

# X-Chromosome Inactivation Analysis

## Indications for Ordering

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- Determine X-chromosome inactivation (XCI) pattern for female carriers of X-linked disorders
- Assess pathogenicity of genetic variant in an X-linked gene

## Test Description

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- Methylation-sensitive restriction enzyme digestion followed by PCR and fragment analysis
- XCI ratio reported for the tissue type tested (ranges from 50:50 to 100:0)

## Tests to Consider

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### [X-Chromosome Inactivation Analysis 2006352](#)

- Does not detect clonality

## Disease Overview

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**Prevalence and/or incidence** – varies by disorder

### Physiology

- Females typically have 2 copies of the X chromosome
  - One copy is randomly inactivated early in the embryonic process 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 or skewed pattern of XCI
  - Skewed defined as XCI ratio of active to inactive <20% or >80%
  - Skewed XCI patterns can result from
    - Secondary cell selection in women who are heterozygous for X-chromosome rearrangements
    - Cell selection bias in females carrying a mutation for an X-linked disorder
    - Neoplasia

## Diagnostic issues

- Skewed 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 mutation
- 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

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**Gene** – CAG repeat in exon 1 of the androgen receptor (*AR*) gene on the X chromosome

### Structure/function

The highly polymorphic CAG repeat is used to distinguish maternally from paternally inherited X-chromosomes

- 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

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### Sensitivity/specificity

Clinical sensitivity – 90%

- 10% of women have skewed XCI by chance
  - Increases with age

### Results

- Positive result – pattern of skewed XCI detected
  - Nonrandom XCI ratios 80:20 to 89:11 – moderately skewed
  - Nonrandom XCI ratios 90:10 to 100:0 – highly skewed
- Negative – no pattern of skewed XCI detected
  - Random – XCI ratio of <80:20 detected
- Uninformative result – XCI ratio cannot be determined
  - Maternally and paternally derived x chromosomes could not be distinguished

**Limitations**

- Testing limited to XX females only
- Test will be uninformative if there is homozygosity at the analyzed *AR* locus (up to 20% of women)
- XCI patterns may differ among tissues
- Will not determine whether the XCI pattern is associated with rearrangements of the X chromosome, gene mutations in X-linked genes, or neoplastic disease
- Not recommended for prenatal diagnosis
- Parental origin of the active X chromosome cannot be determined without parental samples in cases of nonrandom XCI
- Should not be used to predict prognosis for female carriers of X-linked disorders
- Does not detect clonality