Single Gene Analysis

versiti™

Single Gene Analysis

Versiti offers comprehensive genetic analysis to detect sequence variants and large deletions and duplications in over 100 genes known to cause hematologic-associated diseases. Single Gene Analysis can be ordered as:

- Next Generation Sequencing (NGS) only;
- NGS with reflex to array Comparative Genomic Hybridization (aCGH) deletion/duplication analysis if sequencing does not identify clinically significant variants that fully explain the patient's phenotype;
- NGS with concurrent aCGH deletion/duplication analysis (both testing methodologies performed simultaneously); or
- Deletion/duplication analysis by aCGH only.

Please note that *PLAU* is available by aCGH only, and *SERPINA1* and *PRKACG* are not available by Single Gene Analysis. Analysis for the *SERPINA1* Pittsburgh variant, c.1145T>G, is available by Targeted Analysis, 4970, and as included in select panels. Analysis for the *PRKACG* p.I74M allele is available by Targeted Analysis, 4970, and as included in select panels.

Hematology Genetics Single Genes For additional information about genetic panels and more, visit: Versiti.org/HG.

ABCG5	*AP3D1*	*CYCS*	*F7*	*FGG*	*GGCX*	*HPS3*	*LMAN1*	*PLA2G4A*	*RNU4ATAC*	*SMAD4*	*TAFAZZIN*	*VPS13B*
ABCG8	*ARPC1B*	*DIAPH1*	*F8*	*FLI1*	*GINS1*	*HPS4*	*LYST*	*PLAU**	*RUNX1*	*SMARCD2*	*TBXA2R*	*VPS33B*
ACTB	*BLOC1S3*	*DTNBP1*	*F9*	*FLNA*	*GNE*	*HPS5*	*MCFD2*	*PLG*	*SBDS*	*SRC*	*TBXAS1*	*VPS45*
ACTN1	*BLOC1S6*	*EFL1*	*F10*	*FYB1(FYB)*	*GP1BA*	*HPS6*	*MECOM*	*PRKACG* †	*SERPINA1*§	*SRP19*	*TCIRG1*	*VWF*
ACVRL1	*BTK*	*ELANE*	*F11*	*G6PC3*	*GP1BB*	*HRG*	*MPIG6B*	*PROC*	*SERPINC1*	*SRP54*	*THBD*	*WAS*
ADAMTS13	*CDC42*	*ENG*	*F13A1*	*GATA1*	*GP6*	*ITGA2B*	*MPL*	*PROS1*	*SERPIND1*	*SRP68*	*THPO*	*WDR1*
AK2	*CLPB*	*EPHB4*	*F13B*	*GATA2*	*GP9*	*ITGB3*	*MYH9*	*RAC2*	*SERPINE1*	*SRP72*	*TUBB1*	*WIPF1*
ANKRD26	*CSF3R*	*ETV6*	*FERMT3*	*GDF2*	*HAX1*	*JAGN1*	*NBEA*	*RASA1*	*SERPINF2*	*SRPRA*	*USB1*	
ANO6	*CXCR2*	*F2*	*FGA*	*GFI1*	*HOXA11*	*KDSR*	*NBEAL2*	*RASGRP2*	*SLC37A4*	*STIM1*	*VIPAS39*	
AP3B1	*CXCR4*	*F5*	*FGB*	*GFI1B*	*HPS1*	*KNG1*	*P2RY12*	*RBM8A*	*SLFN14*	*STXBP2*	*VKORC1*	

aHUS/DDD Genetic Panel genes *C3, C4BPA, C4BPB, CFB, CFH, CFHR1, CFHR3, CFHR4, CFHR5, CFI, DGKE, MCP* are NOT available as single gene sequencing. **PLAU* available via aCGH only. § *SERPINA1* is targeted for the Pittsburgh allele in exon 5 only.
† *PRKACG* NGS includes only a region to cover the p.Ile74Met variant.

Additional descriptions for our most frequently ordered single gene sequencing requests, *ADAMTS13*, *ELANE*, *F8*, *F9*, and *VWF* are included below.

Frequently Ordered Single Genes

ADAMTS13

ADAMTS13 is a plasma protein that regulates the interaction of platelets with von Willebrand factor by cleavage of VWF multimers. Severe deficiency of ADAMTS13 allows formation of platelet microthrombi, which in turn obstruct arterioles and capillaries, generating the clinical sequelae characterizing thrombotic thrombocytopenic purpura (TTP) of thrombocytopenia, microangiopathic hemolytic anemia, microvascular thrombosis and risk of life-threatening organ dysfunction. In adults, severe ADAMTS13 deficiency is usually an acquired abnormality caused by autoantibody.

Severe congenital ADAMTS13 deficiency is a rare autosomal recessive disorder (also known as familial/inherited/congenital thrombotic thrombocytopenic purpura (TTP) and Upshaw-Schulman syndrome). Patients usually present as children, but adult presentations are reported, often triggered by stress events such as pregnancy. Patients are at risk for recurrent episodes of TTP. Antibody to ADAMTS13 is usually not detected, and patients generally improve with plasma transfusion therapy for ADAMTS13 replacement.

Pathogenic variants have been identified throughout the coding sequence of the *ADAMTS13* gene including missense, nonsense and splice site alterations, as well as nucleotide deletions and insertions. Large deletions have also been reported. Single gene analysis (order code 4855) of *ADAMTS13* is useful in confirming a diagnosis of congenital ADAMTS13 deficiency to guide appropriate treatment and accurate genetic counseling for patients and their families. For family members of individuals with inherited ADAMTS13 deficiency in whom the causative familial variant(s) are unknown, *ADAMTS13* sequencing can be used to determine carrier status or for prenatal diagnosis.

ELANE

Severe congenital neutropenia (SCN) is a disorder of neutrophil production that is characterized by recurrent fever, infections, and inflammation of the mouth, skin, and pharynx. Predisposition to myelodysplastic syndrome and acute myeloid leukemia is also associated with SCN. Diagnosis is based on clinical findings and serial measurement of the absolute neutrophil count (ANC). Cyclic neutropenia is distinguished from SCN by regular oscillations of the ANC, generally milder infectious complications, and no associated risk of malignancy. Both SCN and cyclic neutropenia can be treated with granulocyte colony-stimulating factor.

Pathogenic variants in the *ELANE* gene, which codes for the neutrophil elastase protein, have been reported in 38-80% of SCN patients and in 90-100% of cyclic neutropenia patients. *ELANE*-related neutropenia is inherited in an autosomal dominant manner. In affected individuals with no prior family history, both *de novo* mutations and germline mosaicism in unaffected parents have been reported. Identification of heterozygous pathogenic variants in *ELANE* confirms the clinical diagnosis of either cyclic neutropenia or SCN. Although some *ELANE* variants have been associated with only one or the other diagnosis, other variants have been associated with both phenotypes.

F8

Hemophilia A is an X-linked inherited bleeding disorder caused by pathogenic variants in the *F8* gene that encodes for coagulation factor VIII. The degree of plasma factor VIII deficiency correlates with both the clinical severity of disease and genetic findings. Severe hemophilia A is characterized by plasma factor VIII levels of under 1 IU/dl. Moderate and mild hemophilia A are characterized by factor VIII levels of 1-5 IU/dL and 6-40 IU/dL, respectively.

Approximately 50% of severe hemophilia A cases are attributable to pathogenic inversion variants in *F8* introns 1 or 22. Therefore, specific evaluation with *F8* Inversion Analysis (order code(s) 1400/1401/1402) is recommended prior to *F8* sequencing analysis for initial genetic analysis of male patients with severe hemophilia A, for female patients with factor VIII deficiency in the absence of known family history of hemophilia, and for females with a family history of severe hemophilia A when the familial variant is not known and an affected male relative is not available for testing.

The vast majority of cases of mild and moderate hemophilia A, and of cases of severe hemophilia A in which inversions are excluded, are attributable to missense, nonsense and frameshift variants, with a small percentage due to large structural rearrangements such as large deletions or duplications. *F8* Single Gene Analysis (order code 4855) is recommended for identification of the underlying pathogenic variant in males with mild or moderate hemophilia A, and males with severe hemophilia A in which *F8* intron 1 and 22 inversions have been ruled out. *F8* Single Gene Analysis (order code 4855) can also be used for identification or genetic diagnosis of female carriers and for prenatal diagnosis when the familial variant is not known and an affected male relative is not available for testing, either with family history of mild or moderate hemophilia A, or severe hemophilia A when inversions have been excluded. In cases in which neither a *F8* inversion nor a pathogenic variant is identified by sequence analysis, a large deletion or duplication may be present and *F8* deletion/duplication analysis (order code 4855) by array Comparative Genomic Hybridization (aCGH) may be considered.

F9

Hemophilia B is an X-linked inherited bleeding disorder caused by pathogenic variants in the *F9* gene that encodes for coagulation factor IX. The degree of plasma factor IX deficiency correlates with both the clinical severity of disease and genetic findings. Severe hemophilia B is characterized by plasma factor IX levels of under 1 IU/dl. Moderate and mild hemophilia B are characterized by factor IX levels of 1-5 IU/dl and 6-40 IU/dl, respectively. The vast majority of cases are attributable to missense, nonsense and frameshift variants and a small percentage to large structural rearrangements such as large deletions or duplications. *F9* Single Gene Analysis (order code 4855) is useful for identification of the underlying pathogenic variant in males with hemophilia B or females with factor IX deficiency in the absence of family history of hemophilia. It can also be used for identification or genetic diagnosis of female carriers and for prenatal diagnosis when the familial variant is not known and an affected male relative is not available for testing. In cases in which *F9* sequence analysis does not identify a pathogenic variant, a large deletion or duplication may be present, and *F9* deletion/duplication analysis (order code 4855) by array Comparative Genomic Hybridization (aCGH) may be considered.

VWF

Von Willebrand disease (VWD) is a common inherited bleeding disorder with a reported incidence ranging from 0.01% to 1%. VWD is classified into subtypes of quantitative (types 1 and 3) and qualitative (type 2) defects, caused by pathogenic variants in *VWF*.

Type 1 VWD, characterized by deficiency of von Willebrand factor (VWF), is inherited as an autosomal dominant disorder with variable penetrance. Type 1C VWD is a variant of autosomal dominant type 1 VWD characterized by decreased survival (increased clearance); in addition to VWF antigen and proportionately low VWF activity, patients with this type have an elevated VWF propeptide to VWF antigen ratio and occasionally a multimer abnormality.

The defects observed in type 2 VWD include defects in formation of multimers (type 2A), increased

susceptibility of VWF to degradation by proteases (type 2A), defects in platelet binding with intact multimers (type 2M), enhanced interaction of VWF with platelets (types 2B, and *GP1BA*-related platelet-type VWD), decreased interaction with factor VIII (type 2N), and decreased interaction with collagen (a rare form of type 2M). Types 2B, 2M, and the majority of type 2A cases have an autosomal dominant inheritance pattern, while type 2N is an autosomal recessive disorder.

Type 3 VWD is characterized by severe quantitative deficiency with a virtual absence of VWF and is inherited as an autosomal recessive disorder.

Platelet-type VWD is caused by pathogenic gain-of-function variants in platelet glycoprotein 1b encoded by the *GP1BA* gene (order code 1289), and will not be detected by *VWF* analysis. Complex and sometimes severe phenotypes resulting from compound heterozygosity for qualitative and quantitative defects, such as 2N/1 and 2A/1, have been observed and can be misdiagnosed based on clinical and laboratory phenotype alone.

Genetic testing of the *VWF* gene offers clinical utility in the diagnosis of VWD, in confirming the VWD type to aid in management, and accurately determining recurrence risks. In addition, it can identify the common *VWF* p.D1472H benign variant. This variant decreases ristocetin-mediated VWF-platelet interactions but does not cause type 1 or 2M von Willebrand disease. Individuals who are heterozygous or homozygous for p.D1472H may exhibit reduced ratio of VWF ristocetin cofactor activity to VWF leading to incorrect classification as VWD type 2M. However, p.D1472H does not have functional or clinical consequences and does not result in increased risk for bleeding. Presence or absence of this benign variant will be reported in all tests that include *VWF* genetic analysis by NGS. Types 1 and 3 VWD variants have been identified throughout the *VWF* gene. Although other types, including 1C and 2A, have been associated with variants in certain exons of *VWF*, evolving knowledge has revealed that pathogenic variants causing these phenotypes occur across the gene, and therefore testing of limited exons is no longer a recommended approach; *VWF* Genetic Analysis (All Exons) (order code 4855) is suggested for evaluation of patients with suspected type 1, 1C, 2A, or 3. All type 2B variants and the vast majority of type 2M variants are found in exon 28; for patients with suspected 2B or 2M VWD by specific plasma assays, *VWF* Exon 28 Sequence Analysis (order code 1284) can be considered. Variants causing type 2N are located in specific factor VIII-binding functional domains in exons 17-21 and 24-27; for patients with low factor VIII and suspicion of type 2N VWD, or patients with functional binding assays consistent with this diagnosis, VWD Type 2N Sequence Analysis (order code 1288) is available. In families with a specific VWD diagnosis in whom prior testing has identified a pathogenic variant that fully explains the phenotype, Targeted Familial Variant Analysis (order code 4970) is appropriate for evaluation of at-risk relatives or for prenatal diagnosis.

Among patients without pathogenic variants detected by sequence analysis, large deletions in *VWF* may be detected by array Comparative Genomic Hybridization (aCGH) in approximately 30% of type 1 VWD (VWF antigen less than 30 IU/dL) and 50% of type 1C, as well as 40% of patients with type 3 in whom sequencing did not identify two pathogenic variants (Christopherson et al, 2016). Large deletions or duplications have been reported rarely in type 2 VWD (Goodeve et al, 2009; updated 2017). aCGH for *VWF* (order code 4855) is available for analysis of large deletions and duplications.

Related Testing:

Custom Blood Disorder Panel (NGS and/or aCGH), order code 4850):

- Analysis of 2 to 10 selected genes may also be ordered as a Custom Blood Disorder Panel as dictated by the patient's clinical and laboratory phenotype, as well as their ancestry, family history, or to supplement previous genetic testing.

Targeted Familial Variant Analysis (order code 4970):

- Targeted familial variant analysis (order code 4970) for clinical diagnosis, carrier identification, or prenatal diagnosis can also be performed on any gene when the pathogenic variant(s) is known in the family. If the proband was not tested at Versiti, a control sample may be needed (please call the laboratory to discuss). If the familial variant is a large deletion or duplication, aCGH for the involved gene is required.

Versiti is pleased to support you and your patients' diagnostic journey each step of the way. For clinical questions about laboratory tests and test utilization support, contact Versiti Client Services: (414) 937-6396 or 800-245-3117, Option 1, to be directed to our genetic counselors and clinical support team.

versiti™