Review Article

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Diagnostic approach in 46, XY DSD: an endocrine society of bengal (ESB) consensus statement

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Abstract

Objectives: 46, XY difference/disorder of sex development (DSD) is a relatively uncommon group of heterogeneous disorders with varying degree of underandrogenization of male genitalia. Such patients should be approached systematically to reach an aetiological diagnosis. However, we lack, at present, a clinical practice guideline on diagnostic approach in 46, XY DSD from this part of the globe. Moreover, debate persists regarding the timing and cut-offs of different hormonal tests, performed in these cases. The consensus committee consisting of 34 highly experienced endocrinologists with interest and experience in managing DSD discussed and drafted a consensus statement on the diagnostic approach to 46, XY DSD focussing on relevant history, clinical examination, biochemical evaluation, imaging and genetic analysis.

Content: The consensus was guided by systematic reviews of existing literature followed by discussion. An initial

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draft was prepared and distributed among the members. The members provided their scientific inputs, and all the relevant suggestions were incorporated. The final draft was approved by the committee members.

Summary: The diagnostic approach in 46, XY DSD should be multidisciplinary although coordinated by an experienced endocrinologist. We recommend formal Karyotyping, even if Y chromosome material has been detected by other methods. Meticulous history taking and thorough head-to-toe examination should initially be performed with focus on external genitalia, including location of gonads. Decision regarding hormonal and other biochemical investigations should be made according to the age and interpreted according to age-appropriate norms Although LC-MS/MS is the preferred mode of steroid hormone measurements, immunoassays, which are widely available and less expensive, are acceptable alternatives. All patients with 46, XY DSD should undergo abdominopelvic ultrasonography by a trained radiologist. MRI of the abdomen and/or laparoscopy may be used to demonstrate the Mullerian structure and/or to localize the gonads. Genetic studies, which include copy number variation (CNV) or molecular testing of a candidate gene or next generation sequencing then should be ordered in a stepwise manner depending on the clinical, biochemical, hormonal, and radiological findings.

Outlook: The members of the committee believe that patients with 46, XY DSD need to be approached systematically. The proposed diagnostic algorithm, provided in the consensus statement, is cost effective and when supplemented with appropriate genetic studies, may help to reach an aetiological diagnosis in majority of such cases.

Keywords: 17β-hydroxysteroid dehydrogenase 3 deficiency; 3β-hydroxysteroid dehydrogenase 2 deficiency; 5α-reductase 2 deficiency; 46, XY DSD; androgen insensitivity syndrome; ambiguous genitalia.

Introduction

Differences/disorders of sex development (DSD) are said to be present if the chromosomal sex, gonadal sex, and phenotypic sex (anatomy of the internal and/or external genitalia) of an individual are discordant. DSD is broadly classified into 3 groups: 46, XY DSD, 46, XX DSD, and sex chromosome DSD which includes sex chromosome aneuploidy, mixed gonadal dysgenesis (MGD) and ovotesticular DSD (OTDSD). Patients with DSD possessing 46, XY chromosome complement are collectively referred to as 46, XY DSD, a heterogeneous group of conditions with varying degrees of under-virilization of the external and internal genitalia. The atypical genital organs in 46, XY DSD is attributed to either of the following: (i) disorders of gonad development (testicular dysgenesis [TD], OTDSD, ovarian DSD) (ii) disorders of androgen synthesis, (iii) impaired androgen action or androgen insensitivity syndrome (AIS), and (iv) isolated defect in anti-müllerian hormone (AMH) synthesis or action. The last subgroup, however, is often associated with unilateral/bilateral cryptorchidism or the characteristic 'transverse testicular ectopia' without under-virilization of the male external genitalia.

The clinical presentation of 46, XY DSD varies from ambiguous/atypical genitalia noticed at birth to female external genitalia, when the diagnosis is made during pubertal age group due to primary amenorrhea with/without lack of breast development or new onset virilization. Understandably, patients with 46, XY DSD present at different ages, and to different specialities like paediatricians, endocrinologists, gynaecologists, urologists, and plastic surgeons. It is important to reach a precise diagnosis early in life to decide on the sex of rearing (in children with ambiguous genitalia), avoid life-threatening adrenocortical crisis (in patients with steroidogenic factor [SF1] [also known as NR5A1] mutation and proximal defects in steroid biosynthesis), the timing of gonadectomy in patients with a high risk of gonadal malignancy and schedule a management plan across the lifespan of the individual.

The aetiological diagnosis in 46, XY DSD is based on detailed family history, focussed clinical examination, and relevant biochemical, hormonal, and radiological investigations supplemented by genetic analysis. Though next generation sequencing (NGS) has recently been suggested as a useful and cost-effective frontline test for such patients, confirmed diagnosis is achieved only in less than 1/3 of cases [1]. Moreover, substantial prevalence of genetic variants of 'uncertain significance' underlay the importance of thorough clinical and hormonal assessment in addition to NGS [2]. Despite the fact that hormonal evaluation in 46, XY DSD is often inconclusive, and the diagnosis remains elusive in about half of these patients even after genetic studies, we recommend that a systematic approach should be adopted in all patients with 46, XY DSD aiming to reach a diagnosis. In this consensus statement, we have focussed on relevant investigations, that are available even in resource-restricted settings, and interpretation of different tests across different age groups to reach an aetiological diagnosis.

Methods

The consensus committee consisted of 34 highly experienced endocrinologists with interest and experience in managing DSD (cumulative H-index of 232) who are members of the Endocrine Society of Bengal (ESB), an affiliate of the Endocrine Society of India (ESI). The members realized that unlike 46, XX DSD, 46, XY DSD is a heterogeneous group of diseases and, despite published literature, we lack high-quality evidence on comprehensive diagnostic algorithm in 46, XY DSD, particularly from this part of the world. The committee also realized that developing a comprehensive guideline for diagnosis of the 46, XY DSD is difficult due to regional variations in the availability and affordability of different tests. The gold standard test for steroid hormone estimation liquid chromatography linked with tandem mass spectrometry (LC-MS/MS) is beyond the reach of most endocrinologists working in this part of the world. Similarly, genetic tests like NGS, either focused exome sequencing (FES) or whole-exome sequencing (WES) or whole-genome sequencing (WGS) is relatively expensive. Clinical examination and biochemical evaluation (along with dynamic tests for hormonal ratios) supplemented by genetic tests, thus, remain the mainstay of diagnosis in 46, XY DSD. A literature search was performed using the online database from PubMed using the following MeSH terms: (46, XY DSD) (ambiguous genitalia) (androgen insensitivity syndrome) (17β-hydroxysteroid dehydrogenase 3 deficiency) (5α-reductase 2 deficiency) (3β-hydroxysteroid dehydrogenase 2 deficiency) and relevant articles (original articles, reviews, case series, and case reports) published in peer-reviewed indexed journals were selected. The members of the committee met on 28th April 2022 and the first draft of the recommendation was discussed. All the members provided their critical comments and suggestions on individual components of the recommendation. Relevant modifications were incorporated, and the final draft of the consensus statement was created. The members of the committee unanimously accepted the final draft.

Recommendations

Chapter I: history and clinical examination

R.1 A detailed history should be obtained in all patients with 46, XY DSD focusing on relevant family history, antenatal history, and possible primary adrenocortical insufficiency (PAI) in early infancy.

Comments

A history of failure to thrive, hospital admission for recurrent vomiting or features of salt wasting and need for glucocorticoid administration in the neonatal period or during childhood should be asked for. Birthweight is important to note given the well-reported association of 46, XY DSD with intra-uterine growth retardation and low birth weight; small for gestational age (SGA), however, is extremely unusual in androgen receptor (AR) mutation [3, 4]. History of acne and hirsutism in mother during pregnancy (which would suggest P450 oxidoreductase [POR] deficiency), medications used by the mother during pregnancy which might affect the foetus (e.g., 5α-reductase inhibitors, androgen receptor blockers, progestins, endocrine disruptors), timing, indication (growth restriction and preeclampsia) and mode of delivery should also be asked for [5].

Most of the steroidogenic enzyme deficiencies related to 46, XY DSD are autosomal recessive conditions; hence the history of parental consanguinity should always be sought. On the other hand, a pedigree suggestive of an X-linked inheritance pattern would point towards AIS. Relevant points to be noted in family history include sibling(s) or relative(s) with atypical genitalia, cryptorchidism, or hypospadias; inguinal hernia-unilateral or bilateral, with absent puberty or infertility in 'female' relative(s); genital surgeries; crisis or death in sibling(s) or relative(s).

R.2 Clinical examination in patients with 46, XY DSD should be performed systematically.

R.2.1 Visual inspection of the genital area may reveal peno-scrotal transposition indicating malformative DSD or significant asymmetry of the genitalia suggesting MGD or OTDSD. Hyperpigmentation of scrotal skin could indicate 46, XY DSD with underlying PAI.

Comments

Asymmetric genitalia (discrepancy in scrotal rugosities and/or location of gonads) is highly suggestive of MGD or OTDSD, which is subsequently confirmed by peripheral blood karyotype. Rare patients with TD may also present with asymmetric genitalia. The gonad is bipotential, meaning phenotypically identical both in 46, XX and 46, XY individuals until about 42 days post conception. During this time, the gonadal ridges are sexually undifferentiated with both Wolffian and Müllerian ducts being present, and the urogenital sinus (UGS) and the primordia of the external genitalia being undifferentiated. During this period, i.e., before the differentiation of gonads, defects involving the anlagen of the internal genitalia (Müllerian or Wolffian ducts), the UGS, or the primordia of the external genitalia result in non-endocrine causes of DSD or the so-called 'malformative' DSD. Peno-scrotal transposition (extension of the scrotal folds above the base of the phallus) points towards underlying malformative DSD obviating the need for any hormonal analysis. However, penoscrotal transposition has rarely been reported in patients with AR defects [6, 7]. All patients with malformative DSD should be evaluated for underlying VACTERL (vertebral, anal atresia,

cardiac defect, tracheo-oesophageal fistula, renal anomaly, limb abnormalities) association. Signs/symptoms of coexistent PAI help to narrow down differential diagnoses to conditions affecting both gonads and adrenals, and thus help in more focussed hormonal evaluation.

R.2.2 Genital examination should focus on the position of the gonads, measurement of stretched phallic length (SPL), identification of the position of urethral and/or UGS orifices, and fusion of labioscrotal folds. Meticulous palpation is important to locate the gonads in the scrotal sac, labio-scrotal folds, or inguinal canals. Anogenital distances (AGDs), i.e., the distance from the midpoint of the anus to the junction of the labio-scrotal fold and perineal skin (AGD_L) and the distance from the midpoint of the anus to the base of the phallus (AGD_U) should be measured.

R.2.3 Examination findings should preferably be expressed quantitatively using validated scores for external genitalia examination.

R.2.4 Mean AGD_L: AGD_U ratio at birth in full-term new-borns with 46, XY karyotype and typical male genitalia is 0.48–0.52. A ratio of less than 0.32 should be considered as significant under-androgenization.

Comments

The location of both the gonads is determined by meticulous palpation [8]. A gonad, which is palpable below the inguinal ligaments, contains at least some amount of testicular tissue. Length of the phallus can sometimes be misleading due to excess suprapubic fat and presence of chordee. SPL should be assessed with proper precautions to exclude the foreskin and measure the length along its dorsal aspect in a non-erect state. The phallus should be stretched gently until the point of increased resistance and measurement is taken from the base of the phallus (as close to the pubic bone as possible) to the tip of the glans. Micropenis is defined if the SPL is less than 2.5 standard deviation (SD) from the mean of the available normative data for that ethnicity [9]. Region-specific normative data of SPL and testicular volume are available from different parts of India [10–12]. Based on contemporary data available from a wide range of countries, an SPL of less than 2.5 cm represents a reasonable cut-off for micropenis in the new-born. A pit located on the glans may not necessarily suggest urethral opening. A precise description of external genitalia is facilitated by the use of quantitative scoring systems like the well-established external masculinization score (EMS), or the recently described and validated gender-neutral external genitalia score (EGS), developed by the European Cooperation in Science and Technology (COST) Action BM1303 working group [13, 14]. The more refined, non-dichotomous categorical scale of EGS perhaps better reflects natural variations. EGS uses a graduated scale from female to male (range 0–12) with the same anatomical landmarks as the EMS; the interquartile ranges (IQRs) are smaller for EGS as compared to EMS. The reference values for preterm and term babies up to 24 months of age are available (Table 1). A comparison between EMS and EGS is outlined in Table 2.

Measurement of the AGDs, which correlates with prenatal androgen [dihydrotestosterone (DHT)] exposure before 12 weeks of gestation, has been standardised following the Infant Development and the Environment Study (TIDES), where the fixed end of the calliper is held at the centre of the anus, and the sliding part of the calliper is moved to measure the AGDs [15]. In a recent European multicentre study, the AGD_L: AGD_U ratios in males, females

Table 1: AGD_L:AGD_U ratio and EGS in 46, XY infants with typical male genitalia of different gestational ages and age-groups [adapted from van der Straaten S, et al. [14].

Age groups	AGD_L:AGD_U		EGS		
	Mean	SD	Median	10th percentile	90th percentile
Gestational age					
<28 weeks	0.45	0.1	10	8.6	11.5
28–32.9 weeks	0.44	0.1	11.5	9.2	12
33–36.9 weeks	0.50	0.1	11.5	10.5	12
>37 weeks	0.52	0.1	12	10.5	12
Age group in full-term infants					
0–1 months	0.52	0.1	12	10.5	12
1–6 months	0.48	0.1	12	11.5	12
6–12 months	0.49	0.1	12	11	12
12–24 months	0.49	0.1	12	11.9	12

Table 2: Comparison between EMS and EGS.

EMS	EGS
Components: Scrotal fusion, penile length, position of urethral meatus and position of gonads	Similar anatomic landmarks
Score: 0 to 12	Score: 0 to 12
Gender-specific design and vocabulary (micropenis, scrotal fusion)	Gender neutral terms (labioscrotal folds, phallic length)
Suitable only for 46, XY DSD (karyotype-proven or those with palpable gonads)	Can be used for all
Binary answers for scrotal fusion and micropenis	Quantitative score for phallic length and labioscrotal fold
DSD evaluation is advised if EMS <11	DSD evaluation is advised if EGS >0 and ≤10.5
No gestational age specific normative data available	Normative data is available for premature, low birth weight and full-term babies until the age of two years
	More objective and easier for physicians and general paediatricians
	Better for naturally occurring variations

None of these two takes into account the presence of other atypical genital features such as complete or partial penoscrotal transposition, scrotal anomalies or degree of penile curvature.

and in those with DSD were 0.49 (±0.1), 0.39 (±0.1) and 0.43 (±0.1), respectively [14]. A cross sectional study from Thailand, that evaluated 364 healthy full-term new-borns within 72-h of birth and excluded those with genital ambiguity, dysmorphic features, and known maternal ingestion of androgenic medications or substances, found a mean ratio of 0.48 (±0.08) in 46, XY and 0.39 (±0.08) in 46, XX neonates [16]. The later cut-offs seem clinically more prudent, hence a ratio of less than 0.32 (mean−2 SD) should be considered as significant under-androgenization in 46, XY individuals. Reference ranges for AGD_L: AGD_U ratio in infants born at different gestational weeks and in children up to 2 years of age have also been proposed (Table 1). Low AGD_L: AGD_U in 46, XY infants suggests under-androgenization; the ratio is comparatively lower in the complete form of 17β-hydroxysteroid dehydrogenase3 (17β-HSD3) and 17α-hydroxylase/17, 20-lyase (CYP17A1) deficiencies than in 5α-reductase2 deficiency (5α-R2D). 17β-HSD3 and CYP17A1 enzymes are involved both in classic and backdoor pathways of DHT synthesis whereas 5α-reductase1, and not 5α-reductase2 is involved in the backdoor pathway. DHT, produced by backdoor pathway during foetal life, thus, results in relatively higher AGD_L: AGD_U in 5α-R2D.

R.2.5 Certain extra-genital abnormality (es) may suggest a specific aetiology; hence a thorough systemic examination from head to toe is important. Blood pressure should be measured in all patients of 46, XY DSD using age-appropriate cuffs.

Comments

Systolic and diastolic blood pressure should be interpreted according to the age-specific references, taking into account height percentiles. Unlike 46, XX DSD, PAI is much less common in 46, XY DSD. Hypotension in those with possible history of adrenal crisis and severe under-androgenization should raise the suspicion of proximal defect in steroid biosynthesis (steroidogenic acute regulatory protein [StAR], or cholesterol side-chain cleavage enzyme [CSCCE] deficiency); those with SF1/NR5A1 mutation or defect in 3β-hydroxysteroid dehydrogenase (3β-HSD2) or POR usually present with ambiguous genitalia and may also have hypotension [17]. Hypertension in a severe under-androgenized individual with 46, XY karyotype suggests CYP17A1 defect or underlying nephropathy in Wilms tumor 1 (WT1) mutation. A number of conditions present with 46, XY DSD with one or more associated pathognomonic extragenital manifestations (Table 3); a detailed systemic examination, including those of the heart and limbs in particular, may provide a clue and help focussed investigations.

R.2.6 Hormonal evaluation should be performed in those with an EMS score of less than 11 or an EGS score of ≤10.5.

Comments

The diagnostic yield in neonates with isolated glandular or mid-shaft hypospadias or unilateral inguinal testis is low; hence detailed evaluation is not cost-effective. Those with EMS of less than 11 or EGS of ≤10.5 should be investigated [13, 14].

Chapter II: biochemical and hormonal evaluation

R.3 Investigations in cases of 46, XY DSD are aimed at identifying underlying defect. In resource-constrained settings, biochemical and hormonal investigations may pin-point a diagnosis obviating the need for genetic testing in every patient with 46, XY DSD or may help make more focussed genetic investigations.

Table 3: Extra-genital manifestations in different aetiologies of 46, XY DSD.

Disorder	Extra-genital manifestation
Testicular dysgenesis	
Wilms' tumor (WT1) mutation	
WAGR syndrome	Wilms tumour, aniridia, genitourinary malformations, mental retardation
Denys-drash syndrome	Diffuse proliferative glomerulonephritis, Wilms tumor
Frasier syndrome	Focal segmental glomerulosclerosis, gonadoblastoma
Meacham syndrome	Cardiac defects, diaphragmatic hernia
GATA4	Cardiac defects (septal defects, tetralogy of fallot)
FOG2 (also known as ZFPM2)	Cardiac defects, learning & language disorder, autism spectrum disorder
SOX9	Camptomelic dysplasia
SAMD9	MIRAGE syndrome (myelodysplasia, infections, restriction of growth, adrenal hypoplasia, genital phenotypes, enteropathy)
ATRX	α-thalassemia mental retardation
ARX	Lissencephaly, epilepsy, temperature instability
DHH	Minifascicular neuropathy
WNT4	Mental retardation
DMRT1	Mental retardation
Defects in androgen biosynthesis	
DHCR7	Smith-lemli-opitz syndrome (coarse facies, second- third toe syndactyly, failure to thrive, developmental delay, cardiac and visceral abnormalities)
POR	Skeletal abnormalities resembling antley-bixler craniosynostosis
CYB5A	Methemoglobinemia

R.3.1 Karyotype from peripheral blood leukocyte should be done in all cases. At least 30 cells should be analysed to reduce the chance of missing mosaicism.

Comments

The clinical suspicion of 46, XY DSD should always be confirmed with karyotype from peripheral blood leukocytes. There is data to suggest that to exclude at least 10% mosaicism with 95 and 99% confidence, minimum number of cells, that needs to be evaluated is 29 and 44, respectively [18]. In routine practice, number of analysed cells varies among laboratories and ranges usually between 20 and 50. Absence of Y chromosome warrants repeat karyotype from 100 cells to rule out MGD (45, X/46, XY; 45, X/47, XYY; 45, X/46, XY/47, XYY) or OTDSD (46, XX/46, XY; 45, X/46, XX/47, XYY). Patients, in whom Y chromosome has already been detected by multiplex ligation-dependent probe amplification (MLPA) or quantitative fluorescent polymerase chain reaction (QF-PCR), should also undergo formal karyotyping.

R.3.2 Hormonal evaluation depends on the age of presentation. Basal testosterone (T), Δ4-androstenedione (Δ4-A), luteinizing hormone (LH), follicle stimulating hormone (FSH), and AMH should be assessed in all ages and interpreted according to age-specific reference ranges. Serum sodium (Na), potassium (K) should be measured in those presenting during infancy or childhood. Serum creatinine estimation, and urine analysis should be performed in all patients irrespective of age of presentation. In neonates without signs/symptoms of PAI and normal electrolytes, hormonal evaluation should better be performed at around 10 weeks of age.

Comments

The gonadotropins start to increase from the end of first week after birth, remain elevated during the initial 3–6 months, after which they decline to pre-pubertal concentrations as the hypothalamo-pituitary-gonadal (HPG) axis enters into "juvenile pause" until the onset of puberty. The sex steroids, T and DHT, start declining after birth and may become undetectable soon. They again start to rise around 6–8 weeks, reach a peak around 10 weeks and decline to pre-pubertal levels after 6 months post-partum [19, 20]. Serum FSH level is higher than LH level in prepubertal boys and girls. A striking rise in serum LH amplitude is noted by about a year before clinical evidence of pubertal onset and reaches an early plateau, whereas FSH rises more consistently through male puberty. However, the daytime concentration of serum FSH (expressed in IU/L) is higher than concentration of serum LH (expressed in IU/L) across all stages of puberty and throughout life [21, 22]. In patients with AIS, basal LH, but not FSH, is often above the normal age-specific reference range [23, 24]. FSH in AIS is significantly elevated in patients with undescended testes, secondary to dysgenetic changes or following orchidectomy [25]. LH: FSH ratio of more than 1 (expressed in IU/L) in morning sample, thus, suggests androgen resistance, LH receptor defect or severe degree of testosterone biosynthetic defect. However, a ratio of less than 1 (expressed in IU/L) doesn't exclude these conditions. Elevated FSH (more than 8 IU/L), particularly during minipuberty or puberty suggests testicular dysgenesis or seminiferous tubule dysfunction.

In patients with complete AIS (CAIS) or in those with near complete T deficiency, minipuberty is absent, and gonadotropins are not elevated during minipuberty [26]. Similarly, T is lower than expected and often in the pre-pubertal or early pubertal ranges during minipuberty in CAIS [26]. However, serum T in partial AIS (PAIS) is high-normal or normal when compared with age specific reference ranges during minipuberty or puberty. The HPG axis during minipuberty is most active between days 15 and 90 after birth; therefor, to utilise the hormonal kinetics maximally and to avoid retesting, hormonal evaluation is preferably performed between days 30 and 70 after birth [27, 28].

AMH is strongly expressed in sertoli cells from the time of testicular differentiation. Serum level of AMH in males is low but detectable at birth, rises over infancy, starts to decline around 10 years of age (between Tanner stage II and III), and decreases further during puberty under the influence of T (and possibly DHT) acting through functional AR [29, 30]. FSH and hyperestogenic states stimulate AMH secretion in males [31]. AR is minimally expressed in the sertoli cells during minipuberty. AR expression starts increasing from 2 years of age and reaches adult level at around 8 years of age. In the context of 46, XY DSD, AMH serves two purposes: (1) To diagnose TD (low AMH) across all age groups and (2) to differentiate 5α-R2D (normal AMH) from AIS (high AMH) during peri-puberty [32]. AMH is not a useful test to differentiate AIS from 5α-R2D during minipuberty (lack of AR in sertoli cells) and during childhood (low FSH and low T). The role of AMH estimation in different forms of 46, XY DSD is outlined in Table 4. AMH can be measured by different immunoassay methods like enzyme-linked immunosorbent assay (ELISA) and chemiluminescent immunoassay (CLIA) and, there can be potential discordance between different assay methods. The older AMH assays were standardised for adult women, in whom the values are much lower than in male infants and children. The lower cut-off of the reference range for this age-group often falls beyond the linear part of the dose-response curve even after recommended dilution; hence, measuring AMH in those platforms may not provide the actual concentration of circulating AMH. Even if the values are measurable, older assays are relatively imprecise and inaccurate to detect higher concentrations of AMH. Recently, with standardisation of immunoassay methods and availability of distinctive age-specific normative data (and also for pubertal stage) in males, AMH measurement by these platforms (Roche Elecsys, Beckman Coulter) has become more reliable [33].

Rare patients with 46, XY DSD due to WT1 mutations often have some form of nephropathy like diffuse mesangial sclerosis in Denys-Drash syndrome and focal segmental glomerulosclerosis in Frasier syndrome. Another small subgroup of patients with 46, XY DSD may have associated PAI. Estimation of serum creatinine & urine analysis (may suggest underlying nephropathy) and measurement of Na & K (may suggest underlying PAI) are economical and widely available and therefore should be considered in all. Serum K is relatively higher in neonates and needs to be interpreted accordingly. This approach may help in narrowing down differential diagnoses, avoid unnecessary investigations and help in focussed genetic analysis. We recommend estimating glomerular filtration rate (eGFR) either by bedside Schwartz method (less than 18 years of age) or Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) method (≥18 years of age) rather than relying on serum creatinine alone to detect underlying nephropathy.

R.3.3 Serum T, Δ4-A, and DHT following human chorionic gonadotropin (hCG) stimulation should be measured in all ages. Standard 72 h-hCG stimulation test should initially be performed using age-appropriate hCG dosage. We recommend 1,500 units of hCG intramuscularly

Table 4: Role of AMH in diagnosis of 46, XY DSD.

46, XY DSD condition	AMH level	Age-group in which most useful	Explanation
Testicular dysgenesis	Undetectable to low	All age-groups	Absent or poor sertoli cell reserve
Testosterone biosynthetic defect (including leydig cell hypoplasia[a])	Normal in childhood. Higher than male reference range in peri-puberty	Peri-puberty	Lack of suppressive effect of androgens during peri-puberty
5α-reductase2 deficiency	Normal in childhood. Lower range of normal male reference (<−1 SD)	Peri-puberty	High testosterone suppressed AMH expression
Androgen insensitivity	Normal in childhood. Higher than male reference range in peri-puberty	Peri-puberty	Testosterone cannot act due to defective androgen receptor. Relative hyperestrogenic state may also contribute to high AMH

[a]Post-hCG androgen concentrations overlap in LH/hCG receptor defect and TDS. LH: FSH of >1 and normal AMH suggest the former, while LH:FSH <1 and low AMH is encountered in the latter condition.

for 3 consecutive days and get blood samples on day 4 in children aged 5 years or more.

R.3.4 Prolonged hCG stimulation test (over a period of 3 weeks) should be done if T response following 72 h-hCG stimulation test is inadequate. Prolonged hCG stimulation may be considered as first line test in cases where poor T response to 72-h hCG stimulation is anticipated like in patients with non-palpable gonads.

R.3.5 Post-hCG T: Δ4-A of less than 0.8 and T: DHT of more than 30 is highly suggestive of 17β-HSD3 deficiency (17β-HSD3D) and 5α-R2D, respectively, in all age groups (mini-puberty, pre-puberty, puberty, and post-puberty)

R.3.6 Post-hCG T: Δ4-A of more than 0.8 in pubertal and post-pubertal age-groups does not rule out 17β-HSD3D. Post-hCG T: DHT of less than 30 does not rule out 5α-R2D during mini-puberty and in pubertal and post-pubertal age groups.

R.3.7 Basal (unstimulated) T: Δ4-A and T: DHT ratios are less preferred alternatives to stimulated ratios during minipuberty and puberty.

Comments

Ratios of intermediates following hCG stimulation help classifying 46, XY DSD. hCG stimulation is used to determine whether leydig cells are capable of producing T in response to hCG acting through LH/hCG receptor (LH/hCGR). This is often useful in diagnosing specific enzyme defects, like T: Δ4-A ratio in 17β-HSD3D or T: DHT ratio in 5α-R2D. Pre-hCG T concentration on day 1 might give a clue to underlying AIS (not CAIS) during minipuberty and post-puberty; but baseline measurement of the other androgens usually is not required [23]. There are different stimulatory protocols in addition to what we recommend. One of them is intramuscular administration of 5,000 units of hCG on day 1 and measurement of androgens (T, Δ4-A and DHT) levels on day 4 (72-h after the injection). The other alternative is giving intramuscular hCG 500–2,000 units (<6 months: 500 units, 6 months-5 years: 1,000 unit, 5–10 years: 1,500 units, >10 years: 2,000 unit) on 3 consecutive days and measuring androgens on day 4 (24-h after last injection). Instead of a fixed dose used for 3 consecutive days, body weight or body surface adjusted single hCG injection (100 IU/kg or 5,000 IU/1.7 m^2) has also been used as an effective alternative [34]. Hence, for smaller infants less than 1 year of age, a total dose of 1,500 units may be sufficient [35].

The definition of normal T response has not been clearly defined. Post-hCG T concentration of more than 100 ng/dL or post-hCG T greater than the upper pre-pubertal limit (i.e., 20 ng/dL) or a rise in T to at least twice the baseline value or by at least 100 ng/dL or to lower cut-off of adult reference range (approximately 230–288 ng/dL) have been suggested by various authors [28, 36, 37]. T concentration in pre-pubertal children falls below the detection limit of immunoassays; hence identifying the degree of increment may not always be possible. Moreover, lower cut-off of adult reference range is too high to define a normal response. It has also been suggested that for valid interpretation of the T: DHT ratio, T level should be in the post-pubertal range (i.e., 50 ng/dL). A vast majority of patients with 17β-HSD3 deficiency have post-hCG T concentration of 50 ng/dL. Taking all these issues into consideration, we define post-hCG T of at least 50 ng/dL as a marker of adequate stimulation. Post-hCG T concentration of less than 50 ng/dL makes AIS or 5α-R2D highly unlikely. However, a value of more than 50 ng/dL, particularly between 50 and 100 ng/dL, does not rule out partial TD or partial proximal defects in steroidogenesis. A prolonged hCG stimulation test is indicated when there is poor T response to a standard 72 h-hCG stimulation test. Among different protocols, a 3-week hCG stimulation test seems be more appropriate. The standard 3-day hCG stimulation is followed by further hCG stimulation with 1,000–1,500 units for 2 days a week for the following 2 weeks and T is measured 24-h after the last injection [38, 39].

The typical biochemical profile in 5α-R2D is increased T: DHT ratio at baseline or after hCG stimulation, with lower sensitivity of the former. T is converted to DHT also by 5α-reductase 1, an enzyme which is highly expressed in liver and non-genital skin (hair follicles), particularly from the time of puberty. The typical pubertal onset virilization encountered in 5α-R2D is secondary to conversion of T to sufficient DHT by activity of this 5α-reductase 1; hence post-hCG T: DHT might not be a sensitive test after the onset of puberty. Interestingly, in a cohort of 5α-R2D, T: DHT performed similarly across all age groups including post-puberty [40]. In a different cohort of post-pubertal subjects with PAIS, T: DHT ratio after hCG stimulation, ranged from 5–14 (median, 8) suggesting T: DHT may be useful to differentiate 5α-R2D from AIS even after puberty [24]. The neonatal skin transiently expresses 5α-reductase 1. Moreover, the backdoor-pathway of androgen biosynthesis is active not only in foetus but also during mini-puberty, and 5α-reductase 1 plays an important role in the backdoor pathway. It converts progesterone to 5α-dihydroprogesterone (5α-DHP) and 17α-hydroxyprogesterone (Δ4-17P) to 17α-hydroxydihydroprogesterone and ultimately to DHT by other enzymes like AKR1C2/C4, CYP17A1 and 17β-HSD. Hence, post-hCG T: DHT might lose its sensitivity to detect 5α-R2D during mini-puberty.

Different ratios of T: DHT following hCG stimulation have been suggested in different age groups. T: DHT ratio of 8.5 is suggested to have higher sensitivity during infancy [41]. 5α-R2D is characterized by post-hCG T: DHT greater than 18 in children. A ratio of 30 has a diagnostic sensitivity and specificity of 11 and 99%, respectively, while a cut-off 10 is associated with higher sensitivity (72–78%) but with lower specificity (72%) [40, 42]. High T: DHT may also be encountered in some patients with AIS as T is known to have a positive regulatory effect on 5α-reductase 2 enzyme mediated through AR. In an Indian cohort of PAIS, 36% of patients had post-hCG T: DHT ratio of more than 30 [25]. A cut-off of 20 for post-hCG T: DHT is preferred to make a trade-off between sensitivity and specificity. Higher value increases the likelihood of 5α-R2D. Moreover, for correct determination of T: DHT commercial immunoassays are not sufficient as ≈30% cross-reactivity of DHT with T has been reported [43]. However, T: DHT of more than 30, even if measured in immunoassay appears highly suggestive of the disease. We do not rely much on post-hCG T: DHT to diagnose 5α-R2D, particularly during mini-puberty and after pubertal onset.

A number of different cut-offs for the T: Δ4-A ratio has been proposed for diagnosis of 17β-HSD3D. In a pre-pubertal child T: Δ4-A ratio of <1 has been found to have higher sensitivity than a cut-off of <0.8 [39, 44]. The peri-pubertal virilisation in 17β-HSD3D is due to extra-gonadal conversion of Δ4-A to T by other 17β-HSD isoenzymes during puberty, 17β-HSD5 in particular. 17β-HSD3 & 17β-HSD5 are also involved in the back-door pathway of androgen biosynthesis. However, 17β-HSD5 probably is not expressed during minipuberty [45]. Basal T: Δ4-A values have been found to have nearly 100% sensitivity during infancy and post-puberty with cut-offs of both 0.8 and 1 [39, 44, 46]. However, stimulated T: Δ4-A appears to have higher sensitivity and specificity for 17 β-HSD3D compared to basal ratio [47, 48]. A small proportion of patients with TD and rare patients with mutation negative AIS may demonstrate T: Δ4-A of less than 0.8 after hCG stimulation [49]. Though low AMH may be useful to rule out TD, differentiating 17β-HSD3D from AIS might be challenging without detailed molecular analysis. We recommend T: Δ4-A following hCG stimulation of less than 0.8 across all age groups to suggest underlying 17β-HSD3D.

R.3.8 Serum cortisol, adrenocorticotropic hormone (ACTH), and plasma renin activity (PRA) should be measured in suspected PAI and in patients with low AMH. Cortisol, Δ4-A, dehydroepiandrosterone (Δ5-DHEA), 17-hydroxy-progesterone (Δ4-17P) and 17α-hydroxypregnenolone (Δ5-17P) following tetracosactin should be measured in selected cases with suspected PAI.

Comments

Rare patients with 46, XY DSD (SF1 mutation and defects in steroid biosynthesis like inactivating mutation in StAR, CSCCE, CYP17A1 or 3β-HSD2) may have coexistent PAI. Serum cortisol, ACTH, and PRA should be measured in such cases. Considering the rarity of such cases and the cost involved, we do not recommend evaluation of adrenocortical function in all cases of 46, XY DSD.

Assessment of cortisol, Δ4-A, Δ5-17P and Δ5-DHEA following tetracosactin stimulation should be performed if there is clinical and/or biochemical suspicion of PAI or in cases with poor T response following prolonged hCG stimulation or in patients with low AMH [28, 50]. Unlike 46, XX DSD, Δ4-17P has limited diagnostic role in 46, XY DSD and may be measured in selected patients only. Tetracosactin stimulation test can be done on a different day or can be combined with standard hCG stimulation test wherein tetracosactin injection (250 μg for children above 1 year of age and 125 μg for infants below 1 year of age) is administered intramuscularly/intravenously on morning of day 4, 1 h prior to drawing of blood samples, and cortisol and Δ5--DHEA is measured along with androgens. Δ5-17P measurement is available commercially and should be measured in suspected cases of 3β-HSD2 deficiency. The biochemical markers of classic 3β-HSD2 deficiency are Δ5-17P concentration greater than ≥201 nmol/L or Δ5-17P: cortisol ratio of ≥487 (expressed in nmol/L). Age based cut-offs of post-ACTH Δ5-17P (≥378 nmol/L in neonates and ≥165 nmol/L in children) and Δ5-17P: cortisol ratios (≥434 in neonates ≥216 in children) have also been suggested to diagnose 3β-HSD2 deficiency in children with ambiguous genitalia [51]. Though Δ5-17P to Δ4-17P ratio of more than 18 (expressed in ng/dL) or Δ5-DHEA to Δ4-A ratio of more than 18 (expressed in ng/dL) after ACTH stimulation have also been suggested as markers of 3β-HSD2 deficiency, there is considerable overlap with other causes of ambiguous genitalia [52]. High Δ5-DHEA sulfate (DHEAS) may be used as a surrogate of underlying 3β-HSD2 deficiency.

Deficiency of CYP17A1 is characterized by low androgens (Δ5-DHEA, Δ4-A, and T) with/without hypokalemia and metabolic alkalosis. The diagnosis is reached by low cortisol, high ACTH, high progesterone and suppressed PRA. Patients with isolated 17,20-lyase deficiency have marked reduction in androgen concentration, but with preserved glucocorticoid and mineralocorticoid axes. Cases of isolated 17,20-lyase deficiency also have an increased ratio of C21 deoxysteroids to C19 steroids (Δ5-DHEA, Δ4-A) after tetracosactin stimulation. Both disorders are associated with increased LH, FSH levels after puberty. Circulatory Δ4–17P concentration in

CYP17A1 deficient 46, XY individuals varies from undetectable to high depending on the severity of the defect and age of the patient. In severe degree of enzyme deficiency, Δ4–17P is undetectable or low, while in post-pubertal patients with milder disease and having dominant 17–20 lyase defect, Δ4–17P concentration may be high. This high value reflects testicular origin of Δ4–17P under the influence of elevated LH due to hypergonadotropic hypogonadism. Interestingly, even prepubertal XY patients with CYP17A1 deficiency have been reported to show incremental Δ4–17P response following hCG stimulation, but not with tetracosactin [53].

Patients with cytochrome b5 (CYB5A) deficiency or defect in backdoor pathway of DHT synthesis (3α-HSD or AKR1C2/4 defect) have hormonal/biochemical pictures, that resemble isolated 17,20-lyase deficiency [54].

R.3.9 'Low' basal morning cortisol in early infancy does not confirm PAI. PAI should be suspected only if low/low normal basal cortisol is associated with high plasma ACTH and with/without high PRA.

Comments

The foetal zone begins to involute soon after birth and disappears by about 3–6 months of age. The zona fasciculata starts enlarging simultaneously but is not fully differentiated until about 3 years of age. Serum cortisol measured between 7:00 and 10:00 AM on 3rd day of life in new-born infants ranges from 1.0–18.1 μg/dL (mean 7.65 μg/dL) and 1.7–14 μg/dL (mean 6.2 μg/dL) among different studies [55, 56]. Range of morning cortisol in children aged between 1 and 11 months is 2.8–23 μg/dL [57]. The diurnal rhythms of ACTH and cortisol begin to be established at 6–12 months and often are not well established until after 3 years of age. Cortisol and ACTH can be measured any time of the day in neonates and early infancy. However, there is no established normal range for 'random cortisol' in this age group, hence the above-mentioned cortisol cut-offs may be used both for morning and random cortisol.

R.3.10 Results of stimulation tests (hCG ± tetracosactin) should always be interpreted in the context of clinical presentation and age of the patient. Functional assessment of androgen sensitivity may be attempted in selected cases.

Comments

The two most common aetiologies of 46, XY DSD are PAIS and 5α-R2D. Differentiating these two conditions is often challenging. Results of stimulation tests, though suggestive of a particular disease, is not confirmatory. Gynecomastia at puberty, LH: FSH of more than 1, post-hCG T: DHT of less than 20 and high AMH around puberty point towards possible PAIS. Another investigation which might be helpful is the 'Stanozolol test'. The test, however, is not in common use since the AR gene has been sequenced. Stanozolol, a non-aromatizable androgen, is administered orally as 0.2 mg/kg/day in a single evening dose for 3 consecutive days (D1, D2, D3). Sex hormone binding globulin (SHBG) is measured at baseline (before stanozolol) and on D6, D7, D8 and D9. Samples are to be collected between 1,400 and 1,800 h. AIS needs to be considered if the lowest value of SHBG, obtained between D6 and D9 is more than 63.4% of the baseline value [58]. This test has not been validated during early infancy. This test may be used in selected patients with suspected AIS and negative AR mutational analysis.

Effect of a short course of systemic T therapy on phallic length not only denotes tissue effect of androgen but is also helpful to decide on sex of rearing in PAIS [23]. Our suggested protocol involves monthly intramuscular injection of 25–50 mg of testosterone ester for 3–6 months. An increase in SPL (more than 1.5 cm or more than 50% from baseline) indicates satisfactory response and possibly rules out severe form of AIS. Increased SPL following T in childhood does not predict adequate size of the phallus post-puberty.

R.3.11 LC-MS/MS is the preferred method for analysis of steroid metabolites. However, due to its high cost and lack of widespread availability, immunoassays are an accepted alternative. Clinicians should be aware of the assay method used for a particular analyte and its pitfalls.

Comments

While LC-MS/MS is known to yield more accurate results and analyse multiple steroids simultaneously, availability and cost restricts its universal use in resource-constrained settings. Immunoassays are prone to cross-reactivity and matrix interference. This becomes clinically relevant in conditions associated with altered steroid biosynthesis, particularly in pre-term neonates, since many steroid precursors cross react with cortisol and other analytes of interest in different immunoassay platforms. Immunoassays are reasonable alternative for most of the hormonal assays performed in 46, XY DSD provided clinicians are aware of the assay methods and use method and age-specific reference intervals.

Chapter III: imaging

R.4.1 All patients with 46, XY DSD should undergo abdominopelvic ultrasonography (USG) by a trained radiologist.

Comments

Imaging should be tailored according to the clinical presentation, with minimum possible invasiveness and undue exposure to ionizing radiation. USG is the primary modality since it is easily accessible, cheap, and does not require sedation or the use of radiation or contrast material. USG should focus on mullerian structures in all patients and on identifying both gonads in those with non-palpable gonads. The sensitivity and specificity of USG in determining the presence of gonads were found to be 75–85% and 25–50%, respectively [59, 60]. Streak gonads are difficult to visualize and characterize by USG. The echogenicity of the gonads and the presence of microlithiasis should be looked for in non-scrotal gonads. In addition, both the kidneys, urinary tracts, and prostate should also be assessed. Prostate in healthy children and adolescents is ellipsoid in shape and the volume (0.523 × length × breadth × depth) ranges between 0.4 and 5.2 mL (mean 1.2 mL). Prostate volume is more than 2 mL in pubertal boys and more than 20 mL after 20 years of age. Hypoplastic prostate suggests either insufficient DHT or inadequate DHT activity due to AR defect. Patients with WT1 mutation might harbour renal tumors. USG is also helpful to identify adrenal hyperplasia (limb width more than 4 mm, lobulated or cerebriform surface and abnormal echogenicity [normal adrenal shows central echogenic stripe with a hypoechoic rim]), as seen in proximal defects in steroid biosynthesis (StAR, CSCCE, CYP17A1, or 3β-HSD2 defect) in neonates and in early infancy [61].

R.4.2 Magnetic resonance imaging (MRI) of the abdomen with/without diagnostic laparoscopy should only be performed if mullerian structures and gonads are not identified by USG in patients with non-palpable gonads.

Comments

Though MRI and USG are equally sensitive for the evaluation of intrapelvic structures, MR is more sensitive for identifying intra-abdominal gonads [62]. MRI can capture high-resolution multiplanar images of soft tissues without ionizing radiation and has a sensitivity of 86%, specificity of 79%, and an accuracy of 85% for the detection of nonpalpable gonads in DSD cases [59]. The procedure is time-consuming and ideal for older children; younger children need to be sedated. In addition, it is costly and hence should be reserved as the second-choice imaging technique in 46, XY DSD. The gold standard for evaluation of intra-abdominal structures and gonads is laparoscopy. Moreover, the intra-abdominal gonad(s) can be removed in the same setting for therapeutic or diagnostic purposes (histopathologic examination).

R.4.3 Retrograde urethrocystography/genitography should be done in those with single urethral opening in perineum or presence of two openings (urethra and UGS).

Comments

Precise knowledge of internal anatomy, such as the presence and length of the UGS, relationship of the UGS to the urethra, and position of the external urethral sphincter are necessary for planning surgical strategies. Genitogram may document mullerian structures in those with TD.

Chapter IV: genetic analysis

R.5 Depending on family history, inheritance pattern (if any), clinical phenotype, and hormonal assessment, genetic confirmation needs to be established using the most appropriate molecular diagnostic method.

R.5.1 In patients with suspected TD (low AMH, high FSH) or possible syndromic association (dysmorphism, other organ involvement), single nucleotide polymorphism (SNP) array or array comparative genomic hybridization (aCGH) should be used first to detect pathogenic copy number variation (CNV). In non-syndromic 46, XY DSD with a working diagnosis of PAIS or 5α-R2D, Sanger sequencing of SRD5A2 gene should be performed first followed by the AR gene, if the initial test is negative.

R.5.2 A particular hormonal profile may suggest a specific disease and the target genes can be analysed by Sanger sequencing. At present, NGS (either a designed panel of candidate genes or WES that affords a sequential analysis of a set of candidate genes followed by other possible candidates and a future re-analysis) is no longer a more costly alternative to Sanger sequencing of several genes. NGS may be considered as first-line test in 46, XY DSD, particularly where small genomic rearrangement is suspected.

R.5.3 A negative or inconclusive report does not rule out a defect in the target gene.

Comments

As discussed earlier, the first investigation in suspected 46, XY DSD is confirmation of the chromosomal sex by peripheral blood karyotype. Previously, the molecular investigation of 46, XY DSD involved a stepwise approach

of sequential testing of a small number of target genes by Sanger sequencing, combined with MLPA.

The most common aetiologies of 46, XY DSD are PAIS and 5α-R2D. The SRD5A2 gene is a relatively small gene and a hotspot (p.R246Q in exon 5) has already been described in Indian patients [63]. Patients without dysmorphism and hormonal evaluation suggestive of either of these two diseases should undergo Sanger sequencing of the SRD5A2 gene followed by the AR gene, if the initial test is negative. It needs to be remembered that less than 1/3 of patients with a provisional diagnosis of PAIS have pathogenic variants in the AR gene, suggesting a possible post-receptor defect, epigenetic changes in the AR gene, or involvement of other candidate genes with a similar phenotypic and hormonal presentation.

Several genes have been shown to exert a dose-dependent effect on sex differentiation and development, including duplications (DAX1, WNT4) and deletions (ATRX, DMRT1, EMX2, and WT1). MLPA, SNP array, and aCGH techniques can detect CNVs that can be missed by the conventional G-banded karyotyping. MLPA detects specific target sites whereas the other two examine the entire genome. CNV analysis with SNP array or aCGH should be used as the first-line molecular analysis in patients with suspected TD (low AMH, high FSH) or in those having associated malformations or other system

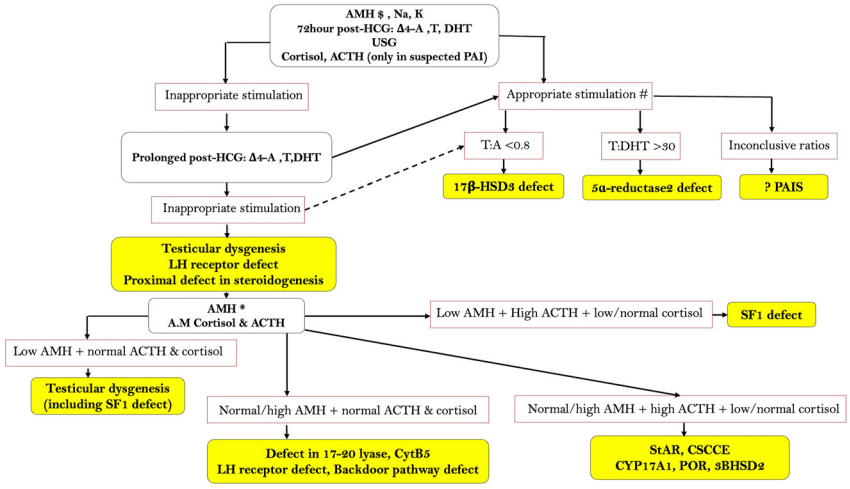

Figure 1: Diagnostic algorithm in 46, XY DSD [$ to be interpreted against age & sex specific reference range; * If not done initially; # Appropriate stimulation is defined as post-hCG testosterone of at least 50 ng/dL] (ACTH, adrenocorticotropic hormone; AMH, anti-mullerian hormone; CSCCE, cholesterol side-chain cleavage enzyme; CYP17A1, 17α-hydroxylase/17, 20-lyase; CytB5, cytochrome B5; DHT, dihydrotestosterone; HCG, human chorionic gonadotropin; K, Potassium; LH, luteinizing hormone; Na, Sodium; PAI, Primary adrenocortical insufficiency; PAIS, partial androgen insensitivity syndrome; POR, P450 oxidoreductase; SF1, steroidogenic factor 1; StAR, steroidogenic acute regulatory protein; T, testosterone; USG, ultrasonography; Δ4-A, Δ4-androstenedione; 3β-HSD2, 3β-hydroxysteroid dehydrogenase 2; 17β-HSD3, 17β-hydroxysteroid dehydrogenase3).

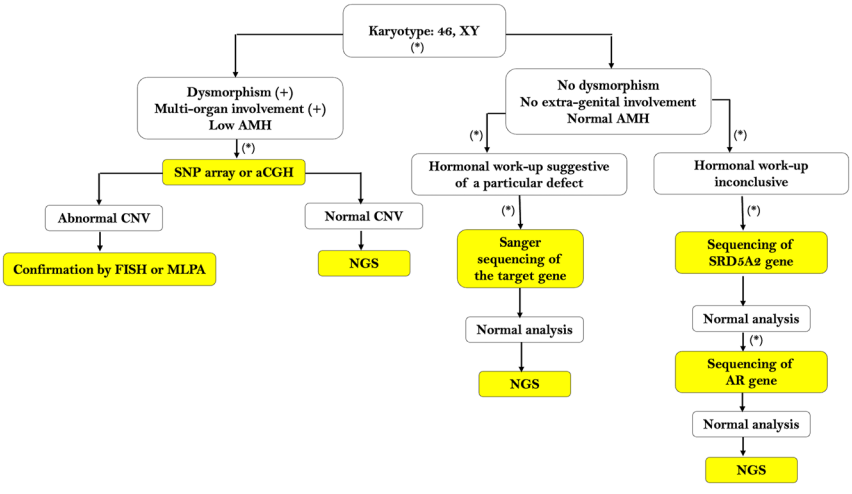

Figure 2: Suggested approach for genetic confirmation in 46, XY DSD ([*] NGS may be considered) (aCGH, array comparative genomic hybridization; AMH, anti-mullerian hormone; AR, androgen receptor; CNV, copy number variation; FISH, fluorescence *in situ* hybridization; MLPA, multiplex ligation-dependent probe amplification; NGS, next generation sequencing; SNP, single nucleotide polymorphism; SRD5A2, Gene encoding 5α-reductase 2).

involvement as pathogenic CNV may be identified in about ≈30% of such cases [27, 64]. Abnormal findings detected by these methods may be confirmed by fluorescence *in situ* hybridization (FISH) or MLPA. While MLPA, aCGH, or SNP array identifies CNVs between 10 kb and 5 Mb in size, smaller genomic rearrangements, like single nucleotide or insertion-deletion variants, can be captured by gene sequencing technology [65]. Candidate gene analysis in 46, XY DSD has shown that mutation in the target gene is more often identified in CAIS (≈93%), 17β-HSD3D (≈90%) and 5α-R2D (≈80%) than in PAIS (≈24%) or TD (≈25%) [42]. In comparison to Sanger sequencing, the development of NGS has enabled simultaneous analysis of a large number of genes in a relatively short time period. With the wider availability of NGS, Sanger sequencing of individual genes have been replaced by panels of DSD candidate genes-FES, WES, or WGS. Potential inconclusive results like identified variants reported as "likely pathogenic" or "variant of uncertain significance" should be considered abnormal if the clinical and hormonal profile is corroborative with that particular disease. In addition, WES fails to identify deep intronic mutations and hence may be reported as normal. At present, only half of those with non-syndromic forms of 46, XY DSD receive a genetic diagnosis.

The phenotypic outcome in 46,XY DSD patients might be determined by variation(s) in multiple genes contributing towards the complex network of sexual development. Phenotypic variability in a particular disease may be attributed to incomplete penetrance, variable sensitivity of partner genes, and coexistent pathogenic mutation in other gene(s). Such oligogenic or polygenic inheritance has been reported in association with genes involved in testicular development, SF1/NR5A1, GATA 4, and MAMLD1, in particular [66–70]. 46, XY DSD patients having heterozygous mutation in SF1/NR5A1 were found to harbour variants of other DSD-related genes like MAP3K1, StAR, AMH and ZFPM2/FOG2 [66, 71]. Such oligogenic mode of inheritance, in which multiple hits, individually non-deleterious, may contribute to the broad spectrum of phenotypes observed in individuals with heterozygous mutation in one of the known DSD-causing genes. 46, XY DSD patients, in whom a clear monogenic cause cannot be demonstrated, should therefore be evaluated for coexistent mutations in other genes.

Conclusion

In this consensus statement we have highlighted the importance of relevant history, focussed clinical examination and appropriate interpretations of hormonal evaluation across different age groups in patients with 46, XY DSD. Our proposed diagnostic algorithm (Figure 1) is cost effective and when supplemented with appropriate genetic studies (Figure 2), may help to reach an aetiological diagnosis in majority of such cases.

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