

# Dopamine D4 Receptor Variant in Africans, D4<sub>Valine194Glycine</sub>, Is Insensitive to Dopamine and Clozapine: Report of a Homozygous Individual

Ivy S.C. Liu, Philip Seeman, Suparna Sanyal, Carla Ulpian, Pamela E.B. Rodgers-Johnson, Graham R. Serjeant, and Hubert H.M. Van Tol

Department of Pharmacology, Medical Sciences Building (P.S., C.U., I.S.C.L.), Molecular Neurobiology Laboratory, Department of Psychiatry, Clarke Institute of Psychiatry (S.S., H.H.M.V.T.), University of Toronto, Toronto, Canada; and Department of Medicine (P.E.B.R.-J., G.R.S.), University of the West Indies, Mona, Kingston, Jamaica, West Indies

The D4<sub>Valine194Glycine</sub> receptor is a variant of the dopamine D4 receptor and is found in 12.5% of the Afro-Caribbean population. Glycine replaces valine at a position one amino acid away from a serine which is critical for the attachment of dopamine. To determine whether this mutation had an effect on the properties of the dopamine D4 receptor, we constructed this variant and tested the sensitivity of the expressed protein with various drugs. We found that the variant receptor was two orders of magnitude less sensitive to dopamine, clozapine and olanzapine. The variant receptor was insensitive to guanine nucleotide, indicating the absence of a high-affinity state or functional state. The one 15-year-old individual found homozygous for this variant also had sickle cell disease. The patient revealed an overall pattern of low weight and no axillary or pubic hair. © 1996 Wiley-Liss, Inc.

**KEY WORDS:** dopamine receptor mutation, clozapine, olanzapine, anti-psychotic drug, schizophrenia, adenylate cyclase

## INTRODUCTION

In searching for an abnormality in the genes for different dopamine receptors, we found a variant of the dopamine D4 receptor, D4<sub>Valine194Glycine</sub>, in 12.5% of 186 Afro-Caribbeans but not in any of 147 Caucasians

Received for publication February 15, 1995; revision received August 9, 1995.

Address reprint requests to Philip Seeman, Department of Pharmacology, Medical Sciences Building, Room 4344, 8 Taddle Creek Road, University of Toronto, Toronto, Ontario, Canada M5S 1A8.

tested [Seeman et al., 1994]. This variant, present in heterozygous individuals, was not associated with schizophrenia or any obvious dopamine-based disease. Recently, however, Nöthen et al. [1994] found a homozygous individual with a nonfunctional dopamine D4 receptor of only 98 amino acids [D4 normally has 387 to 515 amino acids; Van Tol et al., 1992]. This individual was obese, had an acoustic neuroma, excessive sweating, low body temperature, and intermittent sinus tachycardia.

The dopamine D4<sub>Valine194Glycine</sub> receptor variant has a change in one nucleotide wherein T is replaced by G, predicting a substitution of valine by glycine at amino acid position 194 [Seeman et al., 1994]. This amino acid replacement occurs one amino acid away from a serine which is critical for the attachment of dopamine in the dopamine D2 receptor [Cox et al., 1992]. Therefore, in order to examine whether such a major mutation had an effect on the properties of the dopamine D4 receptor, we constructed this variant and tested the sensitivity of the expressed protein with various drugs.

We here report that the dopamine D4<sub>Valine194Glycine</sub> receptor variant is insensitive to dopamine, as well as to clozapine and olanzapine. In addition, we report on an individual who was homozygous for the nonfunctional dopamine D4<sub>Valine194Glycine</sub> receptor variant.

## METHODS

The dopamine D4 receptor variant, D4<sub>Valine194Glycine</sub>, was constructed by means of polymerase chain reaction (PCR)-mediated mutagenesis. The cDNA for the dopamine D4.4 receptor was available from earlier work [Van Tol et al., 1991]. As indicated elsewhere [Van Tol et al., 1992], the dopamine D4 receptor has many variants as a result of a variable repeat unit of 16 amino acids. The D4.2 receptor has 2 repeats, the D4.4 receptor has 4 repeats, the D4.7 receptor has 7 repeats, etc.

The first part of exon 3 of the dopamine D4 receptor variant contains the T-to-G variation. The 5' portion of the D4.4 cDNA (~600 bp) was amplified by PCR, using a primer which flanked the 5' cloning site of pRc/RSV (5'-CATTGGTGTGCACCTCC-3') and a dopamine D4

receptor oligonucleotide containing the introduced mutation (5'-CGGACGAGTAGcCCACGTAGTCGC-3'). The 3' portion of the D4.4 cDNA (~850 bp) was amplified separately by PCR using a dopamine D4 receptor oligonucleotide containing the introduced mutation (5'-GCGACTACGTGGgCTACTCGTCCG-3') and a primer flanking the 3' cloning site of pRc/RSV (5'-AGGCTGATCAGCGAGCT-3'). The two dopamine D4 receptor oligonucleotides described above were complementary to each other. The complete dopamine D4.4 receptor cDNA containing the introduced mutation was then created by a third PCR reaction, using the primers flanking the 5' and 3' cloning sites of pRc/RSV, as described above, to combine the two fragments.

The PCR was carried out in a volume of 50  $\mu$ l containing 1 $\times$  Invitrogen PCR buffer B (Invitrogen Corp., San Diego, CA), 10% DMSO, 0.5 mM of dNTP, 100 ng of each primer, 10–20 ng of plasmid DNA or PCR products and 2.5 units of *Taq* polymerase (Perkin-Elmer Cetus Corp., Foster City, CA). The PCR conditions were as follows: 30 sec denaturation at 95°C, 30 sec annealing at 48°C, 60 sec extension at 72°C, using a GeneAmp PCR System 9600 (Perkin-Elmer Cetus Instruments, Norwalk, CT). Following 20 cycles, the samples were subjected to an additional elongation period of 7 min at 72°C.

The resulting PCR product was digested with Hind III and Eco47 III to produce a unique Hind III-Eco47 III fragment (670 bp) containing the D4<sup>Valine194Glycine</sup> mutation. The wild-type Hind III-Eco47 III fragment in the pRc/RSV D4.4 construct was then replaced by the mutated fragment, thus producing a full-length dopamine D4.4 receptor variant cDNA in pRc/RSV. The Hind III-Eco47 III fragment of this construct was then sequenced [Van Tol et al., 1991]. The DNA sequence (method of Sanger et al., 1977) of D4<sup>Valine194Glycine</sup> was confirmed as containing only a single change in the nucleotide sequence, namely a T-to-G mutation in codon 194. The DNA was transiently transfected into COS cells for expression of the receptor. A stable cell line expressing the dopamine D4<sup>Valine194Glycine</sup> receptor variant was produced in Chinese hamster ovary cells using gentamycin.

The tissue culture cells were pooled and added to buffer (50 mM Tris-HCl, pH 7.4 at 20°C, 1 mM EDTA, 5 mM KCl, 1.5 mM CaCl<sub>2</sub>, 4 mM MgCl<sub>2</sub> and 120 mM NaCl), resulting in a suspension of approximately 200  $\mu$ g protein/ml. The cells were not washed or centrifuged, because these procedures result in a loss of receptors [Seeman et al., 1984]. The cell suspension was homogenized with a Polytron (PT-10 probe, Brinkmann Instruments, Inc., Westbury, NY; setting 5 out of a maximum of 10) for 5 sec.

The competition between the various drugs and [<sup>3</sup>H]spiperone for binding to the receptors was done as follows. Each incubation tube (12  $\times$  75 mm glass) received, in the following order, 0.5 ml buffer (with or without an excess of dopamine, the final concentration of which was 30  $\mu$ M), 0.5 ml [<sup>3</sup>H]spiperone (final concentration of approximately 250 pM; ~100 Ci/mmol; 2.2–3.7 TBq/mmol, from Amersham Life Sciences, Oakville, Ontario, Canada), and 0.5 ml membrane suspension. The tubes were incubated for 2 h at room temperature (20°C), after which the incubates were fil-

tered, using a 12-well cell harvester (Titertek, Skatron, Lier, Norway) and buffer-pres soaked glass fiber filter mats (No. 7034, Skatron, Sterling, VA). After filtering the incubate, the filter mat was rinsed with buffer for 15 sec (7.5 ml buffer). The filters were pushed out and placed in scintillation minivials (Packard Instruments, Chicago, IL). The minivials received 4 ml each of scintillant (Ready Solve, Beckman Co., CA), and were monitored 6 h later for tritium in a Packard 4660 scintillation spectrometer at 55% efficiency. Nonspecific binding to dopamine D4 receptors was defined as that which occurred in the presence of 30  $\mu$ M dopamine [Van Tol et al., 1991]. The  $K_i$  values for the drugs were derived by the Cheng-Prusoff formula [1973], using the appropriate  $K_D$  value for [<sup>3</sup>H]spiperone at the receptor.

The  $K_D$  value (dissociation constant) of [<sup>3</sup>H]spiperone for the dopamine D4 receptors was measured separately. This was done using final concentrations of 10 pM to 2,000 pM for [<sup>3</sup>H]spiperone and using the same procedure outlined above for the competition-type experiments. The dissociation constants were derived by Scatchard analysis.

Cyclic AMP was measured as follows. CHO (Chinese hamster ovary) cells were plated in 6-well, 35 mm dishes 1–2 days prior to the experiment and grown to 80–100% confluency (~1.2  $\times$  10<sup>6</sup> cells/dish). The cells were washed with 1–2 ml HBBS buffer (188 mM NaCl, 4.6 mM KCl, 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 10 mM D-glucose, 20 mM Hepes and 0.3 mM IBMX [3-isobutyl-1-methylxanthine], pH 7.2). The cells were incubated in HBBS buffer with and without dopamine (0.1 nM–3  $\mu$ M) in the presence or absence of 10  $\mu$ M forskolin. The cells were incubated for 30 min at 37°C in a final volume of 1 ml. At the end of the incubation period the medium was removed by aspiration and the cells were harvested in 1 ml permeabilization buffer (1 ml of 2% Triton X-100 in 39 ml HBBS buffer). The samples were vortexed, and centrifuged for 5 min at 13,000 rpm in a microcentrifuge. The supernatant was collected and frozen at –80°C for cyclic AMP measurement by radioimmunoassay, described in the procedure recommended for using the cyclic AMP antiserum (Sigma product A-0670, Sigma Chemical Co., St. Louis, MO). Briefly, the lysates of the permeabilized cells were diluted 20-fold with ice-cold 0.05 M sodium acetate buffer, pH 6.2. The diluted samples (100  $\mu$ l) were transferred in duplicate to polypropylene test tubes and succinylated by addition of 20  $\mu$ l succinylation reagent (200 mg succinic anhydride dissolved in 1 ml dry acetone to which triethylamine was added at a ratio of 25:9 [vol:vol; succinic anhydride:triethylamine]). Ice-cold 0.05 M sodium acetate buffer (1.9 ml) was added and mixed with the sample. Each succinylated sample of 100  $\mu$ l received 100  $\mu$ l of cAMP antiserum. The antiserum was prepared by dissolving 5 mg of lyophilized powder in 1 ml 0.01% bovine serum albumin (BSA) and diluted 10-fold with 0.01% BSA. After adding the antiserum, the samples were vortexed and incubated for 4 h at 0°C. Each sample then received 100  $\mu$ l of adenosine 3',5'-cyclic phosphoric acid 2'-O-succinyl [<sup>125</sup>I]iodotyrosine methyl ester (4.5 nCi/sample, diluted in acetate buffer). The tubes were vortexed and incubated for 18–20 h at 4°C.

Each tube then received 100  $\mu$ l of 10% BSA, followed by the addition of 2 ml of ice-cold ethanol. The tubes were vortexed and centrifuged at  $3,500 \times g$  for 15 min at 4°C. The supernatant was removed by aspiration. The amount of radioactivity retained in the pellet was determined and converted to a corresponding amount of cyclic AMP from a standard curve with a range of 0–5,000 fmol cyclic AMP per 100  $\mu$ l sample volume measured in parallel.

The restriction enzyme, *Acc* I, was used to determine the genotype of patient DF. The DNA sequence of the common form of the dopamine D4 receptor contains a site (corresponding to valine-194) which is sensitive to the cutting action of *Acc* I [Van Tol et al., 1991]. Thus, the DNA sequence of the 259 bases (the first part of exon 3, including the oligonucleotide primer) was cut by *Acc* I into two lengths of 193 and 66 bases. The dopamine D4<sub>Valine194Glycine</sub> receptor variant, however, has a change in one nucleotide wherein T is replaced by G, resulting in the variant being insensitive to the cutting action of *Acc* I [Seeman et al., 1994]. Hence, the enzyme, *Acc* I was used to genotype patient DF by determining whether the action of *Acc* I (acting on the PCR product of exon 3) revealed sequences of 193 or 259 bases on the agarose gel. Thus, a sequence length of 193 bases indicated a homozygous person having the common form of the dopamine D4 receptor, while a sequence length of 259 bases revealed a homozygous individual having the D4 variant, while a pattern of bands at both 193 and 259 indicated a heterozygote having both the common D4 and the D4<sub>Valine194Glycine</sub> receptor variant.

## RESULTS

Compared to the dopamine D4 receptor, the dissociation constants for dopamine, clozapine and olanzapine for the D4<sub>Valine194Glycine</sub> receptor were markedly increased by at least two orders of magnitude (Fig. 1; Table I). The density of the stably expressed D4<sub>Valine194Glycine</sub> receptor averaged  $12.6 \pm 2.2$  fmol/mg protein ( $n = 7$ ), compared to  $54 \pm 8$  fmol/mg protein ( $n = 5$ ) for that of the stably expressed dopamine D4 receptor. The dissociation constant of [<sup>3</sup>H]spiperone,  $K_D$ , for the D4<sub>Valine194Glycine</sub> receptor variant was  $136 \pm 24$  pM ( $n = 5$ ), compared to  $89 \pm 8$  pM ( $n = 88$ ) for the dopamine D4 receptor (Fig. 2 and Table I). The inhibition constant for spiperone,  $K_i$ , was 127 pM on the variant, compared to 160 pM for the D4 receptor (Table I).

The inhibition by dopamine of [<sup>3</sup>H]spiperone binding to the dopamine D4<sub>Valine194Glycine</sub> receptor variant was relatively insensitive to 200  $\mu$ M guanylimidodiphosphate (or Gpp[NH]p) when compared to the common D4 receptor. For example, dopamine inhibited the binding of [<sup>3</sup>H]spiperone at D4 with a  $K_i$  of 148 nM (Table I). Using a curve-fitting procedure [George et al., 1985], this  $K_i$  could be resolved into a high-affinity component at 25 nM dopamine and a low-affinity component at 1,000 nM dopamine, each component consisting of 50% of the total sites (Table I). Guanine nucleotide (i.e., Gpp[NH]p) converted all the high-affinity sites (having a dopamine  $K_i$  of 25 nM) to low-affinity sites having a dopamine  $K_i$  of 413 nM (Table I), a shift of 16-fold. As indicated in Figure 1 and Table I, however, dopamine

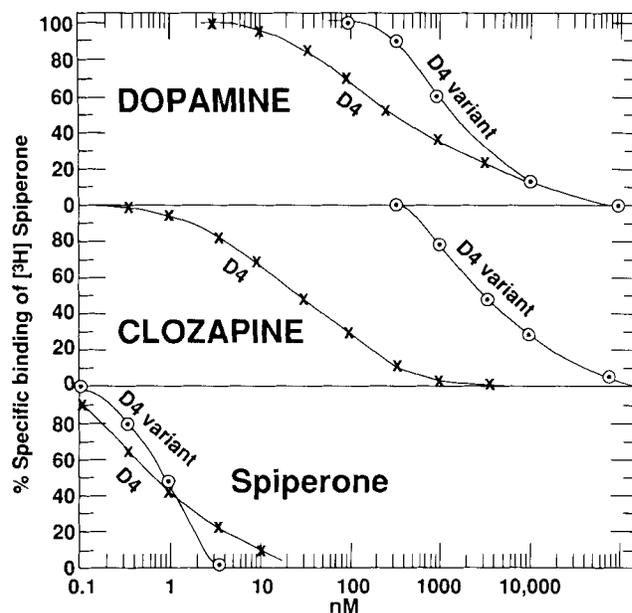


Fig. 1. Representative experiments for various drugs competing against 250 pM [<sup>3</sup>H]spiperone for binding to the common form of the dopamine D4 receptor and to the dopamine D4<sub>Valine194Glycine</sub> receptor variant. The total amount of specific binding was 600 DPM/filter. The  $K_i$  values for the dopamine D4 receptor and the D4<sub>Valine194Glycine</sub> receptor, respectively, were 0.11 nM and 0.13 nM for spiperone, 70 nM and 457 nM for dopamine, and 22 nM and 886 nM for clozapine. Nonspecific binding defined by 30 to 100  $\mu$ M dopamine.

inhibited the binding of [<sup>3</sup>H]spiperone to the D4<sub>Valine194Glycine</sub> receptor variant at only the low-affinity site, as indicated by the high  $K_i$  of 3,700 nM dopamine, comprising 100% of the sites. Because all the sites were in their low-affinity state for dopamine, the effect of guanine nucleotide was relatively small, the dopamine  $K_i$  going from 3,700 nM to 7,100 nM (Table I), a shift of only 1.9-fold.

The data in Figure 3 illustrate that the dopamine D4<sub>Valine194Glycine</sub> receptor was insensitive to dopamine when tested for the inhibition of adenylate cyclase.

Patient DF was found when screening DNA samples for the dopamine D4<sub>Valine194Glycine</sub> receptor variant [Seeman et al., 1994]. The PCR product of exon 3 [280 base pairs] of this patient's dopamine D4<sub>Valine194Glycine</sub> receptor was not sensitive to the cutting action of the restriction enzyme *Acc* I, as shown in Figure 4. This enzyme did, however, cut the PCR product of exon 3 of the regular dopamine D4 receptor (Fig. 4). Figure 4 illustrates that patient DF was homozygous for the dopamine D4<sub>Valine194Glycine</sub> receptor variant because there was only a single uncut band upon exposure to *Acc* I.

Patient DF is a 15-year-old boy who lives in Jamaica. He weighs 81 lbs (36.6 kg) and his height is 5 ft, 2" (157 cm). He was first seen clinically when he was one year old because of anemia. At two years of age, he was diagnosed with homozygous sickle cell disease. Because of this, he has been followed at the Sickle Cell clinic at the University of the West Indies. The patient's height and weight were both below the 3rd percentile for his age. His height had been in the 25th percentile between

TABLE I. Drug dissociation constants

	D4 <sup>b</sup> K, nM (n) [ <sup>3</sup> H]spiperone <sup>c,d</sup>	D4 <sup>Valine194Glycine</sup> K, nM (n) [ <sup>3</sup> H]spiperone <sup>c,d</sup>
Chlorpromazine	9.6 (3)	19 (2)
Clozapine	22.4 ± 1.8 (19)	4,500 (3)
Dopamine, at 50% inhibition	148 (6)	3,700 (4)
at high-affinity state	25 (50% sites)	no high-affinity state
at low-affinity state	1,000 (50% sites)	3,700 (100% sites)
Dopamine + GppNHp <sup>a</sup>		
at 50% inhibition	413 (7)	7,100 (2)
at high-affinity state	no high-affinity state	no high-affinity state
at low-affinity state	413 (100% sites)	7,100 (100% sites)
Haloperidol	2.2 ± 0.3 (24)	47 (3)
Noradrenaline	2,200 (3)	8,000 (2)
Olanzapine	21 (4)	>10,000
Raclopride	2,300 ± 290 (13)	4,300 (2)
Serotonin	4,400	12,000 (2)
Spiperone	0.16 (6)	0.127 (3)
[ <sup>3</sup> H]Spiperone	0.089 ± 0.008 (88)	0.136 ± 0.024 (5)

<sup>a</sup>200 μM guanylimidodiphosphate.

<sup>b</sup>Cloned dopamine D4.4 or D4.2 receptors were used, with identical results.

<sup>c</sup>Final concentration of [<sup>3</sup>H]spiperone was 250 pM.

<sup>d</sup>Nonspecific binding defined by 30 μM dopamine.

ages 8 and 12 years. His weight has always been below the 10th percentile and had recently changed to below the 3rd percentile. His current height is in the 3rd percentile. He attends a regular school, and his grade level is average for his years. He is good at mathematics but is not keen on reading. He sometimes has transient dark spots in his eye fields. At other times he cannot see from the corners of his eyes and has to turn his head to see objects. This complaint has never persisted and he did not seek medical attention for this. He has no other complaints but admits to intermittent joint pains and shortness of breath on running long distances. There were no symptoms of muscle weakness, excessive sweating, episodes of tachycardia, or sensory or cerebellar disturbances.

Neurological development was normal. He lives a life described as "normal" but, because of his slight build, he avoids playing with bigger boys. He is one of two children by the same father, and his sister who is 13 years old is presently asymptomatic. Examination showed him to be of slight build. There was no axillary

or pubic hair. There was no lymphadenopathy. His pulse rate was 80/min with sinus arrhythmia. There was a soft systolic murmur at the apex and a loud pulmonary component to the second heart sound (P2) at the base. The liver was soft and palpable. He had a palpable and moderately enlarged spleen from 2 to 5 cm below the left costal margin; this splenomegaly has been sustained.

He was examined by a neurologist who conducted a formal mental status exam. The clinical findings were not remarkable. He has had no mood swings, depressive episodes, or indications of psychiatric disturbance. He showed no cognitive or personality defects; he was pleasant and cooperative with a good sense of humour.

The neurological examination was normal. The skull, cranial nerves, funduscopy and visual fields were normal. Posture, power, tone, coordination, sensation, and tendon reflexes were all normal. Abdominal reflexes were equal, and plantars were flexor. The current hemoglobin was 5.4 g/dl with no evidence of iron or folate deficiency. The reticulocyte count was 15%.

## DISCUSSION

The insensitivity of this variant receptor to dopamine and to guanine nucleotide suggests that this receptor does not exist in a high-affinity state. Based on these *in vitro* data, it may be speculated that the dopamine D4<sup>Valine194Glycine</sup> receptor variant may also not be functional *in vivo*, because the high-affinity state of dopamine receptors is generally considered to be the functional state [George et al., 1985]. Such speculation suggests that the homozygous patient DF may not have any functional dopamine D4 receptors in his tissues. In addition, this aberrant receptor may affect the clinical action of anti-dopamine drugs. For example, if the dopamine D4 receptor is a primary target in the clinical action of clozapine [Van Tol et al., 1991; Seeman, 1992], it is possible that such homozygous individuals

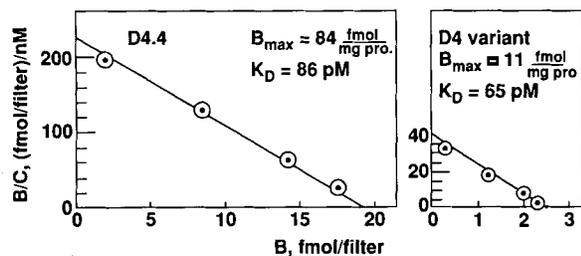


Fig. 2. Representative results (Scatchard analysis) for the dissociation constant of [<sup>3</sup>H]spiperone, K<sub>D</sub>, for the D4<sup>Valine194Glycine</sup> receptor variant (right) and for the dopamine D4 receptor (left, where D4.4 indicates a D4 receptor containing 4 repeat units, as explained in the text). The D4 variant expressed a lower density of receptors compared to the D4.4 receptor (in fmol per mg protein).

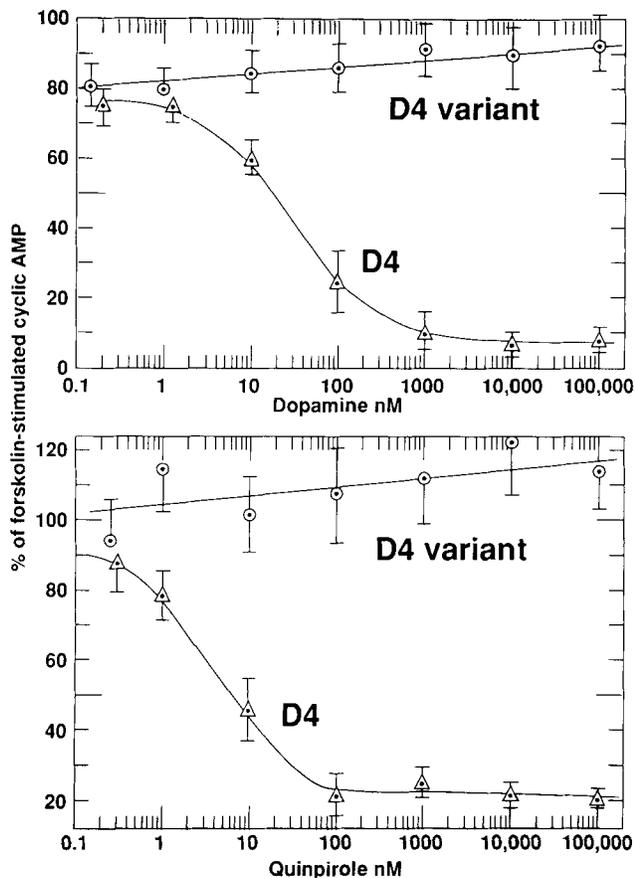


Fig. 3. Dopamine and quinpirole inhibited the production of cyclic AMP (adenosine monophosphate) in the dopamine D4.4 receptor but not in the D4<sup>Valine194Glycine</sup> receptor. The value of 100% represents the amount of cyclic AMP produced by 10  $\mu$ M forskolin alone. The number of fmoles of the D4<sup>Valine194Glycine</sup> receptor per tube was between 10% and 20% of the number of fmoles in the tubes containing the dopamine D4.4 receptors.

who happen to become psychotic may also be resistant to the action of clozapine.

Most or all of his clinical symptoms and signs can be attributed to his sickle cell disease. These include the heart sounds, the very low hemoglobin (ranging from 4.5 to 6.3 g/dl), and the dyspnea on exertion. The clinical picture, however, is not typical of hypersplenism.

The patient's slow growth appears greater than usually found in homozygous sickle cell disease. The overall pattern of low weight and no axillary or pubic hair appears more than can be accounted for by the sickle cell disease. However, before determining the clinical relevance of the dopamine D4<sup>Valine194Glycine</sup> receptor variant, it will be essential to locate a homozygous individual who has no other major genetic illness.

#### ACKNOWLEDGMENTS

We thank Dr. Mary V. Seeman and Dr. Susan R. George for helpful clinical advice, and we thank Dr. H.-C. Guan and Ms. Vera Jovanovic for excellent assistance. We thank Sandoz Pharmaceuticals, Basel (Dr. K. Saameli, Dr. P. Lardi and Dr. H. Friedli) for donating

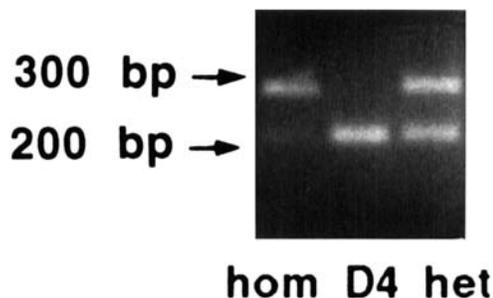


Fig. 4. The first part of exon 3 (259 base pairs, including the oligonucleotide primers) was amplified by the PCR method. The restriction enzyme *Acc* I could cut the PCR product of the dopamine D4 receptor but not the product of the dopamine D4<sup>Valine194Glycine</sup> receptor. Thus, the lower band (193 base pairs) indicates the shorter length of the dopamine D4 receptor (**D4** lane) which was cut by *Acc* I. The upper band (259 base pairs) indicates that the dopamine D4<sup>Valine194Glycine</sup> receptor variant was resistant to the cutting action of *Acc* I (**hom**: homozygous individual DF). For purposes of comparison, a heterozygous pattern is included (**het**).

clozapine (HF 1854), and we thank Dr. David O. Calligaro and Lilly Research Laboratories (Greenfield, IN) for donating olanzapine. We thank Dr. George Holme and Dr. Lorraine Paul of Sandoz Canada Inc. (Montreal, Que., and Toronto, Ont.) for facilitating this research. We thank the following for their generous support of this research: Dr. E. Fuller Torrey and the Stanley Foundation Scholars Program of the National Alliance for the Mentally Ill (Arlington, Virginia); Eva and Leon Adomavicius (Toronto, Ontario); the John William Medland Fund, University of Toronto; the Ontario Mental Health Foundation; and the Medical Research Council of Canada in partnership with Sandoz Canada Inc. (Montreal, Que.). H.H.M. Van Tol is a Career Scientist of the Ontario Ministry of Health.

#### REFERENCES

- Cheng Y-C, Prusoff WH (1973): Relationship between the inhibition constant ( $K_i$ ) and the concentration of inhibitor which causes 50 percent inhibition ( $I_{50}$ ) of an enzymatic reaction. *Biochem Pharmacol* 22:3099-3108.
- Cox BA, Henningsen RA, Spanoyannis A, Neve RL, Neve KA (1992): Contributions of conserved serine residues to the interactions of ligands with dopamine D<sub>2</sub> receptors. *J Neurochem* 59:627-635.
- George SR, Watanabe M, Di Paolo T, Labrie P, Seeman P (1985): The functional state of the dopamine receptor in the anterior pituitary is in the high-affinity form. *Endocrinology* 117:690-697.
- Nöthen MM, Cichon S, Hemmer S, Hebebrand J, Remschmidt H, Lehmkuhl G, Poustka F, Schmidt M, Catalano M, Fimmers R, Körner J, Rietschel M, Propping P (1994): Human dopamine D4 receptor gene: Frequent occurrence of a null allele and observation of homozygosity. *Hum Mol Genet* 3:2207-2212.
- Sanger F, Miklen S, Coulson AR (1977): DNA sequencing with chain termination inhibitors. *Proc Nat Acad Sci USA* 74:5463-5467.
- Seeman P (1992): Dopamine receptor sequences. Therapeutic levels of neuroleptics occupy D<sub>2</sub>, clozapine occupies D<sub>4</sub>. *Neuropsychopharmacology* 7:261-284.
- Seeman P, Ulpian C, Wreggett KA, Wells J (1984): Dopamine receptor parameters detected by <sup>3</sup>H-spiperone depend on tissue concentration: Analysis and examples. *J Neurochem* 43:221-235.
- Seeman P, Ulpian C, Chouinard G, Van Tol HHM, Dwosh H, Lieberman JA, Siminovich K, Liu ISC, Wayne J, Voruganti P, Hudson C, Serjeant GR, Masibay AS, Seeman MV (1994): Dopamine D4 receptor variant, D4194GLYCINE, in Africans, but not in Caucasians: No association with schizophrenia. *Am J Med Genet* 54:384-390.

Van Tol HHM, Bunzow JR, Guan H-C, Sunahara RK, Seeman P, Niznik HB, Civelli O (1991): Cloning of the gene for a human D<sub>4</sub> receptor with high affinity for the antipsychotic clozapine. *Nature* 350:610-614.

Van Tol HHM, Wu CM, Guan H-C, Ohara K, Bunzow JR, Civelli O, Kennedy J, Seeman P, Niznik HB, Jovanovic V (1992): Multiple dopamine D<sub>4</sub> receptor variants in the human population. *Nature* 358:149-152.