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## Next Generation Sequencing Panel for Hereditary Lymphoma

**Clinical Features:**
Lymphomas are cancers originating from the lymphoid tissues, and can be divided into two broad categories, Hodgkin lymphoma and non-Hodgkin lymphoma, which can be differentiated by their cellular pathology. Hodgkin lymphoma (HL) is characterized by the presence of neoplastic binucleated B-cells called Reed-Steinberg cells [1]. Exposure to Epstein Barr virus is associated with increased risk of HL [1]. In addition, familial HL has also been described, and first degree relatives of a patient with HL having an increased risk of developing the condition [2, 3]. Non-Hodgkin lymphoma (NHL) is a diverse group of more than 50 types of lymphoma that arise from the lymphoid tissues but do not contain Reed-Steinberg cells, each with distinct morphological, cytogenetic, genetic and clinical features [1]. Exposure to Epstein Barr virus and HIV are associated with increased risk of NHL; the contribution of other lifestyle factors and exposures remains unclear. Families with high rates of NHL have also been described, indicating a hereditary component in some cases [1]. In addition, first degree relatives of patients with NHL have a 1.5-3 fold increased risk of developing the condition, again indicating a genetic component to disease susceptibility [1]. Chronic lymphocytic leukemia (CLL) is considered a subtype of NHL. A 3- to 8.5-fold increased risk of CLL among first-degree relatives of probands has been reported [1].

*Our Tier 1 Hereditary Lymphoma Panels include sequence and del/dup analysis of the 8 genes listed below.*
*Our Tier 2 Hereditary Lymphoma Panels include sequence and del/dup analysis of the 14 genes listed below.*

| **Tier 1: Hereditary Lymphoma panel** | | | |
|---|---|---|---|
| CHEK2 | KLHDC8B | MLH1 | MSH2 |
| MSH6 | NPAT | PMS2 | TP53 |
| **Tier 2: Hereditary Lymphoma Panel** | | | |
| ADA | CARD11 | PRF1 | TNFRSF13B (TACI) |
| ATM | FAS (TNFRSF6) | RECQL3 (BLM) | WAS |
| BRCA1 | NBN (NBS1) | SH2D1A (SAP) | |
| BRCA2 | NF1 | STXBP2 | |

### Cancer/Tumor Susceptibility Syndromes

| Gene | Clinical Features |
|---|---|
| CHEK2 [OMIM# 604373] | Variants in *CHEK2* have been associated with a phenotype similar to Li-Fraumeni syndrome [11], which is characterized by increased susceptibility to a number of different cancers including breast cancer, soft-tissue sarcoma, brain tumors, adrenocortical carcinoma and leukemias. Other cancers may also be observed. Ruijs *et al* (2009) identified four families with a Li-Fraumeni-like phenotype and a heterozygous mutation in *CHEK2*; one family included two individuals with Hodgkins lymphoma [12]. |
| MLH1 [OMIM# 120436] MSH2 [OMIM# 609309] MSH6 [OMIM# 600678] PMS2 [OMIM# 600259] | Biallelic mutations in DNA mismatch repair genes *MLH1, MSH2, MSH6* and *PMS2* are associated with a disorder called constitutional mismatch repair-deficiency syndrome (CMMR-D) [13]. CMMR-D is associated with increased risk of childhood cancers, including hematological malignancies (15%), brain tumors (48%), and gastrointestinal cancers (32%) [14]. The most prevalent hematological malignancies observed are NHL and acute lymphoblastoid leukemia [13]. The median age of diagnosis for patients who develop lymphoma is 5 years [13]. Patients may also exhibit features typically associated with neurofibromatosis type 1, such as café au lait spots or neurofibromas [13]. Heterozygous mutations in the *MLH1, MSH2, MSH6* and *PMS2* genes are associated with Lynch syndrome, an autosomal dominant condition with increased risk of a number of different types of malignancies, including colon, endometrial, ovarian and stomach cancer [13]. |
| NF1 [OMIM# 613113] | Mutations in *NF1* are associated with neurofibromatosis 1 (NF1), an autosomal dominant neurocutaneous disorder associated with increased risk of both benign and malignant tumors [15]. The most common malignancies include gliomas, peripheral nerve sheath tumors, juvenile myelomonocytic leukemia, pheochromocytoma, gastrointestinal stromal tumors, rhabdomyosarcoma, and malignant triton tumors [15]. Patients also have an increased relative risk of childhood NHL of 10 [16]. |

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|---|---|
| TP53<br>[OMIM# 191170] | Heterozygous mutations in *TP53* are associated with Li-Fraumeni syndrome (LFS), a cancer susceptibility disorder [17]. The most common cancers associated with LFS are soft tissue sarcomas, osteosarcomas, pre-menopausal breast cancer, brain tumors, adrenocortical carcinomas and leukemias [17]. A number of other cancers, including Hodgkin and non-Hodgkin lymphomas, have also been described in families with LFS [17]. |
| NPAT<br>[OMIM# 601448] | A heterozygous mutation in NPAT has been described in fours cousins who all presented between the ages of 22 and 26 with nodular lymphocyte predominant Hodgkin lymphoma [18]. |

## Immunodeficiency and Autoimmune Disorders

| Gene | Clinical Features |
|---|---|
| ADA<br>[OMIM# 102700] | Mutations in the *ADA* gene are associated with autosomal recessive severe combined immunodeficiency, which is associated with defective T and B cell function [4]. This condition has been associated with a 5% risk of NHL [4, 5]. |
| FAS (TNFRSF6)<br>[OMIM# 134637] | Autoimmune lymphoproliferative syndrome type 1A (also called ALPS) is caused by heterozygous mutations in the *FAS* gene [6]. The disorder is caused by defective lymphocyte apoptosis and is associated with chronic lymphadenopathy and splenomegaly, and autoimmune cytopenias [6]. ALPS is associated with a 51-fold increased risk of Hodgkin lymphoma, and a 14-fold risk of non-Hodgkin lymphoma. The average of symptom onset is 5 years, and the average age of lymphoma diagnosis is 28 years [6]. |
| SH2D1A (SAP)<br>[OMIM# 300490] | X-linked lymphoproliferative syndrome-1 (XLP1) is caused by mutations in the *SH2D1A* gene. XLP1 is associated extreme sensitivity to Epstein Barr virus, which leads to severe or fatal infection mononucleosis, acquired hypogammaglobulinemia and a 20% risk of lymphoma [1, 7]. Female carriers are typically asymptomatic [8]. |
| TNFRSF13B (TACI)<br>[OMIM# 604907] | Mutations in *TNFRSF13B* are one cause of common variable immunodeficiency (CVID), which is a heterogeneous disorder characterized by impaired production of antibodies after vaccination or antigen exposure, and reduced serum levels of IgG, IgA and IgM [9]. Symptoms can include chronic sinopulmonary infections [9]. First clinical manifestations typically occur in childhood or adolescence [9]. Mellemkjaer *et al.* (2002), identified 4 cases of NHL out of 176 CVID patients, indicating a 6-fold increase in NHL incidence compared to the general population [10]. Mutations in *TNFRSF13B* may be inherited in an autosomal dominant or recessive manner, and penetrance is incomplete [9]. |
| WAS<br>[OMIM# 300392] | Wiskott-Aldrich syndrome (WAS) is a rare X-linked immunodeficiency caused by mutations in the *WAS* gene. Clinical findings may include thrombocytopenia with small platelets, recurrent otitis media, and eczema. The majority of patients are diagnosed in early childhood. The risk of lymphoreticular malignancies such as lymphoma is 13%. |

## Multisystem Disorders

| Gene | Clinical Features |
|---|---|
| ATM<br>[OMIM# 607585] | Biallelic mutations in *ATM* are associated with ataxia telangiectasia (A-T), which is characterized by childhood onset progressive cerebellar ataxia, telangiectasias of the conjunctivae, immunodeficiency and increased risk of cancer [19]. The overall lifetime risk of cancer is 30-40%, with 40% of tumors being NHL, and approximately 5% being HL. Other associated cancers include leukemia, gastric cancer, breast cancer and medulloblastoma [19]. |
| NBN (NBS1)<br>[OMIM# 602667] | Mutations in *NBN* are associated with the rare autosomal recessive disorder Nijmegen breakage syndrome (NBS). Features of NBS include microcephaly, dysmorphic facial features, immunodeficiency, chromosomal instability, and increased cancer susceptibility. In a series of 55 patients, 29% developed lymphoma, which was the most common cancer observed in the cohort [20]. Age of onset ranged from 1-22 years [20]. Biallelic mutations in *NBN* have also been associated with aplastic anemia [21]. |
| RECQL3 (BLM)<br>[OMIM# 604610] | Mutations in *RECQL3* are associated with Bloom syndrome, an autosomal recessive disorder characterized by growth deficiency, immunodeficiency, sun-sensitive erythema and cancer susceptibility [22]. The lifetime risk of cancer for Bloom syndrome patients is 20% [23], including a 13% risk of NHL and a 1% risk of HL [1]. Other cancer risks include colorectal, breast, larynx and skin cancers [23]. |

## Other disorders

| Gene | Clinical Features |
|---|---|
| BRCA1 | Germline mutations in BRCA1 increase the risks of breast or ovary cancer and all other cancers including Hodgkin's and non-Hodgkin's lymphoma[24, 25] |
| BRCA2 | Germline mutations in BRCA2 increase the risks of breast or ovary cancer and all other cancers including Hodgkin's and non-Hodgkin's lymphoma[24, 25] |
| CARD11<br>[OMIM# 607210] | Recurrent somatic mutations in the *CARD11* gene have previously been identified in tumor samples from diffuse large B-cell lymphoma [26, 27]. A heterozygous germline missense mutation in *CARD11* has also been described in a family with hereditary polyclonal B cell lymphocytosis and splenomegaly [28]. B cell lymphocytosis is a condition that resembles chronic lymphocytic leukemia, and can be difficult to clinically distinguish from lymphoma. |

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| KLHDC8B [OMIM# 613169] | Salipante *et al* (2009) identified a family with multiple individuals with HL, where disease segregated with a chromosomal translocation that disrupted the *KLHDC8B* gene [29]. In three other families with HL a variant in the 5' untranslated region of the *KLHDC8B* gene was found to segregate with the disease phenotype [29]. |
| PRF1 [OMIM# 170280] | Biallelic mutations in *PRF1* are associated with familial hemophagocytic lymphohistiocytosis-2 (FHL2), a life-threatening disorder caused by uncontrolled proliferation of CD25+ T-cells and activation of macrophages that phagocytose blood cells [30]. Symptoms can include fever, hepatosplenomegaly, cytopenias, hypertriglyceridemia, and hypofibrinogenemia [30]. Onset is typically in infancy, however late onset cases have also been described [31]. Clementi *et al* (2005) identified biallelic *PRF1* mutations in 4 out of 29 patients with HL or NHL who also had clinical features of hemophagocytic lymphohistiocytosis [32]. In addition, 4 patients had a heterozygous mutation in the *PRF1* gene. |
| STXBP2 [OMIM# 601717] | Biallelic *STXBP2* mutations have been described in patients with familial hemophagocytic lymphohistiocytosis-5 (FHL5), a genetic disorder of lymphocyte cytotoxicity that typically presents within the first two years of life [33]. Atypical cases with later onset in adulthood have also been described [33]. Associated complications of hemophagocytic lymphohistiocytosis (HLH) can include immunodeficiency, granulomatous lung or liver disease, encephalitis or lymphoma. Rohr *et al* described one individual with biallelic *STXBP2* mutations who presented with Hodkin lymphoma at age 6 years, 2 years prior to first onset of HLH [33]. |

## 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 novel and/or potentially 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. Deletion/duplication analysis of the panel genes is performed by oligonucleotide array-CGH. Partial exonic copy number changes and rearrangements of less than 400 bp may not be detected by array-CGH. Array-CGH will not detect low-level mosaicism, balanced translocations, inversions, or point mutations that may be responsible for the clinical phenotype. The sensitivity of this assay may be reduced when DNA is extracted by an outside laboratory.

### Tier 1: Hereditary Lymphoma Panel (sequence and deletion/duplication analysis of 8 genes)

Sample specifications: 3 to10 cc of blood in a purple top (EDTA) tube
Cost: $3800
CPT codes: 81450
Turn-around time: 4-6 weeks

***Note: We cannot bill insurance for this test.***

### Tier 2: Hereditary Lymphoma Panel (sequence and deletion/duplication analysis of 14 genes)

Sample specifications: 3 to10 cc of blood in a purple top (EDTA) tube
Cost: $2800
CPT codes: 81450
Turn-around time: 4-6 weeks

***Note: We cannot bill insurance for this test.***

### Hereditary Lymphoma Series

Sample specifications: 3 to10 cc of blood in a purple top (EDTA) tube
Cost: $3300-5300
CPT codes: see next page
Turn-around time: 8-10 weeks (Tier 1), 8-10 weeks (Tier 2)

***Note: We cannot bill insurance for this test.***

| Tier | Panel name | CPT code | Cost |
|---|---|---|---|
| 1 | Tier 1: Hereditary Lymphoma Panel (sequencing and deletion/duplication analysis of 8 genes) | 81450 | $3300 |
| 2 | Tier 2: Hereditary Lymphoma Panel (sequencing and deletion/duplication of 14 genes) | 81450 | $2000 |

## 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 Inherited Bone Marrow Sequencing Panel. All abnormal results are reported by telephone.

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