Thiopurine Methyltransferase (TPMT) Genotyping, 4 Variants

Indications for Ordering

- Individuals being considered for thiopurine therapy
- Individuals who have experienced an adverse reaction to thiopurine therapy

Test Description

Polymerase chain reaction (PCR) and fluorescence monitoring

Tests to Consider

Primary test

Thiopurine Methyltransferase (TPMT) Genotyping, 4 Variants 2012233

- Assess risk, due to genetics, for severe myelosuppression with standard dosing of thiopurine drugs
- Appropriate for pre- or posttherapeutic assessments
- Preferred test for patients with recent heterologous blood transfusion
- Can be performed irrespective of thiopurine therapy

Related tests

Thiopurine Methyltransferase, RBC 0092066

- Phenotype test to assess risk for severe myelosuppression with standard dosing of thiopurine drugs
- Can also detect rapid metabolizer phenotype
- Must be performed before thiopurine therapy is initiated

Disease Overview

Prevalence of phenotype

- Very low/absent TPMT activity – ~3/1,000 individuals
- Intermediate TPMT activity – ~10% of Caucasian individuals
- Normal TPMT activity – ~90% of individuals
- High TPMT activity – unknown

Pathophysiology

- Thiopurine drugs are purine antimetabolites and include
  - Azathioprine (AZA, Imuran)
  - 6-mercaptopurine (6-MP, Purinethol)
  - 6-thioguanine (6-TG, Tabloid)
- Thiopurines must be metabolized to 6-thioguanine nucleotides (6-TGN) for activity
  - Proportion of active 6-TGN is regulated by the balance between activation and inactivation mechanisms
- Primary metabolic route for inactivation of thiopurine drugs is catalyzed by TPMT
  - Low TPMT activity – more 6-MP may be converted into active (cytotoxic) 6-TGN
  - Active/cytotoxic metabolites will accumulate when TPMT activity is low
    - Excess 6-TG in the bone marrow inhibits purine synthesis
      - Inhibits cell proliferation
      - Contributes to excessive myelosuppression
- TPMT enzyme can be inhibited by common drugs
  - NSAIDs
    - Ibuprofen
    - Ketoprofen
    - Naproxen
    - Mefenamic acid
  - Diuretics
    - Furosemide
    - Thiazides
  - Ulcerative colitis drugs
    - Mesalamine
    - Olsalazine
    - Sulfasalazine

Treatment issues

- AZA, 6-MP, and 6-TG are inactive prodrugs
  - Used to treat
    - Acute lymphoblastic leukemia
    - Autoimmune diseases
    - Inflammatory bowel disease

- Used to prevent rejection after solid organ transplant
  - For individuals with low to intermediate TPMT activity, myelosuppression is a major issue
  - Individuals with very low/no TPMT enzyme activity universally experience severe myelosuppression when receiving conventional thiopurine doses
  - Reduced drug dosing in these individuals may reduce risk for myelosuppression
- ~30-60% of individuals with intermediate TPMT activity who receive conventional thiopurine doses experience moderate to severe myelosuppression
- Thiopurine dosing should rely on disease-specific guidelines and degree of myelosuppression
- Guidelines for thiopurine dosing by the Clinical Pharmacogenetics Implementation Consortium (CPIC) can be found at www.pharmgkb.org/gene/PA356

Genetics

Gene – *TPMT*

Inheritance – autosomal codominant

Penetrance – drug dependent

Allele variants

- >20 *TPMT* deficiency alleles identified to date
- TPMT deficiency alleles tested
  - *2 (c.238G>C; p.Ala80Pro)
  - *3A (c.[460G>A;719A>G]; p.[Ala154Thr;Tyr240Cys])
  - *3B (c.460G>A ; p.Ala154Thr)
  - *3C (c.719A>G; p.Tyr240Cys)

Allele frequencies

- *TPMT* *2
  - African – 0.000792
  - Asian – 0.0
  - Caucasian – 0.00190
  - Mediterranean – 0.00408
  - Mexican – 0.00592
  - Middle Eastern – 0.00749
- *TPMT* *3A
  - African – 0.00198
  - Asian – 0.0001118
  - Caucasian – 0.0356
  - Mediterranean – 0.0254
  - Mexican – 0.0533
  - Middle Eastern – 0.0114
- *TPMT* *3B
  - African – 0.0
  - Asian – 0.0
  - Caucasian – 0.000461
  - Mediterranean – 0.00426
  - Mexican – 0.00690
  - Middle Eastern – 0.00562
- *TPMT* *3C
  - African – 0.0495
  - Asian – 0.0157
  - Caucasian – 0.004205
  - Mediterranean – 0.00545
  - Mexican – 0.00888
  - Middle Eastern – 0.00562

Test Interpretation

Sensitivity/specificity

- Clinical sensitivity – 95% (Evans, 2004; Yates, 1997)
- Analytical sensitivity/specificity – 99%

Results

- Positive
  - Homozygosity or compound heterozygosity detected for *TPMT* deficiency alleles
    - Associated with very low/no TPMT enzyme activity
    - Very high risk for thiopurine drug-related toxicity with conventional doses
  - Heterozygosity detected for *TPMT* deficiency allele
    - Associated with intermediate TPMT enzyme activity
    - Increased risk for thiopurine drug-related toxicity with conventional doses
- Negative
  - No variants detected
    - Predictive of *1 functional alleles
      - Predicts normal TPMT enzyme activity and normal risk for thiopurine drug-related myelotoxicity
- Inconclusive
  - Phase of identified variants cannot be determined
    - Genotyping cannot distinguish the *1/*3A genotype (intermediate TPMT activity) from the *3B/*3C genotype (low or no TPMT activity)
      - *3B/*3C genotype is very rare (~ 1/500,000 Caucasians)
    - TPMT phenotyping and/or monitoring thiopurine metabolite concentrations is recommended to optimize therapy

Limitations

- Only targeted *TPMT* allele variants will be detected by this panel
- Diagnostic errors can occur due to rare sequence variations
- Genotyping in individuals who have received allogenic stem cell/bone marrow transplant will reflect donor status
- Genotyping cannot distinguish the *1/*3A genotype from the *3B/*3C genotype
- Thiopurine drug metabolism and risk for toxicity may be affected by genetic and nongenetic factors that are not evaluated by this test
- Test does not assess for *TPMT* allele variants associated with ultra-high enzyme activity
- Genotyping does not replace the need for therapeutic drug monitoring or clinical observation

References

- Evans WE. Pharmacogenetics of thiopurine S-methyltransferase and thiopurine therapy. Ther Drug Monit. 2004;26(2):186-191
- Relling MV, Gardner EE, et al. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing: 2013 update. Clin Pharmacol Ther. 2013:93(4):324-325
- Yates CR, Krynetski EY, et al. Molecular diagnosis of thiopurine S-methyltransferase deficiency: genetic basis for azathioprine and mercaptopurine intolerance. Ann Intern Med. 1997;126(8):608-614