Molecular Medicine 4: 14-21, 1998

Molecular Medicine

Original Articles

Comorbid Migraine with Aura, Anxiety, and Depression Is Associated with Dopamine D2 Receptor (DRD2) *Nco*I Alleles

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Communicated by S.H. Snyder. Accepted November 21, 1997.

Abstract

Background: Unrelated individuals (n = 242) were interviewed directly for the presence of migraine, anxiety disorders, and major depression.

Materials and Methods: The data described in this study are derived from a clinical genetic relational database that was developed initially for the genetic analysis of migraine. Genotyping of the DRD2 *Nco*I C to T polymorphism located in exon 6 (His313His) was performed using previously described primers.

Results: A significantly increased incidence of migraine with aura (MWA), major depression, generalized anxiety disorder (GAD), panic attacks, and phobia was observed in individuals with the DRD2 *Nco*I C/C genotype compared with individuals with an DRD2 *Nco*I T allele. Specifically, 69% (91/131) of DRD2 *Nco*I C/C individuals in the present study met criteria for at least one of these neuropsychiatric disorders versus only 22% (4/18) of the DRD2 *Nco*I T/T individuals (Chi-square = 15.29; $p < 0.00005$). The DRD2 *Nco*I C allele frequency is significantly higher (Chi-square = 17.13; $p < 0.00002$) in individuals with MWA, anxiety disorders, and/or major depression (C allele frequency = 0.80) than in individuals who have none of these disorders (C allele frequency = 0.67).

Conclusions: These data indicate that MWA, anxiety disorders, and major depression can be components of a distinct clinical syndrome associated with allelic variations within the DRD2 gene. Clinical recognition of this genetically based syndrome has significant diagnostic and therapeutic implications.

Introduction

Striking similarities exist between the epidemiological characteristics of migraine, anxiety, and depression (1–5). All three disorders afflict approximately 10% to 25% of the general population at some point in life and are approximately twice as common in females as in males. Prophylactic medications for all three disorders have a subacute onset of action, requiring 3 to 6 weeks of therapy to measure clinical improvement. In epidemiological studies, a clinical diagnosis of migraine significantly increases the risk of comorbid anxiety and depression (5–15). Taken together, these data suggest that a common metabolic variation may underlie comorbid migraine, anxiety and depression.

In a recent molecular genetic study, an association was observed between the *Nco*I polymor-

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phism in exon 6 of the dopamine D2 receptor (DRD2) and the clinical susceptibility to migraine with aura (MWA) (16). We therefore performed an association analysis of DRD2 *Nco*I alleles in a group of 242 unrelated individuals, approximately half of whom suffer from MWA, anxiety disorders, and/or major depression. The present study was designed to analyze the possible association of DRD2 *Nco*I alleles with comorbid neuropsychiatric disorders.

Materials and Methods

The data described in this study are derived from a clinical genetic relational database that was developed initially for the genetic analysis of migraine (17). Potential subjects were identified by physician or self-referral for a migraine genetic study. Subjects were evaluated using a semi-structured interview for migraine. Migraine evaluations were conducted by a neurologist and/or trained interviewer. The lifetime presence or absence was determined for each of the criteria in the International Headache Society (IHS) definition of MWA (18).

A semi-structured interview based on the Structured Clinical Interview for DSM-III-R (SCID) (19), modified to include the criteria of the Diagnostic and Statistical Manual of Mental Disorders-IV (DSM-IV) (20,21) was used to evaluate anxiety and depressive disorders in the same individuals interviewed for migraine. The interview included questions that were appropriate to establish a DSM-IV-based diagnosis of generalized anxiety disorder (GAD), phobias, panic attacks, panic disorder, obsessive-compulsive disorder (OCD), and major depression. Interviews were performed by physicians or trained psychiatric nurses. Diagnoses required the concurrence of at least three physicians.

Genomic DNA was isolated using the Puregene DNA isolation kit (Gentra Systems, Research Triangle Park, NC). Genotyping of the DRD2 *Nco*I C to T polymorphism located in exon 6 (His313His) was performed using previously described primers (22). Briefly, 40 ng of genomic DNA was amplified in 10 μL of a solution containing 1× Perkin Elmer PCR amplification buffer, 400 μM each dNTP, 0.5 U *Taq*Gold polymerase (Perkin Elmer, Foster City, CA) and 1 μM primers DRD2.35 (ATCCTGCAGCCATGG) and DRD2.38 (ATTGTCCGGCTTTACC). The enzyme was activated with an initial incubation at 94°C for 10 min, followed by 14 cycles of amplification with denaturation at 94°C for 20 sec, annealing at 63°C for 1 min, elongation at 72°C for 30 sec with a decrease of 0.5°C and 3 sec for each annealing step, and an additional 40 cycles of denaturation at 94°C for 30 sec, annealing at 56°C for 30 sec and elongation at 72°C for 1 min. After amplification, 10 μL of a solution containing 2× NEB4 buffer and 2 U *Nco*I (New England Biolabs, Beverly, MA) were added directly to the amplification reaction and incubated at 37°C for greater than 4 hr. The digested products were separated on a 1.2% agarose SFR gel (Amresco, Solon, OH). Analysis of the genotype was performed by two individuals blinded to the clinical status.

Results

Clinical Characteristics of Individuals in this Study

Direct diagnostic interviews were completed on 242 unrelated individuals in the present study. The ethnic background of the study group was 98% Caucasian, 1% Asian, and 1% African-American A diagnosis was made only if the individual met DSM or IHS criteria for the disorders listed in Table 1. If a clear clinical diagnosis could not be made, the subject was not included in any further statistical analyses for that particular disorder. For each of the conditions analyzed, a diagnosis was made in at least 98% of the individuals. In the overall dataset, 55% (134/242) of individuals were diagnosed with at least one of the clinical disorders analyzed. As shown in Table 1, anxiety disorders are the most common diagnosis, being present in 46% (122/242) of the current dataset. Major depression is the single most common diagnosis (i.e., 38%) amongst the group of analyzed disorders in the present study. The incidences of panic attacks (31%) and phobia (29%) are similar. MWA is present in 21% of the individuals. Panic disorder, GAD, and OCD are present in less than 20% of the current study group.

A distribution of the comorbidities is provided in Table 2. These data are consistent with previous reports on neuropsychiatric comorbidities. For example, approximately 50% of individuals with MWA report a history of major depression (13,13,23). Individuals with panic attacks have the highest incidence of comorbid MWA (78%) in the present study.

Table 1. Incidence of migraine with aura, anxiety disorders, and major depression

	Affected (%)	Unaffected (%)	Not Diagnosed (%)
MWA, anxiety or depression	55	45	0
Any anxiety disorder	46	54	0
Major depression	38	61	1
Panic attacks	31	68	1
Phobia	29	69	2
Migraine with aura	21	79	0
Panic disorder	19	81	1
Generalized anxiety disorder	17	81	2
Obsessive compulsive disorder	14	86	0

Results from current study (n = 242). Clinical diagnoses were based on DSM criteria for anxiety disorders (20) and major depression (21) and on IHS criteria for MWA (18). If a clear diagnosis could not be made, the individual was not diagnosed and was not included in further statistical analyses for the disorder.

Frequency of Neuropsychiatric Disorders Based on DRD2 NcoI *Genotypes*

The incidences of the various clinical diagnoses based on DRD2 *Nco*I genotypes is provided in Table 3. A present or past history of MWA, anxiety disorders, or major depression is present in 69% of the C/C individuals, 53% of the C/T individuals, and 22% of the T/T individuals. The incidence of any of these neuropsychiatric diagnoses is significantly higher in the C/C individuals when compared to either the C/T individuals (Chi-square = 6.53; $p < 0.005$), T/T individuals (Chi-square = 15.29; $p < 0.00005$), or the combined T/any group of individuals (Chi-square = 12.72; $p < 0.0002$).

The presence of an anxiety disorder is significantly more frequent in the C/C individuals than in either the C/T individuals (Chi-square = 3.87; $p < 0.02$), T/T individuals (Chi-square = 8.92; $p < 0.001$), or the combined T/any group of individuals (Chi-square = 7.20; $p < 0.004$). A similar pattern is seen with GAD. Major depression, panic attacks, MWA, and phobia are also all increased significantly in the C/C versus T/any individuals (Table 3). Although both panic disorder and OCD are more frequent in the C/C ver-

Table 2. Comorbid incidence of migraine with aura, anxiety disorders, and major depression

	N	MWA (%)	Major Depression (%)	GAD	Panic Attacks (%)	Panic Disorder (%)	Phobia (%)	OCD (%)
Migraine with aura	51	100	49	24	39	16	43	18
Major depression	92	27	100	36	57	38	53	27
Generalized anxiety disorder	42	31	79	100	71	60	64	38
Panic attacks	76	74	68	39	100	59	57	28
Panic disorder	45	18	80	56	100	100	73	40
Phobia	71	31	31	38	61	46	100	28
Obsessive compulsive disorder	33	27	76	48	64	55	61	100

Results from current study (n = 242). Clinical diagnoses were based on DSM criteria for anxiety disorders (20) and major depression (21) and on IHS criteria for MWA (18).

Table 3. Incidence of migraine with aura, anxiety disorders, and major depression in current database based on DRD2 *Nco*I genotypes

	C/C (%) (n = 131)	C/T (%) (n = 93)	T/T (%) (n = 18)	Chi-square Analysis	
				C/C vs. T/any	p value
MWA, anxiety or depression	69	53	22	12.72	0.0002
Any anxiety disorder	54	41	17	7.20	0.004
Generalized anxiety disorder	23	11	11	6.11	0.007
Major depression	45	33	17	5.18	0.01
Panic attacks	38	26	17	4.96	0.01
Migraine with aura	26	17	6	4.09	0.02
Phobia	34	27	11	2.60	0.05
Panic disorder	22	16	11	1.45	n.s.
Obsessive compulsive disorder	14	16	0	0.01	n.s.

sus T/any individuals, the difference does not reach statistical significance. However, OCD is more frequent in the C/C individuals than in the T/T individuals (Chi-square = 2.84; $p < 0.05$).

DRD2 NcoI *Allele Frequencies in Neuropsychiatric Disorders in this Study*

DRD2 *Nco*I allele frequencies were determined in individuals based on the presence or absence of the neuropsychiatric disorders analyzed in the present study (Table 4). In individuals with MWA, anxiety disorders, and/or major depression, the C allele frequency is 0.80 and the T allele frequency is 0.20. In individuals who have none of these neuropsychiatric disorders, the C allele frequency is 0.63, and the T allele frequency is 0.37. The difference in the DRD2 *Nco*I C allele frequencies between these two groups of individuals is highly significant (Chi-square = 17.13; $p < 0.00002$). These data are consistent with the allele frequencies reported in the general Caucasian population where the C allele frequency was reported to be 0.69 and the T allele frequency was reported to be 0.31 (22).

Discussion

The major finding of the present study is that the incidence of the clinical diagnoses of MWA, anxiety disorders, and major depression are comorbidly associated with the *Nco*I polymorphism within the *DRD2* gene. This is the first known association study of the DRD2 *Nco*I polymorphism in neuropsychiatric disorders. As demonstrated in the present study, individuals with migraine, anxiety, and/or depression display an increased frequency of the DRD2 *Nco*I C/C genotype compared to individuals with the C/T or T/T genotype. These data represent the first direct evidence to suggest that a specific genetic variant may, at least partially, underlie the well-docu-

Table 4. DRD2 *Nco*I allele frequencies in individuals with or without migraine with aura, anxiety disorders, or major depression

	C	T	Chi square	p value
MWA, anxiety or depression	0.80	0.20	17.13	0.00002
No MWA, anxiety or depression	0.63	0.37		

Results from current study. Clinical diagnoses were based on DSM criteria for anxiety disorders (20) and major depression (21) and on IHS criteria for MWA (18).

mented comorbidity of migraine, anxiety disorders, and major depression (5–15).

Multiple previous association studies of other DRD2 polymorphisms have focused on other neuropsychiatric disorders. For example, the DRD2 *Taq*I A1 allele was reported to be a risk factor in the polygenic inheritance of Tourette's syndrome, stuttering, attention deficit hyperactivity, conduct and oppositional defiant disorder (24). In the early 1990s, numerous groups reported an association between the DRD2 *Taq*I A1 allele and alcoholism (25–34), although the putative association was not confirmed by other investigators (35–39). Numerous reviews of all available data have tended to conclude that an association probably does exist between the DRD2 variants and certain forms of alcoholism (40–43).

Significant associations have also been reported between DRD2 polymorphisms and polysubstance substance abuse (43–47). A single report suggested an association between the S311C variant of the DRD2 receptor and schizophrenia (48), although this finding was not confirmed by other investigators (49–51). Individuals with the DRD2 *Hph*I B2 allele were reported to display a decrease in mature, and an increase in neurotic and immature, defense styles compared to individuals without this DRD2 allele (52). Thus, a variety of DRD2 polymorphisms have been associated with numerous neuropsychiatric disorders. A key unanswered question concerns the possible mechanism by which these polymorphisms might affect gene function.

In postmortem brain samples, *Taq* A DRD2 alleles are associated with variations in the density of DRD2 binding sites (26). No data exist at present on the effect of *Nco*I polymorphisms on DRD2 expression. Conceivably, intragenic nucleotide variations may result in functional variations in RNA stability or efficacy in transcription (53), thus resulting in alterations in the density of the expressed receptor protein. For example, recent data suggest that noncoding region variations within the 5-hydroxytryptamine transporter (5-HTT) gene may alter expression of the transporter (54). Specifically, the transcriptional efficiency of the short variant of the 5-HTT gene is reduced compared to the long version of the gene (55). In addition, intragenic nucleotide variations may also exist within a gene that could result in functional variations in splicing and/or RNA stability (53), thus resulting in alterations in the density of the expressed protein. In the case of monoamine oxidase A, for example, noncoding region variations have been shown to result in a 30-fold difference in enzymatic activity (56). Alternatively, the *Nco*I polymorphism analyzed in the present report may lie in linkage disequilibrium with a more directly causative mutation. However, a specific molecular variation within the DRD2 gene which could underlie functional variations in the expressed receptor protein remains unidentified (57). Future studies are needed to more clearly define the possible functional significance of noncoding region variations within the *DRD2* gene.

The association data reported in the present study confirm and extend multiple previous epidemiological studies on migraine and psychiatric comorbidities (5–15). In a study of 133 index cases with major depression versus control index cases and their first-degree relatives (6), a strong association was observed between migraine and depression. Specifically, in the first-degree relatives of control subjects, the incidence of migraine (7%) and depression (10%) was significantly lower than the incidence of migraine (22%) and depression (19%) in the first-degree relatives of depressed subjects. If an index case had both migraine and depression, the individual had a 75% chance of having a comorbid anxiety disorder (6). No association with migraine was found for alcoholism, substance abuse, antisocial personality, obsessive-compulsive disorder or schizophrenia. The authors concluded that "depression may be either a sequela of migraine or the diathesis which results in both migraine and depression." However, they "could not definitely resolve whether the source of correlation between the two disorders was attributable to a transmissible or a non-transmissible common component or both."

An association between migraine and depression was also demonstrated in a study of an unselected epidemiological sample with a standardized assessment of psychiatric diagnoses (7). Migraine patients were noted to have a significantly increased incidence of major depression, bipolar spectrum, general anxiety, panic disorder and phobias. The migraine subjects reported a significantly earlier onset of anxiety symptoms than nonmigraine subjects as evidenced by a high incidence of being more anxious than their peers during childhood. The authors suggested that "migraine with anxiety and depression may constitute a distinct syndrome comprising anxiety, often manifested in early childhood, followed by the occurrence of migraine headaches,

and then by discrete episodes of depressive disorder in adulthood" (7).

A prospective study has also documented an association between migraine and depression/anxiety (23) wherein a history of migraine was found to carry a 4-fold increased incidence of depression. These authors also found a temporal pattern with anxiety preceding migraine, followed by depression (8) and concluded that migraine, anxiety disorders and major depression might share common predispositions, "although the nature of these predispositions remains to be determined" (23). By contrast, the present study provides strong evidence for a shared genetic predisposition to comorbid migraine, anxiety and depression.

In conclusion, the present data indicate that MWA, anxiety disorders and major depression are comorbidly associated with the *Nco*I polymorphism within the *DRD2* gene. As a result, these disorders should not necessarily be considered as independent disorders. Rather, the data indicate that a distinct clinical syndrome may be expressed as a manifestation of a single underlying genetic variation. The clinical recognition that all three disorders may be associated with the same genetic variant has significant diagnostic and therapeutic implications since DRD2 antagonists have been shown to be effective therapeutic agents for a subset of individuals with these common disorders.

Acknowledgments

We thank all of the individuals who participated in this study. We also thank Elizabeth Mahler, M.D., Mary De May, M.D., Greer Murphy, M.D., Ph.D., David A. Greenberg, M.D., Ph.D., Tiffany Howell, Joan Dubose, R.N., Richard Bee, R.N., Michelle Boatwright, Jonathan Haines, Ph.D., Margaret Pericak-Vance, Ph.D. and Le Jin, Ph.D. for their contributions to and comments on various aspects of this work.

References

1. Robins LN, Helzer JE, Weissman MM, et al. (1984) Lifetime prevalence of specific psychiatric disorders in three sites. *Arch. Gen. Psychiatry* **41:** 949–958.
2. Stewart WF, Lipton RB, Celentano DD, Reed ML. (1992) Prevalence of migraine headache in the United States—relation to age, income, race, and other sociodemographic factors. *JAMA* **267:** 64–69.
3. Rasmussen SA, Eisen JL. (1994) The epidemiology and differential diagnosis of obsessive compulsive disorder. *J. Clin. Psychiatry* **55:** 5–14.
4. Kessler RC, McGonagle KA, Zhao S, et al. (1994) Lifetime and 12-month prevalence of DSM-III-R psychiatric disorders in the United States. *Arch. Gen. Psychiatry* **51:** 8–19.
5. Marazziti D, Toni C, Pedri S, et al. (1995) Headache, panic disorder and depression: Comorbidity or a spectrum? *Biol. Psychiatry* **31:** 125–129.
6. Merikangas KR, Risch NJ, Merikangas JR, Weissman MM, Kidd KK. (1988) Migraine and depression: Association and familial transmission. *J. Psychiat. Res.* **22:** 119–129.
7. Merikangas KR, Angst J, Isler H. (1990) Migraine and psychopathology: Results of the Zürich cohort study of young adults. *Arch. Gen. Psychiatry* **47:** 849–853.
8. Breslau N, Davis GC, Andrewski P. (1991) Migraine, psychiatric disorders, and suicide atempts: an epidemiological study of young adults. *Psychiatry Res.* **37:** 11–23.
9. Breslau N, Davis GC. (1993) Migraine, physical health and psychiatric disorder: A prospective epidemiologic study in young adults. *J. Psychiatr. Res.* **27:** 211–223.
10. Merikangas KK, Merikangas JR, Angst J. (1993) Headache syndromes and psychiatric disorders: Association and familial transmission. *J. Psychiatr. Res.* **27:** 197–210.
11. Merikangas KR. (1994) Psychopathology and headache syndromes in the community. *Headache* **34:** 17–26.
12. Breslau N, Davis GC, Schultz LR, Peterson EL. (1994) Migraine and major depression: A longitudinal study. *Headache* **34:** 387–393.
13. Breslau N, Merikangas K, Bowden CL. (1994) Comorbidity of migraine and major affective disorders. *Neurology* **44:** 17–22.
14. Stewart W, Breslau N, Keck PE. (1994) Comorbidity of migraine and panic disorder. *Neurology* **44:** 23–27.
15. Silberstein SD, Lipton RB, Breslau N. (1995) Migraine association with personality characteristics and psychopathology. *Cephalalgia* **15:** 358–369.
16. Peroutka SJ, Wilhoit T, Jones K. (1997) Clinical susceptibility to migraine with aura is modified by dopamine D2 receptor (DRD2) *Nco*I alleles. *Neurology* **49:** 201–206.
17. Peroutka SJ, Howell TA (1996) The genetic analysis of migraine. In: Rose F (ed). *Towards Migraine 2000* Elsevier Science B.V., Amsterdam, pp. 35–48.
18. Headache Classification Committee of the International Headache Society. (1988) Classification and diagnostic criteria for headache disorders, cranial neuralgias and facial pains. *Cephalalgia* **8:** 1–96.
19. Spitzer RL, Eilliams JB, Gibbon M, First MB.

(1992) The structured clinical interview for DSM-II-R (SCID). *Arch. Gen. Psychiatry* **49:** 624–629.
20. American Psychiatric Association. (1994) Anxiety. In: *Diagnostic and Statistical Manual of Mental Disorders*, vol. 4. American Psychiatric Association, Washington, DC, pp. 393–444.
21. American Psychiatric Association (1994) Mood disorders. In: *Diagnostic and Statistical Manual of Mental Disorders*, vol. 4. American Psychiatric Association, Washington, DC, pp. 317–391.
22. Sarkar G, Kapelner S, Grandy DK, et al. (1991) Direct sequencing of the dopamine D2 receptor (DRD2) in schizophrenics reveals three polymorphisms but no structural change in the receptor. *Genomics* **11:** 8–14.
23. Breslau N, Davis GC. (1992) Migraine, major depression and panic disorder: A prospective epidemiologic study of young adults. *Cephalagia* **12:** 85–90.
24. Comings DE, Wu S, Chiu C, et al. (1996) Polygenic inheritance of Tourette syndrome, stuttering, attention deficit hyperactivity, conduct, and oppositional defiant disorder: The additive and subtractive effect of the three dopaminergic genes—DRD2, DBH, and DAT1. *Am. J. Hum. Genet.* **67:** 264–288.
25. Blum K, Noble EP, Sheridan PJ, et al. (1990) Allelic association of human dopamine D2 receptor gene in alcoholism. *JAMA* **263:** 2055–2060.
26. Noble EP, Blum K, Ritchie T, Montgomery A, Sheridan PJ. (1991) Allelic association of the D2 dopamine receptor gene with receptor-binding characteristics in alcoholism. *Arch. Gen. Psychiatry* **48:** 648–654.
27. Blum K, Noble EP, Sheridan RJ, et al. (1991) Association of the A1 allele of the D2 dopamine receptor gene with severe alcoholism. *Alcohol* **8:** 409–416.
28. Comings DE, Comings BG, Muhleman D, et al. (1991) The dopemine D2 receptor locus as a modifying gene in neuropsychiatric disorders. *JAMA* **266:** 1793–1800.
29. Parsian A, Todd RD, Devor EJ, et al. (1991) Alcoholism and alleles of the human D2 dopamine receptor locus. *Arch. Gen. Psychiatry* **48:** 655–663.
30. Amadeo S, Abbar M, Fourcade ML, et al. (1993) D2 dopamine receptor gene and alcoholism. *J. Psychiatr. Res.* **27:** 173–179.
31. Blum K, Noble EP, Sheridan PJ, et al. (1993) Genetic predisposition in alcoholism: Association of the D2 dopamine receptor TaqI B1 RFLP with severe alcoholics. *Alcohol* **10:** 59–67.
32. Noble EP, Syndulko K, Fitch RJ, et al. (1994) D2 dopamine receptor TaqI a alleles in medically ill alcoholic and nonalcoholic patients. *Alcohol Alcohol.* **29:** 729–744.
33. Neiswanger K, Hill SY, Kaplan BB. (1995) Association and linkage studies of the TAQ1 A1 allele at the dopamine D2 receptor gene in samples of female and male alcoholics. *Am. J. Med. Genet.* **60:** 267–271.
34. Noble EP. (1996) The gene that rewards alcoholism. *Sci. Med.* **3:** 52–61.
35. Bolos AM, Dean M, Lucas-Derse S, Ramsburg M, Brown GL, Goldman D. (1990) Population and pedigree studies reveal a lack of association between the dopamine D2 receptor gene and alcoholism. *JAMA* **264:** 3156–3160.
36. Gelernter J, O'Malley S, Risch N, et al. (1991) No association between an allele at the D2 dopamine receptor gene (DRD2) and alcoholism. *JAMA* **266:** 1801–1807.
37. Turner E, Ewing J, Shilling P, et al. (1992) Lack of association between an RFLP near the D2 dopamine receptor gene and severe alcoholism. *Biol. Psychiatry* **31:** 285–290.
38. Cook BL, Wang ZW, Crowe RR, Hauser R, Freimer M. (1992) Alcoholism and the D2 receptor gene. *Alcohol Clin. Exp. Res.* **16:** 806–809.
39. Geijer T, Neiman J, Rydberg U, et al. (1994) Dopamine D2-receptor gene polymorphisms in Scandinavian chronic alcoholics. *Eur. Arch. Psychiatry Clin. Neurosci.* **244:** 26–32.
40. Conneally PM. (1991) Association between the D2 dopamine receptor gene and alcoholism. *Arch. Gen. Psychiatry* **48:** 664–666.
41. Pato CN, Macciardi F, Pato MT, Verga M, Kennedy JL. (1993) Review of the putative association of dopamine D2 receptor and alcoholism: A meta-analysis. *Neuropsychiatr. Gen.* **48:** 78–82.
42. Gelernter J, Goldman D, Risch N. (1993) The A1 allele at the D2 dopemine receptor gene and alcoholism: A reappraisal. *JAMA* **269:** 1673–1677.
43. Uhl G, Blum K, Noble E, Smith S. (1993) Substance abuse vulnerability and D2 receptor genes. *Trends Neurol. Sci.* **16:** 83–88.
44. Smith SS, O'Hara BF, Persico AM, et al. (1992) The D2 dopamine receptor Taq I B1 restriction fragment length polymorphism appears more frequently in polysubstance abusers. *Arch. Gen. Psychiatry* **49:** 723–727.
45. O'Hara BF, Smith SS, Bird G, et al. (1993) Dopamine D2 receptor RFLPs, haplotypes and their association with substance use in black and caucasian research volunteers. *Hum. Hered.* **43:** 209–218.
46. Noble EP, Blum K, Khalsa ME, et al. (1993) Allelic association of D2 dopamine receptor gene with cocaine dependence. *Drug Alcohol Depend.* **33:** 271–285.
47. Noble EP, Jeor STS, Ritchie T, et al. (1994) D2 dopamine receptor gene and cigarette smoking: A reward gene? *Med. Hypotheses* **42:** 257–260.
48. Arinami T, Itokawa M, Enguchi H, et al. (1994) Association of dopamine D2 receptor molecular variant with schizophrenia. *Lancet* **343:** 703–704.
49. Itokawa M, Arinami T, Futamura N, Hamaguchi H, Toru M. (1993) A structural polymorphism of

human dopamine D2 receptor, D2(Ser311Cys). *Biochem. Biophys. Res. Commun.* **196:** 1369–1375.
50. Hattori M, Nanko S, Dai XY, Fukuda R, Kazamatsuri H. (1994) Mismatch PCR RFLP detection of DRD2 SER311CYS polymorphism and schizophrenia. *Biochem. Biophys. Res. Commun.* **202:** 757–763.
51. Higuchi S, Muramatsu T, Murayama M, Hayashida M. (1994) Association of structural polymorphism of the dopamine D2 receptor gene and alcoholism. *Biochem. Biophys. Res. Commun.* **204:** 1199–1205.
52. Comings DE, MacMurray J, Johnson P, Dietz G, Muhleman D. (1995) Dopamine D2 receptor gene (DRD2) haplotypes and the defense style questionnaire in substance abuse, Tourette syndrome, and controls. *Biol. Psychiatry* **37:** 798–805.
53. Maquat LE. (1996) Defects in RNA splicing and the consequence of shortened translational reading frames. *Am. J. Hum. Genet.* **59:** 279–286.
54. Bengel D, Lesch KP, Heils A, Hamer D, Murphy DL. (1996) A gene regulatory region polymorphism alters serotonin transporter expression and function in humans. *Am. J. Hum. Genet.* **59:** A53.
55. Lesch K, Bengel D, Heils A, et al. (1996) Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. *Science* **274:** 1527–1531.
56. Hotamisligil GS, Breakefield XO. (1991) Human monoamine oxidase A gene determines levels of enzyme activity. *Am. J. Hum. Genet.* **49:** 383–392.
57. Gejman PV, Ram A, Gelernter J, et al. (1994) No structural mutation in the dopamine D2 receptor gene in alcoholism or schizophrenia. *JAMA* **271:** 204–208.