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# Gonadoblastoma: Molecular Definition of the Susceptibility Region on the Y Chromosome

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## Summary

**Using sequence-tagged sites we have performed deletion mapping of the Y chromosome in sex-reversed female patients with a Y chromosome and gonadoblastoma. The GBY gene (*gonado*blastoma locus on the *Y* chromosome) was sublocalized to a small region near the centromere of the Y chromosome. We estimate the size of the GBY critical region to be ~1–2 Mb. Our analysis also indicates that copies of two dispersed Y-linked gene families, TSPY (*t*estis-*s*pecific *p*rotein, *Y*-encoded) and YRRM (*Y*-chromosome *R*NA *r*ecognition *m*otif) are present in all patients and that copies of TSPY but not YRRM fall within the GBY critical region as formally defined by deletion mapping. Two tumor samples showed expression of both genes and in one patient this expression was limited to a unilateral gonadoblastoma but absent in the contralateral streak gonad. Although our results do not directly implicate TSPY or YRRM in the etiology of the tumor, they raise the issue of whether there is one GBY gene in the critical region or possibly multiple GBY loci dispersed on the Y chromosome.**

## Introduction

Gonadoblastomas are rare neoplasms composed of aggregates of germ cells intermixed with smaller epithelial cells resembling immature Sertoli and granulosa cells (Scully 1970). These neoplasms arise within dysgenetic gonads of individuals who possess Y-chromosomal material (Verp and Simpson 1987). The majority of these individuals are phenotypic females, otherwise known as sex-reversed females. Sex reversal in these individuals can be caused by deletion or mutation of the testis-determining gene SRY (*s*ex-determining *r*egion, *Y* gene), mosaicism for the Y chromosome, or abnormalities in other genes involved in sex determination (Hawkins et al. 1992).

The frequency of gonadoblastoma in patients who have gonadal dysgenesis and a Y chromosome is ≥30% (Verp and Simpson 1987). In contrast to 46,XY sex-reversed females, 45,X Turner females who also have dysgenetic gonads do not develop gonadoblastoma. The high frequency of gonadoblastoma in sex-reversed females compared to Turner females led to the hypothesis that there is a gene on the Y chromosome that is involved in the development of these tumors (Page 1987). This gene is referred to as *gonado*blastoma locus on the *Y* chromosome, or GBY. Based on deletion mapping in three phenotypic females with gonadoblastoma and partial Y chromosomes, GBY has previously been localized to a region near the centromere either on the short or long arm of the Y chromosome (Magenis et al. 1984, 1987; Disteche et al. 1986*a*; Page 1987; Petrovic et al. 1992). More precise mapping of GBY has been limited not only by the rarity of individuals who have both gonadoblastoma and Y-chromosomal deletions but also by the paucity of Y-specific probes. Recently, Vollrath et al. (1992) constructed a 43-interval deletion map of the human Y chromosome using sequence-tagged sites (STSs) that are distributed along the length of the Y chromosome. Using these STSs, we performed detailed deletion mapping in 10 patients with gonadoblastoma, 6 having a rearranged Y chromosome. These mapping results allowed us to further localize GBY to a small region of the short arm of the Y chromosome.

The TSPY and YRRM genes are both present as dispersed gene families on the Y chromosome, and their expression is testis specific (Arnemann et al. 1991; Zhang et al. 1992; Ma et al. 1993; Manz et al. 1993). Copies of TSPY, but not YRRM, were found to be located within the GBY critical region. However, analysis of individual patients indicates that all patients have some copies of YRRM as well as TSPY. Although the GBY gene may be a single copy sequence within the critical region, the dispersed nature of Y-linked gene families raises the possibility of multiple gonadoblastoma-susceptibility loci. Using reverse-transcription (RT)-PCR we found expression of TSPY and YRRM in gonadoblastomas from two different patients.

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## Patients, Material, and Methods

### Patients

*Patient SA-83.*—Patient SA-83 was evaluated for features of Turner syndrome, including hypoplastic toenails, "puffy" feet, bilateral Simian creases, hydronephrosis of the right kidney, and a possible grade 1/6 heart murmur. At ~2 years of age, the patient underwent bilateral gonadectomy and right nephrectomy, after cytogenetic and DNA analyses revealed a Y-derived marker chromosome (see Results). The kidney showed hydronephrosis with features consistent with obstructive uropathy. Both gonads consisted of streaks, and the left contained small foci of gonadoblastoma. The patient was found to have a 46,X,r(Y) karyotype with a minute ring chromosome that could not be identified by standard cytogenetic techniques. This marker was present in 132 of 133 peripheral blood lymphocytes that were analyzed and stained negative by G-, C-, R-banding and NOR staining. Parental karyotypes were normal, with no evidence of a minute chromosome in 100 cells analyzed.

*Patient SA-91.*—Patient SA-91 had features of Turner syndrome, including short stature, lack of secondary sexual characteristics, increased carrying angle of the forearm, and shield chest. At age 13 years, gonadectomy showed streak gonads and bilateral gonadoblastomas. Cytogenetic analysis revealed that the patient was mosaic for the Y chromosome. Peripheral lymphocytes showed a 46,XY karyotype in 31 cells. Fibroblast cultures from a skin biopsy and from the right gonad showed a mosaic 45,X/46,XY karyotype with 78 of 105 cells and 91 of 100 cells with a 45,X karyotype in each tissue, respectively. The left gonadal culture was 45,X in 100 cells examined.

*Patient SA-92.*—Quick-frozen gonadoblastoma tissue from patient SA-92 was obtained from the Cooperative Human Tissue Network, western division. This individual lacked features of Turner syndrome. At age 14 years, she presented with nausea, vomiting, and abdominal pain, at which time she underwent appendectomy and left salpingo-oophorectomy. The left ovary contained a dysgerminoma. After her karyotype was determined to be 46,XY, the right ovary was removed and was found to have gonadoblastoma with focal dysgerminoma. The karyotype of patient SA-92 is documented as 46,XY according to information provided by the Cooperative Human Tissue Network, western division.

*Other patients.*—Patients SA-6, SA-10, SA-14, SA-20, and SA-40 have been described elsewhere (Disteche et al. 1986*a;* Cantrell et al. 1989) and correspond to patients 2, 4, 3, 5, and 6, respectively, from the previous studies. Patients SA-6 and SA-14 correspond to cases WHT715 and WHT1003, respectively, in the study by Vollrath et al. (1992). Patient SA-39 has also been described elsewhere, as case 2 in Disteche et al. (1986*b*). Patients SA-6, SA-14, and SA-39 had features of Turner syndrome. Patients SA-10, SA-20, and SA-40 had primary amenorrhea and lacked breast development but were of average to tall stature. Patient WHT1297 was previously reported (Vollrath et al. 1992). All patients possessed bilateral streak gonads, and all had unilateral or bilateral gonadoblastoma (Disteche et al. 1986*a,* 1986*b;* Cantrell et al. 1989).

### Nucleic-Acid Preparation

Genomic DNA was prepared by phenol/chloroform extraction. Total cellular RNA from quick-frozen tissues was prepared by the acid guanidinium thiocyanate/phenol/chloroform method (Chomczynski and Sacchi 1987). Concentrations were determined by absorbance at 260 nm for RNA and by a fluorescent method using Hoechst 33258 dye for DNA.

### PCR Analysis

The primer sequences, loci, and PCR conditions for Y-specific STSs have been described elsewhere (Vollrath et al. 1992). The primer sequences for ZFY amplification were 5′ AAC AAG TGA GTT CCA CAG GG 3′ (ZFY1) and 5′ GCA AAG CAG CAT TCA AAA CA 3′ (ZFY2). The PCR conditions for ZFY were the same as the other Y-specific STSs. The first set of STSs used to identify the seven deletion intervals of the Y chromosome were sY14, sY40, sY57, sY70, sY95, sY158, and sY160. Additional STSs were used to refine the breakpoint locations. These STSs included sY43, sY78, sY85, sY149, sY53, sY79, and sY82. Genomic DNA prepared from peripheral blood leukocytes (SA-83), paraffin-embedded gonadal tissue (SA-91), or quick-frozen gonadoblastoma tissue (SA-92) was used as template. For each STS, PCR amplification of genomic DNA from peripheral blood leukocytes of normal male and female controls was performed simultaneously to that of DNA from patient samples. PCR products were resolved on 1% NuSieve agarose/1% SeaKem GTG agarose (FMC) gels in 1 × TBE (0.089 M Tris-borate/0.089 M boric acid/20 mM EDTA, pH 8.3).

Detection of TSPY by PCR amplification of genomic DNA from patient SA-83 was performed by using a primer set (PCR4) specific for the TSPY gene (Zhang et al. 1992). Reactions were performed in 50-μl reaction mixtures (10 mM Tris-HCl, pH 8.3/50 mM KCl/3 mM $MgCl_2$) containing 100 ng of each primer, 100 ng of genomic DNA as template, 2.5 units of *Taq* DNA polymerase (Boehringer Mannheim), and each dNTP at 1 mM. The cycling protocol consisted of 1 min at 94°C, 1 min at 66°C, and 1.5 min at 72°C for 30 cycles followed by a final extension at 72°C for 5 min. Primers

for amplification of a 114-bp stretch of the TSPY gene from DNA that was derived from paraffin-embedded tissue from patient SA-91 were chosen by computer analysis (Primer software, Whitehead Institute for Biomedical Research) of the genomic DNA sequence (Manz et al. 1993). Reactions were performed under the same conditions as for the PCR4 primer set with the exception of using 1 mM $MgCl_2$, an annealing temperature of 58°C, an extension time of 1 min, and 35 cycles of amplification. Genomic DNA from normal male and female individuals was used as template for positive and negative controls with both sets of primers. PCR products were resolved on either 1.2% agarose gels (PCR4) or 1% NuSieve/1% GTG agarose gels.

### *RT-PCR Analysis*

RT-PCR was performed following the method of Kawasaki et al. (1987). The details of the reaction have been described elsewhere (Adler et al. 1991). First strand synthesis was carried out using Superscript reverse transcriptase (Gibco BRL), oligo(dT) as primers, and 1 μg of total cellular RNA from either gonadoblastoma tissue (SA-40, SA-92), streak gonad tissue (SA-40), or normal human testis or ovarian tissues as template. PCR amplification of the resulting cDNA was performed using either the TSPY PCR4 primer set and corresponding conditions described above or the YRRM1 primer set 5′-CTT TGA AAA CAA TTC CTT TTC C-3′ and 5′-ATG CAC TTC AGA GAT ACG G-3′ (modified from Ma et al. 1993) and cycling conditions described in by Reijo et al. (1995). Both sets of primers amplify genomic sequence that contains introns, thus possible DNA contamination of the RNA produces a PCR fragment of larger size than that of the corresponding cDNA. In addition, control RT-PCR reactions omitting reverse transcriptase were performed. RT-PCR for hypoxanthine phosphoribosyltransferase (HPRT) was also performed using the following primers: 5′-GTT TCC AAA CTC AAC TTG AAT TCT CAT C-3′ and 5′-CCG CGC GCT GGC CGG ATC CGT T-3′. First-strand synthesis was carried out as for TSPY and YRRM1. Cycling conditions for PCR amplification of the resulting cDNA were 1 min at 94°C, 1 min at 62°C, and 1 min at 72°C for 30 cycles. The product of these HPRT primers spans many introns of the genomic HPRT sequence; therefore, amplification of any contaminating genomic DNA is not possible. All RT-PCR products were examined on 1.5% agarose gels.

### *Southern Blot Hybridization*

Genomic DNA was subjected to restriction digestion with *Hin*dIII, electrophoresis, and Southern blot hybridization by standard techniques (Sambrook et al. 1989). The 1.6-kb *Hin*dIII insert of the probe pJA36B2 (Arnemann et al. 1987) was labeled with [$^{32}$P]dCTP by random priming. The final wash was carried out in 0.5 × SSC, 0.1% SDS at 65°C. In order to quantitate TSPY copy number, pJA36B2 insert DNA was loaded along with the patient samples in amounts corresponding to 1, 10, 30, and 50 copies.

## Results

### *Deletion Mapping of the Y Chromosome*

The karyotypes of all 10 females with gonadoblastoma and cytogenetically detectable Y chromatin who were used in this study are summarized in figure 1. DNA analysis using a series of Y-specific STSs (Vollrath et al. 1992), including an STS specific for the SRY gene, was carried out to determine the composition of the Y chromosome in each patient.

Patient SA-83, who has a very small Y chromosome, was evaluated for the presence of Y-chromosomal material by using at least one STS from each of the seven deletion intervals defined by Vergnaud et al. (1986). On the basis of these STSs, the small Y chromosome in this patient consists only of intervals 3 and 4 and possibly interval 2 (fig. 2 and table 1). The correct PCR product of each STS was amplified in normal control males, while the absence of PCR products in control females was observed for all STSs used in this study except those in interval 2 (fig. 2). The scoring for interval 2 of the Y chromosome by using STSs is not possible, because this interval lies in a region of XY homology, and PCR products of the same size are present from both the X and the Y chromosomes. The results of all STS analyses performed on patient SA-83 indicate that her breakpoints on the 43-interval deletion map of the Y chromosome (Vollrath et al. 1992) are located between intervals 3C and 1B on the short arm and between intervals 4B and 5A on the long arm (table 1). Patient SA-83 is deleted for interval 1, including sY14, which corresponds to the SRY gene (fig. 1 and table 1). The short-arm breakpoint in patient SA-83 was not further determined, because it lies distal to the breakpoints of other patients (see below) and therefore does not help in further defining the GBY region. The long-arm breakpoint in patient SA-83 is between STS sY78 and sY79 (table 1). STS sY78 recognizes the alphoid repeats of the centromeric region in interval 4B (Foote et al. 1992).

Patients SA-91 and SA-92 were evaluated by using a series of Y-chromosome-specific STSs (fig. 1 and table 1). Patient SA-92 is positive for all STSs tested except for sY14, which corresponds to the SRY locus in interval 1 (fig. 1 and table 1). Patient SA-91 is positive at least for interval 7 and TSPY (fig. 1 and table 1). Scoring for other intervals of the Y chromosome in patient SA-91 was not performed, because of the poor quality of the

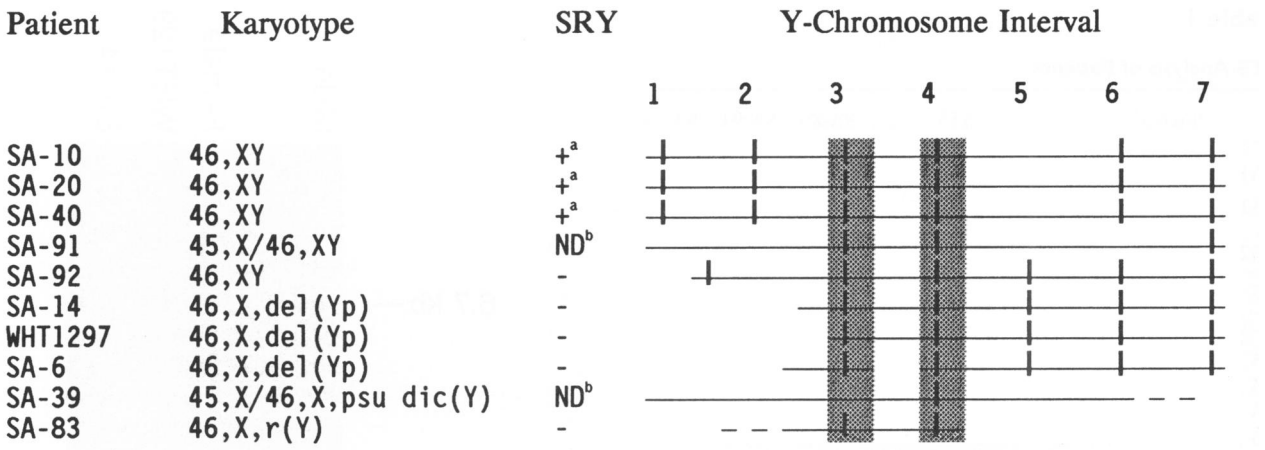


**Figure 1** Karyotype and deletion mapping of patients with gonadoblastoma. The presence or absence of the SRY gene is indicated by a plus sign (+) or a minus sign (−), respectively. *a,* No mutations found by sequence analysis of the coding region of SRY. *b,* Status of SRY not determined. Horizontal lines indicate the presence of a Y-chromosome region defined as deletion interval 1–7 (according to Vergnaud et al. 1986). Small vertical lines indicate that the presence of a specific interval has been checked. Dashed lines represent undetermined breakpoints. Shaded areas indicate Y-chromosome region common to all patients.

DNA obtained from the archival paraffin-embedded tissue. However, the Y chromosome of this mosaic 45,X/46,XY patient appears normal by cytogenetic analysis.

Detailed Y-chromosome analysis by Southern blot hybridization using Y-specific probes and STS scoring has been described elsewhere for patients SA-6 (or WHT715), SA-14 (or WHT1003), and WHT1297 (Disteche et al. 1986*a;* Cantrell et al. 1989; Vollrath et al. 1992). Patients SA-14 and WHT1297 have a terminal deletion of the short arm of the Y chromosome with breakpoints distal to sY66 and sY67, respectively. Patient SA-6 has a terminal deletion of the short arm with a breakpoint distal to pDP1044 (DXYS96) and a second small interstitial deletion between sY69 and sY78 (Disteche et al. 1986*a;* Cantrell et al. 1989; Vollrath et al. 1992) (fig. 1). Patient SA-39, who is mosaic 45,X/46,X,psu dic(Y)(q11.2), is deleted for interval 7 (Disteche et al. 1986*b*). Patients SA-10, SA-20, and SA-40 have apparently normal Y chromosomes (Disteche et al. 1986*b*), and there is no deletion or mutation of SRY by PCR and sequence analysis of the coding region in these three patients (data not shown) (fig. 1).

The only portions of the Y chromosome in common to all of the patients with gonadoblastoma include parts of intervals 3 and 4, depicted as shaded areas in figure 1. Part of interval 4 is absent in patient SA-6, who has an interstitial deletion, in addition to a terminal deletion (Disteche et al. 1986*a;* Cantrell et al. 1989; Vollrath et al. 1992). On the basis of the 43-interval deletion map of the Y chromosome (Vollrath et al. 1992), the intervals

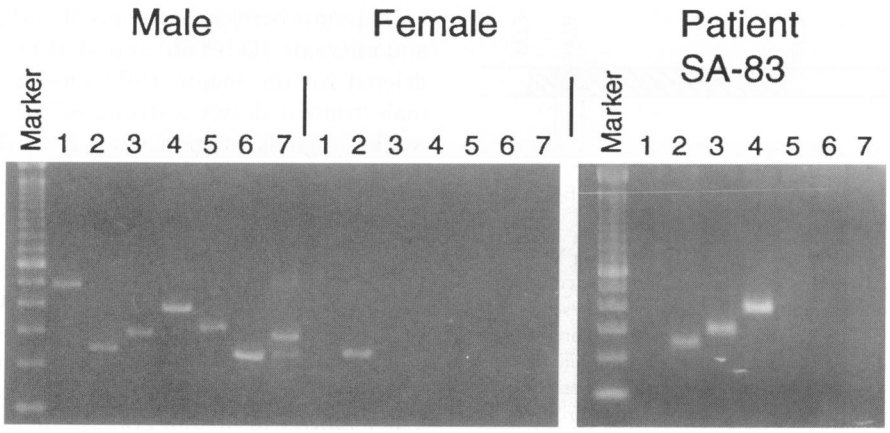


**Figure 2** PCR amplification of STSs for each of the seven deletion intervals of the Y chromosome (1–7 above the lanes), using DNA from a normal male, normal female, and patient SA-83 as template. Size markers consist of a 100-bp ladder.

**Table 1**

**STS Analysis of Patients**

| Interval[a] | STS | SA-83 | SA-91 | SA-92 |
|---|---|---|---|---|
| 1A1A | sY14 (SRY) | – | ... | – |
| 1A1B | sY16 (RPS4Y) / sY17 (RPS4Y) | ... | ... | + |
| 1A2 | ZFY | – | ... | + |
| 3C | sY57 | + | ... | ... |
| 3C | sY53[b] | + | ... | + |
| 3C/proximal to 3D | TSPY | + | + | + |
| 4A | sY70 | + | ... | ... |
| 4B | sY78 | + | ... | + |
| 5A | sY79 | – | ... | + |
| 5B | sY82 | – | ... | ... |
| 5C | sY85 | – | ... | + |
| 5H | sY95 | – | ... | ... |
| 6D | sY149 | ... | ... | + |
| 6F | sY158 | – | ... | ... |
| 7 | sY160 | – | + | + |

[a] Interval as designated in study by Vollrath et al. (1992), except for TSPY, which is mapped in the present study. The majority of TSPY repeats map to interval 3C, but one or a few copies are also present closer to the centromere.

[b] sY53 identifies sequences in intervals 4A, 6E, and 6F, in addition to interval 3C.

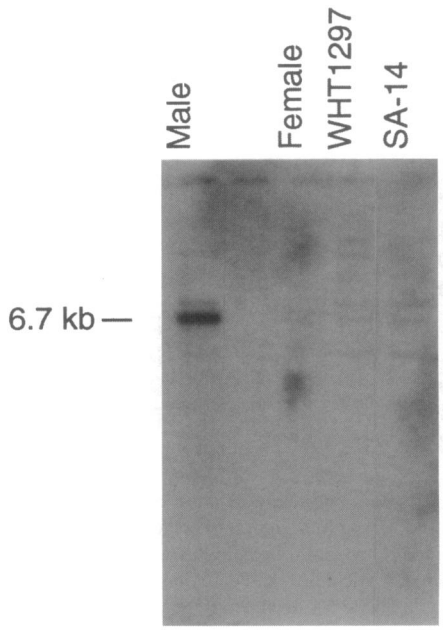


**Figure 4** Southern blot hybridization of *Hin*dIII-digested genomic DNA from patients with Yp deletions, a male control, and a female control, using [$^{32}$P] dCTP–labeled pJA36B2 insert. Patient WHT1297 has breakpoints between intervals 3D (sY66) and 3E (sY67). Patient SA-14 has a breakpoint between intervals 3C (sY57) and 3D (sY66).

common to all of the gonadoblastoma patients are 3E–3G and 4B (fig. 3). Distal interval 3E and proximal intervals 4A and 5A could not be tested, because they contain repetitive DNA and, therefore, could not be excluded from the GBY region.

### *Detection and Expression of TSPY and YRRM*

Southern blot hybridization experiments using a TSPY probe, pJA36B2, hybridized to *Hin*dIII-digested

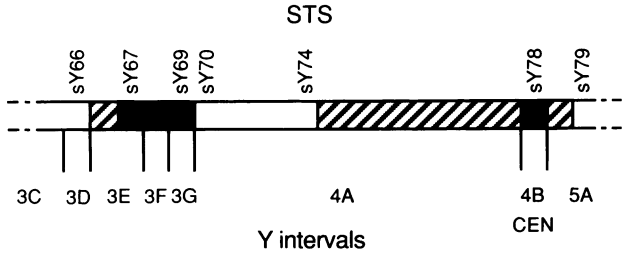


**Figure 3** Portion of the physical map of the human Y chromosome, from Foote et al. (1992), indicating the intervals common to all patients with gonadoblastoma used in this study. These intervals comprise two regions: one from 3E to 3G and another represented by proximal interval 4A, interval 4B, and possibly proximal interval 5A. Distal interval 3E and proximal portions of intervals 4A and 5A contain repeated DNA sequences and could not be tested (crosshatched areas). The STSs that define the boundaries of the critical region are indicated on top and the Y intervals below. CEN denotes the centromere.

DNA from patients SA-14 and WHT1297, together with previously published data on patient SA-6, place a major cluster of TSPY repeats in interval 3C and a minor cluster proximal to interval 3D in a region that appears to be contained in the GBY critical region. Patient SA-6 (also called WHT715 in Vollrath et al. [1992] and LCL43 in Zhang et al. [1992]) was previously shown to contain interval 3C with a breakpoint between 3C and 3B (Vollrath et al. 1992) and also to contain the major repeat locus for TSPY (Zhang et al. 1992). Patients SA-14 and WHT1297, who have Yp deletions and breakpoints between intervals 3C (SY57) and 3D (SY66) and intervals 3D (sY66) and 3E (sY67), respectively, are deleted for the major TSPY cluster (fig. 4). A normal male control shows a strong 6.7-kb signal and several weaker signals ranging from 2–10 kb in size (fig. 4). The intensity of the strong 6.7-kb signal corresponds to ~10 copies of TSPY by comparison of hybridization to dilutions of the pJA36B2 insert (data not shown). Hybridization patterns in patients SA-14 and WHT1297, who are deleted for interval 3C, are the same as the normal male control, but the intensity of the 6.7-kb signal is reduced to only one or a few copies, indicating that these patients are deleted for the major TSPY cluster in 3C but contain the minor locus located closer to the centromere (fig. 4). Mapping of TSPY to YACs also indicates that TSPY copies are present in the

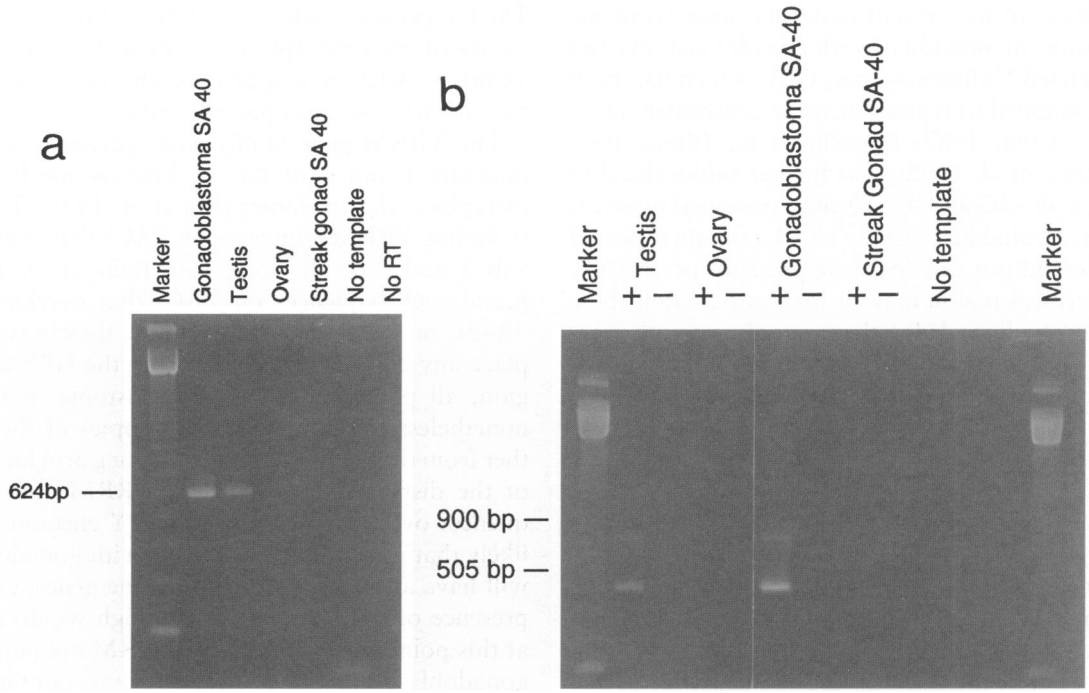


**Figure 5** *a,* RT-PCR for TSPY using RNA from gonadoblastoma and contralateral streak gonad of patient SA-40, control testis and control ovary. Control reactions using either no template or no RT are shown at right. Size marker is a 123-bp ladder. *b,* RT-PCR amplification of YRRM1, using RNA from gonadoblastoma and contralateral streak gonad of patient SA-40, control testis and control ovary. Plus sign (+) indicates reactions with RT, and minus sign (−) indicates reactions without RT. Another control reaction without template is shown at right. Size marker is a 123-bp ladder.

critical region, as YAC yOX233 (Foote et al. 1992), which overlaps interval 4B only, contains TSPY sequences (data not shown).

YRRM sequences are present on YACs that overlap intervals 3B–3C, 5I, 5L, and 6A-6E (Reijo et al. 1995). Although none of these YACs overlap the GBY critical region, comparison of Y-chromosome mapping in the patients versus the YAC mapping of YRRM reveals that all of the patients with gonadoblastoma possess at least some copies of YRRM either from the short arm, as in patient SA-83, or on the long arm, as in patient SA-14.

RT-PCR for TSPY and YRRM1 reveals expression of these genes in gonadoblastomas from two different patients, SA-40 and SA-92. The predicted RT-PCR products for TSPY (624 bp) and YRRM1 (505 bp) are present in control male testis and absent in the control ovary as expected (fig. *5a* and *b*). The correct RT-PCR products for TSPY and YRRM1 are present in the gonadoblastoma from patient SA-40, but the contralateral streak gonad from this patient lacks expression of both genes (fig. *5a* and *b*). To verify that the ovarian and streak gonad RNA was of sufficient quality to amplify, RT-PCR for the X-linked gene HPRT was performed. The correct 707-bp product is observed in both samples (data not shown). Expression of TSPY and YRRM1 was also found in the gonadoblastoma from patient SA-92 (data not shown).

## Discussion

Gonadoblastomas occur almost exclusively in 46,XY, sex-reversed females with gonadal dysgenesis (Verp and Simpson 1987). The etiology of sex reversal in these individuals is diverse. The molecular basis of sex reversal in the patients reported in this study includes mosaicism for the Y chromosome in two patients, deletion of SRY in five patients, and unknown etiology in three patients who had an apparently normal 46,XY karyotype and no apparent mutation in the coding region of the SRY gene. The presence of SRY does not appear to be directly implicated in gonadoblastoma development, since among our series of patients some have deletions of this gene, whereas others do not. However, although sequence analysis of SRY in the three patients with an apparently normal 46,XY karyotype revealed no mutations in the coding sequence, we did not exclude the possibility of deletions or mutations in the promoter region of SRY. Like SRY, the ZFY (*z*inc *f*inger on the *Y* chromosome) gene is not consistently present in our patients, indicating that presence of this gene does not appear to play a direct role in gonadoblastoma.

On the basis of the present molecular analysis of the Y chromosome in individuals with gonadoblastoma and partially deleted Y chromosomes, GBY, which had been previously mapped to regions near the centromere (Magenis et al. 1984, 1987; Disteche et al. 1986*a*; Page 1987; Petrovic et al. 1992), was further sublocalized to subintervals 3E–3G and 4B. Because proximal intervals 4A and 5A, located just distally to 4B, contain repeated DNA, we could not test for their presence or absence. Thus, the critical region may or may not contain these regions. To our knowledge there is only one other reported patient with a minute Y chromosome and gonadoblastoma (Petrovic et al. 1992) whose Y chromosome was shown to contain parts of regions 3 and 4B, because DNA from the patient hybridized to pDP105/A located in interval 3C and pDP97 in 4B. The patient also lacked sequences in interval 4A, as determined by hybridization to pDP34, indicating that there is a complex set of deletions in this patient (Petrovic et al. 1992). Although detailed breakpoints are not available on this patient, the results are in agreement with ours.

Examination of the Y-chromosome map constructed by Foote et al. (1992) shows that the GBY critical region that we defined is covered by only a few overlapping YAC clones. If the average spacing between ordered loci on this map is 220-kb (Foote et al. 1992), the size of the critical region is ~1–2 Mb. On the basis of the reasoning that other protooncogenes have physiological functions in normal cells, a candidate gene for GBY might have some function in normal males, specifically during spermatogenesis. There is evidence that the Y-encoded genes TSPY and YRRM are involved in spermatogenesis (Arnemann et al. 1991; Ma et al. 1993). Expression of TSPY has previously been shown to be limited to testis (Arnemann et al. 1987, 1991; Zhang et al. 1992) and one male tumor cell line (Zhang et al. 1992). In situ hybridization experiments have indicated that TSPY transcripts are found specifically in early spermatids (Arnemann et al. 1991). Similarly, expression of YRRM, a gene proposed as a candidate for the AZF (azoospermia) locus, has also been shown to be testis specific (Ma et al. 1993).

Manz et al. (1993) have demonstrated that TSPY elements are part of the DYZ5 repeat unit, with variability in copy number between individuals and sequence microheterogeneity in both transcribed and nontranscribed elements. The majority of TSPY (also called Y190) repeats map to interval 3C, with either a single TSPY copy or a few homologous but not identical copies, at a locus close to the centromere (Zhang et al. 1992; Tyler-Smith et al. 1993). Our Southern hybridization analysis confirms that the majority of TSPY repeats map to interval 3C with a minor locus proximal to interval 3D close to the centromere. Although this places the majority of TSPY repeats outside of the GBY critical region, a few copies of the gene appear to fall within this region. In addition, deletion mapping results imply that all our patients have some copies of TSPY.

The YRRM gene family was previously mapped to intervals 5 and 6 of the Y chromosome by FISH to metaphase chromosomes (Ma et al. 1993). In addition to finding YRRM sequences on YACS that overlap intervals 5 and 6 on the long arm, Reijo et al. 1995 also found such sequences on YACs that overlap intervals 3B–3C on the short arm. Although these results do not place any copies of YRRM within the GBY critical region, all patients with gonadoblastoma in this series nonetheless presumably possess copies of the gene, either from the short arm loci or the long arm loci. Because of the dispersed nature of the YRRM and TSPY sequences over many regions of the Y chromosome, it is likely that most if not all patients with gonadoblastoma will have at least some copies of the genes, even in the presence of large deletions. Although we do not know at this point whether TSPY or YRRM are implicated in gonadoblastoma, this realization points out the possibility of multiple GBY loci. It will be of importance to determine whether all copies of YRRM and TSPY are expressed, functional genes.

Our results showed expression of TSPY and YRRM in gonadoblastoma tissues from two patients, one who had a contralateral streak gonad that lacked expression of both genes. Expression of these genes may not cause tumor formation but may simply be due to the presence of germ cells in the tumors, since gonadoblastomas appear to recapitulate gonadal development (Scully 1970). Accordingly, the contralateral streak gonad that was devoid of germ cells did not express these genes. Other possibilities are that abnormal TSPY or YRRM products are expressed in dysgenetic gonads or that these genes are pursuing their normal functions, but in the context of dysgenetic gonads these functions may be tumorigenic. Interestingly, a T FLAST A search (Pearson and Lipman 1988) and Bestfit alignment of amino acid sequence (Wisconsin Package; Genetics Computer Group 1994) revealed 30% identity and 56% similarity between the TSPY and SET proteins. The SET gene has been shown to form a fusion gene with a putative oncogene, CAN (CAIN) in a case of acute undifferentiated leukemia (von Lindern et al. 1992).

In order to sort out the role of TSPY and YRRM in gonadoblastoma, more will have to be learned regarding the significance of multiple copies of these genes, interindividual variation in copy number, sequence variation between copies, expression of alternative transcripts, and the presence of untranscribed elements. In addition, other gene(s) yet to be identified may be found in the GBY critical region.

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