

Hypothesis: a Y-chromosomal gene causes gonadoblastoma in dysgenetic gonads

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Summary

The role of the human Y chromosome in the etiology of gonadoblastoma, a gonadal neoplasm, is considered and a two-part model is presented. According to this hypothesis: (1) There is a gene on the Y chromosome that strongly predisposes dysgenetic gonads to develop gonadoblastomas (Page, 1986) and (2) this postulated *GBY* gene (Gonadoblastoma locus on Y chromosome) has some physiological function in normal males. *GBY* may, for example, function in or prior to spermatogenesis in normal testes.

Y-DNA hybridization analysis of individuals with gonadoblastoma and partial deletions of the Y chromosome should be of use in testing this proposal. To date, such studies suggest that *GBY* maps to the region that includes deletion intervals 4B to 7, i.e. it is located near the centromere or on the long arm of the Y chromosome.

Key words: Y chromosome, gonadoblastoma, deletion map.

Introduction: the epidemiology of gonadoblastoma

Gonadoblastomas are neoplasms defined histologically by the occurrence of both germ cells and sex cord elements (cells resembling immature Sertoli and granulosa cells) within well circumscribed nests (Scully, 1953, 1970). Stromal elements resembling Leydig or lutein cells are usually present. Gonadoblastomas are so named because (1) they recapitulate gonadal development (the primitive sex cords and stroma) and (2) they arise in markedly abnormal gonads, often not recognizable as either testis or ovary. Such gonads, often lacking the usual architecture of either an ovary or a testis and usually devoid of normal germ cells, are frequently described as being 'dysgenetic'. Germinomas and other more malignant cancers can arise within gonadoblastomas.

Virtually all gonadoblastomas arise within dysgenetic gonads. Recognizing this fact, Verp & Simpson (1987) suggested two alternative models of the etiology of gonadoblastoma. As one possibility, they suggest that the prevalence of gonadoblastoma in individuals with gonadal dysgenesis simply reflects the heightened neoplastic susceptibility of poorly

differentiated tissue. As a second possibility, they suggest that, in cases where gonadal dysgenesis is the result of a defect in a single X-linked or autosomal gene, an independent and pleiotropic effect of that mutation is to cause gonadoblastoma.

Additional epidemiological data make both models seem unlikely. Gonadoblastomas occur almost exclusively in a very select population, i.e. individuals with dysgenetic gonads and a Y chromosome (reviewed by Verp & Simpson, 1987). Among individuals with dysgenetic gonads who are otherwise phenotypically female, gonadoblastoma occurs in a substantial fraction of those with a 46,XY or mosaic 45,X/46,XY karyotype; it rarely occurs in those with a 45,X or 46,XX karyotype. The tumour also occurs in females with gonadal dysgenesis and a deleted or otherwise structurally abnormal Y chromosome in some or all cells. Gonadoblastomas are sometimes present in individuals with marked sexual ambiguity, a Y chromosome and abnormal, abdominal or inguinal testes (Scully, 1970). Only a very small fraction of gonadoblastomas develop in the apparent absence of Y-chromosomal material and, in these cases, the presence of Y chromatin has been excluded by conventional cytogenetic (chromosome banding)

analysis, not by DNA hybridization. If gonadal dysgenesis alone predisposes to gonadoblastoma, then the tumour should occur at comparable rates in gonadal dysgenesis females with and without Y chromosomal material, which is not the case. If pleiotropic X-linked or autosomal mutations cause, by independent mechanisms, gonadal dysgenesis and gonadoblastoma, then the tumour should be limited mainly to individuals carrying such mutations. However, gonadoblastoma is a frequent occurrence in females without such Mendelian mutations, in whom (1) gonadal dysgenesis is due to mosaicism for a Y-bearing cell line or in whom (2) gonadal dysgenesis (and sex reversal) is due to the presence of a Y chromosome lacking the male-determining region (e.g. case 2 in Disteche *et al.* 1986, and case reported by Magenis *et al.* 1984, 1987).

As mentioned, the occurrence of gonadoblastoma is essentially restricted to individuals with dysgenetic gonads (reviewed by Scully, 1970; Verp & Simpson, 1987). It is rarely, if ever, found in normal males or females; in 46,XY individuals with testicular feminization, who have well-formed, abdominal or inguinal testes; in 46,XY males with undescended but well-developed testes and no sexual ambiguity (undescended testes are prone to other tumours, but not to gonadoblastoma); or in 47,XXY or 46,XX males, whose testes are devoid of germ cells but otherwise well formed.

Hypothesis

How does the Y chromosome foster the development of gonadoblastomas in dysgenetic gonads? It seems unlikely that this ability to provoke neoplasia is a general, nonlocalized characteristic of Y chromatin. It is more likely that the gonad is driven toward neoplasia by the action of a particular Y-chromosomal gene. I postulate that, in the context of a dysgenetic gonad, some structural or regulatory gene of the Y chromosome acts as an oncogene. For purposes of discussion, this postulated gene will be referred to as *GBY* (Gonadoblastoma locus on Y chromosome).

The presence of *GBY* is not a sufficient condition for the development of gonadoblastoma (Fig. 1). First, oncogenic manifestation of *GBY* requires a markedly abnormal gonad. Second, the combination of *GBY* and a dysgenetic gonad constitutes a strong but not absolute predisposition to gonadoblastoma. Some individuals with an intact Y chromosome and gonadal dysgenesis do not develop gonadoblastoma, and gonadoblastomas often appear to be of focal origin within dysgenetic gonads. Thus, additional, unrecognized factors or events may be required for the development of the tumour. It is possible, for

example, that *GBY* becomes oncogenic only after acquiring some somatic mutation. Alternatively, some physiological event not directly involving *GBY* may be necessary.

The first tenet of this hypothesis, then, is that, in the context of dysgenetic gonads, *GBY* acts as an oncogene. The second tenet of the hypothesis is that *GBY* has a physiological function in normal males, likely in the testes, perhaps in or prior to spermatogenesis. The reasoning is as follows. The wild-type alleles (nonmutant forms) of cellular oncogenes have physiological functions in normal cells (Waterfield *et al.* 1983; Doolittle *et al.* 1983; Downward *et al.* 1984). In females with gonadal dysgenesis, *GBY* principally affects the gonads; it does not appear to have pleiotropic effects on other organs. By analogy, its function in normal males may principally involve the testis. Since gonadoblastomas recapitulate germ cell/support cell architecture (i.e. the nesting of germ cells within support cells), *GBY* may well function in or prior to spermatogenesis in normal testes. There is some independent evidence for the existence of one or more Y-chromosomal genes required for spermatogenesis (Tiepolo & Zuffardi, 1976). Thus, as a working hypothesis, it is reasonable to suppose that, while *GBY* induces gonadoblastomas in dysgenetic gonads, it functions in or prior to spermatogenesis in normal testes.

Testing the model by deletion mapping

The model presented here makes a testable prediction. Since gonadoblastoma can occur in the presence of partially deleted Y chromosomes, it should be possible to define the small portion of the Y in which the *GBY* locus is found. Most if not all individuals with gonadoblastoma should carry, in at least some cells, the segment of the Y chromosome that contains

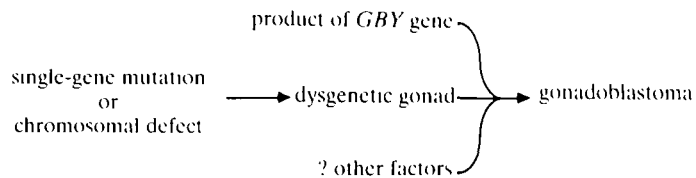


Fig. 1. An hypothesis as to the etiology of gonadoblastoma. Development of gonadoblastoma generally requires both (1) the product of the gonadoblastoma locus (*GBY*), normally present on the Y chromosome, and (2) a dysgenetic gonad. As this combination is not sufficient to cause gonadoblastoma, the existence of other, unidentified factors is invoked. Gonadal dysgenesis itself can have various causes, including but not limited to (1) heritable mutations in X-linked or autosomal genes and (2) structural and numerical defects of the sex chromosomes, including mosaicism for a 45,X cell line.

GBY. Conversely, the absence of the *GBY* locus should prevent gonadoblastoma, even in the presence of other portions of the Y chromosome. Many individuals with gonadoblastoma have an intact Y chromosome. Though such cases underpin the *GBY* hypothesis, they contribute little to testing this particular prediction.

Rapid progress is being made in constructing a detailed deletion map of the human Y chromosome. This progress has stemmed, in part, from advances in the examination of structural abnormalities of the Y chromosome with prometaphase staining techniques (Magenis *et al.* 1984). To a larger degree, however, this progress has been propelled by recombinant DNA cloning of sequences derived from the Y chromosome (Cooke & Hindley, 1979; Page *et al.* 1982; Bishop *et al.* 1983). Cloned Y-DNA sequences are hybridized to gel transfers (Southern, 1975) of genomic DNAs from individuals with structural abnormalities of the Y chromosome, providing data from which a deletion map of the Y chromosome can be deduced (Vergnaud *et al.* 1986; Affara *et al.* 1986; Müller *et al.* 1986; Page, 1986). The precision of this map is limited only by the number of structural abnormalities examined and the number of Y-DNA probes with which they are studied.

The deletion map (Fig. 2) provides a framework for examining the role of the Y chromosome in various biological functions. Indeed, construction of the map has been motivated largely by the desire to understand the role of the Y chromosome in gonadal sex determination. Deletion analysis of 'sex-reversed' individuals (e.g. XX males and XY females) has shown that the presence of a small portion of the short arm of the Y, interval 1, is both necessary and sufficient to induce testicular differentiation of the bipotential gonad (Page, 1986).

The DNA-probe-based deletion map of the human Y chromosome is already finding other uses. Antigenic characterization of B cell lines from patients

with well-defined deletions of the Y has mapped the gene for H-Y transplantation antigen to the long arm or centromeric region of the chromosome (intervals 4B through 7; Fig. 2; Simpson *et al.* 1987), refuting a postulated role for H-Y in gonadal sex determination. More refined deletion mapping of the *H-Y* gene on the Y chromosome may aid in testing its postulated role in spermatogenesis (Burgoyne, Levy & McLaren, 1986).

It is important to distinguish the H-Y transplantation antigen from the serologically detectable male (SDM) antigen. The two antigens are probably not identical (Silvers, Gasser & Eicher, 1982). Nonetheless, SDM is often referred to as H-Y antigen in the literature. SDM, which has been proposed as a risk factor for gonadoblastoma (Warner *et al.* 1985), has not been localized on the DNA-hybridization-based deletion map of the human Y chromosome.

Practical issues and initial observations

Several issues arise in attempting to deletion map *GBY*. First, the phenotype must be identified accurately. The consistent application of defined histological criteria in diagnosing the presence of gonadoblastoma (Scully, 1970) is of critical importance. Second, it must be borne in mind that the presence of Y chromatin merely *predisposes*; gonadoblastomas do not develop in all individuals with dysgenetic gonads and *GBY*. Third, the tumors can first manifest themselves over a wide range of patient ages (Manuel, Katayama & Jones, 1976). Quite appropriately, the gonads of individuals at risk are frequently removed (and examined histologically) in childhood. Consequently, in attempting to map *GBY*, deleted-Y cases with gonadoblastoma can be interpreted with certainty, while cases without gonadoblastoma provide information of more limited usefulness.

The limited evidence presently available suggests that, if there is a single *GBY* locus on the Y

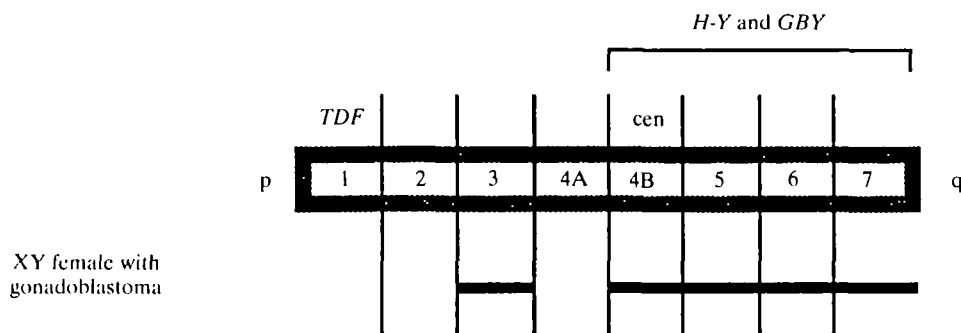


Fig. 2. An 8-interval deletion map of the human Y chromosome (Vergnaud *et al.* 1986; Page, 1986). Beneath the map, the heavy black bar indicates the portion of the Y chromosome (intervals 3 and 4B-7) present in a 46,XY female with gonadoblastoma (case 2 in Disteche *et al.* 1986). Her Y chromosome is deleted for the testis-determining factor (*TDF*) but retains the genes for H-Y antigen (*H-Y*; Simpson *et al.* 1987) and gonadoblastoma (*GBY*). The short arm, centromere and long arm of the Y chromosome are indicated by p, cen, and q.

chromosome, then it maps near the centromere or on the long arm. A female with gonadal dysgenesis, bilateral gonadoblastoma, and a 46.XY karyotype was found to carry DNA sequences specific to intervals 3 and 4B-7 of the Y chromosome (Fig. 2; case 2 in Disteche *et al.* 1986). Her Y chromosome was deleted for intervals 1, 2 and 4A, which can be excluded as sites for *GBY*. DNA hybridization findings in a second female with gonadal dysgenesis, bilateral gonadoblastoma and a 46.XY karyotype (Magenis *et al.* 1984, 1987) suggest that she lacks interval 3 (as well as other intervals on Yp). Together, the findings in these two XY females suggest that *GBY* maps to the region defined by intervals 4B-7, which includes the centromere and all of the long arm (Fig. 2). Since *TDF*, the testis-determining factor, maps to interval 1, *GBY* and *TDF* cannot be one and the same (Page, 1986). The role of the Y chromosome in gonadoblastoma is probably independent of its role in gonadal sex determination.

It will be of interest to map the chromosomal position of *GBY* with more precision. Particularly useful information may come from characterization by Y-DNA hybridization of individuals with gonadal dysgenesis, gonadoblastoma and deletions or translocations with breakpoints on the long arm of the Y chromosome (Yq). Lukusa *et al.* (1986) have suggested that, among individuals with dysgenetic gonads and Y chromosomes of apparently normal length, gonadoblastoma is much more common in those whose Y chromosomes retain the normally intense fluorescence of distal Yq. Though Lukusa, Fryns & Van den Berghe (1986) postulated otherwise, the relative infrequency of gonadoblastoma in those with nonfluorescent Y chromosomes may be due to deletion of *GBY*-bearing portions of Yq. It will also be important to examine, with Y-DNA probes, those individuals in which gonadoblastoma occurs in the apparent absence, as judged by chromosome banding studies, of Y chromatin. Some such individuals may prove to carry all or a portion of the Y chromosome in at least a fraction of their cells. It should be recalled that, in many 46,XX males and 45,X males, Y chromatin was not detected by cytogenetic analysis but was detected by Y-DNA hybridization studies. Alternatively, even if the *GBY* oncogene theory is correct, some gonadoblastomas may arise in its absence as a result of mutations in other genes, leading to tumorigenesis *via* some other pathway.

How many spermatogenesis factors are encoded by the Y chromosome? It has been suggested, from studies in mice, that H-Y transplantation antigen may function in spermatogenesis (Burgoyne *et al.* 1986). I have already argued that *GBY* may also function in or prior to spermatogenesis in normal males. In man,

H-Y, like *GBY*, has been deletion mapped to intervals 4B-7 of the Y chromosome (Simpson *et al.* 1987). This region, which includes the centromere and all of the long arm, probably also includes the spermatogenesis factor postulated by Tiepolo & Zuffardi (1976). The question arises as to whether *GBY*, *H-Y* and this spermatogenesis factor (also known as *AZF*, 'azoospermia factor') are actually three distinct loci. They may be synonyms for a single gene, merely denoting the pleiotropic manifestations of that gene. Deletion mapping may demonstrate that *GBY*, *H-Y*, and *AZF* are distinct from each other (as in the case of *TDF* and *H-Y*), or it may lend credence to their being one and the same. Similarly, it will be interesting to examine the possibility that one or more of these entities, *GBY*, *H-Y* or *AZF*, might be identical to the growth factor, *GCY*, postulated to exist on Yq (Alvesalo & de la Chapelle, 1981).

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