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**ORIGINAL ARTICLE** ***Reproductive epidemiology***

# Only a minority of sex chromosome abnormalities are detected by a national prenatal screening program for Down syndrome

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**STUDY QUESTION:** How does a national prenatal screening program for Down syndrome (DS) perform in detecting sex chromosome abnormalities (SCAs)—Turner syndrome (TS), Klinefelter syndrome, 47,XXX and 47,XYY syndromes.

**SUMMARY ANSWER:** The SCA detection rate resulting from DS screening was below 50% for all four groups of SCAs.

**WHAT IS KNOWN ALREADY:** The detection rates of SCAs are higher in countries with DS screening. TS is associated with greater nuchal translucency (NT) and lower pregnancy-associated plasma protein-A (PAPP-A). However, specific detection rates of SCAs using prenatal DS screening have not been determined. No clear trend in PAPP-A, free beta human chorionic gonadotropin (β-hCG) and NT has been found in the remaining SCAs. Several lines of inquiry suggest that it would be advantageous for individuals with SCA to be detected early in life, leading to prevention or treatment of accompanying conditions. There is limited information about pre- and perinatal status that distinguishes SCA embryogenesis from normal fetal development.

**STUDY DESIGN, SIZE, DURATION:** A register-based case–control study from the Danish Central Cytogenetic Register (DCCR), cross-linked with the Danish Fetal Medicine Database (DFMD), was performed from 2008 to 2012. Groups of SCAs were compared with DS and then matched with non-SCA controls to assess differences between these groups in prenatal markers and birth outcomes.

**PARTICIPANTS/MATERIALS, SETTING, METHODS:** We included cases with prenatal and post-natal SCA karyotypes ($n = 213$), DS ($n = 802$) and 168 056 controls. We screened 275 037 individuals examined prenatally. We retrieved information regarding maternal age, NT, β-hCG and PAPP-A, as well as details regarding maternal and newborn characteristics.

**MAIN RESULTS AND THE ROLE OF CHANCE:** The DS screening procedure detected 87 per 100 000 TS (42% of expected), 19 per 100 000 Klinefelter syndrome (13% of expected), 16 per 100 000 47,XXX (16% of cases) and 5 per 100 000 47,XYY (5% of expected) SCAs, with an overall detection rate of 27%. Compared with controls, all four SCA groups showed significantly higher NT and lower PAPP-A compared with controls (all $P < 0.01$) and similar to DS. The legal abortion rate was high for all four syndromes (47,XXX: 24%; 47,XYY: 29%; Klinefelter syndrome: 48%, TS: 84%). For SCA fetuses carried to term, only TS fetuses had consistently lower birthweights and placenta weights than non-SCA controls (both $P = 0.0001$). A few SCA cases localized in DCCR could not be found in DFMD ($n = 16$).

**LIMITATIONS, REASON FOR CAUTION:** Controls were matched on sex of the fetus of cases, meaning that all electively aborted fetuses (before week 12) were excluded, possibly reducing the diversity in the control group. We were not able to localize all diagnosed cases of SCA and DS in DFMD. Although these cases were present in DCCR, we were not able to account for the discrepancy. In addition, we suspect that several SCA children have not been diagnosed yet and future post-natal diagnosis of these cases would reduce the diagnostic yield reported here even further.

[†] See supplementary data.

**WIDER IMPLICATIONS OF THE FINDINGS:** The prenatal detection rate is below 50% for all SCAs. The approach used for detecting DS cannot be extended to also include SCAs. In addition, all SCAs have low PAPP-A and increased NT, thus probably reflecting an abnormal embryogenesis. Growth retardation of TS fetuses is if anything more pronounced than previously reported, both when evaluating fetus and placenta.

**STUDY FUNDING/COMPETING INTEREST(S):** This study received support from Aarhus University and the Novo Nordisk Foundation. The authors have no competing interests that may be relevant to the study.

**Key words:** pregnancy termination / chromosomal abnormalities / embryology / epidemiology / sex chromosomes

# Introduction

Between 2004 and 2006, Denmark instituted a free prenatal screening program for Down syndrome (DS), in which over 90% of pregnant women in Denmark participate, making it highly representative of pregnancies in the Danish population (Ekelund *et al.*, 2008). Combining maternal age, ultrasonographic nuchal translucency (NT), maternal serum-free beta human chorionic gonadotropin (β-hCG) and pregnancy-associated plasma protein-A (PAPP-A), this screening detects 80–90% of DS fetuses.

Given its ability to detect DS, this procedure has also been proposed to screen for sex chromosome abnormalities (SCAs) (Vaknin *et al.*, 2008). SCAs are common with an estimated prevalence of 250–400 per 100 000 live births and include Turner syndrome (TS), Klinefelter syndrome, Triple-X syndrome and Double-Y syndrome. Currently, most diagnosed SCA children are not detected until adolescence, if detected at all (Abramsky and Chapple, 1997; Bojesen *et al.*, 2003; Stochholm *et al.*, 2006, 2010a, 2010b, 2010c; Boyd *et al.*, 2011). They can be identified prenatally using amnioscentesis or chorion villus sampling, but these procedures are invasive, costly, and carry an increased risk of spontaneous abortion. Accordingly, these procedures are typically reserved for high-risk pregnancies such as advanced maternal age or suspected DS. Several lines of inquiry suggest that it would be advantageous for individuals with SCA to be detected early in life, leading to prevention or treatment of accompanying conditions (Bondy, 2007; Herlihy *et al.*, 2011; Aksglaede *et al.*, 2012; Groth *et al.*, 2013; Arver *et al.*, 2014; Ibarra-Ramírez *et al.*, 2015). Population-based genetic screening can generally be considered if a condition is an important health problem with a latent early symptomatic stage, has a well-understood natural history, and there exists accepted treatments with associated facilities for providing diagnosis and treatment (Grosse *et al.*, 2009), requirements which we consider are fulfilled for at least TS and KS although formal proof of improved long-term adult outcomes may prove challenging to accumulate due to the rarity of the syndromes.

The potential for DS screening to identify SCA fetuses is suggested by higher DS detection rates being positively correlated with those for SCAs (Boyd *et al.*, 2011), and some individual DS screening markers have been related to certain SCAs (Spencer *et al.*, 2000b; Avgidou *et al.*, 2005), although research on diagnosing SCA fetuses using prenatal DS screening is limited (Vaknin *et al.*, 2008). Post-natal studies report that the detection rates for all SCAs is much lower than the known frequency of SCAs based on large chromosome surveys of newborns, and prenatal SCA research is based on small sample sizes. Thus, the use of large representative samples to compare SCA fetus detection rates with established SCA prevalence would be useful for assessing the diagnosticity of prenatal DS screening for fetal SCAs.

Also limited is comprehensive, empirical information about pre- and perinatal status, with emphasis on NT, β-hCG and PAPP-A, that distinguish SCA embryogenesis, including maternal characteristics, prenatal factors and birth outcomes. This information can help describe the fetal development of SCAs, identify empirical SCA predictors and guide parental decisions once a SCA diagnosis is made.

To address these issues, we examined a large, nationally representative sample of pregnancies to assess the ability of prenatal DS screening to detect TS, Klinefelter syndrome, 47,XXX and 47,XYY syndromes. Identified SCA pregnancies were compared with both DS cases and matched non-SCA controls to determine how they differ in terms of maternal age, prenatal DS screening markers and gestational age and birthweight.

# Materials and Methods

The study is designed as a register-based case–control study with the following sampling procedures.

## Sampling procedures and outcome measures

All SCA diagnosed in Denmark between 2008 and 2012 were identified using the Danish Central Cytogenetic Registry (DCCR) and linked to the Danish Fetal Medicine Database (DFMD). We thus screened 275 037 cases, resulting in a total of 213 identified SCA cases. As controls, we matched 100 non-SCA pregnancies to every SCA case, and we also studied 722 cases with DS (see Supplementary data).

### *Statistical analyses*

Descriptive analyses were conducted to examine the gestational development of pregnancies. For each SCA syndrome, detection rates were calculated by comparing the number of SCAs identified in the current sample with known prevalence rates derived from the research literature. Pregnancy complication rates between cases and controls were compared with the $\chi^2$-test. Within each SCA syndrome, differences in prenatal and perinatal outcomes between SCAs and matched controls were assessed using the Mann–Whitney test. Smoking status was tested with Fisher's exact test. All results are shown with 95% confidence intervals (CI) or corresponding ranges, with a significance level of $P < 0.05$. Receiver operating characteristic (ROC) curves were produced for DS risk score, because this variable most significantly discriminated between SCAs and controls. All analyses were conducted using the Stata 12.1 statistical software package (Stata Corp., College Station, TX, USA).

# Results

We screened 275 037 cases (134 768 females and 140 269 males), resulting in a total of 199 identified SCA cases. Of these 199 SCA cases (172 prenatal cases and 27 post-natal cases) identified in DCCR, 172 were present in the DFMD, which constitute the final study group (Supplementary data, Table SI). In addition, we studied 722 and 80

pre- and post-natally diagnosed cases, respectively, with DS and 100 random matched controls for every SCA or DS.

## TS

The DS screening identified TS in 87 out of 100 000 female fetuses. Combined, given an expected rate of 209 TS cases per 100 000, this represents a detection rate of 42% (Table I and Fig. 1); for the 45,X karyotype the expected rate is 130 per 100 000 and thus the detection rate is 43%, and for all other TS karyotypes, the expected rate is 80 per 100 000 and the detection rate is 38%.

Maternal age in TS cases ranged from 22 to 42 years, with a median age of 32. Mothers with 45,X pregnancies were significantly younger than those with other TS karyotypes ($P = 0.03$) (Supplementary data, Table SII). Significantly more mothers with TS pregnancies had another ethnicity than Caucasian ($P = 0.01$). There was no difference in the maternal BMI, height, parity or smoking status between case and control mothers in all karyotype groups, including TS (Supplementary data, Table SII).

**Table I Detection rates for sex chromosome abnormalities and DS pregnancies using prenatal DS screening.**

| Year 2008–2012 | TS | 45,X | Other TS karyotypes | 47,XXY syndrome | 47,XXX syndrome | 47,XYY syndrome | DS |
|---|---|---|---|---|---|---|---|
| Prenatally detected | 117 | 76 | 41 | 27 | 21 | 7 | 722 |
| Attended screening | 275 037 | 275 037 | 275 037 | 275 037 | 275 037 | 275 037 | 275 037 |
| Female fetuses | 134 768 (49%) | 134 768 (49%) | 134 768 (49%) | – | 134 768 (49%) | – | |
| Male fetuses | – | – | – | 140 269 (51%) | – | 140 269 (51%) | |
| Detected prevalence | 87 per 100 000 | 56 per 100 000 | 30 per 100 000 | 19 per 100 000 | 16 per 100 000 | 5 per 100 000 | 263 per 100 000 |
| Expected prevalence | 209 per 100 000 (Gravholt *et al.*, 1996) | 130 per 100 000 (Gravholt *et al.*, 1996) | 80 per 100 000 (Gravholt *et al.*, 1996) | 153 per 100 000 (Bojesen *et al.*, 2003) | 100 per 100 000 (Tartaglia *et al.*, 2010) | 100 per 100 000 (Stochholm *et al.*, 2010a, 2012b) | 308 per 100 000 (Snijders *et al.*, 1999) |
| Detected/expected | 42% | 43% | 38% | 13% | 16% | 5% | 85% |

Observed and expected prevalence of sex chromosome abnormalities per 100 000 screened fetuses, 2008–2012. The expected prenatal prevalence is found in the literature (Gravholt *et al.*, 1996; Bojesen *et al.*, 2003; Stochholm *et al.*, 2010a, 2010c, 2012b; Tartaglia *et al.*, 2010). The column with TS is a total of 45,X syndrome and other TS karyotypes.

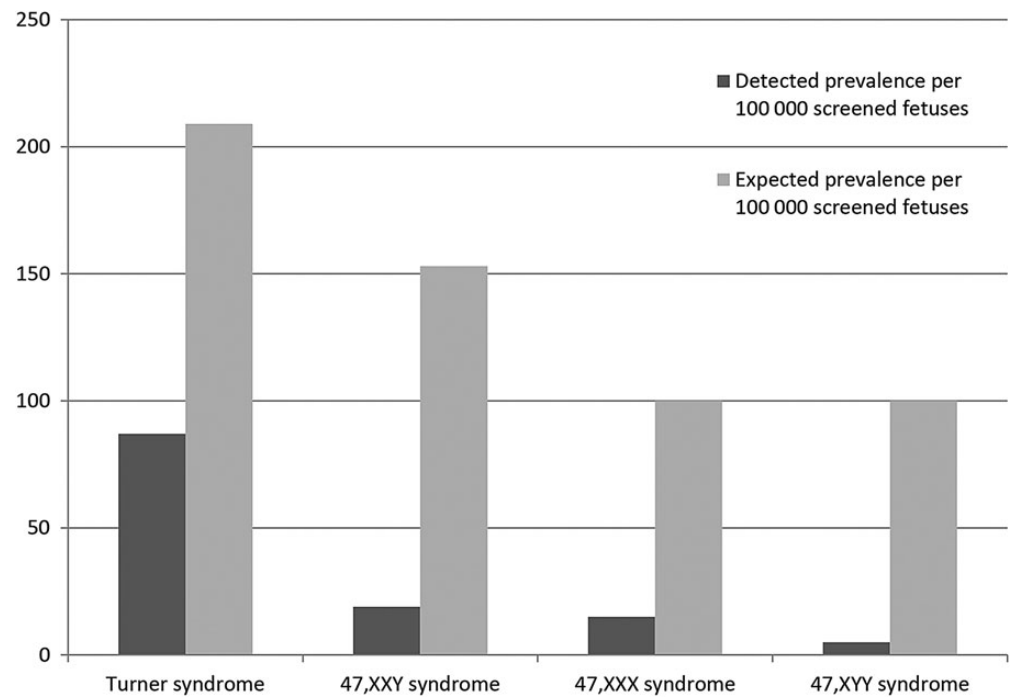


**Figure 1** Detected versus expected prevalence of sex chromosome abnormalities. Observed and expected prevalence of sex chromosome abnormalities per 100 000 screened fetuses, 2008–2012. The expected prenatal prevalence is found in the previous literature (Gravholt *et al.*, 1996; Bojesen *et al.*, 2003; Stochholm *et al.*, 2010a, 2010c, 2012b; Tartaglia *et al.*, 2010). The column with TS is a total of 45,X syndrome, 45,X/46,XX and other TS karyotypes.

The median 45,X NT level was over 2.5 times greater than that for DS cases, and over four times greater than other TS cases and controls (Supplementary data, Table SIII), reflecting that 57 (83%) of 45,X pregnancies—versus 5 (12%) of other TS karyotypes—had a NT in the 99th percentile for control cases (Fig. 2A). Similarly, NT levels were significantly higher in prenatally diagnosed TS fetuses than those diagnosed post-natally (8.0 mm [1.0–14.0 mm] versus 1.6 mm [1.3–2.1 mm], $P = 0.008$), and no post-natally diagnosed TS cases were in the 99th percentile for controls (Supplementary data, Table SIV).

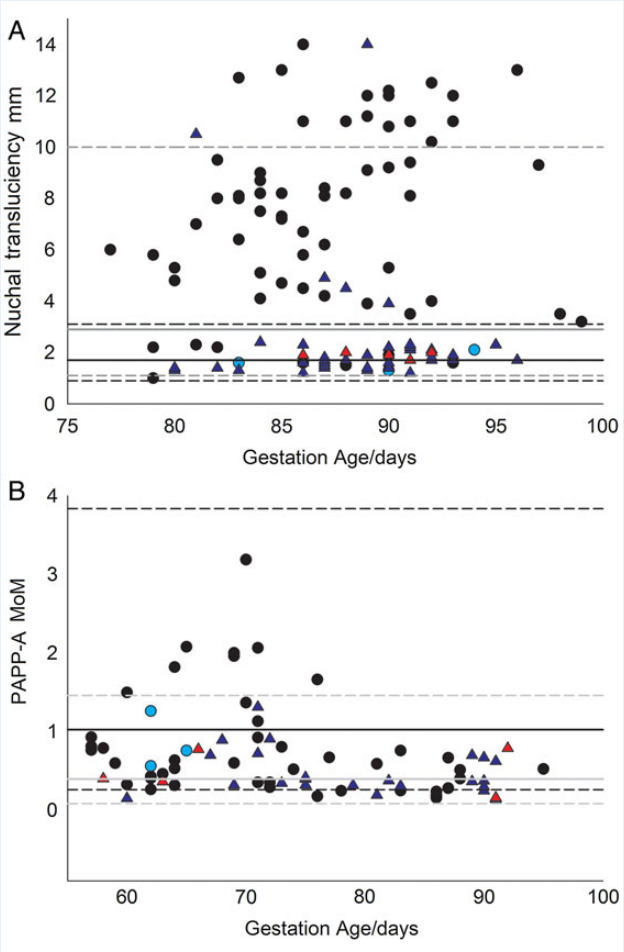


**Figure 2** Gestational age, NT and PAPP-A in TS. (**A**) Black dots indicate prenatal diagnosed 45,X. Cyan dots indicate post-natal diagnosed 45,X. Blue triangles indicate prenatal diagnosed other Turner syndrome karyotypes. Red triangles indicate post-natal diagnosed other Turner syndrome karyotypes. Black solid line indicates the median NT in controls and black dotted lines indicate the 99% prediction interval. Gray solid line indicates the median NT in DS and gray dotted lines indicate the 99% prediction interval. (**B**) Black dots indicate prenatal diagnosed 45,X. Cyan dots indicate post-natal diagnosed 45,X. Blue triangles indicate prenatal diagnosed other Turner syndrome karyotypes. Red triangles indicate post-natal diagnosed other Turner syndrome karyotypes. Black solid line indicates the median PAPP-A in controls and black dotted lines indicate the 99% prediction interval. Gray solid line indicates the median PAPP-A in DS and gray dotted lines indicate the 99% prediction interval. MoM, multiples of the median.

The median PAPP-A level in TS pregnancies was similar to that in DS cases, and ~50% lower than those for controls ($P < 0.001$) (Fig. 2B). In addition, PAPP-A levels in six (10%) of 45,X TS cases—and five (14%) of other TS karyotypes—were in the first percentile for their respective controls (Fig. 2B), while β-hCG levels were comparable to controls. The overall risk of DS based on maternal age, fetal NT, free beta-hCG and PAPP-A was >1:300 in 62 (91.2%) of 45,X TS cases, and in 26 (60.5%) of the other TS karyotypes. A ROC curve analysis of how the DS risk algorithm behaves in detecting 45,X TS can be seen in Fig. 3A, and for other TS karyotypes (Supplementary data, Fig. S1).

## Klinefelter syndrome

Klinefelter syndrome was identified prenatally at a rate of 19 of 100 000 screened male fetuses. With a population prevalence of 153 per 100 000 cases, this represents a detection rate of 13% (Table I and Fig. 1). Maternal age for Klinefelter syndrome cases ranged from 23 to 47 years, with an average of 36 years, being significantly older than their matched controls (Supplementary data, Table SII).

The median NT level among Klinefelter syndrome pregnancies was significantly higher than in controls ($P < 0.001$), with six (26%) of Klinefelter syndrome cases being in the 99th percentile of control NT levels (Supplementary data, Table SIII and Fig. 4A). Both β-hCG and PAPP-A levels were ~50% lower in Klinefelter syndrome than in controls ($P < 0.001$), with 2 (10%) of Klinefelter syndrome cases exhibiting PAPP-A levels in the first percentile of control pregnancies (Fig. 4B), and 2 (10%) of Klinefelter syndrome cases with β-hCG levels in the first percentile. The estimated DS risk was >1:300 in 17 (73.9%) of these Klinefelter syndrome cases (Fig. 3B).

## 47,XXX syndrome

Prenatal DS screening identified 47,XXX in 16 of 100 000 cases, which represents a detection rate of 16% (Table I and Fig. 1). Maternal age for 47,XXX cases was significantly higher than among controls (Supplementary data, Table SII).

The median NT level in the prenatally diagnosed 47,XXX pregnancies was ~50% higher than in controls, and four (24%) 47,XXX cases had NT levels in the 99th percentile for NT among controls (Supplementary data, Table SIII and Fig. 4A). 47,XXX pregnancies had marginally lower levels of β-hCG than control cases ($P = 0.05$), and 50% lower PAPP-A levels than those of controls ($P < 0.001$) (Fig. 4B). Two (13%) of 47,XXX cases had β-hCG levels in the first percentile. Thirteen (76.5%) of 47,XXX pregnancies had a DS risk of >1:300 (Supplementary data, Fig. S2B).

## 47,XYY syndrome

The rate of 47,XYY cases was 5 per 100 000 screened male fetuses, which represents a detection rate of 5% (Table I and Fig. 1). Maternal age for 47,XYY cases was similar to their controls (Supplementary data, Table SII).

The median NT was greater among 47,XYY pregnancies than in controls ($P < 0.001$) (Supplementary data, Table SIII and Fig. 4A). Three (27%) of the 47,XYY case levels were in the 99th percentile for controls, and all three cases were diagnosed prenatally. The median PAPP-A level in 47,XYY pregnancies was lower than in controls ($P < 0.01$) (Fig. 4B). The estimated risk of DS reached 1:300 in 6 (54.5%) of 47,XYY cases (Supplementary data, Fig. S2C).

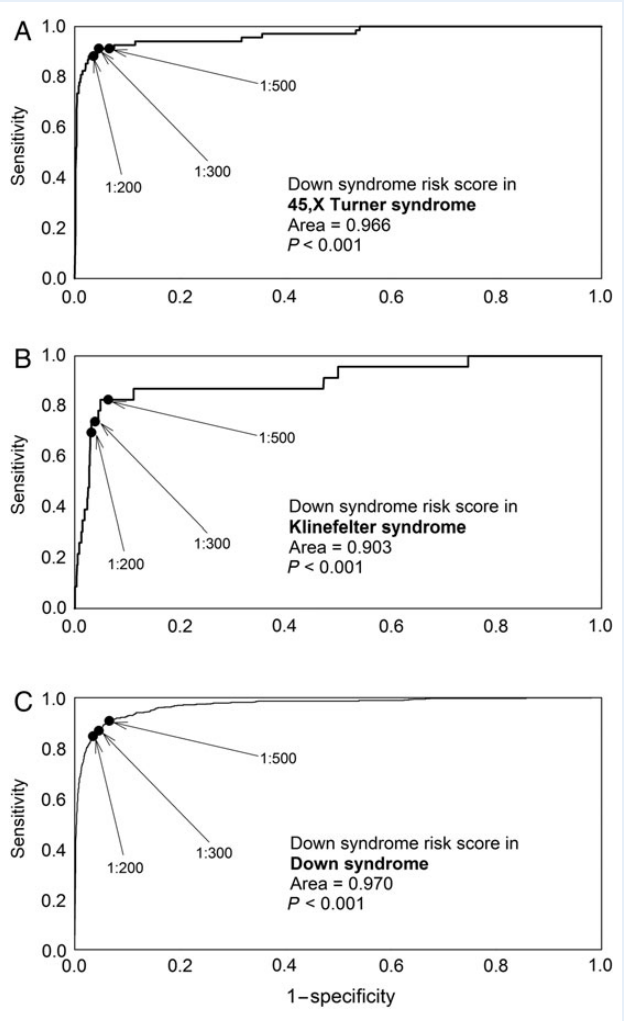


**Figure 3** ROC curves for 45,X TS syndrome, Klinefelter syndrome and DS. ROC curves for 45,X Turner syndrome (**A**), Klinefelter syndrome (**B**) and DS (**C**), using the DS risk score. Arrows indicate a probability of DS of more than 1:200, 1:300 and 1:500, respectively. In each graph is indicated the area under the curve and a *P*-value of whether this distribution is significant.

## DS validation sample

The rate of identified DS cases in the current sample was 263 per 100 000. Given the specific age distribution of the mothers in our study population, we calculated that the prevalence of DS would be 308 per 100 000 (Snijders *et al.*, 1999), and thus the detection rate was 85% (Table I). As expected DS fetuses relative to their controls had higher levels of NT and β-hCG, and lower levels of PAPP-A ($P < 0.001$) (Supplementary data, Table SIII).

## Prenatally detected structural congenital abnormalities

Because many SCA pregnancies were terminated before the anomaly scan in Week 19 (Supplementary data, Table SV), limited data are available on structural abnormalities. Most of this information comes either from pregnancies that were continued despite an abnormal karyotype, or those with abnormalities so severe they were apparent from ultrasound scans prior to 19 weeks of gestation. Two cases of 45,X with severe cardiac anomalies also had an enlarged NT (6.7 and 13.0 mm). Data are too limited to verify a trend between the size of the NT and severe anomalies.

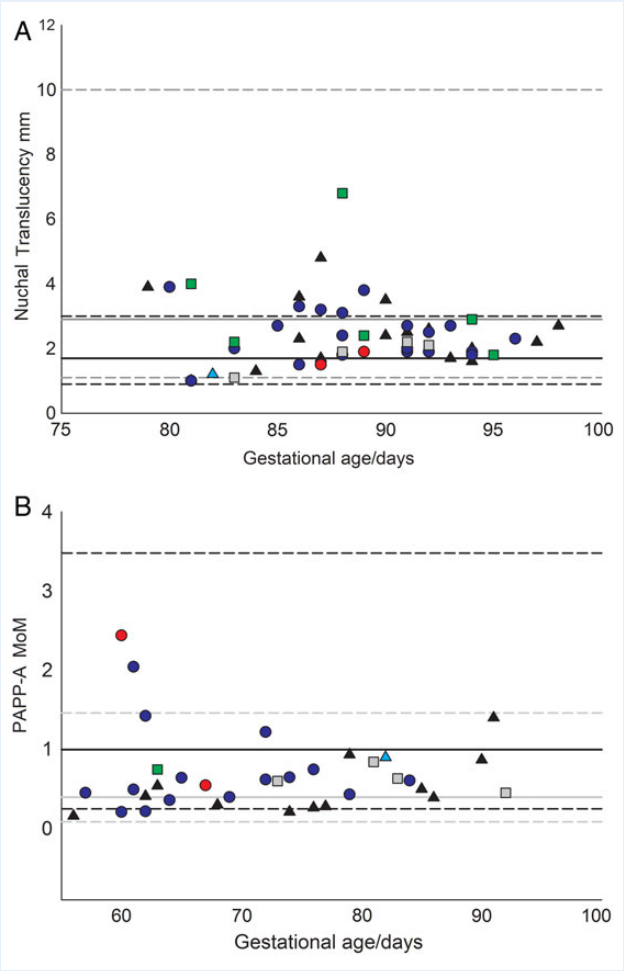


**Figure 4** Gestational age, NT and PAPP-A in 47,XXY, 47,XXX and 47,XYY syndromes. (**A**) Blue dots indicate prenatal diagnosed 47,XXY (Klinefelter syndrome). Red dots indicate post-natal diagnosed 47,XXY (Klinefelter syndrome). Black triangles indicate prenatal diagnosed 47,XXX syndrome. Cyan triangles indicate post-natal diagnosed 47,XXX syndrome. Green squares indicate prenatal diagnosed 47,XYY syndrome. Gray squares indicate post-natal diagnosed 47,XYY syndrome. Black solid line indicates the median NT in controls and black dotted lines indicate the 99% prediction interval. Gray solid line indicates the median NT in DS and gray dotted lines indicate the 99% prediction interval. (**B**) Blue dots indicate prenatal diagnosed 47,XXY (Klinefelter syndrome). Red dots indicate post-natal diagnosed 47,XXY (Klinefelter syndrome). Black triangles indicate prenatal diagnosed 47,XXX syndrome. Cyan triangles indicate post-natal diagnosed 47,XXX syndrome. Green squares indicate prenatal diagnosed 47,XYY syndrome. Gray squares indicate post-natal diagnosed 47,XYY syndrome. Black solid line indicates the median PAPP-A in controls and black dotted lines indicate the 99% prediction interval. Gray solid line indicates the median PAPP-A in DS and gray dotted lines indicate the 99% prediction interval. MoM, multiples of the median.

**Table II Outcome of pregnancy with prenatally diagnosed sex chromosome abnormalities.**

| | Detected prenatally | Induced abortion, *n* (%) | GA at induced abortion, Median (range) | Spontaneous abortion, *n* (%) | Stillborn, *n* (%) | Live born, *n* (%) |
|---|---|---|---|---|---|---|
| 45,X syndrome | 76 | 64 (84) | 99 (94–100) | 3 (4) | 2 (3) | 7 (9) |
| Other TS karyotypes | 41 | 17 (42) | 116 (95, 121) | 4 (10) | 1 (2) | 19 (46) |
| 47,XXY syndrome | 27 | 13 (48) | 99 (92, 107) | 1 (4) | – | 13 (48) |
| 47,XXX syndrome | 21 | 5 (24) | 110 (98, 122) | – | – | 16 (76) |
| 47,XYY syndrome | 7 | 2 (29) | – | 1 (14) | – | 4 (57) |
| Trisomy 21 | 722 | 659 (91) | 98 (97, 98) | 16 (2) | 8 (1) | 39 (6) |

Values are *n* (% of total).
GA, gestational age.

### Pregnancy complications and outcomes

Most DS and 45,X TS pregnancies were terminated (91 and 84%, respectively) (Table II), while the remaining SCAs were terminated in 24–48% of cases (Supplementary data, Fig. S2). Overall, there were nominally fewer complications during pregnancies that were not terminated (placenta insufficiency, placenta previa, abruption, premature rupture of membranes) in SCAs compared with controls ($P < 0.01$) (results not shown). Preterm labor was more common among 47,XXX than matched controls ($P = 0.01$) and among other TS karyotypes, with 6 out of 23 delivered preterm (26%) compared with 247 out of 4084 controls (6%) ($P = 0.001$). No difference was seen in the other SCAs. We found a lower median birthweight in 45,X girls and in other TS karyotypes compared with controls (Supplementary data, Table SVI). Placental weight was ~33% lower in TS 45,X term pregnancies, being more pronounced than for DS (Supplementary data, Table SVI). In addition, Caesarian sections were performed less frequently in the SCAs than in non-SCA controls.

For a more detailed analysis, we examined levels of the most prominent perinatal outcome (NT) for the most prevalent SCA (TS) across each pregnancy outcome. As seen in Supplementary data, Fig. S3, while the frequency of 45,X cases was much higher than other TS karyotypes (and the vast majority of TS cases resulted in induced abortions), the range of NT values for each group was overlapping.

## Discussion

In this large, nationally representative sample, prenatal SCA fetus detection rates using the current DS screening algorithm ranged from 5 to 42%, with an overall detection rate of <30%. This indicates that although the DS screening procedure is successful for identifying DS pregnancies, it is not an effective means of prenatal SCA fetus detection.

This lack of specificity of the DS screening algorithm may be due to DS screening factors not mapping onto SCAs. For example, while prenatal β-hCG levels are significantly elevated in DS relative to controls, they are unrelated to TS and 47,XYY cases and are actually *lower* in Klinefelter syndrome and 47,XXX pregnancies. Low SCA fetus detection rates may also reflect the fact that while many differences in screening factors between SCA fetus and matched controls were statistically significant, they were not necessarily clinically relevant, for most SCA cases fell well within normal ranges for DS screening markers during pregnancy, especially for cases diagnosed post-natally. Finally, the variation in DS markers among SCA fetus pregnancies typically ranged from normal to either increased or decreased levels, making discriminatory thresholds difficult to identify. Taken together, these results suggest that future prenatal SCA fetus screening algorithms, if deemed relevant, should focus on the most clinically relevant DS predictors and consider additional potential prenatal SCA markers, such as sperm count and motility in the fathers (Ferguson *et al.*, 2007). Interestingly, ROC curve analysis shows that, especially for the detected 45,X TS cases, the DS detection algorithm performs almost as well as for DS. However, this good performance has to be evaluated on the backdrop of a detection rate of only 43% of the expected 45,X TS fetuses compared with a detection rate of 85% for DS—in other words, this good performance could well be spoiled when all 45,X TS cases are detected post-natally. For example, it can be appreciated in Fig. 2A that the post-natally detected 45,X cases have a normal NT, explaining why they were not detected during prenatal life.

SCA fetuses differ from non-SCA pregnancies in relation to prenatal markers. SCA fetuses showed consistently greater NT and lower PAPP-A levels, and the growth retardation of TS fetuses was more pronounced than previously reported, both in terms of placental and fetal development. Overall, we report a lower PAPP-A, birthweight and placenta weight and a higher NT, confirming previous observations that prenatally diagnosed 45,X cases are associated with increased fetal NT (Sebire *et al.*, 1998; Spencer *et al.*, 2000b; Avgidou *et al.*, 2005; Maymon *et al.*, 2005; Vaknin *et al.*, 2008). For 47,XXY syndrome, we observed a significantly lower value of serum-free β-hCG than controls, similar to that seen in trisomy 13 (Spencer *et al.*, 2000a). Given the small *n* this result may simply reflect type I error, or one of the many interactions known to involve free β-hCG during pregnancy (Cole, 2012).

We corroborated previous findings that PAPP-A is reduced in TS, while free-serum β-hCG remains unchanged in 45,X. Much less is published on other TS karyotypes and we found less increase in NT, but PAPP-A levels were also low, perhaps as an indication of impaired placental function, as also indicated by the lower placental weight.

Previous studies have pooled hyperploid SCA fetuses due to limited study populations, and no individual comparisons are available for Klinefelter syndrome, 47,XXX and 47,XYY karyotypes. Notably, all three karyotype groups had elevated NT, corroborating previous smaller studies (Sebire *et al.*, 1998; Spencer *et al.*, 2000b). Here PAPP-A was reduced by ~50% in all karyotypes, making it the most consistent

finding across SCA groups, suggesting that PAPP-A is a particularly good indicator of not only autosomal chromosome aberrations like DS, but also for SCAs.

The low levels of PAPP-A in TS pregnancies could be involved in both lower birth and placental weights, as well as higher spontaneous abortion rates, all of which may be related to poor placental function (Wisniewski *et al.*, 2007; Hagman *et al.*, 2010). Similar mechanisms could be involved in the nominally lower birthweight of the other SCAs, although genetic factors may also contribute. Unlike previous research, we did not find more complications during labor of the TS children (Hagman *et al.*, 2010). Prior research using human embryonic stem cells suggests that abnormal placental differentiation may be the cause for the increased spontaneous abortion rate in TS (Urbach and Benvenisty, 2009). PAPP-A is reduced in DS pregnancies, but placental mRNA and protein are not down-regulated here, suggesting that post-translational events might be involved. Thus, the situation in TS pregnancies with similar lowering of PAPP-A could arise from a different background—i.e. one with reduced expression of PAPP-A mRNA (and presumably protein levels).

In the current research, TS cases—especially 45,X pregnancies—had the highest SCA-induced abortion rates (84%), which were only marginally lower than the rate for DS cases (91%), but marginally higher than 45,X abortion rates (76%) found in a recent review of 19 studies (Jeon *et al.*, 2012). Klinefelter syndrome was the second most aborted karyotype in the current study (48%), which is lower than the rate (61%) found in the aforementioned review (Jeon *et al.*, 2012). The induced abortion rates among 47,XYY and 47,XXX pregnancies were 29 and 21%, respectively, and while previous data were not available for 47,XYY cases, these results are broadly consistent with prior results for 47,XXX cases, which had abortion rates of 32% (Jeon *et al.*, 2012). The reason for the large variance in induced abortion rates across karotypes in this research is unclear, since morbidity and mortality rates are similar for all four syndromes (Bojesen *et al.*, 2003; Schoemaker *et al.*, 2008; Stochholm *et al.*, 2010a, 2010c). Of course, there may be explanatory differences in parents' *perceived* morbidity or mortality risk when informed that they are expecting a SCA child. Nor were abortion rates associated with NT levels. More likely, we believe, is that differential abortion rates may be due to identifiable phenotypes, which are more pronounced in TS pregnancies than in the other three SCA karotypes. Follow-up studies of adults with SCAs show that there are significant differences in socioeconomic status, which is significantly higher among females with TS than those with other SCAs (Bojesen *et al.*, 2003; Verlinde *et al.*, 2004; Naess *et al.*, 2010; Stochholm *et al.*, 2010c, 2012a, 2012b, 2013; Gould and Bakalov, 2013), making it difficult to explain the large differences in induced abortion rates. We suggest that lack of knowledge concerning all syndromes, perhaps especially of the improved conditions present for treatment of females with TS, influence the counseling performed by health professionals and may influence the choice of parents to abort the fetus or not (Mortensen *et al.*, 2012).

Advanced maternal age was associated with Klinefelter syndrome, 47,XXX and the other TS karyotypes, but not with 45,X TS and 47,XYY. Previous examinations of the link between maternal age and TS risk were inconclusive (Hook, 1981; Ferguson-Smith and Yates, 1984; Bernasconi *et al.*, 1994; Snijders *et al.*, 1995; Gravholt *et al.*, 1996; Forabosco *et al.*, 2009; Hagman *et al.*, 2010), given the small sample sizes. Thus, the current research supports previous studies indicating that advanced maternal age is indeed a risk factor in 47,XXX and Klinefelter syndrome, but not in 47,XYY (Snijders *et al.*, 1995) and 45,X.

Our research highlights that the finding of a SCA fetus prenatally in many cases leads to legal abortion. Since SCA individuals are not mentally retarded and since a formal discussion amongst clinician, politicians and the public have never agreed on the need to find SCA fetuses prenatally and abort cases, this article shows that our current approach may prove to be a double-edged sword, and as such, should facilitate discussions on the future handling of prenatally detected SCA fetuses.

To our knowledge, at time of writing, this study includes the largest cohort of SCAs involving maternal profile, biochemical serum markers, NT and pregnancy outcomes. The study is based on a nationally representative sample, which increases external validity while reducing the potential for ascertainment bias. Finally, the inclusion of post-natally diagnosed syndromes enabled us to examine the association between postnatal phenotypic characteristics and prenatal biological markers.

This study also has a couple of methodological limitations. First, controls were matched on sex of the fetus, meaning that all electively aborted fetuses (before week 12) were excluded, which in turn reduced the diversity in the control group. Finally, we were not able to localize all diagnosed cases of SCA and DS in DFMD. Although these cases were present in DCCR, we were not able to account for the discrepancy.

In conclusion, the national screening program for DS only detects a minority of SCAs. Our findings showed significantly decreased PAPP-A and increased NT in all karyotype groups. Similarly, free β-hCG was lowered and maternal age increased in 47,XXY. Although the prenatal findings were significantly different from the normal, it was not sufficient to detect the expected amount of SCAs.

## Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

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## Authors' roles

All the authors have participated in study design, data collection and interpretation of results and writing and revising the manuscript. Statistical analyses were performed by M.H.V., K.S. and C.H.G.

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## Conflict of interest

All authors declare no support from any organization with competing interests for the submitted work.

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