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Dopamine D_2-receptor gene polymorphisms in Scandinavian chronic alcoholics

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Abstract Alterations in the dopamine system have been hypothesized as a predisposing factor in alcoholism. The presence of the TaqI A1 and B1 alleles adjacent to the dopamine D_2-receptor gene (DRD2) was studied in Scandinavian alcoholic inpatients (n = 74), alcoholics autopsied at a forensic clinic (n = 19) and controls (n = 81). There were no significant differences between controls and the alcoholics, but a tendency of increased DRD2 TaqI A1 or B1 allele frequencies in alcoholic groups selected for severity (i.e. severity according to DSM-III-R criteria, early onset or severe medical complications due to alcohol abuse) and decreased frequencies in the corresponding less severe alcoholic group. The present study does not yield evidence for the hypothesis of an association between the DRD2 TaqI A1 or B1 alleles and alcoholism.

Key words Dopamine D_2 receptor · Alcoholism · Polymorphism · Dopamine receptors · Dependence

Introduction

The association of alcoholism with the dopamine D_2-receptor (DRD2) TaqI A1 allele defined by the TaqI A restriction fragment length polymorphism (RFLP) adjacent to the DRD2 gene has over the past 3 years been studied by several investigators in USA (Blum et al. 1990; Bolos et al. 1990; Blum et al. 1991; Comings et al. 1991; Gelernter et al. 1991; Parsian et al. 1991; Cook et al. 1992; Turner et al. 1992; Blum et al. 1993; O'Hara et al. 1993; Smith et al. 1993), Finland (Goldman et al. 1992), France (Amadéo et al. 1993) and Japan (Arinami et al. 1993). The underlying concepts are the proposed genetic contribution to the susceptibility of alcoholism (Devor and Cloninger 1989) and the involvement of dopaminergic systems in the actions of addictive substances (Kornetsky et al. 1988; Devor and Cloninger 1989).

Initially Blum et al. (1990) reported that the A1 allele was significantly associated with chronic alcoholism. The finding was challenged by several groups who failed to replicate the proposed association (Bolos et al. 1990; Gelernter et al. 1991; Turner et al. 1992). Parsian et al. (1991) obtained no general association. However, in a subset of 10 alcoholics with severe medical complications a significant association to the A1 allele was found.

The result of this study (Parsian et al. 1991) and later others (Blum et al. 1991; Arinami et al. 1993; Blum et al. 1993) suggested that association between alcoholism and the DRD2 TaqI A1 allele is strengthened by the severity of the disorder. While investigating pedigrees where alcoholism is aggregating two research groups (Bolos et al. 1990; Parsian et al. 1991) were not able to show linkage between the DRD2 TaqI A RFLP and alcoholism. An explanation for association in the general population without linkage in family studies was considered by Parsian et al. (1991) with the DRD2 TaqI A1 allele as a modifying gene, increasing the severity of alcoholism without being causative. This hypothesis was further elaborated by Comings et al. (1991), who besides alcoholism also found associations between the DRD2 TaqI A RFLP and Tourette's syndrome, attention deficit hyperactivity disorder and autism. Thus the data concerning the DRD2 TaqI A1 genotype association with dependence obtained from previous studies are controversial and alternates significant findings with non significant. One main objection against such association is that possible bias may occur due to heterogeneous distribution of the DRD2 TaqI A alleles in the human population. Significant differences in the DRD2 TaqI A allele frequencies are known to exist (Gelernter et al. 1991; Arinami et al. 1993; O'Hara et al. 1993; Uhl et al. 1993), emphasizing that ethnic belonging must be carefully considered in association studies of the DRD2 gene alleles.

An additional TaqI polymorphism in the dopamine D_2 receptor gene, named DRD2 TaqI B RFLP, was described

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in 1991 by Hauge et al. (1991). While the TaqI A RFLP-site is situated downstream, about 10 kb from the 3´-end of the DRD2-gene, the DRD2 TaqI B RFLP-site is situated in the first intron of the DRD2-gene, about 22 kb upstream from the DRD2 TaqI A RFLP-site. The DRD2 TaqI A and B RFLP-sites have been shown to exist in strong linkage disequilibrium (Hauge et al. 1991; O'Hara et al. 1993; Uhl et al. 1993). The TaqI B RFLP has been used as a marker in more recent association studies of alcoholism (Blum et al. 1993) or polysubstance abuse (Smith et al. 1992).

The purpose of the present study was to investigate DRD2 TaqI A and B RFLPs in urban Scandinavian alcoholics.

Material and methods

Patient selection

All probands were caucasians without a history of mood disorder, psychosis or current abuse or dependence of psychoactive substances other than alcohol. They were recruited from the inpatient alcohol detoxification programme at the Magnus Huss Clinic, Karolinska Hospital, Stockholm. All were interviewed and investigated by a physician (JN), specialised in treatment of alcohol and drug abuse. Seventyfour patients gave their informed consent to participate in the study. The patients were asked for the presence of alcoholism among their parents, as well as information concerning age at onset of drinking habits which interfered with their daily life. The severity of alcoholism was assessed according to the criteria of DSM-III-R (American Psychiatric Association 1987) based on interview data and examination of all available hospital records.

The age range of the subjects was 29–71 years (mean 46 years). Sixty-four were men and 10 women. Of the patients, 56 were diagnosed as having severe, and 18 moderate, alcohol dependence (American Psychiatric Association 1987). Twenty-nine developed drinking habits interfering with their daily life before the age of 25. Parental alcoholism (at least one parent with alcohol dependence) was reported by 35 of the subjects. Sixteen subjects were of Finnish origin whereas the remaining 58 had only Swedish antecedents.

An additional group of 19 caucasian alcoholics was obtained by using postmortem brain material. Medical records and data from the criminal and social security registers were collected to verify clinical alcoholism during life and to exclude patients with concurrent mental and nervous system disorders. Subjects who were found to have psychoactive substances in tissues at autopsy were also excluded. Sex, age, autopsy findings and toxicological analyses of the subjects were registered. Alcohol related tissue damage to liver, pancreas, central nervous system (CNS), stomach, kidneys, heart, oesophagus and muscle was graded as light (1p), moderate (2p) and severe (3p).

Of these autopsied subjects, 18 were men, 1 was a woman. They were all of Finnish or Swedish heritage. The average age was 51.1 years, and age range 25–75 years. All had a record or register verifying clinical alcoholism during life. The average tissue damage score was 9.6, range 3–16 (maximum score 24).

Control subjects

Of 106 caucasian ex-control subjects recruited from previous research projects (Sedvall et al. 1980; Wiesel et al. 1982; Oxenstierna et al. 1986) 90 gave informed consent and were given a questionnaire to assess psychiatric morbidity (the survey module of the Swedish version of SCID-I, and where appropriate also other parts of the SCID-I manual) (Spitzer et al. 1986).

The individuals were asked for somatic illness, current alcohol consumption and presence of mental and nervous system disorders among relatives.

If they had been in contact with a mental hospital or outpatient psychiatric service, hospital records were obtained and examined for diagnosis according to DSM-III-R.

Genealogical data were obtained from parish registers to assess the origin of the individuals.

Four control groups were derived from the 90 unscreened control subjects. The first control group (n = 81) consisted of 38 women and 43 men screened for DSM-III-R alcoholism diagnosis. They had an average age of 39.5 years, range 29–56 years. Of the 81 subjects, 70 had only Swedish parents, 3 subjects were of plain Finnish origin whereas 8 subjects had one Swedish and one non-Swedish but Norwegian (n = 1), German (n = 3), Finnish (n = 3) or Dutch (n = 1) parent.

The second control group consisted of 67 subjects screened for any DSM-III-R diagnosis. The 29 women and 38 men had an average age of 39.3 years, range 29–56 years. Of the 67 subjects, 56 had only Swedish parents. Subjects with non-Swedish heritage were as in the first control group.

The third control group (n = 52) consisted of 22 women and 30 men screened for any DSM-III-R diagnosis and first degree relatives with alcohol problems. These subjects had an average age of 39.2 years, range 29–56 years. Both parents were Swedish for 43 subjects of the 52. Two subjects had both their parents Finnish whereas 7 subjects had one Swedish parent and one parent born in Germany (n = 3), Finland (n = 3) or the Netherlands (n = 1).

The fourth control group consisted of 40 subjects screened for any DSM-III-R diagnosis and first- and/or second-degree relatives with alcohol problems. Their average age was 38.9 years, range 30–52 years. Seventeen were women and 23 were men. Of the 40 subjects, 34 had only Swedish parents. For 1 subject both parents were Finnish. Five subjects had 1 Swedish parent and 1 parent born in Germany (n = 2), Finland (n = 2) or the Netherlands (n = 1). The 14 subjects excluded in the second as well as the third and fourth control groups had DSM-III-R diagnoses presenting a variety of different syndromes, mainly anxiety disorders.

Isolation of DNA and endonuclease digestion

Venous blood was taken from all living individuals into citrate or EDTA-containing tubes. DNA isolation was performed according to Luthman and Datta (Holger Luthman and Santanu Datta, Department of Clinical Genetics, Karolinska Hospital, Stockholm, personal communication). Ten ml blood was mixed with 15 ml of buffer A (0.32 *M* sucrose, 10 m*M* Tris-HCl pH 7.6, 5 m*M* EDTA, and 5% Triton X-100) and centrifuged at 3000 r.p.m. for 20 min. The resulting pellet was resuspended in 10 ml of buffer B (0.32 *M* sucrose, 10 m*M* Tris-HCl pH 7.6, 5 m*M* EDTA) and centrifuged at 2500 r.p.m. for 10 min. The suspension in buffer B and centrifugation step was repeated once, followed by suspension of the nuclear pellet in 0.1 ml of buffer B. To the suspension 10 ml of GT-solution (4.2 *M* guanidine-isothiocyanate, 10 m*M* Tris-HCl pH 8.0, 10 m*M* EDTA, 2% sodium-lauroylsarcosine) was added followed by incubation at 37° C for 15 min. With the addition of 0.7 ml 7.5 *M* ammonium acetate, the incubation was continued at 37° C for 2 h with the solution being manually shaken each 30 min. After incubation the DNA was precipitated from the solution by the addition of 12 ml of 99% ethanol. The resulting precipitate was washed in 70% ethanol and dissolved overnight in 2 ml of TE (10 m*M* Tris-HCl pH 7.6, 1 m*M* EDTA) at room temperature. One ml of 5.0 *M* NaCl was added and the samples were rotated (15–20 r.p.m.) at room temperature for 30 min followed by incubation on ice for 30 min, then centrifuged at 2500 r.p.m. for 15 min. The supernatant was transferred to a clean test tube and the DNA precipitated with 300 µl of 3.0 *M* sodium acetate, pH 5.2 and 1.5 ml of 99% ethanol. The DNA precipitate was washed in 70% ethanol and subsequently dissolved in 0.5–1.0 ml of TE pH 8.0 at room temperature overnight.

DNA from postmortem brain tissue was prepared from approximately 1 g of frozen (–80° C) tissue which was thawed and ho-

mogenized in 4 ml lysis buffer (24 m*M* EDTA pH 8.0, 75 m*M* NaCl). Proteinase K (100 ul, 10 mg/ml) and SDS (150 ul, 20% w/v) was carefully mixed into the solution. The resulting mixture was slowly (15–20 r.p.m.) rotated at room temperature for at least 6 h followed by addition of 4 ml phenol (equilibrated with 0.1 *M* Tris-Hcl, pH 8.0). Continued rotation was allowed at 4° C overnight. Centrifugation at 2500 r.p.m. for 10 min resulted in an upper phase which was saved. The rotating step, (this time only 1 h) and the centrifugation were repeated twice, first with 4 ml of fresh phenol and the second time with 4 ml of chloroform/isoamyl-alcohol (24/1, v/v) added to the upper phase. DNA was precipitated from the resulting upper phase with 2 ml of 7.5 *M* ammonium acetate and 3.6 ml of isopropanol. The DNA was washed three times with 70% ethanol, dried and subsequently dissolved slowly (several h to 1 day) in 0.5 ml TE pH 8.0.

Twenty ug of DNA was digested with 120 units of TaqI restriction endonuclease at 65° C overnight. TaqI restriction endonuclease generates a 3.7 kb DNA fragment, detectable with the probes used in this study, defining the A2 allele. It may fail to do so resulting in a 6.6 kb DNA fragment instead, defining the A1 allele. TaqI restriction endonuclease cleavage also generates a 4.6 kb DNA fragment (B1) or a 4.1 kb DNA fragment (B2).

Southern blot and hybridisation

The samples were electrophoresed through a 1% agarose gel at 25 mA for 10 h. The gel was then incubated in "Gelsoak I" (0.2 *M* Na OH, 0.6 *M* NaCl) for 30 min and subsequently washed four times in 25 m*M* Na_2HPO_4/25 m*M* NaH_2PO_4, each wash lasting 15 min. This was followed by transfer to nylon membrane (Hybond N, Amersham) using the same procedure as previously described for the Northern blot (Monstein et al. 1990). Hybridisation was performed in a hybridisation oven (Minioven MK II, Hybaid). Prehybridisation conditions were 45° C for 4 h in 10 ml of BMF (0.12 *M* Tris-HCl, 8 m*M* EDTA, 0.6 *M* NaCl, 1% w/v sodium dodecyl sulphate (SDS), 1% w/v milk powder, 47.5% v/v formamid, pH 7.4). The labelled probe (12.5–25 ng) was added together with 10 ml of fresh BMF (see above) and left to hybridize overnight at 45° C. The hybridized filters were washed three times at 65° C in 0.1 × SSC (SSC = 150 m*M* NaCl, 75 m*M* sodium citrate), 0.1% w/v SDS, each wash lasting 20 min. The hybridised filters were exposed to Hyperfilm™ (Amersham) between intensifying screens for 24–48 h at –80° C.

Probes

A previously described 1.73 kb DRD2 Bam HI fragment (Grandy et al. 1989a; Blum et al. 1991) of the human clone λhD2G1 (Grandy et al. 1989b) was used to detect the TaqI A RFLP. A 3.5 kb Bam HI fragment (Hauge et al. 1991) from the human λhD2G2 clone (Grandy et al. 1989b) was used to detect the TaqI B RFLP. The fragments were obtained through cleavage with Bam HI and subsequent separation by 1% agarose gel electrophoresis followed by purification with "Geneclean" (BIO 101). A portion of the appropriate fragment, 12.5–25 ng, was labelled with α^{32}P-dCTP, 3000 Ci/mmol with a random priming system, "Multiprime" (RPN 1600Z) Amersham, in a 25–50 ul labelling reaction.

Statistical calculations

Testing for significance was performed with the χ^2-test. For derived 2 × 2 contingency tables, continuity correction was applied. Fishers exact test was performed if more than 25% of the cells were expected to contain 5 or fewer individuals. Calculations were based on prevalences of the DRD2 TaqI A1 and B1 alleles, i.e. comparing the A1A1 and A1A2 vs A2A2 genotypes and B1B1 and B1B2 vs B2B2 genotypes for the DRD2 TaqI A and B RFLPs, respectively.

Results

In the inpatient alcoholic group, five of the nine possible genotypes were found, (A1A1B1B1 (*n* = 3), A1A2B1B2 (*n* = 18), A2A2B2B2 (*n* = 49), A1A2B2B2 (*n* = 2), A2A2B1B2 (*n* = 1)). Corresponding numbers for the control group were: (A1A1B1B1 (*n* = 5), A1A2B1B2 (*n* = 24), A2A2B2B2 (*n* = 58), A1A2B2B2 (*n* = 2), A1A1B1B2 (*n* = 1)). The DRD2 TaqI A and B RFLPs were found to be in strong linkage disequilibrium. With the exception of the postmortem alcoholics, who were not

Table 1 DRD2 TaqI A RFLP genotype counts and A1-allele prevalences and frequencies (in percent) in alcoholic and control subjects. P1 = All alcoholic inpatients (*n* = 74), P2 = Inpatients, DSM-III-R severe alcohol dependence (*n* = 56), P3 = Inpatients, onset ≤ 25 years (*n* = 29), P4 = Inpatients with parental alcoholism (*n* = 35), P5 = Autopsied subjects with pathological, anatomical signs of alcoholism (*n* = 19), P6 = Autopsied subjects, tissue damage > 10 p (*n* = 10), P7 = Inpatients, Swedish origin (*n* = 58), P8 = Inpatients, men (*n* = 64), C1 = Controls screened for DSM-III-R alcohol dependence or alcohol abuse (*n* = 81), C2 = Controls screened for any DSM-III-R diagnosis (*n* = 67), C3 = Controls screened for any DSM-III-R diagnosis and first-degree relatives with alcohol abuse or dependence (*n* = 52), C4 = Controls screened for any DSM-III-R diagnosis and first- or second-degree relatives with alcohol abuse or dependence (*n* = 40), C5 = men from C1 (*n* = 43), P1 + P5 = All alcoholics (*n* = 93), P1–P2 = Inpatients, DSM-III-R moderate alcohol dependence (*n* = 18), P1–P3 = Inpatients, onset > 25 years (*n* = 45), P1–P4 = Inpatients without parental alcoholism (*n* = 39), P1–P7 = Inpatients, Finnish origin (*n* = 16), P1–P8 = Inpatients, women (*n* = 10), P5–P6 = Autopsied subjects, tissue damage ≤ 10 p (*n* = 9), C1–C4 = Controls with DSM-III-R diagnosis other than alcoholism or first-or second-degree relative with alcohol abuse or dependence (*n* = 41), C1–C5 = Women from C1 (*n* = 38)

Subjects	*n*	A1/A1	A1/A2	A2/A2	A1-allele prevalence	A1-allele frequency
P1	74	3	20	51	31	18
P2	56	2	18	36	36	20
P3	29	1	12	16	45	24
P4	35	1	8	26	26	14
P5	19	0	9	10	47	24
P6	10	0	6	4	60	30
P7	58	2	17	39	33	18
P8	64	3	19	42	34	20
C1	81	5	24	52	36	21
C2	67	4	18	45	33	19
C3	52	2	12	38	27	15
C4	40	2	8	30	25	15
C5	43	3	13	27	37	22
P1+P5	93	3	29	61	34	19
P1–P2	18	1	2	15	17	11
P1–P3	45	2	8	35	22	13
P1–P4	39	2	12	25	36	21
P1–P7	16	1	3	12	25	16
P1–P8	10	0	1	9	10	5
P5–P6	9	0	3	6	33	17
C1–C4	41	3	16	22	46	27
C1–C5	38	2	11	25	34	20

Table 2 DRD2 TaqI B RFLP genotype counts and B1-allele prevalences and frequencies (in percent) in alcoholic and control subjects. P1 = All alcoholic inpatients (n = 74), P2 = Inpatients, DSM-III-R severe alcohol dependence (n = 56), P3 = Inpatients, onset ≤ 25 years (n = 29), P4 = Inpatients with parental alcoholism (n = 35), P7 = Inpatients, Swedish origin (n = 58), P8 = Inpatients, men (n = 64), C1 = Controls screened for DSM-III-R alcohol dependence or alcohol abuse (n = 81), C2 = Controls screened for any DSM-III-R diagnosis (n = 67), C3 = Controls screened for any DSM-III-R diagnosis and first-degree relatives with alcohol abuse or dependence (n = 52), C4 = Controls screened for any DSM-III-R diagnosis and first- or second-degree relatives with alcohol abuse or dependence (n = 40), C5 = men from C1 (n = 43), P1–P2 = Inpatients, DSM-III-R moderate alcohol dependence (n = 18), P1–P3 = Inpatients, onset > 25 years (n = 45), P1–P4 = Inpatients without parental alcoholism (n = 39), P1–P7 = Inpatients, Finnish origin (n = 16), P1–P8 = Inpatients, women (n = 10), C1–C4 = Controls with DSM-III-R diagnosis other than alcoholism or first- or second-degree relative with alcohol abuse or dependence (n = 41), C1–C5 = Women from C1 (n = 38)

Subjects	n	B1/B1	B1/B2	B2/B2	B1-allele prevalence	B1-allele frequency
P1[a]	73	3	19	51	30	17
P2[a]	55	2	17	36	34	19
P3	29	1	12	16	45	24
P4	35	1	8	26	26	14
P7[a]	57	2	15	40	30	17
P8[a]	63	3	18	42	33	19
C1	81	4	23	54	33	19
C2	67	4	16	47	30	18
C3	52	2	11	39	25	14
C4	40	2	7	31	22	14
C5	43	3	13	27	37	22
P1[a]–P2[a]	18	1	2	15	17	11
P1[a]–P3	44	2	7	35	20	12
P1[a]–P4	38	2	11	25	34	20
P1[a]–P7[a]	16	1	4	11	31	19
P1[a]–P8[a]	10	0	1	9	10	5
C1–C4	41	2	16	23	44	24
C1–C5	38	1	10	27	29	16

[a] One subject who was only genotyped for DRD2 TaqI A RFLP is excluded

typed for the TaqI B RFLP, all statistical calculations were performed for both the DRD2 TaqI A1 and B1 allele prevalences.

The genotype counts, allele frequencies, and allele prevalences are presented in Tables 1 and 2.

Comparisons of the DRD2 TaqI A1 allele prevalences were made between alcoholic and control subjects and within and between subgroups of alcoholics and controls (Table 3). No significant differences could be found when the total sample of alcoholic subjects was compared with the screened control groups. Neither were there any statistically significant differences when the alcoholic subjects were divided with regard to sample (inpatients or autopsied subjects) or the alcoholic inpatients subdivided with respect to degree of alcohol dependence (moderate or severe), age at onset (before or after 25 years), parental alcoholism (presence or absence), or origin (Finnish or Swedish) and compared to the different control subgroups. Dividing the inpatient alcoholics and controls with regard to gender and examining the four relations female controls or male inpatients vs female inpatients or male controls did not reveal significant differences. Examinations between the alcoholic inpatient subgroups comparing moderate vs severe alcohol dependence, age at onset, before vs after 25 year, Finnish vs Swedish origin, and absence vs presence of parental alcoholism, did not show any significant differences. No significant difference was found when comparing autopsied subjects divided with regard to tissue damage score, i.e. more (severe medical complications) or less than 10 p (see methods).

As DRD2 TaqI A and B frequencies became altered when controls were screened, a comparison between controls was performed. The 40 controls screened for any DSM-III-R diagnosis and first- or second-degree relatives with alcohol abuse and dependence, were compared with the remaining 41 controls from the first control group, only screened for DSM-III-R alcoholism diagnosis. No significant difference was found.

The same calculations were performed for the DRD2 TaqI B RFLP. The results were very similar due to the strong linkage equilibrium between the TaqI A and B RFLPs.

Discussion

The present study sample consisted of an ethnically heterogeneous Scandinavian sample. Of the alcoholic inpatients, 22% had Finnish parents, whereas 7–8% of the genes in the control groups were of Finnish origin. If there had been a difference in allele frequencies between Finns and Swedes, this might have caused us to replicate the proposed association between the DRD2 TaqI A1 alleles and alcoholism. However, we could not find any statistically significant difference when we compared Finnish and Swedish alcoholic inpatients or when these subgroups were compared with the control subgroups (Table 3).

Furthermore, we compared our subgroups of Finnish and Swedish alcoholic inpatients with the Finnish alcoholics described in a previous independent investigation (Goldman et al. 1992) without significant differences (χ^2 = 0.009 (1), P = 0.93 and χ^2 = 0.002 (1), P = 0.97, respectively). Neither were there any differences when the Finnish control group (Goldman et al. 1992), assessed for polysubstance abuse or major mental illness, was compared with our different control subgroups (χ^2 = 0.014 (1), P = 0.91 when compared with controls screened for any DSM-III-R diagnosis).

Thus, the ethnical differences between Swedes and Finns do not seem to influence the prevalence of the DRD2 TaqI A1 allele.

No significant association between alcoholics or subgroups of alcoholics and the DRD2 TaqI A or B alleles was observed in our study. These results are in concordance with five previous studies (Bolos et al. 1990; Ge-

Table 3 Obtained *P*-values (χ^2-test or Fishers exact test) comparing DRD2 TaqI A1 allele prevalence in samples of alcoholics and control subjects. P1 = All alcoholic inpatients (n = 74), P2 = Inpatients, DSM-III-R severe alcohol dependence (n = 56), P3 = Inpatients, onset ≤ 25 years (n = 29), P4 = Inpatients with parental alcoholism (n = 35), P5 = Autopsied subjects with pathological anatomical signs of alcoholism (n = 19), P6 = Autopsied subjects, tissue damage > 10 p (n = 10), P7 = Inpatients, Swedish origin (n = 58), P8 = Inpatients, men (n = 64), C1 = Controls screened for DSM-III-R alcohol dependence or alcohol abuse (n = 81), C2 = Controls screened for any DSM-III-R diagnosis (n = 67), C3 = Controls screened for any DSM-III-R diagnosis and first-degree relatives with alcohol abuse or dependence (n = 52), C4 = Controls screened for any DSM-III-R diagnosis and first- or second-degree relatives with alcohol abuse or dependence (n = 40), C5 = men from C1 (n = 43), P1 + P5 = All alcoholics (n = 93), P1–P2 = Inpatients, DSM-III-R moderate alcohol dependence (n = 18), P1–P3 = Inpatients, onset > 25 years (n = 45), P1–P4 = Inpatients without parental alcoholism (n = 39), P1–P7 = Inpatients, Finnish origin (n = 16), P1–P8 = Inpatients, women (n = 10), P5–P6 = Autopsied subjects, tissue damage ≤ 10 p (n = 9), C1–C4 = Controls with DSM-III-R diagnosis other than alcoholism or first-or second-degree relative with alcohol abuse or dependence (n = 41), C1–C5 = Women from C1 (n = 38), F = *P*-value with Fishers exact test

	P1	P2	P3	P4	P5	P6	P1+P5	P1–P2	P1–P3	P1–P4	P5–P6	C1–C4	P7	P1–P7	C5	P1-P8
C1	0.65	0.86	0.52	0.40	0.50	0.25	0.97	0.20	0.17	0.85	0.83		0.85	0.59		
C2	0.97	0.70	0.37	0.61	0.37	0.19	0.97	0.30	0.31	0.91	0.73		0.86	0.76		
C3	0.76	0.44	0.16	0.90	0.18	0.09	0.46	0.58	0.76	0.49	0.99		0.65	0.86		
C4	0.64	0.37	0.14	0.85	0.16	0.08	0.39	0.72	0.96	0.42	0.93	0.08	0.55	0.73		
P1–P2		0.22														
P1–P3			0.07													
P1–P4				0.49												
P5–P6						0.19F										
P1–P7													0.77			
C1–C5															0.96	0.27
P8															0.92	0.24

lernter et al. 1991; Cook et al. 1992; Goldman et al. 1992; Turner et al. 1992) whereas they differ from another nine (Blum et al. 1990; Blum et al. 1991; Comings et al. 1991; Smith et al. 1992; Amadéo et al. 1993; Arinami et al. 1993; Blum et al. 1993; O'Hara et al. 1993; Smith et al. 1993).

Meta-analysis using the results of several different studies, shows a small but significant association between drug abuse (rather than alcoholism specifically) and the prevalence of the DRD2 TaqI A1 (B1) allele (Uhl et al. 1993). However, this view is still controversial (Gelernter et al. 1993). Three models that can be evaluated in the light of existing data are:

1. The A1 (B1) allele constitutes a modifying gene, not causing, but altering the expression of the disorder.
2. Substance abuse is associated with a subpopulation (such as a subgroup with similar ethnic background) with high A1 (B1) allele frequency, but these alleles do not influence the course of the disorder.
3. The A1 (B1) allele (or possibly, another gene linked to this allele) together with other genetic and environmental influences is a causal factor.

The fact that the A1 (B1) allele frequency in most studies and in resulting meta analysis (Uhl et al. 1993) is increased within alcoholic groups or subgroups and the unsuccessful attempts to find linkage in families where alcoholism is aggregating (Bolos et al. 1990; Parsian et al. 1991) support the first model.

In a recent study (Arinami et al. 1993), an increased A1 allele frequency was found in severe and younger Japanese alcoholics. Association of the DRD2 TaqI A1 RFLP with alcoholism in a population with very high A1 allele frequency, such as the Japanese population (Arinami et al. 1993; Gelernter et al. 1993) makes the second model less favorable, as an unintentional selection of subjects from a subpopulation with even higher A1 allele frequency seems unlikely.

This kind of biased selection seems more likely in caucasian populations with generally low A1 allele frequency and heterogeneous ancestry. If the modifying gene hypothesis is valid the same A1 allele frequency would be found for both alcoholics and controls, whereas a subdivision into severe/excessive and moderate alcoholics would create a higher than control A1 allele frequency in the former group and lower than control A1 allele frequency in the latter (Goldman et al. 1992; Gelernter et al. 1993).

Assuming more severe medical complications and a resulting lower life expectancy for severe/excessive alcoholics a lower than control A1 allele frequency in populations of very old alcoholics would also be found.

Increased A1 allele frequency in alcoholic groups selected for severity (i.e. severity according to DSM-III-R criteria, early onset or severe medical complications due to alcohol abuse) and decreased frequencies in the corresponding less severe alcoholic groups as compared to control groups, are tendencies seen in the data of the present study (Table 1). However, the differences were not significant. Interestingly, in the present study the group of 10 alcoholic subjects with severe medical complications due to alcohol abuse was very similar (actually identical

in size and allele distribution) to a group of alcoholic subjects characterised by a history of medical complications (described by Parsian et al. (1991)) found to be significantly different compared to their control group. Also Arinami et al. (1993) found a decrease of the A1 allele frequency in older (> 60 years) alcoholics.

This might be consistent with the idea that medical problems are accentuated for alcoholics carrying the A1 allele and also implicates that age is an important factor. These findings of the Japanese study support the first model and, at the same time, make the second model less likely.

In contrast, the large variation in A1 allele frequency between ethnic groups (Arinami et al. 1993; Gelernter et al. 1993; O'Hara et al. 1993; Uhl et al. 1993) suggests ethnic heterogeneity as a possible explanation of the varying results obtained from the different investigations. However, this does not exclude the first or the third model. The failure to demonstrate linkage and the inconsistent results for association between the A1 allele and alcoholism make the third model less likely. The crucial contrast between the first model and the second is the influence on the disorder postulated in the former model. A possible way of elucidating this problem is to study phenotypic parameters such as molecular interactions and expression in the dopaminergic system or personality traits in homozygotic carriers, associated with the different DRD2 alleles.

Some attempts in this direction have already been made. Noble et al. (1991) described a decrease in the number of dopamine D_2-receptors in striatal tissue from individuals with identified genotypes. Goldman et al. (1992) did not find any relationship between monoamine metabolites and DRD2 TaqI A genotypes in Finnish alcoholics and controls. Nor did Smith et al. (1993) find any association between comorbid psychopathy and DRD2 TaqI A genotype in substance abusers.

The result of this investigation does not support an association between alcoholism and the DRD2 TaqI A1 or B1 alleles. However, considering all the presently available data, the hypothesis of a modifying gene, not causing but infrequently enhancing the severity of symptoms of alcoholism or drug abuse, appears to be the most promising model.

Previous data also suggest that an ethnic or other subpopulation among caucasian subjects might exist and may, in heterogenous populations, enhance a true, but modest, association to a modifying gene. This would explain the alternating statistical outcome of the studies on this topic.

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