Chronic Granulomatous Disease

Indications for Ordering

- Confirm a clinical or laboratory diagnosis of chronic granulomatous disease (CGD)
- Assess carrier status for CGD
- Predictive testing for unaffected at-risk relatives

Test Description

Semiquantitative flow cytometry

Tests to Consider

Typical testing strategy

- A provisional diagnosis of CGD is made using functional tests to detect the absence or reduction of oxidase activity in activated neutrophils (eg, respiratory burst test)
  - Individuals with CGD demonstrate decreased or absent NADPH oxidase activity on functional analysis
- If abnormal oxidase function is noted, molecular testing to confirm the causative variant(s) is necessary for
  - Diagnostic confirmation
  - Genetic counseling
  - Prenatal diagnosis

Primary tests

Neutrophil Oxidative Burst Assay (DHR) 0096657
  - Aid in screening for CGD

Chronic Granulomatous Disease (CYBB Gene Scanning and NCF1 Exon 2 GT Deletion) with Reflex to CYBB Sequencing 2006356
  - Preferred test to assess common molecular causes of CGD

Related tests

Chronic Granulomatous Disease, X-Linked (CYBB) Gene Scanning with Reflex to Sequencing 2006361
  - Molecular test to confirm a diagnosis or assess carrier status for X-linked CGD

Chronic Granulomatous Disease (NCF1) Exon 2 GT Deletion 2006366
  - Tests for a common NCF1 pathogenic variant associated with autosomal recessive CGD

Familial Mutation, Targeted Sequencing 2001961
  - Useful when a pathogenic familial variant identifiable by sequencing is known

Disease Overview

Incidence – 1/250,000 births in the U.S.

- X-linked CGD
  - CYBB – 60-70% of all cases
- Autosomal recessive CGD
  - NCF1 – 25% of cases
  - CYBA – <5% of cases
  - NCF2 – <5% of cases
  - NCF4 – very rare

Pathophysiology

- Primary immunodeficiency disorder that results from changes within genes encoding the essential subunits of the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex
  - NADPH complex produces reactive oxygen species necessary to kill bacterial and fungal microorganisms
  - Pathogenic variants cause defective function of NADPH complex in the leukocytes
  - CGD leukocytes are unable to produce the superoxide, hydrogen peroxide, hydroxyl ion, and hypochlorous acid necessary for intracellular destruction of phagocytized pathogens and results in
    - Recurrent, severe bacterial and fungal infections
    - Dysregulated inflammatory responses leading to granulomas at infection sites

- Common infectious agents include
  - Staphylococcus aureus
  - Burkholderia cepacia
  - Serratia marcescens
  - Nocardia spp
  - Aspergillus spp

Symptoms

- Recurrent severe bacterial and fungal infections of various organs
  - Lymph nodes
  - Liver
  - Lungs
  - Bones
  - Visceral organs
- Granulomas form at infection sites
• Other findings
  o Poor wound healing
  o Hypergammaglobulinemia
  o Splenomegaly
  o Chorioretinitis
  o Colitis/enteritis
  o Obstructions of urinary tract or gastric outlet
• Males with classic X-linked CGD are typically diagnosed before 3 years of age
  o Less severe phenotypes have been observed and may be diagnosed later in life
• Carrier females of X-linked disease (~50%) may have mild symptoms, including
  o Photosensitivity
  o Recurrent mouth ulcers
• Females with skewed X-chromosome inactivation
  o Rare
  o Severe disease presentation
• Individuals with X-linked CGD typically have earlier onset and more severe disease than individuals with variant X-linked or autosomal recessive CGD

Diagnostic issues
• Early diagnosis is essential — disease management relies on lifelong antibiotic and antifungal prophylaxis
• Disease severity can be estimated by the level of NADPH oxidase activity associated with a particular CYBB gene variant
  o Genetic or environmental modifiers may result in variable clinical outcomes

Genetics

Genes — CYBB; NCF1 GT deletion in exon 2

Inheritance
• CYBB – X-linked
• NCF1 – autosomal recessive

De novo variants – 10-20% of CYBB variants

Variants
• >600 pathogenic variants
  o ~90% of CYBB variants are small nucleotide insertions, deletions, or substitutions
  o ~10% are large deletions
• Identical CYBB variants in different individuals can result in variable clinical outcomes

Other non-CYBB variants associated with CGD
• NCF1 – encodes p47-phox
  o GT deletion in exon 2 accounts for majority of variants
• CYBA – encodes p22-phox
• NCF2 – encodes p67-phox
• NCF4 – encodes p40-phox

Test Interpretation

Chronic Granulomatous Disease (CYBB Gene Scanning and NCF1 Exon 2 GT Deletion) with Reflex to CYBB Sequencing

Sensitivity/specificity
• Clinical sensitivity – 86% for CGD
• Analytical sensitivity – 99% for CYBB or homozygous NCF1 GT deletion; 90% for heterozygous NCF1 GT deletion
• Analytical specificity – 99%

Results
• Positive – pathogenic variant was detected
  o CYBB variant
    ▪ In symptomatic male – confirms X-linked CGD
    ▪ In asymptomatic female – confirms carrier status for X-linked CGD
  o NCF1 GT deletion
    ▪ 2 copies confirm autosomal recessive CGD
    ▪ 1 copy confirms carrier status for autosomal recessive CGD
• Negative – CYBB gene pathogenic variant or the common NCF1 GT deletion not detected
  o Reduces, but does not eliminate, the possibility of CGD
• Inconclusive – gene scanning/sequencing may detect novel variants of unknown clinical significance

Limitations
• Diagnostic errors can occur due to rare sequence variations
• Deep intronic variants in CYBB, variants in NCF1 other than the GT deletion in exon 2, and variants in additional genes associated with CGD are not evaluated
• Large CYBB gene deletions/duplications will not be detected in females
• Breakpoints of large CYBB deletions/duplications will not be determined in males
• Lack of GT deletion in exon 2 does not rule out carrier status
  o Due to potential recombination between NCF1 and its pseudogenes