

X-Chromosome Inactivation Analysis

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Females typically have two copies of the X chromosome, with one copy randomly inactivated by lyonization early in embryonic development, which allows females to produce the same amount of gene products from X-linked genes as males. In each somatic cell, either the paternally inherited X chromosome or the maternally inherited X chromosome is inactivated. The majority of genes on the inactivated chromosome are silenced, and many of the CpG islands are methylated. Identification of nonrandom, or skewed, X-chromosome inactivation (XCI) patterns in females may assist in evaluation of X-linked disorders. XCI analysis may be useful to help determine pathogenicity of variants detected in X-linked genes. Nonrandom XCI is defined as a ratio of active to inactive X chromosome greater than 85:15.

Disease Overview

Physiology

- Preferential inactivation of either the paternally or maternally derived X chromosome produces a nonrandom pattern of XCI, which 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.
 - In 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 can be used to distinguish the maternally inherited from the 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.

Test Interpretation

Sensitivity/Specificity

Clinical sensitivity: 90%

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

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Method: Restriction Enzyme Digestion/Polymerase Chain Reaction (PCR)/Fragment Analysis

- Use to determine XCI pattern for female carriers of X-linked 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 is detected, parent of origin of the active X cannot be determined without testing parental samples
- Fetal specimens are not accepted

Results

- Nonrandom XCI ratio: 86:14 to 100:0
 - Suggests nonrandom pattern of XCI in tissue type tested
- Random XCI ratio: 50:50 to 74:26
 - Suggests random pattern of XCI in tissue type tested
- Caution is advised when interpreting XCI ratios between 75:25 and 85:15.
- Uninformative result: XCI ratio cannot be determined.
 - Maternally and paternally derived X chromosomes could not be distinguished.

Limitations

- Testing is limited to XX females only.
- The 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 (SD) of 0.08 for XCI ratios of 50:50-79:50, and an SD of 0.05 for XCI ratios of 80:20 or greater.
- Test 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, the 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; 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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