Hong Kong J Gynaecol Obstet Midwifery 2022;22(2):87-100 | https://doi.org/10.12809/hkjgom.22.2.07

# Pregnancies with positive non-invasive prenatal testing result for sex chromosome abnormalities in a tertiary hospital in Hong Kong

**PL SO[1],** MBBS (HK), MMedSc (Genetic Counselling), MSc (Medical Genetics), FHKCOG, FHKAM (O&G)
**S Lai[1],** BHS (Nursing), M.Soc.Sc (Counselling)
**PP WONG[1],** BSc (Health Studies)
**KH SIONG[1],** MBBS (HK), MSc (Medical Genetics), FHKCOG, FHKAM (O&G)
**HK LEUNG[1],** MBChB, FHKCOG, FHKAM (O&G)
**WK SIN[1],** MBBS (HK), FHKCOG, FHKAM (O&G)
**Anita SY KAN[2],** MBBS (HK), MPH(HK), FRCOG, FHKCOG, FHKAM (O&G)
**HM LUK[3,4],** MBBS (HK), MD(HK), FRCPath(UK), FRCPCH(UK), FRCP(London), FHKCPaed, FHKAM(Paed)
**Ivan FM LO[4],** MBChB, FHKCPaed, FHKAM(Paed)
**KC AU YEUNG[1],** MBBS (HK), FRCOG, FHKCOG, FHKAM (O&G)
**SF WONG[1],** MBBS (HK), FRCOG, FHKCOG, FHKAM (O&G)
[1] Department of Obstetrics and Gynecology, Tuen Mun Hospital
[2] Prenatal Diagnostic Laboratory, Tsan Yuk Hospital
[3] Clinical Genetics Service Unit, Hong Kong Children's Hospital
[4] Clinical Genetic Service, Department of Health, Hong Kong SAR

***Objective:*** To review medical records of pregnant women with positive non-invasive prenatal testing (NIPT) results for sex chromosome abnormalities who attended Tuen Mun hospital between 2015 and 2021. Patient decision after prenatal diagnosis, confirmatory diagnostic testing results, and pregnancy/neonatal outcomes were summarised.
***Methods:*** Medical records of women with abnormal NIPT results for sex chromosome abnormalities who attended Tuen Mun Hospital between January 2015 and December 2021 were retrospectively reviewed.
***Results:*** 56 Chinese women attended our prenatal diagnostic clinic with abnormal NIPT results for sex chromosome abnormalities involving 45,X (n=17), 47,XXY (n=10), 47,XXX (n=6), 47,XYY (n=8), disproportionate level of sex chromosomes (n=9), copy number variants of sex chromosomes (n=3), and suspected maternal sex chromosome imbalance (n=3). 53 had singleton pregnancies and three had dichorionic-diamniotic twin pregnancies. 58.9% had conventional combined Down syndrome screening; 15.2% of them were at high risk for trisomy 21. 33 (58.9%) of the patients opted for invasive diagnostic test: amniocentesis (n=29), chorionic villus sampling (n=3), and chorionic villus sampling followed by amniocentesis (n=1). Confirmatory cytogenetic test results (including postnatal results) were available in 35 cases. The overall positive predictive value of NIPT to detect fetal sex chromosome aneuploidies was 71.4%; the value was 42.9% for detecting 45,X, 100% for detecting 47,XXY, 80% for detecting 47,XXX, and 83.3% for detecting 47,XYY. False positive results were observed in three cases of confined placental mosaicism and three cases of vanishing twin pregnancies. Two women with 47,XXX and one woman with mosaic 45,X/46,XX were also incidentally discovered.
***Conclusion:*** Positive NIPT results for sex chromosome abnormalities can be caused by true fetal sex chromosome abnormalities, confined placental mosaicism/placental mosaicism, vanishing twins, and maternal X chromosome abnormalities. Multidisciplinary management can help prenatal counselling and genetic diagnosis. Follow-up confirmatory cytogenetic analysis prenatally and/or postnatally is useful to characterise the numeric or structural fetal sex chromosome abnormalities and their mosaic patterns, and can maximise the benefits of prenatal genetic screening in obtaining more genetic information to support pregnancy management and clinical care of affected unborn child.

*Keywords: Genetic testing; Noninvasive prenatal testing; Prenatal diagnosis; Sex chromosome aberrations*

*Correspondence to: Dr Po Lam SO*
*Email: so_pauline@yahoo.com.hk*

## Introduction

With the discovery of the presence of circulating cell-free fetal DNA in maternal plasma[1] and the development of high throughput next-generation sequencing, non-invasive prenatal testing (NIPT) for common fetal aneuploidies was introduced in 2011[2]. Compared with traditional prenatal screening, NIPT is superior in detecting fetal trisomies 21, 18, and 13 and reduces the need for invasive diagnostic procedures[3-8]. Since then, NIPT has been implemented as first-tier or second-tier prenatal screening worldwide, using techniques of massively parallel sequencing (shotgun or target) and single nucleotide polymorphism[9]. Since December 2019 in Hong Kong public hospitals, NIPT has been used as second-tier screening for high-risk women with positive traditional prenatal screening result.

Cell-free fetal DNA testing can identify fetal sex and fetal sex chromosome aneuploidies (SCAs)[10], which is one group of sex chromosome abnormalities. The most common SCAs are 45,X, 47,XXY, 47,XXX, 47,XYY, and their various forms of sex chromosome mosaicisms[11]. Collectively, SCAs are the most common chromosomal condition, with estimated prevalence of 1/400 births[11]. Individuals with SCAs display wide spectrum of phenotypes from asymptomatic to serious physical, reproductive, and behavioural presentations[12]. The unpredictability and variable clinical manifestations of SCAs make genetic counselling and parental decision-making towards SCA-affected pregnancy very difficult.

Expanded use of NIPT for SCAs remains a controversy. Nonetheless, NIPT for SCAs has been readily available in the private sector. In a survey in Hong Kong, 98.50% of women preferred to be informed when NIPT results were suspicious of SCAs, and 33% of whom would consider prenatal diagnosis[13]. Post-test counselling by genetic specialists for those with prenatal diagnosis of SCAs may facilitate continuation of pregnancy[14]. There is a need for clinicians to interpret results and provide counselling to those facing unexpected positive results for sex chromosome abnormalities.

We reviewed medical records of pregnant women with positive NIPT results for sex chromosome abnormalities who attended Tuen Mun hospital between 2015 and 2021. Patient decision after prenatal diagnosis, confirmatory diagnostic testing results, and pregnancy/neonatal outcomes were summarised.

## Methods

This study was approved by the Central Institutional Review Board of Hospital Authority (reference: CIRB-2021-011-3). Medical records of women with abnormal NIPT results for sex chromosome abnormalities who attended Tuen Mun Hospital between January 2015 and December 2021 were retrospectively reviewed.

In July 2010, publicly funded first- or second-trimester screening tests for Down syndrome were provided in Hong Kong. In December 2019, publicly funded second-tier NIPT or a conventional diagnostic test was offered for those screened positive for Down syndrome (with a term risk of ≥1:250). The publicly funded NIPT is restricted to reporting trisomies 21, 18, and 13 only. Our unit also receives referrals of cases of abnormal NIPT results from private obstetric care providers and provides genetic counselling by maternal-fetal medicine specialists (Figure).

Patients were explained that NIPT was only a screening test, with varying performance for SCA detection and other limitations. The variable and unpredictable phenotypic expressions of SCA and available intervention strategies were discussed. Baseline ultrasound examination was offered to evaluate the number of fetuses, presence of a vanishing twin, fetal sex, and obvious fetal structural anomalies such as cystic hygroma. An invasive diagnostic test by chorionic villus sampling and/or amniocentesis were also offered; the procedure-related risk of miscarriage is about 0.5%. Compared with chorionic villus sampling, amniocentesis provides more definitive fetal genetic information because of possible confined placental mosaicisms. Before June 2019, rapid screening of common aneuploidies of chromosomes 21, 18, 13, X, and Y was by quantitative fluorescence– polymerase chain reaction (QF-PCR) and then conventional karyotyping. After June 2019, chromosomal microarray is performed if QF-PCR shows normal results. Both chromosomal microarray and conventional karyotyping are performed in those with abnormal QF-PCR for sex chromosomes. For abnormal genetic findings, karyotyping of parental blood samples is offered to establish inheritance. For discordant NIPT results for SCA, maternal karyotyping is performed for biological explanations of the false positive results. All samples are sent to the prenatal diagnostic laboratory of Tsan Yuk Hospital for genetic analysis. Some patients are referred to the clinical genetic service of the Department of Health for further genetic counselling before or after invasive procedures, depending on the NIPT/diagnostic test results and specialists' discretion or patients' preference.

Patients with abnormal diagnostic test results are counselled by maternal-fetal medicine specialists and/or

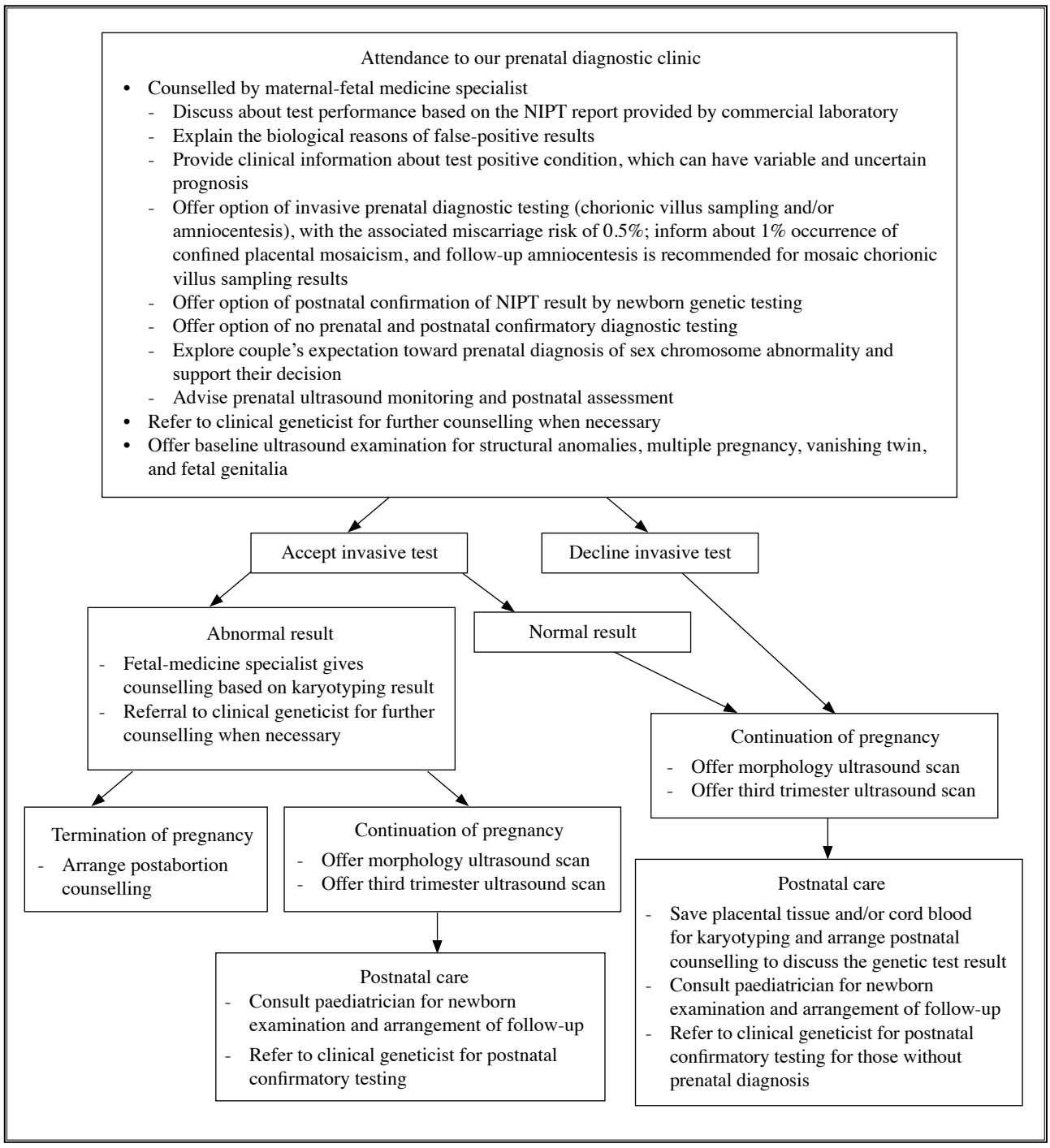


*Figure. Workflow for patients with abnormal non-invasive prenatal testing (NIPT) result for sex chromosome abnormalities*

clinical geneticist regarding the prognosis and pregnancy management. The option of termination or continuation of pregnancy is provided. The legal limit of termination of pregnancy is 24 weeks in Hong Kong. Those who opt for continuation of pregnancy are offered detailed fetal anomaly ultrasound scan and third trimester ultrasound scan. Anomaly ultrasound scan is used to detect any SCA-associated fetal structural abnormalities (such as cardiovascular and renal anomalies in fetuses with 45,X) and other coincidental anomalies. Third trimester ultrasound scan is used to detect any fetal growth restriction related to SCAs and their mosaicism as well as any late presentation of SCA-associated findings. For example, fetuses with 45,X can develop non-immune hydrops fetalis, and ventricular or vascular disproportion (indicating coarctation of aorta) may become more clinically evident in the third trimester. In addition, renal hypoplasia in fetuses with 47,XXX may only be diagnosed in advanced gestation.

Those with normal diagnostic test results are offered further testing after delivery. Karyotyping for the placenta tissue can detect possible placental mosaicism leading to inconsistent findings. For those who declined diagnostic test, both anomaly and growth ultrasound scans are suggested, and their placental tissue or cord/neonatal blood are saved for karyotyping after delivery. All newborns with abnormal NIPT results for sex chromosome abnormalities are examined by our paediatric team in postnatal ward.

Data retrieved included patient demographics, NIPT results, genetic counselling personnel, diagnostic test results, ultrasound findings, karyotyping results, and pregnancy and neonatal outcomes. Small for gestational age is defined as a birthweight below the 10th percentile for the gestational age.

Statistical tests were performed using SPSS (Windows version 23; IBM Corp, Armonk [NY], US). The positive predictive value of NIPT in detecting SCAs was calculated as the number of true positive test results confirmed by amniocentesis or neonatal karyotyping divided by the total number of positive NIPT results.

## Results

Between January 2015 and December 2021, 56 Chinese women attended our prenatal diagnostic clinic with abnormal NIPT results for sex chromosome abnormalities involving 45,X (n=17), 47,XXY (n=10), 47,XXX (n=6), 47,XYY (n=8), disproportionate level of sex chromosomes (n=9), copy number variants of sex chromosomes (n=3), and suspected maternal sex chromosome imbalance (n=3) [Table 1]. The median maternal age was 33.5 years; 39.3% were at an advanced maternal age (≥35 years). 53 had singleton pregnancies and three had dichorionic-diamniotic twin pregnancies. The median gestational age at NIPT was 12.5 weeks. 58.9% had conventional combined Down syndrome screening; 15.2% of them were at high risk for trisomy 21.

The median gestational age at first prenatal diagnostic clinic attendance was 15.6 (range, 12.3-29) weeks. 33 (58.9%) of the patients opted for invasive diagnostic test: amniocentesis (n=29), chorionic villus sampling (n=3), and chorionic villus sampling followed by amniocentesis (n=1) [Table 2]. 36 (64.3%) of the patients received prenatal counselling by clinical geneticists. Confirmatory cytogenetic test results (including postnatal results) were available in 35 cases. The overall positive predictive value of NIPT to detect fetal SCAs was 71.4%; the value was 42.9% for detecting 45,X, 100% for detecting 47,XXY, 80% for detecting 47,XXX, and 83.3% for detecting 47,XYY (Table 3).

**Table 1. Clinical characteristics of 56 patients with abnormal non-invasive prenatal testing (NIPT) results for sex chromosome abnormalities**

| Characteristics | Value* |
|---|---|
| Chinese ethnicity | 56 (100) |
| No. of fetuses | |
| Singleton | 53 (94.6) |
| Twin | 3 (5.4) |
| Conception | |
| Natural | 50 (89.3) |
| Assisted | 6 (10.7) |
| Maternal age, y | 33.5 (23-48) |
| <35 | 34 (60.7) |
| ≥35 | 22 (39.3) |
| Nulliparity | 35 (62.5) |
| Conventional Down syndrome screening test results | |
| Done | 33 (58.9) |
| High risk (≥ 1 in 250) | 5 (15.2) |
| Low risk (< 1 in 250) | 28 (84.8) |
| Not done | 23 (41.1) |
| Gestational age at NIPT, weeks | 12.5 (10-22) |
| 10+0 to 13+6 | 39 (69.6) |
| 14+0 to 15+6 | 10 (17.9) |
| 16+0 to 20+6 | 4 (7.1) |
| ≥21 | 3 (5.4) |
| NIPT platform | |
| Massively parallel sequencing | 55 (98.2) |
| Single nucleotide polymorphism | 1 (1.8) |
| Gestational age at prenatal diagnostic clinic attendance, weeks | 15.6 (12.3-29) |
| 10+0 to 13+6 | 11 (19.6) |
| 14+0 to 15+6 | 18 (32.1) |
| 16+0 to 20+6 | 19 (33.9) |
| 21+0 to 23+6 | 5 (8.9) |
| ≥24 | 3 (5.4) |

* Data are presented as median (range) or No. (%) of patients

17 patients had positive NIPT results for 45,X (Table 4). Four of them had abnormal ultrasound findings. Patients 1 and 2 had findings of hydrops fetalis; chorionic villus sampling confirmed the abnormal karyotype, and they opted for termination of pregnancy. Patient 3 had

**Table 2. Options of invasive diagnostic testing among 56 patients with positive non-invasive prenatal testing (NIPT) results for sex chromosome abnormalities**

| Abnormal NIPT result | No. (%) of patients who declined invasive diagnostic testing | No. (%) of patients who accepted invasive diagnostic testing | No. of patients who had invasive diagnostic testing | | |
|---|---|---|---|---|---|
| | | | Chorionic villus sampling | Amniocentesis | Chorionic villus sampling followed by amniocentesis |
| 45,X (n=17) | 6 (35.3) | 11 (64.7) | 2 | 8 | 1 |
| 47,XXY (n=10) | 5 (50.0) | 5 (50.0) | 0 | 5 | 0 |
| 47,XXX (n=6) | 4 (66.7) | 2 (33.3) | 1 | 1 | 0 |
| 47,XYY (n=8) | 4 (50.0) | 4 (50.0) | 0 | 4 | 0 |
| Others (n=15) [disproportionate level of sex chromosomes (n=9), copy number variants of sex chromosomes (n=3) and suspected maternal sex chromosome imbalance (n=3)] | 4 (26.7) | 11 (73.3) | 0 | 11 | 0 |
| **Total (n=56)** | **23 (41.1)** | **33 (58.9)** | **3** | **29** | **1** |

**Table 3. Performance of non-invasive prenatal testing (NIPT) in detecting fetal sex chromosome aneuploidy**

| Sex chromosome aneuploidy | No. of patients with | | | | | No. of true positive / No. of confirmed karyotype, % |
|---|---|---|---|---|---|---|
| | NIPT positive | Karyotype confirmed | True positive | False positive | Karyotype not confirmed | |
| 45,X | 17 | 14 | 6 | 8 | 3 | 6/14, 42.9% |
| 47,XXY | 10 | 10 | 10 | 0 | 0 | 10/10, 100% |
| 47,XXX | 6 | 5 | 4 | 1 | 1 | 4/5, 80% |
| 47,XYY | 8 | 6 | 5 | 1 | 2 | 5/6, 83.3% |
| **Total** | **41** | **35** | **25** | **10** | **6** | **25/35, 71.4%** |

finding of anencephaly who declined diagnostic test and opted for termination of pregnancy; the karyotype of placental tissue was normal. Patient 4 had findings of ventricular septal defect and echogenic bowel who declined diagnostic test and opted for continuation of pregnancy; neonatal karyotype was normal. 13 patients had normal ultrasound findings. Nine of them opted for diagnostic test through amniocentesis, which confirmed abnormal karyotype in three patients: mosaic 45,X/47,XXX in patients 5 and 6 and de novo isodicentric X chromosome in patient 7. Amniocentesis also identified two cases of confined placental mosaicism. Patient 8 had chorionic villus sampling, which yielded a mosaic karyotype of 45,X[14]/46,XX[16], but follow-up amniocentesis showed normal karyotype. Patient 12 showed normal karyotype after amniocentesis, but the karyotype of the placental tissue showed mos 45,X[3]/46,XY[27], whereas the neonatal karyotype was normal. Four patients with normal ultrasound findings declined diagnostic testing and opted for continuation of pregnancy. Patients 14 to 16 were true positive for mosaic 45,X[17]/47,XXX[33], mosaic 45,X[30]/46,XX[20], or 46,X,i(X)(q10).

10 patients had positive NIPT results for 47,XXY (Table 5). Five of them had amniocentesis, which confirmed the abnormal karyotype (patients 18 to 22). Another five declined diagnostic testing and opted for continuation of pregnancy; neonatal karyotype confirmed the positive NIPT result for 47,XXY in all (patients 23 to 27).

Six patients had positive NIPT results for 47,XXX (Table 6). Patient 28 had chorionic villus sampling, which confirmed the abnormal karyotype, and opted for termination of pregnancy. Patient 29 had amniocentesis,

**Table 4. Ultrasound findings, decision, diagnostic results, and pregnancy/neonatal outcomes of patients with positive non-invasive prenatal testing (NIPT) results for 45,X**

| Patient | NIPT result | Gestational age at NIPT, weeks | Ultrasound findings | Prenatal diagnostic test result | Maternal/ paternal karyotype | Placental tissue for karyotype | Pregnancy/neonatal outcome | Neonatal karyotype |
|---|---|---|---|---|---|---|---|---|
| 1 | 45,X | 10 | Hydrops | Chorionic villus sampling: 45,X | - | - | Termination of pregnancy | - |
| 2 | 45,X | 10 | Hydrops | Chorionic villus sampling: 45,X | - | - | Termination of pregnancy | - |
| 3 | 45,X | 14 | Anencephaly | - | - | 46,XX | Termination of pregnancy, anencephaly | - |
| 4 | 45,X | 20 | Ventricular septal defect, echogenic bowel | - | - | - | Livebirth, ventricular septal defect, atrial septal defect | 46,XX |
| 5 | 45,X | 11 | Normal | Amniocentesis: mos 47,XXX[17]/45,X[13] | - | - | Livebirth | 45,X[33]/ 47,XXX[17] |
| 6 | 45,X | 21 | Normal | Amniocentesis: mos 45,X[6]/47,XXX[55] | - | - | Termination of pregnancy | - |
| 7 | 45,X | 11 | Normal | Amniocentesis: 46,X,idic (X)(p22.3)dn.arr[GRCh37] Xp22.33(168551_1832879) x1,Xp22.33q28(1832912_ 155233098)x3 | Maternal: 46,XX Paternal: 46,XY | - | Livebirth, small for gestational age | 46,X,idic(X) (p22.3) |
| 8 | 45,X | 11 | Normal | Chorionic villus sampling: mos 45,X[14]/46,XX[16] Amniocentesis: 46,XX | - | - | Livebirth | 46,XX |
| 9 | 45,X | 14 | Normal | Amniocentesis: 46,XX | - | - | Unknown decision/ outcome | - |
| 10 | 45,X | 22 | Normal | Amniocentesis: 46,XN | - | 46,XX | Livebirth | - |
| 11 | 45,X | 11 | Normal | Amniocentesis: 46,XX | - | - | Follow-up until 34 weeks, unknown outcome | - |
| 12 | 45,X | 13 | Normal | Amniocentesis: 46,XX | Maternal: 46,XX | mos 45,X[3]/46,X X[27] | Livebirth, small for gestational age, atrial septal defect | 46,XX |
| 13 | 45,X | 10 | Normal | Amniocentesis: 46,XX | Maternal: 46,XX | 46,XX | Livebirth | - |
| 14 | 45,X | 15 | Normal | - | - | 45,X[26]/47,XXX [24] | Livebirth, patent ductus arteriosus, small for gestational age | 45,X[17]/47,X XX[33] |
| 15 | 45,X | 10 | Normal | - | - | 45,X[21]/46,XX[9] | Livebirth | 45,X[30]/46,X X[20] |
| 16 | 45,X | 20 | Normal | - | - | mos 45,X[25]/46, X,i(X)(q10)[5] | Livebirth, developmental dysplasia of the hip | 46,X,i(X)(q10) |
| 17 | 45,X | 14 | Normal | - | Maternal: 46,XX | - | Livebirth | 46,XX |

which confirmed the abnormal karyotype, and opted for continuation of pregnancy and had a livebirth. Four patients declined diagnostic testing and opted for continuation of pregnancy; neonatal karyotype confirmed 47,XXX in three of them (patients 30-32).

Eight patients had positive NIPT results for 47,XYY (Table 7). Four of them had amniocentesis, which confirmed 47,XYY, and opted for continuation of pregnancy. Neonatal karyotype confirmed 47,XYY in patient 34. Another four patients declined diagnostic

**Table 5. Ultrasound findings, decision, diagnostic results, and pregnancy/neonatal outcomes of patients with positive non-invasive prenatal testing (NIPT) results for 47,XXY**

| Patient | NIPT result | Gestational age at NIPT, weeks | Ultrasound findings | Prenatal diagnostic test result | Maternal/ paternal karyotype | Placental tissue for karyotype | Pregnancy/neonatal outcome | Neonatal karyotype |
|---|---|---|---|---|---|---|---|---|
| 18 | 47,XXY | 12 | Normal | Amniocentesis: 47,XXY | - | - | Unknown decision/outcome | - |
| 19 | 47,XXY | 11 | Intrauterine growth restriction | Amniocentesis: 47,XXY | - | - | Livebirth, small for gestational age | - |
| 20 | 47,XXY | 11 | Normal | Amniocentesis: 47,XXY | - | - | Livebirth | - |
| 21 | 47,XXY | 11 | Normal | Amniocentesis: 47,XXY | - | - | Termination of pregnancy | - |
| 22 | 47,XXY | 13 | Normal | Amniocentesis: 47,XXY | - | - | Termination of pregnancy | - |
| 23 | 47,XXY | 14 | Curved penis | - | - | - | Livebirth, buried penis | 47,XXY |
| 24 | 47,XXY | 20 | Normal | - | - | - | Livebirth | 47,XXY |
| 25 | 47,XXY | 12 | Normal | - | - | 47,XXY | Livebirth, small for gestational age | 47,XXY |
| 26 | 47,XXY | 11 | Normal | - | - | 47,XXY | Livebirth, small for gestational age | 47,XXY |
| 27 | 47,XXY | 11 | Normal | - | - | - | Livebirth | 47,XXY |

**Table 6. Ultrasound findings, decision, diagnostic results, and pregnancy/neonatal outcomes of patients with positive non-invasive prenatal testing (NIPT) results for 47,XXX**

| Patient | NIPT result | Gestational age at NIPT, weeks | Ultrasound findings | Prenatal diagnostic test result | Maternal/ paternal karyotype | Placental tissue for karyotype | Pregnancy/neonatal outcome | Neonatal karyotype |
|---|---|---|---|---|---|---|---|---|
| 28 | 47,XXX | 13 | Normal | Chorionic villus sampling: 47,XXX | - | - | Termination of pregnancy | - |
| 29 | 47,XXX | 14 | Normal | Amniocentesis: 47,XXX | - | - | Livebirth | - |
| 30 | 47,XXX | 10 | Normal | - | - | - | Livebirth, atrial septal defect | 47,XXX |
| 31 | 47,XXX | 13 | Normal | - | - | 47,XXX | Livebirth, small for gestational age | 47,XXX |
| 32 | 47,XXX | 10 | Normal | - | - | 47,XXX | Livebirth | 47,XXX |
| 33 | 47,XXX | 13 | Normal | - | - | - | Livebirth | 46,XX |

testing and opted for continuation of pregnancy. Neonatal karyotype confirmed 47,XYY in patient 38. Patient 40 had abnormal ultrasound finding of increased nuchal translucency. Subsequent ultrasound at 33 weeks of gestation revealed right-side pleural effusion with no signs of hydrops or anaemia. Maternal serologic tests for cytomegalovirus and toxoplasma gondii were negative. The baby was born vaginally at term with transient oxygen desaturation, which was self-resolved spontaneously. Chest radiography showed mildly blunted right costophrenic angle, which could be related to previous pleural effusion, and was resolved at day 14 after birth. Sepsis evaluation including skin surface swab, gastric lavage, and blood cultures were negative. The karyotype of placental tissue confirmed 47,XYY. Subsequent follow-up with clinical geneticists was arranged.

Eight patients had NIPT results that showed disproportional low level of Y chromosome (Table 8). Second trimester ultrasound scan suggested female genitalia in four cases. Three of them were dichorionic-diamniotic pregnancies with co-twin demise in early gestation. Two had amniocentesis, which showed 46,XX. Patient 45 had placental abruption and spontaneous preterm delivery at 25 weeks of gestation. The baby girl died at day 2 because of extreme prematurity. Karyotype of cord blood sample showed normal 46,XX, but that of the placental tissue showed 69,XXY[20]/46,XX[10]. Four cases showed

**Table 7. Ultrasound findings, decision, diagnostic results, and pregnancy/neonatal outcomes of patients with positive non-invasive prenatal testing (NIPT) results for 47,XYY**

| Patient | NIPT result | Gestational age at NIPT, weeks | Ultrasound findings | Prenatal diagnostic test result | Maternal/ paternal karyotype | Placental tissue for karyotype | Pregnancy/neonatal outcome | Neonatal karyotype |
|---|---|---|---|---|---|---|---|---|
| 34 | 47,XYY | 12 | Normal | Amniocentesis: 47,XYY | - | 47,XYY | Livebirth | 47,XYY |
| 35 | 47,XYY | 11 | Normal | Amniocentesis: 47,XYY | - | - | Livebirth | - |
| 36 | 47,XYY | 13 | Normal | Amniocentesis: 47,XYY | - | - | Livebirth | - |
| 37 | 47,XYY | 14 | Normal | Amniocentesis: 47,XYY | - | - | Follow-up until 36 weeks, unknown outcome | - |
| 38 | 47,XYY | 11 | Normal | - | - | - | Livebirth | 47,XYY |
| 39 | 47,XYY | 13 | Normal | - | - | 47,XYY | Livebirth | - |
| 40 | 47,XYY | 11 | ↑ Nuchal translucency, pleural effusion | - | - | 47,XYY | Livebirth, pleural effusion | - |
| 41 | 47,XYY | 11 | Normal | - | - | 46,XY | Livebirth | 46,XY |

**Table 8. Ultrasound findings, decision, diagnostic results, and pregnancy/neonatal outcomes of patients with positive non-invasive prenatal testing (NIPT) results for disproportionate level of sex chromosomes**

| Patient | NIPT result | Gestational age at NIPT, weeks | Ultrasound findings | Prenatal diagnostic test result | Maternal/ paternal karyotype | Placental tissue for karyotype | Pregnancy/neonatal outcome | Neonatal karyotype |
|---|---|---|---|---|---|---|---|---|
| 42 | Low level Y | 14 | Dichorionic diamniotic twins: one missed abortion and another normal, female genitalia | Amniocentesis: 46,XX | - | - | Follow-up until 32 weeks, unknown outcome | - |
| 43 | Low level Y | 12 | Dichorionic diamniotic twins: one missed abortion and another normal, female genitalia | Amniocentesis: 46,XX | - | - | Livebirth, small for gestational age | - |
| 44 | Low level Y | 12 | Dichorionic diamniotic twins: one missed abortion and another normal, female genitalia | - | - | 46,XX | Livebirth | - |
| 45 | Low level Y | 21 | Normal, female genitalia | - | - | mos 69,XXY [20]/46,XX[10] | Neonatal death at day 2, placental abruption | 46,XX |
| 46 | Low level Y | 16 | Normal, male genitalia | Amniocentesis: 46,XY | - | 46,XY | Livebirth | - |
| 47 | Low level Y | 13 | Normal, male genitalia | Amniocentesis: 47,XYY | - | - | Unknown decision/ outcome | - |
| 48 | Low level Y | 12 | Normal, male genitalia | Amniocentesis: 46,X,der(X)t(X;Y)(p22.3;p11.2).ish der(X)(SRY+,DXZ1+) | Maternal: 46,XX Paternal: 46,XY | - | Termination of pregnancy | - |
| 49 | Low level Y | 11 | Normal, male genitalia | - | - | 46,XY | Livebirth | 46,XY |
| 50 | Mild ↓ chromosome X DNA | 14 | Normal, female genitalia | - | - | 46,XX | Livebirth | - |

male genitalia. Three of them had amniocentesis, which revealed 46,XY in patient 46, 47,XYY in patient 47, and de novo 46,X,der(X)t(X;Y)(p22.3;p11.2) in patient 48. Patient 48 opted for termination of pregnancy; the abortus showed normal external male genitalia without other apparent abnormalities. The patient refused pathological examination of the fetus. Patient 50 had abnormal NIPT result of mild reduction of X chromosome DNA. A phenotypically normal female baby was delivered, and cytogenetic analysis of placental tissue showed normal 46,XX karyotype.

Three patients had NIPT results that showed copy number variants of X chromosome (Table 9). Amniocentesis confirmed de novo 46,X,idic(X)(p11.21) in patient 51, maternally inherited 46,X,del(X)(p21) in patient 52, and 45,X,inv(19)(p11q13.1)[15]/46,X,r(X)(p22.1q21),inv(19)[10]/46,X,inv(19),+mar[5] in patient 53. They opted for continuation of pregnancy. Neonatal karyotype confirmed amniocentesis findings.

Three patients had abnormal NIPT results that showed increased or decreased chromosome X DNA of possible maternal contribution (Table 10). Second trimester ultrasound scan showed male genitalia, and amniocentesis showed normal 46,XY karyotype. Cytogenetic analysis of maternal blood revealed abnormal 47,XXX karyotype in patients 54 and 55 and mosaic karyotype 45,X[12]/46,XX[18] in patient 56.

18 (85.7%) of 21 patients with prenatally confirmed sex chromosome abnormalities received genetic counselling by clinical geneticists. The remaining three patients were counselled by the maternal-fetal medicine specialist only. They included two cases with hydropic fetuses and 45,X and one case with fetal 47,XXY. Decision for pregnancy was available for 19 patients (Table 11). Seven of them opted for termination. The rates of termination of pregnancy were 75% for 45,X, 50% for 47,XXY, 50% for 47,XXX, 0% for 47,XYY, and 20% for structural sex chromosome abnormalities. The remaining 12 patients opted for continuation of pregnancy; all had livebirths, except for one who was lost to follow-up. For the 12 patients with normal karyotypes after diagnostic testing, all had livebirths, except for three who were lost to follow-up. 23 patients declined invasive diagnostic testing; patient 3 opted for termination of pregnancy based on abnormal ultrasound finding of anencephaly, and patient 45 had extreme preterm birth with early neonatal death.

Birth data were available for 40 infants. The median gestational age at delivery was 38 (range, 25-41) weeks; 92.5% of infants were born at term. 10 (25%) of the infants were small for gestational age. All except two infants with prenatal or postnatal confirmed sex chromosome abnormalities were delivered in private hospitals, had postnatal evaluation and follow-up by the paediatric team and/or clinical geneticists in our hospital.

**Table 9. Ultrasound findings, decision, diagnostic results, and pregnancy/neonatal outcomes of patients with positive non-invasive prenatal testing (NIPT) results for copy number variants of sex chromosomes**

| Patient | NIPT result | Gestational age at NIPT, weeks | Ultrasound findings | Prenatal diagnostic test result | Maternal/ paternal karyotype | Placental tissue for karyotype | Pregnancy/ neonatal outcome | Neonatal karyotype |
|---|---|---|---|---|---|---|---|---|
| 51 | ↓DNA 53.5Mb Xp22.33-Xp11.21, ↑DNA 91.6Mb Xq11.2-Xq28 | 13 | Normal, female genitalia | Amniocentesis: 46,X,idic(X)(p11.21).arr[GRCh37]Xp22.33p11.21(168551_56469081)x1,Xp11.21q28(56474956_155233098)x3 | Maternal: 46,XX Paternal: 46,XY | - | Livebirth, small for gestational age | 46,X,idic(X)(p11.21) |
| 52 | ↓DNA 33.0Mb Xp22.33-Xp21.1 | 12 | Normal, female genitalia | Amniocentesis: 46,X,del(X)(p21) mat.arr[GRCh37] Xp22.33p21.1(168551_35911065)x1 | Maternal: 46,X,del(X)(p21.1) | - | Livebirth | 46,X,del(X)(p21.1) |
| 53 | del (Xp22.33-p22.12, 16.94M) | 13 | Ventricular septal defect, intrauterine growth restriction, female genitalia | Amniocentesis: 45,X,inv(19)(p11q13.1)[15]/46,X,r(X)(p22.1q21),inv(19)[10]/46,X,inv(19),+mar[5].arr[GRCh37]Xp22.33p22.12(168551_20126011)x1,Xp22.12p11.1(20333106_58527155)x1~2,Xq13.1q21.31(67863904_87712575)x1~2,Xq21.31q28(87728897_155233098)x1 | Maternal: mos 45,X,inv(19)(p11q13.1)[4]/46,XX,inv(19)[56] Paternal: 46,XY | - | Livebirth, small for gestational age | mos 46,X,+r(X)[27]/45,X[23] |

**Table 10. Ultrasound findings, decision, diagnostic results, and pregnancy/neonatal outcomes of patients with positive non-invasive prenatal testing (NIPT) results for suspected maternal sex chromosome imbalances**

| Patient | NIPT result | Gestational age at NIPT, weeks | Ultrasound findings | Prenatal diagnostic test result | Maternal karyotype | Placental tissue for karyotype | Pregnancy/ neonatal outcome | Neonatal karyotype |
|---|---|---|---|---|---|---|---|---|
| 54 | ↑chromosome X, ? maternal contribution | 12 | Normal, male genitalia | Amniocentesis: 46,XY | 47,XXX | - | Livebirth | - |
| 55 | ↑chromosome X, ? maternal contribution | 15 | Normal, male genitalia | Amniocentesis: 46,XY | 47,XXX | - | Livebirth | - |
| 56 | ↓chromosome X, ? maternal contribution | 13 | Normal, male genitalia | Amniocentesis: 46,XY | mos 45,X[12]/46,XX[18] | - | Livebirth | - |

**Table 11. Pregnancy decision of patients with confirmed prenatal diagnostic results for sex chromosome abnormalities**

| Abnormal prenatal diagnostic result (mosaic or full-blown) | No. of patients with unknown decision | No. (%) of patients with continuation of pregnancy | No. (%) of patients with termination of pregnancy |
|---|---|---|---|
| 45,X (n=4) | 0 | 1 (25) | 3 (75) |
| 47,XXY (n=5) | 1 | 2 (50) | 2 (50) |
| 47,XXX (n=2) | 0 | 1 (50) | 1 (50) |
| 47,XYY (n=5) | 1 | 4 (100) | 0 (0) |
| Structural sex chromosome abnormalities (n=5) | 0 | 4 (80) | 1 (20) |
| **Total (n=21)** | **2** | **12 (63.2)** | **7 (36.8)** |

## Discussion

About half of our patients with positive NIPT result for sex chromosome abnormalities opted for follow-up invasive diagnostic testing. About 40% of patients affected by sex chromosome abnormalities opted for termination of pregnancy. The overall positive predictive value of NIPT in detecting fetal SCAs in clinical practice was 71.4%.

Prenatal genetic testing empowers women's reproductive autonomy[15]. Women can make informed decision for or against testing for SCA. Genetic counselling for women with positive NIPT results for SCA should provide up-to-date information about SCAs including general characteristics, possible treatments, detection rate, false-positive rate, and positive predictive value of NIPT, and options of prenatal or postnatal follow-up diagnostic testing. Their expectations toward prenatal testing should be explored, including termination of pregnancy and preparation for SCA if confirmed. Although the miscarriage risk of prenatal invasive diagnostic procedure is low (0.20% for chorionic villus sampling and 0.30% for amniocentesis)[16], if the definitive diagnosis is unlikely to affect continuation of pregnancy, diagnostic testing may be deferred until after delivery. Knowing the genetic diagnosis can help timely interventions (such as hormone replacement therapy and educational support) and optimise clinical outcomes[17-19]. Patient autonomy should be respected, and their decisions should be supported. In the literature, the uptake of prenatal diagnostic testing ranged from 34% to 100%[20,21]. In our study, it was about 50%. Acceptance of their children affected with SCAs might be their reason of avoiding prenatal diagnosis.

Multidisciplinary approach is suggested in management of women carrying fetuses with confirmed SCA, because different expertise is needed for advice on neonatal outcomes and provision of long-term care for newborns[10]. Participation of genetic professionals in the counselling can affect reproductive decision-making and facilitate continuation of pregnancy[22,23]. They can give a more accurate, updated, realistic, and positive picture of SCAs. In our study, 64.3% of women had genetic counselling with clinical geneticists. 40% of women opted for termination of pregnancy after diagnostic testing. The percentage is similar to other studies[20,24,25].

In a meta-analysis of 35 studies, the detection rate and false positive rate of NIPT were 95.8% and 0.14%, respectively, for monosomy X and 100% and 0.004%, respectively, for SCAs other than monosomy X[3]. In a systematic review of 13 case series[26], the overall average positive predictive value of NIPT was 48% for SCA, based on 76% of follow-up cytogenetic analysis. The positive predictive value of NIPT was 31% for 45,X, 73% for 47,XXY, 61% for 47,XXX, and 78% for 47,XYY. We achieved higher overall positive predictive value of NIPT for SCAs, particularly 100% for 47,XXY, 80% for 47,XXX, and 83.3% for 47,XYY, compared with 42.9% for monosomy X. NIPT analyses the circulating cell-free DNA from degraded placental cytotrophoblasts (not directly from fetus) and from the mother. The false-positive NIPT results for SCAs can be caused by placental/fetal mosaicism, a vanishing twin, maternal DNA contribution, and maternal neoplastic conditions[10]. NIPT is not always reflective of the fetal karyotype.

Mosaicism is the condition that the conceptus is made up of two or more populations of cells with different genetic constitution[27]. It is much more common with sex chromosomes than autosomal chromosomes[10]. In cases of positive NIPT result for monosomy X with abnormal cytogenetic analysis, the relative frequency was 67% for 45,X, 20% for mosaic 45,X/46,XX, 10% for mosaic 45,X/46,XY, and 3% for X chromosome rearrangement, whereas the relative frequency of mosaicism was 3% for 47,XXY, 7% for 47,XXX, and 12% for 47,XYY[26]. In the present study, in 10 patients with positive NIPT result for monosomy X and with abnormal follow-up cytogenetic analysis, the relative frequency was 20% for 45,X, 60% for mosaic 45,X, and 20% for X chromosome rearrangement. All other SCAs were full-blown. Mosaicism can only be confined to the placenta (confined placental mosaicism) and not extended into the fetal tissue. Confined placental mosaicism can affect about 1% to 2% of chorionic villus samples[28]. In a large study, confined placental mosaicism occurred in 122 (23.4%) of 522 SCAs[29]. In our study, three cases of false-positive results were secondary to confined placental mosaicism. Two with positive NIPT result for 45,X were confirmed normal 46,XX after birth but had mosaic 45,X cell line confined to placenta identified through karyotyping of chorionic villus sample in patient 8 and placental tissue in patient 12. Patient 45 was a confined placental mosaic triploidy with NIPT result showing fetal sex different from ultrasound finding, which resulted in poor perinatal outcomes. To prevent misdiagnosis of fetal genetic condition, any mosaic findings in chorionic villus samples must be confirmed by follow-up amniocentesis[30].

Amniocentesis is the optimal invasive diagnostic procedure to avoid the issue of confined placental mosaicism, because amniotic fluid cells are mainly fetal cells although low level mosaicism in fetal tissue cannot be entirely excluded, and site-specific variations in the proportion of abnormal cells can be present in different fetal tissues. The positive predictive value of NIPT is higher for 45,X with ultrasound abnormalities than 45,X with normal ultrasound finding (99% vs 51%)[29]. Therefore, ultrasound investigation may help the decision-making on the choice of confirmatory diagnostic procedure. Women with abnormal ultrasound findings (such as cystic hygroma or hydrops or increased nuchal translucency or fetal anomalies) can consider chorionic villus sampling for early diagnosis (as in patients 1 and 2). Women with normal ultrasound finding may wait and choose amniocentesis to avoid repeated invasive procedures[29].

Vanishing twin is a biological phenomenon that can cause false-positive NIPT results. The deceased twin is likely to be genetically abnormal (ie, aneuploid) while the viable twin has normal chromosomal constitution[31]. Depending on the individual contribution of each twin to the fetal fraction, the continuous release of DNA fragments from the placenta of demised co-twin into the maternal plasma can influence the NIPT results and mask the actual normal chromosomal condition of the remaining viable twin. The duration of persistence of DNA from a lost twin in maternal circulation is uncertain, but it may be detectable for up to 8 weeks after the co-twin demise[31]. Ultrasound examination in early first trimester can facilitate appropriate pre-test counselling if a failed twin pregnancy is identified before its absorption. In general, NIPT is not recommended for screening in vanishing twin pregnancy. Opposite sex of the vanished and viable twins can also manifest as discordance in fetal sex between NIPT and ultrasound observation of fetal genitalia and/or confirmatory karyotype[32,33]. In our cohort, the discordance of the male fetal sex predicted by NIPT and ultrasound examination findings of female external genitalia was observed in vanishing twin pregnancy in patients 42, 43, and 44. In view of the risk of XY disorder of sexual differentiation, two patients underwent amniocentesis and cytogenetic analysis confirmed normal female karyotype. Co-twin demise of a male fetus can be the reason for the discordant result. Other possible aetiologies are maternal transplantation or transfusion from male donors[34]. Thus, detailed maternal history should be taken. If there is a discrepancy between NIPT reported fetal sex and the ultrasound appearance of fetal external genitalia, options of amniocentesis or newborn genetic assessment should

be discussed for concordance between genotype and phenotype[35,36].

NIPT using massively parallel sequencing cannot distinguish between placental and maternal DNA; false positive results can arise from maternal X chromosome aneuploidy and mosaicism[37-39]. In patients 54 and 55, 47,XXX were incidentally discovered by karyotyping peripheral blood lymphocytes. Somatic age-related X chromosome loss of the women may also lead to high risk NIPT result for monosomy X[40]. This may account for the maternal mosaic 45,X/46,XX in patient 56. Thus, women should be well-informed about the possibility of this unanticipated discovery of maternal genomic information during counselling. Notably, the discovery of maternal SCAs does not exclude fetal SCA, further invasive diagnostic testing may be needed to exclude fetal SCA[10]. The use of single nucleotide polymorphism may potentially allow distinction between fetal (placental) and maternal aneuploidies by analysis of allele polymorphisms[41].

In our study, three women had positive NIPT result for X chromosome copy number variations. Follow-up fetal diagnostic tests confirmed unbalanced structural abnormalities of X chromosome namely deletion (patient 52), ring (patient 53), and isodicentric chromosome (patient 51). Studies have reported patients with an abnormal NIPT result for monosomy X who were eventually diagnosed with structural sex chromosome abnormalities[26,42]. Patient 48 had NIPT result showing disproportionate low level of Y signal. Follow-up diagnostic tests (karyotype and fluorescence in situ hybridisation) confirmed a de novo translocation between the Y chromosome and the X chromosome associated with the diagnosis of nonsyndromic 46,XX testicular disorders of sex development. This condition is characterised by male external genitalia ranging from normal to ambiguous, small testes, gynecomastia, azoospermia, and hypergonadotropic hypogonadism secondary to testicular failure[43].

Standardised management approach for pregnancies with positive NIPT result for SCAs involves collaboration of clinicians, geneticists, paediatricians, and prenatal diagnostic laboratory. Although not all patients opted for diagnostic testing, most had confirmation of positive NIPT results by karyotyping after birth, which provides good estimate of positive predictive value of SCA screening in clinical setting. Nonetheless, the sample is relatively small and is from a single centre. Larger multicentre studies are warranted to evaluate factors affecting the uptake of prenatal diagnostic testing and the clinical impact of the expanded use of NIPT for SCAs in Hong Kong.

## Conclusion

Positive NIPT results for sex chromosome abnormalities can be caused by true fetal sex chromosome abnormalities, confined placental mosaicism/placental mosaicism, vanishing twins, and maternal X chromosome abnormalities. Multidisciplinary management can help prenatal counselling and genetic diagnosis. Follow-up confirmatory cytogenetic analysis prenatally and/or postnatally is useful to characterise the numeric or structural fetal sex chromosome abnormalities and their mosaic patterns, and can maximise the benefits of prenatal genetic screening in obtaining more genetic information to support pregnancy management and clinical care of affected unborn child.

## Acknowledgments

The authors would like to acknowledge the contributions of the clinical geneticists at the Clinical Genetic Service of the Department of Health in provision of genetic counselling service to the parents, and the paediatricians at Tuen Mun Hospital in taking care of the newborns. The authors would also like to thank the laboratory staff at Tsan Yuk Hospital for assisting prenatal diagnosis.

## Contributors

All authors designed the study, acquired the data, analysed the data, drafted the manuscript, and critically revised the manuscript for important intellectual content. All authors had full access to the data, contributed to the study, approved the final version for publication, and take responsibility for its accuracy and integrity.

## Conflicts of interest

All authors have disclosed no conflicts of interest.

## Funding/support

This study received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

## Data availability

All data generated or analysed during the present study are available from the corresponding author on reasonable request.

## Ethics approval

The study was approved by the Central Institutional Review Board of Hospital Authority (reference: CIRB-2021-011-3).

## References


1. Lo YM, Corbetta N, Chamberlain PF, et al. Presence of fetal DNA in maternal plasma and serum. Lancet 1997;350:485-7. Crossref
2. Allyse M, Minear MA, Berson E, et al. Non-invasive prenatal testing: a review of international implementation and challenges. Int J Womens Health 2015;7:113-26. Crossref
3. Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of cell-free DNA in maternal blood in screening for aneuploidies: updated meta-analysis. Ultrasound Obstet Gynecol 2017;50:302-14. Crossref
4. Gil MM, Revello R, Poon LC, Akolekar R, Nicolaides KH. Clinical implementation of routine screening for fetal trisomies in the UK NHS: cell-free DNA test contingent on results from first-trimester combined test. Ultrasound Obstet Gynecol 2016;47:45-52. Crossref
5. Larion S, Warsof SL, Romary L, Mlynarczyk M, Peleg D, Abuhamad AZ. Uptake of noninvasive prenatal testing at a large academic referral center. Am J Obstet Gynecol 2014;211:651.e1-7. Crossref
6. Hui L, Hutchinson B, Poulton A, Halliday J. Population-based impact of noninvasive prenatal screening on screening and diagnostic testing for fetal aneuploidy. Genet Med 2017;19:1338-1345. Crossref
7. Robson SJ, Hui L. National decline in invasive prenatal diagnostic procedures in association with uptake of combined first trimester and cell-free DNA aneuploidy screening. Aust N Z J Obstet Gynaecol 2015;55:507-10. Crossref
8. Warsof SL, Larion S, Abuhamad AZ. Overview of the impact of noninvasive prenatal testing on diagnostic procedures. Prenat Diagn 2015;35:972-9. Crossref
9. Benn P, Borrell A, Chiu RW, et al. Position statement from the Chromosome Abnormality Screening Committee on behalf of the Board of the International Society for Prenatal Diagnosis. Prenat Diagn 2015;35:725-34. Crossref
10. Mennuti MT, Chandrasekaran S, Khalek N, Dugoff L. Cell-free DNA screening and sex chromosome aneuploidies. Prenat Diagn 2015;35:980-5. Crossref
11. Milunsky JM. Prenatal Diagnosis of Sex Chromosome Abnormalities. In: Milunsky A, Milunsky JM, editors. Genetic Disorders and the Fetus: Diagnosis, Prevention and Treatment. 8th ed. Hoboken, New Jersey: Wiley Blackwell; 2021:499- 546. Crossref
12. Pieters JJ, Kooper AJ, van Kessel AG, Braat DD, Smits AP. Incidental prenatal diagnosis of sex chromosome aneuploidies: health, behavior, and fertility. ISRN Obstet Gynecol 2011;2011:807106. Crossref
13. Lau TK, Chan MK, Salome Lo PS, et al. Non-invasive prenatal screening of fetal sex chromosomal abnormalities: perspective of pregnant women. J Matern Fetal Neonatal Med 2012;25:2616-9. Crossref
14. Marteau TM, Nippert I, Hall S, et al. Decision-making after diagnosis of fetal abnormality. Outcomes of pregnancies diagnosed with Klinefelter syndrome: the possible influence of health professionals. Prenat Diagn 2002;22:562-6. Crossref
15. Kater-Kuipers A, de Beaufort ID, Galjaard RH, Bunnik EM. Rethinking counselling in prenatal screening: An ethical analysis of informed consent in the context of non-invasive prenatal testing (NIPT). Bioethics 2020;34:671-678. Crossref
16. Salomon LJ, Sotiriadis A, Wulff CB, Odibo A, Akolekar R. Risk of miscarriage following amniocentesis or chorionic villus sampling: systematic review of literature and updated meta-analysis. Ultrasound Obstet Gynecol 2019;54:442-451. Crossref
17. Ross JL, Quigley CA, Cao D, et al. Growth hormone plus childhood low-dose estrogen in Turner's syndrome. N Engl J Med 2011;364:1230-42. Crossref
18. Tartaglia N, Howell S, Davis S, et al. Early neurodevelopmental and medical profile in children with sex chromosome trisomies: Background for the prospective eXtraordinarY babies study to identify early risk factors and targets for intervention. Am J Med Genet C Semin Med Genet 2020;184:428-443. Crossref
19. Samango-Sprouse CA, Tran SL, Lasutschinkow PC, et al. Neurodevelopmental outcome of prenatally diagnosed boys with 47,XXY (Klinefelter syndrome) and the potential influence of early hormonal therapy. Am J Med Genet A 2020;182:1881-1889. Crossref
20. Ramdaney A, Hoskovec J, Harkenrider J, Soto E, Murphy L. Clinical experience with sex chromosome aneuploidies detected by noninvasive prenatal testing (NIPT): Accuracy and patient decision-making. Prenat Diagn 2018;38:841-848. Crossref
21. Liang D, Cram DS, Tan H, et al. Clinical utility of noninvasive prenatal screening for expanded chromosome disease syndromes. Genet Med 2019;21:1998-2006. Crossref
22. Jeon KC, Chen LS, Goodson P. Decision to abort after a prenatal diagnosis of sex chromosome abnormality: a systematic review of the literature. Genet Med 2012;14:27-38. Crossref
23. So PL, Cheng YKY, Cheuk KY, et al. Health professionals' involvement and information provision in genetic counseling following prenatal diagnosis of sex chromosome aneuploidy in Hong Kong. Int J Gynaecol Obstet 2019;144:314-6. Crossref
24. So PL, Cheng KYY, Cheuk KY, et al. Parental decisions following prenatal diagnosis of sex chromosome aneuploidy in Hong Kong. J Obstet Gynaecol Res 2017;43:1821-9. Crossref
25. Boyd PA, Loane M, Garne E, Khoshnood B, Dolk H; EUROCAT working group. Sex chromosome trisomies in Europe: prevalence, prenatal detection and outcome of pregnancy. Eur J Hum Genet 2011;19:231-4. Crossref
26. Xie X, Tan W, Li F, et al. Diagnostic cytogenetic testing following positive noninvasive prenatal screening results of sex chromosome abnormalities: Report of five cases and systematic review of evidence. Mol Genet Genomic Med 2020;8:e1297. Crossref
27. Engel E. Mosaicism. N Engl J Med 1965;272:34-5. Crossref
28. Malvestiti F, Agrati C, Grimi B, et al. Interpreting mosaicism

in chorionic villi: results of a monocentric series of 1001 mosaics in chorionic villi with follow-up amniocentesis. Prenat Diagn 2015;35:1117-27. Crossref
29. Grati FR, Bajaj K, Zanatta V, et al. Implications of fetoplacental mosaicism on cell-free DNA testing for sex chromosome aneuploidies. Prenat Diagn 2017;37:1017-1027. Crossref
30. Cherry AM, Akkari YM, Barr KM, et al. Diagnostic cytogenetic testing following positive noninvasive prenatal screening results: a clinical laboratory practice resource of the American College of Medical Genetics and Genomics (ACMG). Genet Med 2017;19:845-850. Crossref
31. Curnow KJ, Wilkins-Haug L, Ryan A, et al. Detection of triploid, molar, and vanishing twin pregnancies by a single-nucleotide polymorphism-based noninvasive prenatal test. Am J Obstet Gynecol 2015;212:79.e1-9. Crossref
32. Kelley JF, Henning G, Ambrose A, Adelman A. Vanished twins and misdiagnosed sex: a case report with implications in prenatal counseling using noninvasive cell-free DNA screening. J Am Board Fam Med 2016;29:411-3. Crossref
33. Grati FR. Implications of fetoplacental mosaicism on cell-free DNA testing: a review of a common biological phenomenon. Ultrasound Obstet Gynecol 2016;48:415-423. Crossref
34. Gregg AR, Skotko BG, Benkendorf JL, et al. Noninvasive prenatal screening for fetal aneuploidy, 2016 update: a position statement of the American College of Medical Genetics and Genomics. Genet Med 2016;18:1056-65. Crossref
35. Dhamankar R, DiNonno W, Martin KA, Demko ZP, Gomez-Lobo V. Fetal Sex Results of Noninvasive Prenatal Testing and Differences With Ultrasonography. Obstet Gynecol 2020;135:1198-1206. Crossref
36. Smet ME, Scott FP, McLennan AC. Discordant fetal sex on NIPT and ultrasound. Prenat Diagn 2020;40:1353-1365. Crossref
37. Wang Y, Chen Y, Tian F, et al. Maternal mosaicism is a significant contributor to discordant sex chromosomal aneuploidies associated with noninvasive prenatal testing. Clin Chem 2014;60:251-9. Crossref
38. Yao H, Zhang L, Zhang H, et al. Noninvasive prenatal genetic testing for fetal aneuploidy detects maternal trisomy X. Prenat Diagn 2012;32:1114-6. Crossref
39. Zhang B, Zhou Q, Chen Y, et al. High false-positive non-invasive prenatal screening results for sex chromosome abnormalities: Are maternal factors the culprit? Prenat Diagn 2020;40:463-9. Crossref
40. Russell LM, Strike P, Browne CE, Jacobs PA. X chromosome loss and aging. Cytogenet Genome Res 2007;116:181-5. Crossref
41. Martin KA, Samango-Sprouse CA, Kantor V, et al. Detection of maternal X chromosome abnormalities using single nucleotide polymorphism-based noninvasive prenatal testing. Am J Obstet Gynecol MFM 2020;2:100152. Crossref
42. Sund KL, Khattar D, Boomer T, et al. Confirmatory testing illustrates additional risks for structural sex chromosome abnormalities in fetuses with a non-invasive prenatal screen positive for monosomy X. Am J Med Genet C Semin Med Genet 2020;184:294-301. Crossref
43. Délot EC, Vilain EJ. Nonsyndromic 46,XX Testicular Disorders of Sex Development. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Gripp KW, Mirzaa GM, Amemiya A, editors. GeneReviews® Seattle (WA): University of Washington, Seattle; 1993-2022.