non-transmitted alleles. For the PBA designs, the allele proportions for the ADHD group and the control groups were taken from the tables in the published articles, with adjustments made as indicated in

Table 2
Allele proportions for the DAT1 and DRD4 genes (from eight published studies)

DAT1 allele proportions: "high risk" allele				DRD4 allele proportions: "high risk" allele				
Alleles	10-R	9-R	Other	Alleles	7-R	4-R	2-R	Other
FBA designs				FBA designs				
Cook:				*Swanson:*				
Parents-168	0.768	0.226	0.006	Parents-208	0.226	0.663	0.072	0.039
Probands-84	0.857	0.143	0.000	Probands-104	0.279	0.635	0.077	0.018
Controls-84	0.679	0.321	0.000	Controls-104	0.173	0.692	0.069	0.067
Gill:				*Smalley:*				
Parents-124	0.710	0.290	0.000	Parents-440	0.293	0.439	0.152	0.116
Probands-62	0.839	0.161	0.000	Probands-220	0.350	0.414	0.141	0.095
Controls-62	0.645	0.335	0.000	Controls-220	0.236	0.464	0.164	0.136
Waldman:				*PBA designs*				
Probands-234	0.69	0.29	0.02	*LaHoste:*				
				Probands-78	0.282	0.513	0.154	0.051
				Controls-78	0.115	0.756	0.128	0.000
				Castellanos:				
				Probands-82	0.220	0.683	0.073	0.024
				Controls-112	0.205	0.723	0.027	0.045
				Rowe:				
				Probands-214	0.243	0.617	0.061	0.079
				Controls-116	0.129	0.690	0.112	0.112

Table 3
HRR analysis of DAT1 alleles of 80 parents

	DAT1 Allele		
	9-R (440 bp)	10-R (480 bp)	
Transmitted	$w = 20$	$2n - w = 60$	$2n = 80$
Not-transmitted	$y = 14$	$2n - y = 66$	$2n = 80$
	$w + y = 34$	$4n - w - y = 126$	$4n = 160$

HHR $= 4n(w - y)^2/((w + y)(4n - w - y))$, chi square (1 df) $= 1.34$, $p > 0.05$

Table 4
TDT analysis of the DAT1 alleles of 80 parents

		Non-transmitted alleles		
		440	480	
Transmitted alleles	440	$A = 4$	$B = 16$	$A + B = 20$
	480	$C = 10$	$D = 50$	$C + D = 60$
		$A + C = 14$	$B + D = 66$	$2n = 80$

TDT $= (b - c)^2/(b + c)$, chi square (1 df) $= 1.38$, $p > 0.05$

the text for the separation of "rare alleles" when they had been combined with more frequent alleles in the tables.

DAT1 in DRD4 samples: In the samples described by Swanson et al. [5], DNA was available for 80 parent-proband trios. These probands met the criteria for a refined phenotype that is defined by the overlap of ICD-10 criteria for Hyperkinetic Disorder (HKD) and DSM-IV criteria for Attention Deficit Hyperactivity Disorder (ADHD). This overlap is characterized by ADHD-Combined Type, with no serious comorbidities. Also, due to the recruitment procedures that were used to identify subjects for clinical trails of one stimulant medications, the ADHD subjects were confirmed responders to methylphenidate [5].

The DAT1 alleles were obtained for 80 parent-proband trios. The Haplotype Relative Risk (HRR) analysis, as specified by Ewens and Spielman [14] and as used by Swanson et al. [5], was performed to evaluate the association of the DAT1 gene and ADHD. This analysis is based on the "4*n*" parental alleles. By inspection of the proband alleles—i.e. the 9-R (440 bp) and 10-R (480 bp) alleles—it is deduced which parental alleles were transmitted and not transmitted to the proband. In addition, the Transmission Disequilibrium Test (TDT), as specified by Ewens and Spielman [14], was performed for the 26 parents who were heterozygous for the 440 bp and 480 bp alleles.

3. Results

Allele proportions: In the prior studies of the association of ADHD and the DAT1 gene, the 10-R (480 bp) allele was considered to be the "high-risk" allele. In prior studies of the association of ADHD and DRD4 gene, the 7-R allele was considered to be the high-risk allele. This resulted in some pooling of low-frequency alleles with other alleles that were not the high-risk allele, for analysis.

The Haplotype Relative Risk analyses of the family-based association studies allow for specification of the allele frequency (or proportion) in the parents, the Probands, and the "theoretical controls" (deduced from the non-transmitted parental alleles). The values derived from the eight published studies are shown in Table 2.

These studies provide converging evidence for the association of dopamine genes and ADHD. In the three published studies of the DAT1 gene, the 10-R (480 bp) was higher in the ADHD samples under investigation than in the control groups (even though this allele has the highest proportion in the general population). In four of the five published studies of the DRD4 gene, the 7-R allele (a relative low-frequency allele in the general population) was higher in the ADHD samples under investigation than in the control groups. These converging investigations support the dopamine theory of ADHD.

DAT1 gene in the DRD4 sample: The data from the 80 parent–child trios are presented in Table 3 in the format for the HRR analysis (see Ref. [14, Table 2]). In Table 4, the data are presented in the format for the TDT analysis (see Ref. [14, Table 3]). The 1 df chi square for the HRR test was 1.34, which is not significant at $p < 0.05$. The 1 df chi square for the TDT test was 1.38, which was not significant at $p < 0.05$.

4. Discussion

The analysis of allele frequencies (proportions) reported in the published studies is informative. Across studies, there was considerable variation in allele proportion of the high-risk (10-R) DAT1 allele in the probands (i.e. 0.69–0.768). There was also considerable variation in the proportion of the high-risk (7-R) DRD4 allele in the probands (i.e. 0.220–0.350).

In the studies using Population Based Association designs, the allele proportion of the DRD4 high-risk (7-R) allele varied from 0.115 to 0.205. In the studies using Family Based Association designs, the allele proportion of the DRD4 high-risk (7-R) allele in the "theoretical controls" varied from 0.173 to 0.236.

Thus, in any single study, the expected proportion of the high-risk alleles for the DAT 1 and the DRD4 genes is difficult to specify. Other factors than the presence or absence of ADHD obviously are important determinants of this proportion in any sample.

The result of the test DAT1 association with ADHD is surprising. In a sample defined by a refined phenotype (ADHD-Combined Type with no serious comorbidities, with a demonstrated clinical response to methylphenidate), we did not replicate the association of ADHD with the DAT1 gene reported by Cook et al. [9], Gill et al. [10], and Waldman et al. [15]. In fact, the opposite pattern was observed: the allele designated as the high-risk allele in the prior studies (10-R, 480 bp) was more often "not

transmitted" than "transmitted" in this sample of ADHD children. However, non-replication is expected for small sample sizes (as used here) when investigating a complex psychiatric disorder (such as ADHD) that is likely to be associated with multiple genes each with small effects [12].

This study was a pilot project to evaluate two dopamine genes (the DAT1 and DRD4) genes) in the same children with ADHD diagnosed using a refined phenotype and recruited based on a clinical history of response to methylphenidate. While both are dopamine genes, the differences between the DAT1 and DRD4 genes are important. First, the DAT1 polymorphism is not in a coding region, so the variants do not result in structural differences in the dopamine transporter protein. The DRD4 polymorphism is in a coding region, so the variants do result in a structural difference in the dopamine receptor. Second, the DAT1 allele, suggested by Cook et al. [9] as the high-risk allele, is the allele with the highest frequency in the population. In contrast, the DRD4 allele implicated by Swanson et al. [2–5] is a low-frequency allele in the population.

Both of these dopamine genes have been reported to be associated with ADHD in different samples, but the association has not been strong (i.e. the relative risk as about 1.5–2.0). Clearly, neither of the genes accounts for a large proportion of variance in diagnosis of ADHD. For example, in a comparison of the allele frequencies in the LaHoste et al. [11] study of the DRD4 gene, about half of the diagnosed cases did not carry the high-risk (7-R) allele, and about 20% of the control group did have at least one 7-R allele. Also a genotype comparison indicated that a higher percentage of ADHD cases (about 50%) had at least one 7-R allele (and thus were labeled the 7 + genotype) than the control group (about 20%), but this also showed that the 7 + genotype was not a necessary condition (half of the cases had a 7- genotype) or sufficient condition (about a fifth of the control cases had a 7 + genotype). Thus, it is clear that ADHD has multiple causes, and other factors (genetic or non-genetic) must be specified to understand the etiologies of ADHD.

It is possible that dopamine genes may contribute to a "dopamine deficit" [2,4]. The 7-R allele of the DRD4 gene may produce a receptor that is "subsensitive" to dopamine. The 10-R allele of the DAT1 gene may be associated with a dopamine transporter that is abnormally efficient at the re-uptake process. This in turn may produce underactivity in dopamine pathways—both the mesocorticolimbic pathway (which is rich in D4 dopamine receptors in the frontal lobes) and the nigrostriatal pathway (which is rich in D2 dopamine receptors). These are areas involved in the component processes of attention proposed by Posner and Raichle [16]. Posner and Raichle [16] proposed a neuroanatomical network theory of attention based on the working hypothesis that three distinct neural networks accomplish component processes of Alerting (suppressing background neural noise to establish readiness to react by inhibiting ongoing activity and mental effort), Orienting (mobilizing specific neural resources to prepare for expected type of input by facilitation of one specialized process and inhibition of others), and Executive control (coordinating multiple specialized neural processes to direct behavior toward a goal by detecting the presence of a target, starting and stopping mental operations, and ordering multiple responses). Based on brain imaging work, Posner and Raichle [16] linked the neural network for Alerting to connected brain regions centered in the right frontal lobe, for Orienting to connected brain regions centered in the posterior parietal, and for Executive control to connected brain regions centered in the anterior cingulate gyrus and including the basal ganglia.

The association of dopamine genes with ADHD suggests that the two attentional networks that include brain regions rich in dopamine receptors (the Altering Network and the Executive Control Network) may be involved in the "attentional deficit" that defines this disorder. Genetic or non-genetic (e.g. minimal brain damage) factors could alter these networks. We suggest two possible genetic factors: either a subsensitive D4 receptor or a hyper-efficient dopamine transporter, or both, may result in underactivity of brain regions that are involved in attention and behavior [2]. If this theoretical explanation is correct, it may explain why dopamine agonists drugs, such as methylphenidate, have clinical benefits when used to treat ADHD children. This class of drug (dopamine agonists) may operate to correct dopamine deficit and thus normalize attention and behavior that is governed by activity in brain regions that are rich in dopamine receptors (e.g. the basal ganglia and anterior cingulate gyrus).

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