Phenotypic Variation and Pubertal Outcomes in Males and Females with 46,XY Partial Gonadal Dysgenesis

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Abstract

Background: 46,XY gonadal dysgenesis is classified as complete (CGD) or partial (PGD) subtypes. The phenotype of PGD and the long-term outcome is not clearly defined.

Objective: To evaluate clinical features and pubertal outcome of PGD in a large cohort, using CGD as a comparator for diagnostic clarity.

Methods: Patients with 46,XY GD were identified from the I-DSD Registry and data on phenotype, genetics, biochemistry, gonadal histology and pubertal development were collated in three categories; CGD (n=100), PGD assigned female (PGDf, n=107) and male (PGDm, n=103) at birth.

Results: Most individuals with PGD presented with atypical genitalia in infancy, though, 18% of PGDf presented with delayed puberty and 8% with virilisation. A genetic aetiology was identified in 42% of the cohort, with common gene defects in *SRY* and *WT1* in CGD, and *NR5A1* in PGD. Gonadal pre/malignancy was found in 33.8% in CGD, 19.7% in PGDf and 8.8% in PGDm. Among the PGDm (>13 years) with at least one gonad, 80% had spontaneous pubertal onset and 59% achieved Tanner G5 without hormone treatment. Labio-scrotal gonads at presentation and testosterone response to hCG predicted onset of spontaneous puberty. In PGDf with gonads, 42% developed spontaneous virilisation at puberty. Sex was reassigned in 16.1% and 5.3% of individuals with PGDf and PGDm, respectively.

Conclusions: This study highlights the heterogeneous phenotype of PGD and the consequent diagnostic challenge. Many PGD patients with preserved gonads have the potential to develop puberty spontaneously, though further study is needed to determine the risk of developing gonadal tumours.

Introduction

Gonadal dysgenesis (GD) is a term used when development of the gonad into a testis or an ovary is incomplete or impaired. It is a heterogeneous condition often associated with sex chromosomal anomalies or alterations in various genes involved in the development of the gonad (1). GD is classified as complete (CGD), partial (PGD), and gonadal regression depending on morphology and function (1). 46,XY GD is a common form of GD, in which testis differentiation and development has started but is incomplete.

In contrast to the well characterised presentation of 46,XY CGD with typical female external genitalia and presence of Mullerian structures, the diagnosis of 46,XY PGD is challenging as the phenotype can overlap with other conditions under the umbrella of differences of sex development (DSD) (2). Diagnosis of PGD is based on a combination of clinical features, endocrine investigations, genetic studies, and gonadal histology where necessary (3). However, a recent study using the next generation sequencing approach for known or candidate genes, found alterations in only 40% of partially virilised patients with 46,XY DSD (4). Furthermore, biopsy of the gonads is not always desirable, especially in individuals reared male.

Due to the rarity and heterogeneous presentation of 46,XY PGD, outcome data are limited. Two small studies have reported a high proportion of males (60 - 80%) who attained spontaneous onset of puberty (5) (6). However, data on further pubertal development, gonadal function and gonadal malignancies are limited.

We explored a large international dataset of patients to characterise clinical features of PGD using CGD as a comparator where a firm diagnosis can be made. Other aims of the study were to determine outcome at puberty, including gonadal histology and function and the frequency of sex reassignment.

Patients and Methods

1.Data collection

Data were collected from the I-DSD registry and two additional centres. The I-DSD Registry is an international database established in 2011 and hosted at the University of Glasgow (https://sdmregistries.org/). It has over 6671 cases from 120 centres from 43 countries (June 2022) and the research activity has recently been described (7). In brief, informed consent is obtained prior to enrolment and pseudonymised data from routine clinical care are stored in the registry (7). Centres with cases of 46,XY GD (n=257) were contacted by e-mail between July 2021 and August 2021, with two reminders sent to centres that did not reply. A spreadsheet was used to collect information, containing 98 items for female gender and 121 items for male gender. Participating clinicians returned the completed files in a secured shared folder. The two additional centres were Hospital de Clínicas (HC), UNICAMP (State University of Campinas), Brazil and Hospital das Clinicas, University of São Paulo, Brazil.

2. Patients

We used case definitions as previously reported (8). Inclusion criteria for CGD were: (1) 46,XY karyotype, (2) typical female genitalia, (3) presence of Mullerian duct derivatives or undetectable anti-Mullerian hormone (AMH), and (4) if at pubertal age, elevated levels of luteinising hormone (LH) / follicle stimulating hormone (FSH) (Table 1). Inclusion criteria for PGD were: (1) 46,XY karyotype,

(2) atypical genitalia, and (3) at least one of the following features which suggests GD: (A) testosterone levels after hCG stimulation test less than twice the basal value, low basal testosterone with high gonadotropin levels in minipuberty or puberty, AMH or inhibin B levels below male reference range, or presence of Mullerian duct derivatives, (B) histology consistent with GD, or (C) pathogenic variants in genes associated with GD(8) (Table 1). We assigned patients into three categories: CGD, PGD assigned female (PGDf) and male (PGDm) at birth.

Data were received on 221 patients from 34 centres through the registry and a further 113 patients from the two institutions (Campinas n=48, São Paulo n=65). We excluded 24 subjects from the analysis according to the inclusion criteria (CGD n=6, PGD n=18) for the following reasons: karyotype other than 46,XY (n=4), diagnosed with another DSD condition (n=1), possibility of gene defect due to 46XY trans (1;2)(q23;q11.2) (n=1), insufficient data on phenotype/serum testosterone/genetics/gonadal histology (n=18). A patient with a balanced translocation 46,XY,inv(9)(p11;q13), karyotype considered a normal variant, was included in the study.

A small group of patients (n=10) had typical female genitalia but without a uterus and evidence of gonadal dysfunction from biochemical, genetic or histological investigations. The absence of a uterus was confirmed by laparoscopy or laparotomy in six cases. We included these individuals in the PGDf group, as the phenotype is not consistent with typical CGD, and absence of Mullerian remnants suggested functioning gonads. Therefore, 310 patients were included in the final analysis (CGD n=100, PGDf n=107, PGDm n=103).

3. Data analysis

External masculinization scores (EMS)(9) and external genital scores (EGS)(10) were calculated from the questionnaire data. The EGS was assessed for patients who presented before 2 years of age. For analysis of biochemical data (LH, FSH, testosterone), each institution's threshold was used. Information on assays for these hormone measurements was not available. In cases where a normal range was not provided (66-73% each category), normative values from the Canadian Laboratory Initiative on Pediatric Reference Intervals (CALIPER) were used (11). For AMH analysis, each institution used either Enzyme-Linked Immunosorbent Assay (ELISA) or Chemiluminescence Immunoassay (CLIA) and the age specific male reference was used for assessment (Beckman Coulter Cat# A73818, RRID:AB_3674123; Beckman Coulter Cat# B13127, RRID:AB_2892998; Roche Cat# 06331076, RRID:AB_2895131).

To evaluate onset of puberty, an age threshold of ≥13.0 years was used, as 90% of males would have developed a testis volume ≥4ml at that age (12). We evaluated puberty completion in patients who were either over 17 years of age or had already reached Tanner stage G 5 prior to this age, as 95% of males would have reached stage 5 (12).

4. Histological data

Clinicians were asked to classify reported gonadal histology consistent with one of the following options: "streak gonad", "dysgenetic testis" [including undifferentiated gonadal tissue (UGT)], "normal/almost normal testis" and "replaced by tumour". Clinicians were also asked to submit anonymised histology reports and pathology slide scans for review if feasible. Members of the study team (MC, KV) with expertise in germ cell pathology categorised histology reports into streak, dysgenetic or regressed gonad, UGT, ovotestis, or normal testis. Pathology slides or slide scans were classified as (1) normal testis; (2) dysgenetic testis: poorly developed seminiferous tubules with increased intertubular stroma with or without the following characteristics: fibrosis, intracapsular

growth of testis tubules, diminished tubular diameter, decreased number or absence of germ cells (Sertoli cell only tubules); (3)UGT: germ cells were organised together with Sertoli/granulosa-like cells in cord-like structures or resided without apparent organisation in a background of gonadal stromal cells; (4) streak gonad: fibrous stroma devoid of germ cells; cord like structures, when recognisable, were interpreted as UGT that had lost its germ cells and had undergone a fibromatous involution; or (5) gonadal regression: Mullerian and Wolffian duct derivatives only(13).

5. Ethics

I-DSD Registry is approved by the National Research Ethics Service in the UK as a research database. The State University of Campinas (UNICAMP) and University of São Paulo have local institutional ethics approvals in place to share anonymised data for research studies.

6. Statistics

Data were described as median [first quartile–third quartile] unless specified. Categorical variables were compared using the Fisher exact test and a p value of <0.05 was considered significant.

Results

1. Patients

Patients who fulfilled the inclusion criteria comprised 107 individuals with PGDf and 103 with PGDm. In PGDf, in addition to (1) 46,XY karyotype and (2) atypical genitalia, 74% fulfilled clinical evidence of gonadal dysgenesis (3A), 60% fulfilled histology criteria (3B) and 46% fulfilled (3C) pathogenic gene variant. In PGDm category, 65%, 52% and 38% of the patients fulfilled these criteria respectively (Figure 1). The individuals were born between 1941 and 2020 (median 1997 [1987–2006]: Table 2). The patients were from European centres (n=166, 53.5%), South America (n=118, 38.1%), and Asia (n=26, 8.4%). There was no significant difference in gestational age or birth weight between the categories (Table 1). There was a positive family history of DSD in 17.4% of CGD, 19.0% of PGDf and 25.8% of PGDm. A similar proportion also reported a family history of infertility (Table 2).

2. Presentation

The individuals with CGD presented at age 15.0 [8.4–17.9] years, with the majority (62.5%) complaining of delayed puberty (Figure 2, Table 2). A diagnosis at an earlier age was obtained in 25.0%, where associated features led to karyotype analysis. Symptoms caused by the effects of gonadal tumour such as abdominal pain led to the diagnosis of CGD in 9.1% (n=8/88) at age 12.7 (range 7.8-16.6) years. In contrast, individuals with PGD presented at a younger age (PGDf 1.3 [0.1–13.9] years; PGDm 0.5 [0.1–4.0] years) due to atypical genitalia in all patients with PGDm and 62.1% in PGDf. Delayed puberty was the presenting feature in 17.9% of PGDf patients and in 8.4%, it was associated with virilisation.

3. Phenotype

In individuals who presented before age 2 years, the external genital appearance was more masculinised in PGDm compared to PGDf (EGS 7.0 [5.5–8.3] vs 4.0 [2.0–5.5]; p<0.0001) (Table 2). A uterus was present in a greater proportion of PGDf individuals compared with PGDm (51.0% vs 31.3%, p=0.006) and in all patients with CGD as per case definition. Fallopian tubes were present in 91.5% of individuals with CGD, whereas in PGDf and PGDm, it was 35.5% and 18.8%, respectively.

4. Comorbidities

A high frequency of comorbidities was reported in all categories with no significant differences between groups (CGD 38.0%, PGDf 33.0%, PGDm 33.3%). Detailed comorbidities are listed in Supplementary table 1 (14). Examples of neurodiversity such as developmental delay, learning difficulties, attention deficit hyperactivity disorder (ADHD), autistic spectrum disorder and structural brain anomalies were the most frequent (CGD 28.9%, PGDf 21.3%, PGDm 26.3%). The next frequent group was renal disorders including Wilm's tumour, congenital proteinuria, and structural anomalies (CGD 23.7%, PGDf 12.8%, PGDm 31.6%). While not all patients had *WT1* gene screened, 83% (n=10/12) of individuals with *WT1* mutation had associated renal comorbidities.

5. Biochemical markers of gonadal function

Minipuberty (age 0-6 months):

There was no significant difference in basal serum testosterone levels in individuals with PGDm and PGDf (Table 3). FSH levels were similar between PGDf and PGDm and above the reference range in all individuals with CGD and 67.9% in PGDf and 64.7% in PGDm. LH levels were similar in the groups and above the reference range in 16.7% CGD, 7.1% PGDf and 17.6% PGDm (Table 3).

Age ≥13 years:

In patients who presented at age ≥13 years for the first time, basal serum testosterone levels were similar in PGDf and PGDm (Table 3). LH and FSH levels were also similar in these groups (Table 3). A greater proportion of individuals with CGD had elevated serum LH levels compared to other groups (93.8% in CGD vs 77.1% in PGDf, p=0.022; 76.9% in PGDm, p=0.086). FSH was above the reference range in 95.4% CGD, 80% PGDf and 100% PGDm in this age group (CGD vs PGDf p=0.030, CGD vs PGDm p=ns, PGDf vs PGDm p=ns). Individuals with CGD with low LH and FSH levels were associated with gonadal tumour.

There was no significant difference in day four serum testosterone levels after hCG stimulation (taken at any age) between PGDf and PGDm (Table 3). In these groups, a similar proportion of individuals had low levels of AMH and inhibin B (48% and 58.1% low in AMH and 66.7% and 69.2% low in inhibin B, respectively: Table 3).

6. Genetics

Genetic screening was performed in 78.7% of the cohort with various methods; targeted gene screening (71%), gene panels (13%) and whole exome sequencing (15%). This identified the likely genetic cause (gene or chromosome alterations) in 42.3 % of the cohort (n=131/310). Of those who underwent screening, 48.3% of individuals with CGD, 59.0% with PGDf, and 50.0% with PGDm had a gene or chromosome alteration identified. The most frequent gene alterations in CGD were *SRY* (23.6%; n=21/89) and *WT1* (9.0%; n=8/89). In PGD, alterations in *NR5A1* were the most frequent finding (PGDf: 42.2%; n=35/83; PGDm: 25.6%; n=20/78). Gene alterations are listed in Supplementary table 2 (15).

7. Pubertal development

7.1 PGD Male gender

Information on puberty was available in 45 individuals with PGD who assigned male at age ≥ 13 years, including 9 individuals re-assigned from female to male before age 13 years, with at least one retained gonad (Bilateral gonads n=37, Unilateral n=8). Over half of the individuals in this group

(55.6%, n=25/45) had at least one gonad at the labioscrotal region at the first presentation and 44% (n=20/45) underwent unilateral or bilateral orchidopexy. Spontaneous onset of puberty (Tanner stage G2 or testis volume ≥4 ml) occurred in 80.0% (n=36/45) at age 12 [10.6–12.0] years. Of these, 30.3% (n=10/33) were treated with testosterone to complete puberty (Figure 3). Decision on whether to start testosterone treatment was taken by individual clinicians. In puberty completion assessment, 59.3% (n=16/27) achieved Tanner G5 without hormone treatment at age 18.1 [16.0–20.0] years. In individuals who had spontaneous puberty completion, the Tanner staging was: G5 n=16 (94.1%) and G4 n=1 (5.9%), the median testis volume was 8.0 [4.8–8.5] ml (n=12 gonads) and the stretched penile length was 6.8 [5.9–8.0] cm (n=10) (Table 4). Serum LH and FSH levels were elevated in 94.4% (n=17/18) and 100% (n=18/18), respectively.

Factors which associated with spontaneous puberty were explored. The EMS, EGS or serum basal testosterone levels at the first investigation were not predictive (Supplementary table 3 (16)). However, patients who developed spontaneous puberty were more likely to have at least one labio-scrotal gonad at presentation compared to those with induced puberty (62.9%; n=22/35 vs 12.5%; n=1/8, p=0.017). Also, a higher proportion of individuals in the spontaneous puberty group had a testosterone response to hCG more than double the basal level (88%; n=15/17, vs 33%; n=1/3, p=0.087]. All individuals with gene alterations in *NR5A1* with gonads (n=14; 78.6% uni/bilateral labioscrotal gonad at the first presentation) had spontaneous onset of puberty compared to only 50% (n=4/8; 62.5% uni/bilateral labioscrotal gonad at the first presentation) of the individuals with other gene mutations (p=0.078). Furthermore, 15.4% (n=2/13) of the patients with *NR5A1* gene alteration required subsequent testosterone treatment (one individual started at age 15 years and the other age unknown).

Data beyond puberty (n=16, age ≥ 19 years; [median age 21.5 (range 19–50) years] showed that patients who completed puberty without testosterone treatment did not require further replacement therapy.

7.2 CGD

In women with CGD who presented aged ≥13 years, spontaneous breast development was noted in 18.5% (n=12/65) (Table 5). Puberty induction was started at age 16 [14.0–17.3] years (n=66) and 72.3% (n=34/47) of the individuals aged ≥15 years achieved menarche (Table 5). The median age of menarche was 17.3 [16.5–19.0] years (n=19), following induction treatment of 2.3 [0.7–4.0] years (n=17).

7.3 PGD Female gender

Of individuals with PGD assigned female at age ≥13 years with at least one gonad (n=18/22 individuals with intra-abdominal or impalpable gonad, n=4/22 uni/bilateral inguinal gonad) 42.3% (n=11/26) developed features of virilisation (hirsutism n=5, clitoral enlargement n=7) (Table 5) and had testosterone levels appropriate for boys (9.3 [7.2–13.1] nmol/l, n=6). Spontaneous breast development was noted in 30.8% (n=8/26; Tanner stage B2 n=3, B3 n=3, B5 n=2). Among this group, six patients (75%) presented with virilisation along with breast development. Two individuals with virilisation were treated with a GnRH analogue. Puberty induction with oestrogen was started at age 13.0 [11.0–17.0] years (n=51) and 30% (n=9/30) achieved menarche assessed at age >15 years.

8.Surgical interventions

8.1 Genital surgery

In PGDf, 47.1% (n=33/70) of individuals underwent clitoral reduction surgery and 44.9% (n=31/69) had vaginoplasty (Table 5). About half (n=26/53) and 73.6% (n=39/53) of the individuals with PGDm underwent orchidopexy and hypospadias repair, respectively (Table 4).

8.2 Gonadal biopsy

Gonadal biopsy was performed in 26.0% (n=19/73) of individuals with CGD; all subsequently had gonadectomy (Table 6). In PGDf, biopsy was performed in 22.9% (n=19/83), and 52.6% (n=10/19) subsequently had gonadectomy. Of those who had retained gonads, five individuals reassigned to male sex. In PGDm, 43.5% of the individuals had unilateral or bilateral gonad biopsy (n=40/92; n=12 unilateral, n=28 bilateral) at median age 1.8 years [1.0–4.5] which led to subsequent gonadectomy in 22.5% (n=9/40).

8.2 Gonadectomy

Bilateral gonadectomy was performed in 93.6% of individuals with CGD (n=88/94), and 75% (n=75/100) of the individuals with PGDf, at ages 16.0 [13.1–18.3] years and 6.5 [1.6–15.1] years, respectively (Table 6). The interval between first presentation and gonadectomy was 0.63 [0.3–1] years (n=73) in CGD and 1.42 [0.4–4.4] years (n=51) in PGDf. In PGDm, 19.4% of individuals (n=19/98) had unilateral and 23.5% (n=23/98) had bilateral gonadectomy at age 2.7 [0.87–4.8] (n=19) and 4.0 [3.0–14.6] (n=23) years, respectively (Table 6). In PGDm, all excised gonads were located in the abdomen; about 60% were regressed or streak gonads.

9. Histology of Gonads

Histology diagnosis was reported for 63 patients with CGD, 70 with PGDf and 40 with PGDm. In addition, our expert panel reviewed detailed histology reports on 21 individuals (34 gonads) and whole slides from 17 (28 gonads).

Clinicians' report:

In CGD, 'streak gonad' was the most common reported histological feature (75.7%) followed by 'replaced by tumour' (9.6%) and 'dysgenetic testis' (7.0%). In PGD, 'dysgenetic testis' was more common (PGDf: 51.6%, PGDm: 35.7%) than 'streak gonad' (PGDf: 19.0%, PGDm 28.6%) (Table 6).

Histology report review:

A similar pattern was found from review of the histology reports. Streak gonad, with or without UGT, (55.6%; n=5/9) was more frequent compared to dysgenetic testis (11.1%; n=1/9) in CGD and dysgenetic testis was more common in PGD (35.3%; n=6/17 in PGDf, 62.5%; n=5/8 in PGDm) (Table 6).

Histology slide review:

The common histology features were regressed gonads (60%, n=6/10), streak gonads (30%, n=3/10) and UGT (10%, n=1/10) in CGD. In PGDf, 85.7% were dysgenetic testis (n=6/7) with features of UGT in 33.3% (n=2/6) and 14.3% (n=1/7) were streak gonad. In PGDm, dysgenetic testis (63.6%, n=7/11), normal testis (27.3%, n=3/11; of these n=1 showed UGT) and streak gonad (9.1%, n=1/11) were observed. Features of dysgenetic testis noted were reduced tubular diameter, thickened basal membrane, increased interstitial stroma, intracapsular growth, irregular shaped tubules, Leydig cell micronodules or hyperplasia, Sertoli cell only tubules, reduced germ cell number, absent spermatogenesis and tubular atrophy (Figure 4, Table 6).

Gonadal tumours: Gonadal malignancy or pre-malignancy (germ cell neoplasia in situ; GCNIS) was reported in 33.7% (n=28/83; 18.1% unilateral, 15.7% bilateral) in CGD, 19.7% (n=13/66; 4.5% unilateral, 15.2% bilateral) in PGDf and 8.8% (n=3/34; 5.9% unilateral, 2.9% bilateral) in PGDm (CGD vs PGDf p=0.066, CGD vs PGDm p=0.005). The common types of tumours in all three groups were gonadoblastoma (47.8%, n=32/67), dysgerminoma/seminoma (28.4%, n=19/67) and GCNIS (11.9%, n=8/67). Other examples included mixed germ cell tumour (4.5%, n=3/67), sex cord stromal tumour (4.5%, n=3/67) and yolk sac tumour (1.5%, n=1/67) (Table 6).

10. Sex reassignment

Five individuals (n=5/94; 5.3%) with PGDm were reassigned to female during infancy (n=2), childhood (n=1) or adolescence (n=2) (Table 4). Fifteen individuals (n=15/93; 16.1%) with PGDf were reassigned to male (infancy n=3, childhood n=6, adolescent n=4, adult n=1, unknown n=1) (Table 5).

11. Gonadal tumours and breast development.

In patients with CGD who had spontaneous breast development, gonadal tumours were found in 67% (n=8/12; seminoma/dysgerminoma n=4, mixed germ cell tumour n=2, gonadoblastoma n=2). The risk for gonadal tumours was considerably higher in those with spontaneous breast development compared with those without (67% vs 26% [n=11/42], p=0.014, odds ratio=5.6).
In PGDf, two individuals with breast development had gonadal tumours (25%; n=2/8; bilateral gonadoblastoma, bilateral dysgerminoma), and the risk was not greater than other PGDf patients (30%; n=4/13).

Discussion

The current study provides detailed characteristics in a large cohort of 46,XY PGD individuals and highlights the wide spectrum of this condition. Key observations show that spontaneous onset of puberty occurred in 80% of individuals raised male and 59% achieved Tanner G5 without hormone treatment. In PGD assigned female at puberty, 42% individuals developed features of virilisation.

As 46,XY PGD is a heterogeneous disorder without clearly defined diagnostic criteria, we used the diagnostic criteria from a previous study which requires evidence of testicular dysgenesis, either in a biochemical test (eg. low testosterone response after hCG stimulation, low AMH or inhibin B), histology findings, or a genetic defect associated with GD (8). As we used CGD as a robust comparator, individuals who did not fit in the clinical picture of typical CGD, such as mild virilisation at any age, were classified as PGDf. A small group of individuals with female external genitalia who lacked Mullerian duct structures were difficult to classify. In these cases, absence of uterus was confirmed by laparoscopy in the majority. The variable phenotype likely represents the spectrum of disorder. However, as typical CGD has Mullerian remnants (17), and absence of a uterus suggests anti-Mullerian hormone secretion, we included this group as PGDf. The study design to include CGD patients was instrumental in illustrating the broad range of phenotypes for 46,XY GD.

A genetic cause was identified in approximately half the study cohort which is higher than in recent reports (22-40%) (4,18). Our results were based on historical data with less robust variant classification and possibly a selection bias which may explain the higher rates. Furthermore, standard karyotyping techniques may miss mosaicism such as 46,XY/45,X. It is possible that some of our cohort may have 45,X cell lines as some comorbidities observed, such as short stature, horseshoe kidney and coarctation of aorta are also features of 46,XY/45,X mosaicism. This suggests the need for future

studies in larger cohorts with more extensive genetic analyses. Nevertheless, the common gene alterations we observed were in accordance with previous smaller studies (18).

Disturbed development of gonads increases the risk of germ cell neoplasia (19). The incidence of gonadal neoplasia in CGD observed in this study (33.7%) is similar to other reports (15-40% (13,20)). The prevalence of germ cell neoplasia in PGD is reported to be variable (16-30% (21)). We noted a lower incidence in PGDf (19.7%) as compared to CGD, which may be due to multiple factors such as earlier gonadectomy (age 6.4 years vs 16 years in CGD), higher levels of "testicularisation" of gonads, and an extra-abdominal location (22). In PGDm, there were only three cases of neoplasia reported. However, there was significant variation in monitoring practice amongst centres and only a third (n=34/92) of individuals with PGDm had histology assessment. A collaborative long-term follow up study, ideally including post-puberty biopsy with histology review undertaken centrally in an expert centre, is required to achieve a more accurate risk estimation in boys with PGD.

The frequency of spontaneous or natural completion of puberty was considerably high in our cohort of PGD assigned male, with at least one retained gonad (80% had spontaneous onset and 59% completed puberty spontaneously). Two previous studies from Brazil with limited sample size reported discrepant rates of spontaneous puberty in 46,XY PGD (21,6) Andrade et al. reported higher rates; all (n=10/10) had spontaneous onset and only one individual required testosterone treatment, with normal testosterone levels during puberty but with elevated gonadotropin levels despite relatively low hCG-stimulated testosterone levels (<0.3 to 1.7nmol/l) compared to the present study (median 3.6 nmol/l) (21). Gomes et al. reported 57% (n=12/21) went through puberty spontaneously (6) However, this study included patients assigned female and assessed for puberty from a relatively young age of 9.1 years (6). We found that labioscrotal position of gonad and higher hCG-stimulated testosterone levels are associated with spontaneous pubertal development. Such markers of testis function could be useful when discussing sex assignment of infants. However, EGS at presentation was not related and it is possible that variation in the phenotype as well as limited sample size might explain the lack of correlation. The high rates of spontaneous puberty in patients with *NR5A1* gene alterations confirms findings found in smaller studies (23,24). A plausible explanation is that steroidogenesis in adult Leydig cells is less dependent on *SF-1/NR5A1* compared to fetal Leydig cells (25). We noted high rates of virilisation (44%) with hirsutism and clitoral enlargement in individuals with PGDf who presented with primary amenorrhoea suggesting residual gonadal function. Interestingly, one third displayed breast development (Tanner 2-5) possibly due to estrogen production from aromatisation of androgens (26).

The observation of a high incidence (67%) of gonadal tumours in CGD patients who presented with delayed puberty, but also had breast development, is in keeping with results from smaller studies (27). Germ cell tumours which produce androgens (28,29) and estrogens (28,30) may explain this presentation (31,32). The finding highlights the importance of evaluating these patients for gonadal tumours. This study has included histological findings in a large number of individuals with PGD with the analysis undertaken in a single centre of germ cell tumour expertise. This is the way forward for future prospective outcome studies to determine the precise risk for germ cell neoplasia in later life, particularly in PGD assigned male and retained gonads post-pubertally.

A drawback of this study is its retrospective nature and based on a historical cohort of patients with a median age of 27 years. This possibly contributed to the variety in type of evaluation and patient

management with inconsistent data reporting. Nevertheless, this study, to our knowledge, describes the phenotype and clinical course in the largest group of 46, XY GD patients by virtue of its international collaboration and access to the I-DSD registry.

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Data availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

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Figure 1. Distribution of inclusion criteria. The Venn diagram shows the number of individuals with PGD who fulfilled the inclusion criteria (3A) testosterone levels after hCG stimulation test less than twice the basal value, low basal testosterone with high gonadotropin levels in minipuberty or puberty, AMH or inhibin B levels below male reference range, or presence of Mullerian duct derivatives, (3B) histology consistent with GD, and (3C) pathogenic variants in genes associated with GD, in addition to (1) 46,XY karyotype and (2) atypical genitalia.

Figure 2. Mode of clinical presentation (%). In CGD, 62.5% presented with pubertal delay, followed by incidental karyotype findings and gonadal tumour symptoms such as abdominal pain/discomfort and mild fever. In PGD, the majority presented with atypical genitalia. In PGDf, 17.5% presented with pubertal delay, 8.2% presented with pubertal delay and virilization such as clitoral enlargement and hirsutism.

Figure 3. Pubertal outcome in individuals with PGD assigned male. There were 45 individuals with PGD assigned male aged ≥13 years with unilateral (n=8) or bilateral (n=37) gonads in the cohort. Spontaneous onset of puberty was observed in 80% (n=36/45; n=7 unilateral, n=29 bilateral gonad). In individuals with spontaneous puberty onset, 30.3% (n=10/33) required testosterone treatment to complete puberty. Data on whether testosterone treatment was given was missing in 6 individuals. At the assessment of puberty completion, 94% (n=16/17) of the individuals who did not receive testosterone treatment attained Tanner G5 spontaneously. All patients who received testosterone treatment attained Tanner G5. Data on the final Tanner stage was available in 27 individuals.

Figure 4. Histology of dysgenetic testis. (A) PGDf (hemizygous *NR5A1* deletion), gonadectomy performed aged 6 years. This shows increased interstitial stroma, reduced tubular diameter, mostly Sertoli cell only tubules. Arrows indicate germ cells. (B) PGDf (*MAP3K1* defect) aged 22 months. Panel B.1 shows intracapsular growth. OCT3/4 staining (Panel B.2) shows peripheral preneoplastic cells (grey arrow) and luminal OCT3/4- positive delayed maturation in white arrow. (C) PGDm (unknown genetic cause). Some tubules and the basement membrane (BM) are extremely thickened and interstitial fibrosis is observed.

Table 1. Inclusion criteria for CGD and PGD

	CGD: 1, 2, and either 3 or 4		**PGD: 1, 2, and either 3 or 4, 5 or 6**
1. Karyotype	46XY	1. Karyotype	46XY
2. External genitalia	Female type	2. External genitalia	- Female type with no Mullerian duct derivatives - Female type + virilization at puberty - Atypical genitalia
3. Internal genitalia	Mullerian duct derivatives	3. Internal genitalia	Mullerian duct derivatives
4. Biochemistry	Undetectable anti-Mullerian hormone (AMH)	4. Biochemistry	One of the following; - Testosterone level < twice the basal value after hCG - Low basal testosterone with high gonadotropin levels in minipuberty or puberty - AMH or inhibin B levels below male reference range
	Additional: elevated LH/FSH if pubertal age		
		5. Histology of gonad	Histology consistent with GD
		6. Genetic study	Pathogenic variants in genes associated with GD

Table 2. Characteristics, presentation of the patients and internal/external genital features

Parameter	CGD (n=100)	PGDf (n=107)	PGDm (n=103)	P value
Year of birth (Range)	1941- 2020	1951- 2019	1962- 2019	Total data 1941-2020
Median [1st–3rd quartile]	1995 [1986–2000]	1995 [1985–2006]	2002 [1991–2010]	1997 [1987–2006]
Gestation (weeks)				CGD vs PGDf ns
Median [1st–3rd quartile]	39 [38–40] (n=29)	39 [37.3–40] (n=30)	38.5 [38–40] (n=34)	CGD vs PGDm ns
Preterm	11.5% (n=6/52)	11.3% (n=6/53)	12.8% (n=10/78)	PGDf vs PGDm ns
Term	88.5% (n=46/52)	88.7% (n=47/53)	87.2% (n=68/78)	
Birth weight (g)	3240 [2814–3614] (n=46)	3290 [2790–3775] (n=68)	3185 [2800–3500] (n=81)	CGD vs PGDf ns CGD vs PGDm ns PGDf vs PGDm ns
Positive family history				CGD vs PGDf ns
DSD	17.4% (n=15/86)	19.0% (n=16/84)	25.8% (n=24/93)	PGDf vs PGDm ns
Infertility	2.3% (n=2/86)	6.0% (n=5/84)	5.4% (n=5/93)	CGD vs PGDm ns
Age of the first presentation (year)	15.0 [8.4–17.9] (n=94)	1.3 [0.1–13.9] (n=91)	0.5 [0.1–4.0] (n=94)	CGD vs PGDf p<0.00001 CGD vs PGDm p<0.00001 PGDf vs PGDm p=0.031
External genitalia assessed below 24 months old	n=13	n=53	n=69	
Genital tubercle length	n=8	n=34	n=50	
<10mm	8 (100%)	9 (26.5%)	3 (6%)	
10-20mm	0	13 (38.2%)	21 (42%)	
21-25mm	0	4 (11.8%)	10 (20%)	
26-30mm	0	5 (14.7%)	10 (20%)	
>31mm	0	3 (8.8%)	6 (12%)	
Urethral meatus opening	n=12	n=45	n=69	
Top of the genital tubercle (3)	1 (8.3%)	2 (4.4%)	8 (11.6%)	
Coronal glandular (2.5)	0	1 (2.2%)	4 (5.8%)	
Along the genital tubercle (2)	0	3 (6.7%)	8 (11.6%)	
At the genital tubercle base (1.5)	0	5 (11.1%)	14 (20.3%)	
Labioscrotal (1)	1 (8.3%)	9 (20.0%)	18 (26.1%)	
Perineal (0)	10 (83.3%)	25 (55.6%)	17 (24.6%)	
Labioscrotal fusion	n=13	n=48	n=68	
Fused (3)	0	8 (16.7%)	43 (63.2%)	
Posterior fusion (1.5)	0	16 (33.3%)	13 (19.1%)	
Unfused (0)	13 (100%)	24 (50%)	12 (17.6%)	
Either gonad	n=26	n=122	n=140	
Labioscrotal (1.5)	0	16 (13.1%)	52 (37.1%)	
Inguino-labioscrotal (1.0)	0	2 (1.6%)	2 (1.4%)	
Inguinal (0.5)	1 (3.8%)	31 (25.4%)	34 (24.3%)	
Impalpable (0)	25 (96.2%)	73 (59.8%)	52 (37.1%)	
EGS Median [1st –3rd quartile]	0 [0–0.6] (n=8)	4.0 [2.0–5.5] (n=33)	7.0 [5.5–8.3] (n=51)	CGD vs PGDf <0.0001 CGD vs PGDm <0.00001 PGDf vs PGDm <0.00001
Presence of Uterus	100% (n=96/96)	51.0% (n=52/102)	31.3% (n=30/96)	PGDf vs PGDm 0.0062
Presence of Fallopian tube	91.5% (n=65/71) 1.4% (n=1/71) blind ending in the right	35.5% (n=27/76)	18.8% (n=16/85)	CGD vs PGDf <0.00001 CGD vs PGDm <0.00001 PGDf vs PGDm 0.0003

Footnotes: data presented as median [1st–3rd quartile], NS- not significant. The number of patients available for each analysis are less than the group totals due to missing data.

Table 3. Biochemical data at the first presentation

	CGD (n=100)	PGDf (n=107)	PGDm (n=103)	p value
Basal testosterone 0-6 months (nmol/l)				CGD vs PGDf 0.002 CGD vs PGDm
Median [1st–3rd quartile]	0.30 [0.095-0.82] (n=6)	1.56 [0.45–3.52] (n=23)	2.43 [0.69-4.88] (n=31)	<0.00001
Range	0.087 - 1.1	0.006 - 9.6	0.07 - 8.5	PGDf vs PGDm ns
LH 0-6 months (IU/L)	n=6	n=28	n=34	CGD vs PGDf ns
Median [1st–3rd quartile]	3.25 [1.65–5.6]	2.8 [1.6–4.9]	4.25 [1.25–8.8]	CGD vs PGDm ns
Range	0.1 – 30	0.1 - 18.2	0.1 - 97.1	PGDf vs PGDm ns
LH elevated:0-6 months #	16.7% (n=1/6)	7.1% (n=2/28)	17.6% (n=6/34)	CGD vs PGDf ns CGD vs PGDm ns PGDf vs PGDm ns
FSH 0-6 months (IU/L)	n=6	n=28	n=34	CGD vs PGDf ns
Median [1st -3rd quartile])	28.7 [20.6–38.4]	6.7 [3.3–15.6]	6.8 [2.75–12.1]	CGD vs PGDm ns
Range	11.5 – 120	0.1 – 57	0.7 – 28.5	PGDf vs PGDm ns
FSH elevated :0-6 months #	100% (n=6/6)	67.9% (n=19/28)	64.7% (n=22/34)	CGD vs PGDf ns CGD vs PGDm ns PGDf vs PGDm ns
Basal testosterone >=13yrs (nmol/l)	n=44	n=17	n=14	CGD vs PGDf 0.009 CGD vs PGDm
Median [1st–3rd quartile]	0.8 [0.49–1.18]	3.3 [1.08–11.24]	0.83 [0.49–4.04]	0.00003
Range	0 - 4.56	0.45 - 22.62	0.49 - 20.72	PGDf vs PGDm ns
LH >=13yrs (IU/L)	n=65	n=35	n=13	CGD vs PGDf 0.027
Median [1st–3rd quartile]	27.0 [17.0-37.8]	18.0 [9.9–31.8]	15.8 [10.0–21.0]	CGD vs PGDm ns
Range	0.16 - 112.8 ψ	0.01 - 67.8	0.9 – 149	PGDf vs PGDm ns
LH elevated (>=13 yrs)#	93.8% (n=61/65)	77.1% (n=27/35)	76.9% (n=10/13)	CGD vs PGDf 0.022 CGD vs PGDm 0.086 PGDf vs PGDm ns
FSH >=13 years (IU/L)	n=65	n=35	n=13	CGD vs PGDf
Median [1st–3rd quartile]	85.0 [58.0–119.9]	52.8 [29.5–78.1]	48.9 [44.0–70.0]	0.00006
Range	0.7 - 184.8ψ	2.4 - 170.0	18.1 – 100.0	CGD vs PGDm 0.0003 PGDf vs PGDm ns
FSH elevated # (>=13 yrs))	95.4% (n=62/65)	80.0% (n=28/35)	100% (n=13/13)	CGD vs PGDf 0.030 CGD vs PGDm ns PGDf vs PGDm ns
hCG stimulated testosterone * (nmol/l)	n=15	n=34	n=46	CGD vs PGDf ns CGD vs PGDm
Median [1st–3rd quartile]	0.56 [0.4–1.04]	1.98 [0.59–9.70]	3.6 [1.04–7.29]	0.0026
Range	0.087 - 11	0.035 - 28.8	0.007 - 19	PGDf vs PGDm 0.075
hCG stimulated testosterone *	20% (n=3/15)	47.2% (n=17/36)	66.0% (n=31/47)	CGD vs PGDf ns CGD vs PGDm 0.003
Doubled				PGDf vs PGDm 0.075
AMHΦ				CGD vs PGDf
Low	95.7% (n=22/23)	48.0% (n=12/25)	58.1% (n=18/31)	0.0003
Normal	4.3% (n=1/23)	52.0% (n=13/25)	41.9% (n=13/31)	CGD vs PGDm 0.002 PGDf vs PGDm
				ns
Inhibin B				CGD vs PGDf
Low	100% (n=20/20)	66.7% (n=8/12)	69.2% (n=9/13)	0.014
Normal	0% (n=0/20)	33.3% (n=4/12)	30.8% (n=4/13)	CGD vs PGDm 0.016 PGDf vs PGDm ns

Footnotes: data presented as median [1st–3rd quartile], NS- not significant. The number of patients available for each analysis are less than the group totals due to missing data.

Institution's threshold or CALIPER cut off was used. LH: institution's reference provided in n=24/87, 31/93, 24/85 in CGD, PGDf and PGDm,, respectively. (28-33%). FSH institution's reference provided in n=24/87, 32/93, 23/85 in CGD, PGDf and PGDm, respectively. (27-34%) CALIPER cut-off: LH >10 IU/L, FSH >4 IU/L at 0-6 months; LH >8 IU/L, FSH>7 IU/L at >13 years.

ψ Individuals with CGD with low LH and FSH were associated with gonadal tumour.

* Taken at any age.

Φ Based on age appropriate male AMH reference range in each institution.

Table 4. PGD male pubertal development, surgical outcome and sex reassignment

Information on PGD assigned male at age 13 years with at least one gonad in situ	n=45 (Bilateral gonads n=37, Unilateral n=8)
Spontaneous onset of puberty	80.0% (n=36/45)
Age of spontaneous onset of puberty	12.0 [10.6–12.0] (n=26)
Patients with spontaneous onset of puberty who received testosterone treatment	30.3% (n=10/33)
Frequency of achieving Tanner G5 spontaneously ψ	59.3% (n=16/27)
Puberty assessment of the patients with spontaneous puberty who received no testosterone treatment at completion	Median age 18.1 [16.0–20.0] years (n=13) Tanner stage: G5 94.1% (n=16/17), G4 5.9% (n=1/17) Pubic hair stage: PH5 100% (n=8/8) Testicular volume (ml): 8.0 [4.8–8.5] (n=12 gonads) Stretched penile length (cm): 6.8 [5.9–8.0] (n=10) Gynaecomastia: n=2/16 (12.5%)
Biochemistry data of the patients with spontaneous puberty who received no testosterone treatment at puberty completion	Median age 17.8 [15.3–19.4] years (n=16) Basal testosterone 20.0 [14.3–29.2] nmol/l (n=16) LH 13.2 [9.5–21.5] IU/l (n=16) FSH 28.4 [16.6–49.5] IU/l (n=16)
Orchidopexy (>13years)#	
Unilateral	30.2% (n=16/53)
Bilateral	18.9% (n=10/53)
Hypospadias repair procedure (>13 years)#	
Once	30.2% (n=16/53)
Twice	15.1% (n=8/53)
More than twice	28.3% (n=15/53)
Sex reassignment (Male to Female)*	5.3% (n=5/94)
Age of sex reassignment	Infancy (0-1yr) n=2 Childhood (1-9yrs) n=1 Adolescent (10-19yrs) n=2
Frequency of sex re assignment in geographical regions	Europe 7.9% (n=3/38) South America 3.7% (n=2/54) Asia 0% (n=0/11)

Foot notes: data presented as median [1st–3rd quartile], NS- not significant. The number of patients available for each analysis are less than the group totals due to missing data.

ψ The numbers include subjects who had an assessment at age >17 years or had attained G5 prior to this. Subjects without information on final Tanner stage were excluded from the denominator.

The denominator includes patients who had bilateral gonadectomy before age 13 years.

* Incudes prepubertal individuals

Table 5. Puberty development , surgical outcome and sex reassignment in CGD and PGD assigned female

Individuals with gonad in situ (>13yrs)	**CGD (n=68)**	**PGDf (n=32)**
Breast development	18.5% (n=12/65) B2 n=4, B3 n=1, B4 n=4, B5 n=1, unknown n=2	30.8% (n=8/26) B2 n=3, B3 n=3, B5 n=2 n=6/8 also had virilisation
Virilisation	0% (n=0/65)	42.3% (n=11/26) hirsutism n=5, clitoral enlargement n=7, "virilisation" n=1
Association with gonadal tumour	66.7% (n=8/12) of breast development associated with tumour (gonadoblastoma, seminoma, dysgerminoma, mixed germ cell tumour)	25% (n=2/8) of breast development associated with tumour 36.4% (n=4/11) of virilisation associated with tumour (Gonadoblastoma, dysgerminoma, seminoma)
Age of puberty induction (years)	16.0 [14.0–17.3] (n=66)	13.0 [11.0–17.0] (n=51)

Menarche achieved (at >15y)	72.3% (n=34/47)	30.0% (n=9/30)
Clitoral reduction surgery*	0% (n=0/82)	47.1% (n=33/70)
Vaginoplasty*	0% (n=0/81)	44.9% (n=31/69)
Sex re-assignment (Female to Male)*	0% (n=0/86)	16.1% (n=15/93)
Age of sex reassignment	N/A	Infancy (0-1yr) n=3 Childhood (1-9yrs) n=6 Adolescent (10-19yrs) n=4 Adult (20yrs-) n=1 Unknown n=1
Frequency of sex re assignment in geographical regions		Europe 18.2% (n=6/33) South America 12.7% (n=8/63) Asia 9.1% (n=1/11)

Foot notes: data presented as median [1st–3rd quartile], NS- not significant. The number of patients available for each analysis are less than the group totals due to missing data.

* Incudes prepubertal individuals

Table 6. Biopsy, Gonadectomy and Histology

	CGD (n=100)	PGDf (n=107)	PGDm (n=103)	
Biopsy				
Unilateral	4.1% (n=3/73)	0%	13.0% (n=12/92)	
Bilateral	21.9% (n=16/73)	21.7% (n=18/83)	30.4% (n=28/92)	
Unknown	0%	1.2% (n=1/83)	0%	
Median age [1st–3rd quartile]	11.0 [4.2–15.4] (n=17)	1.8 [0.5–3.7] (n=16)	1.8 [1.0–4.5] (n=39)	
Gonadectomy				
Bilateral	93.6% (n=88/94)	75.0% (n=75/100)	23.5% (n=23/98)	
Unilateral	0%	0%	19.4% (n=19/98)	
No	6.4% (n=6/94)	25.0% (n=25/100)	57.1% (n=56/98)	
Age of gonadectomy (years)			Bilateral gonadectomy	Unilateral gonadectomy
Median [1st–3rd quartile]	16.0 [13.1–18.3] (n=82)	6.5 [1.6–15.1] (n=68)	4.0 [3.0–14.6] (n=23)	2.7 [0.87–4.8] (n=19)
Range	0.42 - 45	0.08 - 43	1.5 - 34	0.08 - 14
Infancy (0-1yr)	1.3% (n=1/79)	18.2% (n=12/66)	4.8% (n=1/21)	28.6% (n=4/14)
Childhood (1-9yrs)	22.8% (n= 18/79)	51.5% (n= 34/66)	57.1% (n= 12/21)	64.3% (n=9/14)
Adolescent (10-19yrs)	63.3% (n=50/79)	25.8% (n=17/66)	33.3% (n=7/21)	7.1% (n=1/14)
Adult (20yrs-)	16.5% (n=13/79)	7.6% (n=5/66)	4.8% (n=1/21)	0% (n=0/14)
Gonadectomy findings: clinicians' multiple-choice question (per gonad)				
Streak gonad	75.7% (n=87/115)	19.0% (n=24/126)	28.6% (n=16/56)	
Dysgenetic testis	7.0% (n=8/115)	51.6% (n=65/126)	35.7% (n=20/56)	
Normal/Almost normal testis	0%	18.3% (n=23/126)	1.8% (n=1/56)	
Replaced by tumour	9.6% (n=11/115)	2.4% (n=3/126)	1.8% (n=1/56)	
Ovary	2.6% (n=3/115)	0%	0	
Ovotestis	0%	0%	1.8% (n=1/56)	
Gonadal regression	5.2% (n=6/115)	8.7% (n=11/126)	30.4% (n=17/56)	
Histology report overview	n=9 gonads from 5 patients	n=17 gonads from 11 patients	n=8 gonads from 5 patients	
Streak +/- UGT	55.6% (n=5/9)	29.4% (n=5/17)	12.5% (n=1/8)	
Dysgenetic	11.1% (n=1/9)	35.3% (n=6/17)	62.5% (n=5/8)	
Testis	0%	11.8% (n=2/17)	0%	
Absent gonad	0%	0%	12.5% (n=1/8)	
Not described	33.3% (n=3/9)	17.6% (n=3/17)	12.5% (n=1/8)	
Whole slide overall features (per gonad)	n=10 gonads from 5 patients	n=7 gonads from 5 patients	n=11 gonads from 7 patients	
Streak gonad	30% (n=3/10 [n=1 +UGT])	14.3% (n=1/7)	9.1% (n=1/11)	
Dysgenetic testis	0%	85.7% (n=6/7 [n=2 +UGT])	63.6% (n=7/11)	
Gonadal regression	60% (n=6/10)	0%	0%	
UGT	10% (n=1)	0%	0%	
Normal testis	0%	0%	27.3% (n=3/11 [n=1 +UGT])	
Whole slide Testicular dysgenesis features	N/A	Reduced tubular diameter n=1, Irregular tubular shape n=2, Intracapsular growth n=3, SCO tubules n=2, Reduced GC number n=4, Increased interstitium n=2	Thickened BM n=4, SCO tubules n=4, LC hyperplasia n=2, Reduced GC number n=2, Intracapsular growth n=2, Interstitial fibrosis n=3, Increased intertubular space n=1	

Pre/Malignancy (per individual) Unilateral Bilateral	18.1% (n=15/83) 15.7% (n=13/83)	4.5% (n=3/66) 15.2% (n=10/66)	5.9% (n=2/34) 2.9% (n=1/34)
Type of malignancy/pre-malignancy (per gonad)	n=41 Gonadoblastoma 19 (46.3%) Germinoma + GB 1 (2.4%) Dysgerminoma (+- GB) 13 (31.7%) Mixed GCT 2 (4.9%) GCNIS 2 (4.9%) Yolk sac tumor 1 (2.4%) Seminoma 1 (2.4%) Unknown 2 (4.9%)	n=23 Gonadoblastoma 12 (52.2%) Dysgerminoma/seminoma 5 (21.7%) Sex cord stromal tumour 3 (13.0%) GCNIS 5 (21.7%)	n=3 Mixed GCT 1 (33%) Gonadoblastoma 1 (33%) GCNIS 1 (33%)
Age of malignancy/pre-malignancy diagnosis # Median age [Range] years	Seminoma 16.8, 15.3 yrs (n=2) Dysgerminoma 15.0[9.6–21.2] (n=9) Germinoma + GB 18.9 yrs (n=1) Mixed GCT 7.8, 16.5 yrs (n=2) Yolk sac tumor 13.5 yrs (n=1) Gonadoblastoma 16.5[0.4–24.8] (n=11) GCNIS 3.8 yrs (n=1)	Seminoma 18.3 yrs (n=1) Dysgerminoma 20 yrs, unknown (n=2) Sex cord stromal tumor 6.4, 9.8 yrs (n=2) Gonadoblastoma 2.5[0.1–17] (n=6) GCNIS 3.8 yrs (n=2)	Mixed GCT 19 yrs (n=1) Gonadoblastoma 2.5 yrs (n=1) GCNIS 8.16 yrs (n=1)

Foot notes: data presented as median [1st–3rd quartile], NS- not significant. The number of patients available for each analysis are less than the group totals due to missing data.

*UGT- undifferentiated gonadal tissue, SCO- Sertoli cell only, GC- germ cell, LC- Leydig cell, GCT- germ cell tumour, GB- gonadoblastoma, GCNIS- germ cell neoplasia in situ

Individuals with two different tumor were counted for more severe form of tumor.

Supplementary data Table 1. List of Comorbidities

	CGD (n=100)	PGDf (n=107)	PGDm (n=103)	p value
Frequency of comorbidities	38.0% (n=35/92)	33.0% (n=32/97)	33.3% (n=31/93)	CGD vs PGDf ns CGD vs PGDm ns PGDf vs PGDm ns
Comorbidities	**Blood & lymphatic 5.3% (n=2/38);** lymphoproliferative disease n=1, Burkitt lymphoma n=1) **CNS 28.9% (n=11/38);** developmental delay n=5, abnormal brain structure n=3, epilepsy n=2, ADHD n=3, learning difficulties n=1, sensorineural deafness n=2, autism n=1 **Craniofacial 2.6% (n=1/38);** Culler-Jones syndrome n=1 **Ear/Nose/Throat 5.3% (n=2/38);** laryngeal cleft n=1, hearing loss n=1 **Heart 7.9% (n=3/38);** ASD n=1, aortic valve stenosis n=1, TAPVR n=1 **Renal 23.7% (n=9/38);** reflux nephropathy, renal transplant n=1, FSGN n=1, renal HTN n=1, proteinuria n=3, Wilms Tumour n=1, double ureter n=1, single kidney n=1 **Short stature 2.6% (n=1/38)** **Skeletal 13.2% (n=5/38);** scoliosis, osteopenia n=4 **Skin 2.6% (n=1/38);** haemangioma n=1 **Other 7.9% (n=3/38);** dysmorphic features n=2, hypothyroidism n=1	**Adrenal 2.1% (n=1/47);** adrenal insufficiency n=1 **Blood & lymphatic 6.4% (n=3/47);** spleen abnormalities n=3 **CNS 21.3% (n= 10/47);** developmental delay n=5, learning difficulty n=2, psychiatric, ADHD, structural anomaly n=2 **Eyes 8.5% (n=4/47);** nystagmus n=1, microphthalmus n=1, vision problem n=1 **GI tract 6.4% (n=3/47);** malrotation n=2, cloacal extrophy n=1 **Heart 6.4% (n=3/47);** hypertension n=2, TGA n=1, anomalous pulmonary vein confluence n=1, situs inversus n=1, CoA n=1 **Renal 12.8% (n=6/47);** Wilms Tumour, proteinuria n=2, hypoplasia n=1, hydronephrosis n=1, single kidney n=1 **SGA 4.2% (n=2/47)** **Short stature 2.1% (n=1/47)** **Skeletal 10.6% (n=5/47);** osteopenia n=3, overgrowth n=1, coxa valgis n=1 **Skin 2.1% (n=1/47);** vitiligo n=1 **Other 17% (n=8/47);** dysmorphic features n=4, hypothyroidism n=1, situs inversus n=1	**Blood & lymphatic 7.9% (n=3/38);** partial spleen deficiency n=1, Congenital Dyserythropoietic Anaemia n=1, lymphocytepaenia n=1 **CNS 26.3% (n=10/38);** seizures n=1, developmental delay n=6, abnormal brain structure n=1, learning impairment n=2, ADHD n=1 **Ear/Nose/Throat 2.6% (n=1/38);** deafness **Eyes 5.3% (n=2/38);** blind n=1, vertical strabismus n=1 **Renal 31.6% (n=12/38);** horseshoe kidney n=1, Wilms Tumour n=6, cystic diverticle n=1, duplex ureter n=1, acute kidney failure n=1, non-nephrotic proteinuria n=2, renal cyst n=2 **Short stature 2.6% (n=1/38)** **Skeletal 7.9% (n=3/38);** club foot n=2, long and slightly curved toes n=1 **Skin 2.6% (n=1/38);** Albinism n=1 **Unidentified syndrome 2.6% (n=1/38)** **Other 10.5% (n=4/38);** precocious puberty, dysmorphic features n=3	

Foot notes: The number of comorbidities higher than the number of individuals as some have multiple comorbidities.

Supplementary data Table 2. Genetic analysis

	CGD (n=100)	PGDf (n=107)	PGDm (n=103)
Screening methods*			
Targeted gene screening	n=66 (66%)	n=52 (48.6%)	n=56 (54.4%)
DSD panel	n=10 (10%)	n=12 (11.2%)	n=11 (10.7%)
WES	n=12 (12%)	n=19 (17.8%)	n=8 (7.8%)
CGH array	n=3 (3%)	n=4 (3.7%)	n=6 (5.8%)
No gene screening	n=13 (13%)	n=26 (24.3%)	n=27 (26.2%)
Genetic cause			
Screened	n=89	n=83	n=78
Gene alterations	41 (46.1%)	46 (55.4%)	35 (44.9%)
Chromosome alterations	2 (2.2%)	3 (3.6%)	4 (5.1%)
VUS or no genetic defect	46 (51.7%)	34 (41.0%)	39 (50.0%)
Genes affected	*SRY* 23.6% (n=21/89) *WT1* 9.0% (n=8/89) *NR5A1* 3.4% (n=3/89) *MAP3K1* 3.4% (n=3/89) Chromosome 2.2% (n=2/89) *SOX9* 2.2% (n=2/89) *PPP2R3C* 1.1% (n=1/89), *DHX37* 1.1% (n=1/89)	*NR5A1* 42.2% (n=35/83) *MAP3K1* 4.8% (n=4/83) *DHX37* 3.6% (n=3/83) Chromosome 3.6% (n=3/83) *MAMLD1*, *DHH*, *MYRF*, *WT1* 1.2% (n=1/83) each Combination 4.8% (n=4/83) (*FGFR2*+*MAP3K1* n=2, *DHX37*+*GATA4*, *NR5A1*+*WWOX*)	*NR5A1* 25.6% (n=20/78) *WT1* 5.1% (n=4/78) *DHX37* 5.1% (n=4/78) *SRY* 5.1% (n=4/78) Chromosome 5.1% (n=4/78) *DAX1*, *ZFPM2*, *GATA4* 1.3% (n=1/78) each

Foot notes: The number of patients available for each analysis are less than the group totals due to missing data.

*****Some patients had multiple screenings, WES: whole exome sequence. VUS: variants of unknown or uncertain significance.

Supplementary data Table 3. PGD assigned male; spontaneous puberty vs induced puberty

	Spontaneous (n=36)	Non-spontaneous (n=9)	p value
EMS at first presentation	5.5 [4.5–8.5] (n=29)	8.0 [4.0–9.0] (n=5)	ns
EGS at first presentation	7.5 [6.0–9.5] (n=29)	7.0 [6.0–7.5] (n=5)	ns
Individuals with one or more gonad at labioscrotal position	62.9% (n=22/35)	12.5% (n=1/8)	p=0.017
Basal testosterone levels at the first presentation at any age (nmol/l)	0.79 [0.35–4.1] (n=33)	2.4 [0.16–15.3] (n=9)	0.094
Testosterone >twice basal value after hCG at the first investigation	88.2% (n=15/17)	33% (n=1/3)	0.087
Testosterone >twice basal value after hCG and >3.5nmol/l	41.2% (n=7/17)	33% (n=1/3)	ns
Basal testosterone (nmol/l) at puberty#	6.52 [3.23–9.82] (n=23)	0.069 [0.043–0.14] (n=3)	<0.00001
LH (IU/L) levels at puberty#	3 [1.22–7.06] (n=26)	0.9 [0.75–4.60] (n=3)	ns
FSH (IU/L) levels at puberty#	11.42 [5.83–26.7] (n=26)	20.68 [11.04–23.34] (n=3)	ns

Foot notes: data presented as median [1st–3rd quartile], NS- not significant. The number of patients available for each analysis are less than the group totals due to missing data.

Age of test: spontaneous group 12.5 [11.7–13.4] years (n=26) and non-spontaneous group 12.0 [10.3–14.0] years (n=3)

Figure 1

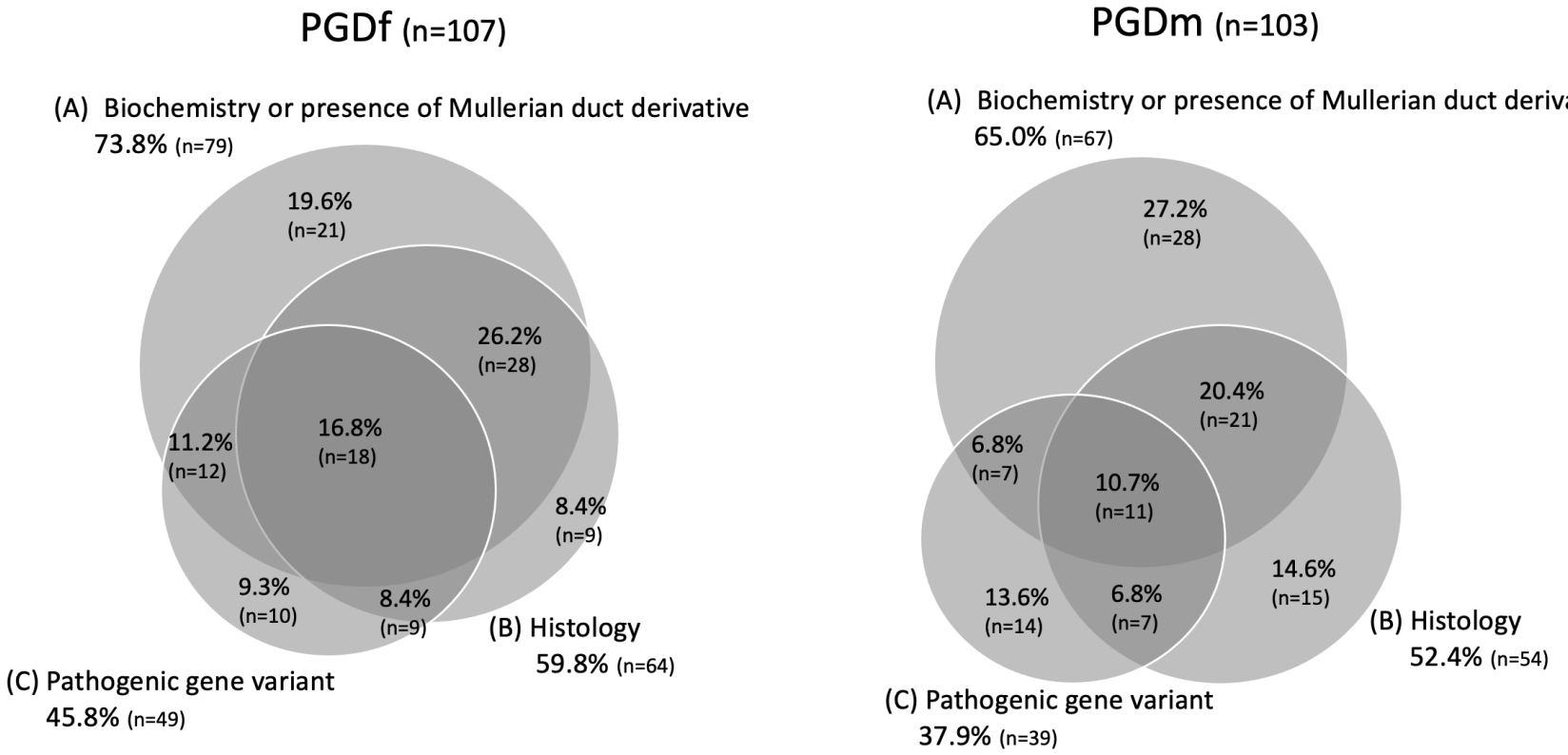

PGDf (n=107)
(A) Biochemistry or presence of Mullerian duct derivative
73.8% (n=79)
19.6%
(n=21)
26.2%
(n=28)
16.8%
(n=18)
11.2%
(n=12)
8.4%
(n=9)
9.3%
(n=10)
8.4%
(n=9)
(B) Histology
59.8% (n=64)
(C) Pathogenic gene variant
45.8% (n=49)
PGDm (n=103)
(A) Biochemistry or presence of Mullerian duct derivative
65.0% (n=67)
27.2%
(n=28)
20.4%
(n=21)
6.8%
(n=7)
10.7%
(n=11)
14.6%
(n=15)
13.6%
(n=14)
6.8%
(n=7)
(B) Histology
52.4% (n=54)
(C) Pathogenic gene variant
37.9% (n=39)

Figure 2

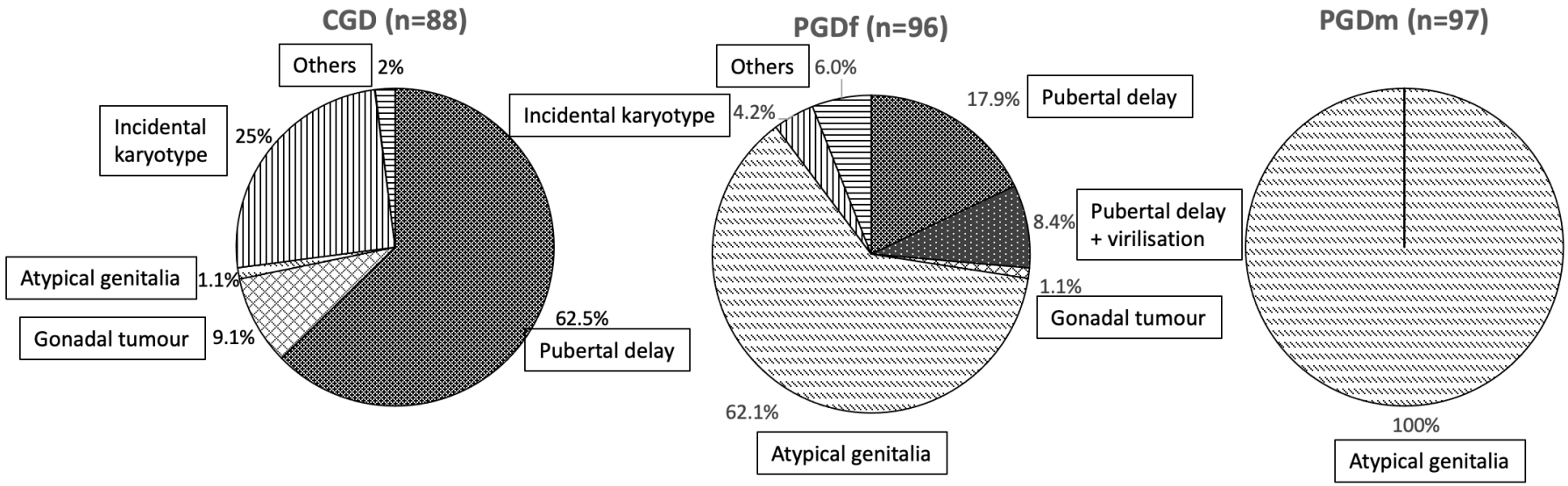

CGD (n=88)
Others
2%
Incidental karyotype
25%
Atypical genitalia
1.1%
Gonadal tumour
9.1%
62.5%
Pubertal delay
PGDf (n=96)
Others
6.0%
Incidental karyotype
4.2%
17.9%
Pubertal delay
8.4%
Pubertal delay + virilisation
1.1%
Gonadal tumour
62.1%
Atypical genitalia
PGDm (n=97)
100%
Atypical genitalia

Figure 3

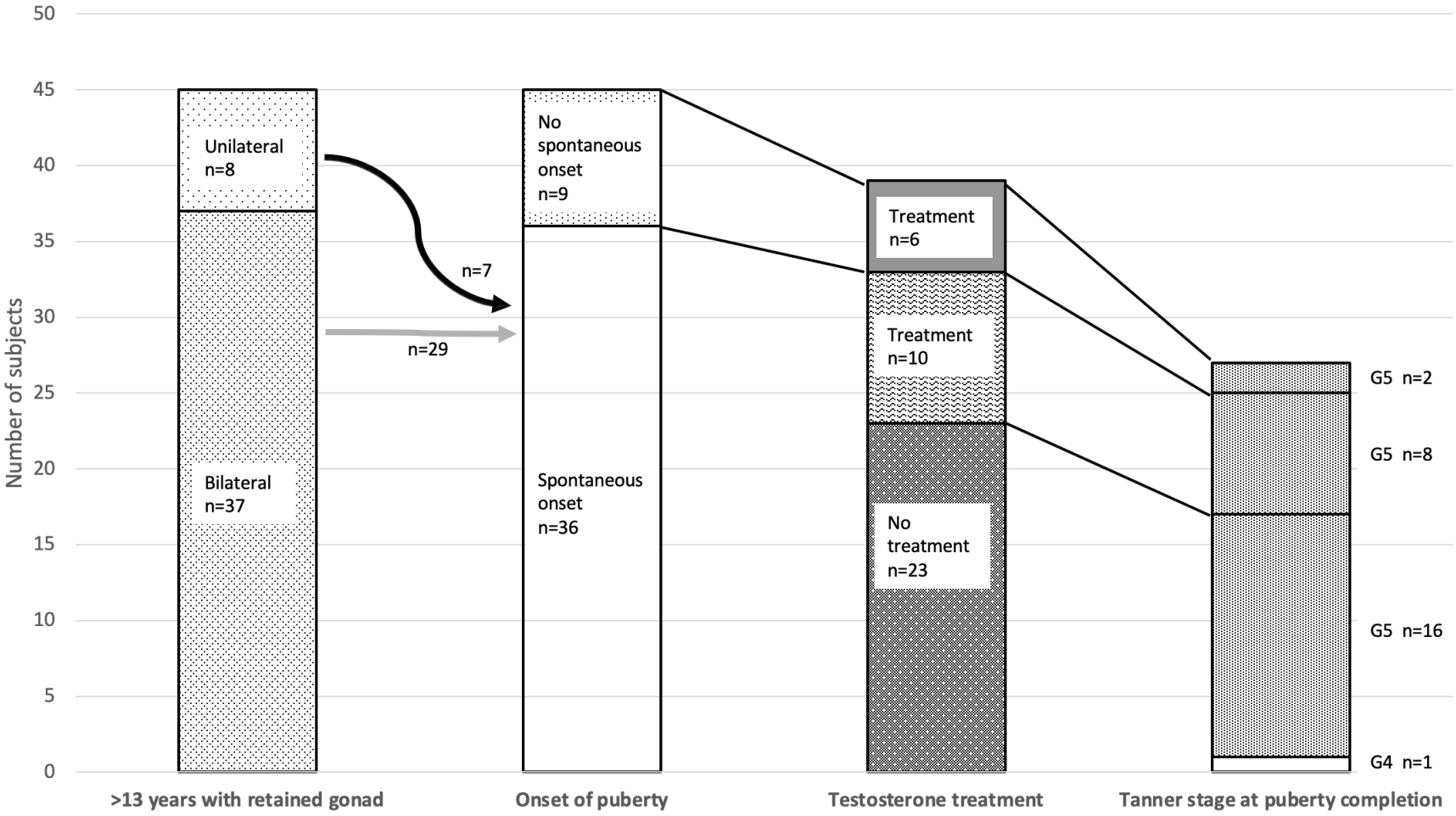
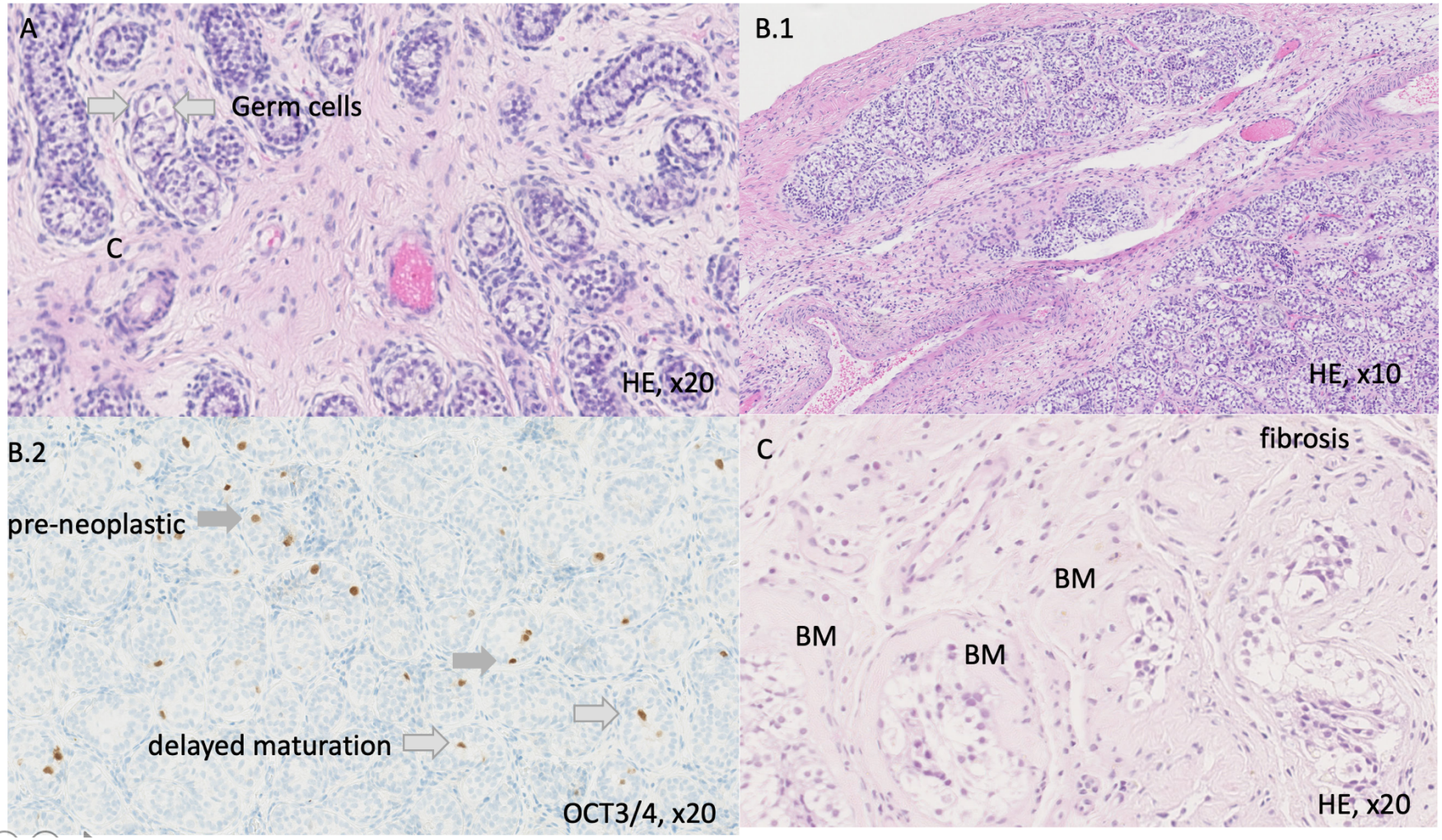

50
45
40
35
30
25
20
15
10
5
0
Number of subjects
Unilateral
n=8
Bilateral
n=37
n=7
n=29
No
spontaneous
onset
n=9
Spontaneous
onset
n=36
Treatment
n=6
Treatment
n=10
No
treatment
n=23
G5 n=2
G5 n=8
G5 n=16
G4 n=1
>13 years with retained gonad
Onset of puberty
Testosterone treatment
Tanner stage at puberty completion

Figure 4


A
Germ cells
C
HE, x20
B.1
HE, x10
B.2
pre-neoplastic
delayed maturation
OCT3/4, x20
C
fibrosis
BM
BM
BM
HE, x20