Blueprint Genetics

Hemolytic Uremic Syndrome Panel

Test code: KI0101

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 atypic 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

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

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

*Some regions of the gene are duplicated in the genome. Read more.

The gene has suboptimal coverage (means <90% of the gene's target nucleotides are covered at >20x with mapping quality score (MQ>20) reads), and/or the gene has exons listed under Test limitations section that are not included in the panel as they are not sufficiently covered with high quality sequence reads.

The sensitivity to detect variants may be limited in genes marked with an asterisk (*) or number sign (#)

Gene refers to the HGNC approved gene symbol; Inheritance refers to inheritance patterns such as autosomal dominant (AD), autosomal recessive (AR), mitochondrial (mi), X-linked (XL), X-linked dominant (XLD) and X-linked recessive (XLR); ClinVar refers to the number of variants in the gene classified as pathogenic or likely pathogenic in this database (ClinVar); HGMD refers to the number of variants with possible disease association in the gene listed in Human Gene Mutation Database (HGMD). The list of associated, gene specific phenotypes are generated from CGD or Mitomap databases.

Non-coding disease causing variants covered by the panel

Gene	Genomic location HG19	HGVS	RefSeq	RS-number

CD46	Chr1:207930564	c.286+27delT	NM_002389.4	rs771669828
DGKE	Chr17:54925466	c.888+40A>G	NM_003647.2	
THBD	Chr20:23030319		NM_000361.2	
THBD	Chr20:23030443	c.-302C>A	NM_000361.2	

Test Strengths

The strengths of this test include:

- CAP accredited laboratory
- CLIA-certified personnel performing clinical testing in a CLIA-certified laboratory
- Powerful sequencing technologies, advanced target enrichment methods and precision bioinformatics pipelines ensure superior analytical performance
- Careful construction of clinically effective and scientifically justified gene panels
- Some of the panels include the whole mitochondrial genome (please see the Panel Content section)
- Our Nucleus online portal providing transparent and easy access to quality and performance data at the patient level
- Our publicly available analytic validation demonstrating complete details of test performance
- ~2,000 non-coding disease causing variants in our clinical grade NGS assay for panels (please see 'Non-coding disease causing variants covered by this panel' in the Panel Content section)
- Our rigorous variant classification scheme
- Our systematic clinical interpretation workflow using proprietary software enabling accurate and traceable processing of NGS data
- Our comprehensive clinical statements

Test Limitations

Due to regions of segmental duplications, the genes *CFHR1*, *CFHR2*, *CFHR3* and *CFHR4* cannot be reliably analyzed with NGS technologies. These genes are not included in this Panel. Please see more information on 'about the disease' section. Genes with partial, or whole gene, segmental duplications in the human genome are marked with an asterisk (*) if they overlap with the UCSC pseudogene regions. The technology may have limited sensitivity to detect variants in genes marked with these symbols (please see the Panel content table above).

This test does not detect the following:

- Complex inversions
- Gene conversions
- Balanced translocations
- Some of the panels include the whole mitochondrial genome but not all (please see the Panel Content section)
- Repeat expansion disorders unless specifically mentioned
- Non-coding variants deeper than ±20 base pairs from exon-intron boundary unless otherwise indicated (please see above Panel Content / non-coding variants covered by the panel).

This test may not reliably detect the following:

- Low level mosaicism in nuclear genes (variant with a minor allele fraction of 14.6% is detected with 90% probability)
- Stretches of mononucleotide repeats
- Low level heteroplasmy in mtDNA (>90% are detected at 5% level)
- Indels larger than 50bp
- Single exon deletions or duplications
- Variants within pseudogene regions/duplicated segments
- Some disease causing variants present in mtDNA are not detectable from blood, thus post-mitotic tissue such as skeletal muscle may be required for establishing molecular diagnosis.

The sensitivity of this test may be reduced if DNA is extracted by a laboratory other than Blueprint Genetics.

For additional information, please refer to the Test performance section and see our Analytic Validation.

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.

The genes on the panel have been carefully selected based on scientific literature, mutation databases and our experience.

Our panels are sectioned from our high-quality, clinical grade NGS assay. Please see our sequencing and detection performance table for details regarding our ability to detect different types of alterations (Table).

	Sensitivity % (TP/(TP+FN)	Specificity %
Single nucleotide variants	99.89% (99,153/99,266)	>99.9999%
Insertions, deletions and indels by sequence analysis		
1-10 bps	96.9% (7,563/7,806)	>99.9999%
11-50 bps	99.13% (2,524/2,546)	>99.9999%
Copy number variants (exon level dels/dups)		
1 exon level deletion (heterozygous)	100% (20/20)	NA
1 exon level deletion (homozygous)	100% (5/5)	NA
1 exon level deletion (het or homo)	100% (25/25)	NA
2-7 exon level deletion (het or homo)	100% (44/44)	NA
1-9 exon level duplication (het or homo)	75% (6/8)	NA
Simulated CNV detection		
5 exons level deletion/duplication	98.7%	100.00%
Microdeletion/-duplication sdrs (large CNVs, n=37))		
Size range (0.1-47 Mb)	100% (37/37)	

The performance presented above reached by Blueprint Genetics high-quality, clinical grade NGS sequencing assay with the following coverage metrics

Mean sequencing depth	143X
Nucleotides with >20x sequencing coverage (%)	99.86%

Performance of Blueprint Genetics Mitochondrial Sequencing Assay.

	Sensitivity % (TP/(TP+FN)	Specificity
ANALYTIC VALIDATION (NA samples; n=4)		
Single nucleotide variants		
Heteroplasmic (45-100%)	100.0% (50/50)	100.0%
Heteroplasmic (35-45%)	100.0% (87/87)	100.0%
Heteroplasmic (25-35%)	100.0% (73/73)	100.0%
Heteroplasmic (15-25%)	100.0% (77/77)	100.0%
Heteroplasmic (10-15%)	100.0% (74/74)	100.0%
Heteroplasmic (5-10%)	100.0% (3/3)	100.0%
Heteroplasmic (<5%)	50.0% (2/4)	100.0%
CLINICAL VALIDATION (n=76 samples)		
All types		
Single nucleotide variants n=2084 SNVs		
Heteroplasmic (45-100%)	100.0% (1940/1940)	100.0%
Heteroplasmic (35-45%)	100.0% (4/4)	100.0%
Heteroplasmic (25-35%)	100.0% (3/3)	100.0%
Heteroplasmic (15-25%)	100.0% (3/3)	100.0%
Heteroplasmic (10-15%)	100.0% (9/9)	100.0%
Heteroplasmic (5-10%)	92.3%(12/13)	99.98%
Heteroplasmic (<5%)	88.7% (47/53)	99.93%
Insertions and deletions by sequence analysis n=42 indels		
Heteroplasmic (45-100%) 1-10bp	100.0% (32/32)	100.0%
Heteroplasmic (5-45%) 1-10bp	100.0% (3/3)	100.0%
Heteroplasmic (<5%) 1-10bp	100.0% (5/5)	>0.9999
SIMULATION DATA /(mitomap mutations)		
Insertions, and deletions 1-24 bps by sequence analysis; n=17		

Homoplasmic (100%) 1-24bp	100.0% (17/17)	99.98%
Heteroplasmic (50%)	100.0% (17/17)	99.99%
Heteroplasmic (25%)	100.0% (17/17)	100.0%
Heteroplasmic (20%)	100.0% (17/17)	100.0%
Heteroplasmic (15%)	100.0% (17/17)	100.0%
Heteroplasmic (10%)	94.1% (16/17)	100.0%
Heteroplasmic (5%)	94.1% (16/17)	100.0%
Copy number variants (separate artifical mutations; n=1500)		
Homoplasmic (100%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (50%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (30%) 500 bp, 1kb, 5 kb	100.0%	100.0%
Heteroplasmic (20%) 500 bp, 1kb, 5 kb	99.7%	100.0%
Heteroplasmic (10%) 500 bp, 1kb, 5 kb	99.0%	100.0%
The performance presented above reached by following coverage metrics at assay level (n=66)		
	Mean of medians	Median of medians
Mean sequencing depth MQ0 (clinical)	18224X	17366X
Nucleotides with >1000x MQ0 sequencing coverage (%) (clinical)	100%	
rho zero cell line (=no mtDNA), mean sequencing depth	12X	

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 corner stone 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 variant databases used 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.

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ICD codes

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

ICD-10	Disease
D58.8	Hemolytic uremic syndrome

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
- Cheong, H. et al. Clinical Practice Guidelines for the Management of Atypical Hemolytic Uremic Syndrome in Korea. J Korean Med Sci. 2016 Oct;31(10):1516-28.
- GeneReviews
- GeneReviews - Genetic Atypical Hemolytic-Uremic Syndrome
- National Organization for Rare Disorder
- Taylor, C.M. et al. Clinical practice guidelines for the management of atypical haemolytic uraemic syndrome in the United Kingdom. Br J Haematol. 2010 Jan;148(1):37-47.
- aHUS Alliance
- aHUS Canada