

1 **A single-center, observational study of 607 children & young people**
2 **presenting with Differences in Sex Development (DSD)**

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1 **ABSTRACT**

2

3 *Context:* Differences in sex development (DSD) represent a wide range of conditions
4 presenting at different ages to various health professionals. Establishing a diagnosis,
5 supporting the family and developing a management plan are important.

6 *Objective:* We aimed to better understand the presentation and prevalence of pediatric
7 DSD.

8 *Design:* A retrospective, observational cohort study was undertaken of all children and
9 young people (CYP) referred to a DSD multi-disciplinary team over 25 years (1995-
10 2019).

11 *Setting:* A single tertiary paediatric center.

12 *Participants:* In total, 607 CYP (520 regional referrals) were included.

13 *Main Outcome Measures:* Data were analyzed for diagnosis, sex-assignment, age and
14 mode of presentation, additional phenotypic features, mortality, and approximate point
15 prevalence.

16 *Results:* Amongst the three major DSD categories, sex chromosome DSD was diagnosed
17 in 11.2% (68/607) (most commonly 45,X/46,XY mosaicism), 46,XY DSD in 61.1%
18 (371/607) (multiple diagnoses often with associated features), while 46,XX DSD
19 occurred in 27.7% (168/607) (often 21-hydroxylase deficiency). Most children (80.1%)
20 presented as neonates, usually with atypical genitalia, adrenal insufficiency, undescended
21 testes or herniae. Those presenting later had diverse features. Rarely, the diagnosis was
22 made antenatally (3.8%, n=23) or following incidental karyotyping/family history
23 (n=14). Mortality was surprisingly high in 46,XY children, usually due to complex
24 associated features (46,XY girls, 8.3%; 46,XY boys, 2.7%). The approximate point
25 prevalence of neonatal referrals for investigation of DSD was 1 in 6,347 births, and 1 in
26 5,101 overall throughout childhood.

27 *Conclusions:* DSD represent a diverse range of conditions that can present at different
28 ages. Pathways for expert diagnosis and management are important to optimize care.

29

30

1 **Introduction**

2

3 Differences in Sex Development (DSD) (also known as disorders of sex development,
4 variations in sex characteristics, or sometimes intersex) represent a wide range of
5 conditions that can be diagnosed at several different stages of life and that can first
6 present to health professionals in diverse disciplines (1-3).

7

8 The most common presentation is in the neonatal period, when a baby is born with
9 atypical (“ambiguous”) genitalia and it is not immediately clear whether the child is a boy
10 or a girl. In other situations, DSD can be diagnosed due to a mismatch between prenatal
11 karyotype and phenotype; due to associated features in childhood (e.g., renal, herniae);
12 during adolescence with absent puberty, virilization, estrogenization or primary
13 amenorrhea; or sometimes in adulthood with infertility or as an incidental finding.

14

15 DSD was originally defined as situations where “chromosomal, gonadal or anatomical
16 sex is atypical” (4). Three major categories are usually considered: sex chromosome DSD
17 (SCDSD, where there is an imbalance in sex chromosome complement), 46,XY DSD,
18 and 46,XX DSD. However, these are only general categories and many specific
19 conditions exist within them. Reaching a specific diagnosis is crucial for initial
20 management, support and education, as well as for planning long-term care through
21 adolescence and into adulthood (3, 5, 6).

22

23 Despite progress at many levels, the debate about what constitutes “DSD” continues and
24 the incidence of different DSD diagnoses and their relative prevalence is not well
25 established. More common genital variations such as distal hypospadias may affect up to
26 1 in 250-300 boys (7), whereas the prevalence of “DSD” requiring further investigation is
27 often cited as approximately 1 in 5,000 children, but robust data are limited (8-10). One
28 of the most important DSD-related conditions, classic congenital adrenal hyperplasia
29 (CAH) (mostly 21-hydroxylase deficiency), affects approximately 1:13,000-18,000 of all
30 children in most countries, and approximately 1 in 30,000-45,000 of all newborns will be
31 a 46,XX infant with atypical genitalia due to CAH (11). In contrast, 46,XY gonadal

1 dysgenesis (sometimes called “Swyer syndrome”) and complete androgen insensitivity
2 syndrome (CAIS) are less common, and often present in teenage years (12). Important
3 global differences in the incidence of different conditions also exist.

4
5 In order to address some of these questions and to obtain a better understanding of DSD
6 demographics, we present an overview of 25 years’ experience of DSD from a single-
7 center pediatric multidisciplinary team (MDT). We provide insight into the referral
8 patterns, range of presentations and diagnoses, associated features and approximate
9 prevalence of conditions.

10 11 12 **MATERIALS AND METHODS**

13 14 **Cohort & setting**

15
16 The study included all children and young people (CYP) discussed at the monthly DSD
17 MDT meeting at Great Ormond Street Hospital (GOSH) NHS Foundation Trust between
18 1st January 1995 and 31st December 2019. This forum brings together paediatric
19 specialists in psychology, urology, endocrinology and allied disciplines (including
20 clinical genetics, biochemistry, gynecology, nursing) to review new referrals, and also
21 provides input at times when follow-up discussions are required. Services for young
22 people first presenting in mid-teenage years (>13 years) are generally provided by
23 University College London Hospitals and these data are not included here.

24
25 A total of 758 case records were reviewed (Fig. 1). Children were excluded if their initial
26 presentation was before 1995 but they were under long-term follow up (n=119), as this
27 subgroup was biased because it included children with more complex diagnoses rather
28 than reflecting the relative proportion of new presentations accurately. Children with
29 cloacal anomalies or bladder exstrophy (n=32) were also excluded as an independent
30 specialist service for these conditions was established during the study period (Fig. 1).

31 Therefore, the final study cohort included 607 CYP who first presented to health
32 professionals between 1995-2019. Of these, 520 CYP were “regional” referrals from

1 London and the South-East of England, 49 were from the rest of the United Kingdom
2 (UK), and 38 were international referrals or children/young people whose families
3 relocated to the UK after the initial diagnosis had been made (Fig. 1, see also
4 Supplemental Fig. 1) (13).

6 **Data acquired**

7
8 Individual records were retrospectively evaluated by two observers (E.M, J.C.A., with
9 input also from C.R.H and K.D). A diagnostic category was assigned to each child based
10 on available data and the template of the Chicago Consensus Meeting (4).

11
12 Key data obtained from records included karyotype, biochemical and molecular genetic
13 diagnosis, sex assignment, age and mode of first presentation, additional clinical features
14 or syndromes, and mortality.

16 **Ethical Review**

17
18 This study was approved as a clinical service evaluation by the GOSH Audit office
19 (1706: Evaluation of clinical pathways for children with disorders of sex development)
20 under NHS Research Guidelines.

22 **Statistical analysis**

23
24 Data trends over time were analyzed using 3-point moving averages and categorical
25 variables were compared using Chi-Square analysis (IBM SPSS Statistics²⁷, IBM Corp.,
26 Armonk, New York, USA). Two-tailed P values of less than 0.05 are considered
27 statistically significant.

29 **Reporting principles**

30

1 As this is a retrospective, cross-sectional observational study, data are presented using
2 STROBE principles.

3

4

5 **RESULTS**

6

7 **Overview of the cohort**

8

9 The median number of new children presenting to the MDT each year was 23 (range 15-
10 40) (total n=607) (Fig. 2A). There were no significant changes over time ($R=0.20$,
11 $p=0.33$). Sex chromosome DSD (SCDSD) accounted for 11.2% (68/607) of subjects,
12 46,XY DSD was found in 61.1% (371/607), and 46,XX DSD occurred in 27.7%
13 (168/607) (Fig. 2B). Overall there was discordance between karyotype and sex in 14.8%
14 of subjects for the study cohort as a whole (13.3% 46,XY children brought up as girls,
15 hereafter termed “46,XY girls”; 1.5% 46,XX children brought up as boys, hereafter
16 termed “46,XX boys”) (Fig. 2B). The percentage of 46,XY girls was slightly lower
17 (11.5%, 60/520) when regional referrals from just London and the South-East of England
18 were considered (Fig. 2B). Of note, many children had a phenotype consistent with the
19 sex they were brought up in and presented for other reasons (e.g. a phenotypic girl with
20 complete androgen insensitivity syndrome and 46,XY karyotype presenting with an
21 inguinal hernia). Less frequently, a child had atypical genitalia and a decision was made
22 by the parents together with the MDT to bring them up in a sex different to their
23 karyotype (see “46,XY girls” below).

24

25 **Categories of DSD**

26

27 An overview of the diagnoses in each major category of DSD is shown in Table 1 and
28 Supplemental Figures 2-4 (13).

29

30 ***Sex Chromosome DSD (SCDSD)***

31

1 Most children presenting with SCDS had 45,X/46,XY mosaicism or variations of this
2 karyotype (56/68, 82.4%) (Table 1, Supplemental Fig. 2) (13). Within this 45,X/46,XY
3 group, 70% (39/56) of children were brought up as boys and 30% (17/56) were brought
4 up as girls. True 46,XX/46,XY chimerism was very rare, being found in only four
5 children in 25 years (5.9% of SCDS, and 0.7% (4/607) of the total cohort overall).
6 Although some studies consider Turner syndrome/monosomy X within a DSD
7 classification, the vast majority of girls with classic Turner syndrome do not present with
8 DSD and did not get referred through a DSD MDT pathway.

9 10 **46,XY DSD**

11
12 The largest and most diagnostically-diverse category was 46,XY DSD (Table 1,
13 Supplemental Fig. 3) (13). Whilst there were many specific diagnoses affecting testis
14 development, androgen synthesis and androgen action, a large number of this cohort were
15 boys with penoscrotal hypospadias without a specific diagnosis (125/371, 33.7%) (Table
16 1). Many children in this group had fetal growth restriction (FGR) (sometimes called
17 intra-uterine growth restriction) (63/125, 50.4%), additional/complex features (18.4%), or
18 isolated cardiac (2.4%) or renal anomalies (2.4%) (Supplemental Fig. 3B) (13). Indeed, in
19 the 46,XY cohort as a whole, 57/371 (15.4%) children had a defined genetic syndrome
20 (35 different conditions) and a further 78/371 (21.0%) had non-specific complex
21 phenotypes (which may represent as yet undiagnosed conditions), giving a total of
22 135/371 (36.4%) children with significant additional features (Supplemental Table 1)
23 (13).

24 25 **46,XX DSD**

26
27 The most common diagnosis within the 46,XX group was CAH (100/168, 59.5%).
28 Within the CAH group, 21-hydroxylase deficiency (21OHD) was by far the most
29 prevalent condition (92/100, 92.0%) (Table 1, Supplementary Fig. 4) (13). Clitoromegaly
30 associated with prematurity was seen in 10.1% (17/168) children, whereas a small but
31 important group of girls who were referred had variations of anatomy considered to be

1 within the “normal” range (11/168, 6.5%). Ovotesticular or testicular DSD
2 (OTDSD/TDSD) was present in 7.7% children (13/168; eight raised female, five raised
3 male). Of note, OTDSD was more common in 46,XX children from an African/Black
4 British background (5/16 [31.3%]) compared to children from a White background (2/79
5 [2.5%]; Chi Sq=16.08, $p<0.0001$). Indeed, OTDSD was at least as common as CAH
6 (21OHD) in 46,XX children of African or Black British background (OTDSD, 5/16
7 [31.3%] versus 21OHD 3/16 [18.8%]; Chi Sq=0.67, $p=0.41$). In contrast, CAH (21OHD)
8 was 24-fold more common than OTDSD in 46,XX children of a White background
9 (21OHD 52/79 [65.8%] versus OTDSD 2/79 [2.5%]; Chi sq=70.33, $p<0.0001$). Only 11
10 children with 46,XX DSD had syndromic associations, most often Mayer-Rokitansky-
11 Küster-Hauser (MRKH) syndrome, a form of uterine agenesis ($n=8$) (Supplemental Table
12 1) (13). MRKH syndrome does not present with atypical genitalia and is usually
13 diagnosed in teenage years, but some girls were referred in childhood with herniae
14 containing descended gonads (ovaries), or because of incidental findings of an absent
15 uterus.

17 **Presentation of DSD**

19 Within the entire cohort, most children (486/607, 80.1%) presented during the neonatal
20 period (< 28 days of age), usually due to isolated atypical genital appearances (371/486,
21 76.3% of neonatal referrals), but sometimes also because of undescended testes and
22 hernia(e) (36/486, 7.4%), or adrenal insufficiency (53/486, 10.9%; 48 with atypical
23 genitalia) (Fig. 3A, *left panel*). An antenatal diagnosis had been made in 3.8% of children
24 in the entire cohort (23/607), 11 of whom had atypical genitalia at birth. Within the
25 London and South-East region, 352/520 (67%) patients presented with atypical genitalia.

27 Those children presenting *after* the first month (121/607, 19.9%) showed a range of
28 different features (Fig. 3A, *right panel*). Genital anomalies were still a surprisingly
29 common reason for presentation, often due to delayed presentation/referral of children
30 with clitoromegaly or a small penis (51/121, 42.1%). Bilateral undescended testes
31 (BUDT)/inguinal herniae were the presenting features in 16/121 (13.2%) of children, of

1 whom five had CAIS (46,XY), one had 17 β -hydroxysteroid dehydrogenase deficiency
2 type 3 (*HSD17B3*) (46,XY), one had a WT1-associated condition (46,XY), five had
3 persistent Müllerian Duct Syndrome (PMDS) (46,XY) and four had Mayer-Rokitansky-
4 Küster-Hauser (MRKH) syndrome (46,XX, inguinal herniae). A diagnosis of DSD after
5 the neonatal period was made in 14 (11.6%) children by karyotyping, either as an
6 incidental finding during genetic testing for other features or because of a known family
7 history of DSD. Other modes of presentation are shown in Fig. 3A.

8
9 Presentation with atypical genitalia in the newborn period was the most common
10 presentation in all major subgroups of DSD except 46,XY girls, who were almost as
11 likely to present later in infancy or childhood as in the first month, or who often
12 presented with BUDT/inguinal herniae rather than atypical genitalia (Fig. 3B & C).

14 **46,XY girls (Girls with a Y)**

15
16 Available data for 46,XY girls were analyzed in more detail, as one of the more
17 challenging situations is when a 46,XY child presents in the newborn period with atypical
18 genitalia and discussions are held with parents whether to bring the child up as a girl or
19 boy. We therefore focused on children referred from London and the South East Region
20 of England to obtain a better reflection of UK practice (Fig. 1, Supplemental Fig. 1) (13).
21 Within this group, 46,XY girls represented 11.5% (60/520) of all children (Fig. 2B).

22
23 Although around half of all 46,XY girls presented early in life (29/60, 48.3%, with three
24 additional children diagnosed prenatally), only 16 of them (16/60, 26.7%) presented with
25 *virilized* atypical genitalia (i.e. clitoral enlargement, partial labioscrotal fusion) in the
26 newborn period, as opposed to palpable inguinoscrotal or labioscrotal testes but no signs
27 of androgenization (Fig. 4A). This represents 4.5% (16/352 of all “regional” children
28 presenting with atypical genitalia in the newborn period (Fig.4B) and 3.1% (16/520) of
29 “regional” DSD referrals overall, so this is not a common scenario. Of note, no 46,XY
30 child with partially androgenized genitalia was raised female in the last 5 years of the
31 study.

1
2 Genetic testing was offered and a specific genetic diagnosis was available in most
3 children (43/60, 71.7%), with the range of conditions shown in Fig. 4C. A prior family
4 history of DSD was present in 9/29 (31.0%) of 46,XY girls who presented in the newborn
5 period and a genetic diagnosis was reached in eight of them.

6 7 **46,XX boys**

8
9 Five children from London/South-East England with a 46,XX karyotype and atypical
10 genitalia in the newborn period were brought up as boys (5/352, 1.4% of all newborns
11 with atypical genitalia; 5/520, 0.96% of all “regional” DSD referrals) (Fig. 4B). Most of
12 these children had OTDSD. An additional two 46,XX boys presented after the first month
13 of life.

14 15 **Mortality**

16
17 Seventeen children died within the follow up period when records were available
18 (SCDSD, n=1; 46,XY DSD, n=16). Median age of death was 5 months (range: 1 month -
19 9 years). All children had established syndromes (e.g. X-linked lissencephaly, ATRX,
20 campomelic dysplasia/SOX9, Scimitar syndrome), chromosomal deletions or complex
21 multisystem involvement, often associated with fetal growth restriction. Mortality was
22 sometimes linked to neurological/brain anomalies (n=5), cardiac defects (n=3), or
23 immune dysfunction and sepsis (n=3), although children often had complex multiorgan
24 dysfunction. Mortality in the first year of life was higher in 46,XY girls (5/60, 8.3%),
25 compared to 46,XY boys (7/256, 2.7%; Chi-Sq 4.46, p<0.05), usually linked to the
26 specific syndromes above which were associated with significant gonadal dysgenesis.
27 Mortality was substantially higher than national rates of mortality for children within
28 these age ranges (Infant Mortality rate, 0.37%; Child Mortality rate (1-15 years), 0.008%;
29 2019 ONS E&W).

30
31

1 **Incidence of DSD**

2

3 Based on Office for National Statistics (ONS) data for birth rates in England & Wales
4 between 1995-2019, and assuming that the “regional” catchment area (London/South-
5 East, Supplemental Figure 1) (13) represents one-seventh of this population, the number
6 of live births “captured” averaged approximately 106,000 per annum (2,653,000 total
7 births over the study period) (14). Based on these data, the estimated point prevalence of
8 DSD referrals *in the neonatal period* (first 28 days) was 1 in 6,347 overall
9 (418/2,653,000; 15.8/100,000); 1 in 32,753 for 21-hydroxylase deficiency (81/2,653,000;
10 3.1/100,000); 1 in 85,906 for 46,XY girls overall (32/2,653,000; 1.16/100,000) and 1 in
11 165,812 for 46,XY girls with virilised genitalia (16/2,653,000; 0.6/100,000)
12 (Supplemental Table 2) (13). The overall prevalence of DSD referrals over the study
13 period was 1 in 5101.

14

1 DISCUSSION

2
3 Here, we present a large, single-center study of the demographics of DSD, involving
4 more than 600 children over a 25-year period between 1995-2019. We used the
5 framework of the Chicago Consensus Meeting to divide children into the three broad
6 categories of sex chromosome DSD (SCDSD), 46,XY DSD and 46,XX DSD, and used
7 available phenotypic, biochemical and genetic data to make a more specific diagnosis
8 wherever possible.

9
10 With this approach, we found that most children referred to specialist services had
11 variations of 46,XY DSD (61.1%), with smaller proportions having 46,XX,DSD
12 diagnoses (27.7%) or SCDSD (11.1%). Referral patterns were remarkably stable over this
13 25-year period, with some year-on-year fluctuations given the small numbers of some
14 categories considered. The predominance of 46,XY DSD has been seen in most other
15 smaller single center studies to date (Table 2) (15-19). In contrast, a recent multi-centre
16 registry study from the USA has described a higher proportion of children with 46,XX
17 CAH (57%), but this finding may reflect differences in referral pathways and a higher
18 threshold for referring some forms of penoscrotal hypospadias to specialist DSD services
19 (20).

20
21 Children with sex chromosome variations (SCDSD) were the smallest group within the
22 cohort (11.1%). Here, the diagnosis can usually be made on karyotype alone. Most of
23 these children had 45,X/46,XY mosaicism, or related variations and 70% were brought
24 up as boys. In fact, based on published antenatal data, more than 90% of children with a
25 45,X/46,XY karyotype are boys who would not present to health professionals, at least in
26 the early years, so those children with atypical genitalia represent a relatively small
27 subgroup of all children with this karyotype (21, 22). Given the phenotypic variability
28 and asymmetry of internal and external anatomy often seen in these children, an
29 individualized approach to management is needed (21, 23, 24), taking into account the
30 degree of gonadal compromise, the likelihood of gonadal tumours, and the use of growth
31 hormone to promote growth. Long-term surveillance is also required for potential

1 features associated with Turner syndrome (25). A similar individualized approach is also
2 required for the small group of children with 46,XX/46.XY chimerism, who can have
3 both ovarian and testicular tissue and who also have variable and often asymmetrical
4 features, although in our series all four children with this karyotype were brought up as
5 girls.

6
7 The largest and most diverse group was 46,XY DSD (61%). Some of these children had
8 specific diagnoses but many were classified with “penoscrotal hypospadias (PSH)”. A
9 large subset of these boys had FGR/IUGR and were born preterm. The association of
10 PSH and FGR is well established and can even occur in the smaller of two 46,XY
11 dizygotic twins (26, 27). Established genetic diagnoses (such as partial androgen
12 insensitivity) are rarely found, but can occur coincidentally (27). The link between FGR
13 and PSH may reflect an epigenetic phenomenon, a shared placental/genital gene, or a
14 hypothesised interplay between placental insufficiency and fetal androgen synthesis.
15 Furthermore, FGR is a key feature of specific conditions such as MIRAGE syndrome
16 (*SAMD9*) and IMAGE syndrome (*CDKN1C*) (28). Our study clearly demonstrated that
17 many children (36.4%) with 46,XY DSD phenotypes had complex additional features or
18 a range of defined syndromes; this finding is becoming well-established (19, 29). Indeed,
19 mortality was especially high in the 46,XY children with complex features, especially in
20 the first year of life (more than 20 times higher than current national data), reflecting
21 often complex neurodevelopmental issues, cardiac defects or immune dysfunction. Thus,
22 MDT support, including specialist clinical genetics input, is important for families.
23 Increased availability of clinical genetic testing using whole genome CGH arrays,
24 targeted gene panels or exome sequencing may play a role in defining these associations
25 further in coming years, leading hopefully to more focused management plans (30, 31).
26 Even in those children without associated features, making a specific diagnosis early on,
27 such as 5 α -reductase deficiency, can have significant implications for management, and
28 genetic analysis has an increasing role to play in this situation as biochemical analysis is
29 unreliable in early life (6).

30

1 One important sub-group of 46,XY DSD is those children with a 46,XY karyotype who
2 are brought up as girls. These children represented 11.5% of the entire “regional” cohort,
3 but only 4.5% of newborn babies presenting with virilised atypical genitalia. Of note,
4 some babies with CAIS had atypical appearing genitalia due to the presence of descended
5 labial testes causing swelling, but without evidence of genuine
6 androgenisation/virilisation. Recent studies have suggested that there may be changes in
7 approaches to sex assignment over time, so that 46,XY children with virilized atypical
8 genitalia are less likely to be brought up as girls than in previous decades (32). The
9 numbers of children in our study are likely to be too small to draw firm conclusions,
10 although a similar trend was seen. Moreover, a specific genetic diagnosis was reached in
11 most 46,XY girls (73.3%), and higher in those with a family history, which has important
12 implications for targeting support, and planning short- and long-term management (30,
13 31).

14
15 Within the 46,XX category (27.7%) the most important and most common diagnosis is
16 CAH, and the most prevalent form of CAH was 21-OHD. CAH should be considered as
17 the diagnosis until proven otherwise in any newborn baby with atypical genitalia and
18 non-palpable testes, as the child is at risk of adrenal insufficiency and a progressive
19 potentially life-threatening salt-losing state that can develop during the first week of life
20 (33). Other rare forms of CAH were diagnosed, often in children whose families originate
21 from regions where there are known geographical hotspots (e.g. 11-hydroxylase
22 deficiency in Turkey). Of note was the observation that OTDSD was especially common
23 in 46,XX newborn of African or Black British ancestry. Although rare overall, OTDSD
24 was at least as common as 21-OHD in children from this background, in keeping with
25 recent data from several centres in Africa (Table 2) (16, 34-36). As the management of
26 OTDSD is markedly different to CAH, this is an important diagnosis to consider.

27 Measuring anti-Müllerian hormone (AMH) can be useful to investigate for the presence
28 of testicular tissue, although CAH should always be the working diagnosis until proven
29 otherwise, as it can be life-threatening if not detected and treated appropriately.

30

1 Several 46,XX newborns were diagnosed with persistent clitoromegaly associated with
2 prematurity. In some children, the clitoromegaly can persist or is associated with excess
3 clitoral skin, but other signs of early *in utero* androgen exposure (e.g., labial fusion,
4 common urogenital sinus) are not present (37). The etiology of this condition is not well
5 understood (38). Furthermore, a small but important group of 46,XX children referred
6 had labial adhesions, excess clitoral skin or genital anatomy within “normal” limits.
7 Labial swelling can occur in some situations (e.g. breech deliveries) and there can be
8 individual variability in genital appearances and ancestral differences in clitoral size in
9 different populations (39-42). Giving reassurance in such situations, after any simple
10 investigations the clinical team feel are necessary, is important.

11
12 Establishing the incidence of DSD is challenging, as there are a diverse range of
13 conditions and a spectrum of severity for each of them, and there is ongoing debate about
14 what constitutes “DSD”. Two recent population-based studies in Turkey and Ghana have
15 reported an incidence of atypical genitalia or “DSD” in newborn populations as 1 in 821
16 (18/14,777) and 1 in 356 (26/9255), respectively (43, 44). However, these studies
17 included all forms of hypospadias (Turkey) or children with variations of their genitalia
18 outside typical ranges (Ghana). Other studies have taken a national registry approach to
19 look at specific subgroups of DSD. In Denmark, where there is a national cytogenetic
20 registry, publications suggest a peak prevalence of women with a 46,XY karyotype
21 (women with a Y) as 1 in 15,625 women (so potentially 1 in 31,000 of the population
22 overall); CAIS as 1 in 24,390 women; and complete gonadal dysgenesis as 1 in 66,667
23 women (12). The peak prevalence of 46,XX men has been reported in Denmark as 1 in
24 28,571 men (so potentially 1 in 57,000 of the population overall) (45). In these studies the
25 median age of presentation for CAIS was 7.5 years, that for complete gonadal dysgenesis
26 was 17.0 years, for 46,XX males 17.0 years and for 46,XX males with testicular DSD
27 25.4 years. These findings highlight the need for long term follow up studies. Only a
28 subgroup of children with these conditions present before teenage years, which explains
29 the lower prevalence in our data (Supplemental Table 2) (13), but, as previously stressed,
30 these are extremely important diagnoses to make so that long-term support and
31 management can be appropriately planned.

1
2 By considering sequential referrals in a single centre over a long time period we have
3 been able to generate approximate prevalence data for childhood DSD overall
4 (approximately 1 in 5,100) and for referrals for further investigation and management in
5 the newborn period (approximately 1 in 6,347). These calculations assume that our centre
6 captures one-seventh of the population in England and Wales; this, however, may be an
7 underestimate as there are inevitable regional biases in referral patterns. Our centre often
8 deals with complex, tertiary referrals, but other centres in the region may see children
9 with DSD and other associated conditions (e.g. hypospadias), in which case the true
10 prevalence of DSD would be higher. Previously, Thyen et al. have suggested a birth
11 prevalence of 1:5000 in Germany, which is similar to our data (9). In contrast, Rodie et
12 al. have recently reported a birth prevalence of children requiring specialist assessment
13 for atypical genitalia of 1:3318 in Scotland, although 68% of these children were
14 assigned at birth before the results of more detailed investigations, so more complex DSD
15 may have a similar prevalence to in our study (10). Specifically focusing on CAH,
16 national registry data from the British Paediatric Surveillance unit (August 2007-August
17 2009) suggested an overall CAH incidence of 1 in 18,000 children (1 in 13,000-15,000 in
18 many countries), and that three-quarters of girls present in the newborn period with
19 genital changes (11). That would lead to an estimated newborn incidence of virilising
20 CAH in 46,XX children of around 1 in 48,000 of all children. Our data suggest a CAH
21 prevalence of 1 in 32,753 children overall, similar to the calculated birth prevalence of
22 CAH of 1 in 35,757 in Scotland (10). Of note, the United Kingdom does not have a
23 newborn screening programme for CAH.

24
25 As with most single center studies, this study has limitations in relation to referral biases
26 and the demographic mix of referral sources. London is a large city with families from
27 diverse backgrounds, so our data inevitably reflect that. Referrals of children from
28 outside the UK or who have relocated here generally involved more “complex”
29 diagnoses, and so we excluded these children for analysis of 46,XY girls and in
30 prevalence estimates, as discussed above. Other limitations are the somewhat restricted
31 use of genetics to investigate the potential etiology of conditions such as isolated

1 penoscrotal hypospadias, and we have not systematically included long-term outcome
2 data, such as gender identity (6). Alternative approaches to studying rare diseases include
3 national and international registry studies (such as iDSD and iCAH) (46, 47).

4
5 This study does also highlight the many different ways that children with DSD can
6 present and the central role the MDT has to play (2, 48). Educating a range of allied
7 professionals in the early approaches to a newborn or child with potential DSD is
8 important, including midwives and nurses, fetal medicine specialists and general
9 surgeons. Increasing emphasis now focuses on early psychological support for families
10 and children and the key value that support groups play in long-term management
11 throughout childhood, transition in teenage years and into long-term adult management
12 pathways (49, 50).

13
14 In summary, we present, to our knowledge, the largest single-center observational study
15 of DSD in newborns, children and young people reported over a 25-year period. These
16 data highlight the range of presentations, associated features and approximate prevalence
17 of these diverse conditions. This work should help in planning management strategies
18 and policy going forward.

19

1 **Contributors**

2 EM, MTD and JCA conceptualized the study. EM, CRH, KD and JCA undertook data
3 curation. EM and JCA undertook formal data analysis. MTD and JCA were involved in
4 funding acquisition. EM, IM, AB, PC, IAH, HK, CEB, MTD and JCA undertook
5 investigation, diagnosis and management, with the considerable support of other authors
6 (HA, RA, CRB, AC, NC, SMC, PGD, EH, PCH, LM, CJP, PGR, NS, HAS, DW). MTD
7 and JCA oversaw project administration and supervision. EM and JCA undertook
8 validation. EM and JCA were responsible for data visualization. EM and JCA wrote the
9 original draft with input from MTD. All authors and members of the GOSH DSD MDT
10 were involved in reviewing and editing the manuscript from an early stage. EM and JCA
11 had full access to all data in the study and had final responsibility for the decision to
12 submit for publication.

13

14 **Acknowledgments**

15

16 We are grateful to the young people and families, and to the many additional clinicians,
17 allied health professionals and trainees for their contributions to the multidisciplinary
18 team over these years. We are grateful to Les Perry and others for support with Chemical
19 Pathology. We also recognise the key role that David Grant played in establishing this
20 MDT.

21

22 **Data availability**

23

24 Most quantitative data generated are presented as in the accompanying tables, figures and
25 supplementary datasets (13). Restrictions apply to the availability of some data generated or
26 analyzed during this study to preserve patient confidentiality or because they were used under
27 license. The corresponding author will on request detail the restrictions and any conditions
28 under which access to some data may be provided.

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1 **FIGURE LEGENDS**

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4 **Figure 1.** Overview of the study cohort.

5

6 **Figure 2. A.** Number of children and young people (CYP) presenting to health
7 professionals and being referred to the multi-disciplinary team each year over 25 years
8 (3-point moving average shown by dashed line). **B.** Major DSD category and sex
9 assignment for the total cohort (n= 607) (*left panel*) and for regional referrals from
10 London and the South-East of England (n=520) (*right panel*). SCDS, sex chromosome
11 DSD.

12

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14 **Figure 3.** Initial presentation of DSD. **A.** Overview of presentation for the total cohort
15 (n=607) (*left panel*) and for children presenting outside the neonatal period (n=121)
16 (*right panel*). **B.** Mode of presentation for major DSD categories (n=607). **C.** Age at
17 presentation for different major DSD categories (n=607). *”Adrenal insufficiency”
18 includes 46,XX children with adrenal insufficiency due to congenital adrenal hyperplasia
19 (with atypical genitalia), and two 46,XY girls with congenital lipoid adrenal hyperplasia
20 (STAR). BU, bilateral undescended testes; SCDS, sex chromosome DSD.

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23 **Figure 4.** Presentation of 46,XY girls referred from London/South-East England. **A.**
24 Mode of presentation for 46,XY girls (n=60). **B.** Major DSD categories and sex
25 assignment for children presenting with atypical genitalia at birth or in the neonatal
26 period (n=352). **C.** Range of genetic diagnoses for 46,XY girls (n=60). Those children
27 with complete or partial gonadal dysgenesis and additional features are grouped under
28 “GD syndrome” and PGD syndrome”, respectively. “PAIS-like” represents children with
29 clinical/biochemical features of PAIS but where no AR or other pathogenic variant was
30 found yet. BU, bilateral undescended testes; CAIS, complete androgen insensitivity

1 syndrome; CYP17A1, combined 17 α -hydroxylase/17,20-lyase deficiency; GATA4,
2 GATA-binding protein 4; GD, gonadal dysgenesis; HSD17B3, 17 β -hydroxysteroid
3 dehydrogenase type 3 deficiency; PAIS, partial androgen insensitivity syndrome; PGD,
4 partial gonadal dysgenesis; POR, P450 oxidoreductase deficiency; SLO, Smith-Lemli-
5 Opitz syndrome; SRD5A2, 5 α -reductase type 2 deficiency; STAR, steroidogenic acute
6 regulatory protein/congenital lipoid adrenal hyperplasia; WT1, Wilms' tumor 1.

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Table 1. Overview of study cohort of children referred

Diagnosis	n	% of cohort (n=607)	Diagnosis	n	% of cohort (n=607)
Sex Chromosome DSD (SCDSD)	68	11.2	46,XY DSD	371	61.1
45,X and variants	2	0.3	Hypothalamic-pituitary disorders	15	2.4
45,X/46,XY and variants	56	9.2	<i>HH with hypopituitarism</i>	6	0.9
47,XXY	5	0.8	<i>HH - isolated</i>	9	1.4
46,XX/46,XY chimerism	4	0.7	Disorders of gonad development	85	14.0
Other (47,XXX)	1	0.2	<i>GD - unknown cause</i>	9	1.4
			<i>GD - syndrome</i>	5	0.8
			<i>Partial GD - unknown cause</i>	20	3.3
46,XX DSD	168	27.7	<i>Partial GD - syndrome</i>	6	1.0
Disorders of gonad development	13	2.2	<i>SRY</i>	2	0.3
<i>(Ovo)testicular DSD</i>	13	2.2	<i>SOX9</i>	1	0.2
Disorders of androgen excess	102	16.8	<i>NR5A1 (SF-1)</i>	8	1.3
<i>CAH - 3β-HSD type 2 deficiency</i>	3	0.5	<i>WT1</i>	12	2.0
<i>CAH - 21-hydroxylase deficiency</i>	92	15.2	<i>GATA4</i>	1	0.2
<i>CAH - 11β-hydroxylase deficiency</i>	5	0.8	<i>Testis regression/anorchia</i>	21	3.5
<i>Aromatase deficiency</i>	1	0.2	Disorders of androgen synthesis	43	7.08
<i>Adrenocortical tumor</i>	1	0.2	<i>Smith-Lemli-Opitz syndrome</i>	1	0.2
Other conditions	42	6.9	<i>Leydig cell hypoplasia</i>	2	0.3
<i>MRKH syndrome</i>	8	1.3	<i>STAR</i>	2	0.3
<i>Syndromes with genital anomalies</i>	3	0.5	<i>CAH - 3β-HSD type 2 deficiency</i>	2	0.3
<i>Ovarian anomaly - isolated</i>	5	0.8	<i>CAH - 17α-hydroxylase/17,20-lyase def.</i>	3	0.5
<i>Vaginal/labial anomaly - isolated</i>	2	0.3	<i>CAH - P450 oxidoreductase def.</i>	1	0.2
<i>Clitoral anomaly - isolated</i>	7	1.2	<i>17β-HSD type 3 deficiency</i>	16	2.5
<i>Clitoromegaly with prematurity</i>	17	2.8	<i>5α-reductase type 2 deficiency</i>	16	2.5
Within "normal" limits	11	1.8	Disorders of androgen action	31	5.1
			<i>Complete AIS</i>	17	2.8
			<i>Partial AIS</i>	8	1.3
			<i>Partial AIS-like</i>	6	1.0
			<i>Persistent Mullerian duct syndrome</i>	7	1.2
			<i>Penoscrotal hypospadias</i>	125	20.6
			<i>Penoscrotal hypospadias - isolated</i>	33	5.4
			<i>Penoscrotal hypospadias - FGR</i>	63	10.4
			<i>Penoscrotal hypospadias - cardiac</i>	3	0.5
			<i>Penoscrotal hypospadias - renal</i>	3	0.5
			<i>Penoscrotal hypospadias - complex</i>	23	3.8
			Other disorders	64	10.5
			<i>Penile anomaly - complex</i>	23	3.8
			<i>Undescended testes - isolated</i>	6	1.0
			<i>Testicular anomaly - isolated</i>	1	1.2
			<i>Testicular anomaly - syndrome</i>	4	0.7
			<i>Syndromes with genital anomalies</i>	30	4.9
			Within "normal" limits	1	0.2

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Abbreviations: AIS, androgen insensitivity syndrome; CAH, congenital adrenal hyperplasia; DSD, difference (disorder) in sex development; FGR, fetal growth restriction (also known as IUGR, intra-uterine growth restriction); GD, gonadal dysgenesis; HH, hypogonadotropic hypogonadism; HSD, hydroxysteroid dehydrogenase; MRKH, Meyer-Rokitansky-Küster-Hausler syndrome; PMDS, persistent Müllerian duct syndrome; SF-1/NR5A1, steroidogenic factor-1; STAR, steroidogenic acute regulatory protein/congenital lipoid adrenal hyperplasia; WT1, Wilms' tumor 1. Other conditions are shown by gene name. For further information on syndromic associations see Supplemental Table 1 and Supplemental Figure 3C (13).

14

1 “Within “normal” limits” most often included 46,XX girls referred for assessment who had labial
2 adhesions, perineal edema or excess clitoral skin.
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1 **Table 2.** Overview of studies reporting the relative prevalence of different DSD categories

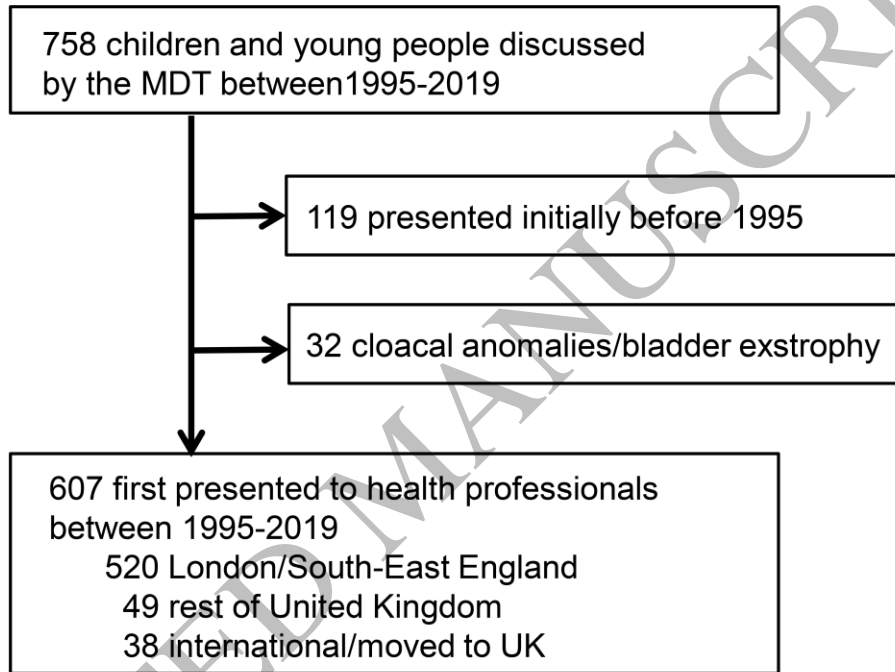
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Study author ^a	Study period	Country	Study design	Total number	Inclusion	SCDSD (%)	46,XY DSD (%)	46,XX DSD (%)
This study	1995-2019	UK	Single centre	607	DSD MDT forum	11.2	61.1	27.7
Ganie et al. (16)	1995-2014	South Africa	Single centre	416	DSD diagnosis in an endocrine clinic	9.5	57.5	33 ^b
De Paula et al. (15)	1989-2011	Brazil	Single centre	408	Atypical genitalia	8.3	61.3	30.4
Ata et al. (18)	2002-2018	Turkey	Single centre	289	DSD diagnosis in an endocrine clinic	29	49.5	21.5
Juniarto et al. (17)	2004-2010	Indonesia	Single centre	286	DSD diagnosis in an endocrine clinic	8.4	68.2	23.4
Heeley et al. (19)	1995-2016	USA	Single centre	128	Atypical genitalia	11.7	53.1	35.2
Finlayson et al. (20)	2013-2017	USA	Multicentre (12 centres)	99	Atypical genitalia (moderate-severe)	9	34	57 ^c
Rodie et al. (10)	2013-2019	Scotland	Multicentre	92	DSD referral at birth	8	70	22

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SCDSD, sex chromosome DSD; MDT, multidisciplinary team. ^aStudies were included where karyotype data were available for all three diagnostic categories and where the number of individuals reported was >90; ^bwithin 46,XX DSD, 60.5% of children had OTDSD; ^cwithin 46,XX DSD, 91% of children had 21-hydroxylase deficiency.

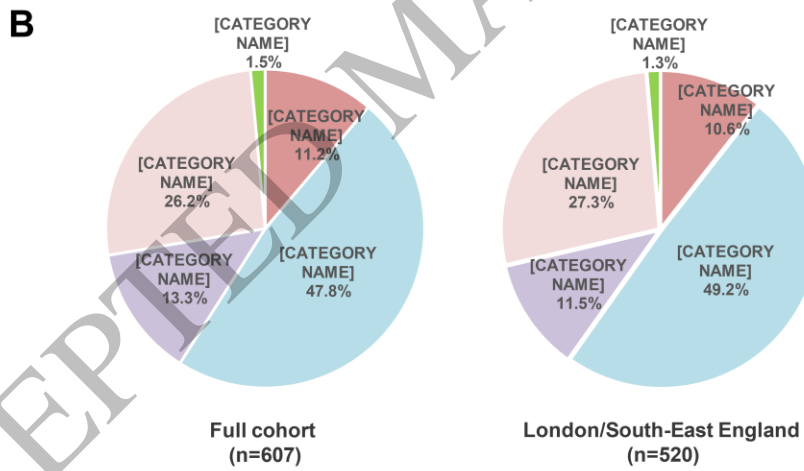
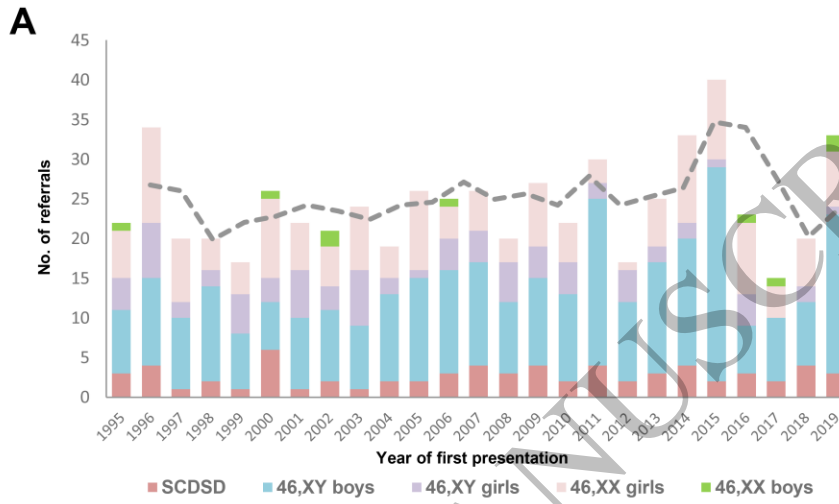
Figure 1



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Figure 1
146x195 mm (.63 x DPI)

Figure 2



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Figure 2
146x195 mm (.63 x DPI)

Figure 3

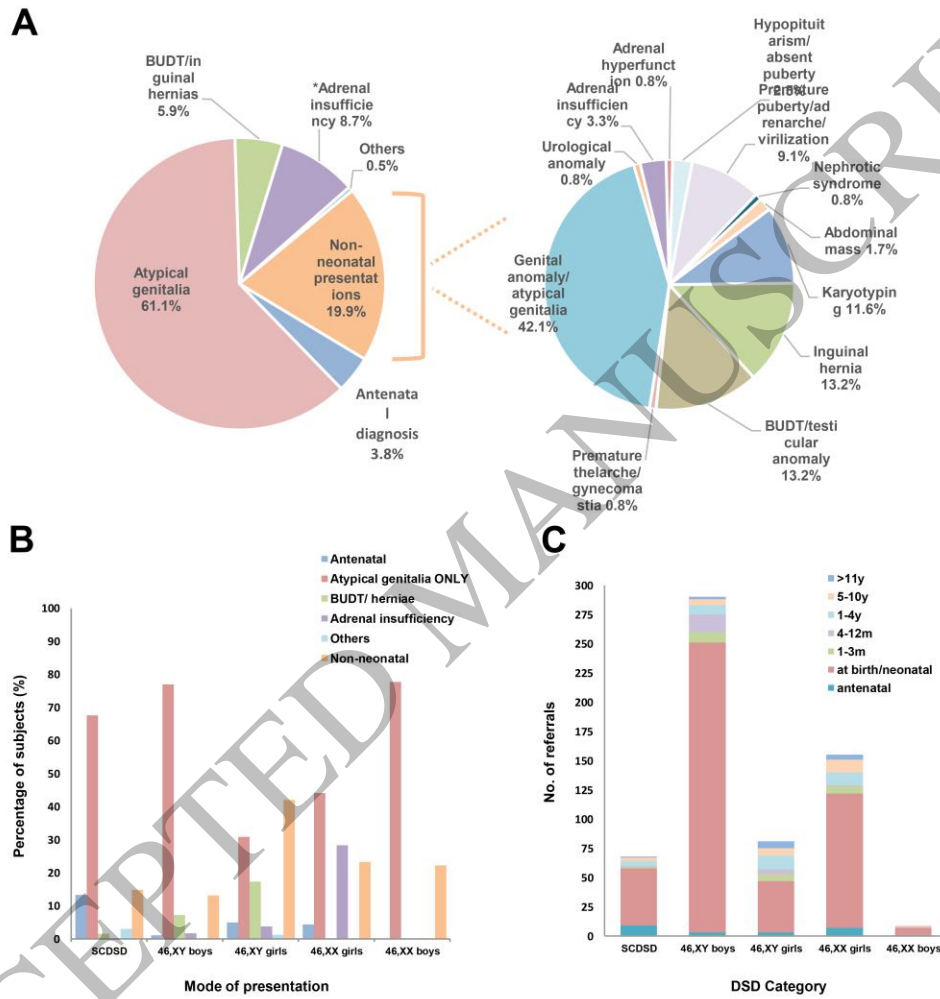
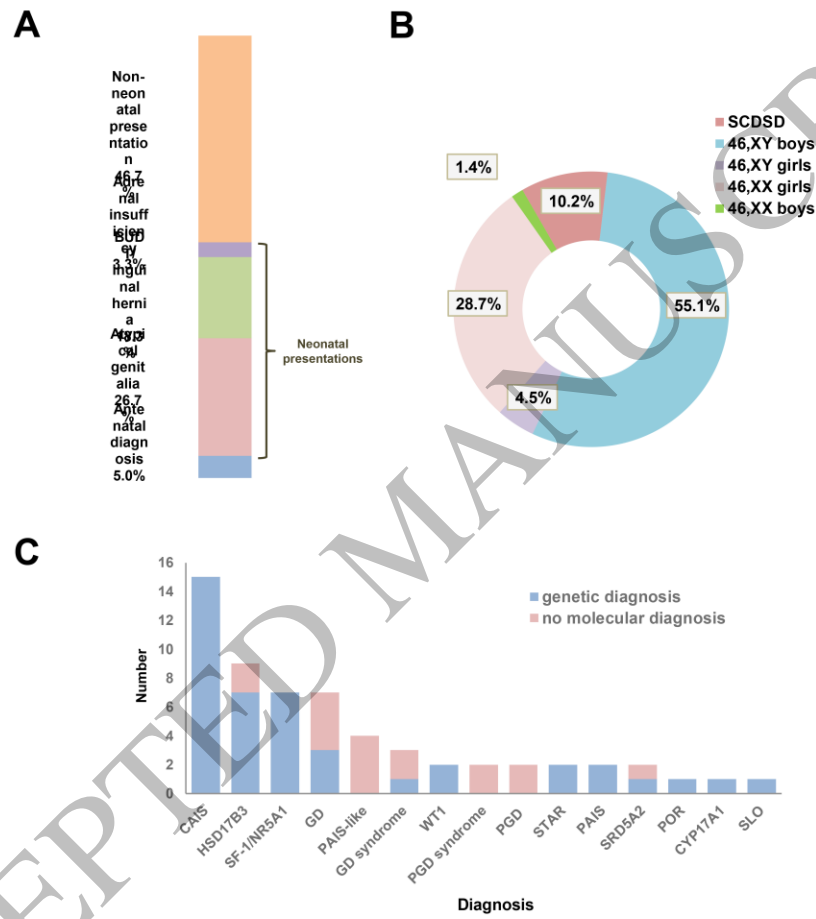


Figure 3
146x195 mm (.63 x DPI)

1
2
3
4

Figure 4



1
2
3

Figure 4
146x195 mm (.63 x DPI)