

Clinical implications of overt and cryptic Y mosaicism in individuals with dysgenetic gonads

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Abstract. The most common germ cell neoplasm in individuals with dysgenetic gonads who have Y-chromosomal material in their genome (45,X/46,XY;46,XY Swyer syndrome) is gonadoblastoma. Gonadoblastoma is considered to be an in situ germ cell neoplasm and recapitulates the embryonic development of the gonad. The clinical significance of gonadoblastoma is that it may progress to dysgerminoma and other highly malignant germ cell neoplasms. The current literature suggests that the overall incidence of germ cell neoplasms in individuals with mosaic 45,X/46,XY karyotypes varies from 10% to 30%. The purpose of this presentation is to review the available literature and the experience at the Medical College of Georgia to characterize and define the risks of germ cell tumors in 45,X individuals who carry a population of overt or cryptic 46,XY cells with or without a morphologically abnormal Y chromosome. The inappropriate and incomplete differentiation of the dysgenetic gonad sets the stage for an unstable situation that may lead to uncontrolled proliferation of primitive germ cells. The potential for these unpredictable transformations is what constitutes the true risk of the dysgenetic gonad and its precursor neoplasm-gonadoblastoma. © 2006 Elsevier B.V. All rights reserved.

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1. Introduction

In the past, the association of Y DNA with dysgenetic gonadal tumors has been confounded by ascertainment biases related primarily to the spectrum of the clinical phenotypes, cryptic Y cell lines, lack of long-term follow-up and misperceptions about the pathogenesis and malignant potential of the tumor types arising in dysgenetic gonads.

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The first part of the presentation will describe the clinical spectrum of 45,X/46,XY individuals who are at risk for dysgenetic tumors. The second part will address the scope and limitations of the current techniques to identify overt and cryptic Y DNA. The third section will discuss the histopathology, morbidity and mortality associated with the different types of tumors that occur in dysgenetic gonads. In the fourth section the literature on this topic will be reviewed and the authors will also describe their experience at the Medical College of Georgia (MCG) with respect to the frequency and tumor types seen in patients with overt and cryptic Y cell lines. Using this body of information the authors will attempt to formulate conclusions concerning the detection of Y DNA, the risk of tumor formation and suggested counseling for those patients judged to be at risk for dysgenetic tumors.

2. Phenotypic and gonadal features seen in 45,X/46,XY individuals

Since the frequency of tumor formation varies across the gonadal spectrum seen in these 45,X/46,XY individuals, attempts to draw tumor associations should start with a firm knowledge of the clinical phenotype and gonadal status of the patient [1,2]. Group 1—normal females with bilateral rudimentary streak gonads; Group 2—females with clitoromegaly, unilateral streak and contralateral intraabdominal testis; Group 3—neonates with labial testis and intraabdominal streak; Group 4—normal males with bilateral scrotal testes.

3. Scope and limitations of current techniques to detect populations of 46,XY cells in 45,X individuals

The inherent limitations in diagnostic methodologies frequently pose a significant challenge in defining the true genotype of the patient with the Turner syndrome (TS). Appropriate clinical decision making is dependent on a firm knowledge of the scope and limitations of the laboratory techniques coupled with precise information regarding the clinical phenotype. Some of the challenging situations encountered by the clinician that require close interaction with the laboratory are as follows: (1) Structurally abnormal X vs abnormal Y with/without a hidden 45,X cell line; (2) non-mosaic 45,X with hidden 46,XY cell line; (3) non-mosaic 46,XY with hidden 45,X cell line; (4) non-mosaic 47,XYY with hidden 45,X cell line.

After counting and analyzing 30–50 metaphases the most practical laboratory strategy to resolve these dilemmas is as follows: (1) Reflex Metaphase FISH: If unknown or marker sex chromosome seen on metaphases, use Reflex Metaphase FISH with X (DXZ1) and Y (DYZ3) centromere specific probes to characterize the unknown fragment as X or Y derived [3]. (2) Reflex Interphase FISH: If non-mosaic 45,X or non-mosaic 46,XY or non-mosaic 47,XYY, perform Reflex Interphase FISH and score 500 nuclei (99% confidence to detect 1% Y mosaicism) [4]. (3) DNA Analysis: Confirm positive or negative FISH for Y material with DNA analysis for DYZ3, SRY, DYZ4 A, DYZ4 B with a series of STSs [5]. Nested PCR may overestimate the frequency of Y sequences and should be avoided [6]. In general Metaphase FISH, Interphase FISH and DNA analysis should complement and validate each other in the detection of covert Y DNA and identification of rearranged X and Y chromosomes [3–5].

4. Histopathology, morbidity and mortality associated with the different types of tumors that occur in dysgenetic gonads

Discussion of the incidence of germ cell tumors in subjects with Gonadal Dysgenesis who carry Y DNA requires a firm knowledge of tumor histopathology and related morbidity. The most common tumor formation is gonadoblastoma. Gonadoblastoma is considered to be an in situ germ cell neoplasm that recapitulates the embryonic development of the gonad. It is usually composed of three principal cell types: (1) large germ cells similar to those of seminoma; (2) small cells resembling immature Sertoli and Granulosa cells; (3) elements resembling Leydig or lutein-like cells may be less frequently present [7].

4.1. Gonads of origin

The rudimentary streak gonads (RSGs) seen in individuals with the Turner syndrome consist almost exclusively of fibrous tissue resembling ovarian stroma. However, when samples from a “totally excised” dysgenetic gonad are studied it is not unusual to see occasional primary oocytes and/or immature seminiferous tubule like structures. The spectrum of histopathology seen in RSGs that have been “totally excised” has been well described by Greenblatt et al. in 1967 [1] and Cools et al. in 2006 [8]. The study of RSGs by Cools et al. includes the immunohistochemical features of the different cellular components of RSGs that have not yet undergone neoplastic transformation compared to those findings in gonadal tissue adjacent to and within gonadoblastoma samples [8].

4.2. Gonadoblastoma

The detailed histology and immunohistochemistry described by Cools et al. suggest that most gonadoblastomas start their growth process within residual primitive, immature seminiferous tubules. On this basis Cools et al. have proposed a model for the development of gonadoblastoma and carcinoma in situ in dysgenetic gonads of patients who carry Y chromosome material in their genome. This model based on their observations and studies of gonadectomy samples from 43 female patients with Y chromosome aneuploidy suggests that the development of gonadoblastoma and its evolution into a broad spectrum of germ cell neoplasm types is a predictable process. Gonadal tissue with gonadoblastomas negative for OCT3/4 expression may participate in a degenerative process. Degeneration and calcification are frequently seen in many gonadoblastomas, whereas in other areas, the cellular elements prevail. Some of these “testicular-like” germ cells may die, but some due to abundant and prolonged expression of testis-specific protein encoded on Y (TSPY) and OCT3/4 will survive. Their continued proliferation leads to clonal expansion and cancer in situ (CIS) formation. This model suggests that the more malignant germ cell neoplasms in these dysgenetic gonads develop on a testicular background [9–12].

Gonadoblastoma is a tumor that is relatively specific for individuals with dysgenetic gonads who have Y chromosome material in their genome (45,X/46,XY;46,XY Swyer syndrome). However, it may occur in patients with Denys–Drash and Frasier syndrome secondary to mutations in WT1. It has been reported on two occasions in both XX and XY

true hermaphroditism. In the latter, consistent with the model proposed by Cools et al., it seems to start in the portion of the gonad where seminiferous tubules are preeminently present [8]. From this model, one can appreciate that any of the cellular components may evolve in an unpredictable fashion into any one of the following invasive germ cell neoplasms (dysgerminoma, chorioepithelioma, embryonal carcinoma, yolk sac tumor, endodermal sinus tumor, malignant teratoma). Equally important for malignant transformation is the uncertain meiotic state of the germ cells that are trapped in a sexually dimorphic environment. Recent data suggest that the retinoic acid stimulated expression of the gene *Stra8* (stimulated by retinoic acid gene 8) is essential for initiating the meiotic process in oocytes. In contrast, retinoic acid in testicular germ cells is actively degraded by a cytochrome *P450* enzyme (*Cyp26b1*) and the premature onset of male germ cell meiosis is prevented. In mouse ovotestis, one can clearly see overexpression of *Stra8* in the ovarian compartment of the gonad and overexpression of *Cyp26b1* in the testicular segment. Any loss of control or misregulation of this process may set up a precarious situation where uncontrolled proliferation of germ cells may occur [13]. Further investigation of these interactions in gonadoblastoma is important to our understanding of malignant transformation in this unregulated and unstable ecology. The ambiguity of the environment and the potential for these unpredictable transformations constitute the true risk of the dysgenetic gonad and its precursor neoplasm—gonadoblastoma.

4.3. Morbidity and mortality

Gonadoblastomas occur almost exclusively in patients with dysgenetic gonads and can be classified as an in situ cancer. However, dysgerminomas and other even more malignant germ cell tumors may occur within or associated with gonadoblastomas. To this day the largest series and most definitive retrospective study looking at the association of gonadoblastoma to virulent germ cell neoplasms come from Robert Scully [14]. In Scully's experience bilateral involvement of the gonads was common. In approximately 50% of the patients the gonadoblastoma was overgrown by dysgerminoma. In six of 74 patients, gonadoblastoma was associated with malignant teratoma, embryonal carcinoma, endodermal sinus tumor or choriocarcinoma. Follow-up was incomplete, but four of the six patients succumbed to their malignancies [14]. The four (4) lethal cases reported by Scully [14] and subsequent three (3) lethal reports from Gallager, Talerman, and Ito [15–17] serve to illustrate that malignant germ cell neoplasms occurring in dysgenetic gonads are just as lethal and have the same potential for morbidity [18] as similar type tumors occurring on a normal genetic background. The prognosis with gonadoblastomas is uniformly good when they are completely removed and do not contain any elements of the more aggressive germ cell tumors.

The association of gonadoblastoma with these highly malignant germ cell neoplasms may also have significant practical implications when it comes to interpreting surgical pathology at the time of laparotomy. The operator unfamiliar with tumors in dysgenetic gonads may remove an ipsilateral gonadoblastoma and leave the contralateral germ cell tumor in place thinking it is normal ovary [15]. This has happened on more than one occasion with subsequent progression of a virulent germ cell tumor leading to death of the patient [15]. These reports serve to emphasize the importance of the associations of

gonadoblastomas with more serious malignancies and the need to carefully review the histopathology of excised gonadoblastomas [16].

At the present time we do not have a way to predict the malignant evolution of gonadoblastoma. Tumor registries are frequently set up to register the diagnosis of cancer and record mortality. If one cross-references the Turner syndrome and gonadoblastoma, one does not identify any mortality. Three registry studies have been carried out in Denmark [19–22] and no cases of gonadoblastoma or death from gonadoblastoma were recorded. This is not surprising since gonadoblastoma is an in situ cancer and patients with a known Y chromosome who are at risk for germ cell neoplasms generally undergo prophylactic gonadectomy at an early age. Virulence with morbidity and mortality only occurs after malignant transformation of the gonadoblastoma. For example, Scully in his series of germ cell neoplasms describes 6 cases of gonadoblastoma associated with highly malignant germ cell neoplasms [14]. Registry searches need to start with all germ cell tumors known to occur in association with dysgenetic gonads. After identifying all registered germ cell neoplasms, one can proceed to select those that have developed on a dysgenetic background. A registry that is appropriately set-up and cross-referenced across the spectrum of germ cell tumors is needed in the United States. An important component of such a registry is an adequate facility to review and validate the coded pathology.

5. Clinical implications of Y mosaicism and association with germ cell tumors in dysgenetic gonads

Review of the literature on this topic suggests that 8–12% of patients with gonadal dysgenesis have 45,X/46,XY cell lines with or without structurally abnormal Y chromosomes. The frequency of cryptic Y DNA in some individuals who appear to be non-mosaic 45,X using standard metaphase karyotyping (30–50 metaphases) varies across the published literature. Some of this variation is a reflection of the numbers of non-mosaic 45,X patients that are studied, the techniques used, and the strategies employed by different investigators to search for small populations of Y containing cells. Judging from the literature, the frequency of covert Y DNA in individuals with non-mosaic 45,X karyotype varies from 0% to 9.3%. The largest series of non-mosaic 45,X patients reported by Canto et al. identified covert Y sequences in 10 of 107 (9.3%) non-mosaic 45,X TS patients [23]. After counting and analyzing 40 metaphase cells the authors used PCR for DYZ3, SRY, ZFY, DYZ1, and PABY (Pseudoautosomal boundary on Y) to detect cryptic Y sequences. Among the 45,X patients ($n=10$) with cryptic Y DNA, 6 underwent bilateral gonadectomy and 2 of the 6 (33%) had dysgenetic tumors (gonadoblastoma with dysgerminoma and gonadoblastoma alone). This large series of non-mosaic 45,X patients suggests that the tumor incidence in patients with cryptic Y DNA is comparable to that among overtly mosaic 45,X/46,XY patients [23]. Screening of all non-mosaic 45,X patients (30–50 metaphases) should be performed using Interphase FISH as a reflex test (500 cells to detect 1% mosaicism with 0.99 confidence) with DNA testing for further confirmation of the FISH results [4].

The overall incidence of germ cell neoplasms in individuals with mosaic 45,X/46,XY karyotypes in the literature varies from 10% to 30%. This wide variation is mainly related to the wide spectrum of the phenotype at risk. This phenotypic heterogeneity creates an

ascertainment bias since the different phenotypes may be seen by different physicians or sometimes overlooked. For example infertile 45,X/46,XY males with bilateral scrotal testes are one of the clinical categories that have not been subject to careful follow-up. Some of these short 45,X/46,XY azoospermic males with scrotal testes may be overlooked if the 45, X cell line is not identified and suspected. Misperceptions about the pathogenesis and evolution of the tumor types arising in dysgenetic gonads may also be a modifying factor.

A review of 13 observational case reports revealed 14 tumors in 60 subjects (23%) with a 45,X/46,XY chromosomal constitution [24–26]. The report by Mazzanti et al. is especially revealing because it demonstrates the instability and toti-potential capabilities of the dysgenetic gonad. One of the 4 tumor patients in this report had co-existing elements of gonadoblastoma, immature teratoma and endodermal sinus tumor within the same primitive gonad [26]. This report serves to illustrate the importance of carefully surveying the histology of the entire excised gonad before arriving at a final diagnosis. A review of 91 patients with 45,X/46,X,idic(Y) reported by Tuck-Muller et al. revealed 13 patients (14%) with dysgenetic tumors [27]. Among 14 patients who had Y chromosome material (Cryptic $n=7$) and underwent adnexectomy ($n=10$), Gravholt et al. identified only 1 dysgenetic tumor (gonadoblastoma) [22]. The low risk of tumor (10%; 95% CI 1–44%) in the Gravholt study is probably a reflection of sample size and the emphasis in the Danish cancer registries on morbidity and mortality end points. Morbidity and mortality are rare with gonadoblastoma, but frequent with other germ cell tumors.

Studies of 45,X/46,XY patients ($n=31$) at risk for germ cell neoplasms at the Medical College of Georgia (MCG) can be compared with the frequency and tumor types in the current literature. The spectrum of 45,X/46,XY patients studied at MCG includes all of the 4 distinct clinical phenotypes that may develop in someone who has a 45,X/46,XY chromosomal constitution. In contrast, the literature on this topic tends to focus on those 45, X/46,XY individuals who are phenotypically females. However, there is no reason to assume that 45,X/46,XY individuals with different degrees of sexual differentiation including 45,X/46,XY males have differential risks for the other features of the TS (tumor formation, cardiovascular anomalies, etc.). A study of all clinical phenotypes derived from a 45,X/46,XY embryos should help to determine if any differential risks exist across the spectrum of clinical phenotypes. The 45,X/46,XY patients ($n=31$) studied at the MCG fall into 4 distinct clinical phenotypes: Group 1: Normal females with bilateral rudimentary streak gonads ($n=15$); Group 2: Females with clitoromegaly, unilateral streak and contralateral intraabdominal testis ($n=8$); Group 3: Neonates with labial testis and intraabdominal streak ($n=2$); Group 4: Normal males with bilateral scrotal testes ($n=6$).

All patients at MCG (newborn–31 years) have been studied clinically, cytogenetically and with DNA analysis to detect and verify the presence of Y DNA. In Group 1, 7/15 patients (47%) had gonadoblastomas and/or dysgerminomas. Included in this group is one patient with cryptic Y and 10 patients with Y derived fragments. Two of the patients in this group both with tumor were studied post-operatively to search for cryptic Y in one patient and clarify an enigmatic Y derived fragment in the second patient. In Group 2, there were 2/8 patients (25%) with dysgenetic tumors and one of these tumors was an embryonal cell carcinoma producing large amounts of AFP. In group 3 there were no tumors, but this group was sexually ambiguous at birth and the gonads were removed neonatally. In Group 4, 1/6 patients (17%) harbored early gonadoblastoma in both testes.

The overall prevalence of all tumor types based on the data at MCG is 10/31 or 32%. Dysgenetic tumors of all types were most prevalent in the Group 1 gonadal phenotype. This may be a reflection of age at gonadectomy, since groups 2 and 3 are more likely to have their gonads removed at early ages. It is equally important to look separately at the prevalence of those tumors exclusive of the precursor tumor, gonadoblastoma. Among the 5/31 patients (16%) with malignant germ cell neoplasms, 4 were with dysgerminoma and 1 with an embryonal cell carcinoma. Three of the four dysgerminomas occurred in those patients who were phenotypic females (Group 1). This group typically has the least differentiated gonads as compared to Groups 2–4. Further studies trying to understand the risk factors for germ cell neoplasm might look more critically at these potential substrate differences.

In all of the discussions it is obvious that sample size is a critical limiting factor in drawing firm conclusions from the literature and the MCG experience. For example, the Gravholt report involves 114 female patients without any signs of virilization, but only 14 with a Y chromosome at risk for tumor. Scully's ascertainment is 74 cases of gonadoblastoma associated with/without other germ cell neoplasms. The MCG experience involves 31 patients with a Y chromosome and 10 tumors.

6. Conclusions

Reviewing the literature and the experience at MCG one can draw some general conclusions concerning the clinical implications of Y DNA in individuals with dysgenetic gonads as contrasted to individuals with X aneuploidy. The prevalence of dysgenetic tumors (gonadoblastoma, dysgerminoma, embryonal cell carcinoma, etc.) in patients with verified Y cell lines range from 14% to 32%. Cryptic Y cell lines appear to be present in approximately 1–9% of non-mosaic 45,X patients (30–50 metaphases). The tumor incidence 2/6 (33%) in this subgroup of patients with cryptic Y DNA is comparable to the prevalence among other mosaic 45,X/46,XY patients.

These findings suggest that screening of all non-mosaic 45,X patients (30–50 metaphases) should be performed using PCR or interphase FISH (500 interphase nuclei). Metaphase FISH, interphase FISH and DNA analysis should complement and validate each other in the detection of covert Y DNA and identification of rearranged X vs Y chromosomes.

It is important to reiterate that gonadoblastoma is a precursor tumor which may undergo malignant transformation into one of the virulent germ cell neoplasms (dysgerminoma, embryonal carcinoma, endodermal sinus tumor, chorioepithelioma or yolk sac tumor). Since the risk for conversion to malignancy is not predictable, and it is still unclear whether gonadoblastoma is always a distinct precursor lesion, prompt prophylactic gonadectomy with careful histopathology of the excised gonads remains the safest approach to avoid the development of an invasive germ cell tumor. The uterus should be left in place, depending on the estimated risks of pregnancy.

If a patient declines gonadectomy, monitoring for germ cell neoplasm is the only option. However, it is unclear whether methods in common use today (vaginal ultrasound, biochemical markers, proteomics, etc.), even with compliant patients, are able to identify germ cell neoplasms at early enough stages to improve the natural history of the disease. It

is possible that a predictable and specific marker of malignant potential may be identified in the future. Until then, physicians will need to be continually updated on these important issues as they relate to the clinical management of patients with Turner syndrome.

References

- [1] R.B. Greenblatt, et al., The spectrum of gonadal dysgenesis: a clinical, cytogenetic and pathologic study, *Am. J. Obstet. Gynecol.* 98 (2) (1967) 151–172.
- [2] P.A. Gantt, et al., A clinical and cytogenetic study of fifteen patients with 45,X/46,XY gonadal dysgenesis, *Fertil. Steril.* 34 (3) (1980) 216–221.
- [3] A.E. Wiktor, D.L. Van Dyke, FISH analysis helps identify low-level mosaicism in Ullrich–Turner syndrome patients, *Genet. Med.* 6 (3) (2004) 132–135.
- [4] A.E. Wiktor, D.L. Van Dyke, Detection of low level sex chromosome mosaicism in Ullrich–Turner syndrome patients, *Am. J. Med. Genet.* 138A (2005) 259–261.
- [5] C.E. Chu, et al., Detection of Y mosaicism in patients with Turner’s syndrome, *J. Med. Genet.* 32 (7) (1995) 578–580.
- [6] M.Y. Nishi, et al., Detection of Y-specific sequences in 122 patients with Turner syndrome: nested PCR is not a reliable method, *Am. J. Med. Genet.* 107 (2002) 299–305.
- [7] R.E. Scully, Gonadoblastoma: a gonadal tumor related to the dysgerminoma (seminoma) and capable of sex hormone production, *Cancer* 6 (3) (1953) 455–463.
- [8] M. Cools, et al., Gonadoblastoma arising in undifferentiated gonadal tissue within dysgenetic gonads, *J. Clin. Endocrinol. Metab.* (April 11, 2006) (Published OnLine).
- [9] A. Horwick, J. Shipley, R. Huddart, Testicular germ cell cancer, *Lancet* 367 (2006) 754–765.
- [10] K. Pauls, et al., Gonadoblastoma: evidence for a stepwise progression to dysgerminoma in a dysgenetic ovary, *Virchows Arch.* 447 (2005) 603–609.
- [11] A.M.F. Kersemaekers, et al., Identification of germ cells at risk for neoplastic transformation in gonadoblastoma. An immunohistochemical study for OCT3/4 and TSPY, *Human Pathol.* 36 (2005) 512–521.
- [12] J. Müller, et al., Carcinoma in situ of the testis in children with 45,X/46,XY gonadal dysgenesis, *J. Pediatr.* 106 (1985) 431–436.
- [13] J. Koubova, et al., Inaugural article: retinoic acid regulates sex specific timing of meiotic initiation in mice, *PNAS* 103 (2006) 2474–2479.
- [14] R.E. Scully, Gonadoblastoma: a review of 74 cases, *Cancer* 25 (6) (1970) 1340–1356.
- [15] H.S. Gallager, R.P. Lewis, Sequential gonadoblastoma and choriocarcinoma, *Obstet. Gynecol.* 41 (1) (1973) 123–128.
- [16] A. Talerma, Gonadoblastoma associated with embryonal carcinoma, *Obstet. Gynecol.* 43 (1) (1974) 138–142.
- [17] K. Ito, et al., Pure yolk sac tumor of the ovary with mosaic 45,X/46,+ mar Turners syndrome with a Y-chromosomal fragment, *Arch. Gynecol. Obstet.* 262 (1998) 87–90.
- [18] T. Ono, et al., 45,XO/46X,dic(Yq) mosaicism in Turner’s phenotype with endodermal sinus tumor of the ovary, *Gynecol. Obstet. Invest.* 27 (1989) 45–47.
- [19] R.W. Naeraa, et al., Mortality in Turner syndrome, in: Albertsson-K. Wikland, M.B. Ranke (Eds.), *Turner Syndrome in a Life Span Perspective: Research and Clinical Aspects*, Elsevier, Amsterdam, 1995, p. 323.
- [20] H. Hasle, et al., Occurrence of cancer in women with Turners syndrome, *Br. J. Cancer* 73 (1996) 1156–1159.
- [21] C.H. Gravholt, et al., Morbidity in Turner syndrome, *J. Clin. Epidemiol.* 51 (2) (1998) 147–158.
- [22] C.H. Gravholt, et al., Occurrence of gonadoblastoma in females with Turner syndrome and Y chromosome material: a population study, *J. Clin. Endocrinol. Metab.* 85 (2000) 3199–3202.
- [23] P. Canto, et al., Gonadoblastoma in Turner syndrome patients with 45,X karyotype and Y chromosome sequences, *Cancer Genet. Cytogenet.* 150 (2004) 70–72.
- [24] L. Telvi, et al., 45,X/46,XY mosaicism: report of 27 cases, *Pediatrics* 104 (1999) 304–308.
- [25] F. Álvarez-Nava, et al., Molecular analysis in Turner syndrome, *J. Pediatr.* 142 (2003) 336–340.
- [26] L. Mazzanti, et al., Gonadoblastoma in Turner syndrome and Y-chromosome-derived material, *Am. J. Med. Genet.* 135A (2005) 150–154.
- [27] C.M. Tuck-Muller, et al., Isodicentric Y chromosome: cytogenetic, molecular and clinical studies and review of the literature, *Hum. Genet.* 96 (1995) 119–129.