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Molecular Mapping of the Putative Gonadoblastoma Locus on the Y Chromosome

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Based on the high incidence of gonadoblastoma in females with XY gonadal dysgenesis or 45,X/46,XY mosaicism, the existence of a susceptibility locus on the Y chromosome (*GBY*) has been postulated. We attempted to map *GBY* by making use of a recently developed dense map of Y-chromosomal sequence-tagged sites (STSs). In two female patients with gonadoblastoma, small marker chromosomes contained portions of the Y chromosome, and a single region of overlap could be defined extending from probe pDP97 in interval 4B, which contains the centromere, to marker sY182 in interval 5E of the proximal long arm. This interval is contained in a YAC contig that comprises approximately 4 Mb of DNA. Our findings confirm the previous localization of *GBY* and greatly refine it. The localization of *GBY* overlaps with the region to which a putative growth determinant, *GCY*, was recently assigned. *Genes Chromosom Cancer 14:210–214 (1995).*

INTRODUCTION

Gonadoblastoma is a neoplasm defined histologically by the occurrence of discrete aggregates of germ cells and epithelial cells resembling immature Sertoli or granulosa cells (Scully, 1953, 1970). Leydig or lutein-type cells are usually present. Dysgerminomas and other malignant tumors can arise from gonadoblastomas.

The incidence of gonadoblastoma is high in female patients with Y-chromosomal material. Gonadal tumors are found in 25–30% of patients with XY gonadal dysgenesis and in 15–20% of 45,X/46,XY individuals (Schellhas, 1974; Verp and Simpson, 1987). Conversely, patients with dysgenetic gonads and no Y chromosome material do not have an elevated risk of gonadoblastoma. The existence of a gene on the Y chromosome predisposing dysgenetic gonads to the development of gonadoblastoma has therefore been suggested and has been named the gonadoblastoma locus on the Y chromosome, or *GBY* (Page, 1987).

Genes can be mapped on the Y chromosome by correlating the phenotype with the presence or absence of portions of structurally abnormal Y chromosomes. A cytogenetically defined deletion of the distal short arm of the Y chromosome in a girl with gonadoblastoma led Magenis et al. (1984, 1987) to suggest that the gonadoblastoma determinant was not located in the deleted part of Yp. Subsequently, a small number of probes recognizing specific loci on the Y chromosome were developed and mapped to create a seven-interval map (Page, 1986; Vergnaud et al., 1986), allowing structurally abnormal Y chromosomes to be characterized via Southern hybridization. Using this approach, Disteche et al. (1986) studied the abnormal Y chromosome of a female patient with gonadoblastoma and found portions of the short arm and the entire long arm to be present. In a further case studied in this way, Petrovic et al. (1992) were able to narrow the candidate region for *GBY* by excluding the distal part of the long arm; the same conclusion was reached in two further reports (Nagafuchi et al., 1992; van der Bijl et al., 1994). These studies can be tentatively interpreted as placing *GBY* in the proximal part of the long arm, including the pericentromeric region.

For the purposes of positional cloning, the physical localization of a gene should be as precise as possible. The recent detailed mapping of the human Y chromosome has provided greatly improved tools to delineate aberrant Y chromosomes (Foote et al., 1992; Vollrath et al., 1992). We used a dense set of mapped markers defining 43 intervals to study two patients with gonadoblastoma who each had a minute Y-chromosome-derived marker chromosome. We reasoned that, if a region was shared by both patients, it might be considered as a candidate region for the localization of *GBY*.

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CASE REPORTS

Case 1

Patient 1 (LGL3315) was born at term, measuring 48 cm long and weighing 3,000 g. No malformations or other major health problems were observed until she was referred at 10 years of age for chromosome analysis because of short stature. Her karyotype was mos45,X/46,X, + mar. The marker was lightly stained by G-banding and was extremely small, dot-like. The marker chromosome was present in 9 of 20 mitoses (45%) derived from phytohemagglutinin-stimulated blood cells, and it was shown to contain Y-chromosomal material when studied with Y-chromosomal probes. An explorative laparotomy was carried out, which showed bilateral streak gonads and a round tumor with a diameter of 8 mm in the left gonad. Gonadectomy was performed. At the age of 11.9 years, her weight was 31.6 kg (+0.8 SD compared to the mean weight of children of the same height) and her height was 135 cm (−2.4 SD), with a markedly delayed bone age. Her mother and father measured 167 cm and 179 cm, respectively. She received testosterone and growth hormone treatment for 1 year. Growth hormone treatment was continued for 2 more years until she was 15 years of age, at which time she measured 162 cm (0 SD) and was still growing. Menarche was hormonally induced at 15 years of age. Mammoplasty was performed at the age of 17 years because of breast hypoplasia. In addition to streak gonads, the patient displayed other features of Turner's syndrome, including widely spaced nipples, mildly webbed neck, and prominent ears.

The right gonad of patient 1 measured 5 × 5 × 15 mm. Histologically it showed blood vessels, stroma, tubular structures, and a few cells resembling Leydig cells. No tumor or germ cells were found. The left gonad contained germ cells resembling dysgerminoma cells and cells of Sertoli or granulosa cell type in well-defined, round clusters. Some of the germ cells showed mitotic activity. In addition, round calcifications and structures resembling Call-Exner bodies were found.

Case 2

Patient 2 (MICY880044) was a 12-year-old girl referred for chromosome analysis because of short stature. She was found to have the karyotype mos45,X/46,X, + mar. The marker chromosome was observed in 58% of her blood lymphocytes. At 13 years of age, a laparotomy showed bilateral streak gonads, and gonadectomy was performed.

TABLE 1. Results of Southern Blotting Analysis in Patient 1 With a Small Marker Chromosome and Gonadoblastoma

Interval	Probe	Reference	Patient 1
1	pDP1007	Page et al., 1987	+
1	pDP132	Bernstein et al., 1987	+
2	pDP61	Bernstein et al., 1987	−
3	50f2/A,B	Guellaen et al., 1984	−
4A	pDP34	Page et al., 1982, 1984	+
4B	pDP97	Bernstein et al., 1987	+
4B	50f2/D	Guellaen et al., 1984	+
6	50f2/C,E	Guellaen et al., 1984	−
7	pY431-HinfA	Bernstein et al., 1987	−

Histological examination showed dysgenetic gonads, with a dysgerminoma arising from a gonadoblastoma in the left gonad. At 17 years of age, she measured 149.5 cm, having still grown after cessation of growth hormone treatment. Menarche had been hormonally induced. Her mother and father measured 161 cm and 179 cm, respectively. This patient has been described previously (Petrovic et al., 1992).

MATERIALS AND METHODS

DNA Extraction

Lymphoblastoid cell lines of both patients were established, and DNA was extracted by standard phenol-chloroform extraction.

Southern Blotting

Restriction digestion, electrophoresis, transfer, and hybridization of DNA were performed by standard methods. Probes detecting Y-specific restriction fragments used in Southern blotting analysis are listed in Table 1. These probes have been assigned to a seven-interval deletion map (Page, 1986; Vergnaud et al., 1986).

Polymerase Chain Reaction Analysis of Sequence-Tagged Sites

The Y-chromosome-derived content of the marker chromosomes was defined by polymerase chain reaction (PCR) analysis of sequence-tagged sites (STSs) as previously described (Foote et al., 1992; Vollrath et al., 1992). DNA from patients was tested for the presence or absence of 31 STSs covering the short and long arms of the Y chromosome except for the pseudoautosomal regions. This set of markers includes assays for the *SRY*, *RPS4Y*, and *ZFY* genes. Intervals 1A1A to 4A correspond to the short arm and intervals 5A to 6F to

the euchromatic q11 band of the long arm of the Y chromosome. Interval 7 corresponds to the distal heterochromatic q12 band.

RESULTS

Southern Blotting

The results of Southern blotting analysis in patient 1 are given in Table 1. The results for patient 2 have been reported previously (Petrovic et al., 1992). Probe pDP97, which detects an alphoid repeated sequence at the centromere of the Y chromosome, hybridized to the DNA of both patients. Thus the centromere in both marker chromosomes was derived from the Y chromosome. The marker chromosome of patient 1 contained intervals 1, 4A, and 4B and was deleted for intervals 2–3 and 6–7. The marker chromosome of patient 2 contained intervals 3 and 4B and was deleted for intervals 1–2, 4A, 6–7, and possibly some of interval 5 (Petrovic et al., 1992).

Polymerase Chain Reaction Analysis of Sequence-Tagged Sites

The results of PCR analysis of STSs are given in Table 2. DNA from neither patient 1 nor patient 2 contains marker sY14 in interval 1A1A, which corresponds to the *SRY* gene involved in male sex determination. This is in keeping with the absence of testicular tissue in both patients.

The marker chromosome of patient 1 contains two blocks of Y chromosomal DNA. One ranges from marker sY15 in interval 1A1B to marker sY19 in interval 1B, including the *RPS4Y* and *ZFY* genes. The other block extends from marker sY72 in interval 4A to marker sY182 in interval 5E. These results are in accordance with the results obtained from Southern blotting analysis. The marker chromosome most probably is a result of a complex rearrangement having arisen as a consequence of more than one break.

The Y-chromosome-derived portion of the marker chromosome of patient 2 extends from marker sY183 in interval 5A to marker sY95 in interval 5H. In addition, the DNA is positive for markers sY57 and sY65 in interval 3C, which has been interpreted as an inversion polymorphism of intervals 3 and 4A (Page, 1986). Thus the findings in this patient suggest that the Y chromosomal DNA is contiguous. These results are in accordance with the previous results obtained from Southern blotting (Petrovic et al., 1992).

TABLE 2. Results of Polymerase Chain Reaction Analysis of Y-Chromosome-Specific Sequence-Tagged Sites (STSs) in Two Female Patients With a Small Marker Chromosome and Gonadoblastoma

Interval	STS	Male	Female	Patient 1	Patient 2
1A1A	sy14	(*SRY*) +	−	−	−
1A1B	sY15	+	−	+	−
1A1B	sY16	+	−	+	−
1A1B	sY17	+	−	+	−
1A1B	*RPS4Y*	+	−	+	−
1A2	sY18	+	−	+	−
1A2	*ZFY*	+	−	+	−
1B	sY19	+	−	+	−
3C	sY57	+	−	−	+
3C	sY65	+	−	−	+
3E	sY67	+	−	−	−
3F	sY68	+	−	−	−
3G	sY69	+	−	−	−
4A	sY72	+	−	+	−
5A	sY183	+	−	+	+
5A	sY81	+	−	+	+
5B	sY82	+	−	+	+
5C	sY84	+	−	+	+
5C	sY85	+	−	+	+
5D	sY87	+	−	+	+
5E	sY182	+	−	+	+
5G	sY94	+	−	−	+
5H	sY95	+	−	−	+
5I	sY161	+	−	−	−
5I	sY97	+	−	−	−
5M	sY113	+	−	−	−
5P	sY124	+	−	−	−
6A	sY129	+	−	−	−
6C	sY142	+	−	−	−
6F	sY157	+	−	−	−
7	sY160	+	−	−	−

DISCUSSION

In this study the Y-chromosomal content of DNA from two patients with gonadoblastoma and a small marker chromosome was defined. A single region of overlap of the marker chromosomes could be found extending from probe pDP97 in interval 4B, which contains the centromere, to marker sY182 in interval 5E of the proximal long arm (Fig. 1). This region is contained in a YAC contig (Foote et al., 1992) that spans approximately 4 Mb of DNA and can be exploited in the positional cloning of the *GBY* gene. One cannot exclude that the marker chromosomes contain material from chromosomes other than the Y chromosome, but this would not change the assignment. Both marker chromosomes contain Y-chromosomal alphoid repeated sequences, indicating that the centromere in both chromosomes is Y derived.

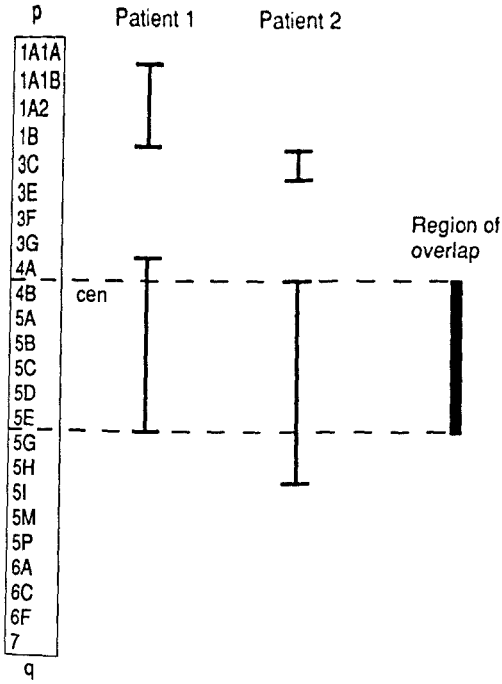

Figure 1. Interval map of the human Y chromosome. At right, the regions remaining in two female patients with a small marker chromosome and gonadoblastoma. The region of overlap is shown separately; p, short arm; q, long arm; cen, centromere.

Previous attempts to localize *GBY* (Magenis et al., 1984, 1987; Disteche et al., 1986; Nagafuchi et al., 1992; Petrovic et al., 1992; van der Bijl et al., 1994) used sets of markers that provided only limited resolution. However, the previous results are in accordance with those presented here, i.e., that the candidate region for *GBY* is close to the centromere, probably on the long arm. This consistency provides added credibility to the hypothesis that the *GBY* locus exists as a specific entity that can be mapped. It remains possible, however, that the marker chromosomes are even more complex than is shown by this set of markers, and they could even share more than one region of overlap.

As far as we know, no known candidate genes map to the defined *GBY* region. Previously we were able to deletion map the presumptive gene affecting stature to the region extending from marker sY78 in interval 4B to marker sY94 in interval 5G of the proximal long arm (Salo et al., 1995), which overlaps with the critical region for *GBY* described herein. We note with interest that our patient 1 and the patients described by Magenis et al. (1984) and Disteche et al. (1986) were not growth retarded even though they showed variable Turner stigmata. Other patients such as our patient 2 and the patient described by van der Bijl et al. (1994) with a deleted Y chromosome and gonadoblastoma have been growth retarded, but the mechanism leading to shortness is not readily interpretable in the presence of mosaicism with a prominent 45,X cell line. These observations suggest that the putative *GBY* and *GCY* genes are located in the same, relatively small interval that we have described. Once these genes and their products are cloned and characterized, it will be possible to determine whether they might be evolutionarily related, whether they share structural and functional properties, or even whether they might be identical. At least one family of transcribed genes that greatly resemble each other has already been found, that is, the *YRRM* genes (Ma et al., 1993), which might indicate that gene families are common on the Y chromosome.

We conclude that a small interval in proximal Yq11 including the centromere is shared in the marker chromosomes of both patients. This is the most precise localization so far suggested for the hypothetical gene conferring susceptibility to gonadoblastoma in dysgenetic gonads.

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