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Author/s:

Narayana, NS;Kean, A-M;Ewans, L;Ohnesorg, T;Ayers, KL;Watson, G;Vasilaras, A;Sinclair, AH;Twigg, SM;Handelsman, DJ

Title:

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Date:

2017-06-01

Citation:

Narayana, N. S., Kean, A. -M., Ewans, L., Ohnesorg, T., Ayers, K. L., Watson, G., Vasilaras, A., Sinclair, A. H., Twigg, S. M. & Handelsman, D. J. (2017). Painful ovulation in a 46, XX SRY - ve adult male with SOX9 duplication. ENDOCRINOLOGY DIABETES AND METABOLISM CASE REPORTS, 2017 (1), <https://doi.org/10.1530/EDM-17-0045>.

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Painful ovulation in a 46,XX SRY –ve adult male with SOX9 duplication

Nandini Shankara Narayana^{1,2}, Anne-Maree Kean¹, Lisa Ewans^{1,3}, Thomas Ohnesorg⁴,
Katie L Ayers^{4,5}, Geoff Watson¹, Arthur Vasilaras¹, Andrew H Sinclair^{4,5},
Stephen M Twigg¹ and David J Handelsman²

¹Royal Prince Alfred Hospital, Sydney, New South Wales, Australia, ²ANZAC Research Institute, University of Sydney, Sydney, New South Wales, Australia, ³Central Clinical School, Sydney Medical School, University of Sydney, New South Wales, Australia, ⁴Murdoch Childrens Research Institute, Melbourne, Victoria, Australia, and ⁵Department of Paediatrics, University of Melbourne, Melbourne, Victoria, Australia

Correspondence
should be addressed
to N Shankara Narayana
Email
nsha7690@uni.sydney.edu.au

Summary

46,XX disorders of sexual development (DSDs) occur rarely and result from disruptions of the genetic pathways underlying gonadal development and differentiation. We present a case of a young phenotypic male with 46,XX SRY-negative ovotesticular DSD resulting from a duplication upstream of *SOX9* presenting with a painful testicular mass resulting from ovulation into an ovotestis. We present a literature review of ovulation in phenotypic men and discuss the role of *SRY* and *SOX9* in testicular development, including the role of *SOX9* upstream enhancer region duplication in female-to-male sex reversal.

Learning points:

- In mammals, the early gonad is bipotent and can differentiate into either a testis or an ovary. *SRY* is the master switch in testis determination, responsible for differentiation of the bipotent gonad into testis.
- *SRY* activates *SOX9* gene, *SOX9* as a transcription factor is the second major gene involved in male sex determination. *SOX9* drives the proliferation of Sertoli cells and activates AMH/MIS repressing the ovary. *SOX9* is sufficient to induce testis formation and can substitute for *SRY* function.
- Assessing karyotype and then determination of the presence or absence of Mullerian structures are necessary serial investigations in any case of DSD, except for mixed gonadal dysgenesis identified by karyotype alone.
- Treatment is ideal in a multidisciplinary setting with considerations to genetic (implications to family and reproductive recurrence risk), psychological aspects (sensitive individualized counseling including patient gender identity and preference), endocrinological (hormone replacement), surgical (cosmetic, prophylactic gonadectomy) fertility preservation and reproductive opportunities and metabolic health (cardiovascular and bones).

Background

DSDs result from disruptions to the delicate balance of the molecular pathways in the male and female sex-determining pathways. They can present at any age ranging from prenatal state, at birth (e.g., hypospadias,

ambiguous genitalia, etc.) to early adulthood (delayed puberty, infertility). They can be extremely challenging owing to the associated diagnostic and ethical dilemmas. DSDs are rare and need a

systematic approach to establish diagnosis through a multidisciplinary approach.

The case

A 17-year-old Caucasian male presented with a two-day history of a painful mass in left testis. There was no history of trauma, fever or weight loss. His pubertal development was normal. His medical history included right cryptorchidism with spontaneous descent at one year of age, Henoch-Schonlein purpura at four years, excision of a benign left extra adrenal ganglioneuroma at five years without recurrence, pyelonephritis at fourteen years and an upper lip capillary haemangioma.

He was the only child to a non-consanguineous couple who had three previous miscarriages. His father died aged 63 years from chronic lung disease secondary to asbestos exposure (Pedigree Chart, Fig. 1). Three first cousins had precocious puberty.

His height was 1.77 m (mother 1.75 m, father 1.68 m), weight 68.8 kg, with Tanner stage 4 pubertal development and small bilateral gynaecomastia, but no acne. By orchidometry, the left testis was 8 mL, containing a tender solid 2 cm mass and a large left hydrocele and the right testis was atrophic and 1 mL.

Investigations

Scrotal ultrasound demonstrated an atrophic right (3 mL) and a larger left testis containing a solid lesion 2 cm in diameter, suggestive of a neoplasm and a 22 mL left hydrocele. CT scan of the chest and abdomen did not show evidence of metastatic disease or abnormal lymph nodes; the seminal vesicles and prostate were normal.

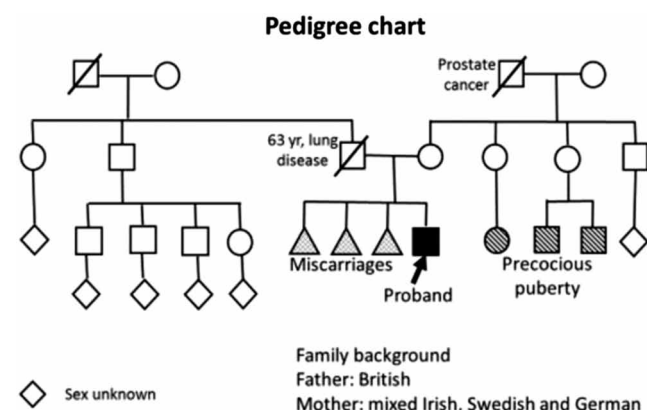


Figure 1
Pedigree chart.

He had elevated serum FSH (17.2 IU/L) and LH (11.7 IU/L), low serum testosterone (5.1 nmol/L) and normal serum SHBG (24 nmol/L). Serum α FP and HCG were negative. Semen analysis showed azoospermia. The working diagnosis was a testicular tumor on a background of possible Klinefelter syndrome.

Treatment and follow-up

Left partial orchidectomy was performed. Macroscopically the mass was a hematoma. Histopathology showed a hemorrhagic corpus luteal cyst within an ovotestis (Fig. 2A, B, C and D). The gonad, including the ovarian tissue, was contained within a tunica albuginea. The testicular tissue contained Sertoli-cell-only seminiferous tubules, interstitial Leydig cells and some Sertoli cell nodules. The ovarian tissue comprised ovarian stroma, a few primordial follicles (the only germ cells in the ovotestis), a hemorrhagic corpus luteum and several corpora albicans.

Karyotype was 46,XX with a notably absent SRY signal on FISH analysis. The ovarian and testicular tissue both had confirmed 46,XX chromosomes without Y signal. Radiological bone age (wrist) was consistent with

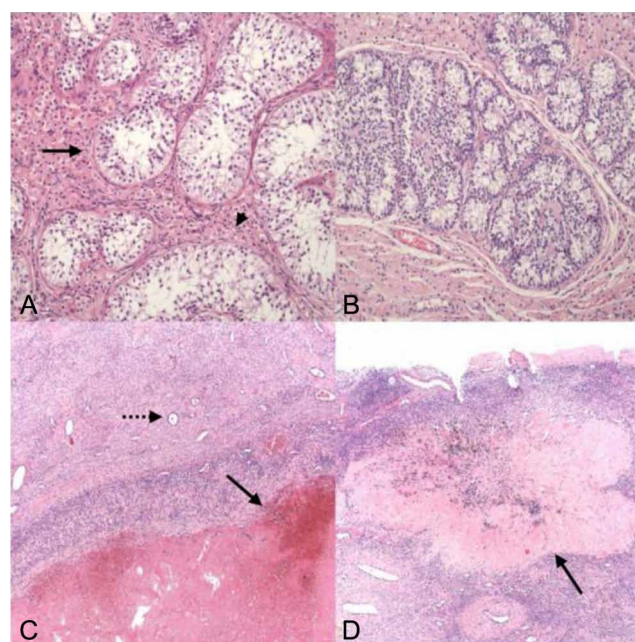


Figure 2
Ovo-testis histology. (A) Seminiferous tubules (arrow), devoid of germ cells or spermatogenesis, with interstitial Leydig cells (arrow head). (B) Sertoli cell nodule in testicular component (Leydig-rich background). (C) Ovarian tissue with an involuting haemorrhagic corpus luteum (arrow) and a primordial follicle (dotted arrow). (D) Ovarian tissue with an old corpus albicans (arrow), the adjacent ovarian stroma with a further primordial follicle. Image dimensions, A and B: 0.9 × 0.7 mm; C and D: 2.2 × 1.7 mm.

chronological age. Post-operatively, both serum FSH (38.2 IU/L) and LH (22.7 IU/L) rose further with a serum total testosterone of 8.7 nmol/L.

Repeat genetic testing on DNA extracted from blood, confirmed the absence of *SRY* and a 46,XX karyotype (1). This classified the diagnosis as 46,XX *SRY* negative (–ve) ovotesticular DSD. Further analysis identified a duplication upstream of the *SOX9* gene, which was confirmed by a second independent method (2).

The proband's mother did not carry the duplication, however there was no paternal family history of DSD or infertility.

Within a multidisciplinary team, as the patient's gender identity was unequivocally male, he was managed as a young man with hypogonadism requiring testosterone replacement and counseling regarding irreversible infertility associated with a missing Y chromosome. He was treated with injectable testosterone undecanoate (1000 mg, per 12 weeks), which achieved Tanner stage 5 virilization with gain of muscle bulk and acne. His most recent trough serum total testosterone concentration was 9.6 nmol/L with suppressed serum LH (<0.1 IU/L) and FSH (0.8 IU/L), indicating adequate replacement therapy.

Discussion

In mammals, the early gonad is bipotential and can differentiate into either a testis or an ovary. *SRY* is the master switch in testis determination (3) having evolved from the ancestral *SOX3* (*SRY* type high-mobility group box gene) gene. *SRY* (sex-determining region on the Y chromosome) is specific to mammals (4). *SRY* upregulation of *SOX9* induces differentiation of the bipotential gonad into a testis and, in its absence, the genital ridge develops by default into ovaries.

The only known function of *SRY* is to upregulate the evolutionarily more conserved *SOX9* (5), an autosomal

(chromosome 17) gene. *SRY*-induced *SOX9* activation drives Sertoli cell differentiation and proliferation, a decisive step in testis development (4, 5). *SRY* is sufficient but not necessary for testis development, as increased *SOX9* expression, is sufficient to induce testis formation, thus substituting for *SRY* function (6). Ectopic expression of *SOX9* in an XX gonad induces testis development in transgenic XX mice (6) while *SOX9* duplication can cause XX sex reversal in humans (7). Furthermore, experimentally replacing *SOX9* for *Sry*, and *Eif2s3x* for *Eif2s3y* (spermatogonial proliferation factor) in mice produced phenotypic males with testes despite the absence of the Y chromosome. These two genes alone were sufficient to produce fertile haploid male gametes that could fertilize oocytes *in vitro* and produce offspring (8).

SOX9 is a pleiotropic gene with an array of gene dosage effects. Inactivation of both *SOX9* alleles leads to the formation of ovaries in XY mice (9). In humans, *SOX9* gene haploinsufficiency causes campomelic dysplasia (CD), an autosomal dominant skeletal dysplasia, as well as male-to-female sex reversal in about 75% of the 46,XY patients (10). *SOX9* is also implicated in pancreatic development with pancreatic hypoplasia reported in patients with CD and in mouse models (11). *SOX9* regulates neural crest development and ectopic expression of *SOX9* in the neural tube progenitors results in neural crest-like properties (12). These links to neural crest development could explain the benign extra adrenal ganglioneuroma in this patient.

Our 46,XX *SRY* –ve patient had a novel duplication in the regulatory region upstream of *SOX9*, leading to a male phenotype (2). He had at least one ovotestis that produced sufficient testosterone to almost complete a phenotypically normal male puberty but not sufficient to suppress ovulation on more than one occasion, as shown by the apparent recent ovulation as indicated by a

Table 1 Hormonal profile pre-operatively, post-operatively and on testosterone replacement therapy.

Hormonal panel	Results					Reference range	
	15 days pre-op	13 days pre-op	2 months post-op	6 months post T Rx	14 months post T Rx	Male	Female
FSH (IU/L)	13	17	38	3.3	0.8	1.0–12	Follicular 3.5–12.5 Mid-cycle 4.7–21.5 Luteal 1.7–7.7
LH (IU/L)	8	12	23	16	<0.1	0.6–12	Follicular 2.4–12.6 Mid-cycle 14–95 Luteal 1–11.4
Testosterone (nmol/L)	4.5	5.1	8.7	9.6	9.7	11.5–32	<2.8
SHBG (nmol/L)		24				15–80	



Table 2 Clinical characteristics, histopathology of the gonad removed and hormonal profile, semen analysis and karyotype of phenotypic men with evidence of ovulation.

Paper	Parvin (1)	Perez-Palacios et al. (2)	Ceci et al. (3)	Kanaka-Gantenbein et al. (4)	Gallegos et al. (5)			
					Case 1	Case 2	Case 3	Our case
Age at presentation (year)	32	16	22	13	15	13	11	18
Presenting symptom	Pain in R testis	BI gynaecomastia	Pain in R testis	L scrotal pain following injury	Familial, siblings			Pain and mass in L testis
Duration	1 week	NR	2 days	NR	NR			2 days
Phenotype	Male	Male	Male	Male	Male		Male	Male
H/o cryptorchidism	+	+	–	–	–		–	+
Fertility	+	NA	NR	NR	NA	NA	NA	Azoospermic
Relevant past history	Testicular pain age 24 years	Thelarche at 14 year	L hypospadias, b/l mammoplasty	L inguinal hernia at 6 months of age	Hypospadias, BI gynaecomastia	Hypospadias, BI gynaecomastia	Hypospadias	Benign extra adrenal ganglioneuroma, lip capillary haemangioma, Henoch-Schonlein purpura
Examination	Small, hard, tender R testis over pubic tubercle, normal Lf testis	BI gynaecomastia, 5.5 cm penis, 2.5 mL R testis and absent Lf testis	Atrophic R testis and rubbery hard large left testis	Acne, gynaecomastia, pubic hair stage 5, normal penis, tender Lf scrotum, R testis 5–16 mL	Scrotal gonads	Scrotal gonads	Scrotal gonads	Tanner stage 4 puberty, small BI gynaecomastia, L testis 8 mL with a tender solid 2 cm mass, large Lf hydrocele, R testis atrophic 1 mL
Primordial follicles	+	+	+	+	+	+	+	+
Corpus luteum	–	Hemorrhagic	+	Hemorrhagic	NR	NR	NR	+
Corpus albicans	+	–	+	NR	NR	NR	NR	+
Fallopian tube	+	+	+	+	–	–	–	–
uterus	–	+	+	+	–	–	–	–
Ovotestis	+	+	+	–	+	+	+	+
Testicular tissue	–	+	Rudimentary epididymis	+	+	+	+	+
Leydig cells	–	+	–	–	–	–	+	+
Sertoli cells	–	+	–	+	+	+	+	+
Seminiferous tubules	–	+	–	+	+	+	–	–
Spermatogenesis	–	Spermatogia	–	Spermatogia	–	–	–	–
FSH (IU/L)	7.6	5.5	26.3	20*				13
LH (IU/L)	7.4	11	9.8	8.2*				8
T (nmol/L)	24.5	2.4	15.5	9.1*				4.5
E2 (pmol/L)	NR	180	135	36.7*				–
P4 (nmol/L)	NR	1.2	NR	NR				–
Semen analysis	15 x 10 ⁶ sperm/mL	NA	NR	NR	NA	NA	NA	Azoospermia
Karyotype peripheral blood	46,XX/46,XY Chimera (81%, 19%)	46,XX/47XXY (72%, 28%)	46,XX/46,XY Chimera (11%, 89%)	46,XX/47,XXY (70%, 30%)	46,XX, no Y detected	46,XX, no Y detected	46,XX, no Y detected	46, XX, SRY –ve
Karyotype gonadal tissue	–	46,XX/47XXY	–	46,XX/47XXY	46,XX/47XXY	46,XX/47XXY	46,XX/47XXY	46,XX, SRY –ve

*2 weeks post gonadectomy.

BI, bilateral; E2, estradiol; L, left; NA, not applicable; NR, not reported; P4, progesterone; R, right; T, testosterone.



Table 3 Summary of case reports with SOX9/upstream duplication.

No	Paper	Age at presentation	Presenting complaint	Histology	Genetics	Comments
1	Huang et al. (6)	1 Infant	Ambiguous genitalia	Not performed	SOX9 duplication	–
2	Kojima et al. (7)	5 Infant	Ambiguous genitalia	BI Wolffian structures + Germinal aplasia, Sertoli and Leydig cells + No ovarian tissue/uterus/tubes	SOX9 upregulation and DAX1 duplication	Sporadic cases
3	Cox et al. (8)	3 Adult	Infertile	Leydig, Sertoli cells + atrophies seminiferous tubules No spermatogenesis	~178 kb duplication 600 kb upstream of SOX9	3 members (2 brothers and paternal uncle) from a family including a 46,XY father who carried the duplication Brothers
4	Vetro et al. (9)	2 Adult	Infertile	Germinal cell aplasia	96 kb triplication 500 kb upstream of SOX9	All 3 were sporadic cases
5	Benko et al. (10)	4 Birth	Ambiguous genitalia	Case 1: ovotestis and fallopian tubes Case 2: L ovary, fallopian tubes, rudimentary vagina and uterus	Case 1: ~605–695 kb duplication 353 kb upstream of SOX9 Case 2: ~148 kb duplication ~595 to ~447 kb upstream of SOX9	Case 2: duplication paternally inherited. Duplication shared by two 46,XY brothers and not the sister Case 3 and 4: Brothers inherited duplication from 46,XY father and 46,XY grandfather
6	Xiao et al. (11)	1 Adult	Infertile	Case 3: ovotestis, primordial follicles, seminiferous tubules, Sertoli cells, fallopian tube + Not reported	Case 3 and 4: ~762–780 kb duplication ~508 kb upstream of SOX9 ~74 kb duplication ~510–584 kb upstream of SOX9	–
7	Lee et al. (12)	1 4 years	Small testis	duplication upstream of SOX9	SOX9 duplication	–
8	Kim et al. (13)	3 Case 1: 25 years	Azoospermia	Not reported	143 kb duplication ~516–659 kb upstream of SOX9	–
9	Hyon et al. (14)	Case 2: At birth Case 3: At birth 3 Adult	Ambiguous genitalia Ambiguous genitalia Infertile	L ovary, fallopian tubes+, R ovotestis primitive seminiferous tubules R ovary, L dysgenetic testis/ovotestis; vagina, rudimentary uterus + Case 1 and 2: atrophic seminiferous tubules containing only Sertoli cells suggestive of testicular dysgenesis, Leydig cell hyperplasia; No spermatogenesis	Case 2: ~444 kb duplication ~259–703 kb upstream of SOX9 Case 3: 480 kb duplication 264–744 kb upstream of SOX9 Case 1: 83 kb duplication ~694 kb upstream of SOX9 Case 2: 83 kb duplication ~694 kb upstream of SOX9 Case 3: 140 kb duplication ~694 kb upstream of SOX9	Paternally inherited – Case 1 and 2 were brothers
10	Vetro et al. (15)	2 Case 1: Adult Case 2: Infant	Infertile Ambiguous genitalia	N/A Ovotestis, numerous oocytes, pre-pubertal seminiferous tubules	Duplication upstream of SOX9 Duplication upstream of SOX9	–
11	Our Case	1 Adult	Mass in L testis	Ovotestis, hemorrhagic corpus luteum and corpus albicans, primordial follicles, Sertoli cell nodules, seminiferous tubules with no spermatogenesis	Duplication upstream of SOX9	

Table 4 Classification of DSDs, in particular 46,XX DSDs that can result in virilization or male phenotype (18).

DSD category	Examples	Karyotype or genes involved
Sex chromosome DSD	Klinefelter's syndrome Turner's syndrome Ovotesticular DSD	47,XXY and variants/mosaics 45,XO and variants/mosaics 46,XX/46,XY chimerism
46,XY DSD	Disorders of androgen action (androgen insensitivity syndrome) Disorders of androgen biosynthesis	AR CYP11A1, HSD3B2, CYP17A1, StAR, HSD17B3, SRD5A2, POR DHH, SRY, SF1, WT1, SOX9
	Disorders of testicular development (ovotesticular DSD, testis regression) Other syndromic associations of male genital development, isolated hypospadias, cryptorchidism	CXorf6, INSL3, GREAT
46,XX DSD	Disorders of ovarian development (ovotesticular DSD, testicular DSD) Disorders of androgen excess – fetal (congenital adrenal hyperplasia), feto placental (aromatase deficiency) and maternal (virilizing tumors e.g. luteomas) Other syndromic associations (e.g. cloacal anomalies), Mullerian agenesis/hypoplasia, uterine abnormalities, vaginal atresia (e.g. McKusick-Kaufman), labial adhesions	SRY, RSPO1, dup SOX3, dup SOX9* HSD3B2, CYP21A2, CYP11B1, POR, CYP19 MKKS

*Gene duplication detected in the current case of 46,XX karyotype in a young phenotypic male.

hemorrhagic corpus luteum as well as a corpus albicans, indicating prior ovulation. Given his male phenotype, no serum estradiol or progesterone concentrations were obtained pre-operatively nor did the hormonal levels (Table 1) confirm ovulation. It cannot be excluded that rather than true ovulation, the histological appearances of a corpus luteum and corpus albicans may have resulted from a collapsed, incompletely mature antral follicle without true LH surge-triggered ovulation. Apparent ovulation in phenotypic men has been reported previously in 46,XX, 46,XX/46,XY, 46,XX/47,XXY DSDs (Table 2, summary of case reports); however, this is the only case of a 46,XX SRY –ve ovotesticular DSD from SOX9 duplication presented in Table 2.

The presence of the Y chromosome is associated with risk of malignancies in a dysgenic gonad. The patient's 46,XX karyotype on all cell lines tested, suggests his risk of gonadoblastoma is low. However, based on a single case report of a Sertoli cell tumor in phenotypic male with 46 XX ovotesticular DSD (13), his follow-up will include regular scrotal ultrasound surveillance.

His genetic diagnosis may have implications for the extended paternal family if the SOX9 duplication was inherited. The lack of paternal DNA precluded distinguishing between a *de novo* germline duplication in the patient or paternal transmission of the SOX9 duplication. The latter has been reported in a fertile 46,XY father carrying a SOX9 or upstream enhancer duplication with transmission to a child with 46,XX karyotype resulting in female-to-male sex reversal (14, 15, 16). As the paternal uncle and all his sons were fertile, there is no evidence to suggest this was paternally inherited.

Previously reported SOX9 duplications are summarized in Table 3, our case is unique in his presentation with a testicular mass from ovulation and with evidence of probable multiple ovulations.

Consensus statement on management of intersex disorders of the Paediatric Endocrine Society and European Society for Paediatric Endocrinology at Chicago International Consensus Conference on Intersex (Chicago consensus) of 2006 (17) defined DSDs as congenital conditions in which development of chromosomal, gonadal or anatomic sex is atypical. The proposed nomenclature emphasizes on the knowledge of karyotype, which is key in categorizing the DSDs as (1) sex chromosome DSDs (variation in the number of sex chromosomes e.g. Turner's syndrome 45,X; Klinefelter's syndrome 47,XXY; mixed gonadal dysgenesis, etc.), (2) 46,XY DSDs and (3) 46,XX DSDs as described in Table 4 (18). Table 4 also demonstrates categories of 46,XX DSDs that can cause virilization or male phenotype in 46,XX individuals as presented in our present case.

Management of DSDs should integrate the medical aspects of care with the psychosocial needs of the patient. Currently, there are no recommendations for sex assignment in neonates who have DSD. Any surgical procedure in children that leads to irreversible change must be considered with utmost caution (18). Ideally, the surgical procedure should also aim to preserve fertility in the most optimal way. In general, individuals with 46,XY DSD have an increased risk of malignancy may need prophylactic gonadectomy if indicated. Treatment is ideal in a multidisciplinary setting with considerations to genetic (implications to family and

including reproductive recurrence risks), psychological aspects (sensitive individualized counseling including patient gender identity and preference), endocrinological (hormone replacement), surgical (cosmetic, prophylactic gonadectomy) fertility preservation, reproductive opportunities and metabolic health (cardiovascular and bones).

There are still uncharacterized genes causing DSD. Such DSDs are very rare and require a careful systematic, sensitive approach to diagnosis and management of the diagnostic and ethical challenges. Clinical assessment with karyotype (including *SRY* expression) and subsequent determination of the presence or absence of Müllerian structures are essential early investigations in any case of DSD. The sophisticated genetic diagnoses now feasible should be undertaken as the basis for genetic counseling of the extended family in any more complex cases.

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.

Funding

This research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Patient consent

A written informed consent has been obtained from the patient for publication of the submitted article and accompanying images.

Author contribution statement

The article was conceived and written by N Shankara Narayana under the supervision of D J Handelsman and S M Twigg. A M Kean, A Vasilaras, L Ewans and G Watson were involved in the clinical care and organized the relevant investigations and revision of the manuscript. T Ohnesorg, K L Ayers and A Sinclair performed the genetics investigations of the diagnosis and contributed to the manuscript.

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Received in final form 11 May 2017

Accepted 15 May 2017