Screening for mutations in 17 β -hydroxysteroid dehydrogenase and androgen receptor in women presenting with partially virilised 46,XY disorders of sex development

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Abstract

Context and objective: The precise diagnosis of partially virilised women with 46,XY disorders of sex development (DSD) is often obscure. In practice, this group often comes under the poorly defined, clinically based label of partial androgen insensitivity syndrome (PAIS). In a previous study, we found that 5 α -reductase 2 (*SRD5A2*) mutations occurred in 43% of women in this subgroup. We expand this work to include biochemical and genetic screening for 17 β -hydroxysteroid dehydrogenase (*HSD17B3*) and androgen receptor (*AR*) mutations.

Methods: Analysis of serum androgens (androstenedione and testosterone) and genetic analyses for *HSD17B3* and *AR* were performed in 42 women from 36 pedigrees with partially virilised 46,XY DSD in whom *SRD5A2* deficiency had been excluded by urine steroid profiling.

Results: Out of 36 unrelated women, 14 (38%) were found to have *HSD17B3* mutations and one (2.7%) to have an *AR* defect. Six novel pathogenic *HSD17B3* mutations were identified: three splice site mutations and three missense changes. Seven patients with *HSD17B3* deficiency tested before gonadectomy had basal testosterone/androstenedione (T/A) ratio <0.8 (sensitivity 100% and specificity 91%).

Conclusions: *HSD17B3* deficiency is prevalent in the adolescent and adult 46,XY female DSD population and is often associated with lesser degrees of virilisation compared with those with 5 α -reductase deficiency. This diagnosis should be considered for individuals labelled as PAIS, particularly, but not exclusively, those who present with virilisation at puberty or primary amenorrhoea. Before gonadectomy, T/A ratio is useful to aid diagnosis, but after gonadectomy sequencing of *HSD17B3* must be performed to confirm the diagnosis.

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Introduction

In clinics for adults with disorders of sex development (DSD), the precise genetic diagnosis of partially virilised women with 46,XY DSD is often unknown. In practice, this group often comes under a poorly defined, clinically based label of partial androgen insensitivity syndrome (PAIS) (1). Candidate genes for the phenotype of women with a 46,XY karyotype, absent uterus and a degree of virilisation include mutations in genes encoding the

androgen receptor (*AR*), steroidogenic factor 1 (*NR5A1*), 5 α -reductase-2 (*SRD5A2*) and 17 β -hydroxysteroid dehydrogenase 3 (*HSD17B3*) enzymes. The clinical presentation depends on the amount of androgen produced, the degree of end-organ responsiveness and the timing of exposure of the external genitalia to androgens. The spectrum of phenotype in this group spans those with micropenis, severe hypospadias and a bifid scrotum (2)

to an appearance more consistent with complete androgen insensitivity (CAIS), where the appearance is that of a female phenotype with a mild degree of clitoromegaly.

The 17 β -hydroxysteroid dehydrogenase type 3 isoenzyme catalyses the conversion of the weak androgen substrate androstenedione into the more biologically active testosterone in the testis, which is essential for normal fetal development of male genitalia (3, 4). Affected individuals have testes and normally developed Wolffian duct derivatives, but show undervirilisation of the external genitalia, which often appear female with clitoromegaly, a blind-ending vagina, and at times, labial fusion. They are, therefore, often raised as females (4). Virilisation tends to occur at puberty, as a consequence of the peripheral conversion of androstenedione by other 17 β -HSD isoenzymes (5) including the extra-testicular 17 β -HSD5 (6, 7) and also because some have residual, albeit reduced, HSD17B3 function (8). The biochemical hallmark of HSD17B3 deficiency in the paediatric setting is the finding of an elevated serum androstenedione concentration and a low serum testosterone/androstenedione (T/A) ratio of <0.8 following an human chorionic gonadotrophin (hCG) stimulation test (9). More than 40 variants have been described in *HSD17B3* (<http://www.hgmd.cf.ac.uk>, accessed January 2015). HSD17B3 deficiency is inherited as an autosomal recessive trait.

Disorders of the *AR* commonly result in CAIS and a female phenotype with no virilisation at puberty, but *AR* mutations causing partial functional deficits have also been found in PAIS (1, 10), where missense mutations rather than deletions or other null mutations predominate (10, 11). More than 1000 *AR* mutations have been described to date (12).

Diagnostic criteria for women with partially virilised 46,XY DSD presenting in an older age group are not established and it is difficult to make a biochemical diagnosis in adults after gonadectomy, especially when the original endocrine data are often not available (13). In these circumstances, genetic testing is required. In a previous study, we found that *SRD5A2* mutations occurred in 43% of women in this clinical subgroup (14). We now extend this work by presenting the results of screening for *HSD17B3* and *AR* mutations in partially virilised women with 46,XY DSD of unknown aetiology. Clinical and biochemical characteristics are described. In addition, we have collated results from all XY DSD patients with a partially virilised phenotype who have attended clinics at University College London Hospitals (UCLH) in order to document the frequency of each disorder in an adult DSD setting.

Subjects and methods

Subjects

Women attending DSD clinics at UCLH were recruited with prerequisite clinical characteristics of an absent uterus, partial virilisation and non-mosaic 46,XY genotype. In those subjects who had presented originally in the paediatric setting, the earlier diagnostic work-up details were not always available in referral letters and historic documents. In addition, some were diagnosed in childhood but were unaware of their diagnoses until later in life. In these cases details of earlier childhood presentations were often unavailable. Prader staging at birth was estimated from available information and cannot be considered perfectly accurate, but is included to convey an idea of virilisation at birth. All subjects had urinary steroid profiles performed to exclude *SRD5A2* deficiency as described previously (14).

From a population of 87 women with this phenotype observed at this centre, 22 were lost to follow-up or did not consent to genetic testing, leaving a study group of 65 subjects.

Study group subset for screening of HSD17B3 and AR genes

Of the 65 subjects, 21 had established genetic diagnoses made either from previous screening of this cohort within our centre or before referral to clinic: 16 with mutations in *SRD5A2*, two with mutations in *HSD17B3*, one with *AR* defect, one with a confirmed *NR5A1* mutation and one with Denys-Drash syndrome. Two additional patients were excluded; one with a family history of *NR5A1* mutation and one with an 45,X;46,XY mosaic karyotype, thus leaving a study group of 42 women from 36 pedigrees with 46,XY DSD of unknown cause. The median age (range) at entry to the study was 27 (15–58) years.

The project was approved by the ethics committee of UCLH and informed consent was obtained from all subjects.

Methods: HSD17B3 and AR mutation screening

DNA was extracted from whole blood collected into EDTA. *HSD17B3* was amplified in four fragments containing exons 1–2, 3–5, 6–9, and 10–11 and *AR* in eight, including three overlapping fragments for exon 1, exons 2–5 amplified separately, then exons 6–8 in a single fragment, followed by unidirectional exon sequencing of purified

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products, with suitably placed internal primers to sequence exons and intron–exon boundaries, on an ABI 310 genetic analyser. Numbering of mutations was based on reference sequence NM_000197.1 (*HSD17B3*) and NM_000044.2 (*AR*), with nucleotide +1 as the A of the ATG translation initiation codon. *In silico* analysis of novel changes was performed using SIFT ((15), <http://sift.jcvi.org/>), Polyphen-2 ((16), <http://genetics.bwh.harvard.edu/pph1/>), and MutationTaster ((17), <http://www.mutation-taster.org/>).

Methods: endocrine evaluation

Serum androstenedione (A) and testosterone levels (T) (nmol/l) were measured by standard immunoassays and collected for comparison where possible. Only patients with levels taken before gonadectomy were included. Basal levels were collected from post-pubertal patients without hCG stimulation and the T/A ratio was calculated.

Methods: collective partially virilised XY DSD population

Collated data from the entire clinic population of partially virilised XY DSD patients, including male and SRD5A2 patients, were reviewed in order to document proportions of diagnostic subgroups according to genetic diagnosis for this broader phenotype. These data were compared with published reference series.

Results

From a study group of 42 individuals from 36 pedigrees, 18 subjects from 14 pedigrees (38%) were found to have *HSD17B3* mutations and one (2.7%) was found to have an *AR* mutation.

Cohort with *HSD17B3* mutations: clinical, genetic, and biochemical characteristics

Clinical details of all patients identified with *HSD17B3* mutation are outlined in Table 1. The median age of diagnosis (range) of the cohort of 18 individuals with *HSD17B3* mutations was 14 (0.5–18) years. The median age (range) of gonadectomy was 14.5 (1–23). At birth, 14/18 subjects were reported to have normal female appearance of the external genitalia with no signs of virilisation and the remaining four had mild ambiguity with labial masses in one subject. Eight patients had

palpable inguinal gonads or had previous surgical treatment for inguinal hernia. Gonadectomy was performed after the age of 10 years in 14 subjects, of whom four did not report virilisation at pubertal age.

Diagnostic labels for this group at the time of referral to our service were diverse; six subjects were referred with no defined diagnosis, three with PAIS, three with vanishing testes syndrome (VTS), three with unspecified AIS, one with CAIS and one as an undefined testosterone biosynthetic defect.

Splice mutations accounted for 16/28 (57%) of pathological variants in *HSD17B3* of which c.[277+4A>T] was the most common, occurring in 11 unrelated alleles (39%), all from Caucasian patients. Three novel splice mutations were found, c.[201+1G>A] and c.[202–1G>A] in the splice donor and acceptor sites, respectively, of intron 2, and c.[277+5G>A] in intron three. All three were predicted to affect splicing. The remainder of the mutations were missense changes including the previously described changes p.Ala56Thr, p.Asn130Ser, p.Met197Lys, p.Ala203Val, p.Val205Glu and p.Pro282Leu. Three novel variants, c.203T>G (p.Leu68Arg), c.599C>T (p.Ala200Val) and c.673G>A (p.Val225Met), were also found, all of which were predicted by *in silico* analysis to be disease causing. The single exception was the SIFT prediction for p.Val225Met that implied a degree of tolerance for the change (score 0.06, cut-off <0.05 for deleterious change). This variant was found in conjunction with a known splice site mutation, c.277+4A>T, in intron 3. A single patient was found to have a known pathological variant, c.1174C>T (p.Pro392Ser) in *AR*.

There were no apparent phenotype/genotype associations among those with mutations in *HSD17B* comparing splice site with missense mutations (Table 1). There was no difference in phenotype between those with *17HSDB3* mutations and the remaining 23 of unknown aetiology (data not shown).

Serum testosterone and androstenedione results were available in seven post-pubertal patients before gonadectomy. Results were also available for eight patients with SRD5A2 deficiency as well as two patients with 46,XY DSD of unknown aetiology. The average basal T/A ratio for all cases of *HSD17B3* deficiency was 0.3 (0.18–0.55). A cut point for the T/A ratio of 0.8 identified all cases with *HSD17B3* deficiency with a specificity of 91% (Fig. 1). The single false-positive subject was found to have a degree of testicular failure as evidenced by a serum FSH level of 26.6 mmol/l and severe atrophy on gonadal histology following gonadectomy.

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Table 1 Clinical features, previous presentation and HSD17B3 mutations in 18 females with HSD17B3 deficiency and in one with AR defect.											
Family/ patient no.	Ethnicity	Consanguinity	Clinical summary	Age Dx	Previous diagnosis	Prader stage	Age Gx	Virilised at puberty	HSD17B3 mutation nucleotide/amino acid		
HSD 1/1	Pakistani	Yes	Hirsutism, clitoromegaly, breaking voice, palpable gonads	12	None	0/1	12	Yes	c.[166G>A][166G>A] p.[Ala56Thr][Ala56Thr]		
HSD 2/2	Pakistani	Yes	Primary amenorrhoea	15	VTS	0	23	Yes	c.[201+1G>A][c.277+5G>A] splice;splice		
HSD 2/3	Pakistani	Yes	Sister of HSD2, screened after sister diagnosed, clitoromegaly noted	14	VTS	0	17	Yes	c.[201+1G>A][c.277+5G>A] splice;splice		
HSD 2/4	Pakistani	Yes	Sister of HSD2, screened after sister diagnosed, primary amenorrhoea, hirsutism, clitoromegaly	14	VTS	0	16	Yes	c.[201+1G>A][c.277+5G>A] splice;splice		
HSD 3/5	Caucasian	Unknown	Primary amenorrhoea	18	None	0	18	Yes	c.[277+4A>T][278–1G>C] splice;splice		
HSD 3/6	Caucasian	Unknown	Sister of HSD5, screened after sister diagnosed	10	None	0	12	No	c.[277+4A>T][278–1G>C] splice;splice		
HSD 4/7	Caucasian	Unknown	Abdominal pain	7	CAIS	0	7	NA	c.[277+4A>T][277+4A>T] splice;splice		
HSD 5/8	Caucasian	No	Clitoromegaly	11	PAIS	0/1	11	Yes	c.[277+4A>T][278–1G>C] splice;splice		
HSD 5/9	Caucasian	No	Sister of HSD8, screened after sister diagnosed, hooded clitoris noted	6	PAIS	0/1	9	NA	c.[277+4A>T][278–1G>C] splice;splice		
HSD 6/10	Caucasian	Yes	Primary amenorrhoea, clitoromegaly noted	16	CAIS	0	18	No	c.[277+4A>T][277+4A>T] splice;splice		
HSD 7/11	Caucasian	No	Primary amenorrhoea, hirsutism, no breast development	16	PAIS	0	17	No	c.[277+4A>T][673G>A] splice;p.[Val225Met]		
HSD 8/12	Caucasian	No	Primary amenorrhoea, hirsutism, deep voice, minimal breast development	15	PAIS	0	15	No	c.[202–1G>A][277+4A>T] splice;splice		
HSD 9/13	Caucasian	No	Clitoromegaly	14	None	0	14	Yes	c.[277+4A>T][845C>T] splice;p.[Pro282Leu]		
HSD 10/14	Afghan	Yes	Clitoromegaly, deepening of voice, hirsutism	12	None	0	13	Yes	c.[590T>A][590T>A] p.[Met197Lys][Met197Lys]		
HSD 11/15	Caucasian	No	Primary amenorrhoea, hirsutism, clitoromegaly	18	PAIS	0	20	Yes	c.[614T>A][614T>A] p.[Val205Glu][Val205Glu]		
HSD 12/16	Caucasian	No	Bilateral labial masses	0.5	Testosterone biosynthetic defect	1	1	NA	c.[277+4A>T][608C>T] splice;p.(Ala203Val)		
HSD 13/17	Caucasian	No	Bilateral inguinal hernias	5	AIS	0	5(R)/10(L)	NA	c.[389A>G][599C>T] p.(Asn130Ser)[Ala200Val]		
HSD 14/18	Caucasian	No	Primary amenorrhoea, deep voice, Adam's apple, clitoromegaly noted	17	None	0	17	Yes	c.[203T>G][277+4A>T] p.(Leu68Arg);splice		
AR 1/1	African	Unknown	Bilateral inguinal hernias, genital ambiguity	2	AIS	1/2	2	NA	c.[1174C>T] p.(Pro395Ser)		

HSD, 17β-hydroxysteroid dehydrogenase deficiency; AR, androgen receptor; Dx, diagnosis; Gx, gonadectomy; AIS, androgen insensitivity syndrome; PAIS, partial androgen insensitivity syndrome; CAIS, complete androgen insensitivity syndrome; VTS, vanishing testes syndrome; NA, information not available.

HSD, 17 β -hydroxysteroid dehydrogenase deficiency; AR, androgen receptor; Dx, diagnosis; Gx, gonadectomy; AIS, androgen insensitivity syndrome; PAIS, partial androgen insensitivity syndrome; CAIS, complete androgen insensitivity syndrome; VTS, vanishing testes syndrome; NA, information not available.

Figure 1

Basal serum testosterone/androstenedione ratios. Serum testosterone/androstenedione (nmol/l) ratio from women with partially virilised 46,XY DSD comparing those with HSD17B3 deficiency (solid circles), 5 α reductase deficiency (open circles) with two of unknown aetiology after screening for these conditions ('x') with case numbers in ascending order for the ratio. Only post-pubertal women with gonads *in situ* are shown. The dotted line indicates the upper border for the ratio based on hCG stimulation test in children (9).

Collective partially virilised XY DSD population

Collated data indicate the prevalence of genetically confirmed causes of partially virilised XY, DSD in all patients attending adult clinics at UCLH. We present a breakdown of diagnostic groups in Fig. 2A. For comparison, we also demonstrate amalgamated data from four predominantly paediatric reports (1, 10, 18, 19) (Fig. 2B).

Discussion

In this study, we have demonstrated that a significant proportion (38%) of unrelated 46,XY DSD females with unknown diagnosis have mutations predicted to cause HSD17B3 deficiency and that this diagnosis should be considered for individuals labelled as PAIS, particularly, but not exclusively, those that virilise post-puberty or those who present with primary amenorrhoea. In this study, six novel pathological variants in *HSD17B3* were identified, three of which are splice site mutations. The most common mutation in our cohort was c.277+4A>T described previously (4). This mutation was restricted to

Caucasian patients but has been described in other ethnic groups (20). This mutation is known to lead to exon skipping (9). Mutations in *AR* were unexpectedly rare (one case) in this adult cohort compared with paediatric series (1, 10, 11).

Compared with our population with *SRD5A2* deficiency, women with *HSD17B3* deficiency were less virilised. For instance, in contrast to those with *SRD5A2* deficiency, none of the subjects in this study were raised as males or underwent a change in sex identity from female to male (14). Out of 14 individuals, four (29%) with *SRD5A2* deficiency were classified as Prader stage 0–1 compared with 18/18 (100%) of those with *HSD17B3* deficiency. Out of 11 individuals, 10 (91%) with *SRD5A2* deficiency who retained their gonads past the age of ten had some degree of virilisation at puberty compared with 10/14 (71%) of those with *HSD17B3* deficiency.

Out of 14 individuals, nine (64.3%) with *SRD5A2* deficiency were of South Asian descent compared with 5/18 (28%) of those with *HSD17B3* deficiency. In families in which the data were known, parental consanguinity was present in 12/21 (57%) of pedigrees with *SRD5A2* mutations and 4/12 (33%) of those with *HSD17B3* mutations.

Boehmer *et al.* (9) described the T/A ratios in cases of *HSD17B3* deficiency following stimulation where the mean ratio was 0.4. They conclude that a T/A ratio

Figure 2

(A) Diagnostic frequency in 75 partially virilised patients with 46,XY DSD at University College London Hospital and (B) amalgamated data for paediatric cohorts (1, 10, 18, 19). *HSD17B3*, 17 β -hydroxysteroid dehydrogenase 3; *SRD5A2*, 5 α -reductase 2; *AR*, androgen receptor; *NR5A1*, steroidogenic factor 1; *WT1*, Wilms tumour suppressor gene; *LHCGR*, luteinizing hormone/choriogonadotropin receptor; *CYP17A1*, cytochrome P450 17A1.

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below 0.8 was able to discriminate those with *HSD17B3* deficiency from controls and those with *AR* mutation. In adults with *HSD17B3* deficiency in whom the gonads remain *in situ*, the T/A ratio can similarly be applied (21, 22). In our series, the basal T/A ratio of 0.8 identified all seven cases of *HSD17B3* deficiency in whom we had samples before gonadectomy with complete separation from those with *SRD5A2* deficiency (Fig. 1). From this figure, it can be observed that one subject with normal gene sequence for both *HSD17B3* and *AR* was found to have a T/A ratio below 0.8, possibly reflecting a degree of testicular failure as reported by other authors (23). Therefore, with the stipulation of including only subjects with normal serum FSH concentrations, an unstimulated T/A ratio below 0.8 in post-pubertal patients before gonadectomy is 100% sensitive and specific in identifying those with *HSD17B3* deficiency and can be used to guide mutational analysis.

Testosterone and androstenedione were measured by routine automated immunoassay. As the world moves to gas chromatography coupled with mass spectrometry (GC–MS) or liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) as the gold standard methods for measuring androgens, it will be interesting to determine whether such methodology alters the utility of T/A ratio in this group of patients (24).

The true incidence of *HSD17B3* deficiency is unknown but has been reported to range from one in 200–300 in Arabs in Gaza, among whom the intermarriage rate is high (25), to one in 147 000 in the Dutch population (9). The prevalence of *HSD17B3* mutation in an adult 46,XY DSD clinic has not been reported previously, but the prevalence in newborns has been reported to be approximately one in 150 000 (26).

Figure 2A presents collated data of 75 patients, both male and female, who have attended the clinics at UCLH with partially virilised XY DSD and in whom a genetic diagnosis has been sought. *HSD17B3* and *SRD5A2* enzyme deficiencies account for the majority of patients in our adult cohort (27 and 31% respectively) with only a small proportion attributed to an *AR* defect (7%). In contrast, *AR* mutations have been found to predominate in 46,XY DSD cohorts of predominantly paediatric populations. For example, in the Cambridge DSD database, *AR* mutations were found in 28% of subjects with PAIS analysed by gene sequencing (11). A study from our Dutch colleagues reported that 13/18 cases previously thought to have AIS were ultimately found to have mutations in *HSD17B3* (9). Amalgamated data from the Dutch nationwide survey (1) and three Spanish reports (10, 18, 19) are shown in Fig. 2B,

estimating diagnostic proportions among paediatric cohorts. Herein, one third of cases are attributable to *AR* defects with much smaller proportions to other causes of partially virilised 46,XY DSD.

There are several areas of potential bias that have to be taken into account when considering these results. First, we note that fewer than expected cases of mutations in *AR* were found in this adult cohort compared with other series. One explanation for this might be that, in the UK, screening for mutations in *AR* has been particularly thorough because of an early interest in this topic by the Cambridge group. We hypothesise that relatively few ‘unknowns’ remained after this process to present to an adult service such as ours. Secondly, clinics in London attract a large proportion of subjects from immigrant communities who often arrive undiagnosed as adults. This subgroup has a high rate of consanguinity, which would favour the diagnoses of the autosomal recessive disorders *SRD5A2* and *HSD17B3* deficiencies over X-linked androgen insensitivity. Finally, the clinic from which this cohort was recruited is based in a Department of Gynaecology which favours a predominantly female presentation, whereas the male end of the DSD spectrum are more likely to present in paediatrics with PAIS. Extrapolating from this last notion, it may be that individuals with mutations in *AR* predominantly present in a younger age group with genital ambiguity, or in an older age group with CAIS, but rarely with virilisation in the middle years.

Conclusions

HSD17B3 and *SRD5A2* deficiencies are relatively common and mutations in *AR* rare in an adult 46,XY female DSD population. A diagnosis of *HSD17B3* deficiency should be considered, particularly in those patients presenting with primary amenorrhoea and/or milder degrees of virilisation. In a post-pubertal patient with intact gonads, an elevated androstenedione level and low basal T/A ratio is suggestive of *HSD17B3* deficiency but requires confirmation by genetic testing. However, the utility of the T/A ratio is diminished in the adult population as many will present following gonadectomy. Definitive diagnosis is of benefit to provide genetic counselling to affected families. Additionally, the natural history of this group of disorders remains largely unknown and the delineation of genetically defined subgroups will allow for the collection of robust long-term outcome data to guide clinical care.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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