Dense Deposit Disease (DDD) and C3 Glomerulonephritis (C3GN)

C3	*CD46 (MCP)*	*CFB*	*CFD*
CFH	*CFHR2*	*CHFR5*	*CFI*

Description:

C3 Glomerulopathy (C3G) defines a group of renal diseases characterized by glomerular accumulation of complement C3 with scant or absent accumulation of immunoglobulins as assessed by immunofluorescence, indicative of activation of the alternative complement pathway (AP). C3G is comprised primarily of two distinct entities, dense deposit disease (DDD) and C3 glomerulonephritis (C3GN), which may be distinguished by electron microscopy. Dense deposit disease is characterized by accumulation of intramembranous sausage-shaped or ribbon-shaped electron-dense deposits. C3GN is a more heterogeneous group of disorders, characterized by isolated or predominant C3 staining on immunofluorescence with ultrastructural evidence of mesangial, subepithelial, or subendothelial electron-dense deposits (Xiao et al., 2014).

Clinical presentations of these disorders may include asymptomatic hematuria and proteinuria, nephrotic syndrome, and acute glomerulonephritis. Outside of renal manifestations, patients with DDD may also develop ocular drusen resulting from retinal deposits and acquired partial lipodystrophy.

Pivotal to the underlying pathogenesis of C3G is dysregulation of the AP C3 convertase C3bBb occuring primarily in the fluid phase, leading to hypocomplementemia from C3 consumption. Such dysregulation occurring at the cell surface instead leads to endothelial cell injury and thrombotic microangiopathy as seen in atypical hemolytic uremic syndrome (aHUS). Dysregulation of the AP C3 convertase is due to acquired factors such as C3 Nephritic Factor (~80% of patients with DDD and ~50% of patients with C3GN) or monoclonal gammopathy, and genetic factors such as pathogenic variants in complement regulatory proteins (Zhang et al. 2012). As pathogenic variants in these regulatory proteins can be identified in either C3G or aHUS, other genetic and environmental factors likely contribute to the predominance of one or the other clinical presentations.

Approximately 50% of patients with DDD progress to end-stage renal disease (ESRD) within 10 years of diagnosis (Smith et al. 2011), whereas the progression to ESRD appears to be slower in C3GN (Xiao et al. 2014). Following renal transplantation, histological recurrence of DDD in the renal allograft is nearly universal, with 50% graft failure in 5 years. Recurrence is also seen in ~70% of patients transplanted with C3GN with graft loss in ~50% of patients due to recurrence.

Immunosuppressive medications and/or plasma exchange may be effective in some patients with DDD and C3GN, particularly if an acquired form of complement dysregulation (i.e. C3NeF) is present. Complement-directed therapy with eculizumab, a humanized monoclonal antibody targeted against the complement protein C5, has been shown to be effective in some patients with DDD and C3GN, particularly if activation of the terminal complement pathway was present (Bomback et al., 2012).

Indications:

Dense deposit diseases/C3 Glomerulonephritis Panel by NGS:

- Confirmation of a genetic diagnosis in patients with dense deposit disease (DDD) or C3 glomerulonephritis (C3GN)

Gene Specific Sequencing:

- Confirmation of genetic diagnosis in a patient with dense deposit disease (DDD) or C3 glomerulonephritis (C3GN) and in whom a specific gene is suspected

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Variant Specific Analysis:

- Carrier testing of parents and other relatives for recurrence risk assessment
- Presymptomatic testing of at-risk family members for medical management
- Prenatal diagnosis of an at-risk fetus, after confirmation of variant(s) in the parent(s) (by prior arrangement only).

Specimen:

At least 3 mls whole blood in a lavender top (EDTA) tube. Label tube with patient's name, birth date, and date of collection.

Note: For post-transplant patients, we prefer pre-transplant samples or post-transplant skin fibroblasts. Culturing of skin fibroblasts is done at an additional charge. For alternate sample types, please contact the laboratory.

Testing Methodology:

DDD Panel by NGS: This test is performed by enrichment of the coding exons, flanking intronic and untranslated regions (5′ and 3′), as well as known pathogenic variants (HGMD 2017.3) in the promoter and deep intronic regions of the genes specified above using oligonucleotide probe hybridization followed by next-generation sequencing with >50X coverage at every target base. All pathogenic and novel variants, as well as variants of unknown significance, as determined bioinformatically, are confirmed by Sanger sequencing.

Gene Specific Sequencing/ Variant Specific Analysis: Sanger sequencing following PCR amplification of the specified coding and exon/ intron boundaries of the specified gene is performed.

Sensitivity:

Clinical Sensitivity: Pathogenic variants in *CFH, CFI,* or CD46 (*MCP*) are identified in approximately 17% of patients with DDD and 13% of patients with C3GN (Servais et al. 2012) and may be identified in *C3, CFB, CFD, CFHR2* and *CFHR5* as well (Pickering et al. 2013).

Analytical Sensitivity: The sensitivity of DNA sequencing is over 99% for the detection of nucleotide base changes, small deletions and insertions in the regions analyzed.

Limitations: Large deletions involving entire single exons or multiple exons, large insertions and other complex genetic events will not be identified using this test methodology. Rare primer site variants may lead to erroneous results.

Note: Single gene sequencing is available for all genes in the panel.
Deletion/ duplication analysis by targeted array comparative genomic hybridization (aCGH) is available for *C3, CFB, CFD* and *CFI*.

Turn-Around Time:

- DDD/C3GN NGS Panel: 28 days
- 28 days for analysis of any gene by Sanger sequencing

CPT Codes:

- **Dense deposit diseases/C3 Glomerulonephritis panel:** 81479 x4
- **Variant specific analysis:** call for information

Please call 1-866-450-4198 for current pricing, insurance preauthorization or with any billing questions.

Results:

Each test report includes a detailed interpretation of the genetic findings, the clinical significance of the result, and specific recommendations for the clinical management and additional testing, if warranted. Results will be reported to the referring physician or health care provider as specified on the test requisition form.

Shipping Instructions:

Please enclose **test requisition** with sample.
All information must be completed before sample can be processed.

Place samples in styrofoam mailer and ship at room temperature by overnight Federal Express to arrive Monday through Friday.

Ship to:

Cytogenetics and Molecular Genetics Laboratories
3333 Burnet Avenue NRB 1042
Cincinnati, OH 45229
513-636-4474

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