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

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- Does not detect clonality

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 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% of women have nonrandom XCI by chance
  - Increases with age

Results

- Nonrandom XCI ratio- 80:20 to 100:0
  - Suggests skewed 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
• Test will be uninformative if there is homozygosity at the analyzed AR locus (up to 20% of women)
• XCI patterns may differ among tissues
• XCI ratio reported is for the tissue type tested with standard deviation 0.09 in random XCI; 0.06 in nonrandom XCI
• Will not determine whether the XCI pattern is associated with rearrangements of the X chromosome, gene variants 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