

## Laboratory guideline for Turner syndrome

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**Disclaimer:** This guideline is designed primarily as an educational resource for health care providers to help them provide quality medical genetic services. Adherence to this guideline does not necessarily ensure a successful medical outcome. This guideline should not be considered inclusive of all proper procedures and tests or exclusive of other procedures and tests that are reasonably directed to obtaining the same results. In determining the propriety of any specific procedure or test, the geneticist should apply his or her own professional judgment to the specific clinical circumstances presented by the individual patient or specimen. It may be prudent, however, to document in the patient's record the rationale for any significant deviation from this guideline.

**Abstract:** Turner syndrome is a disorder that has distinct clinical features and has karyotypic aberrations with loss of critical regions of the X chromosome. Several clinical guidelines on the diagnosis and management of patients with Turner syndrome have been published, but there is relatively little on the laboratory aspects associated with this disorder. This disease-specific laboratory guideline provides laboratory guidance for the diagnosis/study of patients with Turner syndrome and its variants. Because the diagnosis of Turner syndrome involves both a clinical and laboratory component, both sets of guidelines are required for the provision of optimal care for patients with Turner syndrome. *Genet Med* 2010;12(1):52–55.

**Key Words:** Turner syndrome, guideline, cytogenetics, mosaicism

Although clinical guidelines have addressed diagnosis of Turner syndrome,<sup>1–5</sup> laboratory guidelines are lacking. This American College of Medical Genetics (ACMG) laboratory guideline provides information on appropriate pre- and post-natal diagnostic cytogenetic studies for Turner syndrome. Disease-specific statements are intended to augment the current general ACMG Standards and Guidelines for Clinical Genetics Laboratories. Individual laboratories are responsible for meeting the Clinical Laboratory Improvement Amendments/College of American Pathologists quality assurance (QA) standards with respect to appropriate sample documentation, assay validation, general proficiency, and quality control measures.

This guideline is based on peer-reviewed scientific literature to the extent possible. However, there are relatively few articles published on laboratory practice for Turner syndrome, and

hence expert consensus opinion was elicited to obtain best practices. The guideline was reviewed extensively by select experts on Turner syndrome, including Cytogeneticists and Clinical Geneticists, the ACMG Cytogenetic subcommittee of the Laboratory QA Committee, the QA Committee, and the ACMG Board of Directors. In addition, the document was vetted by the entire ACMG membership before adoption as a supplement to the ACMG Laboratory Standards and Guidelines.

### BACKGROUND ON TURNER SYNDROME

#### Chromosome locus

A majority of genes associated with the physical features observed in Turner syndrome are located on Xp (Xp11.2-p22)<sup>6</sup>; loci contributing to ovarian function reside in Xq (Xq24).<sup>7</sup>

#### Disease incidence and karyotypic finding

The disease incidence is approximately 1 in 2500 liveborn females. A 45,X karyotype is observed in ~1% to 2% of conceptuses, 10% of miscarriages and 1% of stillbirths. Greater than 99% of 45,X conceptuses result in spontaneous loss, usually before 28 weeks. The reason why ~1% survive to term with relatively minor somatic abnormalities is not known, although it has been hypothesized that this is due to undetected mosaicism for a cell line with all or part of a second sex chromosome.<sup>8,9</sup>

#### Karyotype findings associated with Turner syndrome

**Prenatal.** Approximately 1% to 2% of conceptuses have a 45,X karyotype. These fetuses typically have ultrasound findings such as cystic hygroma or nuchal thickening. A majority of cases with mosaicism for a 45,X cell line and a cell line with a second structurally normal sex chromosome result in the birth of a child with a normal phenotype.<sup>10,11</sup>

**Postnatal.** Apparently nonmosaic monosomy X is found in ~45% of patients with Turner syndrome postnatally. A structural chromosome abnormality or mosaicism for 45,X and another cell line is found in the lymphocytes of the remaining patients with Turner syndrome (Table 1).

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**Table 1** Turner syndrome karyotypes

Karyotype	Percentage of TS cases <sup>12-14</sup> (%)
45,X	45
46,X,i(X)(q10) w/ or w/o 45,X	15-18
46,X,+mar or +r w/ or w/o 45,X	7-16
45,X/46,XX or 45,X/47,XXX	7-16
46,X,del(Xp) w/ or w/o 45,X	2-5
46,XY or 46,X,del(Y) or 46,X,r(Y) w/ 45,X	6-11
Others	2-8

### Brief clinical description

The features of Turner syndrome include characteristic physical features and complete or partial absence of the second sex chromosome. Phenotypic features vary widely but commonly include short stature, ovarian failure, edema of hands or feet, nuchal folds, left-sided cardiac anomalies, low hairline, low set ears, small mandible, cubitus valgus, nail hypoplasia, hyperconvex nails, multiple pigmented nevi, characteristic facies, short fourth metacarpal, and high arched palate. Females with short stature and deletion of the distal region of Xp including the *SHOX* gene are generally not diagnosed with Turner syndrome. Likewise, individuals with deletions of Xq24 with primary or secondary amenorrhea without short stature are typically diagnosed with premature ovarian failure.<sup>15</sup>

### Mode of inheritance

Turner syndrome is sporadic. A majority of cases ascertained prenatally have a 45,X karyotype. Paternal nondisjunction accounts for ~70% of liveborn cases with a 45,X.<sup>16-17</sup>

### Ethnic association

Turner syndrome is seen in all ethnic groups.

### Special testing considerations

A differential diagnosis that includes Turner syndrome must take into consideration phenotypic features in combination with karyotypic findings. The phenotype varies greatly, therefore, both laboratory and clinical factors must be considered before a diagnosis may be rendered. Mental retardation is not a feature of Turner syndrome. The only sex chromosome structural abnormalities likely to cause mental retardation are a ring X chromosome with loss of *XIST* gene function and certain X-autosome translocations.

### Presence of Y chromosome material

Mosaicism for a cell line with a normal or abnormal Y chromosome is identified in 6% to 11% of patients with Turner syndrome with standard cytogenetic techniques. Identification of Y chromosome material in females with Turner syndrome is important because of the risk of gonadoblastoma.<sup>18</sup> A gonadoblastoma is a neoplasm composed of germ cells and sex cord elements with an excellent prognosis if detected early. However, gonadoblastoma can progress to dysgerminoma with metastatic potential. A gonadoblastoma-susceptibility locus has been proposed for the pericentromeric region of the Y chromosome.<sup>19-20</sup> The neoplasm does not appear to correlate with the presence of *SRY*.

Occult Y chromosome mosaicism detected by techniques other than standard cytogenetics in Turner syndrome varies by study and with methodology used.<sup>13,21-25</sup> A meta-analysis of studies reporting a total of 541 patients with Turner syndrome without Y chromosome material on routine cytogenetic analysis found 5% mosaicism for a Y-containing cell line using molecular techniques (Southern blot and/or polymerase chain reaction [PCR]). The percentage of patients with Y chromosome mosaicism (by molecular or standard cytogenetic techniques) was 8%, and, of these, 12% had gonadoblastoma. Detection of occult Y mosaicism in 45,X subjects using interphase fluorescent in situ hybridization (FISH) with a probe for the Y centromere (DYZ3) has been reported to range from 0% to 4%.<sup>24,25</sup>

### Prenatal testing

Monosomy X is frequently identified by prenatal diagnostic procedures. Ultrasound findings can include nuchal translucency, cystic hygroma, coarctation of the aorta and/or other left-sided heart defects, brachycephaly, renal anomalies, polyhydramnios, oligohydramnios, and growth retardation. Abnormal prenatal serum marker screening results with elevated levels of human chorionic gonadotropin and inhibin and slightly decreased levels of alpha fetoprotein and unconjugated estriol are associated with an increased likelihood of a Turner syndrome diagnosis.<sup>27,28</sup> Prenatal diagnosis may indicate a karyotype consistent with a diagnosis of Turner syndrome; however, the phenotype of the individual cannot be predicted based on the chorionic villus or amniotic fluid cell karyotype, FISH, or microarray results. In the absence of abnormal prenatal ultrasound findings, girls with incidental prenatal karyotype findings associated with Turner syndrome have a less severe phenotype with fewer physical abnormalities compared with those diagnosed due to abnormal ultrasound findings. Because the constitutional karyotype of individuals with prenatal ascertainment of sex chromosome complements consistent with Turner syndrome is uncertain, postnatal chromosome studies are recommended. Failure to confirm the prenatal findings with a blood karyotype should prompt consideration of analysis of another tissue such as buccal or skin cells.

Females with monosomy X or a structurally abnormal X chromosome may manifest X-linked recessive disorders; when an X-linked recessive disorder is identified in a female, karyotyping is warranted.

The detection of a low level of 45,X cells (<10%) during routine cytogenetic analysis of peripheral blood or bone marrow from an adult female can be difficult to interpret. It has been well documented that there is age-related loss of the X chromosome.<sup>29</sup> Laboratories should have procedures for an evaluation that assists with the determination of the significance of 45,X cells detected in women based on age, and for reporting, when appropriate.

## GUIDELINE

### Methodological considerations

#### Prenatal diagnosis

Refer to the ACMG Laboratory Standards and Guidelines (www.acmg.net, 2008) for general laboratory recommendations on prenatal diagnosis.

When multiple cells from a single culture are identified with a 45,X karyotype, a moderate work-up is warranted. This includes examination of an additional 20 cells from cultures other than the one with the initial finding or 12 colonies from coverslips other than the one with the abnormality.<sup>30</sup>

Additional cells or colonies should be evaluated when a structurally abnormal X chromosome is identified to determine if the finding is true or pseudomosaicism. Depending on the aberration (e.g., marker chromosome and unbalanced or balanced structural abnormality in single or multiple cultures), guidelines for moderate or extensive evaluation would apply.<sup>30</sup>

When an apparent sex chromosome marker is identified in a fetus with only a single X chromosome, a moderate (if found in a single cell in culture or on a single coverslip) or extensive work-up (if found in multiple cells in a single culture or multiple colonies from a single coverslip) is recommended.<sup>30</sup>

If the marker is found in every cell or is mosaic, additional testing to identify the origin of the marker using FISH with X and Y centromere probes is recommended.

When a small ring or small marker chromosome is determined to be derived from the X chromosome, FISH with a probe for the XIST gene should be performed. Depending on the size and content of the small ring/marker, lack of the XIST locus may be associated with a more severe phenotype that includes mental retardation.<sup>26,31</sup>

For interphase analysis using X and Y centromere probes, normal cutoff values should be established for a second X chromosome signal using normal male controls, and for a Y chromosome signal using normal female controls.<sup>25</sup> See section E10 for information on establishment of a normal cut-off value (ACMG Standards and Guidelines, www.acmg.net, 2008).

### Postnatal studies

Cases being studied for possible Turner syndrome, in which mosaicism is common, should include a minimum of 30 cells counted, unless mosaicism is documented within the first 20 cells. When low-level mosaicism for 45,X is detected, the age of the female should be taken into consideration to ensure that the 45,X cell line is not due to age-related loss.<sup>29</sup>

Cytogenetic study of a second tissue (e.g., skin biopsy for cell culture or buccal smear for FISH) should be considered in individuals with a 46,XX karyotype only if there is a high level of suspicion for Turner syndrome based on phenotype. Consultation with the referring physician is recommended to determine whether a second tissue should be studied.

Additional studies are warranted if a 30-cell analysis reveals an apparently nonmosaic 45,X karyotype. In patients without virilizing features with a nonmosaic 45,X karyotype, FISH analysis using X and Y probes can identify low-level sex chromosome mosaicism.<sup>25</sup> Although the frequency of gonadoblastoma in Turner syndrome with occult Y chromosome mosaicism without evidence of virilization is not fully appreciated, additional studies are recommended in these cases to help prevent a potential life-threatening cancer.<sup>32</sup>

FISH with X and Y centromere probes should be performed on a minimum of 200 interphase cells. An appropriate database should be created and normal cutoff values should be established for a Y chromosome signal using normal female controls.<sup>25</sup> See section E.10 for information on establishment of a normal cutoff value (ACMG Standards and Guidelines, www.acmg.net, 2008).

If the patient reveals an apparently nonmosaic 45,X karyotype and has clitoromegaly or other masculinizing features, it is very likely that there is mosaicism for a Y chromosome-containing cell line.<sup>15</sup> FISH with probes for the X and Y centromeres should be performed on a minimum of 200 cells to detect low-level Y chromosome mosaicism. Given the high suspicion for Y chromosome material, study of a second cell type may be warranted. Consultation with the referring physician is recommended.

Additional studies should be performed when a small ring/marker chromosome is identified. This is the same as for section "Prenatal Diagnosis" earlier. Note that there is not always a correlation between the presence of XIST in the ring and cognitive phenotype.<sup>33</sup> The size of the active ring appears to have a greater correlation with outcome.<sup>34–36</sup>

### Interpretation and reporting

The following elements should be included in the report, in addition to the items described in the current general Standards and Guidelines.

Referral for genetic counseling and evaluation by a clinical geneticist and/or other appropriate health care provider.<sup>15,37</sup>

When a karyotype consistent with Turner syndrome is found prenatally, postnatal chromosome analysis is recommended to document the child's karyotype.<sup>38</sup>

## ALTERNATIVE TESTING METHODS

### PCR

For detection of Y-chromosomal material using PCR, a high rate of false-positive results has been reported.<sup>25,39</sup> Thus, caution should be exercised in the interpretation of Y-chromosome sequence PCR. FISH confirmation using a Y centromere probe after a positive PCR result is prudent.

Genomic copy number microarray studies can be used to further characterize abnormalities that are detected by cytogenetic studies. However, microarrays may not detect low-level mosaicism and should not be used as the initial screen for sex chromosome abnormalities.

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