A Y-chromosomal DNA Fragment Is Conserved in Human and Chimpanzee

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A human male-specific Y-chromosomal DNA fragment (λYH2D6) has been isolated. By deletion mapping analysis, 2D6 has been localized to the euchromatic portion of the long arm (Yq11) of the human Y chromosome. Among great apes, this fragment was found to be conserved in male chimpanzee but was lacking in male gorilla and male orangutan. No homologous fragments were detected in females of orangutan, gorilla, chimpanzee, or human. Nucleotide sequence analysis indicated the presence of partial-Alu-elements and of sequences similar to the GATA repeats of the snake Bkm sequence.

Introduction

The sex-chromosome pair is thought to have evolved from a common autosomal pair (Ohno 1967, pp. 5–13). Unlike the human X chromosome, which has over 150 genes mapped so far, the Y chromosome is relatively devoid of functional genes (Stewart 1983). The short arm of the human Y chromosome (Yp) contains genes coding for MIC2, ZFY, GM-CSF receptor, and SRY (Darling et al. 1986; Page et al. 1987; Gough et al. 1990; Sinclair et al. 1990). Although many loci have been assigned to the long arm (Yq) of the Y chromosome (Weissenbach et al. 1989), few genes have been mapped there (Anderson et al. 1988). Repetitive elements such as DYZ1 and DYZ2 are known to constitute ≥50% of the human Y chromosome (Willard 1985; Smith et al. 1987). These repeat elements are believed to have originated recently during evolution, because similar sequences have not been found in great apes (Smith et al. 1987).

Because the majority of the human Y chromosome consists of noncoding sequences, there is little selective pressure to eliminate mutations in the Y-chromosomal DNA (Charlesworth 1978). The sequence divergence of the Y chromosome in related species, therefore, would be primarily due to random mutational and transpositional events during evolution. Thus, a comparative analysis of Y-chromosomal DNA sequences among related genera and species can be useful in refining the evolutionary classification and phylogenetic order.

Among primates, the phylogenetic relationship of the human, chimpanzee, gorilla,
and orangutan has been controversial (Templeton 1985; Lewin 1987; Holmquist et al. 1988b). In recent years, molecular hybridization data have suggested a closer relationship between human and chimpanzee (Sibley and Ahlquist 1984, 1987; Sibley et al. 1990). Moreover, comparative analyses of DNA sequences of globin and immunoglobulin genes of human and apes support the conclusions from the DNA hybridization studies (Miyamoto et al. 1987; Ueda et al. 1988). These data, however, are in direct conflict both with the data based on morphological analyses and with the data based on mitochondrial ribosomal genes, data which suggest a closer relationship between gorilla and chimpanzee (Delson et al. 1977; Hixson and Brown 1986). A single clade consisting of orangutans and humans has also been proposed, on the basis of morphological features alone (Schwartz 1984).

The molecular evidence to support the human-chimpanzee clade is derived primarily from the statistical analyses of DNA-DNA hybridization studies and from the globin, immunoglobulin, and mitochondrial DNA sequence data (Sibley and Ahlquist 1984, 1987; Miyamoto et al. 1987; Holmquist et al. 1988a; Ueda et al. 1988). Unfortunately, the conclusions derived from these studies do not lead to an unambiguous determination of the phylogenetic branching order among hominids. The reasons for the difficulty in determining the precise phylogenetic order by using such analyses have been elegantly summarized by Holmquist et al. (1988b).

We have isolated two human Y-chromosomal DNA fragments from an EcoRI-cut chromosome-specific genomic library constructed in λCharon 21A. The evolutionary pattern of these two fragments, 1F5 and 2D6 (DYS128 and DYS129, respectively, in HGM 10), has been examined in primates. Our data indicate that one of these fragments, 1F5, is a Y-specific fragment conserved in great apes (Whisenant et al., in press). We now report that, unlike 1F5, the second fragment (2D6), is conserved only in the human and chimpanzee. Homologous fragments have not been detected in either gorilla or orangutan or Old World monkeys. This fragment, therefore, is a member of a new class of Y-DNA fragments that should prove useful in refining the phylogenetic order in hominids. In addition, sequence analysis of 2D6 revealed the presence of two atypical Alu elements and a Bkm-like sequence.

Material and Methods

Material

Chimpanzee (Pan troglodytes), gorilla (Gorilla gorilla), orangutan (Pongo pygmaeus), gibbon (Hylobates lar), and rhesus (Macaca mulatta) blood were supplied by Yerkes Primate Center (Atlanta). Human DNA was isolated from peripheral blood lymphocytes. The human Y-chromosome library, constructed from flow-sorted, EcoRI-digested DNA in λCharon 21A, was purchased from American Type Culture Collection (Rockville, Md.). All restriction enzymes were from Brisco Ltd. (Winthrop, Mass.). The Sequenase sequencing kit was supplied by U.S. Biochemicals Corporation (Cleveland). Hybond-N membrane and radioisotopes were purchased from Amersham (Arlington Heights, Ill.). The lymphoblastoid cell line, VGD1, carrying a X:Y translocation was a gift from Dr. V. G. Dev. A human cell line, Oxen, carrying multiple Y chromosomes (46, XYYYY) was obtained from Dr. D. Page. Other cell lines, lymphoblasts, and fibroblasts, were obtained from the NIGMS Cell Repository, Coriell Institute (Camden, N.J.).
Isolation of Recombinant Clone

The Y-chromosome library was screened essentially according the method described by Whisenant et al. (in press). In brief, \( \sim 1,000 \) plaques were spotted on Nylon membrane, and the DNA was probed with labeled human female DNA. Positive plaques were discarded, and the rest were probed successively with labeled male mouse total DNA and with cDNA reverse transcribed from mouse testis poly A+ RNA, to obtain clones containing conserved (and possibly transcribed) sequences.

Hybridization

DNA from lymphocytes was prepared by standard procedures (Maniatis et al. 1982). The DNA sample from different species was digested with restriction enzyme according to the manufacturer's protocol, was electrophoresed, and was Southern blotted (Maniatis et al. 1982). Radioactive probes were prepared by labeling the DNA fragment with \( ^{32}P \)-CTP by the random-primer-labeling method (Feinberg and Vogelstein 1983). Hybridization was carried out by standard procedure (Maniatis et al. 1982). The following temperatures were used during washing of Southern blots in 0.1 \( \times \) SSC [saline sodium citrate (0.15 NaCl, 0.015 M sodium citrate)] and 0.1% SDS (sodium dodecyl sulfate): 45°C for low-stringency washes, 50°C for low-moderate-stringency washes, 55°C for moderate-stringency washes, 60°C for moderate-high-stringency washes, and 65°C for high-stringency washes. For rehybridization, the probe was stripped by incubating the blots in boiling distilled water for 2 \( \times \) 15 min.

We estimate that the hybridization conditions used in the present study detect DNA-DNA hybrids of \( >75\% \) sequence similarity at low-stringency washing conditions (45°C), \( >85\% \) sequence similarity at moderate-stringency washing conditions (55°C), or \( >95\% \) sequence similarity at high-stringency washing conditions (65°C).

DNA Sequencing

The Y-chromosomal insert was subcloned into the pTZ series of plasmid vectors, and the nucleotide sequence was determined by the dideoxy chain-termination method (Sanger et al. 1977). Sequencing reactions were carried out in the presence of \( ^{35}S \)-ATP and M13 universal primer, according to the manufacturer's recommendations. The sequence was analyzed by the PCS-DNA sequence-analysis package written by L. M. Lagrimini and S. T. Brentano (University of Iowa, Iowa City). Two DNA-sequence data bases, GenBank and EMBL, were searched for the presence of similar sequences.

Results

The strategy for the isolation of Y-specific DNA led to the identification of two cloned fragments, 1F5 and 2D6. Following are the results of our studies carried out with the cloned 2D6 fragment.

Chromosomal Assignment of 2D6 in Humans

The clone 2D6 contained a 3.1-kb Y-chromosomal insert. DNA from a panel of human males and females, from hamster/human hybrid cells containing human X (GM06318B) or Y chromosome (GM06317), and from an Oxen (49,XYYYY) cell line was digested with EcoRI and hybridized to 2D6. The results of hybridization are shown in figure 1. In blots washed at moderate stringency (fig. 1, panel A), a cognate
3.1-kb band was seen in male genomic DNA. In addition, fainter bands at 3.4 and 3.5 kb were seen. The lane containing hamster/human hybrid genomic DNA (lane Y) showed 3.1- and 3.4-kb bands, indicating their location on the human Y chromosome. In human female DNA, only the 3.5-kb band was observed. The 3.5-kb band was not seen in hamster/human hybrids containing either the X or the Y chromosome, which suggested an autosomal location for this fragment. In blots washed at moderate-high stringency (fig. 1, panel B), the 3.5-kb band was not seen, and only the male-specific 3.1- and 3.4-kb bands were seen. At the high-stringency conditions used in the present study (fig. 1, panel C), 2D6 hybridized to only a 3.1-kb male-specific band. The increased intensity of the 3.1-kb band seen in Oxen DNA compared with normal male DNA further confirmed its location on the Y chromosome. The blot was stripped of its probe and was rehybridized to ZFY (Y-chromosome-linked zinc finger protein; Page et al. 1987) as a control to verify the extent of DNA digestion and hybridization conditions (data not shown). Titration studies revealed the presence of ~5–10 copies of 2D6 in the human male genome (data not shown).

Regional Mapping of 2D6

To identify its subchromosomal location, EcoRI-cut DNA preparations from a panel of cell lines containing deletions or translocations involving the Y chromosome
were Southern blotted and probed with 2D6. Both the karyotype of the cell lines examined and the hybridization results are summarized in Table 1. Control experiments, using ZFY as a probe (Page et al. 1987), showed expected hybridization patterns for all cell lines except GM2730, which lacked the 3.7-kb Y-linked band (data not shown).

The 3.1-kb, 2D6 band was detected in GM09996 and VGD1 (Fig. 2, panel A, lanes 1 and 6). GM09996 contains a deletion in the Yq portion and lacks the entire Yq12 along with a portion of Yq11 (Table 1). VGD1 has a translocation of an almost entire Yq to the X chromosome. The 2D6 fragment was not detectable in DNA from cell line GM2103, which has a translocation of Yq12 and a portion of Yq11. Taken together, these results suggested the location of 2D6 in Yq11.

In addition, the 2D6-hybridizing 3.4-kb band was detected (Fig. 2, panel B) in blots washed at moderate-high stringency, in the cell lines GM2103 (lane 4) and VGD1 (lane 6). These results indicated the location of the 2D6-hybridizing 3.4-kb band in the Yq11-Yqter region distal to the 3.1-kb 2D6 band.

Evolutionary Conservation of 2D6

We have examined the evolutionary conservation of 2D6 in primates. The hybridization pattern of 2D6 in EcoRI-cut DNA of male and female chimpanzee, gorilla, and orangutan is shown in Figure 3, panel A. A 3.1-kb band, similar in size to the band detected in human male (Fig. 3, panel B), is seen in blots of chimpanzee male DNA digests washed at high stringency. No hybridization of 2D6 was observed with other male and female primate DNA preparations (Fig. 3).

To examine the presence of 2D6-related sequences in detail, Southern blots containing male orangutan, gorilla, chimpanzee, and human genomic EcoRI digests were hybridized to 2D6 and were washed at different stringencies (Fig. 4). At low-stringency washing conditions (Fig. 4, panel A), a smear was seen in all the DNA preparations examined, which may be due to the hybridization of 2D6 to repeated DNA elements. At moderately stringent washing conditions (Fig. 4, panel B), the 3.1-kb band was clearly visible in chimpanzee and human. In addition, a very faint 3.5-kb band, similar to the male/female shared band in the human, was visible in chimpanzee. However, no band corresponding to the Y-specific 3.4-kb human band was present in chimpanzee. Curiously, a band at 4 kb in the male gorilla and faint bands at 3.7 and 4.2

<table>
<thead>
<tr>
<th>CELL LINE</th>
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<tr>
<td></td>
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<tr>
<td>GM2103</td>
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<td>M</td>
<td>46,XX</td>
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<td>GM9996</td>
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<td>F</td>
<td>46,X.t(X;Y)(XPter&gt;Xq11::Yq11&gt;Yqter)</td>
<td>+</td>
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* Plus (+) and minus (-) signs indicate, respectively, presence and absence of hybridizing band(s) in Southern blots of EcoRI-digested DNA at moderate-stringency washing conditions. However, at highest stringency conditions only the 3.1-kb band was detectable.
Fig. 2.—Regional assignment of 2D6. Genomic DNA preparations (EcoRI cut) are from human male (lane M), human female (lane F), GM09996 (lane 1), GM2668 (lane 2), GM2730 (lane 3), GM2103 (lane 4), GM2626 (lane 5), and VGD1 (lane 6). Blots washed under moderate-stringency (panel A), moderate-high-stringency (panel B), and high-stringency (panel C) conditions are shown. The autosomal derived 3.5-kb band is lost under moderate-high-stringency washing conditions (panel B), indicating less sequence similarity with 2D6 compared with the 3.4-kb/2D6 hybrid, which is stable under these conditions (panel B). The 3.4-kb fragment is seen in both X:Y translocations (GM2103 and VGD1), suggesting its location in the Yq region (lanes 4 and 6). The 2D6 fragment is present in both GM09996 (lane 1) and VGD1 (lane 6), indicating the overlapping nature of these two cell lines.

kb in the male orangutan were also visible. In blots washed at high stringency (fig. 4, panel C), 2D6 hybridized to only chimpanzee and human DNA. We have repeated these studies with three different orangutans, two gorillas, and two chimpanzees and have consistently observed the same hybridization pattern (data not shown). Rehybridization of the blot with ZFY showed species-specific hybridization patterns (fig. 4, panel D).

Restriction-Enzyme-Site Analysis

Hybridization studies with EcoRI-digested primate DNA indicated that, among the great apes, conservation of 2D6 occurred only in chimpanzee and human. However, it is possible that similar fragments present in gorilla and orangutan may have escaped detection because of alteration of the EcoRI recognition site. To rule out such a possibility, DNA from orangutan, gorilla, chimpanzee, and human were double digested with other restriction enzymes (the enzymes chosen—PstI, TaqI, and MspI—cut within the 3.1-kb sequence defined by the 2D6 probe), were Southern blotted, and were probed with 2D6 under moderately stringent washing conditions (fig. 5). Once again, 2D6-hybridizing bands were seen only in chimpanzee and human (fig. 5, panels A and C). Faint bands at 1.3 and 1.8 kb were visible in gorilla DNA digested with TaqI (fig. 5, panel B).
FIG. 3.—Evolutionary conservation of 2D6. Approximately 5 μg of DNA was digested with EcoRI, was Southern blotted, and was probed with 2D6. The autoradiograph shown was from a blot washed at high stringency. The DNA samples in panel A were from male orangutan (PONGO, lane M), female orangutan (PONGO, lane F), male gorilla (GORILLA, lane M), female gorilla (GORILLA, lane F), male chimpanzee (PAN, lane M), and female chimpanzee (PAN, lane F). The DNA samples in panel B were from human male (HOMO, lane M) and human female (HOMO, lane F). Note the presence of a single 3.1-kb fragment in male human and the chimpanzee genomic digests.

From figure 5 it is apparent that the two PstI restriction sites present in the human 2D6 were also present in the corresponding chimpanzee fragment (panel A). Similar-size fragments were also observed in chimpanzee and human DNA digested with PstI alone, indicating conservation of PstI sites beyond the 3.1-kb EcoRI sites (data not shown). Only one of the two TaqI sites present in human was conserved in chimpanzee (fig. 5, panel B), while the MspI site, present in human, was missing in chimpanzee (fig. 5, panel C). In addition, the XbaI site present in human was also conserved in chimpanzee (data not shown). A restriction map of the human and chimpanzee 3.1-kb fragments that depicts common restriction-enzyme sites is shown in figure 6.
Nucleotide Sequence of 2D6

Because 2D6 represents a new class of Y-chromosomal DNA fragments conserved only in chimpanzee and human, it was important to determine its nucleotide sequence. The complete nucleotide sequence of 2D6 is shown in figure 7. The sequence consists of 3,142 nucleotide pairs, and is AT rich (65.3% AT). Data-base searches (GENBANK and EMBL) failed to identify similar sequence(s). The sequence lacks long open reading frames. A number of interesting sequence regions were identified in the 2D6 sequence: (1) a 146-bp-long region (positions 1175–1320, underlined in fig. 7) and a 95-bp-long region (positions 1731–1825, underlined in fig. 7) which shows \(~\text{75\%}\) sequence similarity to human Alu consensus sequence; (2) a 112-bp-long region (positions 2530–2641, double underlined in fig. 7) which contains 12 noncontiguous GATA repeats and exhibits \(\text{80\%}\) sequence similarity to the GATA repeat region of \textit{Elaphe radiata} W chromosome sex-specific satellite DNA (banded krait minor, or Bkm sequence); (3) a 14 consecutive TC repeats (positions 1316–1343; PUPPY sequence); and (4) eight CA repeats (positions 1343–1358).

1. \textit{Alu}-like Sequences in 2D6

In 2D6, there are two discontinuous regions with similarities to the human consensus Alu element. The first of these, a 146-bp-long (positions 1175–1320) segment
Fig. 5.—Restriction-site analysis. Genomic DNA samples (EcoRI cut) from male orangutan (lanes Po), gorilla (lanes G), chimpanzee (lanes Pa), and human (lanes H) were cut with PstI (panel A), TaqI (panel B), or MspI (panel C) and Southern blotted. The Southern blots were probed with 2D6 under moderately stringent washing conditions, to examine restriction-site conservation. Fragments (>3.1 kb) seen in panels A and C (lanes H) are probably derived from the 3.4-kb fragment. Male chimpanzee DNA lacks MspI sites (panel C, lane Pa). A 600-bp fragment was faintly seen in TaqI digests of male human DNA (panel B, lane H) and is not shown here.

exhibits 72% sequence similarity with the right half of the Alu consensus sequence, while the second element, a 95-bp-long segment (positions 1731–1825), has ~80% sequence similarity with the left half of the Alu consensus sequence (fig. 8). The right-half element is present almost intact, except for the first 14 bp and 3′ A-rich region. By contrast, in the truncated left-half element, the first 40 bp of the consensus Alu element are lacking. A 3′ A-rich region is present in the left-half element, followed by what appears to be a near-direct repeat (TAATAACTACAATA). However, the near-direct repeat flanking the 5′ end (TAATAACTACATA) is located 60 bp upstream from the beginning of the Alu sequence.

2. Polypyrimidine-Polypurine (PUPPY) Sequences

The right half of the Alu element in 2D6 is followed by a 26-bp segment that on one strand is composed solely of pyrimidines (CT) and that on the other strand is composed solely of purine residues (GA). These regions, known as PUPPY sequences, have been shown to form non-B-DNA structures such as triple helices (H-DNA) and are implicated in recombinational events (Wells et al. 1988; Htun and Dahlberg 1989).

3. Bkm-related Sequence in 2D6

A 112-bp-long segment (positions 2530–2641) in the 2D6 sequence showed 80% sequence similarity to the GATA repeats of the colubrid snake W-chromosome sex-
specific satellite DNA (Epplen et al. 1982). There are 12 discontinuous GATA repeats in this region (fig. 9). In a span of 210 bp—positions 2504–2714—there are 21 GATA (quadruplet) repeats. Moreover, the same segment of 2D6 sequence in its reverse orientation was found to contain 22 GATA repeats. The GATA repeats found in 2D6 are not organized as a long series of perfect simple repeats, as present in the snake W chromosome, but are interrupted by other sequences.

Discussion

The studies presented here have led to the isolation and sequence determination of a human Y-linked DNA fragment, 2D6, which is the first member of a new family of fragments conserved only in chimpanzee and human. Identification of such fragments from other chromosomes may help in refining the phylogenetic order of primates.

Alu-like Elements in 2D6

Alu sequences are the major short interspersed repeats found in the human genome and account for ~3%–6% of the total human genome (Jelinek and Schmid 1982). Typically, the Alu sequence exists as a dimeric unit. Although the Alu family members show DNA sequence variations, they exhibit ~87% sequence similarity with the consensus Alu sequence (Jelinek and Schmid 1982). However, the Alu sequences on the human Y chromosome display only ~60% sequence similarity to the consensus Alu element, with a relatively greater similarity to the right half than to the left half of the Alu consensus dimer (Smith et al. 1987). The Alu-like sequences present in 2D6 are ~75% similar to the human consensus Alu element. Unlike the common dimeric organization seen for the Alu family members, the Alu-like sequences in 2D6 occur as partial monomers. Our data are in general agreement with the observation that the human Y-chromosomal Alu sequences are significantly diverged from the average genomic Alu element (Smith et al. 1987).

Human Alu sequences are related to the mouse B1 repetitive elements (Jelinek and Schmid 1982). It is likely, therefore, that the atypical Alu sequences present in 2D6 were responsible for the hybridization with the mouse DNA and cDNA probes used in the screening procedure.
FIG. 7.—Nucleotide sequence of 2D6. Only one strand is shown, although both strands were sequenced. The portions of the 2D6 sequence exhibiting similarity to human Alu sequence are underlined. The region exhibiting similarity to Bkm sequence is double underlined.

Bkm-related Sequence in 2D6

The *Elaphe radiata* (Bkm) satellite DNA contains repetitive sequences hybridizing preferentially to the W chromosome (Singh et al. 1980). Nucleotide sequence analysis of a cloned W-chromosome fragment has shown that such sequences consist of long arrays of GATA quadruplet base repeats (Epplen et al. 1982). The Bkm-like sequences have been shown to be present on the *Drosophila X* chromosome, the mouse Y chromosome (Singh et al. 1984), and the equine Y chromosome (Kent et al. 1988), as
well as on the human X chromosome (Erickson et al. 1988). By contrast, there are conflicting data regarding the presence of Bkm-like sequences on the human Y chromosome (Wolfe et al. 1984; Kiel-Metzger et al. 1985; Arnemann et al. 1986; Singh and Jones 1986). Two studies, one using Bkm sequences to probe a cosmid library containing human Y-chromosome sequences (Wolfe et al. 1984) and one using in situ hybridization (Kiel-Metzger et al. 1985), failed to detect hybridizing sequences. However, Arnemann et al. (1986) have reported the presence of (GATA)4 oligonucleotide-hybridizing clones in a cosmid library constructed from a mouse/human hybrid cell line with multiple Y chromosomes. In addition, in situ hybridization data of Singh and Jones (1986) indicated the presence of Bkm-like sequences on the Y chromosome.
FIG. 9.—Sequence comparison of 2D6 and the Bkm element. The upper strand is the 2D6 sequence, and the lower strand is the GATA repeat region of cloned snake W-chromosome satellite DNA (Bkm sequence taken from Epplen et al. 1982). Identical bases are indicated by an asterisk (*). At some places, blank spaces (−) are inserted to improve the alignment of sequences.

and other acrocentric chromosomes of the human genome. The data presented here provide unequivocal evidence for both the presence of GATA repeats on the human Y chromosome and its sequence similarity with the Bkm sequences (fig. 9). These GATA repeats, however, are not arranged in tandem as seen in snakes and other species. It has been proposed that Bkm-like sequences in Drosophila, snake, and mouse are not homologous but analogous and may have arisen by convergent evolution (Levinson et al. 1985). The 2D6 Bkm-like sequences on the human Y chromosome may have a similar origin. Alternatively, these sequences may represent highly diverged evolutionary remnants of the ancestral sequence.

Evolution of 2D6 in Hominids

DNA-DNA hybridization studies using cloned human Y-chromosomal sequences have shed some light on their evolution in primates (Koenig et al. 1984, 1985; Page et al. 1984; Burk et al. 1985; Casanova et al. 1985; Bickmore and Cooke 1987; Erickson 1987; Pritchard et al. 1987; Yen et al. 1988). Our current understanding regarding the evolution of Y-linked sequences is as follows: (1) The human Y-chromosome-specific repeated sequences, DYZ1 and DYZ2, are of recent origin. The appearance of these repeated elements on the Y chromosome occurred following the divergence of the human lineage from the chimpanzee and/or gorilla lineage (Kunkel and Smith 1982; Smith et al. 1987). (2) Human X and Y shared sequences similar to DXYS1 are found only on the X chromosome of great apes. Again, such sequences on the human Y chromosome arose by transposition from the X chromosome to the Y chromosome, following the divergence of the human lineage from the chimpanzee and/or gorilla lineage (Page et al. 1984; Bickmore and Cooke 1987). (3) Many sequences specific to the Y chromosome in human and great apes are found on the X or autosomes of Old World monkeys. These sequences are more ancient. They were probably transposed, from either the X chromosome or autosomes, to the Y chromosome in the ancestors of modern hominids (Burk et al. 1985).

The cloned fragment, 2D6, described here is present only in the human and chimpanzee Y chromosomes and was not detected in the genomic DNA of other primates. There are at least two possibilities as to why this fragment is only conserved
in human and chimpanzee: (1) The 2D6 fragment became associated with the Y chromosome in the ancestor of modern human and chimpanzee. Its absence in the gorilla would suggest that the common ancestor of the gorilla-chimpanzee-human lineage lacked 2D6. The 2D6 fragment, therefore, would have originated recently during primate evolution. (2) The ancestral 2D6 sequence was present in the ancestor of modern hominids. However, the corresponding sequences present in gorilla and orangutan either have been lost through a deletional event or have diverged so much that they no longer hybridize with the human sequence. Such an explanation would require that 2D6 evolve at a faster rate than do other parts of the genome. Our data do not allow us to distinguish between the alternatives proposed here.

Restriction-site analysis detected six sites conserved between chimpanzee and human fragments, plus two additional sites only in human (fig. 6). These data may be interpreted to mean either (a) that the altered sites represent restriction-fragment-length variations or (b) that the two fragments have become diverged from each other and that their divergence is reflected in the loss of at least two (of eight) restriction sites examined here (fig. 6).

The detection of the 3.5-kb human male-female shared band by 2D6 under moderate-stringency washing conditions provided an important clue regarding the possible origin of the 3.1-kb fragment during recent primate evolution (fig. 1, panel A). The autosomal location of the 3.5-kb fragment has been deduced from the lack of this fragment in the hamster/human hybrids containing either the X or the Y chromosome (fig. 1, panel A). A partial sequence similarity between the 3.5-kb band and the Y-linked 3.1-kb 2D6 fragment (and 3.4-kb band), a similarity inferred from the hybridization data, would imply that the origin of the 2D6 fragment probably involved the 3.5-kb autosomal fragment in a transpositional event, followed by its rearrangement on the primate Y chromosome. Sequence analysis of these three fragments (3.5, 3.4, and 3.1 kb) should provide definitive evidence regarding their exact relationship and the evolution of 2D6 in higher primates.

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