Review Article

Sexual Development

Sex Dev
DOI: 10.1159/000477921

Accepted: March 21, 2017
by M. Schmid
Published online: July 19, 2017

Update on the Pathophysiology and Risk Factors for the Development of Malignant Testicular Germ Cell Tumors in Complete Androgen Insensitivity Syndrome

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Keywords
Carcinoma in situ · Complete androgen insensitivity syndrome · Genetic susceptibility · Germ cell cancer · Germ cell neoplasia in situ · Seminoma · SNP · Testis cancer

Abstract
Prophylactic gonadectomy in young adult women with complete androgen insensitivity syndrome (CAIS) to avoid development of an invasive testicular germ cell tumor (TGCT) is currently advised in most centers. However, women with CAIS increasingly question the need of this procedure. In order to provide optimal counseling and follow-up of these women, insight in the mechanisms underlying TGCT development in androgen insensitivity syndrome (AIS), data regarding the incidence of TGCT in AIS adults specifically, and an overview of existing and novel screening tools for in situ and invasive neoplastic lesions are crucial. The current knowledge regarding these topics is revised in this paper.

Like in other conditions characterized by variant sex development (here referred to as disorders of sex development, DSD), malignant testicular germ cell tumors (TGCT) occur with increased frequency in individuals with complete and partial androgen insensitivity syndrome (CAIS and PAIS, respectively). These tumors belong to the group of the so-called type II germ cell tumors, comprising seminoma of the testis/dysgerminoma of the ovary or dysgenetic gonad and the various nonseminomatous tumors. Their non-invasive counterparts are carcinoma in situ (in a testicular environment) and gonadoblastoma (in the dysgenetic gonad) [Oosterhuis and Looijenga, 2005]. The precursor lesion within the testicular environment is referred to as germ cell neoplasia in situ (GCNIS) according to the newest WHO classification [Moch et al., 2016].

The first larger-scale study reporting on TGCT risk in androgen insensitivity syndrome (AIS) specifically was performed by Rutgers and Scully in 1991, who describe a series of 43 AIS individuals (3 PAIS), aged 14–83 years (mean 27 years) at gonadectomy, of which 4 cases (9%) presented with a TGCT [Rutgers and Scully, 1991]. New studies and a large literature review, published at the beginning of the 21st century, suggested a somewhat lower risk of around 5% in AIS in general, with a particularly low prevalence of <1% in CAIS [Cools et al., 2005, 2006a]. It was recognized, however, that these numbers were based to a large extent on prophylactically removed go-

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nads during early childhood which was at that time considered to be best clinical practice and thus provided limited information on the lifetime risk for TGCT development in AIS. Based on these data, and in line with demands from advocacy groups to restrict surgery in DSD children wherever possible, many DSD centers gradually adopted a policy of postponing prophylactic gonadectomy to late adolescence in individuals with CAIS [Hughes et al., 2012]. Such an approach allows for spontaneous breast development and bone mass accrual during puberty through peripheral conversion of androgens to estrogens [Bertelloni et al., 2010, 2011] without compromising safety regarding tumor development. In addition, informed consent for gonadectomy can be obtained directly from the adolescent girl, alleviating the pressure on parents and promoting psychological adaptation in patients [Siminoff and Sandberg, 2015]. Theoretically, this approach would also open up possibilities for currently available assisted reproductive techniques, such as testicular semen extraction. However, advanced spermatogenesis has never been observed in CAIS gonads [Rutgers and Scully 1991; Cools et al., 2005; Nakhal et al., 2013, Kaprova-Pleskacova et al., 2014; pers. observation].

As this new generation of CAIS girls with retained gonads has now grown up, it becomes increasingly clear that many of them (15% according to Deans et al. [2012]) are reluctant to undergo gonadectomy in adulthood and prefer keeping their testes in place for various reasons. This evolution forces clinicians nowadays to develop protocols for the follow-up of – often abdominally located – testes and to collect new data on TGCT risk in CAIS during adulthood specifically, both of which will be addressed below.

Pathophysiology of TGCT Development in AIS

The risk for TGCT is increased only in DSD individuals who have (part of) a Y chromosome (mainly 46,XY and 45,X/46,XY). The gonadoblastoma on Y (GBY) region, located proximally on Yp, has long been implicated in the pathogenesis of malignant germ cell proliferation, and *TSPY* (testis specific protein, Y-linked) within the GBY region has been proposed as the main candidate gene. Its mechanisms of action are not fully elucidated yet, but in physiologic conditions, TSPY is known to be involved in the mitotic proliferation of germ cells. However, it is thought to act as an oncogene in DSD gonads [Page, 1987; Lau, 1999; Lau et al., 2000, 2003]. Although quantitative expression studies have not been performed so far, surviving germ cells in DSD gonads often display very intense TSPY staining, suggesting upregulation of its expression (and action) in these conditions, except at an invasive stage, where expression tends to decrease or even disappear, likely related to the loss of the Y chromosome in many cases [Kersemaekers et al., 2005].

Another predisposing factor for TGCT development is the prolonged presence of pluripotent germ cells, called gonocytes, in AIS testes. Survival of germ cells and maintenance of their pluripotent state is related to precise expression levels of the octamer binding protein 3/4 (OCT3/4) transcription factor, also known as POU5F1 [Niwa et al., 2000; Kehler et al., 2004]. OCT3/4 is physiologically expressed in testicular germ cells during fetal life and in the first 3 months after birth [Honecker et al., 2004]. Such gonocytes typically reside in the testicular lumen, whereas upon maturation, which is driven by Sertoli cells and possibly androgen signaling, these germ cells migrate to the basal lamina and loose pluripotency (and hence OCT3/4 expression). Delayed maturation of germ cells is a common finding in all conditions characterized by a defective hormonal and/or cellular milieu and can be recognized by the presence of OCT3/4-positive luminal germ cells well beyond birth [Rajpert-De Meyts, 2006]. This is not a premalignant condition per se, as it is believed that most of these cells either mature and lose OCT3/4 expression once they make contact with the tubular basal membrane or undergo apoptosis [Rajpert-De Meyts et al., 2004; Cools et al., 2005, 2006b]. However, gonocytes in contact with the basal membrane that do not downregulate OCT3/4 expression are considered to have gained (pre)malignant characteristics, such as increased survival capacity [Cools et al., 2005]. Therefore, differentiating between these 2 conditions (i.e., maturation delay vs. (pre-)malignancy) is crucial in order to correctly interpret tumor risk and to avoid overdiagnosis of GCNIS. As OCT3/4 is physiologically expressed in the first months after birth and because numerous OCT3/4-positive germ cells can be found in AIS gonads of young children, interpretation of aberrant findings is much easier after the first year of life. Interestingly, it has been shown that (pre-) GCNIS areas but not germ cells with simple maturation delay display aberrant *KITLG* gene expression. Thus, the finding of KITLG positivity in a suspected lesion substantially corroborates the diagnosis of (pre-)GCNIS [Stoop et al., 2008] (Fig. 1).

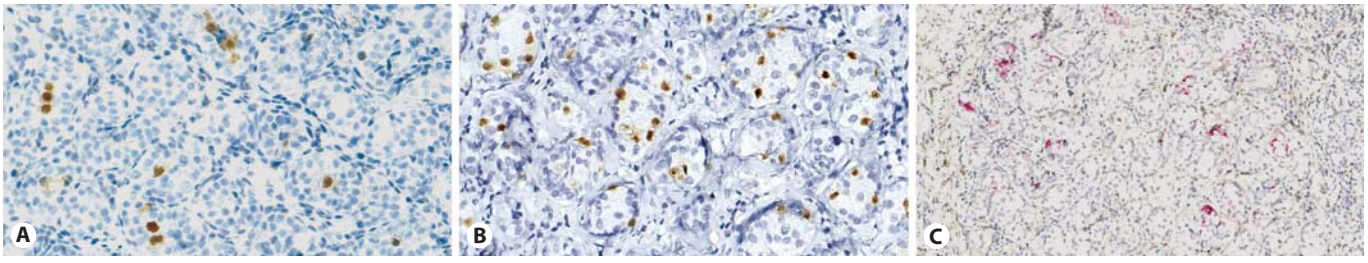

Fig. 1. A Maturation delay of germ cells. All OCT3/4-positive (brown) cells are located in the center of the tubules, germ cells on the basal membrane have lost OCT3/4 expression. OCT3/4 staining. ×400. **B** Pre-GCNIS. Many germ cells have failed downregulation of OCT3/4 (brown staining) while making contact with the basal membrane, some OCT3/4-positive cells are centrally located. OCT3/4 staining. ×400. **C** The same area shows KITLG positivity. KITLG staining. ×200.

Pathological Findings other than Germ Cell Neoplasia in AIS Gonads

In CAIS, Leydig cell hyperplasia is generally present from puberty onwards, provided that the patient is not under hormone replacement therapy. Tubules remain immature, meaning that their caliber is smaller than expected for age and that there is no or limited lumen formation at puberty. Most tubules are Sertoli cell-only, however, some tubular cross-sections, usually clustered together, may contain gonocytes or spermatogonia. Spermatogenesis beyond that stage is not seen. Sertoli cells may display Hürtle cell changes (cytoplasmic eosinophilic granules, corresponding to altered mitochondria). Sertoli cell nodules and hamartomas are frequently encountered. Increased interstitial space, calcifications, thickening of the basal membrane, and tubular fibrosis are generally present in older subjects. Unexpectedly, many CAIS individuals have wolffian and/or müllerian duct derivative structures. Similar findings have been described in PAIS, although somewhat less pronounced [Rutgers and Scully, 1991; Hannema et al., 2004, 2006; Cools et al., 2005; Kaprova-Pleskacova et al., 2014].

New Data Regarding TGCT Risk in AIS Adults

Due to the rarity of the condition and the former practice of prophylactic gonadectomy in childhood, few data exist on the occurrence of TGCT in AIS adults or on the natural outcome of gonads in situ. Although subject to methodological limitations, combining historical patient series provides an opportunity to obtain some larger-scale data. For this purpose, it is crucial to consider only cases in which the diagnosis of AIS has been confirmed with molecular genetic techniques, since tumor risk and type of tumor may considerably vary among the various DSD conditions. In addition, a detailed pathological description of biopsy/gonadectomy material is required for a reliable diagnosis of pre-invasive lesions. Four informative studies could be identified: in 16 postpubertal CAIS woman, GCNIS was diagnosed in 1 case (6.3%) [Cheikhelard et al., 2008]. No GCNIS or invasive tumors were encountered in a series of 20 CAIS and 5 PAIS postpubertal individuals [Audi et al., 2010]. In 24 adult CAIS women who received gonadectomy after magnetic resonance imaging (MRI) of their gonads, GCNIS was histologically confirmed in 3 cases (12.5%) [Nakhal et al., 2013]. Most recently, we identified pre-GCNIS in 6/42 CAIS (14%) and in 1/10 (10%) PAIS individuals aged 14 years or older [Cools et al., submitted]. Combining these series suggests a GCNIS risk of 11/117 adolescent or adult AIS individuals (9.4%), of which 10/102 (9.8%) have CAIS and 1/15 (6.6%) have PAIS. From these data and in contrast to what has previously been thought [Cools et al., 2006a], PAIS individuals do not seem to be at a higher risk as compared to women who have CAIS. Interestingly, none of the patients in these studies had developed an invasive TGCT at the time of gonadectomy, suggesting that either progression of GCNIS to invasiveness is a rare event in AIS or that this takes place at older ages than covered in the mentioned studies. In view of the median age at which seminomas develop in the general male population, i.e., with a peak incidence between 35 and 39 years [Shah et al., 2007], the latter hypothesis is unlikely. Indeed, recently collected epidemiological data support the first hypothesis. We recently performed a survey amongst health care professionals working in DSD expert centers world-

wide. Respondents were questioned about the prevalence of retained gonads in their AIS patient population and about the number of TGCT that had occurred in such gonads. Data were reported on 188 individuals with CAIS, of which 145 (77%) had gonadectomy at the end of puberty, whereas 23 had chosen to further delay gonadectomy (in the remaining, gonadectomy had been performed during childhood). Of these 23, none had developed an invasive (or otherwise symptomatic) TGCT so far. Of the 145 individuals who had gonadectomy in adolescence, only 1 had been diagnosed with GCNIS and 1 with a seminoma [Maris et al., submitted]. These data are in contrast with the current belief that in the general male population, around 70% of GCNIS will become invasive in 7 years, which was extrapolated to 100% in 10 years, although never proven [Giwercman et al., 1991]. The hypothesis that progression to invasiveness of GCNIS lesions in AIS is only seen in a minority of AIS gonads was also launched [Kaprova-Pleskacova et al., 2014].

Four factors are thought to contribute to the low risk for (invasive) TGCT in AIS. Firstly, testicular architecture and Sertoli cell function are relatively normal in AIS, in contrast to e.g., 46,XY gonadal dysgenesis. The capacity of Sertoli cells to push germ cells into further maturational stages is likely a major determinant of TGCT risk. Indeed, we and others have demonstrated that conditions associated with a defective gonadal development in which Sertoli cells are unable to create a sufficiently supportive milieu are characterized by maturational arrest rather than delayed maturation of germ cells. In these conditions, TGCT risk is much higher as compared to the estimated risk in AIS [Cools et al., 2006c; Rajpert-De Meyts, 2006; van der Zwan et al., 2015]. Secondly, most (abnormal) germ cells in AIS are removed by apoptosis already during childhood, safeguarding the testis from malignant degeneration. Germ cell numbers are rapidly decreasing with age in AIS, and most adult AIS gonads have no or only a very limited number of germ cells [Cools et al., 2005; Kaprova-Pleskacova et al., 2014; Cools et al., submitted]. Thirdly, (the lack of) paracrine androgen signaling in AIS may play a crucial role, especially in transformation of GCNIS into invasive TGCT. Androgens are known to be a driving force behind germ cell survival and proliferation, an effect that is mediated via mature but not infantile Sertoli cells [Cools et al., 2011; O'Shaughnessy, 2014; Rey, 2014]. Progression of GCNIS to a more malignant state has been attributed to adaptive genomic changes (i.e., specific chromosomal gains and losses) of transformed gonocytes, equipping these cells with increased survival and proliferation characteristics, following a dramatic increase in testicular hormone production at puberty [Rajpert-De Meyts, 2006]. The deficient androgen signaling in AIS may explain the low frequency of invasive tumors found in this condition, especially in comparison with other forms of DSD. However, it has been shown that even in the complete form of AIS, residual paracrine androgen activity may induce wolffian duct development [Hannema et al., 2004]. It is conceivable that in AIS such a residual androgen activity may in some cases promote invasiveness of GCNIS lesions after puberty. Finally, an individuals' genetic susceptibility, based on pre-identified high-risk single nucleotide polymorphisms (SNPs) for TGCT [Kratz et al., 2011; Chung et al., 2013; Zeron-Medina et al., 2013] may play a crucial role (see below).

Follow-Up of Gonads in situ

As many CAIS women nowadays decline gonadectomy, clinicians need to develop a sensitive and specific screening program for the follow-up of retained gonads. Currently, no written guidelines or evidence-based protocols exist. The strengths and limitations of the various screening tools are discussed below. In our center, we have the policy to discuss current knowledge regarding TGCT risk in CAIS during adulthood and the pros and cons of gonadectomy with patients from adolescence onwards in order to develop an individualized management plan.

Imaging

Due to its low cost, safety, and wide availability, ultrasound (US) is the first choice for imaging retained gonads in a labioscrotal or inguinal position, but this technique will not allow visualization of abdominal gonads [Kim et al., 2007]. Annual follow-up of labioscrotal or inguinal gonads by US is recommended from late puberty onwards. The role of US in the detection of early TGCT lesions has been revised in detail by Hoei-Hansen [2008]. GCNIS cannot be visualized directly by US, but an irregular aspect of the testis parenchyma may be suggestive. Although no specific studies in DSD patients are available, the presence of microlithiasis, especially in combination with an inhomogeneous testis parenchyma, is suggestive but not specific for GCNIS [Elzinga-Tinke et al., 2010].

Although MRI may have a role in the evaluation of suspected testicular lesions, it will not routinely allow detection of testicular GCNIS or microlithiasis [Heinemann et al., 2003; Kim et al., 2007]. However, MRI is believed to

be superior to US in visualizing intraabdominal gonads [Kim et al., 2007] and is crucial in the work-up of an invasive TGCT.

Of interest, a recent study evaluated the role of MRI in 24 postpubertal women with CAIS and retained gonads in an abdominal or inguinal position. MRI was able to reliably detect cysts and Sertoli cell adenomas but could not depict any of the GCNIS lesions found in 3 patients at subsequent gonadectomy and histological analysis. Whether MRI imaging is able to detect early invasive TGCT was not clear from this study as no such cases were available. US data were equally not available for comparison in this study [Nakhal et al., 2013].

Germ Cell Cancer Markers in Serum and Semen

Quantification of tumor markers such as β-HCG and α-fetoprotein (AFP) is often routinely considered in the work-up of suspected germ cell malignancy. However, since these proteins are mostly secreted by nonseminomas (in particular choriocarcinoma and yolk sac tumor), but not seminomas – which is the most frequent malignancy in CAIS – and also not in GCNIS, routine screening for elevated levels of these markers is of limited use in the context of prophylactic follow-up of retained gonads in CAIS [Schmoll et al., 2004].

Serum detection of specific microRNAs, belonging to the miR-371–3 and miR-302/367 clusters, is a promising future biomarker for invasive TGCT in the general male population as well as in DSD patients. The miR-371–3 and miR-302/367 clusters are consistently and specifically overexpressed in TGCT and GCNIS tissue cultures, regardless of histological subtype (except teratoma), tumor site (gonadal or extragonadal), and patient age, and have been shown to play a role in tumorigenesis [Voorhoeve et al., 2006; Looijenga et al., 2007; Palmer et al., 2010; Murray and Coleman, 2012; Novotny et al., 2012; Rijlaarsdam et al., 2015]. It was shown that serum detection of miR-371 is superior to the existing tumor markers AFP and β-HCG for the diagnosis as well as follow-up after orchidectomy of all types of TGCT, including seminomas and clinical stage I disease [Dieckmann et al., 2012]. Based on these results, a quality-controlled test for targeted serum detection of miR-371–3/367 (TSmiR) for the diagnosis and follow-up of TGCT has recently been proposed, yielding a sensitivity of 98%, which largely outperforms traditional tests based on the serum markers AFP and β-HCG [Gillis et al., 2013]. Systematic evaluation of the value of this TSmiR test, now modified including an amplification step (i.e., ampTSmiR) for the diagnosis of TGCT, is very promising [Van Agthoven and Looijenga, 2016], and the test is now ready for commercialization. The fact that specific miRNA clusters have been found to be overexpressed in GCNIS opens the door for a possible application of this test as a screening for GCNIS (and GB) in high-risk populations, such as DSD. Unfortunately however, based on preliminary data, it is unlikely that GCNIS lesions and their precursors secrete such miRNAs in the circulation [Van Agthoven and Looijenga, 2016].

Genetic Screening

Genome-wide association studies have linked numerous SNPs with TGCT development in the general male population. Current data indicate that carriers of a combination of susceptibility alleles have the highest risk, and a risk stratification model has been proposed based on the combined presence of one or more of these risk alleles in an individual's genotype [Kratz et al., 2011; Chung et al., 2013]. Independently, a polymorphic p53 response element within the promotor region of *KITLG* has also been identified as a putative important genetic variant underlying cancer susceptibility in general, possibly including TGCT [Zeron-Medina et al., 2013]. While genetic screening in the general population may not be useful given the low incidence of TGCT, the development of such a screening program in a selected population with other independent risk factors such as DSD might be a meaningful approach [Kratz et al., 2011].

To investigate whether genetic susceptibility adds to the TGCT risk in DSD patients, we performed genotyping of 14 TGCT associated SNPs and applied the risk stratification model as developed by Kratz et al. [2011], assessing TGCT risk in individuals with CAIS and PAIS, aged 14 years or older [Cools et al., submitted]. No invasive tumors were encountered in this study. Individuals who had developed premalignant lesions had a significantly higher predicted SNP-based risk than individuals who had no pre-GCNIS, however, with overlap between both groups. It was concluded that although of relevance in understanding TGCT development in AIS, other factors (e.g., environmental) than genetic susceptibility most likely play a role. In addition, it is conceivable that development of GCNIS is mostly determined by SNPs in other, hitherto unidentified genes as compared to invasive TGCT, which in turn need to be added to the model. Genetic differences amongst ethnicities may add to the complexity of the model. Thus, the genetic approach that has recently been proposed by our group needs to be refined and extended before it can serve as a reliable screening tool that allows identification of those individuals who will ultimately develop a TGCT.

In conclusion, the prevalence of in situ TGCT in adults with AIS is probably around 10%. It is believed that only a very small minority of these lesions will become invasive over time. The idea that individuals with PAIS have a greater tumor risk is not supported by recent data. There are currently no diagnostic tools that allow reliable monitoring of abdominal gonads for the presence of in situ neoplastic lesions, although new and promising micro RNA- and SNP-based screening tests are underway. The decision to perform gonadectomy in adulthood is an individualized one, taken in accordance with patient's expectations and after having discussed all possible outcomes.

Acknowledgement

M.C. is supported by a Senior Clinical Investigator grant from the Research Foundation Flanders – FWO.

Disclosure Statement

The authors have no conflicts of interest to declare.

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