THE UNIVERSITY OF CHICAGO | Genetic Services Laboratories

Next Generation Sequencing Panel for Familial Myelodysplastic Syndrome/Acute Leukemia (MDS/AL)

Clinical Features and Molecular Genetics:

The familial occurrence of myelodysplastic syndrome (MDS) and/or acute leukemia (AL) is rare and heterogeneous. Some families inherit purely AL, and others inherit purely MDS or both disorders within the same pedigree. Many cases of familial MDS/AL also arise in those with particular genetic syndromes with additional clinical findings. Patients with familial MDS/AL are usually younger at presentation than individuals with sporadic disease and are recognized by an unusual family history of more than one first-degree relative with MDS/AL. Most of the families show a pattern of inheritance consistent with a single gene mutation, inherited in an autosomal dominant manner [1, 2].

Pure familial MDS/AL is characterized by multiple cases of MDS and/or AL without bone marrow failure or other phenotypic features in one family. It is due to inheritance of a single abnormal copy of a gene encoding a transcription factor that is critical for hematopoiesis [1]. Mutation carriers can have additional findings in addition to the clinical features than MDS/AL, but these may be subtle or absent [1].

Our Familial Myelodysplastic Syndrome/Acute Leukemia includes mutation analysis of the 30 genes listed below.

Familial MDS/AL Panel				
ANKRD26	CEBPA	MLH1	PAX5	SRP72
ATM	CHEK2 (CHK2)	MSH2	PTPN11	TERC
BLM (RECQL3)	DDX41	MSH6	RTEL1	TERT
BRCA1	ETV6	PMS2	RUNX1	TP53
BRCA2	GATA2	NBN (NBS1)	SAMD9	
CBL	IKZF1	NF1	SAMD9L	

Gene	Inheritance	Clinical Features and Molecular Pathology
ANKRD26	AD	Mutations in the 5'UTR and protein coding regions of *ANKRD26* were reported to cause an autosomal-dominant form of inherited thrombocytopenia, THC2. It has been reported that among 105 people with confirmed or suspected *ANKRD26* mutations, 10 developed hematologic malignancies, including seven with acute leukemias. The overall incidence of development of hematologic malignancies was 240 out of 100,000, and of acute leukemia was 167 out of 100,000, both elevated over expected levels [3-5].
ATG2B/ GSKIP	AD	Germline duplication of *ATG2B* and *GSKIP* predisposes to familial myeloid malignancies, including myeloproliferative neoplasms, frequently progressing to leukemia [6].
ATM	AR	Approximately 10% of patients with ataxia telangiectasia due to biallelic *ATM* mutations develop cancer, mostly of the lymphoid malignancies including Hodgkin's lymphoma, non-Hodgkin's lymphoma, and several forms of leukemia [7, 8]. AML has also been reported in patients with AT [9, 10].
BLM (RECQL3)	AR	Mutations in the gene encoding DNA helicase RecQ protein-like-3 (BLM) cause Bloom syndrome. A 13%-25% lifetime risk of MDS/AML has been reported in patients with Bloom syndrome [11, 12].
BRCA1	AD	Germline mutations in *BRCA1* increase the risks of breast or ovary cancer and many other types of cancer, including myeloid leukemia [13-15].
BRCA2	AD	Germline mutations in *BRCA2* predispose individual to a number of different cancers, including leukemia [13-15].
CBL	AD	Germline mutations of the CBL gene are associated with CBL syndrome with predisposition to juvenile myelomonocytic leukemia [16].
CEBPA	AD	Mutations in the *CEBPA* gene are associated with familial acute myeloid leukemia (AML). Typically the first mutation present in the germline within the 5' end of the gene, and a second 3' mutation is acquired within the leukemia. Though germline 3' *CEBPA* mutations have also been identified. *CEBPA* mutations confer a relatively favorable prognosis. Patients found to have

		biallelic *CEBPA* mutations within their leukemic cells should be tested for germline mutations [17].
CHEK2 (CHK2)	AD	Li-Fraumeni syndrome, which can be caused by an inherited germline mutation in the *CHEK2* tumor suppressor gene, presents with an increased risk of nearly all malignancies, including leukemias [2, 12].
DDX41	AD	Recurrent mutations in the DEAD/H-box RNA helicase gene *DDX41* have been reported in patients with familial and acquired myelodsyplasia and acute myeloid leukemia [18].
ETV6	AD	Germline mutations in *ETV6* are associated with thrombocytopenia, red cell macrocytosis and predisposition to hematological malignancies [19-21].
GATA2	AD	Germline mutations in *GATA2* have been described in association with familial MDS/AML, as well as with several heterogeneous clinical syndromes, including Emberger syndrome and the MonoMAC syndrome which show an overall increased risk of developing MDS/AML [17, 22]. Although the incidence of MDS/AL appears very high, there is incomplete penetrance, with some individuals living into late adulthood without developing malignancy or demonstrating hematologic or infectious abnormalities [1].
IKZF1	AD	A germline *IKZF1* mutation can cause an autosomal dominant form of common variable immunodeficiency that is associated with a striking decrease in B-cell numbers, and is a predisposition to B-cell precursor acute lymphoblastic leukemia, pancytopenia and autoimmune diseases [23, 24].
MLH1 MSH2 MSH6 PMS2	AR/AD	Heterozygous mutations in *MLH1, MSH2*, *MSH6* and *PMS2* are associated with hereditary nonpolyposis colon cancer (HNPCC), which is associated with an increased risk of certain cancers, particularly colon and ovarian cancers. Homozygous or compound heterozygous mutations in these genes lead to a mismatch repair deficiency that can result in a mutator phenotype characterized by early onset gastrointestinal tumors, leukemia and/or lymphoma and features of neurofibromatosis type 1. These features can be summarized with the acronym CoLoN' (Colon tumors or/and Leukemia/Lymphoma or/and Neurofibromatosis features) [25-27].
NBN (NBS1)	AR	Biallelic mutations in the *NBS1* gene are responsible for the Nijmegen breakage syndrome which display an elevated risk to lymphoblastic leukemia [26].
NF1	AD	*NF1* microdeletions are associated with a more severe neurofibromatosis 1 (NF1) phenotype and increased risk for developing malignant tumors, including leukemias (especially juvenile myelomonocytic leukemia, with a risk of progression toward AML) [28, 29].
PAX5	AD	*PAX5* is a member of the PAX family of transcription factors and is required for normal B cell development. Germline mutations in *PAX5* are associated with susceptibility to B cell precursor acute lymphoblastic leukemia (B-ALL) [30, 31].
PTPN11	AD	*PTPN11* mutations are the most common cause of Noonan syndrome, and cause 90% of LEOPARD syndrome cases [32]. Patients with Noonan syndrome and LEOPARD syndrome have a predisposition for leukemia and certain solid tumors [33-35]. Noonan syndrome and a pathogenic *PTPN11* mutation represents a 3.5 times increased risk of developing a cancer compared with the general population[35].
RTEL1	AD/AR	Both dominant and recessive mutations in the *RTEL1* gene have been associated with Hoyeraal Hreidarsson syndrome, a clinically severe variant of DC with cerebellar hypoplasia, severe immunodeficiency, enteropathy, and intrauterine growth retardation [36]. Anticipation has been described in one family where two affected males inherited a heterozygous mutation from a clinically unaffected female with short telomeres [36]. Heterozygous mutations in *RTEL1* have been reported in patients with bone marrow failure and myelodysplastic syndromes [37, 38].
RUNX1	AD	Germline mutations of *RUNX1* can cause familial platelet disorder with propensity to myeloid malignancy (FPD/AML). The clinical presentation is highly variable, but typically includes a lifelong mild to moderate bleeding tendency due to quantitative and/or functional platelet defects. The incidence of MDS/AL in individuals with germline *RUNX1* mutations is over 40% [2, 11]. Patients may present with MDS/AL at any age, with a median age of onset of 33 years and a range of 6 – 76 years [1]. Different FPD/AL families have varying risks of progressing to myeloid malignancy due to different mutations [17].
SAMD9	AD	Mutations in *SAMD9* cause a multisystem disorder including myelodysplasia, infection, restriction of growth, adrenal hypoplasia, genital phenotypes, and enteropathy. Patients carrying a *SAMD9* mutation can develop MDS that was accompanied by loss of the chromosome 7 [39, 40].
SAMD9L	AD	Mutations in *SAMD9L* cause ataxia-pancytopenia syndrome which is characterized by cerebellar ataxia, variable hematologic cytopenias, and predisposition to marrow failure, myelodysplastic syndrome and myeloid leukemia, sometimes associated with monosomy 7. Hematopoietic revertant mosaicism has been reported and was associated with milder disease [41, 42].
SRP72	AD	*SRP72* encodes one of six protein subunits of the signal recognition particle (SRP), part of the cellular apparatus responsible for nascent protein processing and trafficking. To date, only a small number of families have been identified with *SRP72* mutations and aplastic anemia/MDS [17].

TERC	AD	*TERC* mutations cause autosomal dominant dyskeratosis congenita which often presents later in life without classic mucocutaneous symptoms. *TERC* mutations are associated with anticipation, with progressively shorter telomeres passed down through generations [43]. Members of earlier generations often demonstrate mild disease, whereas those of younger generations experience more severe disease manifestations, such as aplastic anemia or MDS/AML [17, 44-46]
TERT	AD	Heterozygous mutations in the *TERT* gene are associated with autosomal dominant dyskeratosis congenita. Penetrance of these mutations appears to be reduced, with some individuals being asymptomatic [47]. Variable expressivity has also been described, with some individuals being mildly affected [47]. More severe disease manifestations may include aplastic anemia or MDS/AML [17, 44-46].
TP53	AD	Li-Fraumeni syndrome, caused by an inherited germline mutation in the *TP53* tumor suppressor genes, presents with an increased risk of nearly all malignancies, including leukemias [2, 12].

Testing Options

Familial Myelodysplastic Syndrome/Acute Leukemia Panel (28 genes)

Sample specifications:	2 T-25 flasks of cultured skin fibroblasts, or DNA extracted from fibroblasts. NOTE: Peripheral blood samples are not accepted for patients with a history of MDS/leukemia.
Cost:	$4000
CPT codes:	81406, 81407
Turn-around time:	4-6 weeks

Note: We cannot bill insurance for this test.

Test methods:

Comprehensive sequence coverage of the coding regions and splice junctions of all genes in this panel is performed. Targets of interests are enriched and prepared for sequencing using the Agilent SureSelect system. Sequencing is performed using Illumina technology and reads are aligned to the reference sequence. Variants are identified and evaluated using a custom collection of bioinformatic tools and comprehensively interpreted by our team of directors and genetic counselors. All pathogenic and likely pathogenic variants are confirmed by Sanger sequencing. The technical sensitivity of this test is estimated to be >99% for single nucleotide changes and insertions and deletions of less than 20 bp.

Our CNV detection algorithm was developed and its performance determined for the sole purpose of identifying deletions and duplications within the coding region of the gene(s) tested. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by this methodology. Regions of high homology and repetitive regions may not be analyzed. This methodology will not detect low level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of our deletion/duplication assay may be reduced when DNA extracted by an outside laboratory is provided.

Results:

Results, along with an interpretive report, are faxed to the referring physician as soon as they are completed. One report will be issued for the entire panel. All abnormal results are reported by telephone.

For more information about our testing options, please visit our website at dnatesting.uchicago.edu or contact us at 773-834-0555.

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