aHUS/DDD Genetic Evaluation

Atypical hemolytic uremic syndrome (aHUS) is a disease characterized by microangiopathic hemolytic anemia, thrombocytopenia, and organ dysfunction, especially renal impairment. Many cases of aHUS are caused by uncontrolled activation of the complement system; some cases of both familial and sporadic forms of aHUS are associated with complement-related genetic variants. Dense deposit disease (DDD) is a subtype of C3 glomerulopathy where uncontrolled activation of the complement alternative pathway leads to renal disease.

Atypical hemolytic uremic syndrome (aHUS) is a thrombotic microangiopathy characterized by hemolytic anemia, thrombocytopenia, and organ dysfunction (mainly renal failure) caused by thrombotic microangiopathy due to dysregulation and/or activation of the alternate complement pathway.

Increased risk for aHUS has been associated with loss of function variants in genes encoding inhibitors of the complement system, including complement factor H (CFH), complement factor I (CFI), membrane cofactor protein (MCP/CD46), complement factor H-related proteins (CFHR1, CFHR3, CFHR4, CFHR5), thrombomodulin (THBD), and C4b binding protein (C4BP), as well as gain of function variants in genes encoding complement factor B (CFB) and complement component 3 (C3), and variants in the gene encoding the epsilon isoform of diacyl glycerol kinase, DGKε, (DGKE). Genetic variants of complement factor H related proteins have also been associated with increased risk for development of autoantibody against CFH, a mechanism well described in the pathogenesis of aHUS.

The age at presentation of aHUS is variable, with some cases manifesting in childhood and others in late adulthood. The broad differential diagnosis includes other primary and secondary thrombotic micoangiopathies (TMA) presenting with overlapping features. In patients with rapidly deteriorating kidney function, the hallmark feature of aHUS, initiation of anti-complement therapies promptly is important in the prognosis for renal recovery. Clinical guidelines suggest that patients presenting with symptomatic thrombotic microangiopathy (as occurs with aHUS) should also be tested for ADAMTS13 deficiency, including evaluation for *ADAMTS13* sequence variants, as findings indicative of acquired and congenital thrombotic thrombocytopenic purpura (TTP) require different therapeutic approaches.

In highly selected disease populations, clinically significant genetic variants have been found in up to 60% of patients with aHUS, the majority in CFH (25-30% of cases) (Osborne et al.), with a smaller proportion in each of the other associated genes. A significant proportion of patients can have variants in 2-3 genes (Fremeaux-Bacchi et al). Inheritance is autosomal dominant with reduced penetrance or autosomal recessive. Disease recurrences are frequently observed in patients with aHUS and a genetic predisposition after renal transplantation. Short-term response to plasma therapy, outcome after kidney transplant, and overall prognosis can correlate with the specific gene involved.

Dense deposit disease (DDD) is a complement-mediated glomerular disease that is characterized by the presence of linear-appearing, electron-dense material in the glomerular basement membrane (GBM). Genetic variants that increase the risk of DDD and related C3 glomerulonephritis have been described in several genes, including *C3, CFH, CFI, CFB, and CFHR5*. DDD typically presents in children and young adults. The treatment is supportive with blood pressure and proteinuria control as well as dyslipidemia treatment. Anti-complement therapy has been used along with immunosuppression in severe cases. DDD can be associated with acquired partial lipodystrophy (APL) and deposits within the retina that can cause vision problems.

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Indications for testing:

The aHUS/DDD Genetic Evaluation should be considered:

- In patients with a clinical presentation compatible with thrombotic microangiopathy (TMA)
- In patients with renal dysfunction who have kidney biopsy findings of or complement deposition in whom there is concern for aHUS, C3GN or DDD
- In patients with a clinical presentation suggestive of aHUS to guide initiation and length of treatment with anti-complement agents
- In patients with a clinical presentation of TMA with renal involvement who are being considered for renal transplantation.
- In patients with personal and family history of unexplained end-stage renal disease

Informed Consent

It is recommended that healthcare providers obtain informed consent from the patient when genetic testing is ordered, consistent with any applicable state laws and regulations, documenting that the patient has been advised of understands the indications for and implications of the genetic test. This panel is designed for clinical detection of germline genetic variants in genes with strong or definitive evidence for causality of atypical hemolytic uremic syndrome (aHUS) and/or dense deposit disease (DDD). Test results may nonetheless yield genetic findings that may be unrelated to the current clinical presentation, and/or may carry individual or familial implications such as risk for syndromic manifestation, predisposition to malignancy, and/or reproductive implications (such as carrier status). If needed, an informed consent form for Versiti Hematology Genetics testing can be found at http://www.versiti.org/hg under *forms*.

Test method:

NGS: This next-generation sequencing assay analyzes the complete coding regions and splice sites of the following genes plus a minimum 30bp of non-coding DNA, including intron-exon boundaries, and is compared to the build GRCh37.p13 reference sequence: *CFH, CFI, MCP (CD46), THBD, C4BPA, C4BPB, CFB, C3, DGKE, ADAMTS13, CFHR1, CFHR3, CFHR4* and *CFHR5*. Specific untranslated, intronic and promoter regions known to be associated with aHUS or DDD are also sequenced. These targeted regions are captured by hybridization, amplified, and sequenced by massively parallel sequencing. Regions will have a minimum coverage of 50x and those regions with less than 50 sequencing reads or low quality coverage are supplemented with Sanger sequencing. All regions are covered by bidirectional analysis. Variants are identified by a customized bioinformatics pipeline, analyzed and comprehensively interpreted by our team of practicing hematologists with expertise in non-malignant hematology and laboratory diagnostics, scientists, and genetic counselors. All reported variants, including pathogenic, likely pathogenic, and variants of uncertain significance, are confirmed by Sanger sequencing. For prenatal testing, analysis of variable number tandem repeats (VNTR) is used to confirm results are not affected by maternal cell contamination.

MLPA: Deletions and duplications in selected exons of *CFH, CFHR1, CFHR3, CFHR4* and *CFHR5* are detected by multiplex ligation-dependent probe amplification (MLPA).

Assay Sensitivity and Limitations:

The analytical sensitivity of the NGS test is >99% for single nucleotide changes and insertions and deletions of less than 20 bp. NGS analysis is not designed to detect large deletions or duplications (>20 bp), or variants that are outside the regions sequenced. Low level mosaicism will not be detected by this sequencing methodology. Copy number variants in the genes/exons not covered by MLPA will not be detected.

Clinical Sensitivity

Clinical sensitivity is greatest in highly selected populations (aHUS and DDD research cohorts) with the highest clinical probability of these specific disorders. In clinical practice, considering the heterogeneity in clinical presentation and overlap with other thrombotic microangiopathies, the diagnostic yield of this is test is expected to be lower.

Reporting of Results

Results are classified and reported in accordance with ACMG next-generation sequencing and copy number variation standards. Sequence variants and large deletions and duplications predicted to be pathogenic, likely pathogenic, and of uncertain significance will be reported; variants classified as likely benign or benign are typically not reported but such data are available upon request. Sequence variants are described using standard Human Genome Variation Society (HGVS) nomenclature (http://hgvs.org); copy number variants are described in accordance with the International System for Human Cytogenomic Nomenclature (ISCN).

Specimen Requirements

Parental/Patient/Pediatric: 3-5 mL Whole blood (EDTA tube, lavender top), 2-5 mL Bone marrow (EDTA tube, lavender top), 3-4 Buccal swabs, or ≥1ug of DNA at ≥50ng/uL of High Quality DNA.

Fetal: 7-15 mL amniotic fluid, 5-10 mg chorionic villi; back up culture of amniocytes or chorionic villi is highly recommended. Cultured: Two T25 flasks cultured amniocytes or chorionic villi ($2x10^6$ minimum). Maternal blood sample of 3-5 mL Whole blood (EDTA tube, lavender top) is requested for all prenatal samples for maternal cell

contamination studies. For questions please contact the laboratory to discuss sample requirements.

SHIP

Shipping Requirements

Ship on an ice pack at room temperature. Protect from freezing. Place the specimen and the requisition into plastic bags and seal. Insert into a Styrofoam container, seal and place into a sturdy cardboard box, and tape securely. Ship the package in compliance with your overnight carrier guidelines. Label with the following address:

Client Services/Diagnostic Laboratory
Versiti
638 N. 18th St
Milwaukee, WI, 53233

ORDER

Required Forms

Please complete all pages of the requisition form. Clinical history (including patient-reported ancestry, clinical diagnosis, family history, and relevant laboratory findings) is necessary for optimal interpretation of genetic test results and recommendations. Clinical and laboratory history can either be recorded on the requisition form or clinical and laboratory reports can be submitted with the sample.

CPT Codes/Billing/Turnaround Time

Test code: 1200

CPT codes: For suggested CPT codes, visit the versiti.org/test-menu

Turnaround time: 28 days

The CPT codes provided are subject to change as more information becomes available. CPT codes are provided only as guidance to assist clients with billing.

For additional information related to shipping, billing or pricing, please contact Versiti Client Services: (414) 937-6396 or 800-245-3117, Option 1, or LabInfo@versiti.org

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Atypical Hemolytic Uremic syndrome and Dense Deposit Disease

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Variant interpretation references

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