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Sequence Variation in the Androgen Receptor Gene Is Not a Common Determinant of Male Sexual Orientation

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Summary

To test the hypothesis that DNA sequence variation in the androgen receptor gene plays a causal role in the development of male sexual orientation, we have (1) measured the degree of concordance of androgen receptor alleles in 36 pairs of homosexual brothers, (2) compared the lengths of polyglutamine and polyglycine tracts in the amino-terminal domain of the androgen receptor in a sample of 197 homosexual males and 213 unselected subjects, and (3) screened the the entire androgen receptor coding region for sequence variation by PCR and denaturing gradient-gel electrophoresis (DGGE) and/or single-strand conformation polymorphism analysis in 20 homosexual males with homosexual or bisexual brothers and one homosexual male with no homosexual brothers, and screened the amino-terminal domain of the receptor for sequence variation in an additional 44 homosexual males, 37 of whom had one or more first- or second-degree male relatives who were either homosexual or bisexual. These analyses show that (1) homosexual brothers are as likely to be discordant as concordant for androgen receptor alleles; (2) there are no large-scale differences between the distributions of polyglycine or polyglutamine tract lengths in the homosexual and control groups; and (3) coding region sequence variation is not commonly found within the androgen receptor gene of homosexual men. The DGGE screen identified two rare amino acid substitutions, ser^{205}-to-arg and glu^{793}-to-asp, the biological significance of which is unknown.

Introduction

Differences in sexual orientation represent one of the most common human behavioral variations. For example, in the United States, approximately 95% of the adult population is predominantly heterosexual, and several percent of the population is predominantly homosexual (Kinsey 1948, 1953). Despite widespread interest in sexual orientation, little is known about the mechanisms underlying its development.

Psychoanalytic and other environment-based theories of the development of sexual orientation emphasize social and family experiences (Friedman 1988). However, several lines of evidence suggest that in some individuals sexual orientation may be significantly influenced by biological variables. Prospective studies show that the majority of boys who display a high level of feminine (i.e., gender-nonconforming) behavior develop a homosexual orientation in adulthood (Zuger 1984; Green 1985), and retrospective studies show that a substantially higher fraction of homosexual than heterosexual men recall gender-nonconforming behavior in childhood (Bell et al. 1981). These behaviors often develop early and persist despite parental and peer disapproval, suggesting an innate predisposition. Neuro-

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anatomic studies have reported differences between heterosexual and homosexual men in the size of three structures—the suprachiasmatic nucleus (Swaab and Hoffman 1990), the third interstitial nucleus of the hypothalamus (LeVay 1991), and the anterior commissure of the corpus callosum (Allen and Gorski 1992). While these findings suggest neuroanatomic correlates for differences in sexual orientation, they do not indicate whether anatomic differences represent a primary biological variable or a secondary response to physiological or behavioral inputs.

Several studies have addressed the question of genetic influences in sexual orientation. A familial clustering of male homosexuality has been reported, a result that is consistent with a genetic component (Pillard and Weinrich 1986). Studies of concordance among MZ and DZ twins are also consistent with a genetic component but have given quantitatively variable results, most likely for methodological reasons. One early study of male MZ twins reported 100% concordance for homosexuality (Kallmann 1952), a result that has not been replicated in subsequent studies. A second study that included both males and females found a concordance rate for homosexuality or bisexuality of 25% among MZ twins and 12% among DZ twins (King and McDonald 1992). In the largest twin studies to date, the concordance rate for homosexuality or bisexuality was 52% among MZ male twins, 48% among MZ female twins, 22% among DZ male twins, and 16% among DZ female twins (Bailey and Pillard 1991; Bailey et al. 1993). These data suggest that the overall heritability for sexual orientation is approximately 50%.

During the past 50 years a large body of work has delineated the influence of gonadal steroids on sexual behavior in humans and in laboratory animals. This work has led to the proposal that gonadal steroids act during a critical period in development to influence adult sexual orientation (Dorner et al. 1975; Dorner 1980). In both male and female rats, perinatal exposure to androgens produces stereotypically male sexual behavior in adulthood (Goy and McEwen 1980). Conversely, low androgen levels during the perinatal period produce stereotypically female sexual behavior in adulthood. In human males, the level of serum testosterone is elevated during the second trimester of gestation and during the first 3 mo of postnatal life, after which it remains at a low level until puberty (Griffin and Wilson 1992). In human females, testosterone levels are low throughout life. Females who are born with congenital adrenal hyperplasia, a disorder that leads to excessive pre- and postnatal testosterone production, show an increased incidence of both gender-nonconforming behavior in childhood and homosexuality in adulthood (Ehrhardt and Meyer-Bahlburg 1981; Money et al. 1984). These behavioral effects are seen even though postnatal testosterone levels are maintained within normal limits by medication. As yet there have been no attempts to correlate fetal or neonatal androgen levels with sexual orientation in males, whereas numerous studies have shown that differences in circulating testosterone levels in adult males do not correlate with sexual orientation (reviewed by Meyer-Bahlburg 1984).

To explore the possibility that inherited variations in androgen action play a causal role in the development of sexual orientation, we have undertaken a genetic analysis of the androgen receptor in homosexual males. The androgen receptor plays a central and essential role in androgen action. A large number of mutations, which lead to various degrees of hormone insensitivity, have previously been described in the androgen receptor (reviewed in McPhaul et al. 1993). The most severe receptor defects lead to the syndrome of complete androgen insensitivity, in which chromosomal males have female external genitalia, secondary sexual characteristics, gender identity, and sexual behavior. The extent to which female gender identity and sexual behavior in these individuals are influenced by social factors or by the lack of androgen receptor function in the brain is not known. Less severe receptor defects lead to intermediate degrees of somatic feminization and, in the mildest cases, infertility in an otherwise normally developed and heterosexual male.

Any model linking variation in androgen receptor function to sexual orientation would require a selective effect within the central nervous system, as there has been no replicable finding that sexual orientation covaries with gonadal function, genital anatomy, or any other morphological secondary sexual characteristic. It is interesting that the recent identification of the molecular basis of spinal bulbar muscular atrophy (SBMA), a disorder in which there is a progressive degeneration of spinal motor neurons, suggests that a subset of androgen receptor sequence variants has effects that are manifest primarily within the central nervous system. SBMA is caused by an expansion of a polyglutamine tract in the androgen receptor, from a normal range of 15–30 amino acids to greater than 40 amino acids (La Spada et al. 1991, 1992). The polyglutamine tract is located within the amino-terminal 550 amino acids of the androgen receptor, a domain that is required for transcriptional activation (Jenster et al. 1992). By contrast, all of the inactivating amino acid substitutions

responsible for the classic androgen-insensitivity syndromes map to the carboxy-terminal DNA- and steroid-binding domains. These data suggest a model in which sequences within the amino-terminal domain modulate receptor activity in a tissue-specific manner.

To test the hypothesis that sequence variation in the androgen receptor plays a causal role in the development of male sexual orientation, we have (1) measured the genetic linkage between the androgen receptor locus and sexual orientation in 36 families in which there are two or more homosexual brothers; (2) compared the lengths of two polymorphic amino acid repeats in the amino-terminal domain of the androgen receptor in a sample of 197 homosexual males and 213 unselected subjects; and (3) screened the entire androgen receptor coding region for sequence variation by PCR and denaturing gradient-gel electrophoresis (DGGE) and/or by single- strand conformation polymorphism analysis (SSCP), in 20 homosexual males with homosexual or bisexual brothers and one homosexual male with no homosexual brothers, and screened the amino-terminal domain of the androgen receptor for sequence variation in an additional 44 homosexual males.

Material and Methods

Recruiting Subjects

Subjects were recruited by word of mouth, through private physicians, through newspaper advertisements, and through the following institutions or organizations: Parents and Friends of Lesbians and Gays, The Whitman Walker Clinic, and the Department of Psychiatry at The Johns Hopkins University School of Medicine. No effort was made to obtain a representative sampling of the homosexual population. Informed consent was obtained from each subject in accordance with the guidelines of the National Institutes of Health or The Johns Hopkins University School of Medicine. Eighteen subjects had a MZ twin, and 10 had a DZ twin; these subjects had participated in an earlier genetic study of male sexual orientation (Bailey and Pillard 1991). The nontwin subjects are likely to have a higher-than-average frequency of homosexual relatives, as several of the advertisements used for recruiting requested only subjects with homosexual relatives. Each subject underwent an extensive interview or filled out a detailed questionnaire covering his psychological and sexual histories. All homosexual subjects had a combined Kinsey scales score of greater than 4, with more than 90% in the "predominantly or exclusively homosexual" range (Kinsey et al. 1948). The subjects also rated the sexual orientations of their immediate relatives. Control subjects were anonymous participants from unrelated studies. The general control group consisted of 213 unrelated subjects recruited from throughout the United States for a study of inherited variation in visual function. The African-American control group consisted of 90 unrelated participants (53 males and 37 females) in a sickle-cell screening study.

PCR Analysis

Ten to 20 cc of venous blood was collected from each participant, and genomic DNA was purified as described elsewhere (Sung et al. 1991). PCR primer sequences are listed in table 1. For DGGE, seven overlapping fragments of approximately 250 bp were synthesized to amplify the first exon, excluding the polyglutamine and polyglycine tracts. Exons 2–8, together with at least 30 bp of flanking intron sequence, were each amplified as a single PCR fragment. In each reaction one primer carried a 40-bp GC-rich segment (a "GC-clamp") at the 5′ end to facilitate detection of mutations by DGGE (Sheffield et al. 1989). Several segments in the first exon amplified poorly using *Taq* polymerase under standard conditions; these segments were amplified with *Pfu* polymerase (Stratagene), using conditions recommended by the manufacturer. To increase the sensitivity of DGGE, heteroduplexes were formed between pairs of samples by an additional cycle of denaturation and renaturation; for those fragments carrying a variant sequence, the mismatched heteroduplexes were observed to migrate considerably more slowly than either homoduplex (Sheffield et al. 1989). PCR products were resolved by DGGE according to methods described elsewhere (Myers et al. 1987; Brown et al. 1990).

SSCP analysis of exons 4–8 was performed essentially as described by Batch et al. (1992) using the primers labeled "DH" in table 1. Exons 6 and 7 were analyzed as single PCR fragments, whereas exons 4, 5, and 8 were digested with restriction endonucleases to reduce the fragment sizes (*Hae*II plus *Hin*fI for exon 4; *Pmi*I for exon 5; and *Msp*I for exon 8). The alpha-^{32}P dCTP–labeled PCR reaction products were analyzed by electrophoresis through Mutation Detection Enhancement gels (AT Biochem, Malvern, PA) containing 10% glycerol and 0.6 × Tris borate/EDTA at 6–8 W for 12–14 h.

Those PCR products that produced a variant pattern

Table 1

PCR Primers Used to Amplify the Androgen Receptor Coding Region

Primer	Exon (strand[a])	Sequence[b]
JN 394	1 (s)	(GC)AAGCTTGGTGGAAGATTCAGCCAAGCTCAA
JM 110	1 (a)	CTGGAATTCCTGCTGCTGCAGCAGCAGCAAACTGGCGCC
JN 395	1 (s)	(GC)AAGCTTCAGCAGCAAGAGACTAGCCCCAGG
JM 104	1 (a)	CAGGAATTCTTTAAGGTCAGCGGAGCAGCTGCTTAAGCC
JN 396	1 (s)	(GC)AAGCTTCAGCAGCTGCCAGCACCTCCGGAC
JN 404	1 (a)	ACGTGAATTCCTCCTTGGCGTTGTCAGAAATGGT
JN 397	1 (s)	(GC)AAGCTTGCTCCCACTTCCTCCAAGGACAAT
JN 405	1 (a)	ACGTGAATTCGGAATACTCAGCAGTATCTTCAGT
JN 398	1 (s)	(GC)AAGCTTGAATGCAAAGGTTCTCTGCTAGAC
JN 406	1 (a)	ACGTGAATTCGATGCGAGCGTGGGGATGGGGAGG
JN 399	1 (s)	(GC)AAGCTTGACTACTACAACTTTCCACTGGCT
JN 407	1 (a)	ACGTGAATTCCCCACCACCACCACACGGTCCATA
JM 117	1 (s)	(GC)CGGCGGCGGCGGCGGCGAGGCGGGAGCTGTAG
JN 409	1 (a)	ACGTGAATTCCCGAAAGGCGACATTTCTGGAAGG
TB 1	2 (s)	(GC)GCCTGCAGGTTAATGCTGAAGACC
TB 2	2 (a)	CCTAAGTTATTTGATAGGGCCTTGCC
TB 3	3 (s)	(GC)TTATCAGGTCTATCAACTCTTGT
TB 4	3 (a)	CTGATGGCCACGTTGCCTATGAA
TB 5	4 (s)	(GC)GATAAATTCAAGTCTCTCTTCCT
TB 6	4 (a)	GATCCCCCTTATCTCATGCTCCC
TB 7	5 (s)	(GC)CAACCCGTCAGTACCCAGACTGACC
TB 8	5 (a)	AGCTTCACTGTCACCTCACCATC
TB 9	6 (s)	(GC)CTCTGGGCTTATTGGTAAACTTCC
TB 10	6 (a)	GTCCAGGAGCTGGCTTTTCCCTA
TB 11	7 (s)	(GC)CTTTCAGATCGGATCCAGCTATCC
TB 12	7 (a)	CTCTATCAGGCTGTTCTCCCTGAT
TB 13	8 (s)	(GC)GAGGCCACCTCCTTGTCAACCCTG
TB 14	8 (a)	GGAACATGTTCATGACAGACTGTACATCA
JM 111	1 (s)	TTCACCTCCCGGCGCCAGTTTGCTGCTGCTGC
JM 112	1 (a)	TTCTGCTGCTGCTGCCTGGGGCTAGTCTCTTG
JM 113	1 (s)	TTCATGGACCGTGTGGTGGTGGTGGGGGTGGT
JM 114	1 (a)	TTCTAGCCGTAGGGGGCTACAGCTCCCGCCTC
DHARD1	4 (s)	AAGTCTCTCTTCCTTCCCAA
DHARD2	4 (a)	GATCCCCCTTATCTCATGCT
DHARE1	5 (s)	CAACCCGTCAGTACCCAGACTGACC
DHARE2	5 (a)	GCTTCACTGTCACCCCATCACCATC
DHARF1	6 (s)	CTCTGGGCTTATTGGTAAACTTCCC
DHARF2	6 (a)	GTCCAGGAGCTGGCTTTTCCCTA
DHARG1	7 (s)	TCAGATCGGATCCAGCTATC
DHARG2	7 (a)	TCTATCAGGCTGTTCTCCCT
DHARH1	8 (s)	GAGGCCACCTCCTTGTCAAC
DHARH2	8 (a)	AAGGCACTGCAGAGGAGTAG

NOTE.—The 14 primer pairs listed at the top were used for PCR and DGGE; the two primer pairs listed in the middle were used to amplify the polyglutamine and polyglycine tracts; and the five primer pairs listed at the bottom were used for SSCP.

[a] s = sense strand; and a = antisense strand.

[b] (GC) represents a 40-bp sequence composed of guanine and cytosine, attached to the 5′ end of one member of each primer pair used for DGGE (a GC-clamp; Sheffield et al. 1989).

on DGGE or SSCP were reamplified and subcloned into a plasmid vector, and multiple subclones were sequenced from each sample. The variant sequences were verified in each case by hybridization with an allele-specific oligonucleotide probe, as described by Sung et al. (1991). For each oligonucleotide hybridization experiment, approximately 50 ng of PCR product, as determined by agarose gel electrophoresis, was denatured and loaded per slot. To measure polyglutamine and polyglycine tract lengths, PCR products were labeled during synthesis with alpha-^{32}P dCTP and resolved on a 6% denaturing acrylamide gel adjacent to a PCR product that had been amplified from a cloned and sequenced androgen receptor cDNA.

Results

Analysis of Concordance among Homosexual Brothers

If sequence variation within the androgen receptor gene were a significant determinant of sexual orientation in a large fraction of males, then there should be nonrandom segregation of particular androgen receptor alleles with either heterosexuality or homosexuality. To test this prediction, the identity or nonidentity of the androgen receptor gene within each of 36 pairs of nontwin homosexual brothers was determined, an analysis that was simplified by the X-chromosomal location of the androgen receptor gene and by the presence of a highly polymorphic polyglutamine tract within the receptor coding region. Omitting heterosexual brothers from this analysis minimizes errors associated with incomplete or delayed expression of a homosexual orientation or an unwillingness to admit homosexual behavior or feelings. Only those pairs in which both brothers were clearly homosexual were included.

For 14 pairs of brothers we were also able to obtain a blood sample from the mother or, in one instance, a sister, and for 13 of these pairs it was possible to distinguish the two maternal androgen receptor alleles by PCR amplification of the polyglutamine tracts. A pair of brothers who carry androgen receptor alleles with identically sized polyglutamine tracts, the length of which corresponds to one but not the other of the polyglutamine tracts present in their mother's DNA, are scored as concordant by descent. In the absence of data on the mother's alleles, a pair of brothers who carry androgen receptor alleles with identically sized polyglutamine tracts are scored as concordant by state. In the latter case, the high degree of polymorphism in polyglutamine tract length predicts a greater than 85% probability that the mother's androgen receptor alleles differ in these lengths (see below). A pair of brothers who carry differently sized polyglutamine tracts are scored as discordant, independent of the availability of maternal data. Finally, a pair of brothers are scored as uninformative if their mother's two androgen receptor alleles have identically sized polyglutamine tracts. Meiotic instability in polyglutamine tract length is an unlikely source of error in this analysis, as La Spada and colleagues (1992) have shown that polyglutamine tracts within the normal range are meiotically stable (no changes in length in 62 meioses examined).

Table 2

Concordance for Maternal Androgen Receptor Alleles in 36 Pairs of Homosexual Brothers

	No. of Pairs
Observed:	
Concordant by descent	8
Concordant by state	10
Discordant	17
Total	35
Uninformative	1
Expected:[a]	
Concordant	18.2
Discordant	16.8
Total	35

NOTE.—For each sample, the polyglutamine tract was amplified by PCR, and its length was determined by polyacrylamide gel electrophoresis. Because the androgen receptor gene is X-linked, only a single allele is scored in each male.

[a] On the basis of the null hypothesis (no linkage) and the allele frequencies of concordant-by-state pairs.

Table 2 summarizes the results of the linkage analysis. The data are consistent with independent segregation of the androgen receptor gene and homosexuality ($P > .5$). As indicated in table 2, the number of concordant pairs may be an overestimate, since some pairs that were scored as concordant by state may carry different maternal alleles that happened to have the same polyglutamine tract length. While these data suggest that sequence variation in the androgen receptor does not play a significant role in determining the sexual orientation of a large proportion of subjects, they do not rule out a significant role for this variation in a smaller proportion of subjects.

Distribution of Trinucleotide Repeats in the Androgen Receptor Gene

As a second approach to analyzing androgen receptor sequence variation, we compared the lengths of the polyglutamine and polyglycine tracts in the androgen

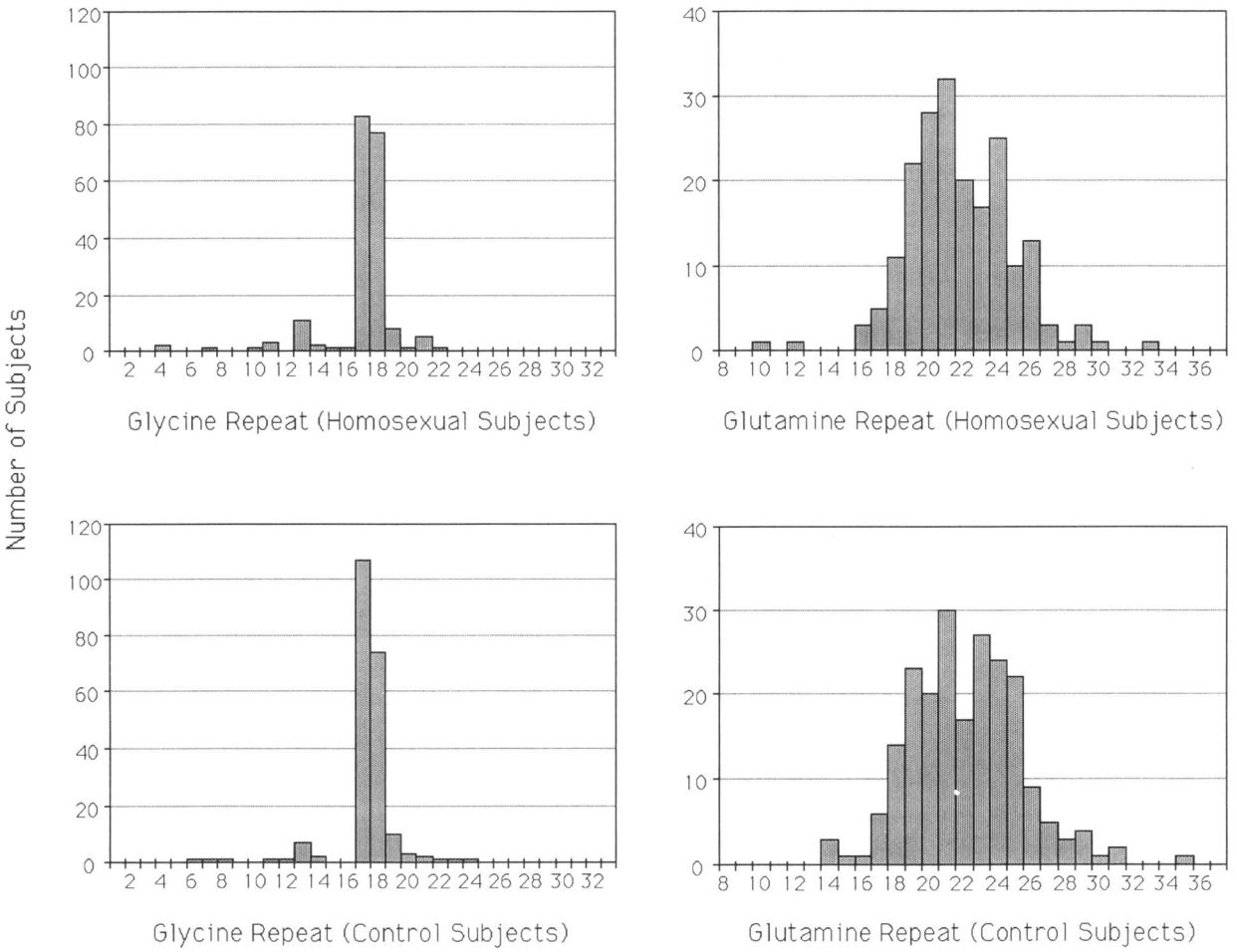

Figure 1 Distribution of polyglutamine and polyglycine repeats in the androgen receptor genes of 197 homosexual males and 213 control males of unknown sexual orientation. Repeat lengths were determined by electrophoresis of PCR products on denaturing 6% acrylamide gels, with PCR products from a cloned and sequenced androgen receptor gene used as a calibration standard.

receptor genes of 197 unrelated homosexual males and 213 unrelated control subjects of unknown sexual orientation. This experiment was stimulated by the observation that both tracts are polymorphic in the population and that expansions in the polyglutamine tract cause a distinctive central nervous system disorder, SBMA. Figure 1 shows the distribution of repeat lengths, determined by PCR amplification followed by electrophoresis on denaturing polyacrylamide gels. The polyglycine tract is significantly less polymorphic than the polyglutamine tract, and the distribution of polyglutamine tract lengths is in good agreement with that described elsewhere for Caucasians (Edwards et al. 1992). There was no correlation between polyglutamine and polyglycine tract lengths. In both cases the homosexual and control groups show a similar distribution of repeat lengths, indicating that there are no major subgroups among the homosexual subjects, which differ significantly from the general population in either polyglycine or polyglutamine tract lengths. Interestingly, each distribution has several outlying points. Among the homosexual subjects, we identified two subjects with a glycine tract length of 4 repeats, and one each with glutamine tract lengths of 10 and 12 repeats. The subject with a glutamine tract length of 12 repeats is unusual for having undergone puberty at age 17. The locations of the two tracts within the amino-terminal domain of the receptor is shown in figure 2.

Point Mutations in the Androgen Receptor

To test the possibility that small variations in the structure of the androgen receptor might influence sex-

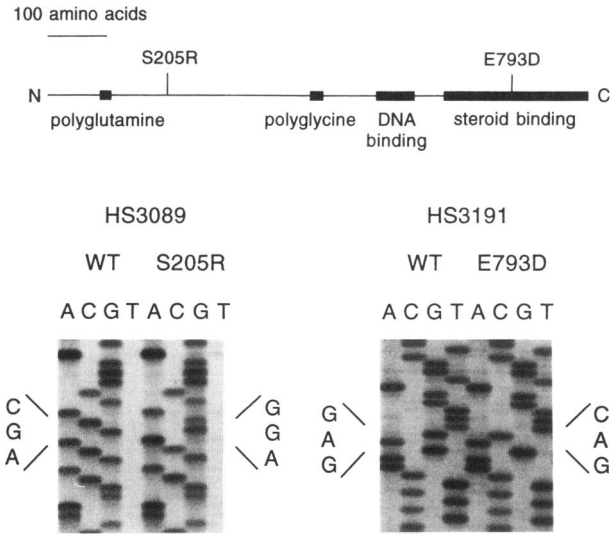

Figure 2 Amino acid substitutions in the androgen receptor genes of two homosexual males, identified by DGGE. *Top,* Map of the androgen receptor, showing the locations of the two amino acid substitutions and the functional and structural landmarks of the protein. *Bottom,* DNA sequences obtained from cloned wild-type and variant PCR products.

ual orientation in a subpopulation of homosexual males, we screened the coding region of the androgen receptor gene for variations in nucleotide sequence by using PCR and either DGGE or SSCP, or, for some segments, by both DGGE and SSCP (table 1). DGGE analysis of the entire coding region was performed on 21 subjects: 8 subjects with homosexual brothers who were genotyped and found to be concordant for the maternal androgen receptor allele (6 concordant by descent and 2 concordant by state), 12 subjects who by family history had a homosexual or bisexual brother (including 4 subjects with a homosexual MZ cotwin), and 1 subject with no known family history of homosexuality. DGGE analysis of exon 1 was performed on an additional 44 subjects: 3 subjects with homosexual brothers who were genotyped and found to be concordant for a maternal androgen receptor allele (1 concordant by descent and 2 concordant by state), 34 with one or more first- or second-degree male relatives who were identified as either homosexual or bisexual, and 7 with no known homosexual or bisexual relatives. SSCP analysis of exons 4–8 was performed on 54 subjects (exon 4), 57 subjects (exon 5), 110 subjects (exon 6), 39 subjects (exon 7), and 57 subjects (exon 8): these subjects included the members of 4–18 families in which two homosexual brothers were concordant for maternal androgen receptor alleles, 4–8 families in which two homosexual brothers were discordant for maternal androgen receptor alleles, 3–12 families in which there were two or more male homosexual relatives, and four individuals without a family history of homosexuality. Of the subjects analyzed by SSCP, six were also analyzed by DGGE. Because the sample screened by DGGE/SSCP contained a disproportionately high number of subjects with homosexual brothers who shared an androgen receptor allele, it should have been enriched for cases in which sexual orientation is associated with sequence variation in the androgen receptor gene, if such cases exist.

The combined DGGE and SSCP screening revealed three band patterns indicative of sequence alterations. One pattern was observed in approximately 10% of the X-chromosomes from both homosexual and control subjects and was derived from the silent substitution guanine^{711}-to-adenine in codon 211 (for numbering system, see Lubahn et al. 1989; the numbering systems differ among the published androgen receptor sequences because of variability in the lengths of the polyglutamine and polyglycine tracts). Two additional band patterns were observed once each among the homosexual subjects: one was found in an African-American subject and derived from cytosine^{693}-to-guanine, which produces a substitution of arginine for serine at position 205; the second was found in a Caucasian subject and derived from guanine^{3410}-to-cytosine, which produces a substitution of aspartate for glutamate at position 793. The ser^{205}- to-arg mutation was not observed in the 196 other homosexual males in this study, nor was it observed in 143 X-chromosomes from unrelated African-American control subjects, as determined by hybridization of immobilized PCR products with an allele-specific oligonucleotide. The glu^{793}-to-asp mutation also appears to be rare in the human population, as it was not present in 196 other homosexual subjects as determined by DGGE or by hybridization of immobilized PCR products with an allele-specific oligonucleotide. However, both of the subjects who carry these mutations report that one or more of their male relatives may be homosexual, and in both cases the relatives in question are related to the probands in a pattern consistent with X-linkage. At present, the significance of these observations is uncertain, as our attempts to contact and recruit these relatives to participate in this study have been unsuccessful.

Discussion

The goal of this study was to test the hypothesis that sequence variation in the androgen receptor plays a

causal role in the development of male sexual orientation. We have taken three complementary approaches: linkage analysis using pairs of homosexual brothers, measurement of repeat lengths in tracts of single amino acids that are known to be highly variable in the population, and direct screening for nucleotide sequence changes.

The linkage analysis revealed that homosexual brothers were as likely to be concordant as discordant for maternal androgen receptor alleles, indicating that sequence variation in the androgen receptor gene is not a common determinant of male sexual orientation. At the 95% confidence level, the linkage analysis indicates that 33% or less of the population shows linkage. Similarly, the analysis of polyglutamine and polyglycine tract lengths revealed no large-scale differences between the distribution of repeat lengths in a sample of 197 homosexual males and 213 unselected control subjects. These data indicate that this most common type of androgen receptor sequence variation is unlikely to be relevant to sexual orientation in the vast majority of males.

The direct search for sequence alterations by using PCR and DGGE/SSCP is consistent with the conclusion reached on the basis of the linkage experiment. However, the finding of two rare amino acid changes suggests the possibility that androgen receptor sequence variation may be relevant in a small fraction of men. Family studies and/or screening experiments on a scale far larger than that reported here will be required to test the relevance of these changes.

In conclusion, we have shown that DNA sequence variation in the androgen receptor gene does not play a major role in the development of male sexual orientation in most of the population studied. It will be of interest to test additional components involved in the action of gonadal steroids to determine whether inherited variation at other points in this system influences sexual orientation.

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