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Deletion Mapping of H-Y Antigen to the Long Arm of the Human Y Chromosome

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A gene encoding or controlling the expression of the H-Y transplantation antigen was previously mapped to the human Y chromosome. We now report the sublocalization of this gene on the long arm of the human Y chromosome. Eight patients with Y-chromosomal abnormalities were examined with a series of existing and new DNA markers for the Y chromosome. The resulting deletion map was correlated with H-Y antigen expression. We conclude that the H-Y antigen gene maps to a portion of deletion interval 6 that is identified by specific DNA markers.

INTRODUCTION

The H-Y transplantation antigen was first discovered by transplanting syngeneic male tissue into female mice (Eichwald and Silmser, 1955). Subsequently, H-Y antigen-specific T-cell responses were generated *in vitro* from mice (Gordon *et al.*, 1975) and humans (Goulmy *et al.*, 1977; reviewed in Simpson, 1982). A gene encoding or controlling the expression of the H-Y antigen was mapped to the human Y chromosome by the analysis of sex-reversed patients. Sex-reversed 46,XX males, who carry a portion of the short arm of the Y chromosome on one of their X chromosomes (Guellaen *et al*, 1984), are H-Y antigen negative, separating the loci for H-Y antigen and sex determination (Simpson *et al.*, 1987). In addition, our previous studies have shown that 46,XY sex-reversed females with deletions of the short arm of the Y chromosome (Disteche *et al.*, 1986a) are H-Y antigen positive (Simpson *et al.*, 1987). The human Y chromosome has been subdivided into seven major deletion intervals (Vergnaud *et al.*, 1986). Because the overlapping deletions of the 46,XY females cover most of the short arm of the Y chromosome (intervals 1, 2, 3, and 4A), the H-Y antigen gene is likely to be located on the proximal short arm or on the long arm of the Y chromosome (Simpson *et al.*, 1987).

In mouse, the H-Y antigen gene (Hya) is also distinct from the testis-determining gene (Tdy). Although both map to the short arm of the Y chromosome, Hya, but not Tdy, is deleted in Sxrb mice (McLaren *et al*, 1984). In XO Sxrb male mice lacking the H-Y antigen, spermatogenesis is blocked, leading Burgoyne *et al.* (1986) to suggest that the H-Y antigen gene or a gene closely linked to it plays a role in spermatogenesis. In humans, a fertility gene has been mapped to the long arm of the Y chromosome (Tiepolo and Zuffardi, 1976). This fertility gene might or might not be identical to the H-Y antigen gene.

In this paper, we report the sublocalization of a gene encoding or controlling the expression of the H-Y transplantation antigen to a portion of interval 6 on the long arm of the human Y chromosome. We describe eight patients whose genetic alterations in the long arm of the Y chromosome allow us to refine the deletion map. Molecular analysis of the deletions in these eight cases results in the ordering of new DNA markers in this interval. The new DNA probes specific for interval 6 of the Y chromosome may prove useful for the isolation of the H-Y antigen gene or other genes in this interval.

MATERIALS AND METHODS

Case Reports and Cytogenetic Analysis

Case 1 (LGL 114) This case was previously reported (de la Chapelle *et al*, 1986, case 2, Andersson *et al*, 1988, case 3). Briefly, this patient had penoscrotal hypospadias Two small left testes appeared histologically normal at 5 months of age At age $8\frac{1}{2}$ years his height was

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FIG. 1. Examples of the X and Y chromosomes stained by G banding from case 2 (**a**) and from his father (**b**)

in the 2nd percentile Case 1 is a 45,X male with Y chromosome material, including the short arm and proximal long arm, translocated on the short arm of chromosome 14 in all lymphocytes and in a portion of fibroblasts, as described previously (Andersson *et al* 1988, case 3) The parents had normal karyotypes

Case 2 (SA 23) This patient was first evaluated at 13 years of age for a slipped left capital femoral epiphysis His height was within the 75–90th percentile and he was overweight Physical examination showed short fourth and fifth metacarpals, delayed sexual maturation, and small teeth with translucent enamel The degree of shortening of his metacarpals suggested a possible diagnosis of Albright's hereditary osteodystrophy, but levels of serum calcium, phosphorus, parathyroid hormone, and urine cyclic AMP were within normal limits Pubertal elevations in serum testosterone and gonadotropin were delayed in onset Testosterone level was 0 3 mg/ml (compared to the normal range of 3 0 to 9 0 mg/ml) at 13 years, but normalized at 15 years of age (3 75 mg/ml) Luteinizing hormone (LH) and follicle stimulating hormone (FSH) were 4 1 mIU/ml (normal is 8 4 to 25 0 mIU/ml) and 4 8 mIU/ml (normal is 3 4 to 19 7 mIU/ml), respectively, at 13 years, with normalization at 15 years of age, LH was 7 7 mIU/ml (normal is 2 to 6 mIU/ml) and FSH was 11 8 mIU/ml (normal is 2 to 10 mIU/ml) At 15 years, testicles measured 3 by 4 cm and pubertal development was Tanner Stage V He has had persistent bilateral gynecomastia with both glandular and fatty tissue present since he was first evaluated at age 13 years His academic progress was hampered by emotional difficulties, with formal intelligence testing demonstrating average intelligence Chromosome analysis showed a small nonfluorescent Y chromosome (Fig 1a) resulting from a deletion of the long arm or the formation of a ring chromosome with deletion of the bright fluorescent Q band of the long arm Because of the very small size of the marker, we could not distinguish between a deletion and a ring The karyotype was described as 46,X,del(Y)(q11) or 46,X,r(Y) The Y chromosome was present in all 122 lymphocytes examined, with one cell having three copies of the deleted Y chromosome, suggesting a tendency to nondisjunction The father's karyotype was normal (Fig 1b)

Case 3 (SA 1) This case was previously published (Disteche *et al*, 1986b) Briefly, the patient was evaluated following exploratory laparotomy for bilateral cryptorchidism at 17 months of age, when the small right testis was removed and the left testis was reimplanted in the scrotum Both testes showed immature seminiferous tubules No mullerian structures were seen The phallus was normal without hypospadias or chordae The patient had been asphyxiated at birth and subsequently had microcephaly and developmental delay Case 3 is a 45,X male with a translocation of part of the Y chromosome, including the short arm and proximal long arm on one chromosome 15

Case 4 (WHT 1373) This male patient was noted at birth to have microcephaly, micrognathia and a small maxilla, a high arched palate, a beaked nose with the septum extending below the nares, and broad thumbs He had an undescended left testis (still undescended at 9 months) and a small right testis The inner canthal and outer canthal distances measured at approximately the 97th percentile and there was synophrys Case 4 has a 46,X,del(Y)(q11 22) karyotype in 20 cells examined His parents had normal karyotypes

Case 5 (WHT 1318) This male patient was diagnosed with hypospadias and an undescended left gonad that was found to be a streak Exploratory laparotomy revealed no evidence of a uterine remnant or other gonadal structures A biopsy of the right testis showed apparently normal histology Case 5 showed a 46,X,psudic(Y)(q11 2) karyotype

Case 6 (LGL 1846) This case was previously reported (Andersson *et al*, 1988, case 1) He is a phenotypically normal male Case 6 is, like case 3, a 45,X male with Y chromosome material, including the short arm and proximal long arm, translocated to the short arm of chromosome 15 This is a familial translocation found in four generations

Case 7 (SA 28) This patient was evaluated at $2\frac{7}{12}$ years of age for mixed gonadal dysgenesis He had hypospadias and an undescended right gonad The patient was otherwise healthy Height was at the 10th percentile At the time of orchiopexy, at $2\frac{4}{12}$ years, he was found to have a streak gonad on the right with a fallopian tube and a unicornous uterus The left gonad, which was descended into the scrotum, was presumed to be a normal testis, it was not biopsied and its adnexae were not evaluated FSH, LH, and testosterone levels were prepubertal Case 7 shows a karyotype of 46,X,psudic(Y)(q11 23) in all 46 metaphase cells examined from a lymphocyte culture The Y chromosome appeared as a pseudodicentric nonfluorescent Y chromosome with two copies of the short arm and proximal long arm The father's chromosomes were not studied

Case 8 (SA 26) This case was previously reported as patient 1 in the report of Drummond Borg *et al* (1988) Briefly, he had penile hypospadias with a normal sized penis, a bifid scrotum, and a right partially descended testis and a left inguinal testis The patient was otherwise healthy Neither vas deferens was seen at the time of orchiopexy, when the patient was 5 years of age On biopsy, the right testis appeared immature, but normal for age Case 8 is mosaic 45,X/46,X,psudic(Y)(q11 23) with 5 of 100 cells being 45,X (Drummond Borg *et al*, 1988) The Y chromosome is nonfluorescent and appears pseudodicentric with two copies of the short arm and proximal long arm The father's karyotype was normal 46,XY

Southern Blot Hybridization

DNA was prepared from peripheral blood lymphocytes or Epstein–Barr virus transformed lymphoblastoid cell lines and then submitted to restriction digestion, electrophoresis, and Southern blot hybridization by standard techniques (Sambrook *et al*, 1989) Hybridization and washing with all previously described DNA probes were performed at high or reduced stringency as described by Cantrell *et al* (1989) and Disteche *et al* (1986b) Probe pMA5 5 identifies the amelogenin gene and recognizes a polymorphic Y specific band (≃15 and 18 kb) on *Taq*I digests (Lau *et al*, 1989) The new interval 6 probes, pMC4, pMC110, pMC118, pMC147, and pJB1, were hybridized and washed at high stringency (Cantrell *et al*, 1989)

Probes pMC4, pMC110, pMC118, pMC147, and pMC50 were isolated from a PERT (phenol enhanced reassociation technique) library (Cantrell *et al* 1989) and contain inserts of less than 100 bp cloned in a pBR322 plasmid vector at the *Bam*HI site The small insert from pMC50 was hybridized to a Y chromosome sorted library (from the Lawrence Livermore Laboratory) to isolate a Charon 21A λ phage clone that contained homologous DNA sequences The 4 4 kb DNA insert from the Charon 21Aλ phage clone was then subcloned into plasmid pGEM 3Z (Promega Corp) at the *Hin*dIII site to generate pJB1 Probe pMC4 recognizes two major DNA fragments identified as pMC4/A, at about 8 kb, and pMC4/B, at 1 2 kb, on an *Eco*RI digest of human genomic DNA Probes pMC110, pMC118, and pMC147 recognize an 8 kb fragment, a 2 kb fragment (Fig 2), and a 3 kb fragment, respectively, on *Eco*RI digested human DNA Probe pJB1 recognizes 18 and 8 kb fragments on an *Eco*RI digest of genomic DNA (Fig 2) Probe pJM77 is a genomic clone isolated directly from the Y chromosome sorted library The 3 kb λ phage insert was subcloned in plasmid pBSSK pJM77 recognizes a 20 kb band on a *Taq*I digest of human genomic DNA

Labeling was performed by random primer hybridization as previously described (Sambrook *et al* 1989), in most instances pMC4, pMC110, pMC118, and pMC147 were PCR amplified from the plasmid with [^{32}P]dCTP present The primers used were pBR322 sequences flanking the *Bam*HI cloning site, which had been destroyed

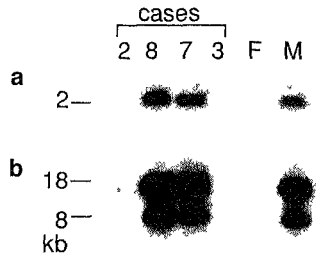

FIG. 2. Examples of Southern blot hybridizations of probes pMC118 (**a**) and pJB1 (**b**) to *Eco*RI-digested genomic DNA from cases 2, 8, 7, and 3, a normal female, and a normal male.

(primer A: 5′-GCGACCACACCCGTCCTGTG-3′ and primer B: 5′-ACGATGCGTCCGGCGTAGAG-3′). The reaction consisted of 10 m*M* Tris, pH 8.2, 5 m*M* NH_4Cl, 1.5 m*M* $MgCl_2$, and 50 or 100 m*M* KCl, approximately 1 pg template DNA, 2 μM dATP, dTTP, and dGTP, 2 μM [^{32}P]dCTP, 38 pmol of each primer, and 2 U *Taq* polymerase. The total volume was 15 μl. The mixture was denatured under oil for 5 min at 94°C and then cycled for 1 min at 94°C, 1 min at 65°C, and 1 min at 72°C for a total of 25 cycles. The final extension totaled 6 min at 72°C. Labeled DNA was purified through Sephadex G50 spin columns prior to blot hybridization.

H-Y Antigen Typing

Prior to H-Y antigen typing, HLA typing was performed to determine whether the patients were of A2 or B7 types, since the T-cell clones available for H-Y typing were restricted by either HLA-A2 or HLA-B7. Standard HLA serotyping was performed at the tissue typing laboratory at the Royal Postgraduate Medical School (Hammersmith, London) by Mr. Nick Davey and by fluorescence-activated cell sorting (FACS) analysis at the Clinical Research Centre (Harrow, UK) using the HLA-A2-specific monoclonal antibody HB82 (BB7.2) and the HLA-B7 (crossreactive on B40) monoclonal antibody HB59 (MB40.2). For cases 7 and 8, FACS analysis only was used. Expression of HLA-A2 and HLA-B7 alloantigens identified by T cells was confirmed by cytotoxic T-cell lysis (CTL) experiments in which the patients' cells were also typed for H-Y (Simpson *et al.*, 1987). Epstein–Barr virus-transformed lines from each of the patients were used as target cells in CTL assays, except for patient 3, for whom a fibroblast line was used. The cytotoxicity was measured in a ^{51}Cr release assay as described in Simpson *et al* (1987). Control normal male and female cell lines were examined with each experiment.

RESULTS

DNA Analysis

Case 1 was previously analyzed using a series of DNA probes as reported in Andersson *et al.* (1988). Reevaluation of this case with new DNA probes for the long arm of the Y chromosome confirms that this patient is deleted for intervals 5, 6, and 7. This patient has the largest deletion of the patients studied here, including a portion of interval 5 recognized by probes pJM77 and p12*f* (Table 1).

Case 2 has the next largest deletion but is positive for probe pJM77, indicating that the new locus recognized by pJM77 is proximal to the locus for p12*f* (Table 1; Fig. 2, case 2). Evaluation of relative hybridization intensities in case 2 compared to those of normal males shows increased hybridization to Y-specific bands that could be quantified relative to X-specific bands for probes specific for intervals 1 (pDP1007 and pDP132) and 2 (pDP61) (Fig. 3, case 2), but not for probes specific for intervals 3 (52d/B and 50*f* 2/A,B) and 4 (pDP34 and pMA5-5). The patient, therefore, appears to have a deletion of intervals 6 and 7 and distal interval 5, as well as a duplication of intervals 1 and 2.

Case 3 was previously shown to be deleted for DNA sequences in intervals 6 and 7 of the Y chromosome (Disteche *et al.*, 1986b). Reevaluation of this patient confirms these findings and allows us to order some of our new DNA probes (Table 1; Fig. 2, case 3). Due to the unavailability of the patient whose deletion allowed Vergnaud *et al.* (1986) to define the border of intervals 5 and 6 (Jean Weissenbach, personal communication), we arbitrarily assigned p12*f* as defining the distal border of interval 5. Thus, purely for simplicity, we will consider all DNA probes located distal to p12*f* to be located in interval 6 or 7.

Case 4 is deleted for interval 7 and has a partial deletion of interval 6 (Table 1). Case 5 is also deleted for interval 7 and shows the smallest deletion of interval 6, including probes pJB1 and pMC4/A (Table 1).

Analysis of cases 6, 7, and 8 by Southern blot hybridizations (Table 1; Fig. 2, cases 7 and 8) shows that these patients contain DNA sequences recognized by all of the probes for interval 6. Absence of the highly fluorescent Q band (Yq12) on their Y chromosomes and lack of hybridization to pY3.4, which recognizes one of the DNA repeats located in interval 7 of the Y chromosome, show them to be deleted for interval 7. Comparison of relative hybridization intensities in cases 7 and 8 shows increased hybridization to all Y-specific bands that could be quantified relative to X-specific bands for probes pDP1007, pDP132, pDP61, 52d/B, pDP34, and 50*f* 2/A,B,E (Fig. 3, case 7). These increased intensities, which were also observed in case 5, support the cytogenetic conclusion that these patients each contain a pseudodicentric Y chromosome, with duplication of most of the Y chromosome except for band q12.

H-Y Antigen Testing

Cells from the patients were examined for expression of H-Y antigen by CTL assays as described in Simpson *et al.* (1987). These data are summarized in Table 2. The lymphoblastoid cell lines derived from cases 1, 2, and 4 were found to be H-Y antigen negative. Case 3 tested H-Y antigen ambiguous in three assays that could not be repeated due to the limited life span of the fibroblast cell line available on this patient. Fibroblasts are much less sensitive than lymphoblastoid lines as targets in CTL assays. Case 7 could not be tested because his HLA type is not A2 or B7. Cases 5, 6, and 8 were found to be positive. These results indicate that a gene encoding or controlling the expression of the H-Y antigen is located in a portion of interval 6 containing loci for probes p50*f* 2/E, pDP105/B, pMC4/B, and pMC118 (Tables 1 and 2). This gene appears to be proximal to loci for probes pJB1 and pMC4/A.

TABLE 1

Identification of Y-Specific DNA Fragments in Eight Patients

DNA Probe	Locus	Deletion interval		Case 1	2	3	4	5	6	7	8
pDP1007	ZFY	1		+[a]	+	+	+	+	+	+	+
pDP132	DXYS23Y	1		+	+	+	+	+	+	+	ND
pDP61	DXYS8Y	2		+	+	+	+	+	+	+	+
pMC23	DYS142	3		ND	+	+	ND	ND	ND	+	+
p52d/B	DYF27	3	p[c]	+	+	ND	+	+	ND	+	ND
pDP105/A	DYZ4	3		+	+	+	+	+	+	+	+
p50f2/A,B	DYS7	3		+	+	+	+	+	+	+	+
pDP34	DXYS1Y	4A		+	+	+	+	+	+	+	ND
pMA5-5	AMGL	4A		ND	+	+	+	+	+	ND	ND
pDP97	DYZ3	4B	cen[c]	+	+	+	+	+	+	+	+
pJM77		5		−	+	+	+	+	+	+	+
p12f	DYS11	5		−	−	+	+	+	+	+	+
pMC110	DYS144	6		−	−	−	+	+	+	+	+
pMC147	DYS146	6		−	−	−	+	+	+	+	+
p50f2/E	DYS7	6		−	−	−	−	+	+	+	+
pDP105/B	DYZ4	6	q[c]	−	−	−	−	+	+	+	+
pMC4/B	DYS143	6		−	−	−	−	+	+	+	+
pMC118	DYS145	6		−	−	−	−	+	+	+	+
pJB1	DYS147	6		−	−	−	−	−	+	+	+
pMC4/A	DYS143	6		−	−	−	−	−	+	+	+
pY3.4	DYZ1	7		−	−	−	−	−	−	−	−
HY typing[b]	HY	6		−	−	?	−	+	+	ND	+

[a] DNA probes were used to test individuals for the presence (+) or absence (−) of the indicated Y-specific restriction fragments. ND, not done.
[b] See Table 2 for details of H-Y antigen testing.
[c] p, short arm; cen, centromere; q, long arm.

DISCUSSION

Our results indicate that a gene encoding or controlling the expression of the H-Y transplantation antigen is located in a portion of interval 6 of the Y chromosome containing probes p50f 2/E, pDP105/B, pMC4/B, and pMC118 and defined by the breakpoints in cases 4 and 5. These data restrict the location of the gene to a proximal portion of interval 6.

The deletion map that we have constructed using previously isolated DNA markers and a series of new Y-chromosome-specific DNA probes assumes that the chromosomal rearrangements of the patients result in simple breaks in the long arm of the Y chromosome and that there are no position effects in these rearrangements. We cannot exclude the possibility that the chromosome breaks are more complex. By cytogenetic analysis, three cases (cases 1, 3, 6) show a Y;autosome translocation that results in the presence of a portion of the long arm of the Y chromosome in the patients. Two cases (cases 2 and 4) show a terminal deletion of the long

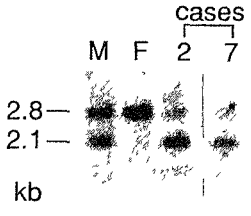

FIG. 3. Example of a Southern blot hybridization of probe pDP61 to *Taq*I-digested genomic DNA from a normal male, a normal female, case 2, and case 7. The 2.8- and 2.1-kb bands represent the X-linked and Y-linked loci of DXYS8, respectively. The 2.1-kb band is increased relative to the 2.8-kb band in cases 2 and 7, whose rearranged Y chromosomes contain two copies of region 2 of the Y chromosome.

TABLE 2

H-Y Antigen Testing

Case	HLA serotype A	HLA serotype B	% lysis with cytotoxic T cells specific for A2	A2/H-Y	B7	B7/H-Y	H-Y type
1	2[a]	27	58[b]	3	ND[c]	ND	−
2	3, 9	7, 12	ND	ND	70	0	−
3	2	ND	15[d]	7[d]	ND	ND	?[d]
4	2, 9	35, 40	21	1	ND	ND	−
5	10, 19	7	ND	ND	30	65	+
6	3	7	ND	ND	63	84	+
7	not 2	not 7	ND	ND	ND	ND	ND
8	2	ND	57	67	ND	ND	+

[a] The identity of the HLA molecule used as a restriction element for the detection of H-Y antigen in the CTL assays is underlined in the HLA serotyping columns.
[b] Figures underlined in the CTL test columns are those showing significant levels of titrating lysis.
[c] ND, *not done*.
[d] Insensitive target, ambiguous results (in each of the three experiments performed with these cells).

arm. Three cases (cases 5, 7, and 8) show a pseudodicentric Y chromosome with deletion of the distal long arm and duplication of the short arm and proximal long arm. In one additional case (case 2), it appears that the chromosome rearrangement is complex, involving both duplication and deletion of Y-chromosome material. The abnormal Y chromosome of case 2 may be a ring chromosome or a short metacentric chromosome derived from an intermediate dicentric Y chromosome. It has recently been shown that some ring chromosomes 21 may derive from an intermediate dicentric chromosome 21 in a similar fashion (Wong *et al*, 1989; McGinniss *et al*, 1992). Further analysis of the breakpoints and the use of pseudoautosomal DNA probes may help to define better the abnormal Y chromosome of case 2.

The majority of patients in this study had genital abnormalities. Four patients had hypospadias (case 1, 5, 7, and 8). Undescended testes were found in cases 3, 4, 5, 7, and 8. Case 2, who has a large deletion, shows few anomalies, including delayed puberty, low testosterone levels, and gynecomastia, but apparently normal genitalia. Case 2 also had short fourth and fifth metacarpals, an anomaly previously seen in a patient with a long-arm deletion of the Y chromosome (Fitch *et al*, 1985) and often reported in cases of Turner syndrome (Hall *et al*, 1982). Where available, testicular biopsies of the patients showed apparently normal histology, although the testes were usually small, except in case 6 who is a normal fertile male. A gene related to fertility has been assigned to the long arm of the Y chromosome on the basis of the observation of an excess number of individuals with deletion of the long arm of the Y chromosome among infertile males (Tiepolo and Zuffardi, 1976). Additional reports support this association between deletion of Yq and azoospermia (Munke *et al*, 1985; Fitch *et al*, 1985). On the basis of the present case 6 and other patients, Andersson *et al* (1988) assigned the fertility factor to interval 6 of the Y chromosome. In our cases (except case 6 as noted above), fertility and sperm production could not be assessed because of the age of the patients. In addition, the aberrations in testicular development of cases 5, 7, and 8 may be secondary either to their Y-chromosome abnormality or to known (in case 8) or possible mosaicism for a 45,X cell line.

The combination of cytogenetic analysis and Southern blot hybridization in the eight patients described has allowed us to assign six new DNA probes to interval 6 of the Y chromosome and to order them at least in part. These probes should be useful for further characterization of the Y chromosome and of specific deletions associated with specific phenotypes. Several other deletion maps of the long arm of the Y chromosome have been reported (Vergnaud *et al*, 1986; Muller *et al*, 1986; Affara *et al*, 1986; Oosthuizen *et al*, 1990; Koteckı *et al*, 1991; Nakahori *et al*, 1991; Bardoni *et al*, 1991), providing tools to isolate genes in this chromosomal region. The present analysis provides evidence for a more precise location of the H-Y antigen gene to a portion of interval 6, which will be useful for its isolation.

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Note added in proof Recent Y-DNA testing of patient CHM018 (DNA kindly provided by Jean Weissenbach), whose Y chromosomal breakpoint historically defined the boundary between intervals 5 and 6 (Vergnaud *et al*, 1986), suggests that the boundary is located more distally on Yq than we had assumed Such a "recalibration" of deletion intervals 5 and 6 would not affect the ordering of breakpoints, DNA loci, and H Y antigen reported here and will be discussed in a subsequent publication

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