X-Chromosome Inactivation Analysis

Indications for Ordering

- 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

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

Tests to Consider

X-Chromosome Inactivation Analysis 2006352
- Does not detect clonality

Disease Overview

Prevalence and/or incidence – varies by disorder

Physiology
- Females typically have 2 copies of the X chromosome
  - 1 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

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 inherited 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

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