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ORIGINAL INVESTIGATION

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Polymorphisms of the dopamine D_4 receptor and response to antipsychotic drugs

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Abstract The dopamine D_4 receptor may be a site through which the clinical effects of antipsychotic drugs are mediated. Polymorphisms of a 48 base pair repeat in the third exon of the DRD4 gene code for different length segments in the third intracytoplasmic loop of the D_4 receptor. The most common long (seven repeat) form of the D_4 receptor has been shown in both physiologic and pharmacologic experiments to respond differently to dopamine agonists and antagonists than do shorter forms of D_4. Thus, variants of D_4 may partly determine patient response to antipsychotic drugs and, in particular, response to typical neuroleptics, which have a relatively low affinity for the D_4 receptor, as compared to clozapine, which has a relatively high affinity for D_4. DRD4 polymorphisms in the third intron were characterized in 28 patients with chronic psychosis who responded well to typical neuroleptics, 32 patients who responded well to clozapine, and 57 healthy comparison subjects. Patients responding to typical neuroleptics carried the allele for the long (seven repeat) form of the D_4 receptor (allele frequency 8.9%) less frequently than patients responding to clozapine (allele frequency 23.4%, $P = 0.046$) or healthy comparison subjects (allele frequency 26.3%, $P = 0.004$). The results of this study suggest that inherited variants of D_4 may explain some of the interindividual variation seen in patient response to different classes of antipsychotic medication.

Key words Dopamine · Polymorphism · Antipsychotic · Receptor

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Introduction

Inherited factors may not only underlie the predisposition to psychotic disorders, they may also determine response to treatment (Cohen and Zubenko 1985). The exact nature of the factors mediating therapeutic response are not known. However, elements of the dopamine neurotransmitter system are likely candidates, as drugs altering dopamine neurotransmission can mimic or ameliorate the symptoms of psychosis (Baldessarini 1996).

An inherited element of particular relevance may be the dopamine D_4 receptor, the product of one of five genes thought to code for functional dopamine receptors in human beings (Van Tol et al. 1991). While less densely expressed in the brain than the closely related D_2 and D_3 receptors, the D_4 receptor is localized to sites in frontal cortex and temporal lobe believed to be associated with symptom production in psychosis (Joyce and Meador-Woodruff 1997). All neuroleptic antipsychotic drugs, the most effective treatments for psychosis, are antagonists at D_4 receptors. In fact, clozapine, an unusually effective antipsychotic drug for acute, chronic, and refractory psychosis (Baldessarini and Frankenburg 1991), is notable for having a high affinity for D_4 relative to other dopamine receptors (Van Tol et al. 1991; Lahti et al. 1993).

In human populations, Van Tol and colleagues (1992) and O'Dowd (1992) observed inherited polymorphisms of the gene, DRD4, which codes for the D_4 receptor. Specifically, they reported a 48 base pair sequence which commonly occurred in 2-, 4- or 7-fold repeats in the third exon of DRD4. This gene segment codes for a 16 amino acid repeated sequence in the putative third intracellular loop of the D_4 protein. This loop of the receptor, in turn, is thought to bind G-proteins, which couple receptor activation to intracellular second messengers. It is not proven that the presence of alternative forms of the D_4 receptor has physiologic consequences in human brain. However, when expressed in

cultured cells, longer forms (notably the form with seven repeats, $D_{4.7}$) have a blunted response to dopamine inhibition of cAMP formation (Asghari et al. 1995). In addition, longer ($D_{4.7}$) forms of the D_4 receptor show a different effect of NaC1 on the relative affinity of clozapine and spiperone than do shorter ($D_{4.2}$, $D_{4.4}$) forms (Van Tol et al. 1992; Asghari et al. 1994). Past studies suggest that the presence of the seven repeat allele of the DRD4 gene may be associated with behavioral changes in human subjects. Specifically, $D_{4.7}$ is more common in children with attention deficit hyperactivity disorder than in healthy comparison subjects (LaHoste et al. 1996). Also, two of three studies (Benjamin et al. 1996; Ebstein et al. 1996; Jonsson et al. 1997) suggest that individuals inheriting the 4.7 allele of the D_4 receptor have more novelty-seeking personality traits. However, for patients with schizophrenic disorders, neither linkage (Coon et al. 1993; Macciardi et al. 1994; Maier et al. 1994) nor association (Nanko et al. 1993; Sommer et al. 1993; Macciardi et al. 1994) studies find that the D_4 receptor, and specifically the inheritance of the 4.7 allele of the D_4 receptor, affects the risk of developing psychosis.

While they may not be strong determinants of the risk for psychotic illness, polymorphisms of DRD4 may modify response to treatment. Although polymorphisms affecting the number of 48 base pair repeats do not appear to correlate with the degree of response to clozapine in patients resistant to typical neuroleptics (Shaikh et al. 1993; Rao et al. 1994; Rietschel et al. 1996), no study has tested whether patients who *do* respond to typical neuroleptics are different in inherited forms of the D_4 receptor from those who do not respond to typical neuroleptics but do respond to clozapine.

This possibility is tested in the current study. Differences in allele frequencies in patients showing a good response to typical agents versus those showing a good response to clozapine would suggest that D_4 might, in part, determine efficacy or side effects produced by different classes of antipsychotic drugs.

Materials and methods

Subjects

Subjects between the ages of 20 and 60 years were recruited from the outpatient clinics of McLean Hospital. Written informed consent, as approved by the McLean Institutional Review Board, was obtained from all study participants. All patients had a chronic psychotic disorder requiring continuous treatment with antipsychotic medication and had been receiving their current antipsychotic medication regimen for at least 1 year. Patients in the group responding to typical neuroleptics were prescribed chlorpromazine (one), fluphenazine (five), haloperidol (five), loxapine (one), mesoridazine (one), perphenazine (seven), thiothixene (five), or trifluoperazine (three). No subjects were receiving risperidone or olanzapine. All subjects receiving clozapine ($n = 32$) had previously received two or more trials on regimens of typical neuroleptics. In the group treated with typical neuroleptics, all but two of 28 subjects were receiving other medications, including combinations of anticholinergics, antidepressants, anticonvulsants, anxiolytics, beta blockers and lithium. In the clozapine-treated group, a larger proportion, nine of 32 subjects, were receiving clozapine alone, but the majority of subjects were receiving one or more other medications from the classes listed above. Not enough subjects were treated with an antipsychotic medication alone to perform a separate analysis of this group.

Subjects received a DSM-IV diagnosis based on SCID standardized interviews (Spitzer et al. 1990). Healthy comparison subjects were staff members or volunteers obtained by advertisements for other approved studies who had no history of psychiatric illness, substance abuse, or treatment with psychotropic medications.

All subjects used in the analysis were Caucasian and from families of European origin. For the 28 subjects receiving typical neuroleptics, average age was 35.0 years (range 20–53), of whom 19 were male and nine were female, with nine having a diagnosis of schizophrenia, 13 schizoaffective disorder, and six bipolar disorder. For the 32 subjects receiving clozapine, average age was 36.6 years (range 20–58), of whom 21 were male and 11 female, with 11 having a diagnosis of schizophrenia, 14 schizoaffective disorder, and seven bipolar disorder. There were 57 healthy comparison subjects, whose average age was 31.4 years (range 20–57), of whom 36 were male and 21 female.

Genotyping

Human blood samples were drawn from an antecubital vein in a 10 ml K_2EDTA containing Vacutainer. Lymphocytes were isolated from 6.0 ml human blood using Histopaque (Sigma Chemical Corp, St Louis, Mo., USA). Genomic DNA was isolated from the lymphocytes by standard methods (Maniatis et al. 1982). A 25 ng sample of the genomic DNA was subjected to PCR amplification in a 20 µl reaction, under the following conditions: 10 mM TRIS-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.001% gelatin, 10% DMSO, 200 µM each of dATP, dCTP, 7-deaza-guanosine and dTTP, 0.55 µM of each primer, and 1 U native Taq DNA polymerase (Perkin-Elmer Corp., Norwalk, Conn., USA). The design of suitable primers was facilitated by using the computer program OSP (Hillier and Greene 1991). The primers [hD_4A717-F (GCCCGCTCATGCTGCTGCTC, sense bp 717–736) and hD_4A1016-R (TCTTGGCACGCCGCCTCCTG, antisense bp 997–1016)] were constructed to amplify a DNA sequence which spans a polymorphic 48 bp repeat originally described for the human DRD4 dopamine receptor sequence HUMD4DOP (Accession no. X58497) (Van Tol et al. 1992). Oligonucleotides were synthesized on an ABI DNA synthesizer (ABI, Foster City, Calif., USA), desalted and used without further purification. The antisense primer hD_4A1016-R was 5′ end-labeled by reaction with T4 polynucleotide kinase (Promega, Madison, Wisc., USA) and r-^{32}P ATP (NEN, Boston, Mass., USA). To reduce the amplification of non-specific products, a "hot start PCR" was used in which the primers and genomic DNA were preheated to 95°C for 5 min prior to the addition of the remaining PCR reagents. Thermal cycling was immediately commenced using the following thermal profile: denaturation at 95°C for 30 s, annealing at 65°C for 1 min, and extension at 72°C for 1 min. This was continued for a total of 30 cycles, followed by a final extension step for 5 min at 72°C. PCR reactions products were separated by gel electrophoresis, under denaturing conditions, using 5% acrylamide. Labeled PCR products were visualized by autoradiography and their molecular weight determined by comparison to a 1 KB DNA ladder (GIBCO/BRL, Bethesda, Md., USA) 5′ end-labeled with ^{32}P.

A representative autoradiogram used to determine the 48 bp D_4 dopamine receptor polymorphism originally described by Van Tol et al. (1991) is presented in Fig. 1. In each case, the genotyping was derived from at least three independent PCR amplification

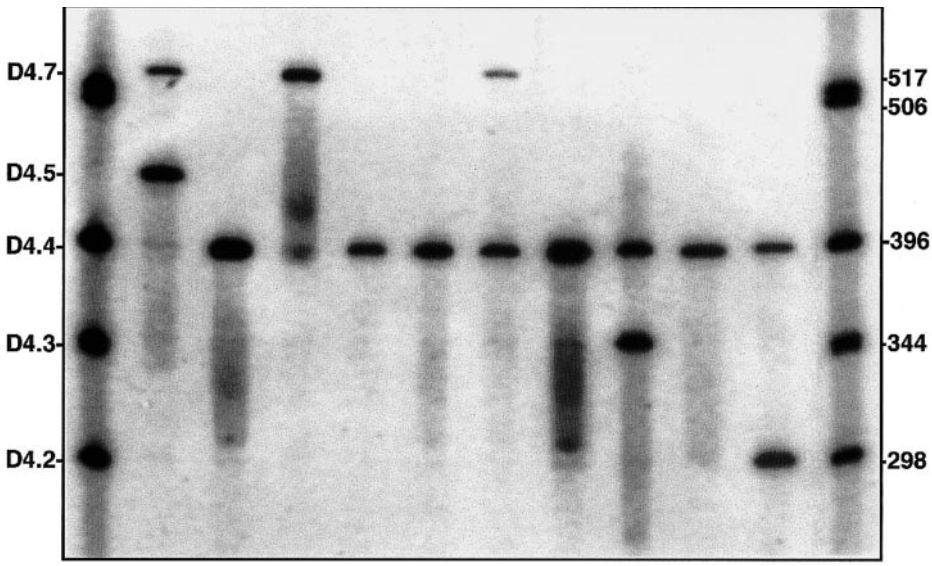

Fig. 1 Results of a single amplification, gel separation and radiolabelling procedure are shown. *Lanes 1* and *12* are 1 kb ladders. Other lanes are subject samples, interpreted as follows: *Lane 2* D4.5, 4.7; *Lane 3* D4.4, 4.4; *Lane 4* D4.7, 4.7; *Lane 5* D4.4, 4.4; *Lane 6* D4.4, 4.4; *Lane 7* 12-D4.4, 4.7; *Lane 8* D4.4, 4.4; *Lane 9* D4.3, 4.4; *Lane 10* D4.4, 4.4; *Lane 11* D4.2, 4.4

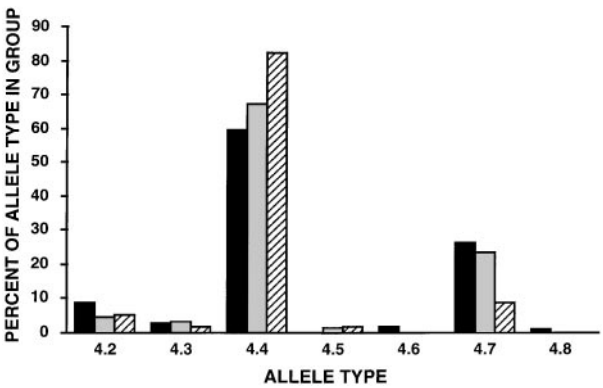

Fig. 2 Distribution of DRD4 alleles with two (*4.2*) to eight (*4.8*) repeats are shown by subject group (■ control, healthy comparison group; ▒ clozapine, patients receiving clozapine; ▨ TYP NLP, patients receiving typical neuroleptics). Allele frequency in TYP NLP's group differs from that in controls ($P = 0.005$) and clozapine ($P = 0.031$) groups: 2×2 chi square analysis

reactions. Scoring of the genotype was confirmed in double blind fashion by agreement of two investigators.

Results

The distribution of alleles of the DRD4 gene for each subject group is shown in Fig. 2. As in past studies (Shaikh et al. 1993; Rao et al. 1994; Ebstein et al. 1996), the forms of D_4 with four and seven repeats are most common in all groups tested. Since past studies in D_4 expressing cells have shown pharmacologic differences between the $D_{4.4}$ and $D_{4.7}$ forms of D_4, and most of the alleles observed were in these classes, statistical tests are reported comparing the relative frequency of these polymorphisms in each group. The results do not change qualitatively if the comparison made is between the prevalence of $D_{4.7}$ and the sum of $D_{4.2}$ and $D_{4.4}$ alleles or between $D_{4.7}$ and all alleles shorter than $D_{4.7}$.

Patients responding to typical neuroleptics had a lower frequency of the $D_{4.7}$ allele relative to $D_{4.4}$ than either the healthy comparison subjects ($P = 0.0043$) or the patients responding to clozapine ($P = 0.046$) (Fisher "exact" test for contingency tables).

In order to estimate the magnitude of these differences, rather than merely assess their statistical significance, we used a method of constructing confidence intervals for 2×2 tables, based on the non-central hypergeometric distribution, that is valid for small cell sizes (Thomas 1971). This method estimates the odds ratio, defined as

$$\frac{p_1/q_1}{p_2/q_2}$$

where, in this context, p_1 is the frequency of the $D_{4.7}$ allele in the first group, q_1 is the frequency of the $D_{4.4}$ allele in the first group, and p_2 and q_2 have the same meanings for the second group.

In the case of the comparison between patients responding to typical neuroleptics and healthy controls, the $D_{4.7}/D_{4.4}$ ratio was 0.109 for the neuroleptic-responders and 0.441 for the controls; the odds ratio (ratio of ratios) was 0.246. The limit of the 95% confidence interval, based on the upper tail of the distribution, was 0.621, which is substantially below the null hypothesis value of 1.0, and suggests a relatively strong effect. In the case of the comparison between neuroleptic-responders and clozapine-responders, the $D_{4.7}/D_{4.4}$ ratio was 0.109 for the neuroleptic-responders and 0.349 for the clozapine-responders; the odds ratio was 0.312. The limit of the 95% confidence interval, based on the upper tail of the distribution, was 0.864, which, while still below the null hypothesis value of 1.0, indicates that a weak but non-zero effect cannot be ruled out.

The strength of the difference between neuroleptic responders and healthy controls made it possible to

detect a statistically significant difference in the distribution of D_4 alleles even when all seven alleles were taken into account ($P = 0.024$, Fisher "exact" test for a 2×7 contingency table). Our planned comparisons were restricted to $D_{4.4}$ and $D_{4.7}$, however, because our small sample size would not have given sufficient power to detect differences in tables with many small cells where we had no prior hypotheses concerning those cells.

No significant differences were observed in the relative frequency of $D_{4.4}$ and $D_{4.7}$ between the group responding to clozapine and the healthy comparison group.

Analysis of the data by genotype rather than allele frequency reveals the same association. That is, subjects who are homozygous or hemizygous for $D_{4.7}$ are less common among patients who respond to typical neuroleptics than among either patients who respond to clozapine or healthy comparison subjects. As there were few homozygotes for $D_{4.7}$ (five in the healthy comparison group, two in the clozapine group, none in the neuroleptic group), it is not possible to comment on the response of $D_{4.7}$ homozygotes to medication.

Discussion

The results suggest that patients with chronic psychotic disorders, requiring continuous treatment with antipsychotic drugs, are less likely to respond to typical neuroleptic antipsychotic agents if they carry a gene for the long form of the dopamine D_4 receptor.

The relative probability of a favorable response to typical neuroleptics is

$$\frac{P(\text{responder}\,|\,D_{4.7})}{P(\text{responder}\,|\,D_{4.4})}$$

where $P(\cdot|\cdot)$ means conditional probability, and "responder" means responder to typical neuroleptics. By Bayes' theorem, this ratio is equal to

$$\frac{P(D_{4.7}\,|\,\text{responder})/P(D_{4.4}\,|\,\text{responder})}{P(D_{4.7}\,|\,\text{chronic psychosis})/P(D_{4.4}\,|\,\text{chronic psychosis})}$$

and since, as noted in the Introduction, the allele ratio for chronic psychotic patients does not differ from that for healthy controls, the denominator can be replaced by

$$\frac{P(D_{4.7}\,|\,\text{control})}{P(D_{4.4}\,|\,\text{control})}$$

and with this change, the ratio is just the odds ratio used in the statistical analysis (Results). In other words,

$$\frac{P(\text{responder}\,|\,D_{4.7})}{P(\text{responder}\,|\,D_{4.4})}$$

with a 95% upper confidence limit of 0.621.

If the observed association is true, it is possible that patients with a long form of D_4 are less sensitive to the therapeutic effects or more sensitive to the side effects of typical neuroleptics than are patients with shorter forms of D_4. Perhaps the D_4 receptor contributes to the antipsychotic effects of neuroleptics, and typical neuroleptics are not adequately potent at $D_{4.7}$ receptors to produce full therapeutic benefits. Alternatively, D_4 receptors may participate in determining the severity of side effects of neuroleptics. In either case, it is plausible that the distinction between typical neuroleptics, with their low relative affinity for D_4 receptors, and clozapine, with its high relative affinity for D_4, would be exacerbated in patients with the longer $D_{4.7}$ form of the receptor.

Early studies with specific antagonists of the D_4 receptor do not demonstrate that D_4 antagonism, alone, produces a clinically significant antipsychotic effect (Kramer et al. 1997). Similarly, the absence of functional D_4 receptors, as observed in a single subject homozygous for a truncated and probably inactive form of D_4, does not appear to produce neuropsychiatric symptoms (Nothen et al. 1994). Thus, the D_4 receptor may not be essential for brain function or sufficient to mediate the actions of antipsychotic drugs. Nonetheless, D_4 antagonism may modify the production of therapeutic or side effects of antipsychotic agents. We do not have reliable information on whether our subjects on clozapine abandoned treatment with typical neuroleptics due to inadequate beneficial effects or excessive side effects and cannot draw conclusions on these competing possibilities.

It is, of course, possible that the findings observed are due to chance alone or sampling bias. Regarding the latter, the samples compared do not differ by ethnic background or, for treated patients, by diagnosis.

On the basis of these preliminary findings, it may be of value for centers with comparable populations to compare genotypes of the DRD4 gene in groups of patients who respond to typical neuroleptics and those who respond to clozapine. Confirmation of these results could provide a clue to one pathway through which the effects of antipsychotic drugs are mediated.

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