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Research Article

Lessons Learned from 17 Years of Multidisciplinary Care for DSD Patients at A Single Indonesian Center

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Short Title: Lesson Learned from DSD Patient Care in Indonesia

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Abstract:

Background: Our Multidisciplinary Team (MDT) is a large specialized team based in Semarang, Indonesia that cares for a wide variety of pediatric and adult individuals with Differences of Sex Development (DSD) from across Indonesia. Here we describe our work over the last 17 years.
Methods: We analyzed phenotypic, hormonal and genetic findings from clinical records for all patients referred to our MDT during the period 2004 to 2020.
Results: Among 1184 DSD patients, 10% had sex chromosome DSD, 67% had 46,XY DSD and 23% had 46,XX DSD. The most common sex chromosome anomaly was Turner syndrome (45,X) (55 cases). For patients with 46,XY DSD under-masculinization was the most common diagnosis (311 cases) and for 46,XX DSD a defect of Müllerian development was most common (131 cases) followed by Congenital Adrenal Hyperplasia (CAH) (116 cases). Sanger sequencing, MLPA and targeted gene sequencing of

257 patients with 46,XY DSD found likely causative variants in 21% (55 cases), with 13 diagnostic genes implicated. The most affected gene coded for the Androgen Receptor. Molecular analysis identified a diagnosis for 69 of 116 patients with CAH, with 62 carrying variants in *CYP21A2* including four novel variants, and seven patients carrying variants in *CYP11B1*. In many cases these genetic diagnoses influenced the clinical management of patients and families.
Conclusions: Our work has highlighted the occurrence of different DSDs in Indonesia. By applying sequencing technologies as part of our clinical care, we have delivered a number of genetic diagnoses and identified novel pathogenic variants in some genes, which may be clinically specific to Indonesia. Genetics can inform many aspects of DSD clinical management, and whilst many of our patients remain undiagnosed, we hope that future testing may provide answers for even more.

Introduction

The development of sex and gender requires an intricate interplay of sex chromosomes, genes and genetic pathways, hormones, anatomy, psychology, and social behavior [Cools et al., 2018; Zhao and Yao, 2019; Garcia-Acero et al., 2020].

While our fundamental understanding of sex development and differentiation is still evolving, we have gained significant insights into these processes through the analysis of individuals with differences of sex development (DSD).

DSDs are conditions with atypical chromosomal, gonadal or anatomical sex, and encompasses a broad range of clinical phenotypes. Individuals born with a DSD were classified into three main groups; 46,XY DSD, 46,XX DSD, and sex chromosome DSD following a consensus meeting in Chicago [Hughes et al., 2006]. The classification of sex chromosomal DSD is subdivided into 45,X (Turner syndrome), 47, XXY (Klinefelter), 45,X/46,XY (mixed gonadal dysgenesis) and 46,XY/46,XX chimeric (ovotesticular DSD) subtypes. Although this classification has its limitations and has been subject of intense discussion, its use prevents misunderstanding and confusion in particular by delineating these conditions from other conditions such as gender dysphoria.

Patients with DSD may present with a wide range of phenotypes; from ambiguous or atypical genitalia, absence of secondary sex characteristics, primary amenorrhea, hypospadias, clitoromegaly to complex congenital malformations, such as cloacal extrophy [Arboleda et al., 2014]. DSD cases can be rare and the rate of occurrence differs for each condition and from country to country, although their incidence is not well established [Lee et al., 2016], especially in developing countries such as Indonesia.

Indonesia is a developing country where tropical-infectious diseases are closely associated with poverty and limited resources, and where non communicable diseases including genetic conditions are not a health care priority. In developed countries, DSD is often diagnosed in new-born babies or even before birth, and clinical management involves large interdisciplinary expert teams in a dedicated center. However, the management of DSD in Indonesia provides some unique challenges including limitations in diagnostic and treatment facilities in addition to poverty, sociocultural factors, religious and traditional beliefs.[Ediati et al., 2015b].

Many patients presenting with DSD in Indonesia do not receive
diagnostic evaluation and options for medical and surgical treatment are limited. The lack of a national standardized management protocol means that DSD care is highly variable and often depends on the experience of the treating clinicians and facilities available in a particular health center/ tertiary hospital.

Additionally, health professionals with less awareness of DSD find it challenging to explain the implications and management options to parents. Diagnostic facilities are usually limited to research centers at the universities or provincial referral hospitals, where some DSD genes can be detected using cytogenetics and simple molecular techniques. However, many patients will not know the cause of their DSD and current clinical management strategies depend on phenotypic appearance, imaging, chromosome analysis and hormonal assays. In response to these challenges, a multidisciplinary team (MDT) for DSD at our center was established in 1989, and it remains the only prominent one of its kind in Indonesia, catering to a total population of >270 million.

Since clinical management of DSD depends on a correct diagnosis, genetic testing has become an essential part of the diagnostic pathway especially where there is limited access to other tools such as detailed endocrine testing. A molecular diagnosis also provides opportunities for understanding disease mechanisms, patterns of inheritance and planning clinical management. Advances in genetics have led to a better understanding of the molecular causes of DSD and have provide new insight into the pathophysiology with a number of genes now known to be essential for sexual differentiation in humans [Délot and Vilain, 2021]. In recent years, next generation sequencing (NGS) technologies have increased the diagnostic yield to between 43% to 69.2% of DSD cases [Abualsaud et al., 2021;

Globa et al., 2022; Zidoune et al., 2022] compared to <20% reported by previous years [Arboleda et al., 2013]. Our previous study using HaloPlex Agilent Technology identified variants in 28 genes from a panel of 64 known diagnostic genes for DSD in just over 300 DSD patients with different conditions.[Eggers et al., 2016] Our earlier research on the etiological spectrum of DSD in a large cohort of underprivileged and undiagnosed patients from Indonesia found that a stepwise diagnostic approach led to a molecular or histologically established diagnosis in 29.4% of patients [Juniarto et al., 2016], indicating the importance of molecular analysis but also highlighting that other genetic causes remain to be discovered.

In this study, we reflect on the large cohort of patients with DSD seen by a MDT at a single Indonesian health center over 17 years. We focus on the genetic analysis, describe the distribution of gene variants among Indonesian DSD patients and the lessons learned along the way.

Materials and Methods

Patient clinical notes and classification

We report here on all patients entered into the study by the MDTand the laboratory of center for biomedical research (CEBIOR) of National Diponegoro University Hospital from 2004 to 2020. This includes patients referred from provincial referral hospitals/ tertiary hospitals, peripheral hospitals, private hospitals or health center by physicians (mostly pediatricians, endocrinologists, urologists and gynaecologists). Clinical notes for patients include physical descriptions such as clinical features of the external genitalia, the presence of gonads, the fusion of the labioscrotal folds, the size of the phallus and the site of the urinary meatus on the phallus scored by the External Masculinization Score (EMS). [Ahmed et al., 2000]. Patients with EMS total scores < 9 were considered as under-virilized males.

Patients were classified as suspected of androgen action disorder (AAD) based on an EMS score <9, while patients with an EMS score ≥9 were classified as under-masculinization of unknown cause (UMU) [Juniarto et al., 2016]. Patients with various DSD phenotypes were referred to CEBIOR for chromosomal analysis. In 46,XY DSD patients, diagnoses were grouped into androgen action disorder (AAD), UMU, and gonadal dysgenesis (GD) based on EMS and serum concentration of LH, FSH and testosterone levels [Juniarto et al., 2016]. The 46,XX DSD patients were grouped based on hormonal diagnosis and physical examination, mainly 17 hydroxy progesterone (17 OHP) for congenital adrenal hyperplasia (CAH). Some patients also had ultrasonographic or radiologic imaging.

MDT and patient clinical care

The MDT consists of specialists representing various departments from the Dr. Kariadi provincial referral Hospital and National Diponegoro University Hospital including urology, pediatric endocrinology, plastic surgery, gynecology, andrology, anesthetics, medical genetics (including a genetic counselor), psychiatry, pathology, psychology, legal medicine and religious leaders. Discussion for 5-10 cases were carried out at bimonthly MDT meetings, their family (mainly parent) are also invited if necessary the genetic testing are done for the extended family. In some cases, the team refered a patients or family to psychological evaluation by the psychologist member of the MDT team who had specialized training on DSD. This was most common where assessment was required to evaluate the gender identity due to patient's doubts over their assigned gender or wishes for gender reassignment. An individual interview was conducted for patients and parents or caregiver regarding gender role behavior, gender stereotypes, and their acceptance of DSD in the family. In pediatric cases where surgery was considered, this was discussed broadly and with the families, only proceeding with informed consent from parents. Regardless of the surgery, sex was assigned based on cytogenetics, internal and external reproductive organs, hormone levels and psychosocial analysis, including cultural and religious issues, and in many cases the wishes of the

parents had a strong bearing on the sex choice of children. The flow chart of patient care is shown in Figure 1.

Molecular testing

Collaborations with Erasmus Medical Centre (Erasmus MC) in Rotterdam and Radboud University Medical Center (Radboudumc) in Nijmegen, Netherlands and the Murdoch Children's Research Institute (MCRI) in Melbourne, Australia provided genetic testing for various conditions and using differing technologies.

Specifically, Radboudumc: CAH patients - *CYP21A2* and *CYP 11B1* Sanger sequencing and MLPA (P050-C1 kit, MRC Holland, Amsterdam, The Netherlands).

Erasmus MC: CAH patients (46,XX DSD with increased 17-OHP level) - Sanger sequencing of the *CYP21A2* and *CYP 11B1* gene. 46,XY DSD patients (with an EMS score <9) for *AR*, *SRY*, *WNT4, NR3C1*and *LHCGR* gene sanger sequencing analysis.

MCRI: Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome - variant analysis for using microarrays and Whole Exome Sequencing. 46,XY DSD - targeted gene sequencing using HaloPlex Agilent Technology on patients [Eggers et al., 2016].

Results

Our cohort consisted of 1184 patients covering the period from 2004 - 2020. Patient number fluctuated each year, with lower numbers in 2014 because our laboratory moved to the main university campus and in 2020 due to limitations imposed by COVID-19 (Figure 2). Patients were grouped into three categories (Sex Chromosome DSD, 46,XX DSD and 46,XY DSD), which were subsequently divided into the appropriate subgroups (Table 1).

A total of 800 patients had 46,XY DSD in total 152 of these patients were evaluated in Erasmus MC while 105 patients were assessed at MCRI. Of those analysed at MCRI, 97 were previously reported in Eggers et al, [2016]. The remaining patients are still without diagnosis except for 5 patients who were found to carry causative AR variants as reported in [Listyasari et al., 2019] Of those analysed at Erasmus MC, a total of 152 case evaluated were previously reported in [Juniarto et al., 2016; Listyasari et al., 2021] The molecular analysis of these 257 patients identified genetic variants considered diagnostic in 55 cases (21 %) from 13 diagnostic DSD genes (Figure 3). The undiagnosed cases included patients with UMU, gonadal dysgenesis or hypospadias.

Patient presented with 46, XX DSD were 274 cases. In 116 of these cases, a diagnosis of CAH was made, which was confirmed by genetic analysis for 69 cases: in 62 patients 28 pathogenic variants were identified in the *CYP21A2* gene (Table 2), while in the *CYP11B1* gene (NM_000497.4) two different pathogenic variants were detected in seven cases i.e. c.1121G>A (2 cases) and c.799G>A (5 cases). The diagnostic yield for DSD cases were available in Figure 4. The most common pathogenic variant in CAH cases is c.1069C>T p(Arg357Trp) in *CYP21A2* (NM_000500.9) which was found in 49 alleles (out of 124 alleles; 15 patients were homozygous for this variant and in two patients this variant was part of a complex allele with several variants (Table 2).[Krone and Arlt, 2009; New et al., 2013; Baumgartner-Parzer et al., 2020] The remaining 47 cases were diagnosed based on clinical and biochemical evaluation only. In addition to the characterized and already registered pathogenic variants in *CYP21A2*, we also discovered four novel variants which were not described in any variant library. In case 1 [c.1155_1157del p.(Ile386del)] has salt wasting type; case 2 [c.739G>T p.(Glu247*)] has simple virilizing type; and case 3 [c.586C>T p.(Gln196)] has salt wasting type all of which were identified at late age, have virilization of external genitalia, short stature and hyperpigmentation. While case 4 [c.65G>A p.(Trp22*)] has salt wasting type which is recognized at early age with normal length/height-for-age and normal puberty due to good medication compliance.

The remaining 158 patients with 46,XX DSD were diagnosed with unknown androgen excess disorders, defect Mullerian development such as Mayer–Rokitansky–Küster–Hauser (MRKH) syndrome, gonadal dysgenesis or Mullerian duct aplasia – renal agenesis – cervicothoracic somite dysplasia (MURCS) or Cloacal malformation. A number of 10 patients with MRKH had genetic testing in MCRI and molecular studies for this group of disorders were not definitive, except for four cases of MRKH type 1 with clinical finding primary amenorrhea and utero vaginal agenesis.[Backhouse et al., 2019]. The other 105 patients in this category were tested for specific genes for *WNT4*, *LHCGR*, *SRY* and *NR3C1* gene using Sanger sequencing in Erasmus MC. The molecular study for this group have identified for 12 cases of *WNT4* defect, 2 cases of glucocorticoid receptor defect, 2 cases of *LH* receptor defects and 6 cases of *SRY* gene translocation as previously reported while the remaining cases were not conclusive. [Juniarto et al., 2012; Juniarto et al., 2016].
In total, 246 patients (aged 2-45 years) were referred for psychological assessment and care by the MDT. Many parents who had girls with DSD voiced concern about future infertility and asked about keeping the DSD diagnosis hidden from others, including extended family. Adolescents' girls and adult women who had a variation of physical appearance (such as a muscular body, masculine behavior, deep voice) experienced more negative reactions and rejection from peers or the community compared to girls with no visible external differences in these characteristics. In most cases, patients and parents received psychological counseling to facilitate better acceptance of their condition in the family and in society. Difficulties in dealing with societal acceptance and understanding were the most common issue reported by parents and patients, particularly when DSD characteristics were visible to others. This included discussion around educating others of their DSD condition, coping with awkward situations in the neighborhood, coping with or anticipating peer or social rejection and marital or infertility issues. We have previously reported similar findings from 118 patients aged 6-42 years. [Ediati et al., 2015a; Ediati et al., 2015c].

Discussion

Clinical care for DSD patients is challenging. Patients with DSD and their families require complex medical and psychosocial care, as well as ongoing follow up throughout their life. Multidisciplinary care, of which psychosocial and peer support are key components, is recommended as best practice [Moran and Karkazis, 2012; Ahmed et al., 2021]. Most importantly, patient management needs to be individualized especially for decisions related to hormonal treatment, sex assignment, surgical interventions, genetic counseling, long-term treatment monitoring and potential fertility preservation.[Ediati et al., 2017; Ediati et al., 2018; Speiser et al., 2018]
In Indonesia, the lack of national guidelines for DSD providers means there is no standardized approach.[Dessens et al., 2017] This is a significant dilemma with serious implications for patients. A standardized network of multidisciplinary DSD teams would provide clinicians with the necessary program planning and team implementation tools.

Late presentations and delayed diagnoses of DSD are also common in Indonesia. This leads to incongruence between expressed gender and the sex assigned at birth which can cause clinically significant distress and impairment in social and emotional function.[Ediati et al., 2015b] The availability of psychological care in addition to medical care is pivotal for patients with DSD and their family. Accepting a diagnosis of DSD can be challenging for patients and families when there is little understanding of these conditions in the community. Affected individuals must make sense of their body and gender when such a discrepancy is present. Having a proper understanding of DSD and its causes can help patients and families come to terms with a diagnosis and educate the wider community about their condition to promote social acceptance. The availability of a multidisciplinary team in our center has been central to providing clinical care for patients with DSD and their families. It has also raised awareness in both the medical and wider community.

In the Indonesian clinical setting, the etiology of DSD remains largely unknown. The integration of phenotypic data, such as detailed anatomical, hormonal, histological data, and genotyping will continue to be crucial.[Ahmed et al., 2022; Rey, 2022] Currently, only some of our many patients have access to genetic sequencing technologies, but in the case of 46,XY DSD it provided a diagnostic finding in 21% of patients. A higher genetic diagnostic rate can be achieved by increasing the availability of NGS technology in diagnostic settings.[Gomes et al., 2022] In Indonesia, the molecular findings showed Androgen Insensitivity (AIS) and 5α-reductase type 2 (5ARD) deficiency as the most common causes of 46,XY DSD, both of which are often inherited. These findings provide genetic counseling opportunities, which can influence family planning and cascade testing.[Juniarto et al., 2016; Listyasari et al., 2019; Marzuki et al., 2019; Listyasari et al., 2021].

In some cases genetic testing can also provide a definitive diagnosis where traditional methods may have failed. One example is AIS and 5ARD which can be phenotypically very similar. Although these can sometimes be distinguished by hormonal testing [Veiga-Junior et al., 2012], this is not always available or accurate. In Indonesia, the DHT test required to make these diagnoses is not frequently provided in clinical settings due to cost considerations. When molecular analysis for the SRD5A2 gene is unavailable, the urinary Etiocholanolone/Androsterone ratio may be a relevant test for identifying 5ARD2 patients and carriers.[Marzuki et al., 2021] 5ARD is autosomal recessive while many cases of AIS we identified had X-linked inheritance, again meaning that a genetic diagnosis may have different implications for families.

In addition to diagnostic changes, molecular sequencing of our patient cohorts also revealed variants of uncertain significance (VUS). Fortunately functional testing has been able to resolve the causality or lack of for several of these VUS, for example in heterozygous missense variants in *GATA4*[Bergen et al., 2020]*, novel* variants in the prodomain of *BMP7* in two pairs of monozygotic concordant twins with proximal hypospadias [Bouty et al., 2019] and *DHH* variants in 46,XY DSD.[Ayers et al., 2019]. Whilst no diagnostic variants were found in MRKH patients (indeed few genes are yet confirmed to cause MRKH), several candidate genes were identified that may contribute to this condition but further analysis will be required to establish whether they are causative [Backhouse et al., 2019]. Further functional analysis of VUS will be essential, especially of variants that may be unique or over-represented in populations like Indonesia.

In our work over 17 years we have seen that the yearly rate of DSD diagnoses remained stable. However, this decreased steeply during the COVID 19 pandemic as many DSD patients/families postponed their appointments as DSD was not considered a medical emergency. However, a large group of 46,XX female patients presenting with ambiguous genitalia at birth have 21-hydroxylase deficiency (CAH). Identification of infants with CAH is urgent because if undiagnosed they may develop salt wasting, which could lead to death. Several other DSD genes cause co-morbidities. A genetic diagnosis can help to monitor these or allow early inteverntion. For example, *GATA4* variants in 46,XY DSD can contribute to congenital heart defects (CHD). Similarly, *WT1*

variants can underlie Wilms tumor. Understanding the pattern of inheritance is essential for genetic counseling and family planning.

Conclusion

For patients with DSD, clinical management by a dedicated MDT and accurate genetic diagnoses are required for optimal care. This is a challenge in developing countries, such as Indonesia, where patients face limited access to diagnostic tests such as hormonal analysis or advanced genetic analysis. We hope that by sharing the last 17 years of experience of our DSD multi-disciplinary team, we can spread awareness in the medical community, and work towards national guidelines for establishing this model of care across Indonesia and other developing countries.

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Statement of Ethics

This study protocol was reviewed and approved by Health Research Ethics Committee, Faculty of Medicine Diponegoro University, Semarang, Indonesia, approval number 31/H.7/KEPK/FK-RSDK/2010. Written informed consent was obtained from participants (or their parent/legal guardian/next of kin) to participate in the study.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Name	Contributions
Sultana MH Faradz:	Substantial contributions to the conception or design of the work; AND Drafting the work or revising it critically for important intellectual content; AND Final approval of the version to be published; AND Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
Nurin Aisyiyah Listyasari:	Substantial contributions to the analysis, and interpretation of data for the work; AND Drafting the work or revising it critically for important intellectual content; AND

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All authors read and approved the final manuscript.

Data Availability Statement
All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

REFERENCES

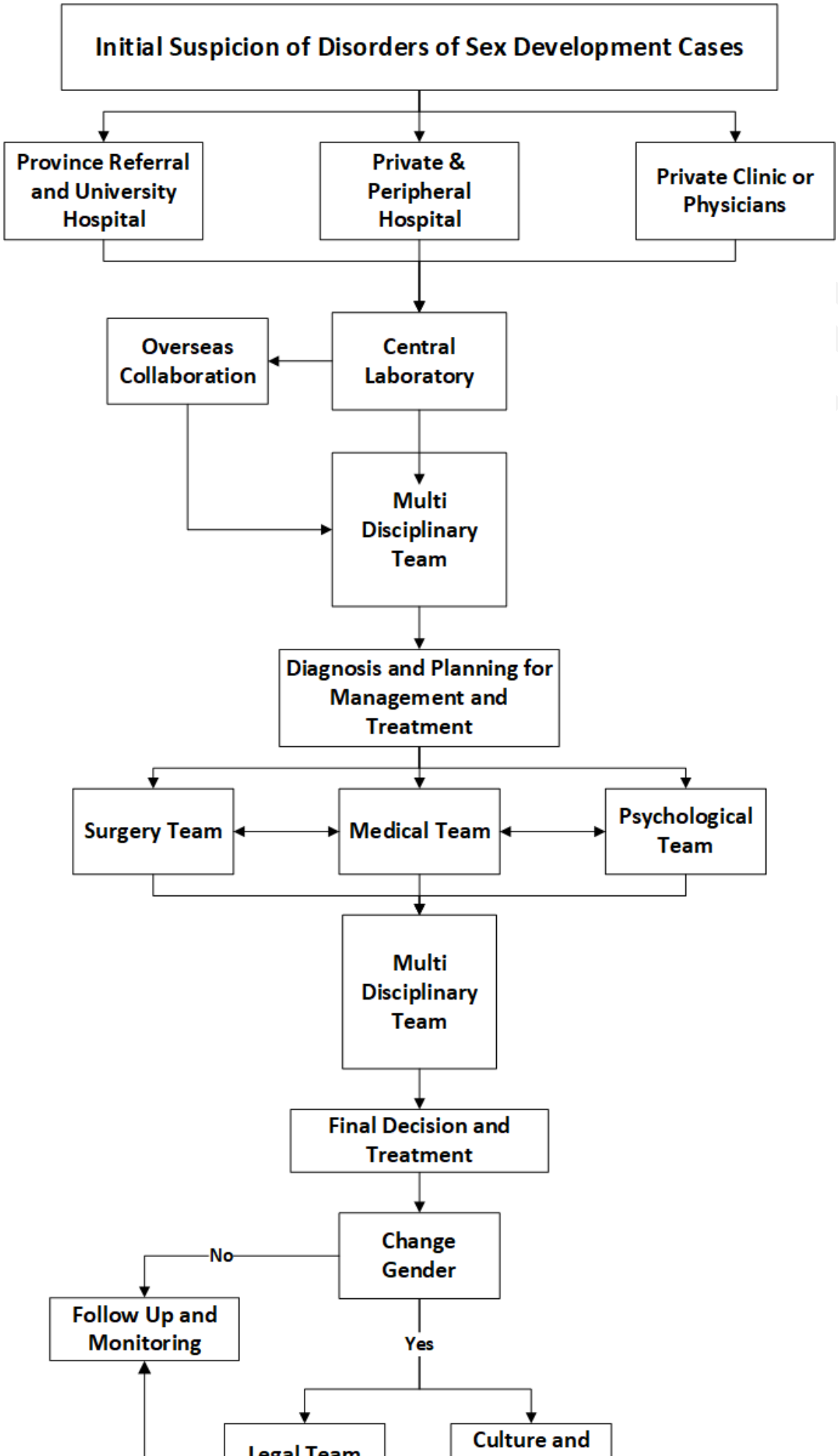
1. Abualsaud D, Hashem M, AlHashem A, Alkuraya FS: Survey of disorders of sex development in a large cohort of patients with diverse Mendelian phenotypes. Am J Med Genet A 185:2789-2800 (2021).
2. Ahmed SF, Achermann J, Alderson J, Crouch NS, Elford S, Hughes IA, Krone N, McGowan R, Mushtaq T, O'Toole S, Perry L, Rodie ME, Skae M, Turner HE: Society for Endocrinology UK Guidance on the initial evaluation of a suspected difference or disorder of sex development (Revised 2021). Clin Endocrinol (Oxf) 95:818-840 (2021).
3. Ahmed SF, Alimusina M, Batista RL, Domenice S, Lisboa Gomes N, McGowan R, Patjamontri S, Mendonca BB: The Use of Genetics for Reaching a Diagnosis in XY DSD. Sex Dev:1-18 (2022).
4. Ahmed SF, Khwaja O, Hughes IA: The role of a clinical score in the assessment of ambiguous genitalia. BJU Int 85:120-124 (2000).
5. Arboleda VA, Lee H, Sanchez FJ, Delot EC, Sandberg DE, Grody WW, Nelson SF, Vilain E: Targeted massively parallel sequencing provides comprehensive genetic diagnosis for patients with disorders of sex development. Clin Genet 83:35-43 (2013).
6. Arboleda VA, Sandberg DE, Vilain E: DSDs: genetics, underlying pathologies and psychosexual differentiation. Nat Rev Endocrinol 10:603-615 (2014).
7. Ayers K, van den Bergen J, Robevska G, Listyasari N, Raza J, Atta I, Riedl S, Rothacker K, Choong C, Faradz SMH, Sinclair A: Functional analysis of novel desert hedgehog gene variants improves the clinical interpretation of genomic data and provides a more accurate diagnosis for patients with 46,XY differences of sex development. J Med Genet 56:434-443 (2019).
8. Backhouse B, Hanna C, Robevska G, van den Bergen J, Pelosi E, Simons C, Koopman P, Juniarto AZ, Grover S, Faradz S, Sinclair A, Ayers K, Tan TY: Identification of Candidate Genes for Mayer-Rokitansky-Küster-Hauser Syndrome Using Genomic Approaches. Sexual Development 13:26-34 (2019).
9. Baumgartner-Parzer S, Witsch-Baumgartner M, Hoeppner W: EMQN best practice guidelines for molecular genetic testing and reporting of 21-hydroxylase deficiency. Eur J Hum Genet 28:1341-1367 (2020).
10. Bergen JA, Robevska G, Eggers S, Riedl S, Grover SR, Bergman PB, Kimber C, Jiwane A, Khan S, Krausz C, Raza J, Atta I, Davis SR, Ono M, Harley V, Faradz SMH, Sinclair AH, Ayers KL: Analysis of variants in GATA4 and FOG2 / ZFPM2 demonstrates benign contribution to 46,XY disorders of sex development. Molecular Genetics & Genomic Medicine 8 (2020).
11. Bouty A, Walton K, Listyasari NA, Robevska G, Van den Bergen J, Santosa A, Faradz SMH, Harrison C, Ayers KL, Sinclair AH: Functional Characterization of Two New Variants in the Bone Morphogenetic Protein 7 Prodomain in Two Pairs of Monozygotic Twins With Hypospadias. J Endocr Soc 3:814-824 (2019).
12. Cools M, Nordenstrom A, Robeva R, Hall J, Westerveld P, Fluck C, Kohler B, Berra M, Springer A, Schweizer K, Pasterski V, group CABw: Caring for individuals with a difference of sex development (DSD): a Consensus Statement. Nat Rev Endocrinol 14:415-429 (2018).
13. Délot EC, Vilain E: Towards improved genetic diagnosis of human differences of sex development. Nature Reviews Genetics 22:588-602 (2021).
14. Dessens A, Guaragna-Filho G, Kyriakou A, Bryce J, Sanders C, Nordenskjold A, Rozas M, Iotova V, Ediati A, Juul A, Krawczynski M, Hiort O, Faisal Ahmed S: Understanding the needs of professionals who provide psychosocial care for children and adults with disorders of sex development. BMJ Paediatr Open 1:e000132 (2017).
15. Ediati A, Faradz SM, Juniarto AZ, van der Ende J, Drop SL, Dessens AB: Emotional and behavioral problems in late-identified Indonesian patients with disorders of sex development. J Psychosom Res 79:76-84 (2015a).
16. Ediati A, Juniarto AZ, Birnie E, Drop SL, Faradz SM, Dessens AB: Gender Development in Indonesian Children, Adolescents, and Adults with Disorders of Sex Development. Arch Sex Behav 44:1339-1361 (2015b).
17. Ediati A, Juniarto AZ, Birnie E, Drop SLS, Faradz SMH, Dessens AB: Body Image and Sexuality in Indonesian Adults with a Disorder of Sex Development (DSD). J Sex Res 52:15-29 (2015c).
18. Ediati A, Juniarto AZ, Birnie E, Okkerse J, Wisniewski A, Drop S, Faradz SMH, Dessens A: Social stigmatisation in late identified patients with disorders of sex development in Indonesia. BMJ Paediatr Open 1:1-9 (2017).

19. Ediati A, Verrips GHW, Juniarto AZ, Faradz SMH, Drop SLS, Dessens AB: Quality of Life in Late-Treated Patients With Disorders of Sex Development: Insights for Patient-Centered Care. Front Pediatr 6:434 (2018).
20. Eggers S, Sadedin S, van den Bergen JA, Robevska G, Ohnesorg T, Hewitt J, Lambeth L, Bouty A, Knarston IM, Tan TY, Cameron F, Werther G, Hutson J, O'Connell M, Grover SR, Heloury Y, Zacharin M, Bergman P, Kimber C, Brown J, Webb N, Hunter MF, Srinivasan S, Titmuss A, Verge CF, Mowat D, Smith G, Smith J, Ewans L, Shalhoub C, Crock P, Cowell C, Leong GM, Ono M, Lafferty AR, Huynh T, Visser U, Choong CS, McKenzie F, Pachter N, Thompson EM, Couper J, Baxendale A, Gecz J, Wheeler BJ, Jefferies C, MacKenzie K, Hofman P, Carter P, King RI, Krausz C, van Ravenswaaij-Arts CM, Looijenga L, Drop S, Riedl S, Cools M, Dawson A, Juniarto AZ, Khadilkar V, Khadilkar A, Bhatia V, Dung VC, Atta I, Raza J, Thi Diem Chi N, Hao TK, Harley V, Koopman P, Warne G, Faradz S, Oshlack A, Ayers KL, Sinclair AH: Disorders of sex development: insights from targeted gene sequencing of a large international patient cohort. Genome Biol 17:243 (2016).
21. Garcia-Acero M, Moreno-Nino O, Suarez-Obando F, Molina M, Manotas MC, Prieto JC, Forero C, Cespedes C, Perez J, Fernandez N, Rojas A: Disorders of sex development: Genetic characterization of a patient cohort. Mol Med Rep 21:97-106 (2020).
22. Globa E, Zelinska N, Shcherbak Y, Bignon-Topalovic J, Bashamboo A, Msmall es CK: Disorders of Sex Development in a Large Ukrainian Cohort: Clinical Diversity and Genetic Findings. Front Endocrinol (Lausanne) 13:810782 (2022).
23. Gomes NL, Batista RL, Nishi MY, Lerário AM, Silva TE, de Moraes Narcizo A, Benedetti AFF, de Assis Funari MF, Faria Junior JA, Moraes DR, Quintão LML, Montenegro LR, Ferrari MTM, Jorge AA, Arnhold IJP, Costa EMF, Domenice S, Mendonca BB: Contribution of Clinical and Genetic Approaches for Diagnosing 209 Index Cases With 46,XY Differences of Sex Development. J Clin Endocrinol Metab 107:e1797-e1806 (2022).
24. Hughes IA, Houk CP, Ahmed SF: Consensus statement on management of intersex disorders. Archives of Disease in Childhood 91:554-563 (2006).
25. Juniarto AZ, van der Zwan YG, Santosa A, Ariani MD, Eggers S, Hersmus R, Themmen AP, Bruggenwirth HT, Wolffenbuttel KP, Sinclair A, White SJ, Looijenga LH, de Jong FH, Faradz SM, Drop SL: Hormonal evaluation in relation to phenotype and genotype in 286 patients with a disorder of sex development from Indonesia. Clin Endocrinol (Oxf) 85:247-257 (2016).
26. Juniarto AZ, van der Zwan YG, Santosa A, Hersmus R, de Jong FH, Olmer R, Bruggenwirth HT, Themmen AP, Wolffenbuttel KP, Looijenga LH, Faradz SM, Drop SL: Application of the new classification on patients with a disorder of sex development in indonesia. Int J Endocrinol 2012:237084 (2012).
27. Krone N, Arlt W: Genetics of congenital adrenal hyperplasia. Best Pract Res Clin Endocrinol Metab 23:181-192 (2009).
28. Lee PA, Nordenstrom A, Houk CP, Ahmed SF, Auchus R, Baratz A, Baratz Dalke K, Liao LM, Lin-Su K, Looijenga LH, 3rd, Mazur T, Meyer-Bahlburg HF, Mouriquand P, Quigley CA, Sandberg DE, Vilain E, Witchel S, Global DSDUC: Global Disorders of Sex Development Update since 2006: Perceptions, Approach and Care. Horm Res Paediatr 85:158-180 (2016).
29. Listyasari NA, Juniarto AZ, Robevska G, Ayers KL, Sinclair AH, Faradz SMH: Analysis of the androgen receptor (AR) gene in a cohort of Indonesian undermasculinized 46, XY DSD patients. Egyptian Journal of Medical Human Genetics 22 (2021).
30. Listyasari NA, Robevska G, Santosa A, Bouty A, Juniarto AZ, van den Bergen J, Ayers KL, Sinclair AH, Faradz SMH: Genetic Analysis Reveals Complete Androgen Insensitivity Syndrome in Female Children Surgically Treated for Inguinal Hernia. J Invest Surg:1-7 (2019).
31. Marzuki NS, Idris FP, Kartapradja H, Renata S, Harahap A, Batubara JRL: Accuracy of Urinary Etiocholanolone/Androsterone Ratio as Alternative to Serum Testosterone/Dihydrotestosterone Ratio for Diagnosis of 5 Alpha-reductase Type 2 Deficiency Patients and Carriers in Indonesia. Int J Endocrinol Metab 19:e109510 (2021).
32. Marzuki NS, Idris FP, Kartapradja HD, Harahap AR, Batubara JRL: Characterising SRD5A2 Gene Variants in 37 Indonesian Patients with 5-Alpha-Reductase Type 2 Deficiency. Int J Endocrinol 2019:7676341 (2019).
33. Moran ME, Karkazis K: Developing a multidisciplinary team for disorders of sex development: planning, implementation, and operation tools for care providers. Adv Urol 2012:604135 (2012).

34. New MI, Abraham M, Gonzalez B, Dumic M, Razzaghy-Azar M, Chitayat D, Sun L, Zaidi M, Wilson RC, Yuen T: Genotype-phenotype correlation in 1,507 families with congenital adrenal hyperplasia owing to 21-hydroxylase deficiency. Proc Natl Acad Sci U S A 110:2611-2616 (2013).
35. Rey RA: Next-Generation Sequencing as First-Line Diagnostic Test in Patients With Disorders of Sex Development? The Journal of Clinical Endocrinology & Metabolism 107:e2628-e2629 (2022).
36. Speiser PW, Arlt W, Auchus RJ, Baskin LS, Conway GS, Merke DP, Meyer-Bahlburg HFL, Miller WL, Murad MH, Oberfield SE, White PC: Congenital Adrenal Hyperplasia Due to Steroid 21-Hydroxylase Deficiency: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 103:4043-4088 (2018).
37. Veiga-Junior NN, Medaets PA, Petroli RJ, Calais FL, de Mello MP, Castro CC, Guaragna-Filho G, Sewaybricker LE, Marques-de-Faria AP, Maciel-Guerra AT, Guerra-Junior G: Clinical and Laboratorial Features That May Differentiate 46,XY DSD due to Partial Androgen Insensitivity and 5alpha-Reductase Type 2 Deficiency. Int J Endocrinol 2012:964876 (2012).
38. Zhao F, Yao HH: A tale of two tracts: history, current advances, and future directions of research on sexual differentiation of reproductive tractsdagger. Biol Reprod 101:602-616 (2019).
39. Zidoune H, Ladjouze A, Chellat-Rezgoune D, Boukri A, Dib SA, Nouri N, Tebibel M, Sifi K, Abadi N, Satta D, Benelmadani Y, Bignon-Topalovic J, El-Zaiat-Munsch M, Bashamboo A, McElreavey K: Novel Genomic Variants, Atypical Phenotypes and Evidence of a Digenic/Oligogenic Contribution to Disorders/Differences of Sex Development in a Large North African Cohort. Front Genet 13:900574 (2022).

40. Figures Legends

41. **Figure 1.** Algorithm of DSD management
42. **Figure 2.** Number of DSD patients per year
43. **Figure 3.** Graph showing the genes responsible for a genetic diagnosis for 46,XY DSD patients. Of a total of 257 46,XY DSD patients sequenced, 55 had variants in a DSD gene that was curated to be likely pathogenic or pathogenic. This illustrates the breakdown of these variants in terms of genes responsible.
44. **Figure 4.** Diagnostic yield of the DSD cohort in this study. A. The molecular analysis of 46,XY DSD patients. From 257 patients who are tested, identified a genetic diagnosis in 21% cases. B. In the 46,XX DSD patient cohort, from 184 patients who had genetic test, 33% were found to have a pathogenic variant in a DSD gene.



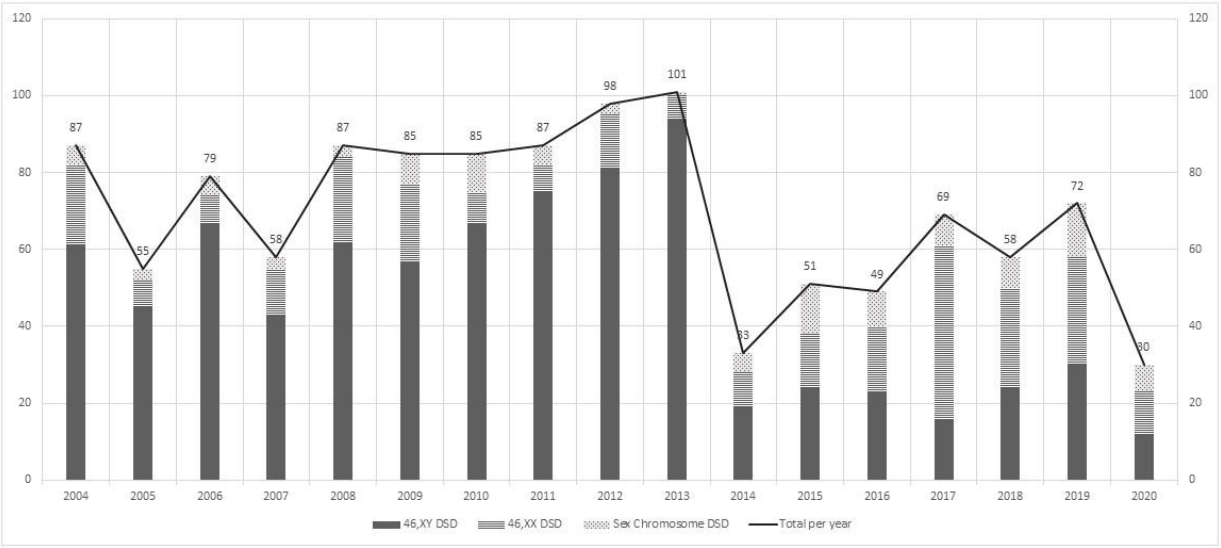
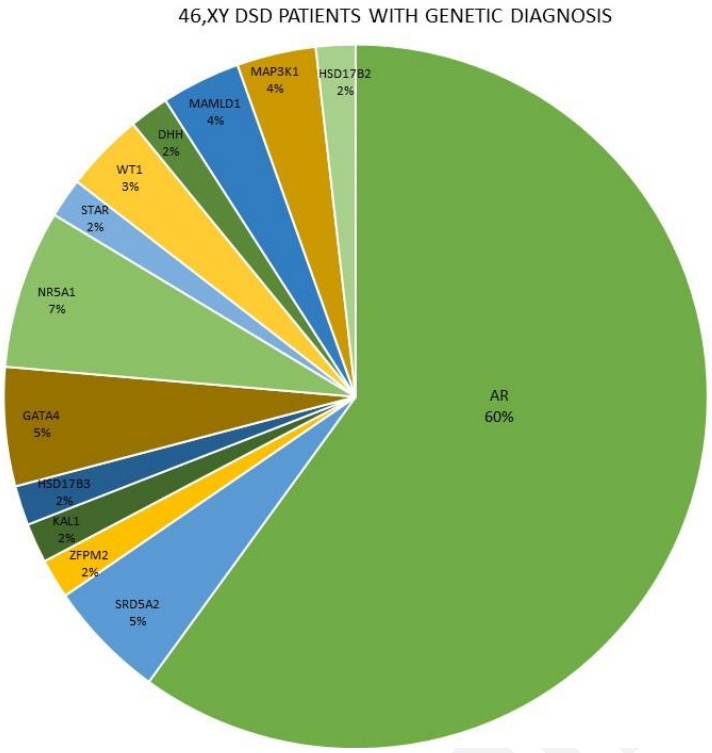


Figure 2. Number of DSD patients per year



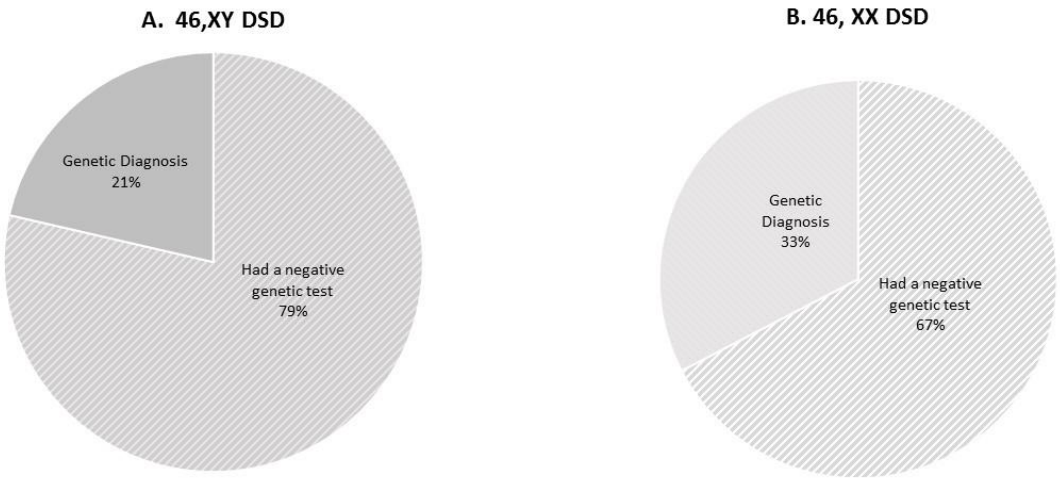


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Table 1. Distribution of DSD cases during 2004-2020 according to DSD classification

No of cases	Sex Chromosome DSD					46,XY DSD				46,XX DSD				
1184	110 (9%)					800 (68%)				274 (23%)				
	Turner and its variant	47,XXY Klinefelter	45,X/46,XY (MGD, Ovotesticular DSD)	46,XX /46,XY ovotesticular DSD	others	Hypospadias	Gonadal dysgenesis	AAD	UMU	CAH	Androgen Excess	Defect of Mullerian Development	Gonadal Dysgenesis	Others
	55 (50%)	12 (10%)	21 (20%)	9 (8%)	13 (12%)	292 (36%)	32 (4%)	157 (20%)	319 (40%)	116 (42%)	11 (4%)	131 (48%)	3 (1%)	13 (5%)

Abbrevation: AAD: Androgen action disorders, UMU: Unknown Male Under masculinization, MGD: Mixed Gonadal Dysgenesis

Table 2. Identified pathogenic variants in the CYP21A2 gene (NM_000500.7) in patients with congenital adrenal hyperplasia and their predicted phenotype based on residual enzyme activity and literature. [Krone and Arlt, 2009; New et al., 2013; Baumgartner-Parzer et al., 2020]

Number of patients	Allele 1				Allele 2				(Assumed) inheritance	Predicted phenotype based on residual enzyme activity
	Nucleotide change	Protein change	Legacy name	(Predicted) enzyme activity	Nucleotide change	Protein change	Legacy name	(Predicted) enzyme activity		
4	c.293-13C>G	p.?	I2G	0-1%	c.293-13C>G	p.?	I2G	0-1%	hom.	SW
5	c.518T>A	p.(Ile173Asn)	I172N	1-5%	c.518T>A	p.(Ile173Asn)	I172N	1-5%	hom.	SV
5	c.518T>A	p.(Ile173Asn)	I172N	1-5%	c.1069C>T	p.(Arg357Trp)	R356W	0%	comp. het.	SW or SV
1	c.955C>T	p.(Gln319*)	Q318X	0%	Not identified yet				het.	unknown
15	c.1069C>T	p.(Arg357Trp)	R356W	0%	c.1069C>T	p.(Arg357Trp)	R356W	0%	hom.	SW
1	c.92C>T	p.(Pro31Leu)	P30L	20-30%	c.955C>T	p.(Gln319*)	Q318X	0%	comp. het.	NC
1	c.844G>T	p.(Val282Leu)	V281L	30-50%	c.955C>T	p.(Gln319*)	Q318X	0%	comp. het.	NC
1	c.92C>T	p.(Pro31Leu)	P30L	20-30%	c.844G>T c.955C>T c.1069C>T	p.(Val282Leu) p.(Gln319*) p.(Arg357Trp)	V281L-Q318X-R356W	0%	comp. het.	NC
2	c.92C>T	p.(Pro31Leu)	P30L	20-30%	c.92C>T	p.(Pro31Leu)	P30L	20-30%	hom.	NC
3	c.293-13A/C>G	p.?	I2G	0-1%	c.1069C>T	p.(Arg357Trp)	R356W	0%	comp. het.	SW
1	c.92C>T	p.(Pro31Leu)	P30L	20-30%	c.1069C>T	p.(Arg357Trp)	R356W	0%	comp. het.	NC
1	**c.1155_1157del**	**p.(Ile386del)**	**unknown**	**unknown**	c.1069C>T	p.(Arg357Trp)	R356W	0%	comp. het.	unknown
1	c.1279C>T	p.(Arg427Cys)	R426C	0%	c.1279C>T	p.(Arg427Cys)	R426C	0%	hom.	SW
1	c.1069C>T	p.(Arg357Trp)	R356W	0%	**c.65G>A**	**p.(Trp22*)**	**unknown**	**0%**	comp. het.	SW
1	c.710T>A c.713T>A c.719T>A	p.(Ile237Asn) p.(Val238Glu) p.(Met240Lys)	E6 cluster or [I236N, V237E, M239K]	0%	c.1069C>T	p.(Arg357Trp)	R356W	0%	comp. het.	SW
1	c.332_339del	p.(Gly111fs)	E3Δ8bp	0%	**c.739G>T**	**p.(Glu247*)**	**unknown**	**0%**	comp. het.	SW
1	c.92C>T	p.(Pro31Leu)	P30L	20-30%	c.1069C>T	p.(Arg357Trp)	R356W	0%	comp. het.	NC
1	c.60G>A	p.(Trp20*)	W19X	0%	c.518T>A	p.(Ile173Asn)	I172N	1-5%	comp. het.	SW or SV
2	c.1069C>T	p.(Arg357Trp)	R356W	0%	c.1218G>A	p.(Trp406*)	W405X	0%	comp. het.	SW
1	**c.586C>T**	**p.(Gln196*)**	**unknown**	**0%**	c.1069C>T	p.(Arg357Trp)	R356W	0%	comp. het.	SW
1	c.1069C>T	p.(Arg357Trp)	R356W	0%	c.1451G>C	p.(Arg484Pro)	R483P	unknown	comp. het.	SV
1	c.844G>T c.923dup c.955C>T c.1069C>T	p.(Val282Leu) p.(Leu308fs) p.(Gln319*) p.(Arg357Trp)	V281L-F306 + 1nt-Q318X-R356W	0%	c.1218G>A	p.(Trp406*)	W405X	0%	comp. het.	SW
6	c.293-13C>G	p.?	I2G	0-1%	c.1069C>T	p.(Arg357Trp)	R356W	0%	comp. het.	SW

1	c.(?_-107)_(939+?)del	p.0	deletion exon 1-7	0%	c.(?_-107)_(939+?)del	p.0	deletion exon 1-7	0%	hom.	SW
1	c.(?_-107)_(738+?)del	p.0	deletion exon 1-6	0%	c.518T>A	p.(Ile173Asn)	I172N	1-5%	comp. het.	SW or SV
1	c.(?_-107)_(738+?)del	p.0	deletion exon 1-6	0%	Not identified yet				het.	unknown
2	c.(?_-107)_(447+?)del	p.0	deletion exon 1-3	0%	c.1069C>T	p.(Arg357Trp)	R356W	0%	comp. het.	SW

In bold variants that have not been described before. Adherence to HGVS nomenclature rules (varnomen.hgvs.org) makes the nucleotide numbers 3 higher and the amino acid numbers 1 higher than the legacy names used in literature.
Abbreviation: Comp. het.: compound heterozygous; het.: heterozygous; hom.: homozygous; NC non-classical, SV simple virilizing, SW salt wasting