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Dopamine D2 receptor gene (*DRD2*) haplotypes in Caucasians

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Abstract

The human dopamine D2 receptor gene (*DRD2*) is considered a candidate gene for neuro-psychiatric diseases. We typed three new DNA sequence variants in *DRD2* intron 4, intron 6 and exon 8, in combination with the known *Taq*I A restriction fragment length polymorphism (RFLP) and exon 7 ^{311}Ser/Cys in 106 unrelated psychiatrically healthy Caucasians. Based on the genotypic data we delineated 10 distinct *DRD2* haplotypes and their genetic relationship. Our data provide evidence that the *Taq* A1 allele and the ^{311}Cys variant are components of different groups of haplotypes though both variants have been speculated to be associated with alcoholism or schizophrenia in recent studies. Therefore we conclude that the prior knowledge of the frequencies and genetic relationships of *DRD2* haplotypes will lead to the selection of more suitable intragenic markers for future association studies.

Keywords: Linkage disequilibrium; Haplotype frequencies; Association studies; Dopamine receptor; Polymerase chain reaction

1. Introduction

The results of numerous studies investigating the association of the *DRD2* variants ^{311}Cys and *Taq* A1 with schizophrenia and alcoholism are conflicting (Pato et al., 1993; Gelernter et al., 1993; Uhl et al., 1993; Suarez et al., 1994; Gejman et al., 1994; Arinami et al., 1994; Nöthen et al., 1994; Higuchi et al., 1994; Finckh et al., 1996b). Possible explanations for such inconsistencies might be population stratification, genetic heterogeneity, the lack of reliable phenotype classification or the usage of unsuitable DNA markers.

The aim of our study was the delineation of *DRD2* haplotypes based on new as well as known DNA markers. The knowledge of the frequencies and genetic relationships of such haplotypes will influence the selection of suitable intragenic markers for genetic association studies and improve the comparability of association studies from different populations.

2. Results and discussion

2.1. Marker and genotype frequencies

PCR based marker systems were established for ^{960}C→G in exon 7 which leads to ^{311}Ser/Cys (Itokawa et al., 1993; Gejman et al., 1994), the *Taq*I A RFLP (Grandy et al., 1993), a single base pair (bp) deletion polymorphism (^{2312}C) in intron 6 (Finckh et al., 1996a), a newly detected A→G substitution polymorphism, 52 bp downstream of the stop codon in exon 8 (^{1412}A/G), and a double substitution polymorphism in intron 4 consisting of the single base substitutions ^{398}C→T and ^{409}A→G laying 11 bp apart (see footnote a of Table 1 for the numbering of the bp positions). We

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Abbreviations: ASP, allele-specific PCR; bp, base pair(s); *DRD2*, dopamine D2 receptor gene; EDTA, ethylenedinitrilo tetraacetic acid; HWE, Hardy Weinberg equilibrium; kb, kilobase(s); PCR, polymerase chain reaction; RFLP, restriction fragment legnth polymorphism.

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Table 1
List of primers and PCR products

Primer No.	Locus	Position[a]	+/−[b] sequence[c] (5′→3′)	Used combined with primer No./ resulting amplicon size (bp)
1	intron 4	355 [a1]	+rev-AGGCTACTGGGCTTCCATCC	6/995
2	intron 4	397	+rev-GCTGTGCGTTTGTG	6/268
3	intron 4	396	+rev-CCGTGCGTTTGTA	6/267
4	intron 4	412	-M13-ATACACAAACGCACA	1/782
5	intron 4	412	-M13-ATATACAAACGCACG	1/782
6	exon 5	620	-M13-AGGAGACGATGGAGGAGTAG	1/995
7	intron 6	1924	+rev-CCTCAGCTGAAGGCAGACTC	12/1195/1196
8	intron 6	2302	+rev-GGGTAGGACGCAGC	12/818
9	intron 6	2302	+rev-GGGTAGGACGAGCA	12/817
10	intron 6	2323	-M13-AGCTGCTCTGCTGC	7/439
11	intron 6	2323	-M13-AGCTGCTCTGCTCG	7/438
12	intron 6	3080	-M13-CCTCATGCAGCCTCAATACC	7/1195/1196
13	intron 6	3237	+CTGATGCCTGGGAACTTGTCCGGCTTTA	18/410
14	exon 7	943	+GACTCTCCCCGACCCGTG	18/252
15	exon 7	943	+GACTCTCCCCGACCCGTC	18/252
16	exon 7	975	-TGGAGACCA/GTGGTGGC	13/191
17	exon 7	975	-TGGAGACCA/GTGGTGGG	13/191
18	intron 7	3647	-CTGTGGCCAGCAGCCAGGGCCGA	13/410
19	intron 7	1590 [a2]	-rev-CCTCCCCGGCTCTGGGGACC	24/494
20	exon 8	1397	+CTCCCTGCCCAGGCCA	24/202
21	exon 8	1397	+CTCCCTGCCCAGGCCG	24/202
22	exon 8	1427	-AAGGGTGAGGCTGGCT	19/325
23	exon 8	1427	-AAGGGTGAGGCTGGCC	19/325
24	exon 8	1583 [a3]	-M13-GGGCTGGTACCATGCCCAGCTC	19/494
25	exon 8	2312 [a3]	+rev-TCTGCCTTAGAGGAGCCCAC	27/∼3500
26	downstream	28 [a4]	+M13-CCTCCTAGAACATCACGCAA	27/298 (127+171)[d]
27	downstream	286 [a4]	-M13/rev-CATCTCGGCTCCTGGCTTA	26

[a] The position of the primers' 5′-base in the *DRD2* gene is indicated. The numbering of the nucleotide positions corresponds to the numbering of fig. 1 in Dal Toso et al. (1989) (EMBL X51645) for the intronic positions and to fig. 2 from the same reference (EMBL X51646) for the exonic positions. [a1]Primer No. 1 has bp position 355 of intron 4 (EMBL Z29558). [a2]Primer No. 19 has bp position 1590 of intron 7 (EMBL Z29564). [a3]The 5′-positions of the primers No. 24 and No. 25 refer to GenBank M29066. [a4]The bp positions from Grandy et al. (1993) (GenBank L22303) are indicated.

[b] '+'=forward-, '-'=backward-orientation of primer.

[c] The forward PCR primers ('+') were synthesized with a 5′-18-mer reverse M13 ('rev') extension (5′-CAGGAAACAGCTATGACC...-3′), and the reverse primers ('-') with a 5′-20-mer-21M13 ('M13') extension (5′-TAAAACCACGGCCAGTGCCA...-3′) for non-radioactive dye primer sequencing of the PCR products. The allele discriminating bases from the allele-specific primers are underlined.

[d] Sizes of the A2-allele fragments after digestion from *Taq*I.

Methods: Genomic DNA was isolated from peripheral blood samples (Miller et al., 1988). The distance of the *Taq*I a RFLP from DRD2 was estimated from a long range PCR product after amplification of genomic DNA with the primers No. 25 and No. 27 with a 5′ universal-21M13 extension. This long range PCR was performed using the Expand Long Template PCR System Kit™ (Boehringer Mannheim, Germany) with a thermoprofile as follows: Initial denaturation 94°C 45 s followed by 5 cycles at 94°C 10 s, 65°C 30 s, a 30 s ramp to 60°C, 68°C 6 min; and 25 cycles of 94°C 10 s, 68°C 6 min with an automatic extension of 20 s in each consecutive cycle; the final extension was 10 min at 68°C. The specificity of this PCR product was evaluated through direct nonradioactive dye primer sequencing of both termini (ABI 373A system). Bidirectional allele-specific PCR amplification of fragments with different lengths flanking the intron 4, intron 6, exon 7 and exon 8 polymorphisms was performed using 4 primers simultaneously in one reaction tube (Ye et al., 1992; Finckh et al., 1996a). This protocol allows for the simultaneous amplification of both alleles through mixture of two flanking non-allele-specific primers with one allele-specific primer that anneals at the plus strand of allele 1 with one allele-specific primer that anneals at the minus strand of allele 2 of the same marker. This allows the amplification of fragments from both alleles in opposite directions respectively. The alleles are differentiated through different lengths of the allele-specific PCR products and visualized by 2.5% agarose gel electrophoresis. The allele-specific fragments were directly amplified from 50-100 ng genomic DNA with 10 pmol of each primer, 1 U *Taq* DNA polymerase (Boehringer Mannheim, Germany), 200 μM dNTP, 1.5 mM $MgCl_2$, 10 mM Tris-HCl, 50 mM KCl, pH 8.3, 12 μl paraffin wax (Paraffin Wax, Aldrich, mp 53-56°C) in a 25 μl reaction volume using a PTC 100 thermocycler (MJ research). As a control, two independent allele-specific PCRs were performed for each marker by using two different primer sets in separate reaction tubes for the allele-specific analysis of both DNA strands respectively. PCR-amplification of the *Taq*I A RFLP-flanking region and *Taq*I restriction digestion of the amplicons was designed according to Grandy et al. (1993). Besides an initial 3 min/95°C denaturation and a final 5 min/72°C extension step the following PCR thermoprofiles for the respective markers were required: Intron 4, primers 1-6.: 10×[95°C/30 s; 58°C/30 s; 72°C/60 s] plus 20×[95°C/30 s; 53°C/30 s; 72°C/30 s]. Intron 6, primers 7-12: 10×[95°C/30 s; 62°C/30 s; 72°C/60 s] plus 20×[95°C/30 s; 53°C/30 s; 72°C/30 s]. Exon 7, primers 13-18: 2×[95°C/30 s; 70°C/30 s; 72°C/20 s] plus 33×[95°C/30 s; 45°C/15 s; 72°C/15 s]. Exon 8, primers 19-24: 3×[95°C/30 s; 70°C/30 s; 72°/30 s] plus 34×[95°C/30 s; 45°C/15 s; 72°C/15 s]. *Taq*I A RFLP, primers 26 and 27: 40×[95°C/20 s; 61°C/20 s; 72°C/20 s].</T1>

always found intron 4 ^{398}C linked with ^{409}A and ^{398}T with ^{409}G.

None of the observed genotype frequencies significantly deviated from Hardy Weinberg equilibrium (HWE). The frequencies of the respective rarer allele from the five diallelic markers were as following: $f_{398T/409G} = 0.415$; $f_{2312C\text{-deletion}} = 0.156$; $f_{311Cys} = 0.024$; $f_{1412G} = 0.302$; $f_{TaqA1} = 0.175$.

The intron 4 and exon 8 markers with frequencies of over 30% of each allele might be of advantage for association studies rather than the rare ^{311}Cys variant and the extragenic *Taq*I A RFLP. Furthermore, the observation of a significant number of homozygotes of both alleles and of some of the delineated *DRD2* haplotypes described in (b) allows the testing of a potential recessive mode of transmission of a postulated *DRD2*-associated genetic vulnerability to neuropsychiatric disorders.

2.2. Haplotype analysis

In the 106 investigated individuals only 21 (from 243 theoretically possible in larger populations) five-marker genotype combinations were observed. Thirty five individuals were homozygous in all five markers and 17 individuals were heterozygous in one of the five markers. The genotype data of these 52 individuals allowed the unambiguous delineation of the eight haplotypes A to F, A_f and A_c. Fifty two of the 54 individuals with more than one heterozygous marker clustered into five genotype combinations, assuming combinations of the unambiguously identified haplotypes whenever this was compatible. Therefore, up to 210 of the 212 investigated chromosomes (99%) cluster into the eight unambiguously delineated haplotypes. The remaining two chromosomes each have one of the two haplotypes A_{ff} and F_a. The haplotype descriptions and the derived frequencies of the haplotypes are listed in Fig. 1. Thus only 10 from 32 theoretically possible haplotypes have been found. None of the haplotype frequencies significantly deviated from HWE (data not shown). The haplotypes A_f, A_{ff}, A_c and F_a probably result either from independent or from single founding recombination events between A and F, A and C and F and A, respectively.

The exclusive occurrence of ^{311}Cys in combination with exon 8 ^{1412}G in haplotype D and the intron 6 ^{2312}C-deletion as well as *Taq* A1 in combination with exon 8 ^{1412}A in haplotypes E, F, A_f, A_{ff} and F_a indicates an independent evolution of ^{311}Ser/Cys and the *Taq*I A RFLP. A retrospective haplotype analysis of a recently identified homozygous ^{311}Cys/Cys-individual (Finckh et al., 1996b) also revealed homozygosity for haplotype D.

By using a computer program, Suarez et al. (1994) described in an US-American Caucasian sample (n = 158) fewer DRD2 haplotypes (n = 18) based on five

Haplotype[b]	Marker[a] 1 intron 4	2 intron 6	3 exon 7	4 exon 8	5 *Taq*I A	n	frequency
A	[C..A]	GCA	TCC	A	TCGA	102	0.481
B	[T..G]	GCA	TCC	A	TCGA	8	0.038
C	[T..G]	GCA	TCC	G	TCGA	42	0.198
D	[T..G]	GCA	TGC	G	TCGA	5	0.024
E	[T..G]	GCA	TCC	A	TCAA	1	0.005
F	[T..G]	GA	TCC	A	TCAA	31	0.146
A_f	[C..A]	GCA	TCC	×[c] A	× TCAA	4	0.019
A_{ff}	[C..A] ×	GA	TCC	A	TCAA	1	0.005
A_c	[C..A] ×	GCA ×	TCC ×	G	TCGA	17	0.080
F_a	[T..G]	GA ×	TCC ×	A ×	TCGA	1	0.005

Fig. 1. Haplotype definition and frequencies. The arrows suggest evolutionary relationships between the haplotypes assuming one ancestral human haplotype. Footnotes: [a]Marker descriptions: 1, double substitution in intron 4 [^{398}C..^{409}A/^{398}T..^{409}G]; 2, 1-bp deletion in intron 6 [G^{2312}CA/GA]; 3, exon 7 ^{960}C/G substitution leading to ^{311}Ser/Cys; 4, exon 8 substitution [^{1412}A/G]; 5, *Taq*I A RFLP, with the alleles A = TCAA and A2 = TCGA. [b]The haplotypes printed in bold have been observed in homozygotes. The haplotypes A_f/A_{ff}, A_c and F_a presumably result from recombination events between haplotype A and F, A and C, and F and A, respectively. [c]×, presumable recombination events. **Study design**: 106 unrelated Caucasian subjects, 55 males, 51 females, mean age (±standard deviation) 54 years (±16) of German origin were included in the study. None of the individuals reported a history of neuropsychiatric disorders in a personal interview. Exclusion criteria were major psychiatric disease, substance abuse or heavy alcohol consumption. Informed consent was obtained from all participants.

diallelic RFLP markers than theoretically possible (n = 32) and – comparable to our haplotype A with $f_A = 0.481$ – there was also one out of eight different *Taq* A2-containing haplotypes with a frequency of approximately 0.5 in contrast to the remaining A2-containing haplotypes with respective frequencies below 0.12. In a recent study from Castiglione et al. (1995) the frequency of the *Taq* A1 allele in Europeans ($f_{TaqA1} = 0.13$) was similar to our sample, and again the frequency of one out of six different A2-containing haplotypes ($f_{B2\text{-}15\text{-}A2'} = 0.457$) exceeded the remaining A2-containing haplotypes with frequencies of up to 0.135.

2.3. Analysis of linkage disequilibria

Table 2 shows the two-locus linkage disequilibria between the five markers based on the observed single-marker data using the LD86 computer program (Weir, 1990). Significant linkage disequilibria were found between intron 4 and exon 8, intron 4 and *Taq* A, intron 4 and intron 6, and intron 6 and *Taq* A. The *Taq*I A RFLP lays approximately 3.3 kb downstream of *DRD2*. The fine mapping strategy of the *Taq*I A RFLP locus is described in the legend of Table 1. The strength of the linkage disequilibria in the *DRD2* locus did not correspond to the physical distance of the markers. The

Table 2
Linkage disequilibria at the *DRD2* locus based on the observed marker frequencies

Markers[a]	Δ^{b}_{xy}	χ^{2c}	D^{b}_{xyy}	χ^2	D^{b}_{xxy}	χ^2	Δ^{b}_{xxyy}	χ^2
1 and 2	0.0925	25.01	0.0034	0.28	0.0014	0.05	−0.0025	0.44
1 and 3	0.0134	2.96	0.0003	0.10	0.0013	0.19	−0.0001	*
1 and 4	0.1032	22.26	0.0122	2.76	0.0045	0.43	0.0018	0.10
1 and 5	0.0910	20.16	0.0032	0.21	0.0002	0.00	−0.0080	3.00
2 and 3	−0.0073	2.02	−0.0007	*	−0.0002	*	−0.0001	0.02
2 and 4	0.0232	2.57	0.0021	0.47	0.0018	0.20	0.0024	*
2 and 5	0.1249	86.68	0.0039	0.21	0.0006	0.01	−0.0031	1.05
3 and 4	0.0141	5.29	0.0003	0.08	0.0009	0.08	−0.0002	0.08
3 and 5	0.0082	2.11	0.0005	*	−0.0002	*	−0.0001	0.02
4 and 5	0.0346	4.76	0.0053	1.87	−0.0006	0.02	0.0033	*

[a] See Fig. 1 for definitions of the markers 1–5.
[b] The pairwise linkage disequilibria Δ_{xy} for the loci were analysed based on the following equations:

$$\Delta_{xy} = D_{xy} + D_{x/y}$$

$$D_{xyy} = P^{xy}_{y} - p_y\Delta_{xy} - p_x D_y p_x P^2_y$$

$$D_{xxy} = P^{xy}_{x} p_x \Delta_{xy} - p_y D_x - p_y p^2_x$$

$$\Delta_{xxyy} = P^{xy}_{xy} - 2p_x D_{xyy} - 2p_y D_{xxy} - 2p_x p_y \Delta_{xy} - p^2_x D_y - p^2_y D_x - \Delta^2_{xy} - D_x D_y - p^2_x p^2_y$$

$$D_x = P^x_x - p^2_x$$

$$D_y = P^y_y - p^2_y$$

$$D_{xy} = P_{xy} - p_x p_y$$

$$D_{x/y} = P_{x/y} - p_x p_y$$

where D_{xy} is a measure of gametic disequilibrium, and $D_{x/y}$ a measure of nongametic disequilibrium; p_x is the frequency of the x-allele of Marker 'X', and p_y the frequency of the y-allele of marker 'Y'; P^x_x and P^y_y are the observed genotypic frequencies of xx and yy homozygotes, respectively, P_{xy} the frequency of the 'coupling' halotype, and $P_{x/y}$ the frequency of genotypes with x on one parental chromosome and y on the other parental chromosome.
[c] Through correction for multiple calculations is $\chi^2 > 7.88$ for $p < 0.05$ and $\chi^2 > 10.83$ for $p < 0.01$.
*, χ^2 cannot be calculated because the variance of the estimate is too small.

strongest disequilibrium was observed between the intron 6 ^{2312}C-deletion and the *Taq*I A RFLP which have a distance of approximately 7.5 kb which is in contrast to the weak linkage disequilibrium between intron 6 and exon 8 ^{1412}A/G with a distance of only 3187 bp. The weakest disequilibria were observed in the marker combinations involving exon 7 ^{311}Ser/Cys. The strong linkage disequilibrium between the intron 6 marker and the *Taq*I A RFLP correlates with the observation that virtually every observed intron 6 ^{2312}C-deletion is accompanied by *Taq* A1, and vice versa, leading to a predominance of two from four possible permutations. Thus, the intron 6 marker may be considered as an equivalent intragenic *DRD2* marker to the 3′-downstream *Taq*I A RFLP.

Taq A1 as well as *Taq* A2 occurred in association with exon 8 ^{1412}A, but we could not identify exon 8 ^{1412}G in association with *Taq* A1. This indicates an extremely small recombination value between different gene arrangements. However, due to the predominance of three of theoretically four possible permutations the composite linkage disequilibrium between exon 8 ^{1412}A/G and the *Taq*I A RFLP was not significant.

In two other studies the *Taq*I A RFLP has been typed simultaneously with a PCR-SSCP polymorphism in *DRD2* exon 8 in American Caucasians ($n = 105$), Finns ($n = 86$) and Cheyenne Indians ($n = 34$) (Goldman et al., 1993; Bolos et al., 1990). In the pooled sample there were only found two from 141 *Taq* A1-bearing chromosomes in which *Taq* A1 was unambiguously associated with SSCP allele '2'. These and our data suggest small recombination values between *DRD2* exon 8 and the *Taq*I A RFLP in different human populations.

3. Conclusions

(1) We identified two diallelic DNA markers in *DRD2* intron 4 and exon 8 with frequencies of over 0.3 of each allele in a sample of German Caucasians.
(2) The combined analysis of five diallelic *DRD2* markers allowed the delineation of ten different haplotypes from which eight were unambiguously observed.
(3) The haplotyping data revealed that the variants *Taq* A1 and ^{311}Cys from the two most frequently used DRD2 markers in association studies (*Taq* A RFLP, ^{311}Ser/Cys) are components of different groups of

haplotypes. Based on the *DRD2* haplotype data more suitable intragenic markers for future association studies may be selected.

(4) Comparison of the haplotypes with data from other authors (Barr and Kidd, 1993; Suarez et al., 1994; Castiglione et al., 1995) suggest a limited number of *DRD2* haplotypes in European Caucasians with the predominance of one particular haplotype with a frequency of approximately 50% in contrast to all remaining haplotypes with respective frequencies below 20%.

(5) In concordance with other authors we found significant linkage disequilibria involving the *Taq*I A RFLP, which lays approximately 3.3 kb downstream of *DRD2*. This raises questions about the existence of structural chromosomal variants which negatively interfere with recombination or a selective disadvantage through recombination events in the 3′ region of *DRD2*.

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