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Pubertal and Gonadal Outcomes in 46,XY Individuals with Partial Androgen Insensitivity Syndrome Raised as Girls

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Keywords
Puberty · Androgen insensitivity syndrome · Partial androgen insensitivity syndrome · Disorders of sex development · Differences of sex development

Abstract

Introduction: Although it was common in the 1970s–1990s to assign female gender of rearing to 46,XY infants with limited virilization of varying etiologies, including those with partial androgen insensitivity syndrome (PAIS), long-term data on outcomes for these individuals are sparse. Therefore, our goal was to use the power of an international registry to evaluate clinical features, surgical management, and pubertal data in patients with a molecularly confirmed diagnosis of PAIS who were born before 2008 and were raised as girls.

Methods: The current study interrogated the International Disorders of Sex Development Registry for available data on management and pubertal outcomes in individuals with genetically confirmed PAIS who were born as girls.

Results: Among the 11 individuals who fulfilled the key criteria for inclusion, the external masculinization score (EMS) at
presentation ranged from 2 to 6 (median 5); 7 girls underwent gonadectomy before the age of 9 years, whereas 4 underwent gonadectomy in the teenage years (≥ age 13). Clitoral enlargement at puberty was reported for 3 girls (27%) who presented initially at the time of puberty with intact gonads. In the 9 individuals (82%) for whom gonadal pathology data were provided, there was no evidence of germ cell tumor at median age of 8.1 years. All girls received estrogen replacement, and 8/11 had attained Tanner stage 4–5 breast development at the last assessment.

**Conclusion:** In general, although it appears that female assignment in PAIS is becoming uncommon, our data provide no evidence to support the practice of prophylactic prepubertal gonadectomy with respect to the risk of a germ cell tumor.

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### Introduction

The androgen insensitivity syndrome results from molecular variants in the gene encoding the androgen receptor (AR) that produce varying degrees of androgen resistance secondary to AR dysfunction [Quigley et al., 1995; Mongan et al., 2015]; reported prevalence ranges from 1 in 20,000 to 1 in 99,000 [Boehmer et al., 2001]. This X-linked condition is classified clinically based on the degree of androgen responsiveness as complete (CAIS, minimal androgen response, and female phenotype); partial (PAIS, wide spectrum of clinical presentation, from female-like genitalia to essentially male-appearing genitalia with hypospadias); and mild (male phenotype with gynecomastia and/or infertility) [Quigley et al., 1995; Mongan et al., 2015]. Although there is no debate regarding female assignment in CAIS, gender assignment is more controversial in individuals with PAIS whose external genitalia are markedly underandrogenized but not typically female [Ahmed et al., 2000a]. Since publication of the 2006 DSD consensus guidelines, practice appears to have shifted toward male assignment in those with PAIS [Kolesinska et al., 2014], possibly based on anecdotal reports of poor long-term outcome in those raised as girls. However, there are no well-conducted outcome studies specifically focused on girls and women with genetically confirmed PAIS, and there is a need for more evidence to inform decision-making.

In girls with PAIS, concerns about both the possibility of virilization during puberty and the potential risk of gonadal neoplasia have contributed to the longstanding practice of and recommendation for early gonadectomy [Lee et al., 2016]. This is contrary to the practice in CAIS, in which gonadectomy is now recommended to be delayed until after puberty, allowing the adolescent to spontaneously develop female secondary sexual characteristics and to be involved in decision-making regarding the eventual fate of her gonads [Tack et al., 2018]. In fact, some patients with CAIS have rejected gonadectomy altogether in favor of long-term gonadal surveillance [Deans et al., 2012; Nakhal et al., 2013]. The aim of the current study was to probe the International Disorders of Sex Development Registry (I-DSD Registry) for available data on management and pubertal outcomes in individuals with genetically confirmed PAIS who were raised as girls.

### Patients and Methods

The I-DSD Registry (www.i-dsd.org) is an internet-based database approved by the National Research Ethics Service in the UK as an archive for non-personally identifiable information collected as part of routine clinical practice and used for secondary research [Ahmed et al., 2010; Ali et al., 2019]. To optimize data quality, the majority of data points are entered as choices among a limited set of predetermined options (e.g., radio buttons for categorical items such as sex at birth; drop-down lists for items such as location of urethral meatus); where needed certain data fields are completed as free text entries (e.g., details of syndromic features, when present; see https://home.i-dsd.org/data-elements/www.i-dsd).

The following terms were used in a search of the database to identify a suitable cohort of subjects: 46,XY; disorder of androgen action; PAIS; year of birth before 2008 (to identify individuals who were at or beyond pubertal age at the time of the study); female sex assignment. At the time of the search (November 2017), 237 individuals were registered as having a diagnosis of PAIS and were born before 2008. Of these, 40 (17%) individuals from 14 clinical centers in 11 countries were identified as having been raised female. As shown in Figure 1, 31 of the 40 cases were excluded for the following reasons: no data provided (n = 5), no puberty data available (n = 15), molecular genetic diagnosis of PAIS not confirmed (n = 11). One center provided data on 2 additional cases who satisfied the inclusion criteria and had not been available in the original search of the registry. Therefore, a total of 11 subjects were included in the study. Data for three of these individuals (subjects C, D, and J) have been published [Evans et al., 1997; Boehmer et al., 2001].

Information on clinical features at first presentation and most recent follow-up, molecular genetic diagnosis, clinical/surgical management, and pubertal development was extracted from the database. The external masculinization score (EMS) and the external genitalia score (EGS) were calculated by the first author (G.G.-F.) based on description of the external genitalia at initial presentation [Ahmed et al., 2000b; Van der Straaten et al., 2020]. Pubertal stages of breast and pubic hair development were described according to Marshall and Tanner [1969]. Data analysis was performed using SPSS version 20.0 (IBM Corporation, Armonk, NY, USA). Continuous variables were reported as medians and ranges. Because of limited subject numbers, the data were not appropriate for statistical testing.
Results

Initial Presentation, Gender Assignment, and Family History

Subjects were born between 1978 and 2005, with 9 of the 11 (82%) being born before 1997. Seven of the 11 individuals presented on the basis of varying degrees of atypical genital development at ≤1 year of age, including 1 subject (A) with isolated clitoral enlargement (Table 1). One subject (K) presented at age 3 with inguinal masses, and 1 subject each was ascertained on the basis of clitoral enlargement at puberty (subject I), absence of müllerian structures discovered during surgery for an unrelated reason (subject D), and positive family history (subject C, the older sibling of subject D, investigated after her sister’s diagnosis). The median EMS at first presentation was 5 (range, 2–6; maximum possible score, representing the greatest degree of masculinization, is 12), with the lowest EMS being for subject I, who presented at puberty with clitoromegaly (Table 1). The EGS was not calculated in subject I due to the lack of precise phallus length information. The median EGS at first presentation in the remaining subjects was 3.75 (range, 3.5–7.0; maximum possible score, representing the greatest degree of masculinization, is 12). The lowest EGS was found in subjects C, D, E, F, and H, with a score of 3.5. Both testes were located in the inguinal region or beyond for 10/11 subjects.

Assignment was female from the outset for 8 (probably 9) of the 11 subjects (for subject I [EMS, 2], the age at female assignment was unavailable, but likely at birth). Subject H was designated as “undetermined” at first presentation (EMS, 4) and later assigned female (presumably within the first month of life, as gonadectomy was performed at 18 days of age), and subject B (EMS, 3) was originally assigned as male and then changed to female at the age of 6 months. At the last assessment, at ages ranging from 12 to 36 years (median 17.5), all subjects were living in the female gender.

Of the 9 individuals for whom information was available, 6 (67%) had a family history of presumed PAIS, two of whom (C and D) were siblings (Table 1); subject B had an affected maternal uncle; subject G had an affected sister; and subjects I and J each had an affected brother [Evans et al., 1997].

Surgical Data (Gonadectomy and Genital Surgery) and Testicular Histology

All 11 girls underwent gonadectomy, with age provided for 10/11 (range: 18 days to 15 years; median: 8.1 years). As shown in Figure 2a, b there was no association between EMS at presentation and age at gonadectomy. However, there appeared to be 3 clusters of age at which gonadectomy was performed: infancy (subjects E, H, and J, aged ≤2 years, all of whom presented with atypical genitalia); mid-childhood (subjects A, F, and K, aged 3.0–8.6 years, with various
Table 1. Clinical features at initial presentation, surgical history, details of pubertal onset and development, hormone therapy, and genetic findings

| ID | DoB  | Age pres. | Reas. | EMS | EGS | Testes (L, R) | Phallus | L-S fusion | Age Gx | Age T2 | Age HT | HT type | Age f/u | Breast f/u | PH f/u | CE pub. | Genital surgery | AR variant |
|----|------|-----------|-------|-----|-----|-------------|---------|------------|--------|--------|--------|---------|---------|---------|------|--------|----------------|------------|
| A  | 1990s| 1         | CE    | 3   | 4   | l-s, l-s   | Perineal | 2.0        | N      | 7.5    | 11     | n/a     | TDE 18 | 18      | 4    | 4      | N Clitoroplasty Introitoplasty | V685E     |
| B  | 1990s| 0.5       | AG    | 3   | 4   | l-s, l-s   | Perineal | 1.0        | N      | 12.2   | 12     | ConE 17 | 17      | 5      | 3    | n/a    | None Vaginoplasty with neovagina | V747M     |
| C  | 1980s| 15        | FHx   | 5   | 3.5 | i, i      | Perineal | 1.0        | Y      | 15     | n/a   | 15     | E2 18   | 18     | 5    | 4      | N Vaginoplasty with neovagina | L769M     |
| D  | 1980s| 13        | AMS   | 5   | 3.5 | i, i      | Perineal | 1.5        | Y      | 14.2   | 14     | E2 18   | 18     | 5    | 4    | N Vaginoplasty with neovagina | L769M     |
| E  | 2000s| <0.1      | AG    | 6   | 3.5 | l-s, l-s   | Perineal | 1.5        | Y      | 0.1    | 13     | E2 14   | 14     | 4    | 4    | N Vaginoplasty | L907V     |
| F  | 1990s| 0.25      | AG    | 5   | 3.5 | i, i      | Proximal | 1.0        | Y      | 8.6    | 12.9   | ConE 17 | 17     | 4    | 3    | N None | I899T      |
| G  | 1970s| 1         | AG    | 6   | 5.5 | l-s, l-s   | Proximal | 3.0        | N      | 13     | 10     | 10.5   | E2 36   | 36     | 5    | 2      | N Clitoroplasty Vaginal dilatation Neovagina | M896V     |
| H  | 1990s| <0.1      | AG    | 4   | 3.5 | i, a      | Proximal | 3.5        | N      | 0.04   | 12.8   | 12.8   | OCP 21  | 21     | 4    | 4      | n/a Clitoroplasty Introitoplasty Vaginoplasty Neovagina | S598G     |
| I  | 1980s| 13        | CE    | 2   | n/a | i, i      | Proximal | n/a        | N      | 13     | n/a   | n/a    | EE2 3    | 3      | n/a | Y Clitoroplasty | G216R      |
| J  | 1980s| <0.1      | AG    | 6   | 7   | l-s, l-s   | Peno-scrotal | 1.5      | Y      | 2      | n/a   | n/a    | ConE 18 | 18     | 2    | 1      | n/a Clitoroplasty Vaginoplasty | R841C     |
| K  | 2000s| 3         | IM    | 5   | 5   | i, i      | Proximal | 1.0        | Y      | 3      | n/a   | 11.7   | TDE 12 | 12     | 2    | 1      | N None | F828V     |

All ages are in years, shown as age at last birthday if no further detail was provided. AR variants L769M (siblings C and D) and R841C (subject J) have been previously reported [Evans et al., 1997; Boehmer et al., 2001]. Subjects C and D are siblings. aPhallus was recorded as below the usual male size, but measurement was not provided. ID, subject identification; DoB, decade of birth; Age pres., age at initial presentation; Reas. pres., reason for presentation; EMS, external masculinization score; EGS, external genital score; Testes (L, R), location of testes (left, right); L-S fusion, labioscrotal fusion; Age Gx, age at gonadectomy; Age T2, age at Tanner stage 2 breast development (15); Age HT, age at start of hormone therapy; HT type, hormone therapy type; Age f/u, age at last follow-up; Breast f/u, breast stage at last follow-up; PH f/u, pubic hair stage at last follow-up; CE pub., clitoral enlargement during puberty; CE, clitoral enlargement; AG, atypical genitalia; FHx, family history; AMS, absent müllerian structures; IM, inguinal mass(es); l-s, labioscrotal; i, inguinal; a, absent; n/a, not available; N, no; Y, yes; PP, prepubertal at unknown age; TDE, transdermal estradiol; ConE, conjugated estrogens; E2, estradiol; OCP, oral contraceptive preparation (dienorgestrol + ethinyl estradiol); EE2, ethinyl estradiol; AR, androgen receptor, variant designation is according to the sequence published based on NCBI reference sequence NM_000044.6 rather than the Genbank mRNA sequence M20132.1.
reasons for presentation); and teenage years (siblings C and D, who presented incidentally; subject G, who presented with atypical genitalia in infancy; and subject I, who presented with clitoral enlargement at puberty). In 9 cases, histological examination of the gonads when gonadectomy was executed did not reveal the presence of germ cell tumor; in two cases, histological information was unavailable.

Eight of 11 girls underwent feminizing genital surgery, with multiple procedures including vaginoplasty or neovaginal creation in 7 and clitoroplasty in 5. As shown in Figure 2c, there was no association between EMS and the performance of genital surgery: EMS at presentation for those who underwent genital surgery ranged from 2 to 6, while EMS for the 3 children who did not undergo genital surgery were 3, 5, and 5. For the 5 girls who underwent clitoroplasty, the clitoral length at presentation (ages <1 month to 13 years) ranged from 1.5 to 3.5 cm (median 2.5 cm) versus 1.0–1.5 cm for those 6 who did not undergo clitoroplasty. Four children who presented with atypical genitalia (subjects B, E, F, and G) and one who presented with clitoral enlargement (subject A) underwent genital surgery in infancy/childhood, and the other 3 surgically treated girls (C, D, and I) underwent their procedures after presentation at puberty.

**Breast Development and Hormone Therapy**

Timing of breast development was available for 6 of 11 girls, two of whom (subjects G and D) had gonads in situ at onset of breast development, at reported ages of 10.0 and 12.4 years, and underwent gonadectomy at ages 13 and 14 years, respectively; subject C, the sister of subject D, also had spontaneous breast development prior to presentation at age 15 with testes in situ, but age at onset was unknown. Gonadectomy had been performed prior to the onset of breast development for 4 girls (subjects A, B, E, H), whose breast development was therefore induced by hormone therapy. Median age at Tanner stage 2 breast development overall was 12.3 years (range, 10–13), with progression of breast development to Tanner stage 4 or 5 for all 6 girls (Table 1).
All girls received estrogen supplementation, beginning at 11.7–13.0 years of age for those who underwent prepubertal gonadectomy and ages 10.5–15.0 years for those who underwent peri- or postpubertal gonadectomy (median age at estrogen start, 12.9 years overall). At the time of their most recent assessment (ages 12–36 years), 9 of 11 individuals (82%) were receiving oral estrogen treatment (4, estradiol; 3, conjugated estrogens; 1, ethinyl estradiol; 1, oral contraceptive) and 2 were receiving transdermal estrogen. For the 5 girls whose age at the onset of breast development was unknown, breast development at the last follow-up (median age 17.5 years) ranged from Tanner stage 2 (a 12-year-old who had received only a few months of transdermal estrogen treatment by that time and an 18-year-old who had received conjugated estrogen for an unknown period) to Tanner 5 (subject B, age 17, receiving conjugated estrogen; subject C, age 18, receiving estradiol; and subject G, age 36, receiving estradiol).

**Androgen Response**

The degree of androgen response at birth (represented by the EMS) was compared with the clinical evidence of androgen response at puberty (clitoral enlargement and/or pubic hair development), albeit hampered by prepubertal orchidectomy in 7 of 11 girls and genital and/or vaginal surgery (likely prepubertal) for 8 of 11 (3 girls had vaginal surgery without surgery to the external genitalia). Of the 4 girls whose testes were present in the teenage years, only 1 girl (subject I) had enlargement of the clitoris during puberty as evidence of moderate androgen responsiveness; she presented for assessment of clitoral enlargement at age 13 with EMS of 2 and underwent clitoroplasty. Two girls (siblings C and D) developed Tanner stage 4 pubic hair, but clitoral size remained within expectations for typical female development. Subject G, who was reported to have Tanner stage 2 pubic hair at the last follow-up, had undergone clitoroplasty (at the age of 19.5 years) so could not have developed clitoral enlargement.

Pubic hair stage at the last follow-up was provided for 10 of 11 individuals. Two subjects (J and K) with EMS 5 and 6 who underwent prepubertal gonadectomy had reportedly no pubic hair at last assessment; for the remaining 8 individuals, reported pubic hair ranged from Tanner stage 2 in subject G, who had EMS of 6 at presentation and underwent gonadectomy at age 13, to Tanner stage 4 for 5 individuals, 2 of whom underwent gonadectomy in infancy (subjects E and H). Figure 2d demonstrates that there was no relationship between EMS at first presentation and pubic hair development at the last assessment.

**AR Gene Variants**

AR gene variants identified in our study subjects all resulted in amino acid (AA) substitutions at residues previously reported as harboring mutations in patients with various forms of AIS [http://androgendb.mcgill.ca/]. Eight of ten AR gene variants affect AAs in the ligand-binding domain, the largest functional domain of the AR and the site of the great majority of reported variants in other studies/databases. The variant for subject H was in the region encoding the DNA-binding domain, and the variant for subject I was in the region encoding the amino-terminal domain. We could not discern an association between the AR variant and EMS or other clinical features.

**Discussion**

Little information is available regarding pubertal development or gonadal neoplasia in individuals with PAIS reared as girls. Therefore, to evaluate puberty and gonadal outcomes, we reviewed the available data for postpubertal patients with molecularly proven PAIS who were registered as female in the I-DSD database. We were particularly interested in learning the relative degree of feminization versus masculinization in girls with PAIS whose testes were retained through puberty. However, the number of female subjects with molecularly proven PAIS in the database was small, and only 11 girls fulfilled our inclusion criteria for assessment. Of the 8 girls who presented in infancy or childhood, 7 underwent prepubertal gonadectomy, precluding assessment of spontaneous puberty. The other 3 girls presented after onset of puberty. The practice of prepubertal gonadectomy in our subjects was likely driven by concern that girls with PAIS would virilize during puberty, based on the assumption that prenatal androgen response would be mirrored by an equivalent response at puberty. However, evidence supporting this assumption is lacking. Furthermore, the majority of girls (73%) also underwent genital/vaginal surgery, although we could not detect an association between prenatal androgenization (as assessed by the EMS) and any postnatal clinical data. There was no evidence of germ cell neoplasia in the 9 individuals for whom testicular histology data were available, and therefore our data provide no evidence to support the practice of prophylactic prepubertal gonadectomy.

Our study has certain limitations, starting with the small number of evaluable subjects, with only 17% of PAIS subjects in the database being registered as female. This finding is possibly explained in part by a bias toward
reporting outcome data for individuals with PAIS assigned male, as in fact a greater number of individuals were raised as girls than boys during the time frame of this study. A detailed study of gender assignment practices indicated that 62–67% of individuals with various 46,XY differences of sex development born before 1999 (the same time frame as 82% of our study subjects) were assigned female [Kolesinska et al., 2014]. Of the 40 female-assigned subjects in the I-DSD Registry, 9 (23%) fulfilled our criteria for evaluation (2 further subjects were added by investigators). Because of these limited subject numbers, our findings may not be representative of the broader population of girls and women living with PAIS. In addition, because all of our subjects were born before 2005, our findings reflect practices that have likely changed substantially, particularly after publication of the 2006 “DSD guidelines” [Lee et al., 2006; Kolesinska et al., 2014].

Although our cohort was small, the fact that we included only individuals with molecularly proven AR variants represents a strength of our study. Earlier studies of cohorts with the presumptive diagnosis of PAIS but without molecular confirmation of AR variants, have led to assumptions based on a mixed bag of diagnoses, ranging from mild forms of gonadal dysgenesis and deficiencies in steroidogenesis [Boehmer et al., 2001], to phenocopies of PAIS involving mutations in other molecular members of the AR signaling pathway [Hornig et al., 2016; Ilaslan et al., 2020]. As a result of low-stringency diagnoses, much of the prognostic information for PAIS to date may have been unreliable, even for key outcomes such as neoplasia risk. Furthermore, clinical tools such as the EMS or hCG stimulation tests have been unable to differentiate between cases with or without genetic variant [Deeb et al., 2005].

In our cohort, 8 of 10 variants affected AAs in the ligand-binding domain of the protein, as expected based on the known distributions of AR gene variants [Deeb et al., 2005; Lek et al., 2018]; 5 of our variants have been reported previously in subjects with PAIS. However, 2 of our identified variants (F828V and 1898T) have been reported only in girls/women with CAIS [Hiort et al., 1998; Chávez et al., 2001]. Phenotypic variation in association with the same mutation is well established within PAIS families, including siblings discordant for gender assignment [Evans et al., 1997; Boehmer et al., 2001]. However, our finding of variants previously reported only in CAIS (i.e., no androgen effect) in two individuals with PAIS and significant androgen effect (EMS of 5 in both) is unusual and expands the list of AR variants associated with variable phenotypes (http://androgendb.mcgill.ca/variable.pdf). Nevertheless, although molecular confirmation of the diagnosis of PAIS is important to ensure accuracy of diagnosis, our finding of further genotype-phenotype heterogeneity confirms that the specific AR variant cannot be used as a basis for gender assignment in PAIS, as previously demonstrated in the large Cambridge cohort [Deeb et al., 2005].

Despite limited numbers, our data offer some insights into the broad variability of phenotype, management, and outcomes in individuals with PAIS assigned female. For example, our results expand the number of individuals with PAIS who have no evidence of gonadal neoplasia. This is important because the gonads of many individuals with AIS (both partial and complete) have been removed over the past 60+ years on the assumption of substantial neoplasia risk. However, detailed retrospective and, more recently, prospective analyses have questioned this long-held assumption [Ahmed et al., 2000a; Audi et al., 2010; Cools et al., 2017]. Given that the testes of individuals with AIS are normally formed and not dysgenetic, the neoplasia risk is now believed to be comparatively low, more likely related to the risk associated with non-scrotal gonadal position and mis-regulation of growth factors than to intrinsic neoplastic potential, similar to the risk in boys and men with cryptorchidism [Pettersson et al., 2007; Ferguson and Agoulnik, 2013; Cools and Looijenga, 2017]. Consistent with this expectation, reports of testicular neoplasia in individuals with PAIS are uncommon. In a comprehensive 2017 study of individuals with PAIS and genetically confirmed AR variants, pre-germ cell neoplasia in situ (pre-GCNIS) was found in only 1 of 17 tests samples from 10 postpubertal individuals (6 male, 4 female) aged 14–54 years, and no invasive neoplasia was detected [Cools et al., 2017]. Similarly, no neoplasia was found in 24 testes of 35 individuals with a clinical diagnosis of PAIS (not molecularly confirmed) in the 2020 European dsd-LIFE study [Słowikowska-Hilczer et al., 2020]. Thus, from these 2 recent PAIS studies, pre-GCNIS was found in only 1 of 41 testes (<3%) of postpubertal individuals, and no invasive neoplasia was detected. In addition, a retrospective survey conducted via the I-DSD network found no reports of neoplasia among 26 men with PAIS [Tack et al., 2018].

No data for testicular neoplasia rates specific to individuals with PAIS reared female have been published, and because data from our study are limited to retrospective analysis of samples from pre- and peri-pubertal girls, they cannot provide reassurance regarding long-term neoplasia risk with testes in situ. Nevertheless, there is no a priori reason to suspect that neoplasia risk would be intrinsically
greater in those living as women compared with those living as men (apart from the risk associated with non-scrotal gonad location), and the overall risk in PAIS appears relatively low – probably fewer than 10% of individuals, depending on age at gonadectomy within the individual series [Cools et al., 2017; Tack et al., 2018]. Additional data collected prospectively on larger cohorts using modern histopathological methods linked with genetic information are needed to evaluate the long-term neoplasia risk associated with the retention of testes in individuals with PAIS, whether living as men or women.

In addition to neoplasia risk, a second clinical factor important to informed decision-making for those with PAIS is the individual degree of retained androgen responsiveness. In typical clinical practice in the 1960s–1990s, the degree of masculinization at birth was assumed to predict the likelihood of further masculinization at puberty and was used as one rationale for gonadectomy in children with PAIS assigned female [Hughes et al., 2006]. The “gestalt” of androgen responsiveness based on genital appearance was supplemented in 2000 by the formalized EMS system, which has proven very useful by allowing consistent comparisons across populations, diagnoses, and studies [Ahmed et al., 2000b]. Androgen responsiveness is relevant to individuals living as girls who may be concerned about the potential increase in masculinization at and beyond puberty with retained testes, and similarly to those living as boys, whose concerns will likely focus on the adequacy of androgen-mediated genital development.

The question of predicting pubertal outcome was examined in a Cambridge study of 27 male-reared subjects with PAIS, in whom the median EMS was 4.7 [Lek et al., 2018], very similar to the median EMS of 5 for our female-reared subjects. In the Cambridge cohort, an EMS of ≥5 at birth was strongly associated with attainment of Tanner stage 4–5 male genital development in late puberty. However, the EMS did not predict breast development/gynecomastia, which was almost universal in this cohort. The EMS was also a good predictor of pubertal masculinization in a cohort of male-reared subjects ascertained from the I-DSD Registry, where gynecomastia was universal [Lucas-Herald et al., 2016]. However, in the present study, the utility of the EMS as well as the EGS for predicting degree and type of pubertal development in girls with PAIS was impeded due to prepubertal gonadectomy in all but 4 girls. Three of the 4 girls who did not undergo prepubertal gonadectomy presented at puberty, and therefore the question of predicting in infancy the degree of future masculinization would not have arisen. Furthermore, concerning the use of the EGS, this information is missing in one subject. As we were unable to demonstrate utility of the EMS or the EGS for predicting pubertal outcome in our female PAIS cohort, this question will need to be addressed in future prospective studies.

Among the additional factors for consideration regarding the fate of the testes is the potential for viable testicular germ cells (spermatogonia) that could be used to generate biological offspring. Reproductive potential for individuals with PAIS has not been studied in detail, but there have been anecdotal reports of fertility in men with AR gene variants [Lucas-Herald et al., 2016; Giwercman et al., 2000; Petrolò et al., 2014; Tordjman et al., 2014], both via natural methods and assisted reproductive strategies. In our study, information was available on presence of germ cells or other relevant testicular findings such as Leydig cells or Sertoli cell hyperplasia for only 2 individuals, whose data were reported by Cools et al. [2017]. In that study, germ cells were present in 15/17 (88%) gonadal samples of individuals with PAIS, and an earlier study found germ cells in 50% of testes from postpubertal women with CAIS [Nakhal et al., 2013]. Although this information suggests that individuals with AIS may have the potential for fertility via assisted reproductive strategies, there is a long way before the viability of these germ cell for this purpose can be stated.

In addition to the questions of neoplasia risk, potential androgen effects at puberty, and opportunities for fertility, other factors such as the impact of hypogonadism on bone strength, sexual function, and quality of life, as well as the non-physiologic nature of available hormone replacement strategies, may raise concerns for affected women [Deans et al., 2012; Nakhal et al., 2013]. At present, there are insufficient data from this or other studies to inform clinical judgment regarding long-term risk-benefit of retention versus removal of testes in individuals with PAIS raised as girls. Therefore, prophylactic gonadectomy should not be a foregone conclusion. Consideration should be given to retaining the gonads on an individual basis, with fully informed consent regarding the known and unknown risks of retention versus removal. Regular surveillance should be provided by the most sensitive means available [Chauhdry et al., 2017; Dohnert et al. 2017; Ali et al., 2019], where feasible with surgical relocation of gonads to a position to facilitate monitoring and biopsy with detailed histological analysis to assess the presence of (pre-) neoplasia and numbers/quality of germ cells.

Of equal importance to the fate of the gonads in the lives of individuals with PAIS is the integrity of their genitalia. In our study, 8 of 11 girls underwent genital/vaginal...
surgery; this was assumed to have been performed during infancy for the 5 girls who presented in the first year of life, as this was standard practice at the time these girls were born, and occurred during the teenage years for the 3 girls who presented around the time of puberty. We have no information on the factors underlying the decision to perform genital surgery in our cohort, but in most cases, this was likely done to create an appearance closer to that of “typical” female genitalia. We also have no long-term follow-up data on the outcomes of these surgeries or their impact on either sexual function or quality of life in this cohort. However, systematic analyses of individuals with various anomalies of sex development have reported significant impacts of surgery in those living in either female or male gender [Cohen-Kettenis, 2010; Kohler et al., 2012]. Furthermore, gender identity disorder has been reported in 12–25% of individuals with PAIS [Babu and Shah, 2020] and self-initiated gender change has been reported in approximately 9%, with similar numbers changing from male to female and from female to male [Mazur, 2005].

These data indicate that up to one-quarter of individuals with PAIS who were managed using protocols and methods in place in the 1990s–2000s may be dissatisfied with their assigned gender. However, outcome data in this area are contentious, and opinions on the prevalence, etiology, and implications of gender issues in those with variations in sex development are conflicting. In the absence of large, well-designed prospective studies, no firm conclusions can be drawn and no guidance can be offered. Nevertheless, the basic ethical principle of keeping options open for the future should be considered when faced with life-altering decisions regarding irreversible gonadal and genital interventions in PAIS and other conditions affecting sex development [Lee et al., 2016].

In conclusion, our study found no evidence of testicular neoplasia in pre- and peri-pubertal girls with molecularly confirmed PAIS and raised questions regarding the rationale for, and/or timing of, gonadectomy in PAIS. The small number of postpubertal women with PAIS in the I-DSD database highlights the importance of increasing enrollment in registries, with data collection following international guidelines for consistency [Flück et al., 2019]. Considering the importance of leaving options open for the future for affected individuals [Lee et al., 2016], such additional prospectively collected data are needed to provide the evidence base for informed decision-making for parents, affected individuals, and care providers.

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Statement of Ethics

The I-DSD Registry is approved for collection of data obtained during routine clinical care following informed consent. This study protocol was reviewed and approved by the West of Scotland Research Ethics Committee, approval number [19/WS/0131]. (Further info: https://home.i-dsd.org/ethicsandirbapproval/). Written informed consent is obtained from participants (or their parent/legal guardian/next of kin) to participate in the study. (Further info: https://home.i-dsd.org/information-sheets-consent-forms/).

Conflict of Interest Statement

The authors have no conflicts of interest/competing interests to disclose.

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Author Contributions

Guilherme Guaragna-Filho, Gil Guerra-Junior, Jillian Bryce, Charmian A. Quigley, and S. Faisal Ahmed conceptualized and designed the study, designed the data collection instruments, coordinated and supervised data collection, performed the initial analyses, drafted the initial manuscript, and approved the final manuscript as submitted. Rieko Tadokoro-Cuccaro, Ieuan A. Hughes, Beatriz A. Barros, Olaf Hiort, Antonio Balsamo, Tulay Guran, Paul M. Holterhus, Sabine Hannema, Sukran Poyrazoglu, and Feyza Darendeliler coordinated and supervised data collection, critically reviewed the manuscript, and approved the final manuscript as submitted. The contribution made by the late Carlo Acerini to the early part of this study is acknowledged.

Data Availability Statement

Data and material are available according to the ethical standards of the I-DSD Registry.
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