Clinical but not histological outcomes in males with 45,X/46,XY mosaicism vary depending on reason for diagnosis

Marie Lindhardt Ljubicic[1,2], Anne Jørgensen[1,2], Carlo Acerini[3], Juliana Andrade[4], Antonio Balsamo[5], Silvano Bertelloni[6], Martine Cools[7], Rieko Tadokoro Cuccaro[3], Feyza Darendeliler[8], Christa E. Flück[9], Romina P. Grinspon[10], Andrea Maciel-Guerra[4], Tulay Guran[11], Sabine E. Hannema[12,13], Angela K. Lucas-Herald[14], Olaf Hiort[15], Paul Martin Holterhus[16], Corina Lichiardopol[17], Leendert H.J. Looijenga[18], Rita Ortolano[5], Stefan Riedl[19,20], S. Faisal Ahmed[14], Anders Juul[1,2]

[1] Dept. of Growth and Reproduction, Rigshospitalet, University of Copenhagen, Denmark
[2] International Center for Research and Research Training in Endocrine Disruption of Male Reproduction and Child Health (EDMaRC), Rigshospitalet, University of Copenhagen, Denmark
[3] Department of Paediatrics, Addenbrooke's Hospital, University of Cambridge
[4] Faculty of Medical Sciences (FCM), Department of Medical Genetics, State University of Campinas (Unicamp), São Paulo, Brazil
[5]Department of Medical & Surgical Sciences, Pediatric Endocrinology Unit, Centre for Rare Endocrine Conditions, S. Orsola-Malpighi University Hospital, Bologna, Italy
[6] Dipartimento Materno-infantile Azienda Ospedaliero, Universitaria Pisana, Pisa, Italy
[7] Department of Paediatric Endocrinology, University Hospital Ghent and Department of Internal Medicine and Paediatrics, Ghent University, Ghent, Belgium
[8] Istanbul University, Istanbul Faculty of Medicine, Istanbul, Turkey
[9] Division of Pediatric Endocrinology, Diabetology and Metabolism, Department of Pediatrics and Department of BioMedical Research, Bern University Children's Hospital, University of Bern, Bern, Switzerland
[10] Centro de Investigaciones Endocrinológicas 'Dr. César Bergadá' (CEDIE), CONICET – FEI, División de Endocrinología, Hospital de Niños Ricardo Gutiérrez, Buenos Aires, Argentina
[11] Marmara University, School of Medicine, Department of Paediatric Endocrinology and Diabetes, Istanbul, Turkey
[12] Dept. of Paediatrics, Leiden University Medical Centre, Leiden, The Netherlands
[13] Dept. of Paediatric Endocrinology, Sophia Children's Hospital, Erasmus MC, Rotterdam, The Netherlands
[14v]Developmental Endocrinology Research Group, University of Glasgow, Glasgow, UK
[15] Division of Paediatric Endocrinology and Diabetes, Department of Paediatrics, University of Luebeck, Luebeck, Germany
[16] Division of Paediatric Endocrinology and Diabetes, Department of Paediatrics, Christian-Albrechts-University of Kiel, Kiel, Germany
[17] Dept. of Endocrinology, University of Medicine and Pharmacy Craiova, University Emergency Hospital Craiova, Romania
[18] Dept. of Pathology, Lab. for Experimental Patho-Oncology, Erasmus MC, University Medical Center Rotterdam, Cancer Institute, Rotterdam, and Princess Maxima Center for Paediatric Oncology, Utrecht, The Netherlands
[19] Pediatric Endocrinology, St. Anna Children´s Hospital, Medical University of Vienna, Vienna, Austria
[20] Department of Pediatric Pulmology, Allergology and Endocrinology, Medical University of Vienna, Vienna, Austria

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Person to whom correspondence and reprint requests should be addressed:
Marie Lindhardt Ljubicic, MD
Department of Growth and Reproduction, GR, 5064
Rigshospitalet
Blegdamsvej 9
DK-2100 Copenhagen Ø
Denmark
Tel no.: +45 3545 5085, fax no.: +45 35456054
E-mail: marie.lindhardt.johansen@regionh.dk

Abstract

Context: Larger studies on outcomes in males with 45,X/46,XY mosaicism are rare.
Objective: To compare health outcomes in males with 45,X/46,XY diagnosed due to either genital abnormalities at birth or non-genital reasons later in life.
Design: A retrospective, multicenter study
Setting: 16 tertiary centers
Patients or other participants: 63 males older than 13 years with 45,X/46,XY mosaicism.
Intervention(s): None.
Main Outcome Measure(s): Health outcomes such as genital phenotype, gonadal function, growth, comorbidities, fertility, and gonadal histology including risk of neoplasia.
Results: 35 patients were in the *genital* group, 28 in the *non-genital*. 80% of all patients experienced spontaneous pubertal onset, significantly more in the *non-genital* group ($p = 0.023$). Patients were significantly shorter in the *genital* group with median adult heights of 156.7 cm and 164.5 cm, respectively ($p = 0.016$). 27% of patients received recombinant human growth hormone. 44 patients had gonadal histology evaluated. Germ cells were detected in 42%. Neoplasia *in situ* was found in five patients. 25% had focal spermatogenesis, another 25.0% had arrested spermatogenesis. 14 out of 17 (82%) with semen analyses were azoospermic; three had motile sperm.
Conclusion: Patients diagnosed due to genital abnormalities have poorer health outcomes than those diagnosed due to non-genital reasons. Most patients, however, have relatively good endocrine gonadal function, but most are also short statured. Patients have a risk of gonadal neoplasia and most are azoospermic, but almost half of patients have germ cells present histologically and up to a quarter have focal spermatogenesis, providing hope for fertility treatment options.

Introduction

45,X/46,XY mosaicism and its variants are rare with a previously reported incidence of 1/15,000 live births (1). The resulting phenotype spans across a wide range of effects including genital anomalies, impaired growth, altered gonadal function and histology, and infertility. The karyotype is covered by the umbrella term Differences (or Disorders) of Sex Development referring to diagnoses in which anatomical, gonadal and/or chromosomal sex are affected (2). The 45,X cell line in these patients probably stems from the loss of a normal or structurally abnormal Y-chromosome in early embryonic mitosis, which produces the mosaicism (3–7).

The phenotypic spectrum of 45,X/46,XY patients varies greatly from females with Turner syndrome to normally androgenized males. Moreover, several studies have reported that 80-95% of prenatally diagnosed cases with a 45,X/46,XY karyotype are born as normally androgenized males (3,5,8,9), whereas postnatally diagnosed pediatric cases present more varied phenotypes including ambiguous genitalia (5,10,11). Furthermore, normally androgenized male patients with a 45,X/46,XY karyotype diagnosed in adulthood are now more frequently identified due to male infertility work-ups, including genetic screening (12). Thus, severity of the patient's phenotype often appears to be directly related to the age at diagnosis and reason for referral.

The wide spectrum of phenotypes in these patients is also reflected in health outcomes such as growth, gonadal function, risk of gonadal neoplasia and comorbidities that are all reported with varying incidences and severities both within the same centers and between centers (5,7,10,13–16). It seems intuitive that the severity of the genital phenotype may be considered a read-out for other health outcomes. Nevertheless, even normally androgenized males diagnosed in adulthood have

been reported to have short stature, declining testicular function with age and infertility, likely related to histologically dysgenetic testes (5,6). However, there is a lack of studies with direct comparisons of outcomes in terms of growth, gonadal function and comorbidities between patients diagnosed at birth due to genital abnormalities and those diagnosed later in life due to other reasons such as short stature, pubertal delay and infertility.

The risk of gonadal neoplasia in patients with 45,X/46,XY mosaicism is reported to be relatively high at around 10-15% (5,16–19). The current practice of early (prepubertal) gonadectomy in girls renders it impossible to evaluate gonadal function and possible fertility potential in women. Moreover, single-center studies on histological outcomes are limited by numbers thus making thorough pathohistological evaluations of larger datasets rare.

Thus, we wanted to investigate and compare health outcomes such as growth, gonadal function, comorbidities, fertility and histology including risk of neoplasia in males with 45,X/46,XY mosaicism and variants diagnosed due to different reasons 1) genital abnormalities and 2) other reasons such as stunted growth, lack of pubertal onset, undervirilization and infertility in a large multicenter study with 16 participating centers including a total of 63 male patients with 45,X/46,XY mosaicism.

Materials and Methods

Subjects

Patients were identified using the I-DSD Registry, which contains pseudoanonymized information on patients with DSD. Information on the registry is available at

https://www.gla.ac.uk/schools/medicine/research/childhealth/researchinterests/i-dsdproject/ and recent uses of the registry have previously been published (14,20).

We identified centers in the registry that had included patients with 45,X/46,XY and its variants (including different aberrations to the Y-chromosome such as deletions and isodicentricism, and a single patient with a 45,X/46,XX(SRY-pos) karyotype) uploaded to the registry. Through the COST network DSDnet (http://www.dsdnet.eu/cost.html), three additional centers with patients not yet uploaded to the registry were identified. A total of 22 centers were contacted, of which 19 centers responded, and 16 centers supplied data on a total of 63 male patients. The inclusion criteria were: male gender of rearing and an age old enough to evaluate height and gonadal function (>13 years of age).

Patients were stratified into two groups according to whether they were diagnosed due to genital abnormalities or other reasons (hereafter '*genital*' and '*non-genital*', respectively). Other reasons included prenatal screening (fetal and maternal factors), growth retardation, gynecomastia, lack of spontaneous pubertal onset, lack of virilization in adulthood and infertility. Two patients in the *non-genital* group underwent hypospadias repairs and thus had genital abnormalities but were not diagnosed due to these and were thus included in the *non-genital* group.

Data collection

Following identification of suitable cases in the I-DSD Registry, each center was contacted to complete a detailed questionnaire that collected the following information: age at presentation, reason for diagnosis, karyotype, sex of rearing, birth weight and length, genital phenotype including External Masculinization Score (EMS, as described by Ahmed et al (21)), renal and cardiac

comorbidities, growth including target height and recombinant human Growth Hormone (rhGH) treatment, pubertal onset, gynecomastia, testosterone (T) treatment, genital and gonadal surgery, gonadal histology including neoplasia, fertility workups, and endocrine biochemistry at presentation and at last available follow-up. Histopathological evaluations were translated locally and added to a predefined table by each participating center. In a few cases attempts were made to get further evaluations, images and/or tissue blocks. However, this was not always possible. Thus, an image of a gonadoblastoma from a patient not included in this study (with a 46,XX/47,XYY/48,XXYY karyotype from the biobank at Dept. of Growth and Reproduction, Copenhagen) is used.

Hormone assays

Sixteen centers participated in this study and different commercially available assays were used to measure follicle-stimulating hormone (FSH), luteinizing hormone (LH) and testosterone (T). All FSH and LH concentrations were reported using IU/L. T concentrations were reported in nmol/L, ng/mL and pg/mL. Based on the molar mass of T of 288 Daltons, all values were standardized to nmol/L (*nmol/L = ng/mL x 3.47 mol/L*). Reference ranges for FSH and LH were based on measurements using the time-resolved immunoflourometric assays (Delfia; PerkinElmer, Boston, MA). The limits of detection (LODs) were 0.05 and 0.05 IU/L, respectively. The intra- and interassay coefficients of variation (CVs) were <5% in both assays. Reference range values for T were measured using the DPC Coat-A-Count RIA kit (Diagnostic Products Corp). LOD was 0.23 nmol/L. Intra- and interassay CVs were 7.6% and 8.6%, respectively. Due to the retrospective and historic nature of the study, assay details were not available from all participating centers.

Hormone reference ranges

Reference ranges and LODs plotted were based on the assays used at the Department of Growth and Reproduction, Copenhagen University Hospital, Copenhagen, Denmark, where this study was based. Any values below the LODs were plotted as (LOD/2). All reference ranges of reproductive hormones and testicular volumes are based on a total of 2095 healthy boys recruited for a cross-sectional study of healthy, Danish children (The Copenhagen Puberty Study) as previously published (22,23). Plotting the reference ranges, despite being from a single center only, was done to enable comparison of normative data and the patient data.

Statistical analyses

The *genital* and *non-genital* groups were compared using the Mann-Whitney U test in terms of external genitalia (EMS), age at referral, final height and height SD scores, while Pearson's Chi-Squared or Fischer's Exact Test (wherever appropriate) was used to compare the binary outcomes of spontaneous puberty, renal and cardiac malformations and disease, genital and gonadal surgeries, fertility, gonadal neoplasia, spermatogenesis, presence of germ cells and distribution (presence) of testicles and streak gonads.

Height was standardized using height-for-age standard deviation scores and plotted against reference ranges from the World Health Organization (WHO) (24).

Ethical considerations

The I-DSD registry is approved by the National Research Ethics Service of the United Kingdom for collection of routine clinical data. In addition, each center obtained necessary approvals and adhered to local laws regarding data collection from patient files. Patient and/or parental consent was obtained prior to registration of cases in the I-DSD Registry. The database from this study is

based in Copenhagen, Denmark, and has received appropriated approvals from the Danish Data Protection Agency (RH-2015-235, I-Suite no.: 04204) and the Danish Health and Medicinal Authorities (3-3013-1376/1). COPENHAGEN Puberty Study was approved by the Danish Data Protection Agency (2015-41-4494) and by the regional ethics committee (KF 01 282214 and V200.1996/90).

Results

In total, 63 males from 16 different centers were included in this study. All patients had missing values for one or more variables, but all were included in the study.

Age and phenotype at diagnosis

Thirty-five (55.6 %) patients were diagnosed due to genital abnormalities and 28 (44.4%) were diagnosed due to other reasons. Ages at first presentation were (median (range)) 0.0 years (0.0-42.7 years) and 24.0 years (0.0 to 49.0 years) in the two groups (n=34 *genital*, 27 *non-genital*), respectively (Table 1).

EMS scores at first examination were (median (range)) 4.0 (1.0 to 9.5) and 12.0 (10.0 to 12.0) in each group, respectively (n=24 *genital*, 20 *non-genital*) Table 1), and significantly differed between the groups ($p<0.001$).

The prevalence of hypospadias repairs ($p<0.001$), orchidopexies ($p=0.006$) and gonadectomies ($p<0.001$) was higher in the genital group (Table 1).

Spontaneous puberty, reproductive hormones and T replacement

The majority of patients in both groups entered puberty spontaneously (n=47 patients (79.7%)) with a significantly higher prevalence in the *non-genital* group (n=22 *genital*, 25 *non-genital*, p=0.023, Table 1). Twenty-one patients (39.6%) were treated with T at some point or continuously during the follow-up period with significantly more in the *genital* group (17 *genital*, 4 *non-genital,* p=0.013). Regardless of the reason for diagnosis, most patients had FSH and LH concentrations at the higher end of the normal reference range (Fig. 1). Likewise, T levels were mostly within the normal reference range. Testicular volumes were typically normal or low within the reference range independent of diagnosis group (Fig. 1).

Growth

Final heights were reduced in the *genital* compared to the *non-genital* group (median (range) 156.7 cm (143.0-169.2 cm) and 164.5 cm (141.1-187.7 cm, p=0.001) (Table 1 and Fig. 2a). However, when the genetic potential was accounted for there was no significant difference. Patients in neither group grew according to genetic potential (height SDS – target height SDS) (median SDS (range)): -2.5 (-4.2 to -1.2) and -2.2 (-3.4 to -1.0) (Table 1 and Fig. 2b).

Seventeen patients (27.0%) were treated with rhGH with no significant difference in the number of treated patients between the two groups (p=0.066). There was no difference in height SD scores 1 year prior to and 1 or 5 years following treatment for all patients overall and when subdivided into the two diagnosis groups or grouped based on treatment (no treatment vs. treated) (all $p > 0.05$) (Fig 2c).

Comorbidities

Cardiac malformations were more frequent (13 patients, 22.4%) than renal malformations (seven patients, (12.3%)) (Table 1). There was no difference in frequencies between the groups.

Gonadal histology, spermatogenesis and neoplasia

Histological features from gonadal biopsies and/or gonadectomies from 44 patients (65.0%), including a total of 61 gonads are summarized in Table 2 and Fig. 3-4. In total, 31 patients from the *genital* group (88.6%) and 13 from the *non-genital* group (46.4%) had histological data available. Patients either had bilateral testicular tissue (51.2%, 10 *genital*, 11 *non-genital*) or testicular tissue on one side and more undifferentiated ovarian-like tissue on the contralateral side, most often in the form of streak gonads (48.8%, 18 *genital*, 2 *non-genital*) (Fig. 3-4).

Sertoli-cell only pattern was evident in 30 patients with available pre- and/or postpubertal histological samples (SCO) (66%). In seven of the post-pubertal patients (35%), the SCO pattern was also associated with spermatocytic arrest in other tubules and in a single case with tubules containing GCNIS (Tables 2-3).

Germ cells were detected in 42.1% of patients (9 *genital*, 7 *non-genital*), whereas no germ cells were found in 55.3% of patients (15 *genital*, 6 *non-genital*). Seven patients did not have information on germ cells available. There was no significant difference between ages at biopsy/gonadectomy in patients with detectable germ cells and patients without detectable germ cells (median (range), 18.77 years (0.10 to 49.40) vs. 13.55 years (0.30 to 47.50), $p = 0.154$).

Folliculogenesis was not detected in any of the included gonadal samples. Moreover, three patients were originally labelled with ovotestes but upon thorough re-examination of the original slides, no follicles could be detected and therefore the presence of ovotestes could not be confirmed in any of the samples from the present study.

Spermatogenesis was evaluated by histology in 20 post-pubertal patients. Spermatids were present in 25.0% (2 *genital*, 3 *non-genital*), while in 50.0% (6 *genital*, 4 *non-genital*) no germ cells were observed (Fig. 3). In the remaining 25.0%, germ cells were present, but with arrest of spermatogenesis at different stages. The five patients with spermatids present had a median EMS score of 11.5 (4.0 to 12.0) (Table 2).

Germ cell neoplasia *in situ* (GCNIS) was present in four patients (two prepubertal, two post-pubertal) and gonadoblastoma in one (total n=5, 11.4%, 4 *genital*, 1 *non-genital*) (Tables 1 and 2). The median EMS score in these patients was 4.8 (1.0 to 12.0) (Table 2).

Tubules with preserved spermatogenesis, including presence of spermatids were present alongside tubules with GCNIS in both of the post-pubertal patients (one patient with an EMS of 4, one patient with an EMS of 12).

Overall, when comparing the included histology parameters between the *genital* and the *non-genital* groups, no differences were found (all $p>0.05$), except for a significant difference in the distribution of predominantly male only and mixed gonads in the two groups with predominantly male gonads being more frequent in the *non-genital* group ($p = 0.009$).

Fertility

Complete azoospermia was observed in 14 (82.4%, 2 *genital*, 12 *non-genital*) of 17 patients who had undergone clinical semen analyses (Table 4). Three (17.6%, 1 *genital*, 2 *non-genital*) had evidence of live spermatozoa (one sample with some live spermatozoa, one sample with a concentration of 0.06 million/mL and few progressively motile, and lastly one small volume sample with a concentration of 114 million/mL). In all of these three cases, spermatids had also been identified in the histological evaluation. One azoospermic patient had spermatids present in a histological sample (Table 4).

One of the three patients with live spermatozoa in their semen sample underwent testicular sperm cell extraction during orchiectomy following a biopsy showing GCNIS. However, none of the patients included in this study fathered biological offspring during the follow-up period.

There was no significant difference in fertility (azoospermia vs. live spermatozoa) between the *genital* and *non-genital* groups ($p = 0.42$).

Discussion

This large, multicenter study of male patients with 45,X/46,XY mosaicism found that most patients are short with varying degrees of gonadal function. Moreover, gonadal histology revealed that the risk of pre-neoplasia was relatively high, but also that the presence of ongoing spermatogenesis was common. Lastly, the risk of pre-neoplasia and presence of spermatogenesis appear to be independent of genital phenotype (degree of virilization).

In general, almost 80% of males with 45,X/46,XY mosaicism had sufficient gonadal function to enter puberty spontaneously, although almost 40% needed subsequent T treatment. However, patients in the genital group had lower rates of spontaneous puberty and higher rates of T treatment. Interestingly, we found that most of the patients appeared to have normal serum concentrations of T. It has previously been reported that many patients with scrotal gonads have some hormone production (25), but it was unexpected that most patients had T levels in the normal range despite their genital phenotype and overall fairly small testicular volumes. However, gonadotropin levels were relatively high indicative of some degree of (early) gonadal failure. Altogether, our findings are in accordance with previous reports on gonadal function in males with a 45,X/46,XY karyotype (5,7,10,16,26).

Most patients in this cohort were short and did not grow according to their genetic potential. Patients in the *genital* group were significantly shorter than those in the *non-genital,* probably reflecting that patients with more severe genital phenotypes (*genital* group) are also more likely to have affected growth. This could in theory be due to a larger degree of 45,X cells and thus a larger degree of SHOX-haploinsufficiency as seen in classic Turner syndrome. Some of the growth trajectories in the adolescent patients in this study appeared to lack the expected pubertal growth spurt. Theoretically, hypogonadism in adolescence could potentiate the effects of the 45,X cell line on growth (5–7,10,15,27,28), thus producing this growth pattern. It is noteworthy that a recent study reports that XY-mosaic Turner patients have less affected growth than classic Turner girls (29)-

One third of patients in this study received rhGH treatment, and the percentage was not influenced by group. Final height SD scores were similar in groups with and without rhGH treatment, but the

retrospective nature of this study does not allow firm conclusions on the efficacy of rhGH treatment. In general, the literature shows contradictory findings on the effects of rhGH treatment on growth (30), and our results as well as those from previous studies raise the critical need for well-designed studies to examine the possible benefits of rhGH in these children. However, none of the existing studies are randomized controlled trials and the opposing results could be due to study design and perhaps also the underlying mechanisms of the growth retardation.

Theoretically, earlier intervention could improve several of the aforementioned clinical outcomes compared to patients with delayed or no intervention. However, a conclusive study will require a larger sample size as well as detailed information on T therapy.

The vast histological material in this study (44 patients, 61 gonads) showed a broad phenotypic spectrum confirming that some patients had one (often scrotal) testis and one intraabdominal streak gonad (48.8%), while others had two testes (often bilaterally scrotal) (51.2%). The distribution was significantly different between the genital and non-genital groups. The 45,X/46,XY karyotype alone therefore does not allude to the histology nor the location of the gonads, and thus, applying the term “mixed gonadal dysgenesis” to this patient population could be deemed as inappropriate.

Most gonads in this study were dysgenetic testes but there was a wide range from relatively normal testes containing tubules with complete/full spermatogenesis to streak gonads at each end of the spectrum. We did not detect the presence of follicles in any of the gonad samples evaluated. This could be due to oogonial loss prior to the formation of primordial follicles or breakdown of formed follicles already in early (fetal) life. Consensus understanding is that it takes two X chromosomes for

primordial follicles to develop. This notion is supported by a study examining the presence of primordial follicles (and number of germ cells) in ovaries from Turner fetuses aged 17-37 weeks, which reported that no primordial follicles could be detected (31). The etiology behind the 45,X/46,XY karyotype has been suggested as one where larger, structural aberrations such as deletions, isodicentricism etc. or minor molecular abnormalities to the Y-chromosome may cause its loss in some cells (3–7). Thus it appears plausible that the vast majority of patients with 45,X/46,XY mosaicism never have had two X chromosomes present and the development of follicles therefore seems unlikely, supported by our current findings. Moreover, it has previously been reported that gonads are relatively often mislabeled as ovotestes in 45,X/46,XY patients (32). The morphology in these samples resembles undifferentiated gonadal tissue and/or streak-like tissue with scattered germ cells. In such cases a higher risk of neoplasia, namely gonadoblastoma instead of GCNIS, has been reported (33). Given all of the above, it seems possible that ovotesticular DSD in 45,X/46,XY patients is a rarity, or maybe even a misconception, as has also previously been suggested (32). Additionally, regarding future fertility preservation potential, the focus should lie on spermatogenesis rather than folliculogenesis in these patients.

In 11.4% of patients we found gonadal neoplasia, specifically four patients with GCNIS and one patient with gonadoblastoma. Given the majority of (dysgenetic) testes in this series, GCNIS is the more likely neoplasia in this group of patients, which is in line with previous reports of gonadoblastoma being more frequent in patients with 45,X/46,XY mosaicism raised as females (18). Interestingly, we found that both post-pubertal patients with GCNIS had spermatogenesis alongside their neoplasia. This is a very important point for clinicians since testicular sperm cell extraction or aspiration should be considered before gonadectomy in these patients, as was done in one patient

in this study. It also indicates that the presence of spermatogenesis should not be interpreted as absence of testicular dysgenesis, and also poses the question of when to biopsy post-pubertal patients in particular.

Interestingly, there did not appear to be a correlation between EMS and risk of germ cell malignancy. A higher risk of neoplasia has previously been found in patients with greater genital ambiguity (5,16), but our current findings are not completely in accordance with this notion. This may be explained by the fact that the study population of this study differs from previous studies, with only men and not females with a Turner syndrome phenotype included. It probably also highlights a dual relationship in which severely dysgenetic gonads do not sufficiently support germ cells regardless of whether these are normal or potentially malignant. Conversely, a low EMS score in men with 45,X/46,XY mosaicism is highly suggestive of dysgenetic testes or undifferentiated gonadal tissue which if germ cells are present has a high risk of GCNIS. Thus, clinicians should be aware of the risk of malignant germ cells in all patients regardless of virilization status.

Surprisingly, a quarter of post-pubertal patients had focal spermatogenesis, while another quarter had spermatogenesis arrested at different stages of germ cell differentiation. Altogether this demonstrates a future fertility potential in up to half of the post-pubertal patients. It was noteworthy that many patients demonstrated focal SCO along with spermatocytic arrest at different stages highlighting that each gonad may be heterogenous and focal spermatogenesis cannot be ruled out based on a single biopsy. Moreover, one azoospermic male had histological evidence of spermatids emphasizing that even males with azoospermia may have focal spermatogenesis.

The implications of the high proportion of patients with spermatogenesis and spermatocytic arrest are important; *in vitro* spermatogenesis, in which germ cells are differentiated *in vitro*, may provide a future fertility treatment option for these patients. However, no verified protocol is currently available for human testis tissue despite few previous reports of successful *in vitro* maturation of germ cells in human tissue (34–37) and even early-stage germ cells (prepubertal) in mice (38). The possibility that a protocol for human *in vitro* spermatogenesis may be established in the near future also raises the question of whether attempts to cryopreserve testicular tissue from 45,X/46,XY patients should be considered. It does, nonetheless, also provide the clinician with ethical dilemmas such as including patients in experimental protocols where the outcome and timeline is still unknown (current patients may not benefit), as well as possible transmission of an aberrant Y chromosome to offspring.

No patients fathered offspring during the follow-up period and over 80% had complete azoospermia (assessed by their semen samples). However, almost 20% did produce semen samples with live spermatozoa. Both findings are in accordance with previous studies reporting low or no fertility in patients with 45,X/46,XY mosaicism (6,12,26,39). It is important to note that the males with live spermatozoa in this study were diagnosed at different ages from birth into adulthood and with varying degrees of genital androgenization. Clinicians should therefore be aware of fertility preservation methods, also in the pediatric setting, and semen sampling should be considered in all patients once they enter a mature age in late adolescence or early adulthood.

The strengths of this study include: 1) the multicenter design which has made it possible to collect data on a large series of males 2) the inclusion of numerous outcomes in a single study, both clinical

and histological, which allows for a thorough understanding of the outcomes and how they relate (or do not) to gonadal histology and karyotypic etiology 3) all patients are old enough to have long-term outcomes such as gonadal function and final height assessed 4) all patients are raised as males allowing for a unified evaluation of their outcomes. There are, however, also limitations and they include: 1) the retrospective design which has led to missing data for some variables for all patients 2) histological data was not available in all patients and conclusions may be skewed by the fact that the most severely affected individuals are far more likely to have gonadal biopsies 3) the use of reference ranges for LH, FSH, T, testicular volumes and growth based on a Danish population and the WHO growth curves, respectively, which do not reflect the composition of this study population, although allowing for the comparison of healthy backgrounds populations with the patients studied 4) some of the patients included in this study have been included in studies previously published (5,16,27) which may alter conclusions if drawn across published data 5) most patients were diagnosed post-pubertally which may skew conclusions towards poorer outcomes than if they had been prenatally diagnosed 6) patients were followed at multiple centers and consequently follow up schemes varied considerably.

In conclusion, in this large, multicenter study of males with 45,X/46,XY mosaicism, we find that patients diagnosed due to genital abnormalities have poorer health outcomes than those diagnosed due to other reasons such as short stature, lack of puberty, and infertility. Overall, patients do, however, have relatively good endocrine gonadal function, but most are also short statured. Moreover, patients regardless of reason for referral have a relatively high risk of gonadal neoplasia and most are azoospermic. Nevertheless, almost half of patients have germ cells present, and up to a quarter have focal spermatogenesis which provides hope for fertility treatment in some patients

and future treatment options in many. In general, the data indicates the importance of highly personalized medical management.

Declaration of Interests

None

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Figure Legends

Fig. 1: LH, FSH and testosterone values along with testicular volumes (largest testicle) at last evaluation according to age. Blue dots represent the *genital* group, green represent the *non-genital* group. Solid lines are reference ranges (mean and ±2 SD for the hormones and mean, ±1 SD and ±2 SD for testicular volumes). Dotted lines signify LODs.

Fig. 2a: Height (cm) according to age stratified according to rhGH treatment and group. Red dots represent rhGH treatment, blue dots represent the genital group, green represent the non-genital. Solid lines represent WHO reference ranges (mean, ±1 SD and ±2 SD).

Fig. 2b: Height and height according to genetic potential (height – target height) expressed in SD scores according to group and rhGH treatment. Dots represent patients in the *genital* group, red have received rhGH treatment, blue have not. Squares represent patients in the *non-genital* group, red have received rhGH treatment, blue have not. Solid lines represent group medians.

Fig. 2c: Height SD scores according to rhGH treatment and stratified according to group. Red dots represent rhGH treatment, blue dots represent the genital group, green represent the non-genital. Dotted lines represent ±2 SD.

Fig. 3: Key histology findings in terms of phenotype, presence of germ cells and germ cell differentiation counted by patients and gonads, respectively.

608 **Fig. 4:** The histological spectrum found in the gonadal samples from males with 45,X/46,XY
609 mosaicism. All images show Haematoxylin Eosin stained sections.
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613 **Table 1:** Differences between the *genital* and *non-genital* group in terms of age, genital
614 phenotype, growth, comorbidities, surgeries and gonadal neoplasia.
615 **Table 2:** Histological findings in samples from 61 gonads grouped according to reason for diagnosis
616 and including ages at the time of biopsy/gonadectomy and EMS scores.
617 **Table 3:** Tally of patients with Sertoli cell only pattern alongside spermatocytic arrest, full
618 spermatogenesis and/or GCNIS.
619 **Table 4:** Reproductive hormones, clinical features and gonadal histology in patients with available
620 semen analyses.