Consanguinity and Disorders of Sex Development

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Abstract

Disorders of sex development (DSD) are defined as ‘congenital conditions in which the development of chromosomal, gonadal, or anatomical sex is atypical’ [Lee et al., *Pediatrics* 2006;118:e488–e500]. Studies conducted in Western countries, with low rates of consanguinity, show that truly ambiguous genitalia have an estimated incidence of 1:5,000 births. There are indications that the prevalence of DSD is higher in endogamous communities. The incidence of ambiguous genitalia in Saudi Arabia has been estimated at 1:2,500 live births; whilst in Egypt, it has been estimated at 1:3,000 live births. This may be due in part to an increase in disorders of androgen synthesis associated with 46,XX DSD. There is clearly a need for further studies to address the frequency of DSD in communities with high levels of consanguinity. This will be challenging, as an accurate diagnosis is difficult and expensive even in specialized centres. In developing countries with high levels of consanguinity, these limitations can be compounded by cultural, social and religious factors. Overall there is an indication that consanguinity may lead to an increase in incidences of both 46,XY and 46,XX DSD, and a co-ordinated study of populations with higher incidences of consanguinity/endogamy is needed to resolve this.

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Introduction

Disorders of sex development (DSD) are defined as ‘congenital conditions in which the development of chromosomal, gonadal, or anatomical sex is atypical’ [1]. DSD has recently been coined to encompass terms such as intersex, pseudohermaphroditism, hermaphroditism and sex-reversal, which can be confusing to clinicians, patients and parents. The definition provides a rational basis for designating each phenotype. 46,XY DSD includes errors of testis determination or undermasculinization of an XY male due to errors in either androgen synthesis or androgen action. 46,XY gonadal dysgenesis is an error of testis determination that is either complete (complete gonadal dysgenesis; CGD) or partial (partial gonadal dysgenesis; PGD). 46,XY CGD is characterized by completely female external genitalia, well-developed Müllerian structures and a gonad composed of a streak of fibrous tissue. On the other hand, 46,XY PGD is characterized by partial testis formation, usually a mixture of Wolffian and Müllerian ducts and varying degrees of masculinization of the external genitalia. 46,XX DSD includes overvirilization or masculinization of an XX individual due to androgen excess, and the vast majority of cases of 46,XX DSD are associated with congenital adrenal hyperplasia (CAH) [2]. The much rarer 46,XX testicular DSD refers to a male with testes and a normal male habitus, whereas 46,XX ovotesticular DSD refers to individuals that have

both ovarian and testicular tissue in the gonads, usually as ovotestes but also less commonly as separate gonads [1].

There is very limited data available on the incidence rate and prevalence of DSD. In the newborn, truly ambiguous genitalia have an estimated incidence rate of 1:4,500–5,500 births [3, 4]. However, a concern about the development of the external genitalia may exist in 1:300 newborn infants. This includes undescended testes or anomalies of the opening of the urethra on the penis (hypospadias). Most of the published data on the incidence of DSD is available from Western countries with low rates of consanguinity, and it may not be a true reflection of the worldwide prevalence. In a study conducted in Germany, the incidence of ambiguous genitalia in infants of non-German background was four times higher compared to the general population [3]. This was considered to be due to higher rates of consanguinity in migrant populations, since several conditions in DSD have an autosomal recessive inheritance. One hindrance in defining the prevalence of DSD is the lack of an accurate and sometimes even any diagnosis. In the German study, almost half of the children did not have a definitive diagnosis by the age of 6 months [3]. Indeed, excluding cases where the biochemical profile indicates a specific error in steroidogenesis, it has been estimated that a specific molecular diagnosis is obtained in about 20% of cases of DSD and that only 50% of 46,XY DSD children will ever receive a definitive clinical diagnosis [1]. In this review, we will describe the biology of sex determination and the conditions associated with errors in these processes along with the role that consanguinity may play in the incidence of atypical gonad development.

Genetic and Hormonal Control of Sexual Development

The urogenital ridge, the common precursor of the urinary and genital systems, develops at approximately 4 weeks' fertilization in the human embryo as a thickening of the mesodermic mesonephros covered by coelomic epithelium [5]. At 5 weeks, the human gonadal ridge is bipotential and must choose between two mutually opposing fates, developing either into an ovary or a testis. At 7 weeks in the XY gonad, SRY is expressed in pre-Sertoli cells and this, in synergy with NR5A1, results in the up-regulation of SOX9 expression beyond the critical threshold, leading to the initiation of definitive Sertoli cell differentiation [6]. Sertoli cells act as the organizing centre of the testis. SOX9 functions by regulating the production of anti-Müllerian hormone from Sertoli cells and represses (directly or indirectly) genetic pathways involved in

ovarian development. Once Sertoli cells are formed, they induce the development of fetal Leydig cells, via a hedgehog signalling pathway. At 8–9 weeks of development, Leydig cells produce androgens and insulin-like factor 3. Testosterone and anti-Müllerian hormone cause the regression of Müllerian structures and differentiation of the Wolffian duct into the epididymis, vas deferens and seminal vesicles, whereas insulin-like factor 3 is required for testicular descent. Testosterone is the most abundant androgen in serum [7]. Approximately 97% of testosterone is bound to albumen and sex-hormone-binding globulin, the remaining 3% are free and biologically active. In male fetuses, it stimulates the differentiation of the Wolffian duct into male internal genitalia (epididymis, vas deferens and seminal vesicles) and at puberty it stimulates the libido, enlargement of the vocal cords, skeletal muscles, penis and scrotum and the initiation of spermatogenesis [7]. Intracellular testosterone is converted into dihydrotestosterone, the preferred ligand for androgen receptor transactivation, by the enzyme 5 α reductase. Upon ligand binding and transactivation, the dihydrotestosterone-androgen receptor complex moves from the cytoplasm to the nucleus and activates the transcription of androgen regulated genes [7].

In the XX gonad, the absence of SRY results in the inability of SOX9 expression to reach a critical threshold. This, together with the expression of factors such as RSPO1/WNT4 and FOXL2, leads to the formation of the ovary, at least in part through suppressing the activity of 'testis' genes [8]. Similarly, at least in the mouse, the Sertoli cells' fate is constantly reinforced since the loss of the testis-determining gene *Dmrt1* in the Sertoli cells of the adult testis allows their transformation into female granulosa cells, which are the female equivalent of Sertoli cells [9]. Thus, the development and maintenance of the mammalian gonad is regulated by a double repressive system, where equilibrium of mutually antagonistic pathways must be attained for the normal development of either the testis or ovaries [10].

Consanguinity and Genetic Errors in Testis Determination

There has been a considerable increase in our understanding of the genetic networks controlling testis determination, but a surprising number of cases of 46,XY gonadal dysgenesis still remain unexplained [11]. 46,XY CGD and PGD have been reported in association with mutations involving a number of genes that are impor-

tant for the Sertoli cells' development, including *SRY*, *SOX9*, *NR5A1*, *GATA4*, *FOG2*, *WT1*, *CBX2*, *ATRX*, *MAP3K1* and *TSPYL1* genes [11]. Recessive mutations involving these genes are rare. In the vast majority of cases, the mutations associated with the phenotype are heterozygous or hemizygous (*SRY*, *ATRX*). This indicates that testis formation is sensitive to the gene dosage of autosomal transcription factors. *NR5A1* (also known as SF-1) has originally been identified as a master-regulator of steroidogenic enzymes in the early 1990s which controls many key aspects of adrenal and reproductive function [12]. Mutations involving *NR5A1* are one of the most common causes of 46,XY DSD and they are associated with a range of phenotypes including 46,XY gonadal dysgenesis and adrenal failure, 46,XY DSD with apparently normal adrenal function, 46,XY infertile males and 46,XX females with ovarian insufficiency [12–15]. Although the vast majority of reported mutations are heterozygous, a few rare homozygous mutations have been identified. A homozygous p.R92Q mutation has been reported in association with gonadal dysgenesis and adrenal insufficiency [16]. The effects of this homozygous change are variable in functional assays, but the mean functional activity was in the order of 30–40% of wild-type. A further homozygous mutation (p.D293N) has been reported in association with 46,XY PGD [15]. In this case, the mutant protein retained about half the biological activity of the wild-type *NR5A1* protein. In both of these examples, the residual activity of the mutated protein in the homozygous state was around 50% of wild-type, a situation similar to heterozygous mutants that have a complete loss of function. A limited number of studies have described mutations involving *NR5A1* in North African communities that have a relatively high degree of consanguinity, but these are heterozygous with endogamy having no apparent influence on the phenotype [14, 17].

Approximately 80% of 46,XX individuals with testicular DSD and 10% with ovotesticular DSD carry *SRY* in their genome that may explain the phenotype [18]. The remaining *SRY*-negative cases may be explained by either hidden mosaicism of a 46,XY cell line in the gonad, mutations in a sex-determining gene downstream from *SRY* or neomorphic mutations. Rare cases of 46,XX *SRY*-negative testicular or ovotesticular DSD are due to rearrangements of the regulatory regions of the *SOX9* or *SOX3* genes [11]. The majority of cases appear to be sporadic, but familial cases have been described that are indicative of a monofactorial recessive mode of inheritance [19–21]. Many of these pedigrees have been reported in families of North African/Middle Eastern origin, suggesting that there is a

recessive deleterious allele in these populations [19–21, and unpubl. data]. 46,XX *SRY*-negative ovotesticular DSD is also relatively common in South Africa. As many as 51% of South African patients investigated for ambiguous genitalia are 46,XX ovotesticular DSD [22, 23]. The mode of inheritance of the phenotype and the genetic cause have not yet been established in this population.

Rare recessive mutations in two genes that function at least in part by repressing the testis pathway have been described. Two Italian families have been identified that carry homozygous mutations in *RSPO1* associated with a rare recessive syndrome characterized by XX testicular DSD, palmoplantar hyperkeratosis and a predisposition to squamous cell carcinoma of the skin [24]; and a further case of 46,XX ovotesticular DSD has been described with a splice-site mutation involving *RSPO1* [25]. Four heterozygous mutations in *WNT4* have been reported in association with androgen excess, abnormal development of Müllerian ducts but normal female genitalia [26]. A homozygous *WNT4* mutation has been identified in a consanguineous family of Arab Muslim origin with an embryonic lethal syndrome encompassing 46,XX testicular DSD and the dysgenesis of kidneys, adrenals and lungs (SERKAL syndrome) [27].

Consanguinity and Disorders of Androgen Synthesis or Action

Anomalies of Leydig Cell Formation and Differentiation

Several genes are known to be involved in the specification and differentiation of Leydig cells, including the X-linked aristaless-related homeobox gene (*ARX*), desert hedgehog (*DHH*) and platelet-derived growth factor receptor alpha (*PDGFR*). Umehara et al. [28] reported a homozygous missense mutation at the initiating codon, which resulted in failure of translation of the *DHH* gene in a patient presenting with 46,XY PGD associated with minifascicular neuropathy. In this case of Japanese ancestry, the parents were first cousins. Four other independent homozygous mutations have been reported in association with CGD from India and Mexico without details about consanguinity or the families/communities [29, 30].

The human LH/CG receptor, a member of the G-protein-coupled receptor family, has two natural ligands: luteinizing hormone (LH) and human chorionic gonadotropin. Human chorionic gonadotropin induces fetal Leydig cell differentiation and testosterone production, whereas LH promotes testosterone production by Leydig

cells [31]. Homozygous or compound heterozygous inactivating mutations of the *LHCGR* gene encoding the LH receptor can cause Leydig cell hypoplasia in 46,XY individuals [32]. These mutations are rare and the phenotype ranges from completely female external genitalia, a lack of breast development, low testosterone and high LH levels and primary amenorrhoea (type I) to normal male sex differentiation with hypospadias or a micropenis (type II). The most severe form is caused by mutations that completely inactivate the *LHCGR* and thereby block testosterone production. In both forms (type I and II), the internal genitalia of an affected 46,XY individual typically consists of small undescended testes with seminiferous tubules but no Leydig cells. The uterus and fallopian tubes are usually absent. Homozygous and compound heterozygous mutations of the *LHCGR* gene have been identified, but these may explain only around 50% of cases, suggesting a contribution of other genetic factors to the phenotype [33]. Although consanguinity has been widely reported in association with Leydig cell hypoplasia, particularly in cases from Brazil and North Africa, this remains a very rare phenotype with an incidence rate of around 1:1,000,000 individuals [34–36].

Cholesterol Synthesis Defects

Smith-Lemli-Opitz syndrome (SLOS) is characterized by distinctive facial features, microcephaly, intellectual disability and behavioural problems [37]. Malformations of the heart, lungs, kidneys, gastrointestinal tract and genitalia are also common. SLOS is a recessive cholesterol deficiency syndrome caused by mutations in the *DHCR7* gene. The enzyme *DHCR7* catalyzes the reduction of 7-dehydrocholesterol to cholesterol. Although the phenotype is due, at least in part, to deficiencies in hedgehog signalling (as cholesterol is an activator of sonic hedgehog), the gonadal phenotype is due to a reduced testosterone production. The first step of testosterone biosynthesis is the uptake of cholesterol from the extracellular space and/or the endogenous synthesis of cholesterol by Leydig cells. A blockage or reduction in this can lead to undervirilization of the genitalia in an XY male. 25% of 46,XY SLOS patients have female external genitalia, with a further 50% presenting with cryptorchidism and/or hypospadias [38]. SLOS is most prevalent in populations of Northern and Central European descent and rare in populations of African or Asian descent. Population surveys have found that the carrier frequency of the most common SLOS mutation (a splice-site mutation; c.964–1G>C) is approximately 1% [39, 40]. Given that this mutation represents <30% of alleles in surveys of af-

ected individuals, the carrier rate for SLOS mutations may be as high as 1:30 [39]. This carrier rate predicts a hypothetical SLOS rate in live births of 1:2,500–1:4,500, whereas the recorded SLOS incidence is 1:10,000–1:16,000 births. This discrepancy between the calculated and observed incidence rates could be due to undiagnosed mild cases, misdiagnosed severe cases, death prior to diagnosis or fetal loss. The high carrier frequencies for *DHCR7* mutations suggest the possibility of a heterozygote advantage. These include increased vitamin D levels, providing protection against vitamin D-deficient rickets and autoimmune diseases, or increased resistance to certain infections resulting from an increased 7-dehydrocholesterol concentration in cell membranes. In Northern European populations, heterozygosity for a null *DHCR7* mutation may have provided protection against the development of vitamin D-deficient rickets [41].

Lipoid CAH

StAR (the steroidogenic acute regulatory protein) is required for the transport of substrate cholesterol from the cytosol to the inner membrane of the mitochondria, where the steroid biosynthesis is initiated [42]. Lipoid CAH is a rare autosomal recessive DSD characterized by greatly reduced or absent synthesis of all adrenal and gonadal steroids [43]. The resultant adrenal deficiency of mineralocorticoid and glucocorticoid hormones usually presents during the first weeks of life with significant failure to thrive [43]. Affected individuals are phenotypic females or rarely have slightly virilized external genitalia with or without cryptorchidism. Lipoid CAH caused by mutations in *StAR* is most frequent in East Asian and Palestinian communities with evidence of founder mutations, and it is relatively rare in Europe and America. The Q258X mutation accounts for 70% of mutations in *StAR* observed in Japanese and Korean cohorts [44, 45]. In the latter population, the carrier frequency has been estimated at 1:250 and may represent a founder mutation [45]. A p.R182H mutation has been reported in 5 apparently unrelated families from Eastern Saudi Arabia and in a patient from Qatar [46]. Interestingly, the patients were homozygous for the p.R182H mutation and showed substantial variation in disease severity and their age at symptom onset (1–14 months). The cause of the dramatic spectrum of clinical presentations in the same ethnic group with the same mutation is unclear. In 4 apparently unrelated Palestinian families with lipoid CAH, a homozygous c.201_202delCT has been detected that was predicted to result in the premature truncation of the protein [47]. Informative markers at the *StAR* locus indicated

that the c.201_202delCT mutation is a Palestinian founder mutation. Although the 4 consanguineous families were not aware of any common ancestry, they identified their ancestral backgrounds to Hebron, Jerusalem and Ramallah.

Congenital Adrenal Hyperplasia

One of the most commonly inherited metabolic disorders is CAH. The most common form of CAH is due to a deficiency of 21-hydroxylase, which is caused by mutations in the 21-hydroxylase gene (*CYP21A2*), and accounts for 90–95% of all cases [48, 49]. 21-hydroxylase-deficiency-induced CAH represents the most frequent cause of 46,XX DSD. The condition can be divided into classic (severe salt wasting and less severe simple virilizing) and non-classic (or mild/late onset) forms with hyperandrogenism being the most common clinical manifestation [49]. Classic 21-hydroxylase deficiency is characterized by an impaired cortisol production leading to the accumulation of metabolic intermediates (progesterone and 17-hydroxyprogesterone) that results in an excessive androgen production leading to varying degrees of virilization. In the classic form, progressive postnatal virilization can be seen that may include progressive penile or clitoral enlargement, precocious pubic hair and hirsutism. The non-classic form of CAH results from a mild deficiency of 21-hydroxylase that does not induce ambiguity of the external genitalia at birth in females, but may postnatally result in varying degrees of symptoms associated with androgen excess [49].

The *CYP21A2* gene is located on chromosome 6p21.3, 30 kb from the *CYP21A1P* pseudogene. Both *CYP21A2* and *CYP21A1P* show sequence identity of 98% between exons and 96% between introns. The high sequence identity and close proximity of *CYP21A2* and *CYP21A1P* may result in unequal crossing-over events during meiosis or gene conversion, thus predisposing the locus to numerous rearrangements resulting in the transfer of deleterious mutations from the pseudogene to *CYP21A2* [50]. De novo mutations in *CYP21A2* associated with CAH are rare (3–5%), with the vast majority of these mutations identified either in single families or small populations [50].

The overall prevalence at birth of the classic form of CAH in the USA is estimated at 1:20,800 and in the UK at 1:18,000 [51, 52]. However, the distribution of the condition varies with ethnicity. For example, in the UK 25% of all cases concerned individuals of Asian ethnic origin [52]. The highest frequencies of CAH are found in isolated populations. The highly endogamous Yupik Eskimos of Southwestern Alaska carry an A>G transition in

the second intron of *CYP21A2*, and the frequency of CAH in this population is 1:282 [53]. The frequency of the classic form amongst the people of the island of La Reunion, France, is 1:2,100 [54]. Differences in the incidence rates of the classic form are highlighted by African Americans, who have a lower rate than the white US population (1:42,000 vs. 1:15,500 [55]). Lower incidence rates have also been reported in Asia [56]. In Western Australia, the overall incidence of classic CAH was estimated to be similar to figures for white Americans at around 1:15,000; however, the ratio of the Aboriginal rate compared with the non-Aboriginal rate for CAH was 2.45 [57]. The basis for this increased rate is unknown since no genetic data is available [57]. In a Tunisian cohort, a single Q318X mutation in *CYP21A2* had a large prevalence of 35.3% [58]. This is likely to be a founder mutation, since linkage disequilibrium was found between this mutation and a *CYP21* gene polymorphism in 83.3% of alleles [58]. Consanguinity was high in this study cohort; it was present in 31 families (60.8%), absent in 15 and unknown in 5. The consanguineous families were unrelated and originated from different regions of Tunisia [58].

The estimated incidence rate of the non-classic form is about 1:1,000 in the general Caucasian population, but it might be much more frequent in certain ethnic groups such as East European Jews that carry ancestry-specific mutations [59]. In one study, 63% of Ashkenazi Jews with non-classic CAH carried the V281L (1685G>T) mutation in *CYP21A2* [60].

CAH due to 11 β -hydroxylase deficiency is an autosomal recessive disorder of corticosteroid biosynthesis resulting in androgen excess, virilization and hypertension [50]. It accounts for approximately 5–8% of all cases worldwide; however, the incidence rate can be much higher depending on the study population. In affected XX individuals, the phenotypes vary from an enlarged clitoris in the mildest forms to a severely hypertrophied clitoris with penile urethra and fused labial-scrotal folds [49]. Patients with this disorder are unable to convert 11-deoxycortisol to cortisol. Elevated levels of adrenocorticotrophic hormone (ACTH) cause steroid precursors to accumulate proximal to the blocked step which are then shunted into the pathway for androgen biosynthesis as occurs in 21-hydroxylase deficiency [49].

There are considerable differences in the ethnic distribution of 11 β -hydroxylase deficiency. 11 β hydroxylase has been noted at high frequencies in Israeli families of Moroccan, Tunisian, Turkish and Iranian origin [61]. The incidence in Jews of Moroccan origin is estimated to be 1:5,000–1:7,000 births [62], whereas it is estimated to

be 1:100,000 in Caucasians [63]. White et al. [64] identified a homozygous p.R448H mutation in 6 independent Israeli families of Moroccan ancestry and postulated that this was a potential founder mutation in this relatively endogamous community with a carrier rate of 1:40. However, further studies have suggested a much lower carrier frequency, suggesting that other gene mutations may be involved [65]. The R448H mutation may not be limited to Jews of Moroccan ancestry, since a Caucasian patient has been reported homozygous for this mutation [66]. Furthermore an Iranian and a Turkish patient were each found to be homozygous for a different mutation at the same codon, R448C, suggesting that this codon may be a mutational hotspot [66, 67].

In 15 unrelated Tunisian patients with classic 11 β -hydroxylase deficiency, two homozygous mutations have been detected in the *CYP11B1* gene: p.Q356X (26.6%) and p.G379V (73.3%) [68]. Consanguinity was reported for 12 of the 15 apparently unrelated families from different parts of the country. Similar to the situation with *CYP21A2* mutations in Tunisia, these mutations are considered to be founder mutations [68].

Recessive or compound heterozygous mutations in *CYP11A1* and *HSD3B2* (3 β -hydroxysteroid dehydrogenase type 2 deficiency) are relatively rare causes of CAH [50]. There is a lack of data regarding population-based incidence rates of mutations in these genes. The *CYP17A1* gene encodes steroid 17 α hydroxylase, an enzyme involved in cortisol and sex steroid synthesis in the adrenal glands and gonads. Mutations in *CYP17A1* result in mild to severe undervirilization of 46,XY individuals. Although there is a lack of evidence for founder mutations or mutational hotspots within *CYP17A1*, in the Brazilian population, p.W406R and p.R362C represent 50 and 32% of mutations, respectively [69]. However, the majority of all affected individuals carrying the p.W406R or p.R362C mutations are of Spanish or Portuguese ancestry, respectively, therefore indicating a founder effect [69]. Recurring mutations have also been described in unrelated individuals of Friesian origin from the Netherlands. A 4-base duplication within exon 8 of the *CYP17A1* gene has been identified in 6 families with 17 α -hydroxylase/17,20-lyase deficiency residing in Dutch Friesland [70]. The same duplication has been found in 2 unrelated Canadian Mennonite individuals [71], who derive their name from Menno Simons, an early leader of a sect in Friesland. This indicates that this 4-base duplication may represent a founder effect originating in Friesland.

CAH due to P450 oxidoreductase deficiency is an autosomal recessive disorder, due to mutations in the *P450*

oxidoreductase (POR) gene [53]. In contrast to all other forms of CAH, POR deficiency (PORD) results in severe sexual ambiguity in both sexes (46,XX and 46,XY DSD). Affected girls may present with significant virilization of the external genitalia, indicating prenatal exposure to androgen excess. Affected boys show varying degrees of undervirilization ranging from a borderline micropenis to severe perineoscrotal hypospadias. On the other hand, normal genital development in both 46,XX and 46,XY individuals has also been reported in cases of PORD [72]. Affected children can also present with bone malformations, similar to the pattern seen in patients with Antley-Bixler syndrome. POR is the crucial electron donor to all microsomal cytochrome P450 (CYP) enzymes including the steroidogenic enzymes CYP21A2, CYP17A1 and P450 aromatase (CYP19A1). The mutations in POR result in deficiencies in steroid synthesis and thus in the observed phenotype. The incidence rate of PORD in the general population is unknown as the phenotype shows considerable variability and can be difficult to diagnose accurately [50, 72].

In PORD patients of Caucasian origin, the mutation p.A287P is most frequently observed [72]. In one study on a large cohort of PORD patients, this mutation was observed in 23 of 54 alleles (43%) [72]. The frequency of this variant (rs121912974) in the general population is 4:8,410 alleles in the European American population (NHLBI Exome Sequencing Project: <http://evs.gs.washington.edu/EVS/>). In Japanese patients with PORD, a p.R457H variant has been observed in 47 of 70 alleles studied (67%) [73]. No POR patient carrying non-sense mutations on both alleles has been described so far, suggesting incompatibility of such genotype with postnatal life. Major loss-of-function mutations on one of the affected alleles are associated with severe malformations, whereas homozygosity or compound heterozygosity for missense mutations is associated with a mild to moderate phenotype. Importantly, homozygosity for the most common mutation in Caucasians, p.A287P, allows for prediction of the genital phenotype and is associated with mild to moderate malformations [72].

Consanguinity and Disorders of Androgen Function

5 α -Reductase Deficiency

In Salinas, a remote village in the Dominican Republic, 2% of live births in the 1970s were male pseudohermaphrodites, known locally as ‘guevedoche’ or ‘guevedoce’ from the Spanish phrase ‘huevos a las doce’ meaning ‘eggs

at twelve'. These children, who appeared to be female or male with ambiguous genitalia at birth, developed a penis, testicles and all of the typical male physical characteristics at the time of puberty. Most were found to be descendants of a single common ancestor: Altagracia Carrusco [74, 75]. Studies showed that these individuals carried a mutation in the 5 α -reductase type 2 (*SRD5A2*) gene that converts testosterone to dihydrotestosterone; the androgen with the highest affinity for the androgen receptor [74]. Other independent mutations have been reported in the *SRD5A2* gene in affected individuals from the Dominican Republic suggesting multiple founder mutations [76].

SRD5A2 deficiency is a rare condition and apart from the studies in the Dominican Republic the exact incidence rate is largely unknown, although consanguinity has been observed in around 40% of all reported cases [77]. A cluster of male pseudohermaphrodites in the Simbari Anga linguistic group in the Eastern Highlands of Papua New Guinea [78] and large consanguineous families with affected members have been reported in Turkey and Egypt [79, 80]. These and other studies are case reports on limited numbers of consanguineous families or patient clusters from the same geographical area and/or ethnic background that resulted in the belief that *SRD5A2* mutations were present in specific and isolated populations. However, recently 5 α -reductase deficiency has also been identified in populations not considered at risk such as those from Belgium, France, Italy, Poland, Spain or Quebec [81].

Conclusions

Data on the actual prevalence of DSD in developing countries with high rates of consanguinity or endogamy is largely unavailable. The limited numbers of studies that are published describe, for the most part, small series of patients and may not be reflective of a population as a whole. There is, however, some evidence for a higher rate of DSD in societies with a higher rate of consanguinity. The incidence of ambiguous genitalia in Saudi Arabia has been estimated at 1:2,500 live births, whilst in Egypt it has been estimated at 1:3,000 live births [82, 83]. Both figures are higher than the reported frequency of 1:4,500–1:5,500 in European countries, indicating that consanguinity may be a contributing factor to an increase in the incidence rate of DSD. In one recent retrospective study from Sudan, of 122 DSD cases, 69 had 46,XX DSD and 45 had 46,XY DSD. The most common cause of 46,XX DSD was CAH

21-hydroxylase deficiency (52 of 62 cases with a proven aetiology), whereas androgen insensitivity was the most frequent cause of DSD in 46,XY individuals. 70% of all 122 cases were born to first-cousin marriages [84]. A further small study of 26 DSD cases from Sudan observed parental consanguinity in 70% of cases, 10% reported no consanguinity, whilst 20% did not provide sufficient family history to ascertain consanguinity [85]. This frequency of consanguinity is higher than in individuals of Arab descent in Khartoum, which was estimated at around 52% [86]. Similarly, consanguinity has been proposed as the cause of a high frequency of 46,XX DSD (CAH in 65.4% of all DSD cases) in one referral centre in Saudi Arabia over a 20-year period [87]. Other studies from Saudi Arabia have reported similar findings [88]. However, Mazen et al. [83] suggest a somewhat different relationship between consanguinity and DSD. They reported on consanguinity and parental origins of 208 DSD patients from Egypt; the consanguinity rate was 62.8% for all DSD cases (72% in 46,XX DSD and 59.5% in 46,XY DSD) [83]. However, in contrast to other studies of Arab populations, 46,XY DSD was more common than 46,XX DSD, constituting 65.9% of the total cases [83]. The higher rates of 46,XY DSD may reflect an unidentified selection bias or may represent regional differences in the general incidence and nature of pathogenic mutations that could give rise to DSD. The rate of consanguinity associated with DSD (62.8%) is also higher than in the general Egyptian population, where studies have reported that consanguinity rates during the last 40 years have been ranging between 29 and 39% with considerable differences between urban and rural populations [89–92]. Although some of this increase may be due to the CAH cases, it is more difficult to explain the high degree of consanguinity in 46,XY DSD cases, unless there are novel recessive mutations responsible for the phenotype in this population.

There is clearly a need for further studies to address the prevalence of DSD in communities with high levels of consanguinity. This will be challenging for several reasons, many of which could apply equally to communities with low levels of consanguinity. Until very recently, there has been a multitude of terms used to describe DSD and, consequently, in many older publications the precise clinical phenotype is confusing. This makes it very difficult to accurately assess older datasets. As mentioned earlier, only around 50% of all 46,XY DSD children will ever receive a definitive clinical diagnosis even at a specialized clinical centre [93–95]. Although a number of diagnostic algorithms exist, no single evaluation protocol is suitable for all circumstances and some basic tests, such as hor-

mone measurements, are not only expensive but need specialized interpretation of the specific assay characteristics compared to normal values for gestational and chronological age. In some cases serial measurements may be needed to enable an accurate interpretation of laboratory tests. In developing countries with high levels of consanguinity, these limitations of diagnostic and treatment facilities are compounded by cultural, social and religious factors that can affect the management of patients. In many developing societies, female infertility precludes marriage, which also affects employment prospects, and this drives the DSD patient's family to choose the male sex. Indeed, several studies have indicated the preference of the male sex of rearing despite the severity of genital ambiguity [83, 96, 97]. Furthermore in some traditional communities, parents feel that their child's condition is spiritual rather than medical and do not seek detailed clinical evaluation of the condition [98].

There are indications that consanguinity may lead to an increase in the incidence rates of both 46,XY and 46,XX DSD, depending on the population studied. A coordinated study of worldwide or region-wide populations with a higher incidence of consanguinity/endogamy may provide a definitive answer.

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