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 Descriptions

Neutrophil Oxidative Burst Assay (DHR)
- Semiquantitative flow cytometry

CYBB Sequencing
- Bidirectional sequencing of the *CYBB* coding region and intron-exon boundaries

CYBB Sequencing and *NCF1* Exon 2 GT Deletion
- Bidirectional sequencing of the *CYBB* coding region and intron-exon boundaries
- Polymerase Chain Reaction/High-Resolution Melt Analysis to assess for the common pathogenic *NCF1* c.75_76delGT variant

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 Panel (*CYBB* Sequencing and *NCF1* Exon 2 GT Deletion) 3000544
- Preferred test to assess common molecular causes of CGD

Related tests

Chronic Granulomatous Disease, X-Linked (*CYBB*) Sequencing 3000541
- 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 pathogenic *NCF1* 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, leading to
  - 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
- Approximately 50% of carrier females of X-linked disease may have mild symptoms, including
  - Photosensitivity
  - Recurrent mouth ulcers
- Females with skewed X-chromosome inactivation may have 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
  - Genetic or environmental modifiers may result in variable clinical outcomes

Genetics

Genes tested – *CYBB*; *NCF1*

Inheritance

- *CYBB* – X-linked
- *NCF1* – autosomal recessive

De novo variants – 10-20% of *CYBB* variants

Variants

- >600 *CYBB* pathogenic variants
  - ~85% are small nucleotide insertions, deletions, or substitutions (Roos, 2010b)
  - ~15% are large deletions (Roos, 2010b)

Genes associated with autosomal recessive CGD

- *NCF1* – encodes p47-phox
  - GT deletion in exon 2 (c.75_76delGT) accounts for ~85% of causative variants in *NCF1* (Vázquez 2001)
- *CYBA* – encodes p22-phox
- *NCF2* – encodes p67-phox
- *NCF4* – encodes p40-phox

Test Interpretation

Chronic Granulomatous Disease Panel (*CYBB* Sequencing and *NCF1* Exon 2 GT Deletion)

Sensitivity/specificity

- Clinical sensitivity – Up to 78% for CGD (Roos, 2010a; Roos, 2010b; Vázquez, 2001)
- 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
  - *CYBB* variant
    - In symptomatic male – confirms X-linked CGD
    - In asymptomatic female – confirms carrier status for X-linked CGD
  - *NCF1* GT deletion (c.75_76delGT)
    - 2 copies confirms autosomal recessive CGD
    - 1 copy confirms carrier status for autosomal recessive CGD
- Negative – *CYBB* gene pathogenic variant and the common pathogenic *NCF1* GT deletion not detected
  - Reduces, but does not eliminate, the possibility of CGD
- Inconclusive – gene sequencing may detect novel variants of unknown clinical significance

Limitations

- Diagnostic errors can occur due to rare sequence variations
- Regulatory region variants, deep intronic variants, and large duplications in *CYBB* will not be detected in patients of either sex
- Large *CYBB* gene deletions will not be detected in females
- Variants in *NCF1* other than c.75_76delGT are not evaluated
- Lack of detection of *NCF1* c.75_76delGT does not rule out carrier status
  - Due to potential recombination between *NCF1* and its pseudogenes, false negative results may occur for c.75_75delGT heterozygotes
- This assay does not interrogate all genes associated with CGD (eg, *CYBA*, *NCF,2* and *NCF4*)

References

- Roos, D. et al. Hematologically important mutations: the autosomal recessive forms of chronic granulomatous disease (second update). Blood Cells Mol Dis. 2010. Apr 15;44(4):291-9.
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- Vázquez N. et al. Mutational analysis of patients with p47-phox-deficient chronic granulomatous disease: The significance of recombination events between the p47-phox gene (NCF1) and its highly homologous pseudogenes. Exp Hematol. 2001. Feb 29;(2):234-43.