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Review Article

Is it Time to Start NewbornScreening forTurner Syndrome?

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Abstract

Turner syndrome (TS), is the most common chromosomal abnormality in females. TS is caused by haploinsufficiency of the short arm of X-chromosome, and is usually diagnosed by karyotyping which is time-consuming, expensive and unfeasible for population screening. Neonatal diagnosis of TS permits detection of associated malformations, appropriate therapy for short stature and puberty, thus improving patient quality of life and minimizing sequels. However fewer than 20% of the cases are diagnosed during the neonatal period. TS is not currently part of newborn screening. A considerable delay in diagnosing girls with TS is obvious. The use of combined molecular-cytogenetic approaches in different tissues of different embryological origins would be of help for thedetection of mosaic TS patients. Simpler, faster and less expensive new methods are needed for population screening programmes.

INTRODUCTION

Chromosome aneuploidies, mostly characterized by trisomy 21,18,13 and monosomy X. The aneuploidies usually occur in one out of every 160 live births and account for 6-11% of all stillbirths and newborn deaths. Sex chromosome abnormalities (SCAs) are the most common genetic disorder with a frequency of 1/400 or 1/500 live births.Turner syndrome (TS), is the most common chromosomal abnormality in females. Itoccurs in one in 2500 live-born females andpresent in 3% of conceptions and in as many as 10% of all miscarriages [1]. The prenatal prevalence is much higher than the postnatal prevalence [2].

Neonatal diagnosis of TS is very important for the detection of associated malformations, improving patient quality of life. But TS is not currently part of newborn screening. A considerable delay in diagnosing girls with TS is present.

Conventional cytogenetic karyotype analysis is still the standard test in genetic diagnosis. Karyotyping for neonatal screening is time-consuming, expensive, unfeasible and need for specialized personnel, mosaicism detection rate is low. Simpler, faster and less expensive new methods are neededfor diagnosis or neonatal screening of TS.

CLINICAL MANIFESTATION

TS can cause a wide variety of symptoms, but short stature and gonadal dysgenesis are almost invariably present.Somatic manifestations can be divided into three categories:

- **Skeletal anomalies:** short metacarpals, Madelung's deformity, cubitus valgo, short neck,narrow, high-arched palate, retrognathia, broad chest scoliosis, flat feet

- **Soft-tissue abnormalities**: related to lymphatic obstruction and consequent lymphedema, ungueal dysplasia, webbed neck, low implantation of the hair line

- **Visceral anomalies**: disorders of renal rotation, pielo-calicial duplicity and horse-shoe kidney; cardiac congenital defects, such as bicuspid aortic valve and aortic coarctation

The presence and severityof clinical manifestation is related to the type of chromosomal abnormalities, the time at which chromosome disjunction failed and the proportion of compromised cells in each tissue.

Short Stature: The primary characteristics of turner syndrome and its pathogenesis

The most common feature of TS is short stature.It is important to accurately assess the incidence of TS in growth-retarded girls, even in the absence of other dysmorphisms. The growth decrease can be present at birth, and proceeds to become more obvious by two to three years and gradually more obvious thereafter. At the time of puberty, the growth spurt is much less than average. On average the girl with TS will grow to be 20 cm shorter than she would have been and do not lack pituitary growth hormone [3].

Loss of one copy of **S**hort stature homeobo**x** **(**SHOX gene) is the main cause of short stature in TS. The SHOX gene is located in the pseudoautosomal region (PAR1) of the X and Y chromosome which is associated with short stature in humans if mutated or present in only one copy (haploinsufficiency). The gene was first found during a search for the cause of short stature in women

with TS.SHOX related haploinsufficiency disorders range from Leri-Weill dyschondrosteosis (LWD) at the more severe end to SHOX-related short stature at the mild end of spectrum [4].

Contiguous Gene Syndrome (CGS)

CGS is caused by interstitial or terminal deletions of several adjacent genes. The phenotype results in a combination of 2 or more monogenic disorders, and clinical findings are correlated with corresponding genotypes

Several important genes have been identified in the distal part of Xp; Xp22.2-p22.3 region, including 6 contiguous disease genes:

- short stature (SS, P- growth gene)
- X-linked recessive chondrodysplasia punctata (CDPX1)
- X-linked nonspecific mental retardation (MRX)
- X-linked ichthyosis (XLI)
- Kallmann syndrome (KAL1)
- Type 1 ocular albinism (OA1)

In 1985, a contiguous gene phenotype mapped the X-chromosomal locus for Duchenne muscular dystrophy (DMD) and sub-sequently, the dystrophin gene was identified (1,2). Con-tiguous gene phenotypes involving Xp22.3 deletions in-clude variable combinations of mental retardation (MR), congenital ichthyosis, short stature, Kallmann syndrome, skeletal abnormalities, and epilepsy Contiguous gene phenotypes involving Xp22.3 deletions include variable combinations of mental retardation congenital ichtiyosis, short stature, Kallman syndrome, skeletal abnormalities and epilepsy

GENETIC OVERVIEW OF TURNER SYNDROME

Complete monosomy of X chromosome is responsible for almost 50% of all cases of TS, and mosaicism and X anomaly are detected in the other half.It has been claimed that the pure 45,X karyotype does not exist, because such an individual could not survive *in utero*. This claim is supported by meticulous studies examining more than one tissue (i.e. other than lymphocytes) for the presence of mosaicism. A woman with TS will have one of three X chromosome problems:

A. X chromosome monosomy(50%) (45XOAbsence of the X chromosome in all cells)

B. X chromosome defects in all cells (15%)The most common structural aberrations are the long arm isochromosome (46XiXq), the short arm deletion (46XdelX) and the X-ring chromosome (46XrX)

C. Mosaic TS(30%) (Absence of the X chromosome in some cells 45XO/46XX or 45XO/47XXX) is usually caused by paternal meiotic non- disjunction.

D. X-autosome translocation

X chromosome monosomy

Mortality is increased in TS and women with the 'pure' 45,X karyotype do seem to be most severely affected. Cardiac malformations are more prevalent among the subgroup of patients with 45,X (39%), than among those with karyotypes that include an isochromosome (Xq) (11-12%) [5].

Turner syndrome and X chromosome defects

Another cause of TS involves X chromosome defects, rather than complete loss. For example, one X chromosome may be fragmented, have portions deleted, or have other structural problems, such as ring formation preventing the normal expression of X chromosome genes. In a woman having one normal and one defective X chromosome, the symptoms vary widely.A small deletion on the X chromosome may result in a single TS feature, such as ovarian failure or short stature, and no other effects. Larger deletions or deletions on the X chromosome, affecting critical areas regulating the whole chromosome, may result in a full spectrum of TS features.

Mosaic turner syndrome

Many, perhaps most, girls and women with TS are actually mosaic. There is a mix of 46,XX and 45,X cells, and the resulting clinical abnormalities tend to be milder than in 45,X TS. Girls with TS and Y chromosome mosaicism are at increased risk of developing the gonadal tumors and should have their nonfunctioning ovaries removed.

It is suggested that in most cases 45,X/46,XX mosaicism in Turner females arises through loss of one of the X chromosomes in some cell lines in originally 46, XX conceptuses, rather than through mitotic non-disjunction during early embryogenesis in originally 45, X conceptuses. A high sensitivity of the modified assay based on PCR-amplification of the (CAG)n repeat within androgen receptor gene proves its usefulness as a tool for studying mosaicism in TS [6].

The features of mosaic TS correlate with the relative percentage of 45, X cells within the body, compared to 46, XX cells. Recently it is also suggested that submicroscopic X-linked and autosomal copy number variations (CNVs) as an important genetic risk category for premature ovarian insufficiency and may be involved in modulating the TS ovarian phenotype [7,8].

Ovarian dysfunction in TS women is likely caused by accelerated oocyte-loss in the early stages of the meiotic prophase after the 18th week of fetal life, later resulting in ovarian dysgenesis and streak ovaries [9]. Two distinct loci on the Xq (Xq13-q21 and Xq23-q27) and one region on the Xp22.1-p11.2 are significantly associated with the ovarian phenotype [10]. 30% or more of females with TS show signs of puberty and 2–5% will have spontaneous menses and may have the potential to achieve pregnancy without medical intervention. Spontaneous puberty has been observed in 15– 20% of 45,X patients and in 32% of mosaic patients, inversely correlating with the severity of chromosomal anomalies at conventional karyotype [11].

Subjects with 45,X frequently have serum follicle-stimulating hormone (FSH) levels already in the post-menopausal range during infancy, whereas FSH levels are generally normal in patients of the same age with mosaic TS [12]. The antimullerian hormone is generally low in infants with 45,X or structural abnormalities of the second X, but similar to controls in mosaic Turner patients [13].

Castronovo et al., performed new generation genetic (Copy Number Assay) and molecular-cytogenetic (aCGH and iFISH) investigations in a cohort of TS patients in order to better clarify the synergistic burden of X chromosome mosaicism and rare X-linked and autosomal submicroscopic CNVson the observed ovarian phenotypes of these patients. Submicroscopic CNVsaffecting several X-linked and autosomal loci with a possible role in female fertility have been detected by means of array comparative genomic hybridization (aCGH) and single-nucleotide polymorphism (SNP) array. The aCGH could detect structural abnormalities of the second X in the euploid cell lines, which were not identified by standard karyotype, and precisely maps the breakpoints.The use of combined molecular-cytogenetic approaches in different tissues would be of help also in unveiling cryptic 45,X/ 46,XY mosaics. In contrast with the SNP array, a quantitative estimation of X chromosome mosaicism rate cannot be performed directly by analysing the aCGH profile shift and an ad hoc mosaicism scale must be applied [15-17] (Table 1).

X chromosome inactivation (XCI) patterns in 45,X/46. XX mosaics have been investigated by the BstXI restriction endonuclease detection of an X-linked phosphoglycerate kinase (PGK) gene polymorphism following digestion of the DNA with methylation-sensitive HpaII, or with methylation-insensitive AfaI as a control and the detection of a CAG triplet repeat polymorphism in the X-linked androgen receptor (AR) gene after sodium bisulfite treatment. 36% showed extremely skewed XCI for at least one of the polymorphisms, which was a much higher incidence than previously reported for normal females [13]. For determining of diparental/uniparental origin of X chromosomes in mosaic Turner females, An (CAG)n repeat within the AR genewas selected as a genetic marker. PCR-amplification of the

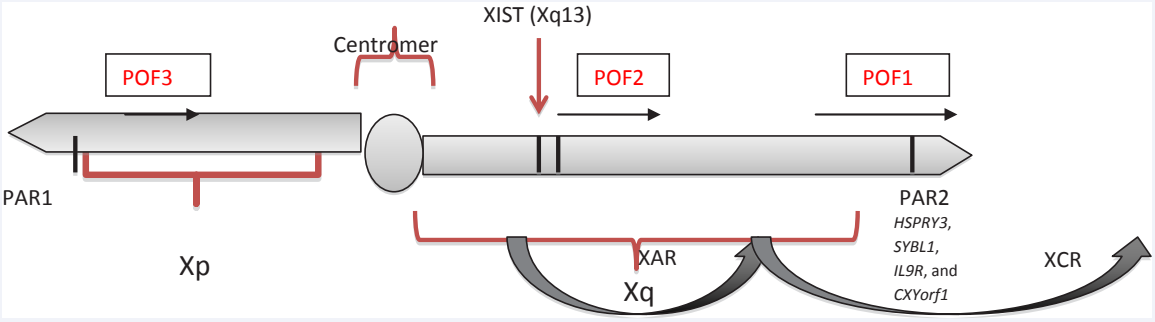

Figure 1 X Chromosome schematic structure. Evolutionary domains of the X chromosome.
Abbreviations: PAR1, PAR2: Pseudoautosomal region; XAR: X added region; XCR: X conserved region

CNV[a]	Physical position[b]	Chromosomal band	Size (kb)	Genes included in the CNV[c]	Patient ID
Rare					
Loss	chr9:118971743-119187538	9q33.1	216	***PAPPA***, *ASTN2*	PA5
Gain	chr15:85591615-85666309	15q25.3	75	***PDE8A***[d]	PA4
Gain	chrX:50400649-50955142	Xp11.22	554	***BMP15***, *SHROOM4*	SM1
Common					
Gain	chr3:100347505-100419624	3q12.2	72	***GPR128***[e]	SM3
Gain	chr3:100380047-100419624	3q12.2	40	***GPR128***[e]	PA7
Gain	chr5:69711984-70314582	5q13.2	603	*SERF1A*, *SMN2*, ***NAIP***[e]	PA26
Gain	chr5:69705562-70587018	5q13.2	881	*SERF1A*, *SMN2*, ***NAIP***, *GTF2H2*	PA37
Loss	chr5:69705562-70309855	5q13.2	604	*SERF1A*, *SMN2*, ***NAIP***	PA40
Loss	chr6:266079-307998	6p25.3	42	***DUSP***	SM3
Loss	chr6:259528-293493	6p25.3	34	***DUSP***	PA17
Loss	chr6:283968-375949	6p25.3	92	***DUSP***	PA31
Gain	chr6:259528-317679	6p25.3	58	***DUSP***[e]	PA33
Gain	chr6:259528-293493	6p25.3	34	***DUSP***[e]	PA34
Loss	chr8:15952011-16015454	8p22	63	***MSR1***	PA41
Loss	chr22:39359112-39385485	22q13.1	26	***APOBEC3A***, ***APOBEC3B***	SM5
Loss	chr22:39359112-39385485	22q13.1	26	***APOBEC3A***, ***APOBEC3B***	PA18
Loss	chr22:39359112-39385485	22q13.1	26	***APOBEC3A***, ***APOBEC3B***	PA38

[a]Copy number variations classification has been performed according to the Database of Genomic Variants (http://projects.tcag.ca/variation/).
[b]According to the genome assembly hg19 (UCSC Genome Browser, release February 2009, http://genome.cse.ucsc.edu, hg19).
[c]Genes likely to be implicated in female fertility are indicated in bold.
[d]Intragenic duplication likely perturbing gene expression.
[e]Partial gene duplications involving either 5' or 3' end must be further molecularly characterized to clarify the actual perturbation of gene expression.

Table 1 Detailed list of the identified autosomal and X linked CNVs related to ovarian function in patients with TS by Castronovo et al. 2013.

(CAG)n repeat within AR gene proves its usefulness as a tool for studying mosaicism in TS [6].

X-autosome translocation

Females who carry structural rearrangements of the X chromosome, including balanced translocations, show a non-random distribution of inactivation patterns. These females are also at risk for X-linked recessive disorders normally expressed only in males. For example, Duchenne muscular dystrophy (DMD) The gene locus for DMD is on Xp21.

Balanced de novo t(X;12) (X;9) (X;1) (X;2) (X;4) (X;6) (X;21) translocations were reported in the literature in patients with progressive Duchenne muscular dystrophy (DMD),TS, epilepsy and mental retardation. The involvement of the paternal X is suggested [14].

Gonadal mosaicism for the DMD locus was discussed by Wood and McGillivray (1988), who described a family in which a female ancestor of an individual with DMD seemed to have transmitted 3 distinct types of X chromosome to her offspring, as indicated by RFLP analysis. The authors postulated that in this individual the mutation arose as a postzygotic deletion, resulting in germinal mosaicism [15].

TS, associated with a DMD mutation in the remaining X chromosome is another reported genetic mechanism for female DMD. Chelly et al. (1986) for the first time observed a girl with typical DMD and typical 45,XO TS. The one X chromosome in the girl was normal by high resolution banding, but DNA analysis by Southern blotting and hybridization with 7 cloned probes mapping in the Xp21 region showed a deletion of 3 of the probes. In this case, the paternal chromosome was lost and the maternal X chromosome suffered a deletion mutation in the Xp21.2 region [16].

DIAGNOSIS OF TURNER SYNDROME

Prompt and effective diagnosis of TS is very important for appropriately monitoring the comorbidities.

Antenatal diagnosis of turner syndrome

Is there a unique pattern of second-trimester maternal serum analyte for fetal Turner syndrome, with or without hydrops? The second trimester maternal serum screening test is performed between 16-20 weeks of gestation. Different researches have been made for the detection of maternal serum analysis results in genetically and phenotypically different TS patients. The results are summarized in Table (2).

Saller et al. has found that in both hydropic and nonhydropic cases, alpha-fetoprotein (AFP) levels were slightly reduced, and unconjugated estriol levels were markedly reduced. In hydropic pregnancies human chorionic gonadotropin (hCG) levels were elevated, and nonhydropic pregnancies had low human chorionic gonadotropin levels [17].

Fourteen cases of TS (45, X), two cases of mosaic TS (45, X/47, XXX and 45,X/ 46,XX), and one case of TS involving an isochromosome X [46,X,i(X)(q10)] were ascertained by prenatal maternal serum alpha-fetoprotein (MSAFP) and free hCG screening by Laundon et al. Eleven of the 17 cases had hydrops and presented with an increased Down syndrome risk based on MSAFP and free beta hCG screening. The median MOM level was 0.98 and 4.04 for MSAFP and free beta hCG, respectively. Three cases had hydrops but screened negative. The two cases of mosaic TS were non-hydropic and screened positive. The 46, X, i(X)(q10) case was non-hydropic but had elevated MSAFP and free beta hCG levels. The free beta hCG alone was the most effective screening marker for TS pregnancies [18].

Second-trimester levels of maternal serum inhibin A levels were investigated in 10 cases of TS without hydrops and 12 cases of TS with hydrops. Inhibin A levels were modestly, but significantly reduced in cases of TS without hydrops (median = 0.64 MoM). In contrast, inhibin A levels were markedly increased in cases of TS with hydrops These data for TS are similar to those for hCG [19-21].

Table 2: Pattern of second-trimester maternal serum analyte for fetal Turner syndrome.

	TS with hydrops	TS without hydrops	Trisomy 21	Trisomy 18
Inhibin A	elevated	significantly reduced	elevated	N/A
Hcg	elevated*◊	reduced/elevated	elevated	reduced
uE_3	markedly reduced*◊	markedly reduced*	reduced	reduced
AFP	elevated/ slightly reduced*◊	slightly reduced*	reduced	reduced

*Saller et al.1992
◊Laundon et al.1996
⍟Lambert-Messerlian GM et al. 1998,

Table 3: Pattern of first-trimester maternal serum analyte for fetal sex chromosome abnormalities (SCA)s.

	NT	PAPP-A
TS 45X0	increased	low
Klinefelter syndrome 47, XXX	increased	low
47, XYY	increased	low
Trisomy 21	in some	low
Trisomy 18	in some	low

First trimester screening pattern for fetal Turner Syndrome

First Trimester Screeningincludes a sonogram and maternal blood work performed between 11 4/7 – 13 6/7 weeks of pregnancy. The sonogram is performed for pregnancy date, measurement of the nuchal translucency (NT) and presence of the baby's nasal bone. A maternal blood sample is used to analyze free beta- hCG and pregnancy associated plasma protein-A (PAPP-A) (Table 3).

Only a minority of SCAs are detected by first trimester prenatal screening program for Down syndrome (DS); 42% of TS, 13% of Klinefelter syndrome 16% of 47, XXX and 5% of 47,XYY SCAs, with an overall detection rate of 27%. The prenatal detection rate is below 50% for all SCAs. About 2/3 of the Turner and 47, XYY cases had either abnormal DS screening tests or sonographic findings, such as: increased nuchal translucency, mainly cystic hygroma and fetal hydrops [21].

For SCA fetuses carried to term, only TS fetuses had consistently lower birthweights and placenta weights than non-SCA controls. Growth retardation of TS fetuses is if anything more pronounced than previously reported, both when evaluating fetus and placenta [22].

Noninvasive prenatal test (NIPT)

Cell-free DNA is the small amount of DNA that is released from the placenta into a pregnant woman's bloodstream. The cell- free DNA in a sample of a woman's blood can be screened for trisomy 21, 13, 18, and SCAs. It can be done starting at 10 weeks of pregnancy. It takes about 1 week to get the results. A positive cell-free DNA test result should be followed by a diagnostic confirmation test with amniocentesis or chorion villus sampling (CVS). Cell-free DNA testing is not recommended for a woman carrying more than one fetus.

To explore the feasibility of high-throughput massively parallel genomic DNA sequencing technology for the NIPT for the detection of fetal SCAs; fetal karyotyping has made to 33 pregnant women with NIPT result of SCA (27 with TS (45,X), eight with Triple X syndrome (47,XXX), 12 with Klinefelter syndrome (47,XXY) and three with 47,XYY). 18 had results consistent with NIPT, while 15 patients received a normal karyotype result. The overall positive predictive value of NIPT for detecting SCAs was 54.54% and for detecting TS was 29.41% [23].

The potential explanations for NIPT fetal karyotype discordance include CPM, maternal mosaicism, co-twin demise, maternal malignancy, and even laboratory error. The circulating cfDNA in maternal plasma is a combination of maternal and fetal DNA, among which the fetal fraction is only 3-6%. So it is highly recommended that, for the cases with abnormal results in NIPT, maternal karyotype should be confirmed to eliminate the influence of maternal mosaicism [24].

NIPT should not be recommended for the genetic evaluation of the aetiology of ultrasound anomalies, as both resolution and sensitivity, or negative predictive value, are inferior to those of conventional karyotyping and microarray analysis [25].

Although PCR-based approaches have been proposed for prenatal detection of TS, they do not effectively detect individuals with mosaicism or partial X-chromosome deletions, which account for more than 40% of karyotypes in TS [26,27].

NEWBORN SCREENING OF TURNER SYNDROME

Neonatal diagnosis of TS permits detection of associated malformations, appropriate therapy for short stature and puberty, thus improving patient quality of life and minimizing sequels.However fewer than 20% of the cases are diagnosed during the neonatal period. Nearly one quarter present at adult services with primary or secondary amenorrhoea and short stature. Average diagnostic delay is 7.5 years during childhood and adolescence.

Conventional cytogenetic karyotype analysis is still the standard test in genetic diagnostics. The main early clinical features of TS include; smaller size than expected for the gestational age, lymphedema of the hands and feet, redundant posterior cervical skin, and low hair implantation.

- **Signs that indicate a karyotype is needed:** Fetal cystic hygroma
- Webbed neck or lymphedema in newborns
- Specific heart problems in infants
- Slow growth and/or short stature Any girl who is very short for her age or who is growing at less than two inches per year
- Delayed puberty or amenorrhea
- Infertility or menstrual irregularities.

TS screening is recommended for all short girls at or below the 5% threshold for height. It is estimated that one in 50–100 girls with short stature have TS [28,29].

Instead of conventional cytogenetic karyotyping, various molecular methods have been proposed for diagnosis or neonatal screening of TS, including Southern blotting, polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP), fluorescent PCR genotyping, GeneScan-based genotyping, pyrosequencing, and real-time PCR [30,31].

8. Which probe which Genes?

FISH probes are labeled either directly using fluorochrome-

Table 4: Comparison of the conventional cytogenetic karyotyping with molecular methods.

	Cytogenetic analysis	Molecular analysis
Cell culture need	yes	no
Results	20 days	4 days
Suitable for screening	no	yes
Cost	Expansive	cheap
Parental maternal origin info	Could not give	Could give
Mosaisizm detection rate	low	high
Need special personnel	yes	no

conjugated nucleotides or indirectly using reporter molecules by nick-translation, random priming, PCR, or various other molecular genetic techniques. Whole chromosome painting probes are specific for each chromosome. The X-chromosome contains 11,232 probes. Only 13 probes on the 450K array are located in a pseudoautosomal region on the X-chromosome, and they are all in pseudoautosomal region 1 (PAR1) (Xp22). For chromosome enumeration, probes detecting chromosome-specific satellite regions near the centromeres are preferred.

For the investigation of specific submicroscopic chromosomal regions, a wide spectrum of so-called locus-specific identifiers (LSI) are used for the detection of the classical microdeletion syndromes (contiguous gene syndromes) or specific chromosome translocations followed by chimeric gene fusion. To scan metaphase spreads for cryptic aberrations at the gene-rich ends of the chromosomes special subtelomeric probes were established [32].

9. Use of Various X Chromosome Genes Have Been Suggested for Diagnosis or Neonatal Screening of Ts

CAG repeats of the androgen receptor gene(Xq11-12),ARSE gene (arylsulfatase E-OMIM 300180) located in the telomerİc pseudoautosomal region (ARSE-Xp22.3), the MAGEH1 gene (melanoma antigen, H1-OMIM protein: 300548) located in the pericentromeric region (MAGEH1-Xp11.21), ARSE : GAPDH ratio, SHOX, VAMP7, XIST, UBA1, and SRY genes, X-chromosome inactivation specific differentially methylated CpG sites (XIDMSs), Fragile X-related epigenetic element 2, for sex-determining region Y (SRY), and FMR1 CGG repeat analyses [32-40].

10. Epigenome-Wide Association Studies (EWAS)

In humans, DNAmethylation is an important epigenetic mark occurring at CpG dinucleotides, which is implicated in gene silencing. In 2011, Illumina released the Human Methylation 450 bead array, also known as the 450k array. This array has enabled population-level studies of DNA methylation by providing a cheap, high-throughput and comprehensive assay for DNA methylation. Applications of this array to population-level data include epigenome-wide association studies (EWAS) [41,42].

11. Genome-Wide Expression Analysis

Regarding TS, only few studies exist which performed genome-wide expression analysis in fibroblast and in cell free DNA derived form amniotic fluid. Sharma et al., analyzed DNA methylation in whole blood samples and they studied the DNA methylation status of CpG islands and promoters in blood samplesGene ontology analysis reveals clusters of genes, which are expected to be involved in the clinical phenotype.Interestingly they have found that methylation of mainly autosomal loci is affected in both Turner and Klinefelter syndromes. Most of them are found in the monosomic state of the X chromosome (45,X).

Differences at autosomal genes, such the ones verified by pyrosequencing HOXA4, HOXB6, DIRAS3, ZNF593, AMT, SCRL, TP53INP1, ITGB1BP1, ENTPD1 TRPM2, and SEPTIN D1 the hypermethylation status of HOXA4 and HOXB6 suggests a strong effect on developmentally important genes. Apart from that, genes involved in immune system processes, immune and defense response to bacteria, cytokine production, and meiosis were highly enriched in Turner samples. SERPINB10 and LRRC17and LEP, USP10, HPS4, SYNE2, PILRA, MST1, TRIP6, NPR2, CLEC2D [43].

12. Whole Exome Sequencing

Given the potential use of next generation sequencing for newborn screening in many different diseases and syndromes, WES can be used as a screening test for TS in newborns [44].

The Newborn Screening Biobanks can be used for genome-wide association studies. Frost storage generally serves to preserve the overall sample quality compared to samples stored at room temperature [45].

DNA extracted from a fraction (2×3.2mm discs) of an archived neonatal dried blood spot samples (DBSS) can be whole genome amplified (wgaDNA) and used for accurate array genotyping. The genotyping accuracy of neonatal wgaDNA has previously been questioned. However, several studies have shown that the wgaDNA performs equally well compared to high-quality DNA of other sources. wgaDNA from DBSS can be used for accurate whole genome sequencing (WGS) and exome targeted next-generation sequencing (WES). The validation of samples by NGS typically involve the parallel sequencing of a high-quality DNA reference to be used as a gold standard for comparison. wgaDNA of neonatal DBS samples performs equally to high-quality reference DNA in WES [46,47].

Personalized Genomic Research study at the National Institutes of Health performed whole-exome sequencing (WES) and evaluated the copy number variation using EXCAVATOR2 and B allele frequency was calculated from informative single nucleotide polymorphisms (SNPs). Simulated WES data were generated for detection of low-level mosaicism and complex structural chromosome abnormalities. Sensitivity and specificity were both 100% for the diagnosis of TS with no false positive or false negatives. Using simulated WES data, detection rate of isochromosome Xq and low-level mosaicism was as low as 5%. WES can be used as a screening test for TS in newborns [44].

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