DR NATHALIA LISBOA GOMES (Orcid ID : 0000-0002-1072-7903)

Article type : 4 Original Article - Americas

Long-Term Outcomes and Molecular Analysis of a Large Cohort of Patients with 46,XY Disorder of Sex Development due to Partial Gonadal Dysgenesis

Nathalia Lisboa Gomes[1], Antônio Marcondes Lerário[2], Aline Machado Zamboni[1], Daniela Rodrigues de Moraes[1], Thatiana Evilen da Silva[1], Ivo Jorge Prado Arnhold[1], Rafael Loch Batista[1], José Antônio Diniz Faria Júnior[1], Elaine F. Costa[1], Mirian Y. Nishi[1], Marlene Inacio[1], Sorahia Domenice[1], Berenice B. Mendonca[1]

[1]Unidade de Endocrinologia do Desenvolvimento, Laboratório de Hormônios e Genética Molecular/LIM42, Hospital das Clínicas, Disciplina de Endocrinologia, Faculdade de Medicina da Universidade de São Paulo, São Paulo, Brazil;
[2]Department of Internal Medicine, Division of Metabolism, Endocrinology and Diabetes, University of Michigan, Ann Arbor, MI 48109, USA

Running title: Follow-up of patients with 46,XY DSD due to PGD

Disclosure: **The authors report no conflicts of interest in this work.**

Corresponding author: Berenice B. Mendonca, M.D.,

Divisão de Endocrinologia, Hospital das Clínicas, FMUSP
Rua Dr Eneas de Carvalho 255
01060-970 São Paulo, Brazil.
Phone: +55 11-26617564
Email: beremen@usp.br; nathalialisboa.endocrino@gmail.com

Abstract

Background: Follow-up data on patients with 46,XY partial gonadal dysgenesis (PGD) until adulthood are scarce, making information on prognosis difficult.

Objective: To analyse the long-term outcomes of patients with 46,XY PGD regarding testosterone production, germ cell tumour risk, genotype, and psychosexual adaptation.

Methods: A retrospective longitudinal study of 33 patients (20 assigned male and 13 patients assigned female at birth). Molecular diagnosis was performed by Sanger sequencing or by targeted massively parallel sequencing of 63 genes related to disorders of sex development (DSDs).

Results: Age at first and last visit ranged from 0.1 to 43 and from 17 to 53 years, respectively. Spontaneous puberty was observed in 57% of the patients. During follow-up, six of them had a gonadectomy (four due to female gender, and two because of a gonadal tumour). At last evaluation, five of six patients had adult male testosterone levels (median 16.7 nmol/L, range 15.3–21.7 nmol/L) and elevated LH and FSH levels. Germ cell tumours were found in two postpubertal patients (one with an abdominal gonad and one patient with Frasier syndrome). Molecular diagnosis was possible in 11 patients (33%). *NR5A1* variants were the most prevalent molecular defects (n = 6), and four of five patients harbouring them developed spontaneous puberty. Gender change was observed in four patients, two from each sex assignment group; all patients reported satisfaction with their gender at final evaluation. Sexual intercourse was reported by 81% of both gender and 82% of them reported satisfaction with their sexual lives.

Conclusion: Spontaneous puberty was observed in 57% of the patients with 46,XY PGD, being *NR5A1* defects the most prevalent ones among all the patients and in those with spontaneous puberty. Gender change due to gender dysphoria was reported by 12% of the patients. All the patients reported satisfaction with their final gender, and most of them with their sexual life.

Key-words: Disorder of sex development, gonadal dysgenesis, atypical genitalia, puberty

1. Introduction

The 46,XY disorder of sex development (DSD) due to gonadal dysgenesis is a congenital disorder caused by alterations in the complex process of gonadal determination[1]. There is a wide phenotype spectrum ranging from a partial form, characterised by variable degrees of external genitalia undervirilisation, development of Mullerian derivatives, and testosterone production to a complete form with female external and internal genitalia.

There are scarce data on long-term follow-up of 46,XY partial gonadal dysgenesis (PGD) patients, regarding spontaneous puberty[2], risk of a gonadal tumour development[3] and gender adjustment, making it difficult to provide comprehensive information to parents.

Our aim was to describe the phenotype, genotype, and long-term outcomes of a large cohort of patients with 46,XY PGD followed until adulthood.

2. Subjects and Methods

Thirty-three patients with 46,XY PGD were included in this retrospective longitudinal study conducted at Hospital das Clínicas of São Paulo (HCFMUSP). Twenty-six patients were initially evaluated at our service, and 7 had already had a previous genitoplasty and/or gonadectomy elsewhere. Written informed consent was obtained from all the patients. The clinical and molecular data from 8 patients were previously reported[4-7]. Inclusion criteria were as follows: ≥17 years of age at last evaluation, a 46,XY karyotype in a G-banded karyotyping analysis of at least 30 peripheral blood lymphocytes, atypical genitalia associated with the presence of Mullerian derivatives, and/or at least one gonad with histopathological features compatible with testicular dysgenesis. Data regarding sex assignment, age at first and last evaluation, external genitalia appearance, the hormonal profile throughout the follow-up and at last visit, pubertal development, and gonadal tumour incidence were collected from medical records. Patients were assumed to be at prepubertal age if they were younger than 9 years, at pubertal age if they were 9.1–16 years old, and at adult age if they were older than 17 years. Micropenis is defined as a normally

structured penis which in its fully stretched length is less than 2.5 standard deviations (SDs) below the mean for age[8] and microphallus is defined as a micropenis associated with hypospadias.

The external masculinisation score (EMS) was calculated as previously described[9]. To determine the hormonal profile, luteinising hormone (LH), follicle-stimulating hormone (FSH), and testosterone were measured by immunoradiometric or immunofluorimetric assays at the first and at each semi-annual follow-up visit. Spontaneous puberty was assumed if virilisation was observed in pubertal patients or was reported by the patients that came in adulthood in conjunction with the presence of pubertal signs (presence of secondary sex characteristics, such as increased penile length and testis diameter >2.5 cm, when they were palpable) and with male serum testosterone levels without the use of exogenous testosterone.

For prepubertal patients, a human chorionic gonadotropin (hCG) test was performed by means of 4 intramuscular injections at 50 to 100 IU/kg each, with a 4-day interval between the injections. A single dose of 5000 IU of hCG was administered to adult patients. Serum levels of testosterone were measured before and 72 hours after the last hCG injection.

Data on a gonadal histology were collected from medical records.

Physical evaluation of the palpable scrotal testes of male patients was performed at every medical visit (each semester or annually), and testicular ultrasonography was performed once every 2 years. If a suspicious nodule was identified, tumour markers (β-HCG, α-fetoprotein, and carcinoma embryonic antigen) were tested, and a gonadectomy was indicated if needed.

Continuous variables were described as median and range. Differences in the categorical variables among the groups were analysed by the Chi-square test or Fisher's exact test, when appropriate. The Mann–Whitney *U* test served as a nonparametric test, and data with $p < 0.05$ were considered statistically significant. All analyses were performed in SPSS Statistics 24.0 software (Chicago, IL).

Evaluation of psychosexual characteristics was performed on 21 patients by a psychologist specializing in DSDs. Self-reported gender identity, the self-reported gender

role in childhood, the desire to change gender, and satisfaction with gender and with their sexual life were analysed via a questionnaire (see Appendix 1).

For molecular diagnosis, genomic DNA was obtained from peripheral blood leukocytes by the proteinase K–SDS salting-out method[10]. Six genes involved in testicular dysgenesis (*SRY, NR5A1, CBX2, MAPK3, FGF9,* and *FGFR2)* were previously sequenced by the Sanger method in patients 1, 3, 10, 14, 26, and 30. Patients 15 and 31 had only *WT1* variants screened, considering their phenotypic features, as previously reported in great detail[5,6]. The entire coding region and the exon–intron boundary areas of each gene were PCR-amplified with specific primers. The PCR products were sequenced according to the protocol of the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Life Technologies Corporation, CA, USA) on an ABI Prism Genetic Analyzer 3130XL (Life Technologies Corporation, CA, USA).

Twenty-seven patients were analysed during the 2010s by targeted massively parallel sequencing. An amplicon-based capture panel was designed against exonic regions of 63 genes, including 43 genes already associated with human DSDs and 20 candidate genes involved in gonadal determination pathways or with a DSD phenotype in rats[1,11-18] (see Appendix 2). Capture of the target sequences was performed using a custom Sure Select Target Enrichment System Kit (Agilent). Sequencing was performed on the Illumina MiSeq platform. Paired-end reads (2 × 300) were aligned to the hg19 assembly of the human genome with BWA-MEM[19]. The aligned reads were sorted and converted to the BAM format using the bamsort tool from the biobambam2 suite (https://launchpad.net/biobambam2). Mean coverage was over 95× for all the samples, and more than 96% of the RefSeq gene coding regions was covered at 20× or greater. Single-nucleotide variants and small insertions or deletions (indels) were simultaneously called in all samples in the Freebayes software (https://github.com/ekg/freebayes). Annotation of the variants was performed in ANNOVAR[20]. For prioritizing the most likely pathogenic variants, we filtered out those with a minor allele frequency >0.5% in available population databases Genome Aggregation Database (gnomAD)[21], 1000 Genomes[22], and in the Brazilian population database ABraOM[23]. To assess the possible impact of the novel nonsynonymous variants on protein structure and function, we employed *in silico* algorithms (SIFT, PolyPhen2, Mutation Assessor, and CADD) and conservation scores

(GERP++, PhyloP). These variants were considered deleterious when predicted as pathogenic by at least three algorithms. The variants were classified according to the American College of Medical Genetics and Genomics guidelines (ACMG)[24].

3. Results

The patients' age at first visit ranged from 10 days to 43 years (median 13 years), and at last visit, from 17 to 53 years (median 26.5 years). Follow-up ranged from 3 to 26 years (median 13.2 years). Nineteen patients had Mullerian derivatives (57%). Histological analysis identified testicular dysgenesis in 18 patients (67%) and absence of gonadal tissue in 9 (33%).

Sex assignment and the EMS

Thirteen patients were assigned female and 20 were assigned male at birth. Twenty-six patients were assigned before the year 1990 (16 patients to male and 10 patients to female), and seven patients were assigned between 1990 and 1999 (4 patients to male and 3 patients to female). The median EMS at first evaluation in patients without a previous genitoplasty was 3.5 (1.0 to 5.5) for the patients assigned female and 6.0 (3.0 to 7.5) for the patients assigned male at birth (p = 0.002). This difference in the EMS between the two sexes was observed in the patients assigned before and after the year 1990.

Four patients changed their gender in adulthood, two from male to female and two from female to male. These four patients visited our hospital at an adult age.

The patients were grouped on the basis of their gender and not on their sex assignment.

Female gender group (n = 13; Table 1)

Two patients came at prepubertal age (patients 1 and 2), four at pubertal (patients 3–6), and seven after pubertal age (patients 7–13). Three patients had already had a gonadectomy and genitoplasty (patients 6, 7, and 9). Amongst the 10 patients without previous genital surgery, external genitalia ranged from normal female (patient 12), female with clitoromegaly (patients 1–5, 8, and 11) to micropenis (patient 11). Six patients had two perineal openings (patients 1, 3, 4, 5, 6, and 13), and five patients had a single perineal opening (patients 2, 8, 10, 11, and 12). All the patients had bilateral cryptorchidism. These patients had elevated serum gonadotropin levels with predominance of FSH levels (range from 38 to 77 IU/L) over LH levels (range from 5 to 32 IU/L).

All female patients had undergone feminizing genitoplasty and bilateral gonadectomy. Oestrogen replacement was started at a median age of 14 (10 to 31 years) with normal breast development.

Male gender group (n = 20; Tables 2 and 3)

Twelve patients were evaluated at prepubertal age (patients 14–18, 24–30), three at pubertal age (patients 19, 20 and 31), and five after puberty (patients 21–23, 32 and 33). At first evaluation, three patients had a previous genitoplasty (patients 20, 21, and 29), two had undergone bilateral gonadectomy (patients 20, 21), and one unilateral gonadectomy (patient 29).

Regarding the 17 remaining patients, 12 had microphallus and proximal hypospadias (71%), 14 had bilateral cryptorchidism (82%), had unilateral cryptorchidism, and one patient had both testes lying inside the scrotum.

The patients with low basal and/or hCG-stimulated testosterone levels had undergone bilateral gonadectomy. All of them received testosterone replacement at a median age of 15 (11.7 to 48 years). Their median phallus size at first visit was –3.4 SD (–6.1 to –1.7 SD), and in adulthood, after testosterone replacement, the phallus size reached a median of 9 cm (range 6.7 to 12 cm), corresponding to –2.7 SD (range –4.1 to –0.9 SD).

Patients with preserved testosterone secretion with one atrophic cryptorchid testis had had unilateral gonadectomy. The median phallus size of these patients at first visit was –3.3 SD (range –4.3 to –0.3 SD). In adulthood, phallus size reached a median of 8 cm (6.5 to 9.2 cm), corresponding to –3.3 SD (–4.3 to –2.6 SD).

There was no statistically significant difference in phallus size SDs between the patients with preserved testosterone secretion and those who received testosterone replacement and also at their first and last evaluation.

Testosterone production in patients with 46,XY PGD

At first evaluation, 28 patients who did not undergo bilateral gonadectomy in childhood were evaluated regarding testosterone production (Figure 1). Fourteen of them were at prepubertal age (patients 1, 2, 14–18, and 24–30). Eight of them had normal hCG-stimulated testosterone levels (patients 1, 2, and 25–30), median of 13.8 nmol/L (6.2 to 22.1 nmol/L), and one patient at minipuberty had normal basal testosterone levels (patient 24). The other five individuals (patients 14–18) showed very low hCG-stimulated

testosterone levels (undetectable to 2.9 nmol/L). All the male patients with impaired testosterone secretion and two female patients (patients 1 and 2) had had bilateral gonadectomy.

Among the 21 patients without bilateral gonadectomy, 12 went through spontaneous puberty (patients 3, 4, 5, 13, 25–29, and 31–33), including four female patients (patients 3, 4, 5, and 13; Figure 1). These female patients and the two male patients who developed a gonadal tumour (patients 31 and 32) underwent bilateral gonadectomy. Patient 29 progressively lost testosterone secretion and started testosterone replacement at the age of 34 (Figure 1).

At last evaluation, five male patients maintained normal male adult testosterone levels (median 16.7 nmol/L; Table 4). Four patients had high LH (median 11 IU/L) and FSH levels (median 24 IU/L), and one subject had normal gonadotropin levels (patient 25) at 17 years of age.

Altogether, twelve patients (57%) had gone through spontaneous puberty. At last evaluation, five of eight patients with male social sex who had gone through spontaneous puberty still had normal testosterone production.

Psychosexual follow-up according to gender

Ten patients assigned female and 11 patients assigned male at birth were evaluated. Gender dysphoria and gender change were observed in four patients, two from each gender group. None of the patients reported non-binary or gender-fluid feelings.

Patients 8 and 11 were assigned male at birth. The former had atypical genitalia, and the latter had severe micropenis and bilateral cryptorchidism. They clearly displayed female behaviour, preferring female activities and clothes since childhood. They received proper medical and psychological assistance at the ages of 19 and 30, respectively. At the time, their hormonal profile showed hypergonadotropic hypogonadism without pubertal signs. Their psychological analysis revealed female gender identity and gender dysphoria. They changed their gender to female, had feminizing genital surgery, and were treated with conjugated oestrogens.

Patients 21 and 33 had atypical genitalia and were first assigned female at birth. They had manifested male behaviour since childhood, preferring boys' hobbies and clothes.

Patient 21 had a feminizing genitoplasty and gonadectomy at 1.6 years of age elsewhere and had no psychological evaluation and follow-up. At age 19, he changed his gender to male, and testosterone replacement was started. At 27 years of age, he came to our institution looking for neophallus surgery.

Patient 33 never had medical assistance. He had had male gender identity since he was 9 years old. Virilisation due to spontaneous puberty was noticed when he was 15 years of age. At the time, he changed his gender to male. He was first seen at our service at age 26, when masculinizing genitoplasty was performed.

At final evaluation, all the 21 patients had gender identity concordant with their self-reported gender role in childhood and were satisfied with their gender. Four females (40%) and eight males (73%) had a steady partner. Penetrative sexual intercourse was reported by eight females (80%) and by nine males (81%), among whom six females (75%) and eight males (81%) reported satisfaction with sexual life and orgasm.

None of the patients from both genders have offspring or adopted children.

Testosterone production and gender

There was no relation between postnatal testosterone levels and gender considering the highest serum testosterone level at baseline or after the hCG stimulation test observed at the follow-up ($p = 0.9$).

Incidence of gonadal tumours

During follow-up, patients underwent bilateral gonadectomy due to female gender, for impaired testosterone secretion, for an atrophic cryptorchid testis, or because of a gonadal tumour. Thirteen patients had bilateral or unilateral gonadectomy at prepubertal age at a median age of 4 years (1.2 to 8.8), and no evidence of germ cell neoplasia was found. Fifteen patients had a gonadectomy at pubertal age or in adulthood at a median age of 21 (9.9 to 47.9), and a testicular tumour was found in two subjects (patients 31 and 32).

Patient 31 had bilateral gonadoblastoma at ages 18 and 20 and an *in situ* germ cell neoplasia in the right testis, despite the scrotal position of both testes, due to a *WT1* (Wilms' tumour 1) mutation, as previously reported[6]. Patient 32 had a mixed germ cell tumour (80% embryonal carcinoma, 15% yolk sac tumour, 5% choriocarcinoma) with a gonadoblastoma in the left abdominal gonad at 23 years of age associated with very high

levels of hCG (536 IU/L; reference level <3 IU/L). He underwent bilateral gonadectomy and chemotherapy with a good response.

Molecular diagnosis

Pathogenic or likely pathogenic variants were found in nine sporadic cases and in two familial cases, eight identified by Sanger sequencing and three by targeted massively parallel sequencing *(Table 5)*. All the identified variants are heterozygous and located in genes previously associated with gonadal dysgenesis phenotypes (*NR5A1*, *SRY*, *WT1*, *MAP3K1*, and *FGFR2)*. Nine variants had already been described[4-7,25], and two variants are novel (in *MAP3K1* and *FGFR2*, the familial cases). None of the variants was found in population databases, including the Brazilian ABraOM[23]. *NR5A1* defects were the most common, being responsible for 18% of the cases, and *in silico* and *in vivo* studies corroborated the deleteriousness of *NR5A1* variants, as previously reported by our group[4,7]. None of these patients had adrenal failure. Segregation analysis by Sanger sequencing was possible in eight out of ten families and confirmed segregation with the phenotype in five families and *de novo* status of the two *WT1* variants.

4. Discussion

The DSD due to 46,XY PGD is a rare disorder. It represents 19.6% in our cohort of 250 patients with a 46,XY DSD. The current study is the largest 46,XY PGD cohort showing clinical outcomes and molecular analysis.

Sex assignment is the most controversial issue of DSD management. In our cohort, most patients were assigned before the 1990s, and male sex assignment was significantly more frequent in patients with a higher EMS, in a ratio of 1.5 males to 1.0 female. The International Disorder of Sex Development (I-DSD) Registry reported an increase in the male-to-female sex assignment ratio in 46,XY dysgenetic DSD babies with time, starting from a ratio of 0.4 before the 1990s to a ratio of 1.5 in children born after 1999, regardless of the EMS [26].

Despite this increasing trend on male sex assignment, there are scarce data on pubertal development of those patients. In our cohort, spontaneous puberty was observed in 57% of the patients who did not undergo bilateral gonadectomy in childhood. In adulthood, all the male patients maintained testosterone secretion, except for the oldest, who showed a decrease in testosterone secretion at the age of 34. They all had high LH levels (>10 IU/L),

with the exception of the youngest, indicating partial Leydig cell dysfunction. Regarding reproductive function, high FSH levels (>20 IU/L) were found in most patients manifesting compromised spermatogenesis although the sperm count was not performed.

In one retrospective study on pubertal development of 46,XY PGD patients, 9 out of 10 patients had gone through spontaneous puberty with high FSH levels and progressive elevation of LH[2]. These patients had a mild-gonadal-dysgenesis phenotype as 60% had a penile urethra opening and all of them were assigned male at birth[2].

In humans, the process of gonadal determination is quite complex[1] and a molecular defect was identified in 20% and 40% of the 46,XY gonadal dysgenesis patients who were studied by Sanger[27] and target massively parallel sequencing, respectively. *NR5A1* and *MAP3K1* allelic variants were the most frequent molecular diagnosis[28].

In our study, likely pathogenic or pathogenic allelic variants were identified in 33% of the patients, in one of the following genes: *NR5A1*, *SRY*, *WT1*, *MAP3K1*, and *FGFR2*. *NR5A1* defects (n=6) were the most frequent in the whole cohort and also among the 12 patients who developed spontaneous puberty (n=4). Moreover, three of these patients were assigned female at birth owing to their severely undervirilised genitalia. This finding is in agreement with other reports[29-35]. In those cases, the severe undervirilisation of external genitalia could not predict virilisation in adulthood because testosterone secretion recovered during puberty for unknown reasons[29-36].

SRY defects have been mostly associated with complete gonadal dysgenesis and rarely with partial gonadal dysgenesis[25,37-39]. The *SRY* p.Arg30Ile pathogenic allelic variant was identified in one of our patients with spontaneous puberty. This same variant was also found in another Brazilian family, including affected members with various phenotypes, ranging from complete to partial gonadal dysgenesis[25]. *In vitro* studies proved the deleteriousness of the variant[25]. None of the reported patients with PGD due to *SRY* variants had preserved testosterone secretion[25,37-39].

Missense defects in Wilms' tumour suppressor gene 1 (*WT1)* cause Frasier and Denys–Drash syndromes[40,41]. One of our patients with normal-size testes and spontaneous puberty harboured the most common allelic variant in intron 9 of *WT1*, which is associated with Frasier syndrome[6]. This syndrome is generally characterised by bilateral gonadal dysgenesis, female external genitalia, renal failure in the second decade of life, and high

risk of testicular gonadoblastoma. Instead, our patient had a predominantly male phenotype, with normal penile length, and perineal hypospadias resembling the Denys–Drash phenotype. Even though there are five other cases of Frasier syndrome with a male phenotype[42-44], including one with a normal male phenotype[45], there are no reports of patients with spontaneous puberty.

The novel heterozygous variants *MAP3K1*p.Leu639Pro and *FGFR2* p.Ser453Leu were found simultaneously in two 46,XY sisters within our cohort, both inherited from their unaffected mother. Both had severe undervirilised genitalia at first evaluation, though normal male testosterone levels were reached after hCG stimulation in the 1.2-year-old child, and her 9.4-year-old sister had pubertal male testosterone levels.

MAP3K1 was first associated with 46,XY DSDs by Pearlman *et al*.[46]. Targeted massively parallel sequencing has revealed previously reported and novel *MAP3K1* mutations not only in patients with complete gonadal dysgenesis but also in patients with PGD[28,47,48].

FGFR2 variants most commonly cause craniosynostosis syndromes without any gonadal phenotype. Although there is one report of a heterozygous *FGFR2* p.Cys342Tyr variant that was associated with complete gonadal dysgenesis and no report of patients with PGD[49]. The *FGFR2* p.Ser453Leu allelic variant found in one of our families is located in the hotspot region for pathogenic variants responsible for craniosynostosis phenotypes; however, our patients and their mother did not have any skull problems.

The mechanism by which the gonadal FGF signal is transduced intracellularly remains unclear, but *FGFR2* and *MAP3K1* are members of the RAS/RAF/MEK/ERK signalling pathway, and these patients may have a digenic inheritance cause of gonadal dysgenesis.

The prevalence of germ cell tumours in PGD is variable. Reported rates range between 16% and 30%[3] and for Denys–Drash and Frasier syndromes is as high as 40–60%[50]. In our cohort, two patients (7%) had a germ cell tumour: one had an invasive seminoma, and the other had bilateral gonadoblastoma associated with *in situ* germ cell neoplasia. Both patients had additional factors for germ cell tumour development: one had an abdominal gonad, and the other had both testicles lying within the scrotum but carried a *WT1* mutation.

Pre- and postnatal androgen exposure seems to contribute to male gender identity in patients with the 46,XY DSD due to 5alpha-reductase type 2 and 17beta-hydroxysteroid dehydrogenase type 3 deficiencies. In those patients, despite the severe undervirilisation and female rearing, a high rate of gender change to male is observed, ranging from 50% to 63% and from 39% to 64%, respectively[51]. In 46,XY PGD, testosterone production is quite variable during foetal and adult life. In our cohort, postnatal testosterone levels were not related to the gender. In addition, among the 5 patients assigned female at birth who virilised at puberty, 3 harboured *NR5A1* variants, and gender change to male was observed in one patient, who reported gender dysphoria since childhood.

Gender dysphoria had rarely been observed in patients with PGD. To our knowledge, only one case of female-to-male gender change has been reported[34]. This patient harboured a *NR5A1* variant, was assigned female at birth, presented with virilisation at puberty and changed his gender at 18 years of age[34]. Nonetheless, no gender change was observed among another six PGD patients with *NR5A1* defects already described, who had gone through spontaneous virilisation[29-31,33,35,36]. Together with the already published cases of *NR5A1* defects, among the 10 patients assigned female at birth with virilisation at puberty, two patients changed their gender to male. This data does not suggests that testosterone production at puberty is a determinant of gender change, but it should play a role in 46,XY PGD patients' gender, although the small sample size does not allow us to make any conclusion.

The psychosexual evaluation in adulthood revealed that the patients were satisfied with their final social sex, and ~80% of the patients reported satisfaction with their sexual life.

5. Conclusion

The present study represents the largest 46,XY PGD cohort showing clinical outcomes and molecular analysis. Spontaneous puberty was observed in 57% of the patients with 46,XY PGD, being *NR5A1* defects the most prevalent among all patients and among those with spontaneous puberty. A germ cell tumour was detected only after puberty in 7% of the patients. Gender change due to gender dysphoria was reported by 12% of the patients. All the patients reported satisfaction with their gender and most of them with their sexual life.

6. Acknowledgments: This work was supported by grants from Conselho Nacional de Desenvolvimento Cientifico e Tecnológico – CNPq (301339/2008-9 to B.B.M.) and from Fundação de Amparo à Pesquisa do Estado de São Paulo (2013/02162-8 to B.B.M.).

6. References

1. Ono M, Harley VR. Disorders of sex development: new genes, new concepts. *Nat Rev Endocrinol.* 2013;9(2):79-91.
2. Gabriel Ribeiro de Andrade J, Marques-de-Faria AP, Fabbri HC, de Mello MP, Guerra-Júnior G, Maciel-Guerra AT. Long-term follow-up of patients with 46,XY partial gonadal dysgenesis reared as males. *Int J Endocrinol.* 2014;2014:480724.
3. Abacı A, Çatlı G, Berberoğlu M. Gonadal malignancy risk and prophylactic gonadectomy in disorders of sexual development. *J Pediatr Endocrinol Metab.* 2015;28(9-10):1019-1027.
4. Domenice S, Zamboni Machado A, Moraes Ferreira F, et al. Wide spectrum of NR5A1-related phenotypes in 46,XY and 46,XX individuals. *Birth Defects Res C Embryo Today.* 2016;108(4):309-320.
5. da Silva TE, Nishi MY, Costa EM, et al. A novel WT1 heterozygous nonsense mutation (p.K248X) causing a mild and slightly progressive nephropathy in a 46,XY patient with Denys-Drash syndrome. *Pediatr Nephrol.* 2011;26(8):1311-1315.
6. Melo KF, Martin RM, Costa EM, et al. An unusual phenotype of Frasier syndrome due to IVS9 +4C>T mutation in the WT1 gene: predominantly male ambiguous genitalia and absence of gonadal dysgenesis. *J Clin Endocrinol Metab.* 2002;87(6):2500-2505.
7. Correa RV, Domenice S, Bingham NC, et al. A microdeletion in the ligand binding domain of human steroidogenic factor 1 causes XY sex reversal without adrenal insufficiency. *J Clin Endocrinol Metab.* 2004;89(4):1767-1772.

8. Lee P, Mazur T, Danish R, Amrhein J, Blizzard R, Money J. *Micropenis. I – Criteria, etiologies, and classification.* Jo hns Hopk Med J 1980.
9. Ahmed SF, Khwaja O, Hughes IA. The role of a clinical score in the assessment of ambiguous genitalia. *BJU Int.* 2000;85(1):120-124.
10. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 1988;16(3):1215.
11. Baetens D, Güran T, Mendonca BB, et al. Biallelic and monoallelic ESR2 variants associated with 46,XY disorders of sex development. *Genet Med.* 2017.
12. Fitzpatrick SL, Sindoni DM, Shughrue PJ, Lane MV, Merchenthaler IJ, Frail DE. Expression of growth differentiation factor-9 messenger ribonucleic acid in ovarian and nonovarian rodent and human tissues. *Endocrinology.* 1998;139(5):2571-2578.
13. Williams LS, Demir Eksi D, Shen Y, et al. Genetic analysis of Mayer-Rokitansky-Kuster-Hauser syndrome in a large cohort of families. *Fertil Steril.* 2017;108(1):145-151.e142.
14. Zhao L, Du X, Huang K, et al. Rac1 modulates the formation of primordial follicles by facilitating STAT3-directed Jagged1, GDF9 and BMP15 transcription in mice. *Sci Rep.* 2016;6:23972.
15. Cheng Y, Kawamura K, Takae S, et al. Oocyte-derived R-spondin2 promotes ovarian follicle development. *FASEB J.* 2013;27(6):2175-2184.
16. Feng CW, Bowles J, Koopman P. Control of mammalian germ cell entry into meiosis. *Mol Cell Endocrinol.* 2014;382(1):488-497.
17. Bhandari RK, Schinke EN, Haque MM, Sadler-Riggleman I, Skinner MK. SRY induced TCF21 genome-wide targets and cascade of bHLH factors during Sertoli cell differentiation and male sex determination in rats. *Biol Reprod.* 2012;87(6):131.
18. Nakamura Y, Hornsby PJ, Casson P, et al. Type 5 17beta-hydroxysteroid dehydrogenase (AKR1C3) contributes to testosterone production in the adrenal reticularis. *J Clin Endocrinol Metab.* 2009;94(6):2192-2198.

19. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics.* 2009;25(14):1754-1760.
20. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. *Nucleic Acids Res.* 2010;38(16):e164.
21. Lek M, Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature.* 2016;536(7616):285-291.
22. Auton A, Brooks LD, Durbin RM, et al. A global reference for human genetic variation. *Nature.* 2015;526(7571):68-74.
23. Naslavsky MS, Yamamoto GL, de Almeida TF, et al. Exomic variants of an elderly cohort of Brazilians in the ABraOM database. *Hum Mutat.* 2017;38(7):751-763.
24. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med.* 2015;17(5):405-424.
25. Assumpção JG, Benedetti CE, Maciel-Guerra AT, et al. Novel mutations affecting SRY DNA-binding activity: the HMG box N65H associated with 46,XY pure gonadal dysgenesis and the familial non-HMG box R30I associated with variable phenotypes. *J Mol Med (Berl).* 2002;80(12):782-790.
26. Kolesinska Z, Ahmed SF, Niedziela M, et al. Changes over time in sex assignment for disorders of sex development. *Pediatrics.* 2014;134(3):e710-715.
27. Hughes IA, Houk C, Ahmed SF, Lee PA, Group LWPESESfPEC. Consensus statement on management of intersex disorders. *J Pediatr Urol.* 2006;2(3):148-162.
28. Eggers S, Sadedin S, van den Bergen JA, et al. Disorders of sex development: insights from targeted gene sequencing of a large international patient cohort. *Genome Biol.* 2016;17(1):243.

29. Sıklar Z, Berberoğlu M, Ceylaner S, et al. A novel heterozygous mutation in steroidogenic factor-1 in pubertal virilization of a 46,XY female adolescent. *J Pediatr Adolesc Gynecol.* 2014;27(2):98-101.
30. Cools M, Hoebeke P, Wolffenbuttel KP, et al. Pubertal androgenization and gonadal histology in two 46,XY adolescents with NR5A1 mutations and predominantly female phenotype at birth. *Eur J Endocrinol.* 2012;166(2):341-349.
31. Warman DM, Costanzo M, Marino R, et al. Three new SF-1 (NR5A1) gene mutations in two unrelated families with multiple affected members: within-family variability in 46,XY subjects and low ovarian reserve in fertile 46,XX subjects. *Horm Res Paediatr.* 2011;75(1):70-77.
32. Tantawy S, Lin L, Akkurt I, et al. Testosterone production during puberty in two 46,XY patients with disorders of sex development and novel NR5A1 (SF-1) mutations. *Eur J Endocrinol.* 2012;167(1):125-130.
33. Pedace L, Laino L, Preziosi N, et al. Longitudinal hormonal evaluation in a patient with disorder of sexual development, 46,XY karyotype and one NR5A1 mutation. *Am J Med Genet A.* 2014;164A(11):2938-2946.
34. Camats N, Pandey AV, Fernández-Cancio M, et al. Ten novel mutations in the NR5A1 gene cause disordered sex development in 46,XY and ovarian insufficiency in 46,XX individuals. *J Clin Endocrinol Metab.* 2012;97(7):E1294-1306.
35. Werner R, Mönig I, August J, et al. Novel Insights into 46,XY Disorders of Sex Development due to NR5A1 Gene Mutation. *Sex Dev.* 2015;9(5):260-268.
36. Barbaro M, Cools M, Looijenga LH, Drop SL, Wedell A. Partial deletion of the NR5A1 (SF1) gene detected by synthetic probe MLPA in a patient with XY gonadal disorder of sex development. *Sex Dev.* 2011;5(4):181-187.
37. Domenice S, Yumie Nishi M, Correia Billerbeck AE, et al. A novel missense mutation (S18N) in the 5' non-HMG box region of the SRY gene in a patient with partial gonadal dysgenesis and his normal male relatives. *Hum Genet.* 1998;102(2):213-215.

38. McElreavey K, Vilain E, Barbaux S, et al. Loss of sequences 3' to the testis-determining gene, SRY, including the Y pseudoautosomal boundary associated with partial testicular determination. *Proc Natl Acad Sci U S A.* 1996;93(16):8590-8594.
39. Paris F, Philibert P, Lumbroso S, et al. Primary amenorrhea in a 46,XY adolescent girl with partial gonadal dysgenesis: identification of a new SRY gene mutation. *Fertil Steril.* 2007;88(5):1437.e1421-1435.
40. Little M, Wells C. A clinical overview of WT1 gene mutations. *Hum Mutat.* 1997;9(3):209-225.
41. Barbaux S, Niaudet P, Gubler MC, et al. Donor splice-site mutations in WT1 are responsible for Frasier syndrome. *Nat Genet.* 1997;17(4):467-470.
42. Denamur E, Bocquet N, Baudouin V, et al. WT1 splice-site mutations are rarely associated with primary steroid-resistant focal and segmental glomerulosclerosis. *Kidney Int.* 2000;57(5):1868-1872.
43. Kohsaka T, Tagawa M, Takekoshi Y, Yanagisawa H, Tadokoro K, Yamada M. Exon 9 mutations in the WT1 gene, without influencing KTS splice isoforms, are also responsible for Frasier syndrome. *Hum Mutat.* 1999;14(6):466-470.
44. Kitsiou-Tzeli S, Deligiorgi M, Malaktari-Skarantavou S, et al. Sertoli cell tumor and gonadoblastoma in an untreated 29-year-old 46,XY phenotypic male with Frasier syndrome carrying a WT1 IVS9+4C>T mutation. *Hormones (Athens).* 2012;11(3):361-367.
45. Tajima T, Sasaki S, Tanaka Y, et al. 46,XY phenotypic male with focal segmental glomerulosclerosis caused by the WT1 splice site mutation. *Horm Res.* 2003;60(6):302-305.
46. Pearlman A, Loke J, Le Caignec C, et al. Mutations in MAP3K1 cause 46,XY disorders of sex development and implicate a common signal transduction pathway in human testis determination. *Am J Hum Genet.* 2010;87(6):898-904.

47. Baxter RM, Arboleda VA, Lee H, et al. Exome sequencing for the diagnosis of 46,XY disorders of sex development. *J Clin Endocrinol Metab.* 2015;100(2):E333-344.
48. Kim JH, Kang E, Heo SH, et al. Diagnostic yield of targeted gene panel sequencing to identify the genetic etiology of disorders of sex development. *Mol Cell Endocrinol.* 2017.
49. Bagheri-Fam S, Ono M, Li L, et al. FGFR2 mutation in 46,XY sex reversal with craniosynostosis. *Hum Mol Genet.* 2015;24(23):6699-6710.
50. Pleskacova J, Hersmus R, Oosterhuis JW, et al. Tumor risk in disorders of sex development. *Sex Dev.* 2010;4(4-5):259-269.
51. Furtado PS, Moraes F, Lago R, Barros LO, Toralles MB, Barroso U. Gender dysphoria associated with disorders of sex development. *Nat Rev Urol.* 2012;9(11):620-627.

Figure legends:

Figure 1: Flowchart of patients´ follow-up regarding testosterone secretion and gonadal tumour incidence

Appendix 1: Questionnaire applied to 46,XY DSD patients due to partial gonadal dysgenesis regarding gender identification and sexual life quality

Social sex and gender identity

- At birth, your assigned social sex was: 1 – Female 2 – Male 3 – Undefined
- Have you ever wished to change your gender? 1 – yes 2 – no
- Have you changed your gender: 1 – yes 2 – no
- How old were you when you started thinking about to change your gender?
- How old were you when you changed your gender?
- Define your gender identification: 1- female 2- male 3- both 4- none

Self reported gender role at childhood

- At childhood, you used to behave like you were: 1 – a girl 2 – a boy 3 – Both

- **At childhood, how you used to feel about your gender**

  - **If assigned female at birth, you used to feel like a girl:**

    1- Never
    2- Almost never
    3- Sometimes
    4- Often
    5- Always

  - **If assigned male at birth, you used to feel like a boy:**

    1- Never
    2- Almost never
    3- Sometimes
    4- Often
    5- Always

- **Which were your preferable toys at childhood**

  Classification of the preferable toys at childhood according gender:

Typically female toys	Typically boys toys	Neutral toys

Dolls, make-ups, drawing, reading, costumes, board games	Cars and trucks, building games (like Lego)	Plays with ball, running, group activities

Sexual life aspects

- Do you have regular sexual intercourses? 1 – yes 2 – no
- Do you have a steady partner? 1 – yes 2 – no
- Do you have penetrative sexual intercourses? 1 – yes 2 – no
- Are you satisfied with your sexual life? 1 – yes 2 – no
- Do you have orgasm during intercourse? 1 – yes 2 – no

Gene	Associated phenotype already related to human DSDs reported in OMIM (OMIM number) or in the literature (L)[1] (n=43)	Inheritance
	Gonadal development genes	
BMP15	Ovarian dysgenesis 2 (300510); Premature ovarian failure 4 (300510)	XL
CBX2	46,XY sex reversal 5 (613080); 46,XY complete gonadal dysgenesis (L)	AR
DHH	46XY partial gonadal dysgenesis, with minifascicular neuropathy (607080); 46XY sex reversal 7 (233420)	AR
DMRT1	Dysgenetic testis or ovotestis (L);	AD
DMRT2	Haploinsufficiency 9p sex-determining gene leads to gonadal dysgenesis (L)	NA
FGFR2	46,XY sex reversal with craniosynostosis (L)	AD, AR
FOXL2	Premature ovarian failure 3 (608996); blepharophimosis, epicanthus inversus, and ptosis, type 1 and 2 (110100)	AD
GATA4	Testicular anomalies with or without congenital heart disease (615542)	AD
MAP3K1	46XY sex reversal 6 (613762)	AD
NR0B1	46XY sex reversal 2, dosage-sensitive (300018)	XL
NR5A1	46, XX sex reversal 4 (617480); 46XY sex reversal 3 (612965); premature ovarian failure 7 (612964)	AD
RSPO1	Palmoplantar hyperkeratosis and true hermaphroditism (610644); palmoplantar hyperkeratosis with squamous cell carcinoma of skin and sex reversal (610644)	AR
SOX9	Campomelic dysplasia with autosomal sex reversal (114290)	AD
SOX3	46,XX testicular or ovotesticular DSD (L); 46,XX Sex Reversal 3 (300833)	XL
SRY	46XX sex reversal 1 (400045); 46XY sex reversal 1 (400044)	Y-linked
STAG3	Premature ovarian failure 8 (615723)	AR
WNT4	Mullerian aplasia and hyperandrogenism (158330)	AD
WT1	Denys-Drash syndrome (194080); Frasier syndrome(136680)	AD
WWOX	46,XY gonadal dysgenesis (L)	Y-linked
ZFPM2	46XY sex reversal 9 (616067)	AD
	Sexual differentiation genes	
AKR1C2	46XY sex reversal 8 (614279)	AR
AKR1C4	46XY sex reversal 8, modifier (614279)	AR

AMH	Persistent Mullerian duct syndrome, type I (261550)	AR
AMHR2	Persistent Mullerian duct syndrome, type I (261550)	AR
AR	Androgen insensitivity (300068); Hypospadias 1, X-linked (300633)	X-L
CYP17A1	17,20-lyase deficiency, isolated (202110)	AR
CYP19A1	Aromatase deficiency (613546); aromatase excess syndrome (139300)	AD
CYP21A2	Adrenal hyperplasia, congenital, due to 21-hydroxylase deficiency (201910)	AR
DHCR7	Smith-Lemli-Opitz syndrome (270400)	AR
FSHR	Ovarian dysgenesis 1 (233300)	AR
HSD11B1	Cortisone reductase deficiency 2 (614662)	AD
Continuing		
HSD17B3	Pseudohermaphroditism, male, with gynecomastia (264300)	AD
HSD3B2	3-beta-hydroxysteroid dehydrogenase, type II, deficiency (201810)	AD
LHCGR	Leydig cell hypoplasia with pseudohermaphroditism (238320); Leydig cell hypoplasia with hypergonadotropic hypogonadism (238320)	AR
POR	Antley-Bixler syndrome with genital anomalies and disordered steroidogenesis (201750); Disordered steroidogenesis due to cytochrome P450 oxidoreductase (613571)	AR
SRD5A2	Pseudovaginalperineoscrotal hypospadias (264600)	AR
STAR	Lipoid adrenal hyperplasia (201710)	AR
	Other (syndromic DSD, isolated hypospadias)	
ARX	Hydranencephaly with abnormal genitalia (300215)	X-L
ATRX	ATR-X syndrome with gonadal abnormalities (301040)	X-LD/X-LR
CDH7	CHARGE syndrome (214800); hypogonadotropic hypogonadism with or without anosmia (612370)	AD
HNF1B	Mayer-Rokitansky-Kuster-Hauser syndrome (L)	AD
LHX1	Mayer-Rokitansky-Kuster-Hauser syndrome (L)	AD
MAMLD1	Hypospadias, X-linked (300758)	XL

Gene	Candidate genes associated with human DSDs selected from the literature (L) and in OMIM (O) (n=20)	Inheritance

AKR1C3	Testosterone production in the adrenal reticularis[18]	NA
AXIN1	Wnt-beta-catenin signaling (O)	NA
CITED2	An upstream regulator of NR5A1 (L)[1]	NA
ESR1	Sex reversal in ESRA/ESRB knockout males (O)	NA
ESR2	46,XY DSD candidate gene (L)[11]	AR;AD
FGF9	XY mice KO results in male-to-female sex reversal (L)[12]	NA
GDF9	Ovarian development (L)[12]	NA
GSK3β	Wnt-beta-catenin signaling (O)	NA
LHX9	Gonadal formation in mouse model (L)[1]	NA
NANOS2	Expressed in adult and fetal testis (O)	NA
NANOS3	Nanos3-null mice present reduced spermatogenesis (O)	NA
PAPPA	Expressed in ovarian follicles and in the seminal vesicles and fluid (O)	NA
PAX2	WT1 pathway (L) [13]	NA
PBX1	Müllerian development in the mouse (L) [13]	NA
PTDGS	Required for testis formation (L)[1]	NA
RAC1	Formation of primordial follicles in mouse (L)[14]	NA
Continuing		
RSPO2	Essential for primary follicle development (L) [15]	NA
STRA8	Pre-meiotic DNA replication (L) [16]	NA
TCF21	SRY pathway (L) [17]	NA
TES	Testis-specific enhancer of Sox9 (L) [1]	NA

Table 1 Phenotypes at First Evaluation of 46,XY PGD Female Gender Patients

Patient	1	2	3	4	5	6[a]	7[a]	8[b]	9[a]	10	11[b]	12	13[c]
Age (yr.)	1.2	3.7	9.4	11	12.3	16	18.9	19	27	31	30	38	43
EM score	2	4	1	4	5	NA	NA	6	NA	3	6	3	NA
Clitoris/Phallus size (cm)	2.5x1.0	3.0x0.9	3.0x0.9	6.0x2.0	3.0x1.0	Previous surgery	Previous surgery	5.5x1.7	Previous surgery	4.1x1.0	Micropenis	0.8x1.0	Previous surgery
Perineal openings	2	1	2	2	2	2	1	1	2	1	1	1	2
Gonads location	RG: labial LG: ABD	ABD	ABD	RG:ING LG:ABD	ING	LG:ING	ABD	Not found	NA	Not found	Not found	Not found	ABD
Mullerian Derivatives	Present	Present	Present	Absent	Present	Present	Absent	Absent	NA	Absent	Present	Absent	Present
Age at gonadectomy (yr.)	1.4	3.9	9.9	12	15.2	14	5	9	14	31	30	38	43
Gonadal histology	Dysgenetic	Dysgenetic	Dysgenetic	Dysgenetic	Dysgenetic	NA	RG:Dysgenetic LG:NGT	NGT	NGT	NGT	NGT	NGT	RG: NGT LG:Dysgenetic
Basal T (nmol/L)	2.6*	0.9†	4.0†	14.4*	10.3*	NA	NA	<0.9†	NA	1.2†	1.0*	<0.9†	11.2*
T after hCG (nmol/L)	19.9*	11.7†	NA	NA	NA	NA	NA	NA	NA	1.3	NA	NA	NA
LH (IU/L)	<1.0	NA	5.1	13	24	NA	NA	23	NA	18	10	11	32

FSH (IU/L)	<0.6	NA	50	77	69	NA	NA	62	NA	57	40	38	52
Affected genes	MAP3K1/ FGFR2	-	MAP3K1/ FGFR2	NR5A1	NR5A1	NR5A1	-	-	-	NR5A1	-	-	-

Abbreviations: PGD: partial gonadal dysgenesis; NA: not available; EM score: External masculinization score; RG: right gonad; LG: left gonad; ABD: abdominal; ING: inguinal; NGT: no gonadal tissue;

Notes: a = Previous gonadectomy and genitoplasty in another hospital; incomplete data; b = Patients that changed from male to female social sex and underwent previous testosterone replacement; c = Previous genitoplasty in another hospital; †Radioimmunoassay (RIA), normal male value: prepubertal age <0.9 nmol/L; adults: 8.7–35.7 nmol/L *Immunofluorometric assay (IFMA), normal male value: prepubertal age <0.65 nmol/L; adults 9.4–33.5 nmol/L;

Table 2. Phenotypes at First Evaluation of 46,XY PGD Male Gender Patients and Impaired Testosterone Secretion

Patient	**14**	**15**	**16**	**17**	**18**	**19**	**20** [a]	**21** [a b]	**22**	**23**
Age (yr.)	1.9	2.0	7	7.4	9	14.9	16	27	34	47.9
EM score	4	7	6	7	6	6	NA	NA	3	3
Phallus size (cm)	2.0x1.0	3.0x1.0	3.0x1.0	4.5X2.0	3.4x1.4	3.6X1.2	Previous surgery	Previous surgery	5.0x1.2	8.5x3.0
Phallus size (SD)	-3.4	-2.3	-3.4	-1.7	-2.9	-6.1	NA	NA	-5.2	-3.0
Urethra location	Perineal	Perineal	Topic	Perineal	Topic	Topic	NA	NA	Perineal	Perineal
Gonad location	ABD	ABD	Not found	ABD	Not found	Not found	Cryptorchidism	NA	Not found	Not found
Mullerian Derivatives	Absent	Absent	Absent	Absent	Absent	Present	Present	Present	Present	Present
Age at gonadectomy (yr.)	9.9 and 10.5	2.5 and 4.7	15	4 and 11	9.0	15	NA	1.7	34	48
Gonadal hystology	Dysgenetic	Dysgenetic	Dysgenetic	Dysgenetic	NGT	NGT	NA	Infantile testes	NGT	NGT
Basal T (nmol/L)	0.9†	<0.9†	NA	<0.9†	<0.9†	<0.9†	NA	NA	2.6*	0.9*
T after hCG (nmol/L)	0.9†	2.9†	NA	<0.9†	<0.9†	NA	NA	NA	4.1*	NA
LH (IU/L)	NA	NA	NA	NA	NA	20	NA	NA	10	16
FSH (IU/L)	NA	NA	NA	NA	NA	41	NA	NA	78	57
Affected genes	-	WT1	-	-	-	-	-	-	-	-

Abbreviations: PGD: partial gonadal dysgenesis; NA: not available; EM score: external masculinization score; ABD: abdominal; ING: inguinal NGT: no gonadal tissue;

Notes: a=Previous gonadectomy and genitoplasty in another hospital; incomplete data; b=Patient that changed from female to male social sex.

†Radioimmunoassay (RIA), normal male value: prepubertal age <0.9 nmol/L; adults: 8.7–35.7 nmol/L *Immunofluorometric assay (IFMA), normal male value: prepubertal age <0.65 nmol/L; adults 9.4–33.5 nmol/L;

Table 3. Phenotypes at First Evaluation of 46,XY PGD Male Gender Patients and Preserved Testosterone Secretion

Patient	**24**	**25**	**26**	**27**	**28**	**29** [a]	**30**	**31**	**32**	**33** [b]
Age (years)	Newborn	0.5	0.6	0.9	1.7	4.0	4.3	13	23	26
EM score	6	6	7.5	7.5	6	NA	4	5	4.5	5.5
Phallus size (cm)	2.0x1.0	3.0x0.9	2.5x1.0	3.5X1.2	2.0 x1.0	Previous surgery	2.5 X 1.0	8.7X4.2	8.0x2.7	6.5x2.5
Phallus size (SD)	-3.75	-1.12	-2.25	-0.34	-3.3	NA	- 3.5	-2.8	-3.3	-4.3
Urethra location	Penile	Perineal	Perineal	Perineal	Topic	NA	Perineal	Perineal	Perineal	Perineal
Gonad Location	RG: ABD LG: topic	Topic	RG: ING LG: ABD	RG: ABD LG: ING	RG: ING LG: not found	RG: NA LG: topic	ABD	ING	RG: ING LG: ABD	RG: ING LG: topic
Mullerian Derivatives	Present	Absent	Present	Present	Absent	Present	Present	Absent	Present	Present
Age at gonadectomy (yr)	8	NA	1.1	1.2	2	4	4 and 6	19 and 21	23	NA
Gonadal histology	NA	NA	LG: dysgenetic	RG: dysgenetic	LG: dysgenetic	RG: dysgenetic	Dysgenetic	Bilateral gonadobastoma RG: in situGCT	RG:Dysgenetic LG: mixed GCT	NA
Basal T (nmol/L)	10.7†	<0.9†	<0.9†	NA	2.6†	1.5†	<2.9†	18.5*	14.6*	13.7*

T afterhCG (nmol/L)	NA	15.9†	6.2†	22.1†	7.3†	17.1†	6.6†	21.4*	NA	17.3*
LH (IU/L)	NA	NA	<0.6	<0.6	NA	NA	<1.0	13.3	34	10.8
FSH (IU/L)	NA	NA	<1.0	<1.0	NA	NA	3.0	39.6	75.5	20.7
Affected genes	-	NR5A1	SRY	-	-	-	-	WT1	-	NR5A1

Abbreviations: PGD: partial gonadal dysgenesis; NA: not available; EMS: external masculinization score; ABD: abdominal; NGT: no gonadal tissue; NA, not available; GCT, germ cell tumor

Notes: a= Previous unilateral gonadectomy and genitoplasty perfomed in another hospital; b=Patient that changed from female to male social sex

†Radioimmunoassay (RIA), normal male value: prepubertal age <0.9 nmol/L; adults: 8.7–35.7 nmol/L *Immunofluorometric assay (IFMA), normal male value: prepubertal age <0.65 nmol/L; adults 9.4–33.5 nmol/L;

Table 4. Phenotypes of 46,XY PGD Male Gender Patients and Preserved Testosterone Secretion at Final Evaluation

Patient	25	26	27	28	33
Age (years)	17	17	17.6	21	28
Penile length (cm)	8.0x3.0	8.0x3.0	7.5x2.5	9.2x2.5	6.5x2.5
Z Phallus (SD)	-3.3	-3.3	-3.6	-2.6	-4.3
Testis final size (cm)	4.5x2.5	6.7x2.1	4.0x2.5	4.3x2.0	6.5X2.5
Basal T* (nmol/L)	441	561	625	482	396
LH (IU/L)	5.6	14	11	11	10.8
FSH (IU/L)	3.7	26	25	24	20.7

Abbreviations: PGD: Partial gonadal dysgenesis

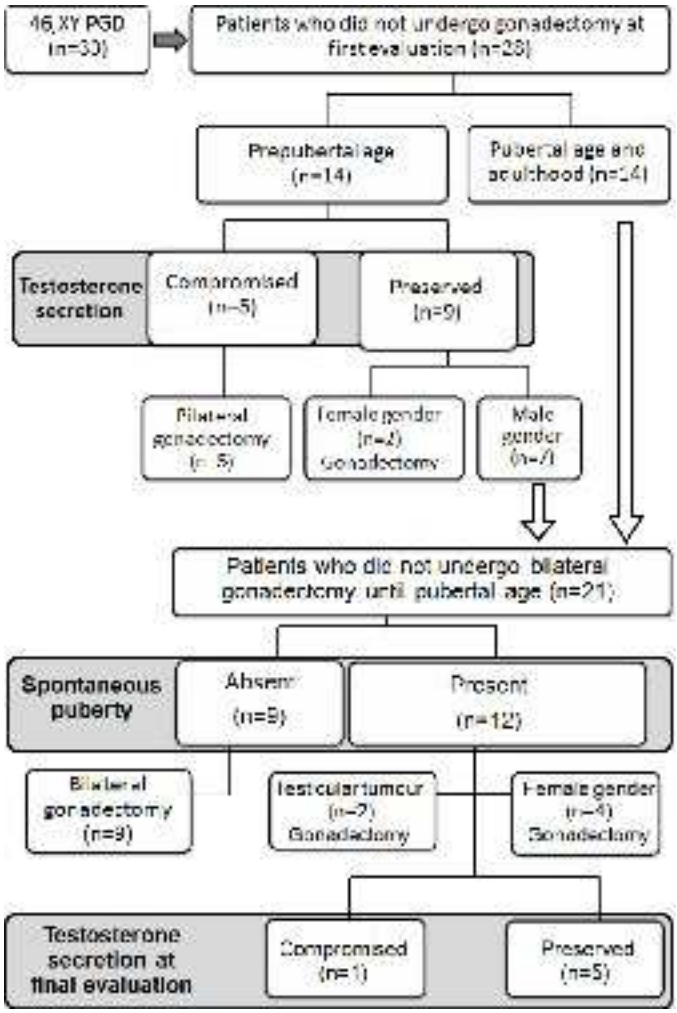
*Immunofluorometric assay (IFMA), normal male value: prepubertal age <0.65 nmol/L; adults 9.4–33.5 nmol/L;

Table 5: Deleterious Variants´ Description and Testosterone Production at Puberty in Patients with 46,XY Partial Gonadal Dysgenesis

Patient	T secretion at puberty	Gene	Transcript ID	Allelic Variant [d]	Protein alteration	Previous published/ Reference	Variant Segregation in family members	ACMG Classification
4	Preserved	NR5A1	NM_004959	c.T1073C	p.Leu358Pro	Yes/2	M: C with POI	Likely Pathogenic
5	Preserved	NR5A1	NM_004959	c.C633G	p.Tyr211*	Yes/2	M: AC; F:NA	Pathogenic
6	Previous gonadectomy	NR5A1	NM_004959	c.G77A	p.Gly26Glu	Yes/2	NA	Likely Pathogenic
10	Absent	NR5A1	NM_004959	c.1058_1065del	p.Glu353Ala fs*31	Yes/5	NA	Pathogenic
25	Preserved	NR5A1	NM_004959	c.1183_1185del	p.Glu395del	Yes/2	NA	Pathogenic
33	Preserved	NR5A1	NM_004959	c.C741A	p.Cys247*	Yes/2	NA	Pathogenic
26	Preserved	SRY	NM_003140	c.G89T	p.Arg30Ile	Yes/23	F:AC	Likely Pathogenic
15	Previous gonadectomy	WT1	NM_024426	c. A742T	p.Lys248*	Yes/3	De novo	Pathogenic
31	Preserved	WT1	NM_024426	IVS 9+4C>T	Splice site change	Yes/4	De novo	Pathogenic
1 and 3 (sisters)	Previous gonadectomy [e]	MAP3K1 and	NM_005921	**c.T1916C**	**p.Leu639Pro**	Novel	M:AC; P:WT	Likely Pathogenic
1 and 3 (sisters)	Previous gonadectomy [e]	FGFR2	NM_000141	**c.C1358T**	**p.Ser453Leu**	Novel	M:AC; P:WT	Likely Pathogenic

Abbreviations: M, mother; F, father; NA, not available; WT, wild type; C, carrier; POI, premature ovarian failure; AC, asymptomatic carrier;

Notes: The references of the previously reported variants were included. The novel variants are highlighted in bold; d=All the variants are in heterozygous state and are absent in population database; e= These female social sex patients had preserved testosterone secretion at childhood and underwent bilateral gonadectomy



cen_13717_f1.tiff