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 mutation(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* mutation 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
  - Mutations 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
  - Poor wound healing
  - Hypergammaglobulinemia
  - Splenomegaly
  - Chorioretinitis
  - Colitis/enteritis
  - Obstructions of urinary tract or gastric outlet
- Males with classic X-linked CGD are typically diagnosed before 3 years of age
  - 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
  - Photosensitivity
  - Recurrent mouth ulcers
- Females with skewed X-chromosome inactivation
  - Rare
  - 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 mutation
  - 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 mutations – 10-20% of *CYBB* mutations

Mutations

- >600 pathogenic mutations
  - ~90% of *CYBB* mutations are small nucleotide insertions, deletions, or substitutions
  - ~10% are large deletions
- Identical *CYBB* mutations in different individuals can result in variable clinical outcomes

Other non-*CYBB* mutations associated with CGD

- *NCF1* – encodes p47-phox
  - GT deletion in exon 2 accounts for majority of mutations
- *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 mutation was detected
  - *CYBB* mutation
    - In symptomatic male – confirms X-linked CGD
    - In asymptomatic female – confirms carrier status for X-linked CGD
  - *NCF1* GT deletion
    - 2 copies confirm autosomal recessive CGD
    - 1 copy confirms carrier status for autosomal recessive CGD
- Negative – *CYBB* gene mutation or the common *NCF1* GT deletion not detected
  - 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 mutations in *CYBB*, mutations in *NCF1* other than the GT deletion in exon 2, and mutations 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
  - Due to potential recombination between *NCF1* and its pseudogenes