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* Present address: Department of Microbiology, North Carolina State University, Raleigh 27607.
† Address correspondence to W.J.B.

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Mapping the Locus of the *H-Y* Gene on the Human Y Chromosome

Abstract. *The* H-Y *locus is on the short arm of the human Y chromosome in most individuals but on the long arm in at least one of 17 individuals with structural abnormalities of the Y.*

When female mice are sensitized with skin grafts or lymphoid cells from males of the same inbred strain, they produce antibody which identifies a plasma membrane component called H-Y (histocompatibility-Y) antigen (*1*). Recently, we have shown that the gene governing expression of H-Y antigen is widespread among vertebrates, occurring in several divergent species including man (*2*). In all species so far examined, H-Y antigen is associated with the heterogametic (XY) sex. H-Y antigen is not present in XO female mice but is present in XXY male mice (*3*) and in *Tfm* mice, that is, XY intersexual mice with testicular feminization (*4*). These findings support the hypothesis that a gene on the Y chromosome is necessary for the expression of H-Y antigen and that androgen responsiveness, which is absent in testicular feminization, is not required. A structural or regulatory gene for H-Y antigen expression is known to be located on the Y chromosome of man; white blood cells from human males with two Y chromosomes express more H-Y antigen than cells from normal XY males (*5*). However, the exact position of the human *H-Y* gene remains to be determined.

In view of the possible role of H-Y antigen in the differentiation of the mammalian testis (*6*), precise localization of the Y-linked *H-Y* gene may prove crucially important (i) in the detection of virilized gonads in phenotypic females and (ii) in understanding the etiology of intersexual phenotypes associated with structural abnormalities of the Y chromosome. Therefore as a first step in localizing the *H-Y* gene, we have studied H-Y antigen expression in patients exhibiting deletions or other structural modifications of the Y. A note on the findings in a few of these patients has been published (*7*).

Serological detection of H-Y antigen was based on the ability of white blood cells (WBC) to absorb H-Y antibody. H-Y antiserums were collected from inbred female mice that had been sensitized with serial inoculations of male spleen cells. Before reaction with mouse sperm, H-Y antiserms were pooled and subdivided, and portions were absorbed with WBC from normal human males (46,XY), normal human females (46,XX), and patients (for example, male, 46,XYq-). Positive absorption (indicating the presence of H-Y antigen on the absorbing WBC) was manifested as a decrease in the reaction of H-Y antiserums with target sperm. Thus WBC from human males (H-Y$^+$) specifically absorbed (bound) H-Y antibodies from H-Y antiserums and thereby decreased the ability of these serums to react with mouse sperm in both the sperm cytotoxicity test (*5*) and the mixed hemadsorption hybrid antibody test (*8*).

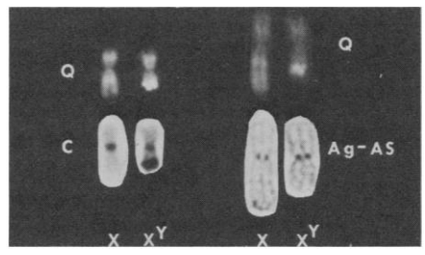

Fig. 1. The sex chromosomes from two cells of case 8. Those on the left were photographed first to show quinacrine banding (*Q*) and then to show C banding (*C*). Those on the right were photographed first to show Q banding and then to show silver-staining (*Ag-AS*).

Of the 17 individuals tested, 15 were H-Y antigen positive (Table 1). The correlations of H-Y antigen expression with karyotype, sexual phenotype, and appearance of the gonads are summarized in Table 1.

The *H-Y* locus is not on the intensely quinacrine fluorescent distal region of the Y chromosome. H-Y antigen was present in cases 1 to 4 and 9 to 16 despite the absence of this region in all 12 of these cases. Case 1 had a deletion of approximately the distal half of the Y, including almost all of the quinacrine-bright and 5-methylcytosine-rich materials. Cases 2 to 4 had Y-derived chromosomes of average size and typical morphology, but in each case the distal portion failed to show intense fluorescence after quinacrine staining, and no portion of the Y showed the intense binding of antiserum to 5-methylcytosine that is characteristic of normal Y chromosomes (*9*). On the other hand, in case 5 the quinacrine-bright portion of the Y was present, but the absence of H-Y antigen confirmed the exclusion of this region as the locus of the *H-Y* gene.

Evidence that the *H-Y* locus may be on the short arm of the Y chromosome was provided by several cases. In the first of these, case 5, the abnormal Y chromosome was symmetrical and had the characteristics of an isochromosome of the long arm of the Y (*10*); that is, only the short arm was absent. Since H-Y antigen was also absent from this female, who had Turner syndrome and streak gonads, the *H-Y* locus must have been on the short arm of the Y. Case 6 also had Turner syndrome (although the gonads were not examined) and was H-Y antigen-positive with no evidence of the increased amount of antigen seen in individuals with two Y chromosomes (*5*). This patient, a female, had a slightly asymmetrical Y chromosome with duplicated long arms, which was not an isochromosome because there was additional material on one arm forming a quinacrine-dull band adjacent to the centromere. The simplest explanation for these results is that the extra band represents material from the short arm of the Y and that it contains the *H-Y* locus.

The location of the *H-Y* locus on the short arm of the Y is also indicated in case 7. This male had testes and was

H-Y antigen-positive despite the presence of only 45 chromosomes, including a single X, in each cell. However, in two-thirds of his cells one of the chromosomes 22 had an additional band, as was evident by Q- or G-banding methods. These findings suggest that part or all of the short arm of the Y, including the *H-Y* locus, had been translocated to the distal end of one chromosome 22. If the translocated segment had come from the long arm of the Y, at least two breaks would have been required to free it from the rest of the Y chromosome.

In case 8, the *H-Y* locus could not have been on the short arm of the Y. This patient had a Y-to-X translocation in which almost the entire long arm of the Y appeared to have been translocated to the long arm of an X chromosome. One arm of the translocation chromosome was made up of the short arm of the X. The centromere was also derived from the X as shown by the presence of C banding in the centromeric region (Fig. 1), and the quinacrine banding pattern was compatible with an X chromosome origin of a small segment adjacent to the centromere opposite the short arm. Silver staining, which, in contrast to the C banding, labels the centromeric region of the Y chromosome, did not reveal a Y-derived centromeric region (Fig. 1). Therefore, the abnormal chromosome appeared to contain the entire long arm of the Y but no short arm material. Nevertheless, H-Y antigen was present, indicating that the *H-Y* gene was located on the long arm of the Y chromosome in this case.

A series of patients was studied who had, in addition to a 45,X complement, a minute metacentric chromosome (cases 9 and 10) or a dotlike or obvious ring chromosome (cases 11 to 17). Eight of these patients (cases 9 to 16) were H-Y antigen positive, and the minute metacentric or ring chromosomes are therefore thought to have been derived from a Y chromosome. In one individual (case 17), H-Y antigen could not be detected, and it is uncertain whether the ring chromosome arose from an X or a Y chromosome.

In our study of H-Y mapping assignments, 15 individuals who lacked a normal Y chromosome but had an abnormal chromosome—presumably containing Y chromosome material—were H-Y antigen positive, while one individual with an abnormal chromosome clearly derived from a Y had no H-Y antigen. The presumptive breakpoints in the Y chromosome represented by these cases are shown in Fig. 2, which also shows the tentative location(s) of the *H-Y* locus close to the centromere on either the short arm or the long arm of the Y.

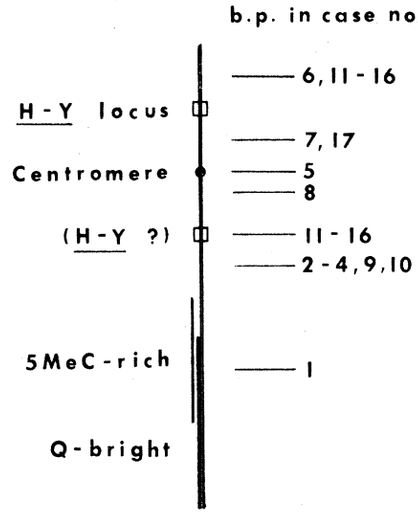

Fig. 2. Cytogenetic map of the human Y chromosome. The approximate position of the cytological breakpoints (*b.p.*) in the 17 cases are indicated by lines to the right of the chromosome map. The position of the centromere, the 5-methylcytosine-rich (*5MeC-rich*) region and the quinacrine-bright (*Q-bright*) region on the chromosome are shown. The approximate position of the *H-Y* locus on the short arm, as inferred from the presence or absence of H-Y antigen in most of the 17 cases, is indicated. The approximate location of the *H-Y* locus on the long arm, as inferred from the presence of H-Y antigen in case 8, is also indicated.

Of the 17 cases included in this study, 12 had a 45,X cell line in addition to the line with the abnormal Y chromosome (Table 1). An intersexual or female phenotype (with streak gonads) was seen in 9 of the 12, and 8 of these were H-Y antigen positive. The discrepancy between the H-Y antigenic status and the phenotype of these 9 individuals is presumably due to the presence of the 45,X cell line, a point which has been discussed (*11*). These cases are useful in mapping the *H-Y* locus.

Two informative cases, numbers 5 and 8, gave contradictory results. Case 5 had an isochromosome of the long arm of the Y. This chromosome was symmetrical and had a functioning centromere. It must therefore have contained the entire long arm of the Y. The absence of H-Y antigen in this case is most easily explained by postulating that the *H-Y* locus is on the short arm of the Y, which is absent from the isochromosome. This location is compatible with the results obtained in all the other cases, with one striking exception, case 8.

In case 8, H-Y antigen was present despite the apparent absence of the short arm of the Y chromosome. The abnormal chromosome contained the short arm, centromeric heterochromatin (C band) region, and probably the proximal part of the long arm of the X, suggesting that its centromere was derived from the X. The centromere of the Y chromosome was probably not present because only one centromeric region, that derived from the X, was silver-stained, and there was no other sign of a second centromere such as a constriction. It is therefore likely that only the long arm of the Y chromosome was present in this chromosome, which can be designated der(X)t(X;Y)(Xpter→Xq1::Yq11→Y qter). No chromosome resembling the reciprocal translocation product was present. We must therefore conclude that the short arm of the Y was

Table 1. Karyotype, expression of H-Y antigen, sex appearance of gonads in individuals with an abnormal Y chromosome.

Case No.	Karyotype*	H-Y antigen	Sex	Gonads
1	46,X,Yq-	+	Male,	Testes
2	45,X/46,X,der(Y)t(Y;?)	+	Male, first-degree hypospadias	Testes
3	45,X/46,X,der(Y)t(Y;?)	+	Intersex	Undifferentiated
4	45,X/46,X,der(Y)t(Y;?)	+	Female	Streaks
5	46,X,i(Yq)	−	Female Turner	Streaks
6	46,X,dup(Yq)	+	Female Turner	?
7	45,X/45,X,der(22)t(22;Y)	+	Male	Testes
8	46,X,der(X)t(X;Y)	+	Female	?
9	45,X/46,X,Yq-	+	Male, fourth-degree hypospadias	Testes
10	45,X/46,X,Y minute	+	Female Turner	?
11	46,X,r(Y)	+	Male	Testes
12	45,X/46,X,r(Y)	+	Male	Testis, right, ? left
13	45,X/46,X,r(Y)	+	Intersex	?
14	45,X/46,X,r(Y)	+	Intersex	Abdominal testes
15	45,X/46,X,r(Y)	+	Female Turner	?
16	45,X/46,X,r(Y)	+	Female Turner	?
17	45,X/46,X,r(X or Y)	−	Female Turner	?

*Symbols:Yq-, deletion of long arm (q) of Y chromosome; 45,X/46,X, and the like, mosaic karyotypes: one cell line having 45 chromosomes including the X, another having 46 chromosomes including the X and . . .; der(Y)t(Y;?), derivative of Y translocation involving Y and unknown chromosome; i(Yq), isochromosome of long arm of Y chromosome; dup(Yq), duplication involving Yq; (22), autosome 22; and r(Y), ring chromosome (of Y-chromosomal origin).

absent, and that the *H-Y* locus in this case must have been on the long arm of the Y chromosome. This location, if near the centromere, is compatible with the results obtained in most of the other cases studied, with the exception of case 5 and (probably) case 7.

These discrepancies need to be reconciled. Perhaps a pericentric inversion of the Y chromosome altered the position of the locus in either cases 5 and 7 or in case 8. The Y chromosome in man, as in most animals, does not show the same type of meiotic pairing with the X, involving chiasma formation and crossing-over, as do the autosomes with their homologs (*12*). Therefore, inversions involving the Y chromosome need have little, if any, effect on fertility unless a breakpoint falls within the *H-Y* locus, and a population of individuals could contain a variety of Y chromosomes with the position of the *H-Y* locus different in each one. In this regard, it is interesting that in each of our cases with an abnormal Y chromosome the findings indicate a locus relatively close to the centromere despite discrepancies regarding which arm it is on.

Our finding can be explained by a simple mechanism: the existence of two regions highly susceptible to breakage, one in each arm of the Y chromosome, distal to the *H-Y* locus. Rejoining of the broken ends could produce either an inversion or a ring chromosome. The inversion would have no phenotypic effect unless there was a position effect due to transfer of a gene from a euchromatic region to a heterochromatic one (*13*). Whether the *H-Y* gene is more commonly located in the long arm or the short arm can be determined only by further study.

We have proposed that H-Y antigen is the product of the primary male-determining gene in mammals (*14*). According to this hypothesis, male differentiation of the initially indifferent embryonic gonad occurs in the presence of H-Y antigen and female differentiation occurs in its absence. Localization of the *H-Y* gene to a region on the short arm of the human Y is consistent with this hypothesis because the male determining gene or genes has been assigned to this region on the basis of findings in a number of cases (*15*). However, some cases are known in which a long arm location of male determining genes seems likely. For example, Siebers *et al.* (*16*) found a female phenotype and streak gonads in a patient with a presumptive 45,X,i(Yp) karyotype. The minute metacentric chromosome was said to have identical banding on the two arms and was thus interpreted as an isochromosome of the short arm of the Y. There is insufficient evidence at this time to rule out a long arm location of the *H-Y* locus in some individuals or even to hazard a guess at the proportion of human Y chromosomes with an *H-Y* gene at a given location.

GLORIA C. KOO, STEPHEN S. WACHTEL
KAREN KRUPEN-BROWN
LILLIAN R. MITTL
Memorial Sloan-Kettering Cancer Center, New York 10021
W. ROY BREG, MYRON GENEL
Department of Pediatrics, Yale University School of Medicine, New Haven, Connecticut 06520
IRA M. ROSENTHAL
Department of Pediatrics, University of Illinois, Chicago 60637
DIGAMBER S. BORGAONKAR
Department of Medicine, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205
DOROTHY A. MILLER
RAMANA TANTRAVAHI,
RHONA R. SCHRECK
Department of Human Genetics and Development, College of Physicians and Surgeons, Columbia University, New York 10032
BERNARD F. ERLANGER
Department of Microbiology, College of Physicians and Surgeons
ORLANDO J. MILLER
Departments of Human Genetics and Development and *Obstetrics and Gynecology, College of Physicians and Surgeons*

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Plant Crops as a Source of Fuel and Hydrocarbon-Like Materials

Abstract. *Chemical analyses have been made of a number of plant species in order to assess their suitability as renewable sources of hydrocarbon-like photosynthetic products. Yields of rubber and wax, glycerides, isoprenoids, and other terpenoids were estimated. Individual sterols were identified in latex from some species.*

It has been suggested (*1*) that certain plants rich in polyisophrenes and other hydrocarbon-like materials might be cultivated and grown as renewable sources of highly reduced photosynthetic products. Two distinctly different agricultural methods can be applied in approaching this problem. Either we can harvest whole plants as suggested in a biomass plantation (*2*) or we can tap latex-containing plants as is done in the production of natural rubber (*Hevea brasiliensis*).

In order to evaluate the prospects of this idea, we have started a program of chemical analysis of both whole plant extracts (Table 1) and of plant latex (Table 2) (*3*). As can be seen from Table 1, as