Hemolytic Uremic Syndrome Panel

Is ideal for patients with a clinical suspicion of atypical hemolytic uremic syndrome.

The panel covers genes associated with autosomal recessive and autosomal dominant forms of the disease.

About Hemolytic Uremic Syndrome

Hemolytic uremic syndrome (HUS) is characterized by hemolytic anemia, thrombocytopenia, and renal impairment. Atypical HUS (aHUS) is genetic, whereas typical HUS is triggered by infectious agents, not by genetic predisposition. Age at onset of aHUS ranges from prenatal to adulthood. Patients with the familial form of aHUS have a poor prognosis, with a rate of either end-stage renal disease (ESRD) or death of 50 to 80% (PMID: 19846853). Individuals with genetic aHUS frequently experience relapse even after complete recovery following the presenting episode. Sixty percent of genetic aHUS progress to ESRD. Mutations in CFH account for approximately 30% of the cases, CD46 for 12%, CFI for 5%-10%, C3 for 5%, and THBD for 3%-5%. In early onset aHUS, defined as disease onset before age 1 year, mutations in DGKE explain 27% of cases. Predisposition to aHUS is inherited in an autosomal recessive or autosomal dominant manner with incomplete penetrance. Treatment can be highly optimized with genetic testing. Live renal transplantation from related persons should be avoided as also they might be at increased genetic risk of the disease. Evidence suggests that kidney graft outcome is favourable in those with CD46 and DGKE mutations, but not in those with CFH, CFI, C3, THBD, or CFB mutations; however, simultaneous kidney and liver transplantation in young children with aHUS and CFH mutations may correct the genetic defect and prevent disease recurrence. OTHER INFORMATION ON CFH AND CFHR1-4 GENES

CFH gene have multiple exons that are pseudogenic (exons 8-9, 11, 21-23). Moreover, the function of CFHR1, CFHR2, CFHR3 and CFHR4 has not been established and they are highly homologues (see below chapter 'CFHR1-4 genes'). Genetics of atypical hemolytic uremic syndrome (aHUS) Mutations in CFH account for approximately 30% of the cases, CD46 (also known as MCP) 12%, CFI 5%-10%, C3 5%, THBD 3%-5%. In early onset aHUS, disease manifesting before age 1 year, mutations in DGKE explain 27% of the cases. Inheritance mode is difficult to determine for most of the genes related to aHUS due to low penetrance but the predisposition to disease is commonly autosomal dominant. In the ClinVar mutation database, vast majority of the novel disease associated variants in major aHUS genes such as CFH, CD46, CFI and C3 are classified as risk factors but not pathogenic or likely pathogenic. In most of the families where probands has novel variant in aHUS genes, some of the unaffected parents or other family members carry the same variant. However, one study showed fully penetrant recessive aHUS relating to homozygous CFH mutations in a large Bedouin pedigree with 10 aHUS cases (PubMed: 9811382). In addition, deletions in CFHR1 to CFHR5 genes have shown to increase slightly a risk for aHUS. The Newcastle cohort of 66 aHUS patients showed deletions in CFHR1 were more frequent in aHUS patients compared to controls (zero copies 10% vs. 2%; one copy 35%vs 9% and two copies 55% vs.89%) indicating odds ratios (OR) 6.3 for homozygous deletion and 3.8 for heterozygous. Absence of CFHR1 and/or CFHR3 was shown to contribute to the defective regulation of complement activation on cell and tissue surfaces (PubMed: 17367211). Hofer et al evaluated 116 aHUS patients and 118 control. Homozygous deletion in CFHR1 was detected in 32% of the patients with aHUS tested and in 2.5% of controls. CFH antibodies were present in 25% of the patients and none of the controls. CFH antibodies were detected in 82% of patients with homozygous CFHR1 deletion and in 6% of patients without. CFH antibody-positive patients with aHUS showed a significantly lower platelet nadir at disease onset and significantly less frequent involvement of the central nervous system than did antibody-negative patients. Antibody-positive patients also received plasma therapy more often (PubMed: 23243267). It is noteworthy that disease activity appears to correlate better with immune complex titers than FHAA titers (PubMed: 22922817). In 2016, Challis et al described novel CFH/CFHR3 hybrid gene in a patient with aHUS secondary to a de novo 6.3-kb deletion that arose through microhomology-mediated end joining rather than nonallelic homologous recombination. Secreted protein product lacked the recognition domain of factor H and exhibits impaired cell surface complement regulation. The fact that the formation of this hybrid gene arose as a de novo event suggests that this cluster is a dynamic area of the genome in which additional genomic disorders may arise (PubMed: 26490391). CFHR1-4 genes In August 25 2017, Blueprint Genetics excluded CFHR1, CFHR2, CFHR3, CFHR4 genes from three diagnostic NGS panels including Primary Immunodeficiency Panel, Complement System Disorder Panel and Hemolytic Uremic Syndrome Panel. This was done due to extensive homology between these genes making it difficult or even impossible to determine copy number reliably from these genes with short read length NGS methods. Moreover, homozygous or heterozygous deletions involving these gene are common in population even though enriched in patients with aHUS. By relying on three estimates: 1) higher end of aHUS prevalence (9 per 1,000,000), 2) frequency of homozygous CFHR1 deletion (2%) and 3) assuming that all aHUS cases would be

https://blueprintgenetics.com/
caused by this defect (over estimating the effect), we are left with the fact that 99.95% of the individuals with homozygous CFHR1 deletion will never get aHUS. Thus, we consider releasing copy number from CFHR1-4 genes may be misleading, and is not considered helpful in clinical practice. We believe that fusion genes between CFH and CFHR1-4 may be the mechanism that explain the association between CFHR1-4 gene deletions and aHUS. However, this kind of alterations are not reliably detected by targeted sequencing approaches.

### Availability

**Results in 3-4 weeks**

### Gene set description

Genes in the Hemolytic Uremic Syndrome Panel and their clinical significance

<table>
<thead>
<tr>
<th>Gene</th>
<th>Associated phenotypes</th>
<th>Inheritance</th>
<th>ClinVar</th>
<th>HGMD</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADAMTS13</td>
<td>Schulman-Upshaw syndrome, Thrombotic thrombocytopenic purpura, familial</td>
<td>AR</td>
<td>30</td>
<td>183</td>
</tr>
<tr>
<td>C3</td>
<td>Hemolytic uremic syndrome, atypical, Complement component 3 deficiency, Macular degeneration, age-related</td>
<td>AD/AR</td>
<td>6</td>
<td>87</td>
</tr>
<tr>
<td>CD46*</td>
<td>Hemolytic uremic syndrome, atypical</td>
<td>AD/AR</td>
<td>5</td>
<td>81</td>
</tr>
<tr>
<td>CFB</td>
<td>Complement factor B deficiency, Hemolytic uremic syndrome, atypical</td>
<td>AD/AR</td>
<td>2</td>
<td>26</td>
</tr>
<tr>
<td>CFH*</td>
<td>Hemolytic uremic syndrome, atypical, Complement factor H deficiency, Basal laminar drusen</td>
<td>AD/AR</td>
<td>18</td>
<td>305</td>
</tr>
<tr>
<td>CFHR5</td>
<td>Atypical hemolytic-uremic syndrome with anti-factor H antibodies, C3 glomerulonephritis</td>
<td>AD/AR</td>
<td>4</td>
<td>32</td>
</tr>
<tr>
<td>CFI</td>
<td>Hemolytic uremic syndrome, atypical, Complement factor I deficiency</td>
<td>AD/AR</td>
<td>10</td>
<td>143</td>
</tr>
<tr>
<td>DGKE</td>
<td>Nephrotic syndrome</td>
<td>AR</td>
<td>17</td>
<td>38</td>
</tr>
<tr>
<td>THBD</td>
<td>Thrombophilia due to thrombomodulin defect, Hemolytic uremic syndrome, atypical</td>
<td>AD</td>
<td>5</td>
<td>28</td>
</tr>
</tbody>
</table>

*Some regions of the gene are duplicated in the genome leading to limited sensitivity within the regions. Thus, low-quality variants are filtered out from the duplicated regions and only high-quality variants confirmed by other methods are reported out. Read more.*

Gene, refers to HGNC approved gene symbol; Inheritance to inheritance patterns such as autosomal dominant (AD), autosomal recessive (AR) and X-linked (XL); ClinVar, refers to a number of variants in the gene classified as pathogenic or likely pathogenic in ClinVar ([http://www.ncbi.nlm.nih.gov/clinvar/](http://www.ncbi.nlm.nih.gov/clinvar/)); HGMD, refers to a number of variants with possible disease association in the gene listed in Human Gene Mutation Database ([HGMD](http://www.hgmd.cf.ac.uk/ac/)). The list of associated (gene specific) phenotypes are generated from CDG ([http://research.nhgri.nih.gov/CGD/](http://research.nhgri.nih.gov/CGD/)) or Orphanet ([http://www.orpha.net/](http://www.orpha.net/)) databases.

### Non-coding disease causing variants covered by the panel

<table>
<thead>
<tr>
<th>Gene</th>
<th>Genomic location HG19</th>
<th>HGVS</th>
<th>RefSeq</th>
<th>RS-number</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD46</td>
<td>Chr1:207930564</td>
<td>c.286+27delT</td>
<td>NM_002389.4</td>
<td>rs771669828</td>
</tr>
</tbody>
</table>
Test performance

The Blueprint Genetics hemolytic uremic syndrome panel covers classical genes associated with hemolytic uremic syndrome. The genes on the panel have been carefully selected based on scientific literature, mutation databases and our experience.

Our panels are sliced from our high-quality whole exome sequencing data. Please see our sequencing and detection performance table for different types of alterations at the whole exome level (Table).

Assays have been validated for different starting materials including EDTA-blood, isolated DNA (no FFPE), saliva and dry blood spots (filter card) and all provide high-quality results. The diagnostic yield varies substantially depending on the assay used, referring healthcare professional, hospital and country. Blueprint Genetics’ Plus Analysis (Seq+Del/Dup) maximizes the chance to find a molecular genetic diagnosis for your patient although Sequence Analysis or Del/Dup Analysis may be a cost-effective first line test if your patient’s phenotype is suggestive of a specific mutation type.

Bioinformatics

The target region for each gene includes coding exons and ±20 base pairs from the exon-intron boundary. In addition, the panel includes non-coding variants if listed above (Non-coding variants covered by the panel). Some regions of the gene(s) may be removed from the panel if specifically mentioned in the ‘Test limitations’ section above. The sequencing data generated in our laboratory is analyzed with our proprietary data analysis and annotation pipeline, integrating state-of-the art algorithms and industry-standard software solutions. Incorporation of rigorous quality control steps throughout the workflow of the pipeline ensures the consistency, validity and accuracy of results. Our pipeline is streamlined to maximize sensitivity without sacrificing specificity. We have incorporated a number of reference population databases and mutation databases such as, but not limited, to 1000 Genomes Project, gnomAD, ClinVar and HGMD into our clinical interpretation software to make the process effective and efficient. For missense variants, in silico variant prediction tools such as SIFT, PolyPhen, MutationTaster are used to assist with variant classification. Through our online ordering and statement reporting system, Nucleus, the customer has an access to details of the analysis, including patient specific sequencing metrics, a gene level coverage plot and a list of regions with inadequate coverage if present. This reflects our mission to build fully transparent diagnostics where customers have easy access to crucial details of the analysis process.

Clinical interpretation

We provide customers with the most comprehensive clinical report available on the market. Clinical interpretation requires a fundamental understanding of clinical genetics and genetic principles. At Blueprint Genetics, our PhD molecular geneticists, medical geneticists and clinical consultants prepare the clinical statement together by evaluating the identified variants in the context of the phenotypic information provided in the requisition form. Our goal is to provide clinically meaningful statements that are understandable for all medical professionals regardless of whether they have formal training in genetics.

Variant classification is the cornerstone of clinical interpretation and resulting patient management decisions. Our classifications follow the Blueprint Genetics Variant Classification Schemes based on the ACMG guideline 2015. Minor modifications were made to increase reproducibility of the variant classification and improve the clinical validity of the report. Our experience with tens of thousands of clinical cases analyzed at our laboratory allowed us to further develop the industry standard.

The final step in the analysis of sequence variants is confirmation of variants classified as pathogenic or likely pathogenic using bi-directional Sanger sequencing. Variant(s) fulfilling the following criteria are not Sanger confirmed: the variant quality score is above the internal threshold for a true positive call, and visual check-up of the variant at IGV is in-line with the variant call. Reported variants of uncertain significance are confirmed with bi-directional Sanger sequencing only if the quality score is below our internally defined quality score for true positive call. Reported copy number variations with a size <10 exons are
confirmed by orthogonal methods such as qPCR if the specific CNV has been seen less than three times at Blueprint Genetics.

Our clinical statement includes tables for sequencing and copy number variants that include basic variant information (genomic coordinates, HGVS nomenclature, zygosity, allele frequencies, in silico predictions, OMIM phenotypes and classification of the variant). In addition, the statement includes detailed descriptions of the variant, gene and phenotype(s) including the role of the specific gene in human disease, the mutation profile, information about the gene’s variation in population cohorts and detailed information about related phenotypes. We also provide links to the references used, congress abstracts and mutation databases to help our customers further evaluate the reported findings if desired. The conclusion summarizes all of the existing information and provides our rationale for the classification of the variant.

Identification of pathogenic or likely pathogenic variants in dominant disorders or their combinations in different alleles in recessive disorders are considered molecular confirmation of the clinical diagnosis. In these cases, family member testing can be used for risk stratification within the family. In the case of variants of uncertain significance (VUS), we do not recommend family member risk stratification based on the VUS result. Furthermore, in the case of VUS, we do not recommend the use of genetic information in patient management or genetic counseling.

Our interpretation team analyzes millions of variants from thousands of individuals with rare diseases. Thus, our database, and our understanding of variants and related phenotypes, is growing by leaps and bounds. Our laboratory is therefore well positioned to re-classify previously reported variants as new information becomes available. If a variant previously reported by Blueprint Genetics is re-classified, our laboratory will issue a follow-up statement to the original ordering health care provider at no additional cost.

## ICD codes

Commonly used ICD-10 codes when ordering the Hemolytic Uremic Syndrome Panel

<table>
<thead>
<tr>
<th>ICD-10</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>D58.8</td>
<td>Hemolytic uremic syndrome</td>
</tr>
</tbody>
</table>

## Accepted sample types

- EDTA blood, min. 1 ml
- Purified DNA, min. 3μg*
- Saliva (Oragene DNA OG-500 kit)

Label the sample tube with your patient’s name, date of birth and the date of sample collection.

Note that we do not accept DNA samples isolated from formalin-fixed paraffin-embedded (FFPE) tissue.

## Resources

- Atypical HUS Foundation
- GeneReviews
- GeneReviews - Genetic Atypical Hemolytic-Uremic Syndrome
- National Organization for Rare Disorder
- aHUS Alliance
- aHUS Canada

https://blueprintgenetics.com/