

X-Chromosome Inactivation Analysis

Females typically have two copies of the X chromosome with one copy randomly inactivated by Lyonization early in embryonic development. In each somatic cell, either the paternally inherited X chromosome or the maternally inherited X chromosome is inactivated, which silences gene expression of most genes on that chromosome. Identification of nonrandom, or skewed, X chromosome inactivation (XCI) patterns in females may assist in evaluation of X-linked disorders. For example, the expression of X-linked disease in a female may depend on whether the X chromosome with the disease-causing variant is preferentially activated or silenced. Females with a pathogenic variant in an X-linked recessive gene may develop manifestations of the disorder if the X chromosome harboring the variant is preferentially active. XCI analysis may be useful to help determine pathogenicity of variants detected in X-linked genes. Nonrandom XCI is often defined as a ratio of active to inactive X chromosome of 80:20 or greater.

DISEASE OVERVIEW

Physiology

- Females typically have two copies of the X chromosome
 - One copy is randomly inactivated early in embryonic development by lyonization
 - Allows females to produce same amount of gene products from X-linked genes as males
 - Majority of genes on the inactivated chromosome are silenced
 - Many of the CpG islands are methylated
- Preferential inactivation of either the paternally or maternally derived X chromosome produces a nonrandom pattern of XCI
 - Nonrandom defined as XCI ratio 80:20 or greater
 - Nonrandom XCI patterns can result from
 - Secondary cell selection in women who are heterozygous for X-chromosome rearrangements
 - Cell selection bias in females carrying a variant for an X-linked disorder
 - Neoplasia

Diagnostic Issues

- Nonrandom XCI may influence expression of X-linked disorders
 - Female carriers may be symptomatic in X-linked recessive disorders if the affected X chromosome is preferentially activated
 - Female carriers may be asymptomatic in X-linked dominant disorders if the affected X chromosome is preferentially inactivated
 - For some X-linked diseases, there is a strong selection bias for XCI in favor of cells with the variant
- Assessing XCI in a carrier mother may help to determine the pathogenicity of a genetic variant in an X-linked gene detected in her offspring

GENETICS

The highly polymorphic CAG repeat in exon 1 of the androgen receptor (AR) gene on the X chromosome is used to distinguish the maternally inherited from paternally inherited X chromosome.

- At least 80% of women are heterozygous at the analyzed AR locus, allowing for differentiation between maternal and paternal X chromosomes
- Restriction sites near the AR gene are methylated on the inactive X chromosome and unmethylated on the active X chromosome
- Methylation-sensitive restriction enzymes are able to digest DNA only on the active X chromosome
- Methylation is correlated with XCI

Tests to Consider

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Method: Restriction Enzyme Digestion/Polymerase Chain Reaction/Fragment Analysis

- Use to determine XCI pattern for female carriers of XCI disorders and assess pathogenicity of genetic variant in an X-linked gene
- XCI ratio reported for the tissue type tested (ranges from 50:50 to 100:0)
- Does not detect clonality
- Testing limited to XX females
- When nonrandom XCI detected, parent of origin of the active X cannot be determined without testing parental samples
- Fetal specimens are not accepted

TEST INTERPRETATION

Sensitivity/Specificity

Clinical sensitivity: 90%

- 10-15% of females have nonrandom XCI by chance
 - Increases with age

Results

- Nonrandom XCI ratio: 80:20 to 100:0
 - Suggests nonrandom pattern of XCI in tissue type tested
- Random XCI ratio: 50:50 to 79:21
 - Suggests random pattern of XCI in tissue type tested
- Uninformative result: XCI ratio cannot be determined
 - Maternally and paternally derived X chromosomes could not be distinguished

Limitations

- Testing limited to XX females only
- Assay will be uninformative in up to 20% of females due to homozygosity for the polymorphic AR gene locus analyzed
- XCI patterns may differ among tissues
- XCI ratio reported is for the tissue type tested with a standard deviation 0.09 in random XCI; 0.06 in nonrandom XCI
- Will not determine if the X-inactivation pattern is associated with rearrangements of the X chromosome, pathogenic variants in X-linked genes, or neoplastic disease
- If nonrandom XCI pattern is present, parent of origin of the active X cannot be determined without testing parental samples
- XCI ratios should not be used to predict prognosis for female carriers of X-linked disorders as variable expressivity may result due to other genetic or environmental modifiers
- Test is not recommended for prenatal diagnosis because XCI levels may differ in prenatal specimens and whole blood
- Diagnostic errors can occur due to rare sequence variations

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