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ORIGINAL ARTICLE ***Reproductive epidemiology***

Incidence, prevalence, diagnostic delay, morbidity, mortality and socioeconomic status in males with 46,XX disorders of sex development: a nationwide study

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STUDY QUESTION: What is the epidemiology and trajectory of health and socioeconomic status in males with 46,XX disorders of sex development (DSD)?

SUMMARY ANSWER: 46,XX DSD males had an increased overall morbidity compared to male background population controls, and the socioeconomic status was inferior on outcome parameters such as education and long-term income.

WHAT IS KNOWN ALREADY: 46,XX DSD males are rare and estimates of prevalence and incidence are limited. An increased morbidity and mortality as well as a negatively affected socioeconomic status are described in males with Klinefelter Syndrome. However, this has never been systematically studied in 46,XX DSD males.

STUDY DESIGN, SIZE, DURATION: In this nationwide registry study including 44 males with a verified diagnosis of 46,XX DSD we aimed to estimate incidence, prevalence and diagnostic delay. Further, we aimed to study morbidity, mortality and socioeconomic outcome parameters using the Danish registries. The socioeconomic outcome parameters were education, income, retirement, parenthood and cohabitation. 46,XX DSD males were born during 1908–2012 and follow-up started at birth or at start of registration and ended in 2014.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Potential cases (n = 69) were identified in the Danish Cytogenetic Central Registry and the diagnosis was verified by medical record evaluation (n = 44). A randomly selected age-matched control group of 100 males and 100 females per case was identified by Statistics Denmark.

MAIN RESULTS AND THE ROLE OF CHANCE: Among newborn males the prevalence of diagnosed 46,XX DSD males was 3.5–4.7 per 100 000. Median age at diagnosis was 17.0 years (range: 0.0–62.8). Overall morbidity was increased compared to male controls (hazard ratio [HR] = 2.4, 95% CI: 1.8–3.3) but not when excluding endocrine and urogenital diseases as well as congenital malformations (HR = 1.2, 95% CI: 0.8–1.6). Mortality was not increased (HR = 0.6, 95% CI: 0.2–2.5) compared to male controls. 46,XX DSD males had poorer education (HR = 0.1, 95% CI: 0.0–0.9) and fewer fatherhoods (HR = 0.4, 95% CI: 0.2–0.7) than male controls, and their income was reduced for the following age groups; 45–49 years: odds ratio [OR] = 0.4 (95% CI: 0.2–0.7); 50–54 years: OR = 0.1 (95% CI: 0.0–0.6).

LIMITATIONS, REASONS FOR CAUTION: The study cohort is rather small, although it is large in comparison to other studies on 46,XX DSD males. Some 46,XX DSD males may have been excluded from the study owing to lack of data in medical records, making the

diagnosis impossible to verify. As in all epidemiologic studies a risk of misclassification must be considered when interpreting the study results, and as the study included diagnosed 46,XX DSD males only, conclusions cannot be extended to non-diagnosed 46,XX DSD males.

WIDER IMPLICATIONS OF THE FINDINGS: This study provides a new insight into trajectory of health and socioeconomic status of 46,XX DSD males.

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Introduction

Congenital conditions in which phenotypic males have a female karyotype are rare with a reported prevalence of approximately four per 100 000 newborn males (four males with a 46,XX karyotype identified among 96 121 newborn males) (de la Chapelle, 1981). The first known case was reported by de la Chapelle *et al.* in 1964 (de la Chapelle *et al.*, 1964). The conditions were previously termed intersex, pseudohermaphroditism or male sex reversal. However, the current terminology for individuals in whom the chromosomal, gonadal or anatomical sex is atypical was introduced with the 'Chicago Consensus' in 2006 (Hughes *et al.*, 2006), and phenotypic males with a female karyotype were gathered under the umbrella term 46,XX disorders of sex development (DSD). We use the term 46,XX DSD males in this manuscript.

The classical form of 46,XX DSD males is testicular DSD, and approximately 80% of these males present Y-chromosomal material including the sex-determining region Y (*SRY*) – gene, primarily owing to a chromosome Y to chromosome X translocation during paternal meiosis (Andersson *et al.*, 1986; Zenteno-Ruiz *et al.*, 2001; Ono and Harley, 2013). In both *SRY* positive and *SRY* negative 46,XX DSD males with testicular DSD, the bipotential gonads differentiate to testes, and because of the hormonal secretion of androgens and anti-Müllerian hormone from the gonads, the Wollfian structures develop, the Müllerian structures regress and the external genitalia virilize (Ono and Harley, 2013). Other forms of 46,XX DSD are ovo-testicular DSD with coexistence of testicular and ovarian tissue, congenital adrenal hyperplasia (CAH) caused by fetal enzymatic defects in steroidogenesis (e.g. 21-hydroxylase deficiency or 11-hydroxylase deficiency), and androgen excess owing to either a maternal (e.g. androgen secreting luteoma) or an exogenous source during the critical time for sex differentiation in fetal life (Hughes *et al.*, 2006).

The clinical presentation of 46,XX DSD males shows considerable variation. Genital ambiguity occurs in approximately 15%, primarily in those with *SRY* negativity (Boucekkine *et al.*, 1994), whereas the remaining have completely virilized external genitalia (de la Chapelle, 1972; Vilain *et al.*, 1994). 46,XX DSD males with testicular DSD may have normal-for-age testosterone during puberty but hypergonadotrophic hypogonadism in adulthood; thus small testes are a characteristic trait, and gynecomastia occurs in approximately one-third of the patients (Vorona *et al.*, 2007; Majzoub *et al.*, 2017). Axillary hair and body hair are scanty, and pubic hair shows a female pattern of distribution (de la Chapelle, 1972; Majzoub *et al.*, 2017).

Other syndromes categorized as sex chromosome DSD, such as Klinefelter syndrome and Turner syndrome, are associated with an increased morbidity and mortality (Bojesen *et al.*, 2006, 2011; Stochholm *et al.*, 2006), and the socioeconomic profile of patients with these syndromes is inferior on certain parameters compared to the background population (Bojesen *et al.*, 2011; Stochholm *et al.*, 2012). Recently we showed that phenotypic females with 46,XY DSD are more frequent than previously thought and present quite differently (Berglund *et al.*, 2016). In 46,XX DSD males, however, none of these aspects have ever been studied systematically.

With this study of a nationwide cohort of 46,XX DSD males we therefore aimed to estimate incidence, prevalence, diagnostic delay and clinical presentation of 46,XX DSD males. Furthermore, we aimed to study morbidity, mortality and the socioeconomic profile of 46,XX DSD males in a comparison to the male background population.

Materials and Methods

Since 1968, all persons residing in Denmark have been assigned a unique 10-digit civil personal registration (CPR) number, from which date of birth and sex can be identified. The CPR number allows accurate matching of data from different sources.

Identification of patients and controls

The Danish Cytogenetic Central Registry (DCCR) was established in 1966 and comprises data of all individuals being karyotyped in Denmark since 1960. From the DCCR, all male individuals recorded with a female karyotype during 1960–2015 were identified, and information regarding CPR number, date of birth, karyotype and date of karyotyping was retrieved in November 2015.

All somatic hospital admissions have been recorded in the Danish National Patient Registry (DNPR) since 1977, and all out-patient contacts have been recorded since 1995. For all individuals identified in the DCCR the following information was retrieved from the DNPR: Diagnoses according to the International Classification of Diseases (8th edition until 1993 [ICD-8], and thereafter 10th edition [ICD-10]), dates of admission and discharges, and institutional origin of the ICD diagnoses.

Medical records were identified and reviewed in order to confirm or reject a patient as a 46,XX DSD male. If a medical record for some reason did not exist (e.g. destroyed because of archiving regulations or simply disappeared) or if the information in the medical record was insufficient to confirm the diagnosis, DSD specialists evaluated the ICD diagnoses from the DNPR, and the status of the patient was decided by consensus. Thus,

if a patient had one or more ICD diagnoses that could be associated with 46,XX DSD (e.g. diagnoses of traits characteristic for 46,XX DSD males, such as cryptorchidism or delayed puberty) the diagnosis was considered as verified.

By Statistics Denmark, all verified 46,XX DSD males were matched with 100 male and 100 female background population controls on age (month and year of birth). All controls were alive on the date of diagnosis of their relevant case. All comparisons are solely between 46,XX DSD males and the male background population controls except when comparing overall morbidity.

Medical record review

The 46,XX DSD males were categorized as having testicular DSD if *SRY* positivity was shown at genetic testing. *SRY* negative 46,XX DSD males, or 46,XX DSD males without further genetic testing, were categorized according to their clinical presentation in the medical records: first, testicular DSD if both gonads were localized in the scrotum or if gonads were macroscopically described as testes at orchiopexy or other surgical procedures; second, ovotesticular DSD if both ovarian and testicular tissue were present in the gonads; third, CAH if both gonads were ovaries, which was further substantiated by a hormone profile consistent with CAH. In cases where no firm diagnosis could be determined, 46,XX DSD males were categorized as unclassified 46,XX DSD males. These males could be suspected of having testicular DSD, but other causes of 46,XX DSD could not be ruled out based on the existing evidence.

Age at diagnosis was defined as age at diagnosis evaluated from the medical record review (clinically or genetically) or as age at karyotyping (retrieved from the DCCR), whichever came first.

From the medical records the following information was retrieved, if available: Symptoms leading to referral and subsequent diagnosis; *SRY* positivity or *SRY* negativity; degree of virilization at diagnosis was retrospectively scored according to the Prader Scale (Prader, 1954) owing to descriptions of the external genitalia (e.g. if a patient, at diagnosis owing to infertility, presented with genitalia described as complete male, and no genital surgery had been performed, then the Prader stage was scored as 5); localization of gonads; testosterone treatment and age at start of treatment.

Morbidity, mortality and socioeconomic outcome parameters

We retrieved data for all 46,XX DSD males and controls regarding morbidity (primary diagnoses and the corresponding date of diagnosis according to all in-and out-patient contacts), mortality (date of death and primary cause of death), socioeconomic parameters (education, income, retirement, cohabitation and parenthood) and dates of emigration, if relevant.

Education

Data regarding educational category and dates of achieved education were available from 1970 to 2014. Educational categories were primary school, high school, vocational training, a bachelor's degree (e.g. nurse or school teacher), a candidate degree and a PhD degree. A completed bachelor's degree or higher was categorized as an education, and the registered event was the first achieved bachelor's degree in persons between 18 and 45 years of age.

Income

Information on gross annual taxable income was obtained from 1987 to 2014. Analyses were performed in persons aged between 18 and 70 years.

Retirement

Retirement was defined as retirement owing to sickness or age. The registered event was the first year of retirement regardless of a later return to the labor market in persons with a minimum age of 18 years. Data were retrieved annually from 1980 to 2013.

Cohabitation

Information regarding cohabitation status was retrieved for each January 1 from 1980 to 2015. The event was the first recorded cohabitation with a partner. Analyses were performed for individuals aged between 18 and 70 years.

Parenthood

All children, born or adopted, were recorded until 2012. The event was the first registration of a child either born or adopted in individuals aged between 15 and 50 years. We have no records for mode of achieving pregnancy in the study group.

Statistics

For the analyses, we describe two cohorts of 46,XX DSD males: first, all 46,XX DSD males (the combined cohort of 46,XX DSD males) and second, the 46,XX DSD males with testicular DSD.

The incidence was estimated as the average number of annually diagnosed 46,XX DSD males per million males in the background population. Observation periods started the year the first individual in the relevant cohort was diagnosed. Accordingly, periods started in 1970 for the combined cohort of 46,XX DSD males and in 1972 for the cohort of testicular DSD.

The prevalence was estimated in 5-year intervals as the average number of 46,XX DSD males born per 100 000 newborn males in the background population. Observation periods started the year the first individual in the relevant cohort was born, thus in 1908 for the combined cohort of 46,XX DSD males and in 1933 for the cohort of testicular DSD. All observation periods ended in 2015. Background population data were retrieved from Statistics Denmark (www.dst.dk).

Time trend in incidence and prevalence was analyzed using Poisson regression, and time trend in age at diagnosis was analyzed using linear regression.

Morbidity, mortality, education, retirement, cohabitation and parenthood were analyzed using stratified Cox regression, where each 46,XX DSD male and his controls constituted one stratum. Follow-up time for morbidity and mortality started at birth. Morbidity analyses were also performed with a follow-up time starting on the date of the 46,XX DSD male diagnosis. Follow-up time for education, retirement and cohabitation started at the 18th year birthday or at the start of registration of the event. Follow-up time for parenthood started at the 15th year birthday. Follow-up time ended at the first occurrence of the event, for example birth of first child, first date of emigration, death or at end of registration, whichever came first. Analyses were performed for the combined cohort of 46, XX DSD males.

The overall morbidity analysis was also performed adjusting for cohabitation status and education.

Ages at specific events were compared using Kruskal–Wallis test.

Income was analyzed using conditional logistic regression, in which each 46,XX DSD male and his controls constituted one stratum. All retired persons were excluded from the first year of retirement. The median annual income for controls was calculated in 5-years intervals. For each calendar year, a dichotomous variable indicated whether the income for a 46,XX DSD male was higher or lower than the median income in his respective controls in the 5-year interval.

CIs were estimated as 95% CI. *P*-values less than 0.05 were considered statistical significant. All analyses were performed using Stata 13.1 and Stata 14.1 (StataCorp, USA).

Ethics

The study was approved by the Danish Data Protection Agency (journal number: 2012-41-0047) and the Danish Health Authorities (journal number: 3-3013-472/1/).

Results

From the DCCR, 76 persons with a male CPR number and a 46,XX karyotype were identified. Initially, seven persons were excluded as they had an invalid CPR number, which could be due to death before 1968, where the Civil Registration System was established. For the remaining persons ($n = 69$), medical records were accessible for 72% (50/69). Of these, 40 persons were verified as 46,XX DSD males, whereas ten persons were non-46,XX DSD males [46,XX karyotype in a male subsequent to bone marrow transplant with a female donor ($n = 4$), registration error ($n = 3$), transgender ($n = 2$) and a female CAH individual with clitoral hypertrophy raised as male, despite the fact that her phenotype was clearly feminine ($n = 1$)]. Of the 19 persons for whom medical records were inaccessible, ICD diagnoses from the DNPR were evaluated and four persons were verified as 46,XX DSD males. In total, 44 persons were categorized as 46,XX DSD males. Of these, 32 had testicular DSD, and the remaining had ovotesticular DSD ($n = 1$) and CAH ($n = 3$) or were categorized as unclassified 46,XX DSD males ($n = 8$) (Fig. 1).

Incidence and prevalence

During 1970–2015, an average of 2 600 000 males were at risk of receiving a 46,XX DSD diagnosis each year. Hence, the average minimum incidence for the combined cohort of 46,XX DSD males and for testicular DSD was 0.4 and 0.3 per million males, respectively (Fig. 2A). No change in incidence was observed during 1970–2015 ($P = 0.85$).

During 1908–2015, the average prevalence for the combined cohort of 46,XX DSD males was 1.1 per 100 000 newborn males. The average prevalence of testicular DSD during 1933–2015 was 0.8 per 100 000 newborn males. A marked increase in the prevalence of 46,XX DSD males was observed during 1961–1990, where the average prevalence for the combined cohort was 3.5 per 100 000 newborn males and the average prevalence of testicular DSD was 2.7 per 100 000 newborn males. During 1976–1990, the prevalence of the combined cohort of 46,XX DSD males reached a maximum of 4.7 per 100 000

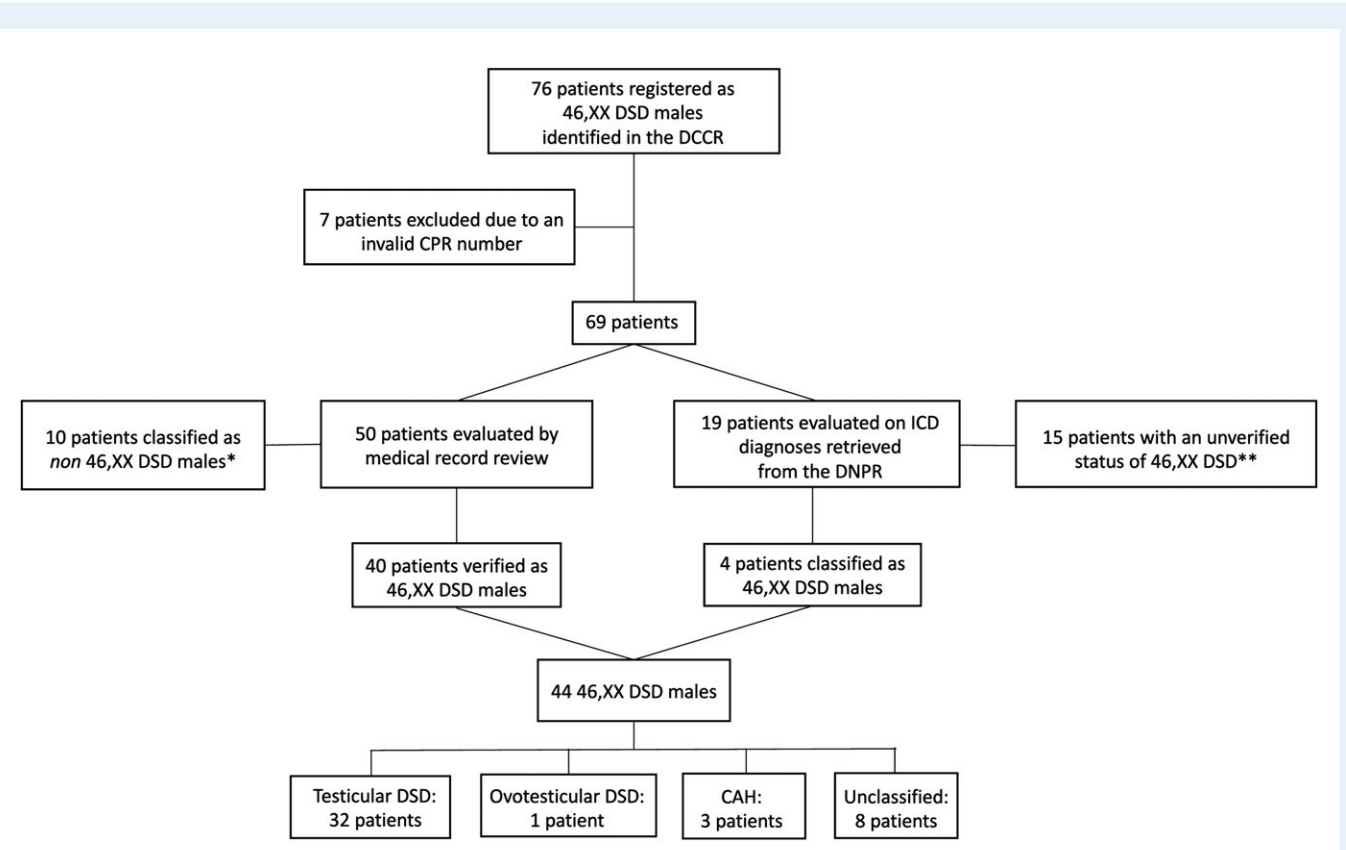


Figure 1 Identification of 46,XX DSD males. DSD, disorders of sex development; DCCR, Danish Cytogenetic Central Registry; CPR number, civil personal registration number; DNPR, Danish National Patient Registry; ICD, International Classification of Diseases (8th and 10th edition); CAH, congenital adrenal hyperplasia. *46,XX karyotype in males subsequent to bone marrow transplant with a female donor ($n = 4$), registration error ($n = 3$), transgender ($n = 2$), female individual with clitoral hypertrophy raised as male ($n = 1$). **Confirmation of a 46,XX DSD male diagnosis was not possible owing to lack of information.

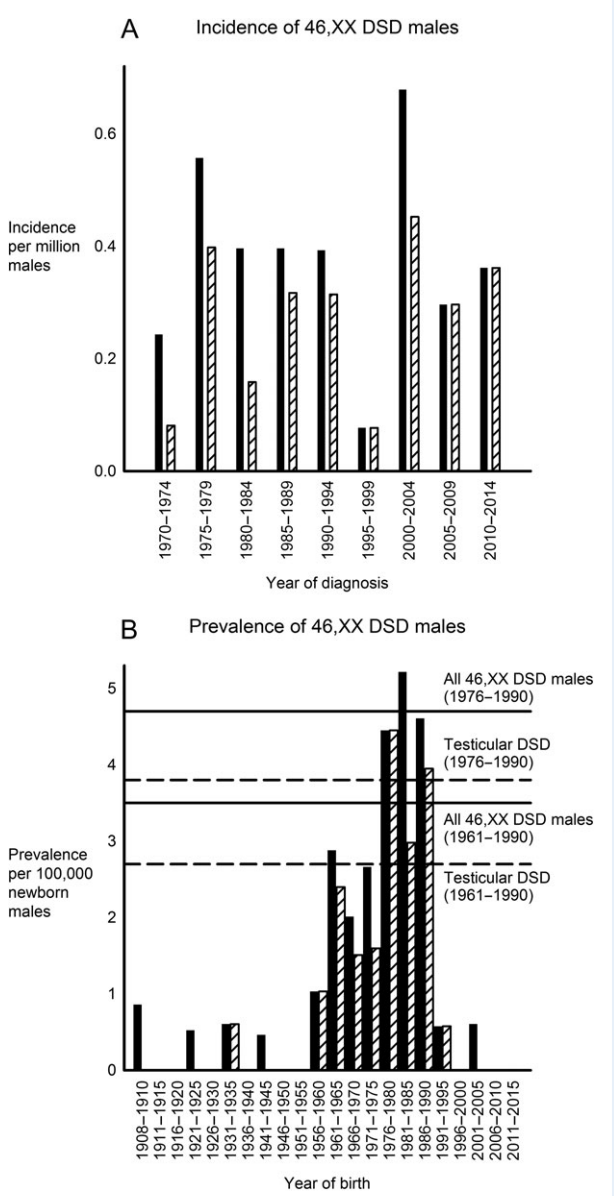


Figure 2 Incidence and prevalence of 46,XX DSD males. (**A**) Incidence of 46,XX DSD males per million males in the background population. Black bars: combined cohort of 46,XX DSD males; shaded bars: testicular DSD. (**B**) Prevalence of 46,XX DSD males per 100 000 newborn males. Black bars: combined cohort of 46,XX DSD males; shaded bars: testicular DSD. Horizontal lines indicate the average prevalence of the combined cohort of 46,XX DSD males (solid lines) and of testicular DSD (dashed lines) during 1961–1990 and 1976–1990.

newborn males, and the corresponding prevalence of testicular DSD was 3.8 per 100 000 newborn males (Fig. 2B). The prevalence of 46,XX DSD males increased substantially during 1908–2015 ($P < 0.0001$).

Including individuals without a verified 46,XX DSD diagnosis born during 1961–1990 or 1976–1990 ($n = 5$ and $n = 1$, respectively), the estimates of prevalence were 3.9 and 5.0 46,XX DSD males per 100 000 newborn males, respectively.

Age at DSD diagnosis

Median age at diagnosis of 46,XX DSD males was as follows: for the combined cohort of 46,XX DSD males it was 17.0 years (range: 0.0–62.8), and for those with testicular DSD it was 25.4 years (range: 0.0–44.8) (Table I). In the combined cohort of 46,XX DSD males, 25% were diagnosed at 0.0 years and 75% at 34.0 years. Age at diagnosis of 46,XX DSD males is illustrated in Supplementary Fig. S1.

Age at diagnosis of 46,XX male DSD increased with borderline significance during 1970–2015 ($P = 0.05$) in the combined cohort of 46, XX DSD males, whereas it increased significantly in the cohort of 46, XX DSD males with testicular DSD ($P = 0.0004$).

Symptoms leading to the 46,XX DSD diagnosis

Males with testicular DSD were primarily diagnosed owing to infertility (48%, 13/27) and a discrepancy between prenatal karyotype and phenotype at birth (22%, 6/27). The remaining males were diagnosed owing to a wide variety of symptoms (Table II).

SRY gene

The *SRY* status was known in 28 males, with 24 being *SRY* positive. The four SRY negative males were classified as ovo-testicular DSD ($n = 1$), CAH ($n = 1$) and as unclassified 46,XX DSD males ($n = 2$), respectively (Table I).

Localization of gonads and degree of virilization

The localization of gonads was known in 32 46,XX DSD males, of whom 28 had testicular DSD. Among these 28 males with testicular DSD, cryptorchidism was present in 29% (8/28) (Table II), and among those was bilateral cryptorchidism present in 63% (5/8). The one male with ovo-testicular DSD had unilateral cryptorchidism with the ovary localized inguinally. An abdominal localization of both gonads was present in the three males with CAH (Table I).

Among 31 males where the Prader stage could be scored retrospectically, 87% (27/31) were classified as Prader stage 5. Among males with testicular DSD, this applied to 89% (25/28). The one male with ovo-testicular DSD was classified as Prader stage 5. Patients with CAH were classified as Prader stage 5 ($n = 1$) and Prader stage 4 ($n = 1$). No males were classified with Prader stages below 4 (Table I).

Testosterone treatment

Testosterone was prescribed for 73% (19/26) of 46,XX DSD males and seventeen of those were classified as testicular DSD with either *SRY* positivity ($n = 14$) or an unknown *SRY* status ($n = 3$). Two males with CAH had testosterone prescribed. In males with testicular DSD 26% (6/23) had no testosterone prescription, and of those one had not yet reached pubertal age at the last visit (Table I). The male with ovo-testicular DSD had no testosterone prescription. Median age at first hormone prescription was 19.0 (range: 13.0–44.0) years.

Morbidity

Overall, morbidity was increased in 46,XX DSD males (HR = 2.4, 95% CI:1.8–3.3) compared to controls, and it was significantly

Table I Clinical presentation of 46,XX DSD males.

	Testicular DSD	All 46,XX DSD males
	n = 32	*n* = 44
Median age at diagnosis (years, range)	25.4 (0.0–44.8)	17 (0.0–62.8)
SRY status		
Positive	24	24
Negative	0	4
Unknown	8	16
Localization of gonads		
Scrotum	20	20
Scrotum and inguinal canal	2	3
Inguinal	5	5
Abdomen	0	3
Abdomen and scrotum	1	1
Unknown	4	12
Degree of virilization at diagnosis		
Prader stage 5	25	27
Prader stage 4	3	4
Unknown	4	13
Prescribed testosterone		
Yes	17	19
No	6	7
Unknown	9	18

SRY, Sex determining region Y gene; DSD: disorders of sex development

Table II Symptoms leading to diagnosis of 46,XX DSD males with testicular DSD and other forms of 46,XX DSD.

	Testicular DSD	All 46,XX DSD males
	n = 32	*n* = 44
Infertility	13	13
Discrepancy between prenatal genetic sex and phenotype at birth	6	6
Cryptorchidism	3	4
Gynecomastia	2	2
Dysmorphic signs	2	2
Short stature	1	1
Delayed puberty	1	1
Hypospadias	1	1
Family history of congenital adrenal hyperplasia	0	1
Multiple congenital malformations	0	1
Menarche	0	1
Clinical signs of congenital adrenal hyperplasia	0	1
Autopsy	0	1
Unknown	5	11

increased within congenital malformations and endocrine and urogenital system diseases (Fig. 3A and B). Adjusted for cohabitation and education, overall morbidity remained increased in 46,XX DSD males (HR = 2.2, 95% CI: 1.6–3.1).

Excluding the diagnoses of congenital malformations and endocrine and urogenital system diseases there was no difference in morbidity (HR = 1.2, 95% CI: 0.8–1.6).

Comparing 46,XX DSD males to the female control population the overall morbidity was increased as well (HR = 2.2, 95% CI: 1.6–3.0) (Fig. 3B), however after exclusion of the diagnoses of congenital malformations and endocrine and urogenital system diseases it was not (HR = 1.0, 95% CI: 0.8–1.4)

One male (testicular DSD) had a record of type two diabetes. No records of osteoporosis were observed, nor were records of testis or breast cancer. Ischemic heart disease was recorded for half of the 46, XX DSD males within the cardiovascular diagnosis group (3/6, two with testicular DSD and one unclassified 46,XX DSD male). Four 46, XX DSD males had records within the group of psychiatric diagnoses, and three of those had records of either depression (*n* = 1, testicular DSD) or anxiety disorders (*n* = 2, testicular DSD), whereas the last (*n* = 1, CAH) had a record of gender dysphoria.

Stratifying overall morbidity on the time before and after the 46,XX DSD diagnosis, the HR was significantly increased after (HR = 2.8, 95% CI: 2.0–3.9) but not before (HR = 1.4, 95% CI: 0.9–2.0).

The median age at first registered hospital admittance was lower in 46,XX DSD males (12.5 years, range: 0.0–74.6) than in controls (14.0 years, range: 0.0–89.9) (*P* = 0.01).

Mortality

In total, six 46,XX DSD males died during the study period, and four of those (CAH, *n* = 1 and unclassified 46,XX DSD males, *n* = 3) died within one day of birth. Thus, the median age at death in 46,XX DSD males was 0.0 (range: 0.0–83.6) years. The median age at death in controls was 74.4 (range: 1.1–98.8) years.

The primary cause of death for the male classified as CAH was 'other specified syndromes', and the three unclassified 46,XX DSD males all died owing to congenital malformations ('unspecified anomalies of the heart', 'other anomalies of the lung' and 'unilateral renal agenesis'). Two 46,XX DSD males died from pneumonia at the ages of 68 and 83 years.

The overall mortality in 46,XX DSD males was insignificantly increased compared to controls (HR = 1.9, 95% CI: 0.8–4.2). Excluding 46,XX DSD males and controls dying at an age of 0 years no significant difference in mortality was observed as well (HR = 0.6, 95% CI: 0.2–2.5).

Socioeconomic parameters

The incidence of achieving a bachelor's degree was significantly decreased in 46,XX DSD males compared to controls (HR = 0.1, 95% CI: 0.0–0.9), with only one 46,XX DSD male being censored achieving a bachelor's degree.

There was no significant difference in the incidence of retirement comparing 46,XX DSD males and controls (HR = 0.6, 95% CI:

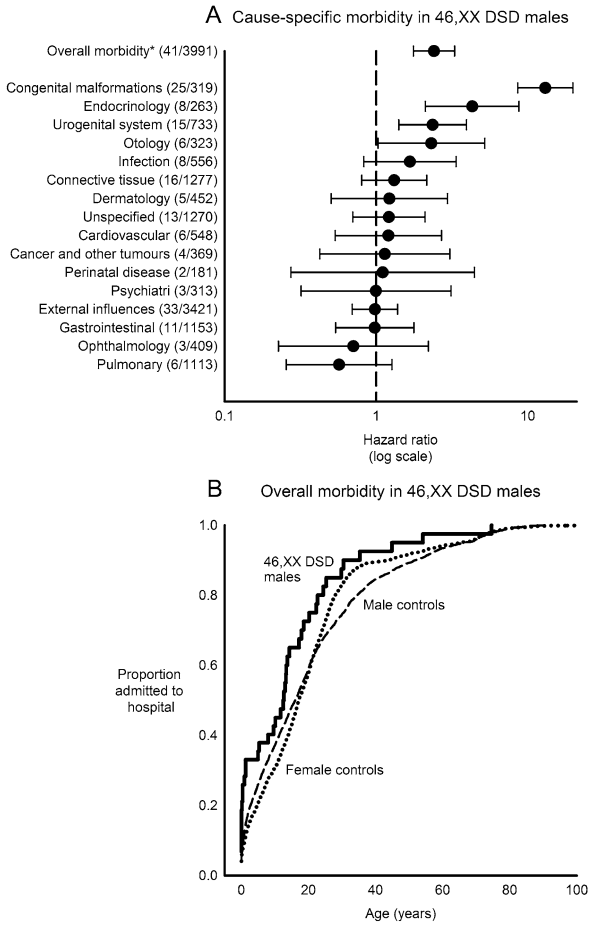


Figure 3 Morbidity in 46,XX DSD males. (**A**) Overall morbidity and cause-specific morbidity in 46,XX DSD males compared to male background population controls. *HR of first registered hospital admission due to any diagnosis. Only ICD chapters with at least one registration for both 46,XX DSD males and controls are shown. In parentheses: Numbers of 46,XX DSD males/male controls with at least one registration. All HRs are unadjusted for socioeconomic status. (**B**) Proportion of 46,XX DSD males (bold line), male controls (dashed line) and female controls (dotted line) having their first registered hospital admission.

0.2–2.0), nor was there a significant difference in the incidence of cohabitation comparing 46,XX DSD males and controls (HR = 0.7, 95% CI: 0.4–1.0). The median age at first cohabitation was 24.8 (range: 20.8–57.9) years in 46,XX DSD males and 24.2 (range:18.0–65.9) years in controls ($P = 0.02$) (Fig. 4A).

The incidence of parenthood was significantly decreased in 46,XX DSD males compared to controls (HR = 0.4, 95% CI: 0.2–0.7) (Fig. 4B), and the median age at parenthood was 34 (range: 27–46) years in 46,XX DSD males and 28 (range: 15–48) years in controls ($P = 0.0001$).

Income was significantly increased in 46,XX DSD males compared to controls in the age groups between 20 and 29 years, whereas it was significantly decreased in the age groups between 45 and 54 years (Fig. 4C).

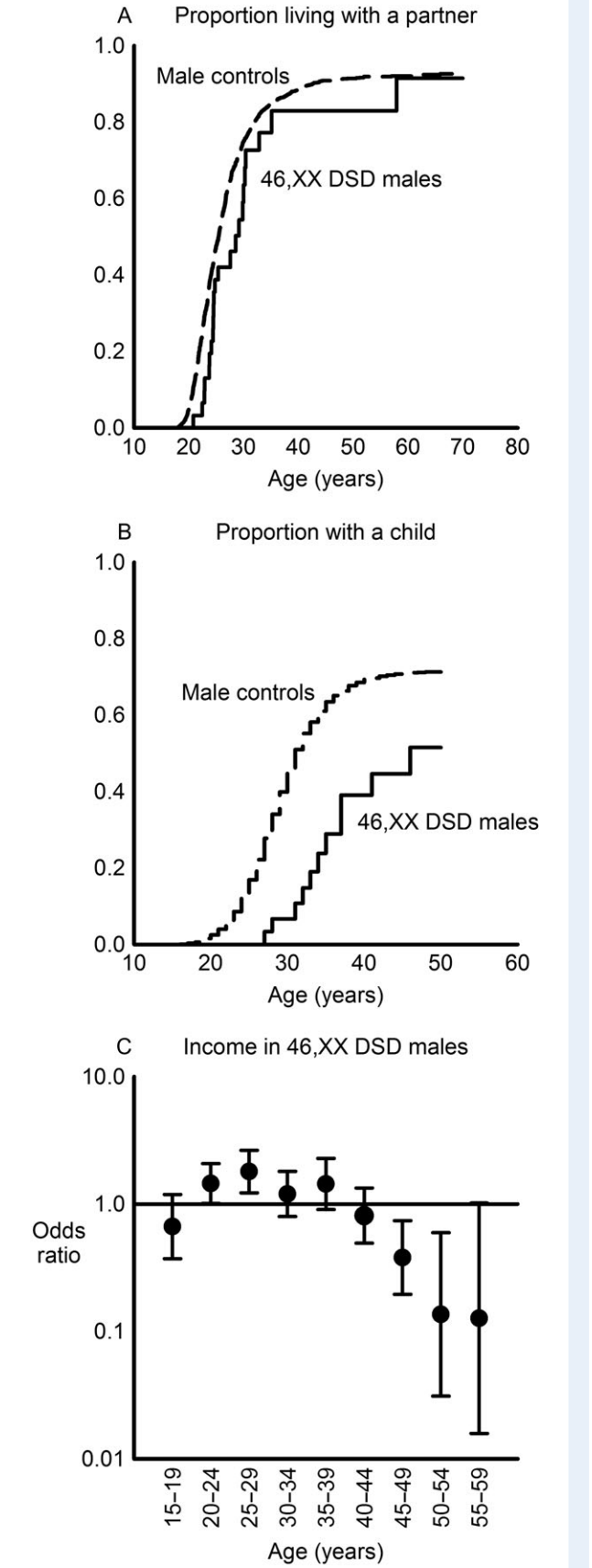
Discussion

In this nationwide study including all males with a verified diagnosis of 46,XX DSD we show that 3.5–4.7 males per 100 000 newborn males were diagnosed as 46,XX DSD males. Diagnosis was made throughout life with infertility being the most frequent reason for diagnosis. In comparison with an age-matched male background population, a significantly increased overall morbidity was identified in 46,XX DSD males. Further, these novel data show an inferior socioeconomic profile in 46,XX DSD males with poorer education and a reduced long-term income as well as a decreased frequency of parenthood despite no substantial difference in the incidence of cohabitation between 46, XX DSD males and the male background population.

The prevalence of diagnosed 46,XX DSD males in the birth cohorts observed during 1908–2015 varied widely with a substantial increase during 1961–1990, reaching a maximum during the 15 year period 1976–1990. Karyotyping as a diagnostic procedure did not become available in Denmark before 1960, explaining the low prevalence of diagnosed 46,XX DSD males in the birth cohorts until this point in time. The low prevalence of diagnosed 46,XX DSD males in the birth cohorts from 1991 and onwards likely reflects that a number of individuals are awaiting diagnosis owing to the often delayed clinical presentation of the 46,XX DSD male phenotype. Elective abortions following a prenatal diagnosis could theoretically also cause the decrease of diagnosed 46,XX DSD males in the recent years, but we consider this unlikely. Therefore, we consider the prevalence of 3.5–4.7 diagnosed 46,XX DSD males per 100 000 newborn males during 1961–1990/1976–1990 as the 'true' prevalence of diagnosed 46,XX DSD males in Denmark.

Previously, the prevalence of testicular DSD has been reported as approximately four per 100 000 newborn males (de la Chapelle, 1981). We pooled data from a number of cytogenetic surveys and estimated a prevalence of 6.7 (95% CI: 2.9–13.2) 46,XX DSD males per 100 000 newborn males (eight 46,XX DSD males among 119 523 newborn males) (Maclean *et al.*, 1964; Lubs and Ruddle, 1970; de la Chapelle, 1972; Friedrich and Nielsen, 1973; Bochkov *et al.*, 1974; Jacobs *et al.*, 1974; Hamerton *et al.*, 1975; Nielsen and Sillesen, 1975; Goad *et al.*, 1976; Hansteen *et al.*, 1982; Nielsen and Wohlert, 1990; Maeda *et al.*, 1991). It must be stressed that the prevalence estimates in the present study are conservative estimates as 15 patients were excluded from the analysis as their 46,XX DSD diagnosis could not be verified (Fig. 1). However, including individuals without a verified 46,XX DSD diagnosis who were born during 1961–1990 or 1976–1990, the corresponding estimates of prevalence were 3.9 and 5.0 46,XX DSD males per 100 000 newborn males, respectively. Thus, it is possible that non-diagnosis of 46, XX DSD males exists – a phenomenon which is also known from studies on sex chromosome DSD conditions such as Turner and Klinefelter syndromes (Bojesen *et al.*, 2003; Stochholm *et al.*, 2006). In contrast, we reported a high diagnostic rate in females with 46,XY DSD in a recent study, perhaps illustrating that their phenotype is more likely to lead to a diagnosis (Berglund *et al.*, 2016). The increasing age at diagnosis of males with testicular DSD, as well as the overall increase in the prevalence of diagnosed 46,XX DSD males during the observation period, suggests that the detection rate of 46,XX DSD males may increase and reflects catch-up of patients that previously were undiagnosed.

Infertility was the most common reason for diagnosis. It can be speculated whether non-diagnosed 46,XX DSD males have a more



severe phenotype with hypogonadism reducing their chances of cohabitation, directly or indirectly, and as a consequence reducing their chances of referral to fertility clinics and subsequent diagnosis. On the other hand, a less severe phenotype in the non-diagnosed 46,XX DSD males may also result in undiagnosed cases.

Overall, 46,XX DSD males had an increased morbidity compared to controls, and it was significantly increased in the diagnostic groups of congenital malformations and endocrine and urogenital system diseases. The increased morbidity in these diagnostic groups was expected as they encompass either the diagnoses of sex chromosome disorders or the diagnoses of the distinctive traits of 46,XX DSD males. Considering this the overall morbidity analysis was performed with exclusion of these diagnostic groups, and encouragingly this led to an estimate where no difference in morbidity was observed between 46,XX DSD males and controls.

Testicular DSD is often associated with hypergonadotrophic hypogonadism. Hypogonadism is, besides loss of libido and vigor, and a risk of depression (Bhasin and Basaria, 2011), related to a negatively altered metabolic profile leading to an unfavorable body composition with increased visceral fat and decreased muscle mass, and an increased risk of the metabolic syndrome, type two diabetes and ischemic vascular diseases. Furthermore, hypogonadism is associated with reduced bone density and increased fracture risk (Ding *et al.*, 2006; Corona *et al.*, 2011). Therefore, it was surprising that no increased risk of either type two diabetes or osteoporosis was observed in the present study. We have previously reported a four-fold increased risk of type two diabetes and an increased risk of osteoporosis among males with Klinefelter syndrome in a similar study setting (Bojesen *et al.*, 2006). We also did not detect an increased risk of ischemic vascular diseases, albeit half of the 46,XX DSD males within the cardiovascular diagnosis group had a diagnosis of ischemic heart disease. This may be related to a lack of statistical power. Additionally, no records of breast cancer were observed, although breast cancer has been reported in 46,XX DSD males (Giammarini *et al.*, 1980; Decker *et al.*, 1982; Hado *et al.*, 2003).

Adequate testosterone replacement therapy can ensure proper masculinization with normal development of bone and muscle mass, and testosterone treatment may thus diminish the risk of diseases associated with hypogonadism. In the present study, 73% were receiving testosterone from a median age of 19 years, which might explain both the lack of increased occurrence of diseases related to hypogonadism as well as the similarity in overall morbidity when comparing 46,XX DSD males and controls without inclusion of diagnoses related to congenital malformations and endocrine and urogenital system diseases in the analysis. However, we cannot rule out that since the present population of 46,XX DSD males is comparatively young, being primarily born during 1961–1990, they may not have reached an age, where the consequences of hypogonadism

Figure 4 Socioeconomic outcome parameters in 46,XX DSD males and male controls. (**A**) Proportion of persons living with a partner, (**B**) proportion of persons becoming fathers, (**C**) odds ratios of income above the median in 46,XX DSD males versus controls in 5-year intervals. All retired persons are excluded from first year of retirement and onwards.

have become manifest. Additionally, the study cohort is rather small, even though it is to our knowledge one of the largest cohorts of 46,XX DSD males ever studied.

Mortality was insignificantly increased in 46,XX DSD males compared to controls, and excluding neonatal deaths (4/6) mortality was insignificantly decreased. This may indicate that mortality in 46,XX DSD males is similar to controls, at least if the neonatal period is survived. However, it must be stressed that three of the four observed neonatal deaths were observed in unclassified 46,XX DSD males, and as such we cannot extend this consideration to any specific subgroup of 46,XX DSD males.

Generally, 46,XX DSD males are reported as having a normal neurocognitive status (de la Chapelle, 1981; Delot *et al.*, 1993). In the present study there was a strong association between being a 46,XX DSD male and poor educational achievement. In fact, only one 46,XX DSD male was censored with an achieved bachelor degree. This finding may substantiate the few previous reports that actually suggest some neurocognitive impairment in 46,XX DSD males (LaFranchi *et al.*, 1980; Van Dyke *et al.*, 1991; Bayramov *et al.*, 2015). Income differed during life, with 46,XX DSD males having an increased income in the younger years and a reduced income in the older years. While studying, most Danish students receive study grants, yielding a lower income than even low-income jobs. As relatively more controls than 46,XX DSD males did study, the increased income in 46,XX DSD males in the younger years may well reflect relative more 46,XX DSD males being on the labor market during that period. The reduced income in 46,XX DSD males in the older age groups is most likely a consequence of reduced educational level with subsequent lower profiled jobs and thus lower salary. The lower income in the older age groups could however also be considered as being related to earlier retirement of 46,XX DSD males than controls. However, this was not the case, as no difference in retirement was observed comparing 46,XX DSD males and controls.

Studies of both Klinefelter and Turner patients have revealed lower frequencies of cohabitation (Bojesen *et al.*, 2011; Stochholm *et al.*, 2012). As self-esteem and a person's expectations could be influenced by the 46,XX DSD diagnosis, we did expect similar findings in the present study. Encouragingly, no significant difference in cohabitation was observed between 46,XX DSD males and controls, but the age at first cohabitation was higher than in controls. As expected the incidence of becoming a father was significantly reduced.

46,XX DSD males with CAH differ from 46,XX DSD males with testicular DSD especially taking the adrenal insufficiency into account. Theoretically, they also have the opportunity to achieve pregnancy as ovaries and Müllerian structures are present. Here, however, we aim at describing phenotypic males with a female karyotype. Interpreting the results of the present study it must be stressed that morbidity, mortality and socioeconomic parameters were studied for the combined cohort of 46,XX DSD males. Thus, conclusions are drawn for 46,XX DSD males as a whole and not for specific subgroups of 46,XX DSD males, although individuals with 46,XX testicular DSD clearly comprise the largest group.

The strengths of this study are the nationwide approach in a uniform public health care system with complete follow-up combined with a thorough review of the majority of patient files and the close matching with the male background population controls. Further, we only include 46,XX DSD males whom we are certain fulfilled the diagnostic criteria.

The limitations of the study are the exclusion of potential 46,XX DSD males in whom lack of data made us unable to verify a diagnosis of 46,XX DSD. Additionally, some 46,XX DSD males were included as unclassified 46,XX DSD males as an exact diagnosis could not be determined from the present data. Thus, an effect on the estimates concerning the testicular DSD subgroup of 46,XX DSD males cannot be ruled out. A risk of misclassification must also be considered when interpreting the study results, which is applicable to all epidemiologic studies. Further, as the study included diagnosed 46,XX DSD males only, the conclusions of the study cannot be extended to non-diagnosed 46,XX DSD males.

In summary, the prevalence of diagnosed 46,XX DSD males was 3.5–4.7 per 100 000 newborn males. Overall morbidity was significantly increased in 46,XX DSD males, however, not after exclusion of diagnosis groups including 46,XX DSD related diagnoses. There was no difference in mortality between 46,XX DSD males and the background population. The socioeconomic profile of 46,XX DSD males was poor with a negative association to education, parenthood and long-term income, whereas there was no significant difference in the incidence of cohabitation. Whether these findings are caused by the condition *per se* or current treatment regimens remains unclear.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

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Authors' roles

A.B., T.H.J. and J.F. did the medical record review. A.B. and M.H.V. retrieved the data from Statistics Denmark. A.B. did the statistical analyses with support from K.S. A.B. drafted the manuscript. K.S. and C.H.G. designed the study and contributed to interpretation of data. All authors contributed with critical revision of the manuscript.

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Conflict of interest

A.B., T.H.J., K.S., J.F., L.A., M.H.V., K.M.M. and C.H.G. have nothing to declare.

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