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Anorexia Nervosa, Perfectionism, and Dopamine D4 Receptor (*DRD4*)

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The dopamine D4 receptor (*DRD4*), a well-characterized, polymorphic gene, is an attractive candidate for contributing risk to disordered eating and anorexia nervosa (AN). We tested association using UNPHASED for 5 *DRD4* polymorphic loci, 3 promoter region SNPs (C-521T, C-616G, A-809G), the 120 bp promoter region tandem duplication and the exon III repeat, in 202 AN trios and 418 control families. Since perfectionism characterizes AN, we tested these five loci for association with the Child and Adolescent Perfectionism Scale (CAPS) in the AN and control groups. Single locus analysis showed significant association between the 'C' C-521T allele and AN. Haplotype analysis also showed significant association, particularly a 4-locus haplotype (exon III&120 bp repeat&C-521T&A-809G). Association was also observed between *DRD4* and CAPS scores both for AN and control subjects. The insulin-like growth factor 2 (*IGF2*) and the arginine vasopressin receptor 1a[Q2] (*AVPR1a*), previously shown to be associated with disordered eating, were also associated with CAPS scores. Three genes associated with AN were also associated with perfectionism. Personality traits are potential endophenotypes for understanding the etiology of eating disorders and one of the several pathways to eating pathology may be mediated by the impact of DNA sequences on perfectionism.

KEY WORDS: anorexia nervosa; perfectionism; *DRD4*; *IGF2*; *AVPR1a*; endophenotype

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INTRODUCTION

Dopamine (DA) is a neurotransmitter critical to food intake and much animal research shows that this catecholamine is essential for feeding [Meguid et al., 2000]. DA release seems to have a site-specific action in food-intake regulation, especially in the nucleus accumbens, the brain region associated with the reinforcing effect of feeding [Salamone et al., 2003]. In the hypothalamus, DA release is associated with the duration of meal consumption, a factor in determining the pattern of feeding behavior. Hence, DA is required to initiate each meal and is associated with meal number and duration [Meguid et al., 1997]. The role of DA in feeding behavior is no doubt an ancient one, as suggested in a recent study showing that DA mediates reinforcement for appetitive associative conditioning of feeding in the invertebrate, Aplysia [Reyes et al., 2005].

Both D1 and D2 DA receptor types participate in food intake and feeding regulation [Terry et al., 1995]. A mouse study provides evidence for the involvement of the DA D4 receptor (*DRD4*) in satiety [Huang et al., 2005]. In humans, the *DRD4* receptor is one of the most studied and best characterized of common polymorphisms [Tarazi et al., 2004]. It is highly polymorphic and several functional variants are known, including an exon III repeat [Van Tol et al., 1992; Lichter et al., 1993; Asghari et al., 1994, 1995; Van Craenenbroeck et al., 2005], a promoter region 120 bp tandem duplication [Seaman et al., 1999; D'Souza et al., 2004], and a promoter region SNP, C-521T [Okuyama et al., 2000].

Widespread interest in the *DRD4* gene and the importance of DA in eating behavior prompted us to examine this gene as a risk factor in anorexia nervosa (AN) by examining a group of 202 trios (AN daughter plus parents), who were genotyped for five *DRD4* polymorphisms (three SNPs in the promoter region including the functional C-521T [Okuyama et al., 2000], the functional 120 bp tandem duplication [Rogers et al., 2004] and the exon III repeat [Van Craenenbroeck et al., 2005]). Additionally, in an attempt to understand the psychological mechanisms that constitute the substrate for gene effects in conferring risk for AN, we also examined association between *DRD4* with scores on the Child and Adolescent Perfectionism Scale (CAPS) [Hewitt et al., 1991], both in the AN trios as well as in a group of control subjects with no history of an eating disorder (418 families of which 378 have at least one sibling forming more than one trio in each family with a total of 542 daughters) whom we have studied across a range of behavioral

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phenotypes [Bachner-Melman et al., 2005a,b,c,d; Kremer et al., 2005]. Testing association in a control group also avoids the conundrum of the effect of illness that may obscure relationships between personality traits such as perfectionism and common polymorphisms. Perfectionism is a trait that characterizes women with AN not only during acute illness, but also after recovery, suggesting that premorbid perfectionism may be a risk factor for AN [Bulik et al., 2003]. Heritability estimates between 22% and 42% (see Bulik et al. [2003]) also indicate that perfectionism is a probable endophenotype in eating disorders. We also thought it of interest to test two additional genes that we have found to be linked to disordered eating for association with CAPS scores: the insulin-like growth factor 2 (*IGF2*) [Bachner-Melman et al., 2005d] and the arginine vasopressin receptor 1a (*AVPR1a*) [Bachner-Melman et al., 2005d].

METHODS

AN Subjects

The AN women were recruited from the community via announcements on college campuses, in newspapers, and on the internet. The vast majority of participants were currently students who had achieved various levels of recovery. A community sample was preferred since there is evidence that this yields a more representative sample of affected individuals than those recruited via treatment centers, who are usually included in research projects and bias samples towards more seriously compromised individuals [Braun et al., 1994]. Pasberg and Wang [1994] found that psychiatric registers included less than one-half of the AN cases detected via local newspapers. Based on a comprehensive review of eating disorders epidemiological research, Hoek and van Hoeken [2003] estimated that community studies of AN produce a 10-fold increase in estimated cases compared with studies based on psychiatric inpatients.

Altogether, 322 women who replied to announcements were initially screened by telephone for past or present anorexic symptoms by the first author, a psychologist. Subsequently, full DSM-IV criteria for a lifetime diagnosis of AN were confirmed using the eating disorders section of the Structured Clinical Interview for DSM-IV [First et al., 1996] by two psychologists (Rachel Bachner-Melman and Elad Lerer) for 251 of them. Interviews were conducted face to face, and in a minority of cases by telephone, when distance and/or circumstances prevented participants from traveling to the research center. The exact protocols of the interviews were read by a psychiatrist (Ilana Kremer), who recontacted the participants by phone where necessary; a lifetime diagnosis of AN was confirmed for 202 women (DNA obtained from both parents). Current AN status and symptomatology was also determined in the interview using an extended version of the eating disorders section of the SCID.

For probands, ascertainment rules were age between 14 and 36 and an unequivocal lifetime diagnosis of AN by DSM-IV criteria, including primary or secondary amenorrhea. Exclusion criteria, determined upon screening, were organic brain syndrome, mental retardation, or insufficient Hebrew language proficiency to complete the battery of questionnaires.

Although we relied on self-reported clinical information, current and lifetime diagnoses of AN based on evidence have been shown to be highly reliable [Williams et al., 1992]. Similarly, height and weight were also self-reported based on evidence that anorexic, bulimic and underweight women report their weights remarkably reliably [Rowland, 1990; Keel et al., 1999; McCabe and Ricciardelli, 2001]. Healthy control participants tend to slightly underreport their weight, especially as BMI rises [Fortenberry, 1992; McCabe and Ricciardelli, 2001]. Overall, the correlation between actual weight and reported weight is typically above 0.95 [Cash et al., 1989].

Genotypes for the *DRD4*, *AVPR1a*, and *IGF2* genes were available and verified for 178 of these women and their parents.

Control Subjects

Respondents were primarily college students at Israeli institutions of tertiary education and their families, recruited by word of mouth and advertisements on campus notice boards. We have previously studied this group of women [Bachner-Melman et al., 2004, 2005b,c,d; Kremer et al., 2005].

All control subjects who initially contacted us were screened for history of an eating disorder using the following criteria: a BMI of 17.5 or less or over 30 currently or since reaching current height, an ideal BMI of 17.5 or less, amenorrhea, an EAT-26 score of above 20, or body dissatisfaction scores in the highest percentile (EDI body dissatisfaction score >38). Respondents were also asked whether "eating has ever been problematic or a source of distress" and the responses of those replying in the positive were examined. Women who described symptoms compatible with eating disorders, or who fulfilled at least one of the other criteria above, were contacted and interviewed with the SCID-IV. Those for whom a lifetime diagnosis of the full clinical syndrome of AN was confirmed were transferred to the AN group. Those with a lifetime diagnosis of bulimia nervosa or eating disorder not otherwise specified, including subthreshold AN (all criteria except amenorrhea or body weight under 85% of ideal weight) and subthreshold BN (frequency of bingeing and purging under twice a week for 3 months), and those who refused to be interviewed were excluded.

Although the AN group was significantly older than the control group (21.5 ± 4.4 vs. 23.5 ± 4.2; $P < 001$), the correlations between age and questionnaire scores were all insignificant at the 0.05 level. There were no significant between-group differences between religion, ethnicity, age of menarche, or the level of education of mothers or fathers.

Each contact person received questionnaires and two sterile test tubes per family member for DNA sampling, each containing 10 cc of "Aquafresh" mouthwash. Siblings completed questionnaires and provided DNA samples, whereas parents provided DNA samples but did not complete questionnaires. After a complete description of the study to all subjects, written informed consent was obtained. Completed questionnaires and DNA mouthwashes were returned by mail or hand-delivered to an office. The contact person received a modest monetary incentive, and the study was approved by the local IRB and by the Israeli Ministry of Health Genetics Committee. In the interest of confidentiality, personal information (name, phone, address, etc.) did not appear on questionnaires or test tubes, and was not entered into the database. Each family and each family member were assigned unique identification numbers that were used to track all data linked to that person.

Instruments

EAT-26. The EAT-26 is a 26-item self-report factor-analytically derived scale, originally validated on a sample of 160 women with eating disorders and 140 female non-clinical controls [Garner et al., 1982].

Eating disorders inventory (second edition; EDI-2)—body dissatisfaction and drive for thinness subscales. The EDI-2 is a self-report measure of symptoms generally related to eating disorders [Garner, 1991]. It contains 11 standardized subscales, each independently derived and representing a unique trait. In the present study, the EDI-2

was used to assess participants' Body Dissatisfaction and Drive for Thinness.

CAPS. The CAPS [Hewitt and Flett, 1991] consists of 22-items. Its two subscales are the Self-Oriented Perfectionism Scale (CAPSso) and the Socially Prescribed Scale (CAPSsp). It was translated into Hebrew, back translated, corrected and validated with an adult population in previous research [Zohar et al., 2005]. Both the full scale ($\alpha = 0.94$) and the two subscales ($\alpha = 0.93$ and 0.85) were highly reliable.

As noted by Hewitt and Flett [1991], the CAPS is closely modeled after the adult version (MPS; [Hewitt et al., 1991]), and measures self-oriented and socially prescribed perfectionism, but in terms more relevant to adolescents and children. We are aware that the CAPS is intended for use with participants somewhat younger than the groups we examined (anorexics= 23 years; non-clinical= 21 years). An examination of the items in the scale (Appendix I) reveals the reasons that this scale is recommended for use with children and adolescents: questions refer to parents (item 3), teachers (item 17), and grades (items 6, 20). In the current study, all control participants and almost all the AN subjects were participating together with both their parents in the study, so that the question about parents is indeed relevant in this study. In addition, the great majority of participants were students (Israeli University students are relatively older due to army service), so that teachers and grades were also relevant in their lives. For these reasons we chose to employ the CAPS rather than the very similar adult version of the perfectionism scale.

Genotyping

DNA was obtained from all family members including parents. The DRD4 exon III and promoter region SNPs and the 120 bp repeat were genotyped as previously described [Bachner-Melman et al., 2005b]. The two AVPR1a promoter region microsatellite markers were assayed as previously described [Bachner-Melman et al., 2004, 2005a]. The IGF2 SNPs were genotyped as previously described [Bachner-Melman et al., 2005d]. All the polymorphisms currently examined in this population are in Hardy–Weinberg equilibrium.

Statistical Analysis

We also used the logistic based variant of the transmission disequilibrium test (TDT), the so-called ETDT [Sham and Curtis, 1995], to assess association (and linkage) without confounding effect of population stratification. For each allele, the TDT, in its simplest version, compares the number of times it is transmitted to the number of times when it is not transmitted to an affected offspring. Note that only heterozygous parents are informative. This approach can be extended to haplotypes. The various tests are implemented in the program UNPHASED [Dudbridge, 2003] (http://www.rfcgr.mrc.ac.uk/~fdudbrid/software/unphased/).

RESULTS

We first examined five DRD4 polymorphisms for association with AN. As shown in Table I presenting the results from single locus analysis, significant association is only observed for the C-521T SNP in the combined AN sample. The C allele, which is associated with less transcriptional activity [Okuyama et al., 2000], is preferentially transmitted to AN subjects. An association with the 120 bp tandem repeat polymorphism was observed in the bingeing/purging subtype, but not in the restrictive group, where only a trend of association was observed for the C-521T SNP.

We next proceeded to haplotype analysis and overall significance (global *P*-value) is observed for two, three, four, and five locus haplotypes (Table II). Especially significant association is revealed for two of the four locus haplotype (C-521T & C-616G & A-809G & 120 bp tandem repeat, $P = 0.0001$; C-521T & A-809G & exon III & 120 bp tandem repeat, $P = 0.007$). The 120 bp single duplicate is transcriptionally more active [D'Souza et al., 2004]. Haplotype analysis for the sample grouped by bingeing/purging and restrictive subtypes of AN is shown in Table III. Overall, similar levels of significance are observed for both subtypes for single to five locus haplotypes.

The observation that the DRD4 gene contributes risk to AN encouraged us examine this gene in our group of control women for association with scores on the EAT-26 and EDI questionnaires. Scores for the EDI were categorized to two groups;

TABLE I. Association Between DRD4 Polymorphisms and AN: Single Marker Analysis

Polymorphism	Allele	T	NT	X^2	*P*-value
D4pr C(521)T	C	101	67	**6.84**[Q3]	**0.009**
	T	67	101		
D4pr C(616)G	C	77	88	0.736	0.390
	G	88	77		
D4pr A(809)G	A	89	82	0.297	0.586
	G	82	89		
D4pr 120 repeat	1	49	47	0.040	0.839
	2	47	49		
D4 exon III repeat	2	28	28	0	1
	3	12	14	0.149	0.699
	4	76	69	0.328	0.566
	5	4	3	0.145	0.704
	6	1	1	0	1
	7	44	50	0.371	0.542
		Bingeing purging			
D4pr 120 repeat	1	28	13	5.617	**0.018**
	2	13	28		
		Restrictive			
D4pr C(521)T	C	66	46	3.591	0.058
	T	46	66		

Stratification by subtypes of AN (only significant results showed).
T, number of transmitted alleles; NT, not transmitted; includes uncertain haplotypes.

TABLE II. Association Between DRD4 and Anorexia Nervosa: Haplotype Analysis (Global *P*-Value)

UNPHASED window	Haplotype	LRS	df	*P*-value
2	C(521)T–C(616)G	7.64	3	0.054
	C(521)T–A(809)G	8.02	3	**0.046**[Q4]
	C(521)T–exon III	11.17	10	0.345
	C(521)T–120 bp	5.20	3	0.157
	C(616)G–4(809)	6.93	3	0.074
	C(616)G–exon III	8.61	10	0.566
	C(616)G–120 bp	1.34	3	0.719
	A(809)G–exon III	10.52	10	0.396
	A(809)G–120 bp	3.40	3	0.333
	Exon III–120 bp	9.43	9	0.398
3	C(521)T–G(616)G–A(809)G	14.61	7	**0.041**
	C(521)T–C(616)G–exon III	26.97	19	0.105
	C(521)T–C(616)G–120 bp	9.024	7	0.251
	C(521)T–A(809)G–120 bp	17.44	7	**0.014**
	C(521)T–exon III–120 bp	29.01	16	**0.023**
	C(521)T–A(809)G–exon III	26.07	19	0.128
	C(616)G–A(809)G–exon III	22.40	19	0.264
	C(616)G–A(809)G–120 bp	8.92	7	0.258
	C(616)G–exon III–120 bp	15.92	17	0.529
	A(809)G–exon III–120 bp	27.47	16	**0.037**
	C(616)G–A(809)G–exon III	22.41	19	0.265
4	C(521)T–C(616)G–A(809)G–exon III*	14.55	6	**0.024**
	C(521)T–C(616)G–A(809)G–120 bp	42.72	15	**0.0001**
	C(521)T–C(616)G–exon III–120 bp*	14.81	6	**0.022**
	C(521)T–A(809)G–exon III–120 bp*	25.09	7	**0.0007**
	C(616)T–A(809)G–exon III–120 bp*	8.983	6	0.174
5	C(521)T–C(616)G–A(809)G–exon III–120 bp*	31.11	8	**0.0001**

LRS, likelihood ratio statistic; UNPHASED window: 2 = two locus, 3 = 3 locus, 4 = 4 locus, 5 = five locus analysis. Includes certain haplotypes.
*Dropped rare haplotypes (<0.05).

High, top 20% ("affected") and Low, low 80% ("non-affected"). As shown in Table IV, especially significant is the preferential transmission for the two most common exon III repeats, the 4 and the 7 with the EDI DT subscale. The two locus haplotype (A-809G & exon III; C-616G & exon III) shows very significant preferential transmission overall (global $P = 0.002$) as does the three locus haplotype (A-809G & exon III & 120 bp tandem repeat, $P = 0.004$).

Since perfectionism is a personality feature that underlies AN, we examined association in the AN group between the *DRD4* gene and scores on the self-orientated (SO) and the socially proscribed (SP) perfectionism subscales of the CAPS, towards understanding by which pathways sequence variants in this gene may be translated into pathological eating behavior. Single locus analysis which is presented in Table Va revealed overall significance (global *P*) between

TABLE III. Association Between DRD4 and Anorexia Nervosa: Stratification by Subtypes of AN

UNPHASED window	Haplotype	LRS	df	*P*-value
	Bingeing purging			
2	Exon III–120 bp*	11.48	4	**0.021**[Q5]
	A(809)G–120 bp*	7.572	2	**0.023**
	C(521)T–120 bp	11.41	3	**0.009**
3	A(809)G–exon III–120 bp	25.33	14	**0.031**
	C(616)G–exon III–120 bp	26.96	17	0.059
	C(616)G–A(809)G–120 bp	13.59	6	**0.034**
4	C(521)T–C(616)G–A(809)G–exon III	40.41	28	0.061
	C(616)G–A(809)G–exon III–120 bp	39.82	22	**0.011**
	C(521)T–C(616)G–exon III–120 bp	37.15	24	**0.042**
	C(521)T–C(616)G–A(809)G–120 bp	28.65	13	**0.007**
5	C(521)T–C(616)G–A(809)G–exon III–120 bp	53.04	32	**0.011**
	Restricting			
3	C(521)T–C(616)G–exon III	28.51	17	**0.043**
4	C(521)T–C(616)G–A(809)G–120 bp*	27.51	10	**0.002**
	C(616)G–A(809)G–exon III–120 bp	38.40	24	**0.032**
5	C(521)T–C(616)G–A(809)G–exon III–120 bp*	27.42	8	**0.0005**

Haplotype analysis (global *P*-value). Only significant results showed.
LRS = likelihood ratio statistic; UNPHASED window: 2 = two locus, 3 = 3 locus, 4 = 4 locus, 5 = five locus analysis.
*Dropped rare haplotypes (<0.05).

TABLE IV. Association[Q6] Between DRD4 and EDI DT in Control Women (High/Low Affected = Top 20%)

Polymorphism	Allele	T	NT	X^2	*P*-value
Single locus analysis					
D4pr C(521)T	C	100	106	0.154	0.694
	T	106	100		
D4pr C(616)G	C	110	98	0.634	0.4259
	G	98	110		
D4pr A(809)G	A	96	123	3.264	0.078
	G	123	96		
D4pr 120 repeat	1	84	77	0.309	0.5784
	2	77	84		
D4 exon III repeat	2	28	47	5.146	**0.023**[Q7]
	3	17	14	0.282	0.594
	4	131	84	10.3	**0.001**
	5	12	10	0.186	0.666
	6	2	1	0.341	0.5593
	7	57	90	7.263	**0.007**
Global *P*-value*	LRS = 14.05, DF = 2, *P*-value = **0.00089**				

UNPHASED window	Haplotype	LRS	df	*P*-value
Haplotype analysis (only significant results showed, global *P*-value)				
2	C(521)T–exon III**	13.74	4	**0.008**
	C(616)G–A(809)G**	8.72	3	**0.033**
	C(616)G–exon III**	17.48	4	**0.002**
	A(809)G–exon III**	16.11	4	**0.002**
	Exon III–120 bp**	11.12	3	**0.011**
3	C(521)T–C(616)G–exon III**	16.13	7	**0.024**
	C(616)G–A(809)G–exon III	35.86	21	**0.023**
	C(616)G–A(809)G–120 bp**	12.97	5	**0.023**
	A(809)G–exon III–120 bp	42.12	21	**0.004**
4	C(521)T–A(809)G–exon III–120 bp	51.81	36	**0.043**
	C(616)T–A(809)G–exon III–120 bp**	16.83	7	**0.018**
5	C(521)T–C(616)G–A(809)G–exon III–120 bp	78.79	56	**0.024**

T = number of transmitted alleles; NT = not transmitted; includes uncertain haplotypes; LRS = likelihood ratio statistic.
LRS = likelihood ratio statistic; UNPHASED window: 2 = two locus, 3 = 3 locus, 4 = 4 locus, 5 = 5 locus analysis.
*Dropped rare alleles (<0.05).
**Dropped rare haplotypes (<0.05).

CAPS SP and the C-616G SNP ($P = 0.006$) as well as the 120 bp tandem repeat ($P = 0.029$). Haplotype analysis revealed significant two locus and three locus association especially with the C-521T, C-616G, and A-809G promoter region SNPs. Overall similar results were observed for the CAPS SO subscale which were categorized to two groups; High, top 20% ("affected") and Low, low 80% ("non-affected") (Table Vb). We also examined association between *DRD4* and CAPS in the female control subjects. As shown in Table VI, significant association is observed between the exon III repeat region and CAPS SO and CAPS SP (scored divided to High/Low). The four repeat is preferentially transmitted to women with high scores and the opposite is observed for the two allele.

We have previously observed association between *IGF2* SNPs, especially the *ApaI* polymorphism [Bachner-Melman et al., 2005d], and the *AVPR1a* (arginine vasopressin 1a) gene

TABLE Va. Association between DRD4 and CAPS SP in Anorexia Nervosa Subjects

UNPHASED window	Polymorphism/haplotype	LRS	df	*P*-value
1	D4pr C(521)T	1.18	1	0.277
	D4pr C(616)G	7.68	1	**0.006**[Q8]
	D4pr A(809)G	0.16	1	0.687
	D4pr 120 repeat	12.38	5	**0.029**
	D4 exon III repeat	0.81	1	0.366
2	C(521)T–C(616)G	11.67	3	**0.008**
	C(521)T–exon III	20.14	9	**0.017**
	C(616)G–4(809)	8.478	3	**0.037**
	C(616)G–exon III	25.52	10	**0.004**
	C(616)G–120 bp	8.09	3	**0.044**
	Exon III–120 bp	23.69	9	**0.004**
3	C(521)T–G(616)G–A(809)G	15.14	7	**0.034**
	C(616)G–A(809)G–exon III	35.68	17	**0.005**
	A(809)G–exon III–120 bp	26.68	15	**0.031**

Single locus and haplotype analysis (only significant results). Global *P*-value.
LRS = likelihood ratio statistic; UNPHASED window: 1 = one locus, 2 = two locus, 3 = 3 locus analysis.

TABLE Vb. Association Between DRD4 and CAPS SO in Anorexia Nervosa Subjects

UNPHASED window	Haplotype	LRS	df	*P*-value
1	D4pr C(521)T	4.63	1	**0.031**[Q9]
	D4pr C(616)G	2.27	1	0.131
	D4pr A(809)G	1.75	1	0.185
	D4pr 120 repeat	9.74	5	0.082
	D4 exon III repeat	0.01	1	0.894
2	C(521)T–C(616)G	9.05	3	**0.028**
3	C(521)T–C(616)G–exon III	23.95	14	**0.046**
	C(521)T–exon III–120 bp	22.90	15	0.086
	C(616)G–exon III–120 bp	23.66	16	0.097
4	C(521)T–C(616)G–A(809)G–120*	14.33	8	0.073
	C(521)T–C(616)G–exon III–120	35.28	22	**0.036**
	C(521)T–A(809)G–exon III–120 bp	38.15	24	**0.033**
5	C(521)T–C(616)G–A(809)G–exon III–120 bp	59.74	36	**0.007**

Single locus and Haplotype analysis (only significant results). High/Low affected = top 20%. Global *P*-value. LRS = likelihood ratio statistic; UNPHASED window: 1 = one locus, 2 = two locus, 3 = 3 locus, 4 = 4 locus, 5 = five locus analysis.
*Dropped rare haplotypes (<0.05).

[Bachner-Melman et al., 2004; Bachner-Melman et al., submitted] and eating disorders, suggesting these two genes might also be associated with CAPS scores. Significant association was observed between the *IGF2 ApaI* SNP (Table VII) but not for the two other SNPs in the *IGF2* gene, 2207 and 1156 [Gaunt et al., 2001]; nor did two or three locus haplotype analysis add any additional significance to the single *ApaI* association. The G allele for the *IGF2 ApaI* polymorphism is preferentially transmitted. Direct association was not observed for the *AVPR1A* RS1 and the RS3 microsatellites and CAPS SP/SO in the non-clinical female subjects (data not shown). Haplotype analysis, however, revealed a strong association (CAPS SO: $P = 0.0319035$; CAPS SP: $P = 0.0075536$, global *P*-value) for the RS1 and

TABLE VI. Association Between DRD4 and Child Adolescent Perfectionism Scale (CAPS) in Control Female Subjects

Polymorphism	Allele	T	NT	X^2	*P*-value
CAPS SP (socially prescribed) High/Low affected = top 20%					
D4pr C(521)T	C	111	79	45.81	**0.024**[Q10]
	T	79	111		
D4pr C(616)G	C	87	101	47.74	0.312
	G	101	87		
D4pr A(809)G	A	93	104	48.44	0.435
	G	104	93		
D4pr 120 repeat	1	73	89	46.55	0.213
	2	89	73		
D4 exon III repeat	2	21	44	8.249	**0.004**
	3	11	9	0.205	0.651
	4	102	64	8.052	**0.004**
	5	4	5	0.112	0.737
	7	48	63	1.787	0.181
Global *P*-value		LRS = 13.1, DF = 5, *P*-value = **0.02243**			
CAPS SO (self oriented) High/Low affected = top 20%					
D4pr C(521)T	C	124	92	4.84	**0.027**
	T	92	124		
D4pr C(616)G	C	78	108	4.46	**0.034**
	G	108	78		
D4pr A(809)G	A	101	115	0.90	0.326
	G	115	101		
D4pr 120 repeat	1	72	75	0.058	0.808
	2	75	72		
D4 exon III repeat	2	24	43	5.312	**0.021**
	3	9	5	1.177	0.277
	4	93	67	3.739	0.053
	5	5	5	0	1
	7	48	57	0.641	0.379
Global *P*-value		LRS = 10.4, DF = 5, *P* = 0.06464			

Single locus analysis.

TABLE VII. Association Between IGF2 and Child Adolescent Perfectionism Scale (CAPS SP, Socially Prescribed) in Control Female Subjects

Polymorphism	Allele	T	NT	X^2	*P*-value
IGF2 *ApaI*	A	78	112	6.467	**0.011**[Q11]
	G	112	78		
IGF2 *2207*	C	35	33	0.059	0.807
	T	35	33		
IGF2 *1156*	C	85	107	2.776	0.096
	T	107	85		

T = number of transmitted alleles; NT = not transmitted; includes uncertain haplotypes.

RS2 haplotype and both subscales of perfectionism, divided to two groups (high/low) as described previously (data not shown).

DISCUSSION

Despite intense study of the *DRD4* receptor polymorphisms for association with both normal and abnormal behavior, we are aware of only four previous investigations that have addressed the role of this gene in eating disorders. Levitan et al. [2004] observed an association between *DRD4* exon III 7 repeat allele and binge eating. Similarly, a second study found a significant increase in the frequency of the *D4DR* exon III long alleles in individuals at high risk for obesity [Poston et al., 1998]. In contrast, two early studies failed to observe association between *DRD4* and AN [Hinney et al., 1999; Karwautz et al., 2001]. The investigation by Hinney et al. [1999] examined 57 trios. The negative study by Karwautz et al. [2001] compared 45 sister-pairs, one of who had AN and the other did not; a power calculation by the authors showed insufficient power to detect the expected effect on risk with this sample size.

The finding in the current study that the *DRD4* gene contributes risk for AN suggested that this gene may also contribute to dysfunctional eating in individuals who have never suffered from a DSM-IV eating disorder. We therefore examined association between *DRD4* and the EAT-26 [Garner et al., 1982] and EDI [Garner, 1991] questionnaires that assess eating habits in a group of female controls. Association was observed as we expected following the positive association between *DRD4* and AN, especially between the EDI Drive for Thinness scale and the *DRD4* gene. The mechanisms by which variations in the sequence of genes and their regulatory regions work their way up to affecting behavioral traits generally remain obscure. The pathway from DNA → individual nerve cell function → nerve pathway → integration → behavior that eventually is manifested in disordered eating or clinical illness is complex. One such pathway may be mediated by the contribution of genes to personality traits [Cloninger, 1999; Ebstein et al., 2000; Benjamin et al., 2002a]. Personality or temperament traits may be closer to the molecular substrate of gene action than are clinical diagnostic categories. We hypothesized that increased *DRD4* transcription, as suggested by the current findings, may be contributing to individual variance in personality features that predispose some individuals to disordered eating.

The strong relationship between perfectionism and AN [Bulik et al., 2003] prompted us to examine the genes that confer risk for AN and this personality trait in women both with and without a lifetime diagnosis of DSM-IV AN that we have genotyped. As we conjectured, *DRD4* was associated with perfectionism scores in both AN and independently recruited control subjects.

In our control population, we initially studied two additional genes, *IGF2* [Bachner-Melman et al., 2005d] and *AVPR1a*, for risk in dysfunctional eating [Bachner-Melman et al., 2004]. We now found the *IGF2* gene to be associated with perfectionism scores in these women with no history of an eating disorder. Interestingly, the *IGF2 Apa*I 'G' allele found to be associated with disordered eating in the female controls is the same allele associated with high perfectionism scores in this group. Although the *AVPR1a* RS3 marker has been found to be associated in this group with the EDI Drive for Thinness subscale [Bachner-Melman et al., 2004], direct association was not observed for the RS1 and RS3 markers and perfectionism, while haplotype analysis showed a strong association. We have also shown the *IGF2* and *DRD4* genes to be associated with self-report measures of selflessness or altruism [Bachner-Melman et al., 2005b] in this sample of control women, another trait that tends to characterize women with eating disorders [Bachar et al., 2002].

In summary, it is significant that three polymorphic genes that we have shown to be associated with dysfunctional eating and AN also appear to be associated with personality traits characteristic of patients with AN, especially perfectionism. We and others have theorized that personality traits are useful endophenotypes for major mental illness [Cloninger, 1987; Benjamin et al., 2001, 2002b; Van Gestel and Van Broeckhoven, 2003; Gottesman and Gould, 2003] and the current findings strengthen the notion that one of the several pathways to serious behavioral disorders is mediated by the impact of variations in DNA sequences on personality traits.

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APPENDIX I: ITEMS IN THE CHILD AND ADOLESCENT PERFECTIONISM SCALE

1. I try to be perfect in everything that I do.[+]
2. I want to be the best at everything that I do.[+]
3. My parents do not always expect me to be perfect at everything that I do.[*] (R)
4. I feel I have to do my best the whole time.[+]
5. Some people in my life expect me to be perfect.[*]
6. I always try to get the highest grade in an exam.[+]
7. It bothers me if I do not do everything I can all the time.[+]
8. My family expects me to be perfect.[*]
9. I do not always try to be the best.[+] (R)
10. People expect from me more than I am capable of giving.[*]
11. I get angry at myself every time I make a mistake.[+]
12. Others think that I have failed if I do not do my best the whole time.[*]
13. Others always expect me to be perfect.[*]
14. I get sad if there is even one mistake in my work.[+]
15. People around me expect me to be good at everything.[*]
16. When I do something, it has to be perfect.[+]
17. My teachers expect my work to be perfect.[*]
18. I do not have to be the best at everything that I do.[+] (R)
19. I am always expected to do things better than others.[*]
20. Even if I work, I feel that I have failed if I did not get one of the highest grades.[+]
21. I feel that people ask too much of me.[*]
22. I cannot stand being less than perfect.[+]

In the above list, + denotes self-orientated perfectionism subscale; * denotes socially proscribed perfectionism subscale; and R stands for reversed.

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