Clinical Policy Bulletin:  
Genetic Testing

Revised February 2015

Number: 0140

Policy

Aetna considers genetic testing medically necessary to establish a molecular diagnosis of an inheritable disease when all of the following are met:

- The member displays clinical features, or is at direct risk of inheriting the mutation in question (pre-symptomatic); and
- The result of the test will directly impact the treatment being delivered to the member; and
- After history, physical examination, pedigree analysis, genetic counseling, and completion of conventional diagnostic studies, a definitive diagnosis remains uncertain, and one of the following diagnoses is suspected (this list is not all-inclusive):

<table>
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<th>Genetic Condition</th>
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<td>Achondroplasia (FGFR3)</td>
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<td>Albinism</td>
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<td>Alpha-1 antitrypsin deficiency (SERPINA1)</td>
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<td>Alpha thalassemia/Hb Bart hydrops fetalis syndrome/HbH disease* (HBA1/HBA2, alpha globin 1 and alpha globulin 2)</td>
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<td>Angelman syndrome (GABRA, SNRPN)</td>
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<td>Charcot-Marie Tooth disease (PMP-22)</td>
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<td>Classical lissencephaly</td>
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<td>Congenital adrenal hyperplasia/21 hydroxylase deficiency (CYP21A2)*</td>
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<td>Congenital amegakaryocytic thrombocytopenia</td>
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<td>Congenital central hypoventilation syndrome (PHOX2B)</td>
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<tr>
<td>Congenital muscular dystrophy type 1C (MDC1C) (FKRFR (Fukutin related protein))</td>
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<td>Crouzon syndrome (FGFR2, FGFR3)</td>
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<td>Cystic fibrosis (CFTR) (see below)</td>
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<td>Dentatorubral-pallidoluysian atrophy</td>
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<td>Duchenne/Becker muscular dystrophy (dystrophin)</td>
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<td>Dystrophia myopathica</td>
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<td>Ehlers-Danlos syndrome</td>
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<td>Emery-Dreifuss muscular dystrophy (EDMD1, 2, and 3)</td>
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<td>Fabry disease</td>
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<td>Factor V Leiden mutation (F5 (Factor V))</td>
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<td>Factor XII deficiency, congenital (F13 (Factor XII beta globulin))</td>
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<td>Familial adenomatous polyposis coli (APC) (see below)</td>
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<td>Familial dysautonomia (IKBKAP)</td>
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<td>Familial hypocalciuric hypercalcemia</td>
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<td>Genetic Testing</td>
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<tr>
<td><strong>Familial Mediterranean fever (MEFV)</strong></td>
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<td><strong>Fanconi anemia (FANCC, FANCD)</strong></td>
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<td><strong>Facioscapulohumeral muscular dystrophy (FSHMD1A)</strong></td>
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<td><strong>Fragile X syndrome, FRAXA (FMR1)</strong> (see below)</td>
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<td><strong>Friedreich’s ataxia (FRDA (frataxin))</strong></td>
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<td><strong>Galactosemia (GALT)</strong></td>
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<td><strong>Gaucher disease (GBA (acid beta glucosidase))</strong></td>
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<td><strong>Gitelman’s syndrome</strong></td>
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<td>**Hemoglobin E thalassemia ****</td>
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<td>**Hemoglobin S and/or C ****</td>
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<tr>
<td><strong>Hemophilia A/VWF (F8 ( Factor VIII))</strong></td>
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<td><strong>Hemophilia B (F9 (Factor IX))</strong></td>
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<td><strong>Hereditary amyloidosis (TTR variants)</strong></td>
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<td><strong>Hereditary ataxia</strong></td>
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<td><strong>Hereditary hemorrhagic telangiectasia (HHT)</strong></td>
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<td><strong>Hereditary hemochromatosis (HFE) (see below)</strong></td>
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<tr>
<td><strong>Hereditary neuropathy with liability to pressure palsies (HNPP)</strong></td>
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<tr>
<td><strong>Hereditary non-polyposis colorectal cancer (HNPPC) (MLH1, MSH2, MSH6, MSI) (see below)</strong></td>
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<tr>
<td><strong>Hereditary paraganglioma (SDHD, SDHB)</strong></td>
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<td><strong>Neimann-Pick disease, type A (SMPD1, sphingomyelin phosphodiesterase)</strong></td>
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<td><strong>Nephrotic syndrome, congenital (NPHS1, NPHS2)</strong></td>
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<tr>
<td><strong>Neurofibromatosis type 1 (NF1, neurofibromin)</strong></td>
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<td><strong>Neurofibromatosis type 2 (Merlin)</strong></td>
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<td><strong>Neutropenia, congenital cyclic</strong></td>
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<td><strong>Phenyketonuria (PAH)</strong></td>
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<td><strong>Pfeiffer syndrome (FGFR1)</strong></td>
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<tr>
<td><strong>Prader-Willi-Angelman syndrome (SNRPN, GABRA5, NIPA1, UBE3A, ANCR, GABRA)</strong></td>
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<tr>
<td><strong>Primary dystonia (TOR1A (DYT1))</strong></td>
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<tr>
<td><strong>Prothrombin (F2 (Factor II, 20210G&gt; A mutation))</strong></td>
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<td><strong>Pyruvate kinase deficiency (PKD)</strong></td>
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<td><strong>Retinoblastoma (Rb)</strong></td>
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<td><strong>Rett syndrome (FOXG1, MECP2)</strong></td>
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<td><strong>Saethre-Chotzen syndrome (TWIST, FGFR2)</strong></td>
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<tr>
<td><strong>Saethre-Chotzen syndrome (TWIST, FGFR2)</strong></td>
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<tr>
<td><strong>Smith-Lemli-Opitz syndrome</strong></td>
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<td><strong>Spinal muscular atrophy (SMN1, SMN2)</strong></td>
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<tr>
<td><strong>Thanatophoric dysplasia (FGFR3)</strong></td>
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<tr>
<td><strong>Von Gierke disease (G6PC, Glycogen storage disease, Type 1a)</strong></td>
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<td><strong>Von Hippel-Lindau syndrome (VHL)</strong></td>
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<tr>
<td><strong>Walker-Warburg syndrome (POMGNT1)</strong></td>
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<td><strong>22q11 deletion syndromes (DCGR (CATCH-22))</strong></td>
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</tbody>
</table>

* Medically necessary if results of the adrenocortical profile following cosyntropin stimulation test are equivocal or for purposes of genetic counseling.

** Electrophoresis is the appropriate initial laboratory test for individuals judged to be at-risk for a hemoglobin disorder.

In the absence of specific information regarding advances in the knowledge of mutation characteristics for a particular disorder, the current literature indicates that genetic tests for inherited disease need only be conducted once per lifetime of the member.

**Note:** Genetic testing of Aetna members is excluded from coverage under Aetna's benefit plans if the testing is performed primarily for the medical management of other family members who are not covered under an Aetna benefit plan. In these circumstances, the insurance carrier for the family members who are not covered by Aetna should be contacted regarding coverage of genetic testing. Occasionally, genetic testing of tissue samples from other family members who are not covered by Aetna may be required to provide the medical information necessary for the proper medical care of an Aetna member. Aetna covers genetic testing for heritable disorders in non-Aetna members when all of the following conditions are met:

- The information is needed to adequately assess risk in the Aetna member; and
- The information will be used in the immediate care plan of the Aetna member; and
- The non-Aetna member's benefit plan, if any, will not cover the test (a copy of the denial letter*** from the non-Aetna member's benefit plan must be provided).

*** Aetna may also request a copy of the certificate of coverage from the non-member's health insurance plan if: (i) the denial letter from the non-member's insurance carrier fails to specify the basis for non-coverage; (ii) the denial is based on a specific plan exclusion; or (iii) the genetic test is denied by the non-member's insurance carrier as not medically necessary and the medical information provided to Aetna does not make clear why testing would not be of significant medical benefit to the non-member.

**Medical Necessity Criteria for Specific Genetic Tests:**

**I. Adenosis polyposis coli (APC):**

Aetna considers adenosis polyposis coli (APC) genetic testing medically necessary for either of the following indications:

- A. Members with greater than 10 colonic polyps; or
- B. Members with a desmoid tumor; or
C. Members with 1st-degree relatives (i.e., siblings, parents, and offspring) diagnosed with familial adenomatous polyposis (FAP) or with a documented APC mutation. The specific APC mutation should be identified in the affected 1st-degree relative with FAP prior to testing the member, if feasible. Full sequence APC genetic testing is considered medically necessary only when it is not possible to determine the family mutation first.

Aetna considers APC genetic testing experimental and investigational for all other indications because its effectiveness for indications other than the ones listed above has not been established.

II. CADASIL:

Aetna considers DNA testing for CADASIL medically necessary for either of the following indications:

A. Pre-symptomatic individuals where there is a family history consistent with an autosomal dominant pattern of inheritance and there is a known mutation in an affected member of the family; or
B. Symptomatic individuals who have a family history consistent with an autosomal dominant pattern of inheritance of this condition (clinical signs and symptoms of CADASIL include stroke, cognitive defects and/or dementia, migraine, and psychiatric disturbances).

Aetna considers CADASIL genetic testing experimental and investigational for all other indications because its effectiveness for indications other than the ones listed above has not been established.

III. Catecholaminergic polymorphic ventricular tachycardia (CPVT):

Aetna considers genetic testing for CPVT medically necessary for the following indications:

A. Children or young adults (less than 40 years of age) with a 1st degree relative with a clinical diagnosis of CPVT, or a 1st or 2nd degree relative with a defined CPVT mutation; or
B. Persons who display exercise- or emotion-induced PVT or ventricular fibrillation, occurring in a structurally normal heart.

IV. Cystic fibrosis:

Aetna considers genetic carrier testing for cystic fibrosis medically necessary for members in any of the following groups:

A. Couples seeking prenatal care; or
B. Couples who are planning a pregnancy; or
C. Persons with a family history of cystic fibrosis; or
D. Persons with a 1st degree relative identified as a cystic fibrosis carrier; or
E. Reproductive partners of persons with cystic fibrosis.

Aetna considers genetic carrier testing for cystic fibrosis experimental and investigational for all other indications because its effectiveness for indications other than the ones listed above has not been established.

Aetna considers a core panel of 25 mutations that are recommended by the American College of Medical Genetics (ACMG) medically necessary for cystic fibrosis genetic testing. The standard CF transmembrane regulator (CFTR) mutation panel is as follows (Available at: http://www.acmg.net):

<table>
<thead>
<tr>
<th>ΔF508</th>
<th>ΔI507</th>
<th>G542X</th>
<th>G551D</th>
<th>W1282X</th>
<th>N1303K</th>
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<tbody>
<tr>
<td>R553X</td>
<td>621+1G→T</td>
<td>R117H</td>
<td>1717-1G→A</td>
<td>A455E</td>
<td>R560T</td>
</tr>
<tr>
<td>R1162X</td>
<td>G85E</td>
<td>R334W</td>
<td>R347P</td>
<td>711+1G→T</td>
<td>1898+1G→A</td>
</tr>
<tr>
<td>2184delA</td>
<td>1078delT</td>
<td>3849+10kbC→T</td>
<td>2789+5G→A</td>
<td>3659delC</td>
<td>I148T</td>
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<tr>
<td>3120+1G→A</td>
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Aetna considers experimental and investigational screening for cystic fibrosis mutations that extend beyond the standard mutation panel recommended by the ACMG.

V. Factor V Leiden:

Aetna considers Factor V Leiden genetic testing medically necessary for members with any of the following indications:

A. Age less than 50, any venous thrombosis; or
B. Myocardial infarction in female smokers under age of 50; or
C. Recurrent venous thrombosis; or
D. Relatives of individuals with venous thrombosis under age of 50; or
E. Venous thrombosis and a strong family history of thrombotic disease; or
F. Venous thrombosis in pregnant women or women taking oral contraceptives; or
G. Venous thrombosis in unusual sites (such as hepatic, mesenteric, and cerebral veins).

Aetna considers Factor V Leiden genetic testing experimental and investigational for all other indications because its effectiveness for indications other than the ones listed above has not been established.

Aetna considers Factor V HR2 allele DNA mutation analysis experimental and investigational because its effectiveness has not been established.

VI. Familial nephrotic syndrome (NPHS1, NPHS2):

A. Aetna considers genetic testing for an NPHS1 mutation medically necessary for children with congenital nephrotic syndrome (nephrotic syndrome appearing within the first month of life) who are of Finnish descent or who have a family history of congenital nephrotic syndrome. Genetic testing for NPHS1 mutations are considered experimental and investigational for screening other persons with nephrotic syndrome and for all other indications because its effectiveness for other indications other has not been established.

B. Aetna considers genetic testing for an NPHS2 mutation medically necessary for children with steroid resistant nephrotic syndrome (SRNS) and for children who have a family history of SRNS. Genetic testing for NPHS2 is considered experimental and investigational for persons with steroid-responsive nephrotic syndrome and for all other indications because its effectiveness for indications other than the ones listed above has not been established.

Aetna considers genetic testing for familial nephrotic syndrome experimental and investigational for all other indications.

VII. Fragile X:

Aetna considers genetic testing for fragile X syndrome medically necessary for members in any of the following risk categories where the results of the test will affect a member's clinical management or reproductive decisions:

A. Individuals with mental retardation, developmental delay, or autism; or
B. Individuals planning a pregnancy who have either of the following:
   1. A family history of fragile X syndrome, or
   2. A family history of undiagnosed mental retardation; or
C. Fetuses of known carrier mothers. Prenatal testing of a fetus by amniocentesis or chorionic villus sampling is indicated following a positive Fragile X carrier test in the mother.

Aetna considers Fragile X DNA testing medically necessary for members with a negative cytogenetic test for fragile X if they have any physical or behavioral characteristics of fragile X syndrome and have a family history of fragile X syndrome or undiagnosed mental retardation.

Aetna considers Fragile X DNA testing medically necessary for members with a phenotype that is not typical for fragile X syndrome who have a cytogenetic test that is positive for fragile X.

Aetna considers population-based fragile X syndrome screening of individuals who are not in any of the above-listed risk categories experimental and investigational because its effectiveness for indications other than the ones listed above has not been established.

VIII. Hereditary hemochromatosis:

Aetna considers genetic testing for HFE gene mutations medically necessary for persons who meet all of the following criteria:

A. Member who has symptoms consistent with iron overload; and
B. Member who has 2 consecutive transferrin saturations of 45% or more.

Aetna considers genetic testing for HFE gene mutations medically necessary for 1st degree relatives of persons homozygous for HFE gene mutations. Genetic testing for hereditary hemochromatosis is considered experimental and investigational for all other indications because its effectiveness for indications other than the ones listed above has not been established.

IX. Hereditary non-polyposis colorectal cancer (HNPPC)/Lynch syndrome (LS):

A. Aetna considers genetic testing for HNPPC (MLH1, MSH2, MSH6, PMS2, EPCAM, sequence analysis) medically necessary for members who meet any one of the following criteria:
   1. Member meets Amsterdam II criteria or revised Bethesda guidelines (see appendix); or
   2. Member is diagnosed with endometrial cancer before age 50 years; or
   3. Member has a 1st- or 2nd-degree relative with a disease confirmed to be caused by a HNPPC mutation (genes MLH1, MSH2, MSH6, PMS2, EPCAM) upon testing of the 1st- or 2nd-degree relative; or
   4. Member has ≥5% risk of LS on a validated mutation prediction model (eg, MMRpro, PREMM[1,2,6], MMRpredict). The PREMM[1,2,6] model can be used online at http://premm.dfcio.harvard.edu/ and the HNPPC predict model is available for online use at http://hnppcpredict.hgu.mrc.ac.uk/. MMRpro is available...
B. Aetna considers microsatellite instability (MSI) testing or immunohistochemical (IHC) analysis of tumors medically necessary as an initial test in persons with colorectal or endometrial cancer in order to identify those persons who should proceed with HNPPC mutation analysis.

See also CPB 0516 - Colorectal Cancer Screening.

X. Hereditary pancreatitis (PRSS1):

Aetna considers genetic testing for hereditary pancreatitis (PRSS1 mutation) medically necessary in symptomatic persons with any of the following indications:

A. A family history of pancreatitis in a 1st-degree (parent, sibling, child) or 2nd-degree (aunt, uncle, grandparent) relative; or
B. An unexplained episode of documented pancreatitis occurring in a child that has required hospitalization, and where there is significant concern that hereditary pancreatitis should be excluded; or
C. Recurrent (2 or more separate, documented episodes with hyper-amylasemia) attacks of acute pancreatitis for which there is no explanation (anatomical anomalies, ampullary or main pancreatic strictures, trauma, viral infection, gallstones, alcohol, drugs, hyperlipidemia, etc.); or
D. Unexplained (idiopathic) chronic pancreatitis.

This policy is based upon guidelines from the Consensus Committees of the European Registry of Hereditary Pancreatic Diseases, the Midwest Multi-Center Pancreatic Study Group and the International Association of Pancreatology (Ellis et al, 2001).

Aetna considers genetic testing for hereditary pancreatitis experimental and investigational for all other indications because its effectiveness for indications other than the ones listed above has not been established.

XI. Long QT syndrome:

Aetna considers genetic testing for long QT syndrome medically necessary for either of the following:

A. Persons with a prolonged QT interval on resting electrocardiogram (a corrected QT interval (QTc) of 470 msec or more in males and 480 msec or more in females) without an identifiable external cause for QTc prolongation (such as heart failure, bradycardia, electrolyte imbalances, certain medications and other medical conditions); or
B. Persons with 1st-degree relatives (siblings, parents, offspring) with a defined LQT mutation, or long QT syndrome in sudden death (1st or 2nd degree) close relatives.

Aetna considers genetic testing for long QT syndrome experimental and investigational for all other indications because its effectiveness for indications other than the ones listed above has not been established.

XII. Malignant Hyperthermia Susceptibility:

Aetna considers genetic testing for malignant hyperthermia susceptibility (MHS) medically necessary for either of the following indications:

- screening clinically confirmed MHS patients for variants in the RYR1 gene that are considered causative for MH by the European Malignant Hyperthermia Group (EMHG) to facilitate predictive testing in at-risk relatives.
- screening at-risk relatives of patients with clinically confirmed MHS for a known familial variant in the RYR1 gene that is considered causative for MH by the EMHG.

Aetna considers genetic testing for malignant hyperthermia susceptibility (MHS) experimental and investigational for all other indications.

Aetna considers genetic testing for central core disease (CCD) experimental and investigational because there is inadequate evidence in the peer-reviewed published literature regarding its effectiveness.

XIII. MUTYH-associated polyposis:

Aetna considers testing for MUTYH mutations medically necessary for the following indications:

A. Members with greater than 10 colonic polyps; or
B. Members meeting criteria for serrated polyposis syndrome (SPS) (see below) with at least some adenomas; or
C. Members 1st-degree relatives (i.e., siblings, parents, and offspring) with a documented deleterious biallelic MUTYH mutation.

A clinical diagnosis of SPS is considered in an individual who meets at least one of the following empiric criteria:

1) At least 5 serrated polyps proximal to the sigmoid colon with 2 or more of these being greater than 10 mm; or
Aetna considers MUTYH mutations testing experimental and investigational for any other indications because its effectiveness for indications other than the ones listed above has not been established.

XIV. Primary dystonia (DYT1):

Aetna considers genetic testing for DYT1 medically necessary for the following indications:

A. Parents of children with an established DYT1 mutation, for purposes of family planning; or
B. Persons with onset of primary dystonia other than focal cranial-cervical dystonia after age 30 years who have a affected relative with early onset (before 30 years); or
C. Persons with primary dystonia with onset before age 30 years.

Aetna considers DYT-1 testing experimental and investigational for all other indications, including the following because its effectiveness for indications other than the ones listed above has not been established:

A. Asymptomatic individuals (other than parents of affected children), including those with affected family members (genetic testing for dystonia (DYT-1) is not sufficient to make a diagnosis of dystonia unless clinical features show dystonia); or
B. Persons with onset of symptoms after age 30 years who either have focal cranial-cervical dystonia; or
C. Persons with onset of symptoms after age 30 years who have no affected relative with early onset dystonia.

This policy is adapted from guidelines from the European Federation of Neurological Societies.

XV. Spinal Muscular Atrophy

Aetna considers genetic testing for spinal muscular atrophy (SMA) medically necessary for the following indications:

■ the diagnosis of persons with hypotonia and muscle weakness who are suspected of having spinal muscular atrophy; or
■ the identification of SMN1 deletion carriers in the families of persons with SMA; or
■ the prenatal diagnosis or preimplantation genetic diagnosis of SMA in the pregnancy of two known carriers

Aetna considers genetic testing for spinal muscular atrophy (SMA) experimental and investigational for the identification of SMN1 deletion carriers in the general population and for all other indications because there is inadequate evidence in the published peer-reviewed clinical literature regarding its effectiveness.

XVI. SHOX-related short stature:

Aetna considers genetic testing for SHOX-related short stature medically necessary for children and adolescents with any of the following features:

A. Above-average body mass index (BMI); or
B. Cubitus valgus (increased carrying angle); or
C. Dislocation of the ulna at the elbow; or
D. Increased sitting height/height ratio; or
E. Madelung deformity of the forearm; or
F. Muscular hypertrophy; or
G. Reduced arm span/height ratio; or
H. Short or bowed forearm.

Aetna considers genetic testing for SHOX-related short stature experimental and investigational for all other indications because its effectiveness for indications other than the ones listed above has not been established.

XVII. Hypertrophic cardiomyopathy (HCM):

Aetna considers genetic testing for HCM medically necessary for individuals who are at-risk for development of HCM, defined as having a close (1st or 2nd degree) relative with established HCM, when there is a known pathogenic gene mutation present in an affected relative. Aetna considers genetic testing for HCM experimental and investigational for all other indications because its effectiveness for indications other than the one listed above has not been established.

XVIII. Thoracic aortic aneurysms and dissections (TAAD)

Aetna considers genetic testing for thoracic aortic aneurysms and dissections (TAAD) medically necessary for asymptomatic blood relatives of persons with genetically confirmed TAAD. Genetic testing for thoracic aortic aneurysms and dissections (TAAD) is considered experimental and investigational for any other indication, including but not limited to patients clinically diagnosed with TAAD, with a positive family history of the disorder, and for whom a genetic syndrome has been excluded.

XIX. Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy (ARVD/C)
Genetic testing for Arrhythmogenic Right Ventricular Dysplasia/Cardiomyopathy (ARVD/C) is considered medically necessary for the following indications:

- Testing for sequence variants in the DSG2, DSP, and PKP2 genes in probands with ITF-confirmed ARVD/C to facilitate genetic screening for ARVD/C in at-risk relatives; or
- Testing for a known familial sequence variant in the DSG2, DSP, and PKP2 genes for at-risk relatives of probands with ITF-confirmed ARVD/C, who are either asymptomatic or have ARVD/C symptoms that fail to meet the ITF diagnostic criteria.

Genetic testing for ARVD/C is considered experimental and investigational for all other indications.

XX. Osteogenesis imperfecta

Genetic testing for COL1A1 and COL1A2 gene sequencing in the management of osteogenesis imperfecta types I to IV, is considered medically necessary for the following indications:

- Genetic testing for sequence variants in COL1A1/2 to confirm the presence of mosaicism in the asymptomatic parent of a child with OI caused by sequence variants in COL1A1/2 for reproductive decision making purposes; or
- Preimplantation genetic diagnosis or prenatal diagnosis for sequence variants in COL1A1/2 in couples in which 1 or both members have OI caused by sequence variants in COL1A1/2.

Genetic testing for COL1A1 and COL1A2 gene sequencing is considered experimental and investigational in any other circumstances, including, but not limited to:

- testing for sequence variants in COL1A1/2 to confirm diagnosis of OI when clinical and radiological examination and family history provide adequate information for diagnosis of OI.
- Genetic testing for sequence variants in COL1A1/2 for the diagnosis of OI when clinical and radiological examination and family history provide inadequate information for diagnosis of OI.
- Genetic testing for sequence variants in COL1A1/2 in children diagnosed with OI to aid in reproductive planning for unaffected couples seeking to have additional children.

Genetic testing for COL1A1/2 is considered experimental and investigational for all other indications.

XXI. Neurofibromatosis

Genetic testing for neurofibromatosis is considered medically necessary for persons who meet all of the following criteria:

- Displays a sign of or has clinical features of the NF; and
- Has a 50% risk of inheriting NF (pre-symptomatic); and
- A definitive diagnosis remains uncertain despite a complete family/personal history, physical examination and conventional diagnostic studies; and
- Confirmation of the diagnosis will impact treatment.

Genetic testing for neurofibromatosis is considered experimental and investigational for all other indications.

XXII. Marfan syndrome

Aetna considers genetic testing for Marfan syndrome (MFS) medically necessary for the following indications:

- the use of FBN1 gene testing to facilitate the diagnosis of Marfan syndrome in patients who do not fulfill the Ghent diagnostic criteria but have at least one major feature of the condition; or
- the use of Marfan syndrome gene testing in patients fulfilling the Ghent diagnostic criteria for the purpose of obtaining information for reproductive decision making or facilitating the diagnosis of Marfan syndrome in at-risk relatives; or
- the use of Marfan syndrome gene testing in the evaluation of at-risk relatives of patients carrying known disease-causing variants; or
- the prenatal diagnosis or PGD of Marfan syndrome in the offspring of patients with known disease-causing variants.

Genetic testing for Marfan syndrome (MFS) is considered experimental and investigational for any other indications, including but not limited to:

- the use of FBN1 gene testing in the diagnostic evaluation of Marfan syndrome in patients exhibiting only minor features of the condition, according to the Ghent diagnostic criteria.
- the use of TGFBR2 gene testing to facilitate the diagnosis of Marfan syndrome in patients testing negative for FBN1 gene variants
- the use of TGFBR1 gene testing to facilitate the diagnosis of Marfan syndrome in patients testing negative for FBN1 and TGFBR2 gene variants.
- the use of Marfan syndrome gene testing in patients fulfilling the Ghent diagnostic criteria who will not be using the information for reproductive decision making or facilitating the diagnosis of Marfan syndrome in at-risk relatives.

XXIII. Cadherin-1 for hereditary diffuse gastric cancer

http://qawww.aetna.com/cpb/medical/data/100_199/0140_draft.html
Cadherin-1 (e-cadherin, CDH1) for hereditary diffuse gastric cancer (HDGC) is considered medically necessary for persons who meet the following criteria:

- Individuals with HDGC for the purpose of identifying a CDH1 gene sequence variant that may be used to screen at-risk first- and second-degree relatives; or
- Presymptomatic testing of first- and second-degree at-risk relatives for a known familial variant in the CDH1 gene.

Cadherin 1 testing is considered experimental and investigational for confirming the clinical diagnosis of HDGC and for other indications because there is inadequate evidence in the peer-reviewed published clinical literature regarding its effectiveness.

XXIV. Maturity Onset Diabetes of the Young (MODY)

Genetic testing for maturity-onset diabetes of the young (MODY) is considered medically necessary for the diagnosis of MODY2 or MODY3 in persons with hyperglycemia or non-insulin-dependent diabetes who have a family history of abnormal glucose metabolism in at least 2 consecutive generations, with the patient or ≥ 1 family members diagnosed before age 25.

Genetic testing for maturity-onset diabetes of the young (MODY) is considered experimental and investigational for all other indications.

XXV. Huntington disease

Aetna considers genetic testing for Huntington disease (HD) medically necessary for either of the following indications:

- Predictive testing for CAG repeat length in asymptomatic individuals from families in which there is a history of HD to define risk of transmission; or
- Prenatal testing for CAG repeat length in fetuses from families in which there is a history of HD.

Genetic testing for Huntington disease is considered experimental and investigational for indications other than those listed above.

XXVI. Familial hypocalciuric hypercalcemia:

Aetna considers familial hypocalciuric hypercalcemia (FHH) medically necessary in any of the following:

A. Atypical cases where no family members are available for testing; or
B. Families with familial isolated hyperparathyroidism; or
C. Infants or children under 10 years of age in whom neonatal hyperparathyroidism, neonatal severe hyperparathyroidism, and FHH are the commonest causes of parathyroid hormone-dependent hypercalcemia; or
D. Individuals with overlap in the calcium/creatinine (Ca/Cr) clearance ratio, namely between 0.01 and 0.02; or
E. Individuals with the phenotype of FHH whose parents are both normocalcemic (i.e., FHH possibly caused by a de novo CaSR mutation).

XXVII. PTEN Gene Testing

Based upon guidelines from the National Comprehensive Cancer Network, PTEN gene testing is considered medically necessary in individuals with a suspected or known clinical diagnosis of Cowden syndrome or Bannayan-Riley-Ruvalcaba syndrome (BRR), or a known family history of a PTEN mutation who meet any of the following criteria:

A. A relative with a known deleterious PTEN gene mutation; or
B. Personal history of any of the following:
   1. Bannayan Riley-Ruvalcaba syndrome; or
   2. Adult Lhermitte-Duclos disease (LDD); or
   3. Autism-spectrum disorder and macrocephaly; or
   4. At least 2 biopsy-proven trichilemmomas; or
   5. Macrocephaly plus one other major criteria*; or
   6. Three major criteria* without macrocephaly; or
   7. One major and at least three minor criteria*; or
   8. Four or more minor criteria*.

C. Family history of both of the following:
   1. At-risk relative (includes 1st-degree relative or more distant relatives if the 1st degree relative is unavailable or unwilling to be tested) with a clinical diagnosis of Cowden syndrome or BRR (no previous genetic testing); and
   2. One major or two minor criteria* in the at-risk relative.

* Criteria for PTEN genetic testing purposes are:

Major:
Breast cancer
Mucocutaneous lesions

One biopsy-proven trichilemmoma
Multiple palmpoplantar keratoses
Multi-focal or extensive oral mucosal papillomatosis
Multiple cutaneous facial papules (often verrucous)
Macular pigmentation of glans penis

Macrocephaly (97th percentile or greater; 58 cm in adult women, 60 cm in adult men)
Endometrial cancer
Non-medullary Follicular thyroid cancer
Multiple GI hamartomas or ganglioneuromas

Minor:

Colon cancer
Esophageal glycogenic acanthosis (>3)
Papillary or follicular variant of papillary thyroid cancer
Testicular lipomatosis
Vascular anomalies (including multiple intracranial developmental venous anomalies).
Other thyroid lesions (e.g., adenoma, nodule(s), goiter)
Mental retardation (IQ < 75)
Autism spectrum disorder
Single gastrointestinal hamartoma or ganglioneuroma
Lipomas
Renal cell carcinoma

Note: Insufficient evidence exists in the literature to include fibrocystic disease of the breast, fibromas, and uterine fibroids as diagnostic criteria.

I. Li-Fraumeni syndrome (TP53 gene)

TP53 gene testing is considered medically necessary for individuals with a suspected or known clinical diagnosis of Li-Fraumeni syndrome (LFS) or Li-Fraumeni-Like syndrome, or a known family history of a TP53 mutation. Testing is considered medically necessary in individuals whose medical and/or family history is consistent with any of these:

A. A relative with a known deleterious TP53 gene mutation; or
B. A diagnosis of classic Li-Fraumeni syndrome, defined by all of the following:

1. Diagnosis of sarcoma before the age of 45 years; and
2. A parent, child, or full sibling diagnosed with cancer before the age of 45 years; and
3. An additional 1st- or 2nd-degree relative in the same lineage with cancer diagnosed before age 45 years, or a sarcoma at any age; or

C. Persons meeting Chompret criteria:

1. Persons with a tumor from the LFS tumor spectrum (e.g., soft tissue sarcoma, osteosarcoma, brain tumor, breast cancer, adrenocortical carcinoma, leukemia, lung bronchoalveolar cancer) before age 46 years AND at least one first- or second-degree relative with any of the aforementioned cancers (other than breast cancer if the proband has breast cancer) before the age of 56 years or with multiple primaries at any age; or
2. Persons with multiple tumors (except multiple breast tumors), two of which belong to the LFS tumor spectrum with the initial cancer occurring before the age of 46 years; or
3. Individuals with adrenocortical carcinoma or choroid plexus carcinoma at any age of onset, regardless of family history; or

D. A diagnosis of breast cancer before age 30 35 years with a negative BRCA1/2 test especially if there is a family history of sarcoma, brain tumor, or adrenocortical carcinoma.

**** Cancers associated with Li-Fraumeni syndrome include but are not limited to premenopausal breast cancer, bone and soft tissue sarcomas, acute leukemia, brain tumor, adrenocortical carcinoma, choroid plexus carcinoma, colon cancer, and early onset of other adenocarcinomas or other childhood cancers. Note that Ewing sarcoma is less likely to be related to Li-Fraumeni as compared to other sarcomas.

II. Peutz-Jeghers syndrome

STK11 (LKB1) gene testing may be considered for individuals with a suspected or known clinical diagnosis of Peutz-
Jeghers syndrome, or a known family history of a STK11 (LKB1) mutation. Testing may be considered for individuals whose medical and/or family history is consistent with any of the following:

A. A relative with a known deleterious STK11 (LKB1) gene mutation; or
B. A clinical diagnosis of PJS based on at least 2 of the following features:
   1. At least 2 PJS-type hamartomatous polyps of the small intestine;
   2. Mucocutaneous hyperpigmentation of the mouth, lips, nose, eyes, genitalia, or fingers;
   3. A family history of PJS.

III. Ashkenazi Jewish Testing Panel

Aetna considers medically necessary preconception or prenatal carrier screening for couples of Ashkenazi Jewish ancestry with a panel of genetic tests recommended by the American College of Medical Genetics (ACMG):

A. Tay Sachs disease
B. Canavan disease
C. Cystic fibrosis
D. Familial dysautonomia
E. Bloom syndrome
F. Fanconi anemia
G. Niemann-Pick disease
H. Gaucher disease
I. Mucolipidosis IV

If only one partner is of Ashkenazi Jewish ancestry, then testing of that partner is considered medically necessary. Testing of the other partner is considered medically necessary only if the result of testing of the Ashkenazi Jewish partner is positive.

IV. Juvenile Polyposis Syndrome

Genetic testing for juvenile polyposis syndrome (JPS) (BMPR1A and SMAD4) is considered medically necessary for persons who meet any of the following criteria:

A. More than five pathologically confirmed juvenile polyps of the colorectum; or
B. Multiple pathologically confirmed juvenile polyps throughout the GI tract; or
C. Any number of pathologically confirmed juvenile polyps and a family history of juvenile polyps.

Genetic testing for SMAD4 is considered medically necessary for infants with first degree relatives with JPS because of the risk of hereditary hemorrhagic telangiectasia.

V. Aetna considers genetic testing experimental and investigational for any of the following:

A. Age-related macular degeneration
B. Brugada syndrome
C. Choroidal neovascularization (e.g., Retnagene)
D. Congenital stationary night blindness
E. Coronary artery disease
F. Costello syndrome (HRAS gene)
G. Diamond-Blackfan anemia
H. Dilated cardiomyopathy [CMD1A]
I. Epidermolytic hyperkeratosis
J. Essential tremor
K. Familial Alzheimer disease
L. Familial amyotrophic lateral sclerosis (SOD1 mutation)
M. Familial cold urticaria/familial cold autoinflammatory syndrome
N. Familial partial lipodystrophy (FPLD2)
O. Genetic testing panels for aortic dysfunction or dilation
P. Genetic testing panels for colon cancer syndromes
Q. Genetic testing panels for nonsyndromic hereditary hearing loss (e.g., OtoScope, OtoGenome, OtoSeq)
R. Genetic testing panels for X-linked intellectual disability
S. Glioblastoma multiforme
T. Hemiplegic migraine (HM)
U. Hemophilia C (F11 [Factor XII])
V. Heterotaxy
W. Klippel-Feil syndrome
X. Lactose intolerance
Y. Legius syndrome (SPRED1 gene)
Z. Malignant melanoma
AA. May-Hegglin anomaly
AB. McCune-Albright syndrome
AC. Mowat-Wilson syndrome (ZEB2 gene)
AD. Multiple mitochondrial respiratory chain complex deficiencies
AE. Myoclonus-dystonia (epsilon-sarcoglycan gene (SCGE) deletion analysis)
AF. Migrainous vertigo
AG. Narcolepsy
AH. Oculopharyngeal muscular dystrophy (OPMD) (PABPN1 gene)
AI. Osteoporosis
AJ. Parkinson disease
AK. Polycystic kidney disease
AL. Prostate cancer
AM. Seizure disorders (e.g., creatine transporter 1 sequencing for testing parents of individuals with seizures; GABRG2 mutations and SCN1A deletion test for infantile febrile seizures; Generalized epilepsy with febrile seizures plus (GEFS+))
AN. Sleep-walking
AO. Townes-Brocks syndrome (SALL1 gene)
AP. Type 2 diabetes (other than MODY)
AQ. Very long chain acylCoA dehydrogenase deficiency (VLCADD)
AR. von Willebrand factor gene testing.

VI. Aetna considers the following tests experimental and investigational:

- deCODE AF
- deCODE BreastCancer
- deCODE Glaucoma
- deCODE MI
- deCODE PrCa
- deCODE T2
- EpiSEEK test for epilepsy/seizures
- Exome sequencing
- Home genetic tests
- Nuclear encoded mitochondrial genomic sequencing panel
- OncoVue breast cancer risk test
- POLG1 for mitochondrial recessive ataxia syndrome
- SLCO1B1 testing for statin induced myopathy
- SLIT1 testing for Asperger syndrome
- Whole genome sequencing
- Whole mitochondrial genome sequencing


Background

According to the American College of Medical Genetics (ACMG), an important issue in genetic testing is defining the scope of informed consent. The obligation to counsel and obtain consent is inherent in the clinician-patient and investigator-subject relationships. In the case of most genetic tests, the patient or subject should be informed that the test might yield information regarding a carrier or disease state that requires difficult choices regarding their current or future health, insurance coverage, career, marriage, or reproductive options. The objective of informed consent is to preserve the individual's right to decide whether to have a genetic test. This right includes the right of refusal should the individual decide the potential harm (stigmatization or undesired choices) outweighs the potential benefits.

DNA-based mutation analysis is not covered for routine carrier testing for the diagnosis of Tay-Sachs and Sandhoff disease. Under accepted guidelines, diagnosis is primarily accomplished through biochemical assessment of serum, leukocyte, or platelet hexosaminidase A and B levels. The literature states that mutation analysis is appropriate for individuals with persistently inconclusive enzyme-based results and to exclude pseudo-deficiency (non-disease related) mutations in carrier couples.

Testing of a member who is at substantial familial risk for being a heterozygote (carrier) for a particular detectable mutation that is recognized to be attributable to a specific genetic disorder is only covered for the purpose of prenatal counseling under plans with this benefit (see CPB 0189 - Genetic Counseling).

Confirmation by molecular analysis of inborn errors of metabolism by traditional screening methodologies (e.g., Guthrie microbiologic assays) is covered. Rigorous clinical evaluation should precede diagnostic molecular testing.

In many instances, reliable mutation analysis requires accurate determination of specific allelic variations in a proband (affected individual in a family) before subsequent carrier testing in other at-risk family members can be accurately performed. Coverage
of testing for individuals who are not Aetna members is not provided, except under the limited circumstances outlined in the policy section above.

Hereditary non-polyposis colon cancer

Hereditary non-polyposis colon cancer (HNPCC, Lynch syndrome) is one of the most common cancer predisposition syndromes affecting 1 in 200 individuals and accounting for 13 to 15% of all colon cancer. HNPCC is defined clinically by early-onset colon carcinoma and by the presence of other cancers such as endometrial, gastric, urinary tract and ovarian found in at least 3 first-degree relatives. Two genes have been identified as being primary responsible for this syndrome: hMLH1 at chromosome band 3p21 accounts for 30% of HNPCC patients, and hMSH2 or FCC at chromosome band 2p22 which together with hMLH1 accounts for 90% of HNPCC.

Unlike other genetic disorders that are easily diagnosed, the diagnosis of HNPCC relies on a very strongly positive family history of colon cancer. Specifically, several organizations have defined criteria that must be met to make the diagnosis of HNPCC.

Although HNPCC lacks strict clinical distinctions that can be used to make the diagnosis, and therefore diagnosis is based on the strong family history, genetic testing is now available to study patient's DNA for mutations to one of the mismatch repair genes mutation. A mutation to one of these genes is a characteristic feature and confirms the diagnosis of HNPCC. Identifying individuals with this disease and performing screening colonoscopies on affected persons may help reduce colon cancer mortality.

Microsatellite instability (MSI) is found in the colorectal cancer DNA (but not in the adjacent normal colorectal mucosa) of most individuals with germline mismatch repair gene mutations. In combination with immunohistochemistry for MSH2 and MLH1, MSI testing using the Bethesda markers should be performed on the tumor tissue of individuals putatively affected with HNPCC. A result of MSI-high in tumor DNA usually leads to consideration of germline testing for mutations in the MSH2 and MLH1 genes. Individually with MSI-low or microsatellite stable (MSS) results are unlikely to harbor mismatch repair gene mutations, and further genetic testing is usually not pursued.

HNPCC is caused by germline mutation of the DNA mismatch repair genes. Over 95% of HNPCC patients have mutations in either MLH1 or MSH2. As a result, sequencing for mismatch repair gene mutations in suspected HNPCC families is usually limited to MLH1 and MSH2 and sometimes MSH6 and PMS2. In general, MSH6 and PMS2 sequence analysis is performed in persons meeting aforementioned criteria for genetic testing for HNPCC, and who do not have mutations in either the MLH1 or MSH2 genes. In addition, single site MSH6 or PMS2 testing may be appropriate for testing family members of persons with HNPCC with an identified MSH6 or PMS2 gene mutation.

HNPCC is a relatively rare disease, which making screening the entire populace burdensome and ineffective. The incidence of this disease, even among the families of patients with colon cancer, is too small to make screening effective. (See also CPB 0189 - Genetic Counseling and CPB 0227 - BRCA Testing, Prophylactic Mastectomy, and Prophylactic Oophorectomy).

Familial adenomatous polyposis (FAP)

Familial adenomatous polyposis (FAP) is caused by mutation of the adenomatous polyposis coli (APC) gene. According to guidelines from the American Gastroenterological Association (AGA, 2001), adenomatous polyposis coli gene testing is indicated to confirm the diagnosis of familial adenomatous polyposis, provide pre-symptomatic testing for at-risk members (1st degree relatives 10 years or older of an affected patient), confirm the diagnosis of attenuated familial adenomatous polyposis in those with more than 20 adenomas, and test those 10 years or older at risk for attenuated FAP.

The AGA guidelines state that germline testing should be performed on an affected member of the family to establish a detectable mutation in the pedigree. If a mutation is found in an affected family member, then genetic testing of at-risk members will provide true positive or negative results. The AGA guidelines state that, if a pedigree mutation is not identified, further testing of at-risk relatives should be suspended because the gene test will not be conclusive: a negative result could be a false negative because testing is not capable of detecting a mutation even if present. When an affected family member is not available for evaluation, starting the test process with at-risk family members can provide only positive or inconclusive results. In this circumstance, a true negative test result for an at-risk individual can only be obtained if another at-risk family member tests positive for a mutation.

MYH-associated polyposis

MYH is a DNA repair gene that corrects DNA base pair mismatch errors in the genetic code before replication. Mutation of the MYH gene may result in colon cancer. In this regard, the MYH gene has been found to be significantly involved in colon cancer, both in cases where there is a clear family history of the disease, as well as in cases without any sign of a hereditary cause.

The National Comprehensive Cancer Network (NCCN)’s practice guidelines on colorectal cancer screening (2006) recommended testing for MYH mutations for individuals with personal history of adenomatous polyposis (more than 10 adenomas, or more than 15 cumulative adenomas in 10 years) either consistent with recessive inheritance or with adenomatous polyposis with negative adenomatous polyposis coli (APC) mutation testing. The guideline noted that when polyposis is present in a single person with negative family history, de novo APC mutation should be tested; if negative, testing for MYH should follow. When family history is positive only for a sibling, recessive inheritance should be considered and MYH testing should be done first. In a polyposis family with clear autosomal dominant inheritance, and absence of APC mutation, MYH testing is unlikely to be informative. Members in such family are treated according to the polyposis phenotype, including classical or attenuated FAP.

Factor V Leiden mutation

http://qawww.aetna.com/cpb/medical/data/100_199/0140_draft.html 04/22/2015
Factor V Leiden mutation is the most common hereditary blood coagulation disorder in the United States. It is present in 5% of the Caucasian population and 1.2% of the African-American population. Factor V Leiden increases the risk of venous thrombosis 3 to 8 fold for heterozygous individuals and 30 to 140 fold for homozygous individuals. Factor V Leiden mutation has been associated with the following complications:

- Cerebrovascular accident and myocardial infarction
- Deep venous thrombosis
- Gallbladder dysfunction
- Preeclampsia and/or eclampsia
- Pulmonary embolism
- Unexplained miscarriage
- Venous thrombosis.

According to the American College of Medical Genetics, Factor V Leiden genetic testing is indicated in the following patients:

- Age less than 50, any venous thrombosis; or
- Myocardial infarction in female smokers under age 50; or
- Recurrent venous thrombosis; or
- Relatives of individuals with venous thrombosis under age 50; or
- Venous thrombosis and a strong family history of thrombotic disease; or
- Venous thrombosis in pregnant women or women taking oral contraceptives; or
- Venous thrombosis in unusual sites (such as hepatic, mesenteric, and cerebral veins).

The ACMG does not recommend random screening of the general population for factor V Leiden. Routine testing is also not recommended for patients with a personal or family history of arterial thrombotic disorders (e.g., acute coronary syndromes or stroke) except for the special situation of myocardial infarction in young female smokers. According to the ACMG, testing may be worthwhile for young patients (less than 50 years of age) who develop acute arterial thrombosis in the absence of other risk factors for atherosclerotic arterial occlusive disease. The ACMG does not recommend prenatal testing or routine newborn screening for factor V Leiden mutation.

The ACMG does not recommend general screening for factor V Leiden mutation before administration of oral contraceptives. The ACMG recommends targeted testing prior to oral contraceptive use in women with a personal or family history of venous thrombosis.

Factor V Leiden screening of asymptomatic individuals with other recognized environmental risk factors, such as surgery, trauma, paralysis, and malignancy is not necessary or recommended by the ACMG, since all such individuals should receive appropriate medical prophylaxis for thrombosis regardless of carrier status. When Factor V Leiden testing is indicated, the ACMG recommends either direct DNA-based genotyping or factor V Leiden-specific functional assay (e.g., activated protein C (APC) resistance). Patients who test positive by a functional assay should then be further studied with the DNA test for confirmation and to distinguish heterozygotes from homozygotes. According to the ACMG, patients testing positive for factor V Leiden or APC resistance should be considered for molecular genetic testing for prothrombin 20210A, the most common thrombophilia with overlapping phenotype for which testing is easily and readily available. The prothrombin 20210A mutation is the second most common inherited clotting abnormality, occurring in 2% of the general population. It is only a mild risk factor for thrombosis, but may potentiate other risk factors (such as Factor V Leiden, oral contraceptives, surgery, trauma, etc.).

A factor V gene haplotype (HR2) defined by the R2 polymorphism (A4070G) may confer mild APC resistance and interact with the factor V Leiden mutation to produce a more severe APC resistance phenotype (Bernardi et al, 1997; de Visser et al, 2000; Mingozzi et al, 2003). In one study, co-inheritance of the HR2 haplotype increased the risk of venous thromboembolism associated with factor V Leiden by approximately 3-fold (Faioni et al, 1999). However, double heterozygosity for factor V Leiden and the R2 polymorphism was not associated with a significantly higher risk of early or late pregnancy loss than a heterozygous factor V Leiden mutation alone (Zammiti et al, 2006). Whether the HR2 haplotype alone is an independent thrombotic risk factor is still unclear. Several studies have suggested that the HR2 haplotype is associated with a 2-fold increase in risk of venous thromboembolism (Alhenc-Gelas et al, 1999; Jadaon and Doshi, 2005). In contrast, other studies (de Visser 2000; Luddington et al, 2000; Dindagur et al, 2006) found no significant increase in thrombotic risk (GeneTests, University of Washington, Seattle, 2007).

CADDASIL

CADDASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) is a rare, genetically inherited, congenital vascular disease of the brain that causes strokes, subcortical dementia, migraine-like headaches, and psychiatric disturbances. CADDASIL is very debilitating and symptoms usually surface around the age of 45. Although CADDASIL can be treated with surgery to repair the defective blood vessels, patients often die by the age of 65. The exact incidence of CADDASIL in the United States is unknown.

DNA testing for CADDASIL is appropriate for symptomatic patients who have a family history consistent with an autosomal dominant pattern of inheritance of this condition. Clinical signs and symptoms of CADDASIL include stroke, cognitive defects and/or dementia, migraine, and psychiatric disturbances. DNA testing is also indicated for pre-symptomatic patients where there is a family history consistent with an autosomal dominant pattern of inheritance and there is a known mutation in an affected member of the family. This policy is consistent with guidelines on CADDASIL genetic testing from the European Federation of Neurological Societies.

Cystic fibrosis
Cystic fibrosis is the most common potentially fatal autosomal recessive disease in the United States. It is characterized by chronic progressive disease of the respiratory system, malabsorption due to pancreatic insufficiency, increased loss of sodium and chloride in sweat, and male infertility as a consequence of atresia of the vas deferens. Pulmonary disease is the most common cause of mortality and morbidity in individuals with CF. The incidence of this disease ranges from 1:500 in Amish (Ohio) to 1:90,000 in Hawaiian Orientals, and is estimated to be 1:2,500 newborns of European ancestry. It occurs less frequently in people with other ethnic and racial backgrounds. About 1:25 persons of European ancestry is a carrier (or heterozygote), possessing one normal and one abnormal CF gene. Because of recent advances in clinical management of CF, babies born today are expected to live well into middle age.

Currently, the most frequently employed test for CF is the quantitative pilocarpine iontophoresis sweat test. Sweat chloride is more reliable than sweat sodium for diagnostic purposes with a sensitivity of 98 % and a specificity of 83 %. However, this test can not detect CF carriers because the electrolyte content of sweat is normal in heterozygotes (Wallach, 1991). The gene for CF (cystic fibrosis trans-membrane conductance regulator, CFTR) was cloned, and the principal mutant gene in white people (DF508) was characterized in 1989. This mutation is due to a 3-base-pair deletion that results in the loss of a phenylalanine at position 508 from the 1,480-amino acid coding region (Riordan et al, 1989). This mutation is found in approximately 70 % of carriers of European ancestry, but the relative frequency varies from 30 % in Ashkenazi Jews to 88 % in Danes (Cutting et al, 1992). Available evidence indicates that CFTR functions as a chloride channel, although it may also serve other functions. Since then, more than 200 CF mutations have been described. Five of the most common mutations (DF508, G542X, F551D, R553X, N1303K) constitute approximately 85 % of the alleles in the United States (Elias et al, 1991). Thus, screening procedures that test for these 5 mutations will detect approximately 85 % of CF carriers. The genetic screening test for CF is usually based on mouthwash samples collected by agitating sucrose or saline in the mouth. The DNA of these cells are amplified, digested, and subjected to separation techniques that identify 3 to 5 common mutations.

A National Institutes of Health consensus panel (1997) recommended that genetic testing for CF should be offered to adults with a positive family history of CF, to partners of people with the disease, to couples currently planning a pregnancy, and to couples seeking prenatal testing. However, the panel did not recommend genetic testing of CF to the general public or to newborn infants. The American College of Obstetricians and Gynecologists (2001) has issued similar recommendations on genetic carrier testing for CF. ACOG recommends that obstetricians should offer CF screening to:

- Couples in whom one or both members are white and who are planning a pregnancy or seeking prenatal care;
- Individuals with a family history of CF; and
- Reproductive partners of people who have CF.

ACOG also recommends that screening should be made available to couples in other racial and ethnic groups. To date, over 900 mutations in the CF gene have been identified. As it is impractical to test for every known mutation, the ACMG Accreditation of Genetic Services Committee has compiled a standard screening panel of 25 CF mutations, which represents the standard panel that ACMG recommends for screening in the U.S. population (Grody et al, 2001). This 25-mutation panel incorporates all CF-causing mutations with an allele frequency of greater than or equal to 0.1 % in the general U.S. population, including mutation subsets shown to be sufficiently predominant in certain ethnic groups, such as Ashkenazi Jews and African Americans. This standard panel of mutations is intended to provide the greatest pan-ethnic detectability that can practically be performed.

The ACOG's update on carrier screening for CF (2011) provided the following recommendations.

- If a patient has been screened previously, CF screening results should be documented but the test should not be repeated.
- Complete analysis of the CFTR gene by DNA sequencing is not appropriate for routine carrier screening.

Fragile X syndrome

Fragile X syndrome is the most common cause of inherited mental retardation, seen in approximately one in 2,100 males and one in 2,500 females. Phenotypic abnormalities associated with Fragile X syndrome include mental retardation, autistic behaviors, characteristic narrow face with large jaw, and speech and language disorders. Fragile X syndrome was originally thought to be transmitted in an X-linked recessive manner; however, the inheritance pattern of fragile X syndrome has been shown to be much more complex.

Standard chromosomal analysis does not consistently demonstrate the cytogenetic abnormality in patients with fragile X syndrome, and molecular diagnostic techniques (DNA testing) have become the diagnostic procedure of choice for fragile X syndrome.

Aetna's policy on coverage of fragile X genetic testing is based on guidelines from the ACMG (1994) and the ACOG (1995).

Lactose intolerance

Lactase-phlorizin hydrolase, which hydrolyzes lactose, the major carbohydrate in milk, plays a critical role in the nutrition of the mammalian neonate (Montgomery et al, 1991). Lactose intolerance in adult humans is common, usually due to low levels of small intestinal lactase. Low lactase levels result from either intestinal injury or (in the majority of the world's adult population) alterations in the genetic expression of lactase. Although the mechanism of decreased lactase levels has been the subject of intensive investigation, no consensus has yet emerged.
The LactoTYPE Test (Prometheus Laboratories) is a blood test that is intended to identify patients with genetic-based lactose intolerance. According to the manufacturer, this test provides a more definitive diagnosis and scientific explanation for patients with persistent symptoms.

There is insufficient evidence that the assessment of the genetic etiology of lactose intolerance would affect the management of patients such that clinical outcomes are improved. Current guidelines on the management of lactose intolerance do not indicate that genetic testing is indicated (NHS, 2005; National Public Health Service for Wales, 2005).

Long QT Syndrome

Voltage-gated sodium channels are transmembrane proteins that produce the ionic current responsible for the rising phase of the cardiac action potential and play an important role in the initiation, propagation, and maintenance of normal cardiac rhythm. Inherited mutations in the sodium channel alpha-subunit gene (SCN5A), the gene encoding the pore-forming subunit of the cardiac sodium channel, have been associated with distinct cardiac rhythm syndromes such as the congenital long QT syndrome (LQT3), Brugada syndrome, isolated conduction disease, sudden unexpected nocturnal death syndrome (SUNDS), and sudden infant death syndrome (SIDS). Electrophysiological characterization of heterologously expressed mutant sodium channels have revealed gating defects that, in many cases, can explain the distinct phenotype associated with the rhythm disorder.

The long QT syndrome (LQTS) is a familial disease characterized by an abnormally prolonged QT interval and, usually, by stress-mediated life-threatening ventricular arrhythmias (Priori et al, 2001). Characteristically, the first clinical manifestations of LQTS tend to appear during childhood or in teenagers. Two variants of LQTS have been described: a rare recessive form with congenital deafness (Jervell and Lange-Nielsen syndrome, J-LN), and a more frequent autosomal dominant form (Romano-Ward syndrome, RW). Five genes encoding subunits of cardiac ion channels have been associated to LQTS and genotype-phenotype correlation has been identified. Of the 5 genetic variants of LQTS currently identified, LQT1 and LQT2 subtypes involve 2 genes, KCNQ1 and HERG, which encode major potassium currents. LQT3 involves SCN5A, the gene encoding the cardiac sodium current. LQT5 and LQT6 are rare subtypes also involving the major potassium currents.

Guidelines on sudden cardiac death from the European College of Cardiology (Priori et al, 2001) state that identification of specific genetic variants of LQTS are useful in risk stratification. The clinical variants presenting association of the cardiac phenotype with syndactyly or with deafness (Jervell and Lange-Nielsen syndrome) have a more severe prognosis. Genetic defects on the cardiac sodium channel gene (SCN5A) are also associated with higher risk of sudden cardiac death. In addition, identification of specific genetic variants may help in suggesting behavioral changes likely to reduce risk. LQT1 patients are at very high risk during exercise, particularly swimming. LQT2 patients are quite sensitive to loud noises, especially when they are asleep or resting.

Genetic testing for LQTS may be indicated in persons with close relatives that have a defined mutation. Genetic testing may also be indicated in individuals with a prolonged QT interval on resting electrocardiogram (a corrected QT interval (QTc) of 470 msec or more in males and 480 msec or more in females) without an identifiable external cause for QTc prolongation. Common external causes of QTc prolongation are listed in the table below.

<table>
<thead>
<tr>
<th>Table: Common External Causes of Prolongation of QTc Interval</th>
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<tbody>
<tr>
<td>Bradycardia</td>
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<td>Heart disease (heart failure, ischemia)</td>
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<td>Hypocalcemia</td>
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<td>Hypomagnesemia</td>
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<td>Hypokalemia</td>
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<td>Hypothyroidism</td>
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<td>Antiarrhythmic medications (quinidine, procainamide, amiodarone, sotalol, and dofetilide)</td>
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<td>Tricyclic and tetracyclic antidepressants (e.g., amitriptyline)</td>
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<td>Erythromycin</td>
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Genetic testing for long QT syndrome has not been evaluated in patients who present with a borderline QT interval, suspicious symptoms (e.g., syncope), and no relevant family history (Roden, 2008). In these patients, the incidence of false positive and false negative results and their implications for management remain unknown.

Genetic testing may also be necessary in persons with long QT syndrome in sudden death close relatives.

**Brugada syndrome**

Brugada syndrome is an inherited condition comprising a specific EKG abnormality and an associated risk of ventricular fibrillation and sudden death in the setting of a structurally normal heart. Brugada syndrome is characterized by ST-segment abnormalities on EKG and a high risk of ventricular arrhythmias and sudden death. Brugada syndrome presents primarily during adulthood but age at diagnosis ranges from 2 days to 85 years. Clinical presentations may also include sudden infant death syndrome and sudden unexpected nocturnal death syndrome, a typical presentation in individuals from Southeast Asia.

Brugada et al (2005) reported that Brugada syndrome and LQTS are both due to mutations in genes encoding ion channels and that the genetic abnormalities causing Brugada syndrome have been linked to mutations in the ion channel gene SCN5A. Brugada stated that the syndrome has been identified only recently but an analysis of data from published studies indicates that the disease is responsible for 4 to 12 % of unexpected sudden deaths, and up to 50 % of all sudden death in patients with an apparently normal heart. Brugada explained that Brugada syndrome is a clinical diagnosis based on syncopal or sudden death episodes in patients with a structurally normal heart and a characteristic ECG pattern. The ECG shows ST segment elevation in the primordial leads V1-V3, with a morphology of the QRS complex resembling a right bundle branch block; this pattern may also be caused by J point elevation. When ST elevation is the most prominent feature, the pattern is called "coved-type". When the most prominent feature is J point elevation, without ST elevation the pattern is called "saddle-type". Brugada pointed out that it is important to exclude other causes of ST segment elevation before making the diagnosis of Brugada syndrome. Brugada syndrome is inherited in an autonomic dominant manner with variable penetrance. Most individuals diagnosed with Brugada syndrome have an affected parent. The proportion of cases caused by de novo mutations is estimated at 1 %. Each child of an individual with Brugada syndrome has a 50 % chance of inheriting the mutation. According to Brugada, antiarrhythmic drugs do not prevent sudden death in symptomatic or asymptomatic individuals with Brugada syndrome and that implantation of an automatic cardioverter-defibrillator is the only currently proven effective therapy.

To date the great majority of identified disease-causing mutations have been located in the SCN5A gene encoding the a subunit of the human cardiac voltage-gated sodium channel but such mutations can be identified in, at most, 30 % of affected people. Moreover, a positive genetic test adds little or nothing to the clinical management of such a person (HRUK, 2007). The identification of an SCN5A mutation does, of course, allow screening of family members but the usefulness of genetic screening may be less than for other familial syndromes, however, given that the routine 12-lead EKG (with or without provocative drug testing) appears to be a relatively effective method of screening for the condition.

**Hypertrophic cardiomyopathy**

Hypertrophic cardiomyopathy (HCM) is a disease of the myocardium in which a portion of the myocardium is hypertrophied without any obvious cause; it is among the most common genetically transmitted cardiovascular diseases.

The genetic abnormalities that cause HCM are heterogeneous. Hypertrophic cardiomyopathy is most commonly due to a mutation in one of 9 genes that results in a mutated protein in the sarcomere. Some of the genes responsible for HCM have not yet been identified, and among those genes that have been identified, the spectrum of possible disease-causing mutations is incomplete. As a result, a thorough evaluation of known genes requires extensive DNA sequencing, which is onerous for routine clinical testing. Less rigorous methods (such as selective sequencing) reduces the likelihood of identifying the responsible mutation.

Population studies have demonstrated that some patients are compound heterozygotes (inheriting 2 different mutations within a single HCM gene), double heterozygotes (inheriting mutations in 2 HCM genes), or homozygotes (inheriting the same mutation from both parents). To be certain of detecting such genotypes, sequencing of candidate genes would need to continue in a given patient even after a single mutation was identified.

In many persons with HCM mutations, the disease can be mild and the symptoms absent or minimal. In addition, phenotypic expression of HCM can be influenced by factors other than the basic genetic defect, and the clinical consequences of the genetic defect can vary. There is sufficient heterogeneity in the clinical manifestations of a given gene mutation that, even when a patient's mutation is known, his or her clinical course can not be predicted with any degree of certainty.

In addition, the prognostic impact of a given mutation may relate to a particular family and not to the population at large. Many families have their own "private" mutations and thus knowledge of the gene abnormalities can not be linked to experience from other families.

Family members with echocardiography evidence of HCM should be managed like other patients with HCM. In general, genetically affected but phenotypically normal family members should not be subjected to the same activity restriction as patients with HCM.

<table>
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<th>Cisapride</th>
<th>Pimozide</th>
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Genetic testing for long QT syndrome may also be necessary in person with long QT syndrome in sudden death close relatives.
Bos and colleagues (2009) stated that over the past 20 years, the pathogenic basis for HCM, the most common heritable cardiovascular disease, has been studied extensively. Affecting about 1 in 500 persons, HCM is the most common cause of sudden cardiac death (SCD) among young athletes. In recent years, genomic medicine has been moving from the bench to the bedside throughout all medical disciplines including cardiology. Now, genomic medicine has entered clinical practice as it pertains to the evaluation and management of patients with HCM. The continuous research and discoveries of new HCM susceptibility genes, the growing amount of data from genotype-phenotype correlation studies, and the introduction of commercially available genetic tests for HCM make it essential that cardiologists understand the diagnostic, prognostic, and therapeutic implications of HCM genetic testing.

Hudecova et al (2009) noted that the clinical symptoms of HCM are partly dependent on mutations in affected sarcomere genes. Different mutations in the same gene can present as malign with a high-risk of SCD, while other mutations can be benign. The clinical symptomatology can also be influenced by other factors such as the presence of polymorphisms in other genes. Currently, the objective of intensive clinical research is to access the contribution of molecular genetic methods in HCM diagnostics as well as in risk stratification of SCD. It is expected that genetic analyses will have an important consequence in the screening of the relatives of HCM patients and also in the prenatal diagnostics and genetic counseling.

Shephard and Semsarian (2009) stated that genetic heart disorders are an important cause of SCD in the young. While pharmacotherapies have made some impact on the prevention of SCD, the introduction of implantable cardioverter-defibrillator (ICD) therapy has been the single major advance in the prevention of SCD in the young. In addition, the awareness that most causes of SCD in the young are inherited, means family screening of relatives of young SCD victims allows identification of previously unrecognised at-risk individuals, thereby enabling prevention of SCD in relatives. The role of genetic testing, both in living affected individuals as well as in the setting of a "molecular autopsy", is emerging as a key factor in early diagnosis of an underlying cardiovascular genetic disorder.

The Heart Failure Society of America's practice guideline on "Genetic evaluation of cardiomyopathy" (Hershberger et al, 2009) stated that genetic testing is primarily indicated for risk assessment in at-risk relatives who have little or no clinical evidence of cardiovascular disease. Genetic testing for HCM should be considered for the one most clearly affected person in a family to facilitate family screening and management. Specific genes available for testing for HCM include MYH7, MYBPC3, TNNT2, TNNI3, TPM1, ACTC, MYL2, and MYL3. MYH7 and MYBPC3 each accounts for 30 % to 40 % of mutations; TNNT2 for 10 % to 20 %. Genetic cause can be identified in 35 % to 45 % overall; up to 60 % to 65 % when the family history is positive.

The BlueCross BlueShield Association Technology Evaluation Center (TEC)’s assessment on genetic testing for predisposition to inherited HCM (2010) concluded that the use of genetic testing for inherited HCM meets the TEC criteria for individuals who are at-risk for development of HCM, defined as having a close relative with established HCM, when there is a known pathogenic gene mutation present in an affected relative. In order to inform and direct genetic testing for at-risk individuals, genetic testing should be initially performed in at least 1 close relative with definite HCM (index case) if possible. This testing is intended to document whether a known pathologic mutation is present in the family, and optimize the predictive value of predisposition testing for at-risk relatives. Due to the complexity of genetic testing for HCM and the potential for misinterpretation of results, the decision to test and the interpretation of test results should be performed by, or in consultation with an expert in the area of medical genetics and/or HCM.

The TEC assessment also concluded that genetic testing for inherited HCM does not meet the TEC criteria for predisposition testing in individuals who are at-risk for development of HCM, defined as having a close relative with established HCM, when there is no known pathogenic gene mutation present in an affected relative. This includes:

- Patients with a family history of HCM, with unknown genetic status of affected relatives; and
- Patients with a family history of HCM, when a pathogenic mutation has not been identified in affected relatives.

Arrhythmogenic right ventricular dysplasia/cardomyopathy (ARVD/C)

Arrhythmogenic right ventricular dysplasia/cardomyopathy is a condition characterized by progressive fibro-fatty replacement of the myocardium that predisposes individuals to ventricular tachycardia and sudden death. The prevalence of ARVD/C is estimated to be 1 case per 10,000 population. Familial occurrence with an autosomal dominant pattern of inheritance and variable penetrance has been demonstrated. Recessive variants have been reported. It is estimated that half of the individuals have a family history of ARVD/C and the remaining cases are new mutations.

Genetic testing has not been demonstrated to be necessary to establish the diagnosis of ARVD/C or determine its prognosis. Twelve-lead ECG and echocardiography can be used to identify affected relatives.

The genetic abnormalities that cause ARVD/C are heterogeneous. The genes frequently associated with ARVD/C are PKP2 (plakophilin-2), DSP2 (desmoglein-2), and DSP (desmoplakin). A significant proportion of ARVD/C cases have been reported with no linkage to known chromosomal loci: in one report, 50 % of families undergoing clinical and genetic screening did not show linkage with any known genetic loci (Corrado et al, 2000).

Most affected individuals live a normal lifestyle. Management of individuals with ARVD/C is complicated by incomplete information on the natural history of the disease and the variability of disease expression even within families. High-risk individuals with signs and symptoms of ARVD/C are treated with anti-arrhythmic medications and those at highest risk who have been resuscitated or who are unresponsive to or intolerant of anti-arrhythmic therapy may be considered for an ICD.

According to the Heart Failure Society of America’s Practice Guideline on the genetic evaluation of cardiomyopathy (2009), the clinical utility for all genetic testing of cardiomyopathies remains to be defined. The guideline stated, “because the genetic knowledge base of cardiomyopathy is still emerging, practitioners caring for patients and families with genetic cardiomyopathy
are encouraged to consider research participation.” The Multidisciplinary Study of Right Ventricular Dysplasia (North American registry) is a 5-year study funded by the National Institutes of Health to determine how the genes responsible for ARVD/C affect the onset, course, and severity of the disease. Enrollment in the study was completed in May 2008 and the study is currently in the follow-up period.

Catecholaminergic polymorphic ventricular tachycardia (CPVT)

Catecholaminergic polymorphic ventricular tachycardia (CPVT) is a highly lethal form of inherited arrhythmogenic disease characterized by adrenergically mediated polymorphic ventricular tachycardia (Liu et al, 2007). Mutations in the cardiac ryanodine receptor (RyR2) gene and the cardiac calsequestrin (CASQ2) gene are responsible for the autosomal dominant and recessive variants of CPVT, respectively. The clinical presentation encompasses exercise- or emotion-induced syncopal events and a distinctive pattern of reproducible, stress-related, bi-directional ventricular tachycardia in the absence of both structural heart disease and a prolonged QT interval.

CPVT typically begins in childhood or adolescence. The mortality rate in untreated individuals is 30 to 50 % by age 40 years. Clinical evaluation by exercise stress testing and Holter monitoring and genetic screening can facilitate early diagnosis. Beta-blockers are the most effective drugs for controlling arrhythmias in CPVT patients, yet about 30 % of patients with CPVT still experience cardiac arrhythmias on beta-blockers and eventually require an implantable cardioverter defibrillator. Liu et al (2008) stated that molecular genetic screening of the genes encoding the cardiac RyR2 and CASQ2 is critical to confirm uncertain diagnosis of CPVT.

Katz et al (2009) noted that CPVT is a primary electrical myocardial disease characterized by exercise- and stress-related ventricular tachycardia manifested as syncope and sudden death. The disease has a heterogeneous genetic basis, with mutations in the cardiac RyR2 gene accounting for an autosomal-dominant form (CPVT1) in approximately 50 % and mutations in the cardiac CASQ2 gene accounting for an autosomal-recessive form (CPVT2) in up to 2 % of CPVT cases. Both RyR2 and calsequestrin are important participants in the cardiac cellular calcium homeostasis. These researchers reviewed the physiology of the cardiac calcium homeostasis, including the cardiac excitation contraction coupling and myocyte calcium cycling.

Although the clinical presentation of CPVT is similar in many respects to the LQTS, there are important differences that are relevant to genetic testing. CPVT appears to be a more malignant condition, as many people are asymptomatic before the index lethal event and the majority of cardiac events occur before 20 years of age. Affected people are advised to avoid exercise-related triggers and start prophylactic beta-blockers with dose titration guided by treadmill testing.

Genetic testing has been recommended in individuals with clinical features considered typical of CPVT following expert clinical assessment (HRUK, 2008). Clinically the condition is difficult to diagnose in asymptomatic family members as the ECG and echocardiogram are completely normal at rest. Exercise stress testing has been advised in family members in order to identify exercise-induced ventricular arrhythmias, but the sensitivity of this clinical test is unknown. Although the diagnostic yield from genetic testing is less than that for the LQTS (about 50 %) in patients with typical clinical features, a positive genetic test may be of value for the individual patient (given the prognostic implications) and for screening family members (given the difficulties in clinical screening methods) (HRUK, 2008). The RyR2 gene is large and a “targeted” approach is usually undertaken, in which only exons that have been previously implicated are examined.

The 2006 guidelines from the American College of Cardiology on management of patients with ventricular arrhythmias and the prevention of sudden cardiac death (Zipes et al, 2006) included the following recommendations for patients with CPVT:

There is evidence and/or general agreement supporting the use of beta blockers for patients clinically diagnosed on the basis of spontaneous or documented stress-induced ventricular arrhythmias.

There is evidence and/or general agreement supporting the use of an implantable ICD in combination with beta blockers for survivors of cardiac arrest who have a reasonable expectation of survival with a good functional capacity for more than 1 year.

The weight of evidence and/or opinion supports the use of beta blockers in patients without clinical manifestations who are diagnosed in childhood based upon genetic analysis.

The weight of evidence and/or opinion supports the use of an ICD in combination with beta blockers for patients with a history of syncope and/or sustained ventricular tachycardia while receiving beta blockers who have a reasonable expectation of survival with a good functional capacity for more than 1 year.

The usefulness and/or efficacy of beta blockers is less well established in patients without clinical evidence of arrhythmias who are diagnosed in adulthood based upon genetic analysis.

Hereditary hemochromatosis

Hemochromatosis, a condition involving excess accumulation of iron, can lead to iron overload, which in turn can result in complications such as cirrhosis, diabetes, cardiomyopathy, and arthritis (Burke 1992; Hanson et al, 2001).

Hereditary hemochromatosis (HHC) is characterized by inappropriately increased iron absorption from the duodenum and upper intestine, with consequent deposition in various parenchymal organs, notably the liver, pancreas, joints, heart, pituitary gland and skin, with resultant end-organ damage (Limdi and Crampton, 2004). Clinical features may be non-specific and include lethargy and malaise, or reflect target organ damage and present with abnormal liver tests, cirrhosis, diabetes mellitus, arthropathy, cardiomyopathy, skin pigmentation and gonadal failure. Early recognition and treatment (phlebotomy) is essential to prevent irreversible complications such as cirrhosis and hepatocellular carcinoma.

HHC is an autosomal recessive condition associated with mutations of the HFE gene. Two of the 37 allelic variants of the HFE gene, C282Y and H63D, are significantly correlated with HHC. C282Y is the more severe mutation, and homozygosity for the
C282Y genotype accounts for the majority of clinically penetrant cases. Hanson et al (2001) reported that homozygosity for the C282Y mutation has been found in 52 to 100 % of previous studies on clinically diagnosed index cases. Five percent of HHC probands were found by Hanson et al to be compound heterozygotes (C282Y/H63D), and 1.5 % were homozygous for the H63D mutation; 3.6 % were C282Y heterozygotes, and 5.2 % were H63D heterozygotes. In 7 % of cases, C282Y and H63D mutations were not present. In the general population, the frequency of the C282Y/C282Y genotype is 0.4 %.

HHC is a very common genetic defect in the Caucasian population. C282Y homozygosity ranges from 9.2 % in Europeans to nil in Asian, Indian subcontinent, African, Middle Eastern, Australian and Asian populations (Hanson et al, 2001). The H63D carrier frequency is 22 % in European populations.

Accurate data on the penetrance of the different HFE genotypes are not available. But current data suggest that clinical disease does not develop in a substantial proportion of people with this genotype. Available data suggest that up to 38 % to 50 % of C282Y homozygotes may develop iron overload, with up to 10 % to 33 % eventually developing hemochromatosis-associated morbidity (Whitlock et al, 2006). A pooled analysis found that patients with the HFE genotypes C282Y/H63D and H63D/H63D are also at increased risk for iron overload, yet overall, disease is likely to develop in fewer than 1 % of people with these genotypes (Burke, 1992). Thus, DNA-based tests for hemochromatosis identify a genetic risk rather than the disease itself.

Whether it is beneficial to screen asymptomatic people for a genetic risk of iron overload is a matter of debate. To date, population screening for HHC is not recommended because of uncertainties about optimal screening strategies, optimal care for susceptible persons, laboratory standardization, and the potential for stigmatization or discrimination (Hanson et al, 2001; Whitlock et al, 2006). A systematic evidence review prepared for the U.S. Preventive Services Task Force concluded: "Research addressing genetic screening for hereditary hemochromatosis remains insufficient to confidently project the impact of, or estimate the benefit from, widespread or high-risk genetic screening for hereditary hemochromatosis" (Whitlock et al, 2006).

**Familial nephrotic syndrome (NPHS1, NPHS2)**

Nephrotic syndrome comes in 2 variants: (i) those sensitive to treatment with immunosuppressants (steroid-sensitive), and (ii) those resistant to immunosuppressants (steroid-resistant). Familial forms of nephrotic syndrome are steroid resistant (Niaudet, 2007). Mutations in two genes, NPHS1 and NPHS2, have been associated with a familial nephrotic syndrome. Mutations in the gene for podocin, called NPHS2, also known as familial focal glomerulosclerosis, are observed in patients with both familial and sporadic steroid-resistant nephrotic syndrome (SRNS).

Identifying children with nephrotic syndrome due to NPHS2 mutations can avoid unnecessary exposure to immunosuppressive therapy, because immunosuppressive therapy has not been shown to be effective in treating these children (Niaudet, 2007). Thus, authorities have recommended testing for such mutations in those with a familial history of steroid resistant nephrotic syndrome and children with steriod-resistant disease.

Some have suggested that, to avoid unnecessary exposure to steroid therapy, all children with a first episode of the nephrotic syndrome should be screened for NPHS2 mutations (Niaudet, 2007). However, given that over 85 % of children with idiopathic nephrotic syndrome are steroid-sensitive and only approximately 20 % of steroid-resistant patients have NPHS2 mutations, screening for abnormalities at this genetic locus would identify less than 5 % of all cases. However, screening a child with a first episode of the nephrotic syndrome with a familial history of steroid-resistant nephrotic syndrome has been recommended because they are at increased risk for having a NPHS2 gene mutation.

Mutations in the gene for nephrin, called NPHS1, cause the congenital nephrotic syndrome of Finnish type (CNF) (Niaudet, 2007). CNF is inherited as an autosomal recessive trait, with both sexes being involved equally. There are no manifestations of the disease in heterozygous individuals. Most infants with the CNF are born prematurely (35 to 38 weeks), with a low birth weight for gestational age. Edema is present at birth or appears during the first week of life in 50 % of cases. Severe nephrotic syndrome with marked ascites is always present by 3 months. End-stage renal failure usually occurs between 3 and 8 years of age. Prolonged survival is possible with aggressive supportive treatment, including dialysis and renal transplantation.

The nephrotic syndrome in CNF is always resistant to corticosteroids and immunosuppressive drugs, since this is not an immunologic disease (Niaudet, 2007). Furthermore these drugs may be harmful due to affected individuals' already high susceptibility to infection.

The CNF becomes manifest during early fetal life, beginning at the gestation age of 15 to 16 weeks. The initial symptom is fetal proteinuria, which leads to a more than 10-fold increase in the amniotic fluid alpha-fetoprotein (AFP) concentration (Niaudet, 2007). A parallel, but less important increase in the maternal plasma AFP level is observed. These changes are not specific, but they may permit the antenatal diagnosis of CNF in high risk families in which termination of the pregnancy might be considered. However, false positive results do occur, often leading to abortion of healthy fetuses.

Genetic linkage and haplotype analyses may diminish the risk of false positive results in informative families (Niaudet, 2007). The 4 major haplotypes, which cover 90 % of the CNF alleles in Finland, have been identified, resulting in a test with up to 95 % accuracy.

Authorities do not recommend screening for NPHS1 mutations for all children with the first episode of nephrotic syndrome, for the reasons noted above regarding NPHS2 mutation screening. However, genetic testing may be indicated for infants with congenital nephrotic syndrome (i.e., appearing within the first months of life) who are of Finnish descent and/or who have a
family history that suggests a familial cause of congenital nephrotic syndrome. The primary purpose of this testing is for pregnancy planning. Detection of an NPHS1 mutation also has therapeutic implications, as such nephrotic syndrome is steroid resistant.

**Primary dystonia (DYT-1)**

Dystonia consists of repetitive, patterned, twisting, and sustained movements that may be either slow or rapid. Dystonic states are classified as primary, secondary, or psychogenic depending upon the cause (Jankovic, 2007). By definition, primary dystonia is associated with no other neurologic impairment, such as intellectual, pyramidal, cerebellar, or sensory deficits. Cerebral palsy is the most common cause of secondary dystonia.

Primary dystonia may be sporadic or inherited (Jankovic, 2007). Cases with onset in childhood usually are inherited in an autosomal dominant pattern. Many patients with hereditary dystonia have a mutation in the TOR1A (DYT1) gene that encodes the protein torsinA, an ATP-binding protein in the 9q34 locus. The role of torsinA in the pathogenesis of primary dystonia is unknown. DNA testing for the abnormal TOR1A gene can be performed on individuals with dystonia. The purpose of such testing is to help rule out secondary or psychogenic causes of dystonia, and for family planning purposes.

**Malignant melanoma**

An estimated 8 to 12 % of persons with melanoma have a family history of the disease, but not all of these individuals have hereditary melanoma (Tsao and Haluska, 2007). In some cases, the apparent familial inheritance pattern may be due to clustering of sporadic cases in families with common heavy sun exposure and susceptible skin type.

A melanoma susceptibility locus has been identified on chromosome 9p21; this has been designated CDKN2A (also known as MTS1 (multiple tumor suppressor 1)) (Tsao and Haluska, 2007). There is a variable rate of CDKN2A mutations in patients with hereditary melanoma. The risk of CDKN2A mutation varies from approximately 10 % for families with at least 2 relatives having melanoma, to more than 40 % for families having multiple affected 1st degree relatives spanning several generations.

Persons at increased risk of melanoma are managed with close clinical surveillance and education in risk-reduction behavior (e.g., sun avoidance, sunscreen use). It is unclear how CDKN2A genetic test information would alter clinical recommendations (Tsao and Haluska, 2007). The negative predictive value of a negative test for a CDKN2A mutation is also not established since many familial cases occur in the absence of CDKN2A mutations. It is estimated that the prevalence of CDKN2A mutation carriers is less than 1 % in high incidence populations. Thus, no mutations will be identifiable in the majority of families presenting to clinical geneticists.

The American Society of Clinical Oncology (ASCO) has issued a consensus report on the utility of genetic testing for cancer susceptibility (ASCO, 1996), and recommendations for the process of genetic testing were updated in 2003 (ASCO, 2003). The report notes that the sensitivity and specificity of the commercially available test for CDKN2A mutations are not fully known. Because of the difficulties with interpretation of the genetic tests, and because test results do not alter patient or family member management, ASCO recommends that CDKN2A testing be performed only in the context of a clinical trial.

The Scottish Intercollegiate Guidelines Network (SIGN, 2003) protocols on management of cutaneous melanoma reached similar conclusions, stating that “*genetic testing in familial or sporadic melanoma is not appropriate in a routine clinical setting and should only be undertaken in the context of appropriate research studies.*”

The Melanoma Genetics Consortium recommends that genetic testing for melanoma susceptibility should not be offered outside of a research setting (Kefford et al, 2002). They state that “[u]ntil further data become available, however, clinical evaluation of risk remains the gold standard for preventing melanoma. First-degree relatives of individuals at high risk should be engaged in the same programmes of melanoma prevention and surveillance irrespective of the results of any genetic testing.”

**Charcot-Marie Tooth disease type 1A (PMP-22)**

Charcot Marie Tooth disease, also known as peroneal muscular atrophy, progressive neural muscular atrophy, as well as hereditary motor and sensory neuropathy, is 1 of the 3 major types of hereditary neuropathy. With an estimated prevalence of at least 12,500 (autosomal dominant), CMT is one of the most common genetic neuromuscular disorders affecting approximately 125,000 persons in the United States. This hereditary peripheral neuropathy is genetically and clinically heterogeneous. It is usually inherited in an autosomal dominant manner, and occasionally in an autosomal recessive manner. Sporadic as well as X-linked cases have also been reported. In the X-linked recessive pattern, only males develop the disease, although females who inherit the defective gene can pass the disease onto their sons. In the X-linked dominant pattern, an affected mother can pass on the disorder to both sons and daughters, while an affected father can only pass it onto his daughters. The clinical manifestations can vary greatly in severity and age of onset. The clinical features may be so mild that they may be undetectable by patients, their families and physicians.

Charcot-Marie-Tooth disease is usually diagnosed by an extensive physical examination, assessing characteristic weakness in the foot, leg, and hand, as well as deformities and impaired function in walking and manual manipulation. The clinical diagnosis is then confirmed by electromyogram and nerve conduction velocity tests, and sometimes by biopsy of muscle and of sural cutaneous nerve. Since CMT is a hereditary disease, family history can also help to confirm the diagnosis. Based on studies of motor nerve conduction velocity, CMT can be further classified into 2 types: (i) CMT Type I -- slow conduction velocity (less than 40 meters/second for the median nerve or less than 15 meters/second for the peroneal nerve) which accounts for 70 % of all CMT cases, and (ii) CMT Type II -- normal or near normal nerve conduction velocity with decreased amplitude which accounts for the remaining 30 % of CMT cases. Charcot Marie Tooth Type I disease is a demyelinating neuropathy with hypertrophic changes in peripheral nerves, and has its onset usually during late childhood. On the other hand, CMT Type II is a non-demyelinating neuronal disorder without hypertrophic changes, and has its onset generally during adolescence.
Both CMT Types I and II are characterized by a slow degeneration of peripheral nerves and roots, resulting in distal muscle atrophy commencing in the lower extremities, and affecting the upper extremities several years later. Symptoms include foot drop or clubfoot, paresthesia in legs, sloping gait, later weakness and atrophy of hands, then arms, absence or reduction of deep tendon reflexes, and occasionally mild sensory loss. Charcot Marie Tooth disease is not a fatal disorder. It does not shorten the normal life expectancy of patients, and it does not affect them mentally. As stated earlier, there is a wide range of variation in the clinical manifestations of CMT -- the degree of severity can vary considerably from patient to patient, even among affected family members within the same generation. The condition can range from having no problems to having major difficulties in ambulation in early adult life, however, the latter is unusual. Most patients are able to ambulate and have gainful employment until old age. Currently, there is no specific treatment for this disease. Management of the majority of patients with CMT disease consists of supportive care with emphasis on proper bracing, foot care, physical therapy and occupational counseling. For example, the legs and shoes can be fitted with light braces and springs, respectively, to overcome foot drop. If foot drop is severe and the disease has become stationary, the ankle can be stabilized by arthrodeses.

The underlying genetic basis for CMT Type I has been characterized. A point mutation in the PMP22 gene which encodes a peripheral myelin protein with an apparent molecular weight of 22,000 or a DNA duplication of a specific region 5 megabases) including the PMP22 gene in the proximal short arm of chromosome 17 (band 17p11.2-p12) has been identified in 70 % of clinically diagnosed patients --- CMT Type IA. Thus, patients with CMT Type IA represent approximately 50 % of all CMT cases. Other CMT Type I patients (CMT Type IB) exhibit an abnormality (Duffy locus) in the proximal long arm of chromosome number 1 (band 1q21-22). Presently, no test is available for the dominant CMTIB gene on chromosome 1. On the other hand, a CMT Type IA DNA test is available commercially. The test is accomplished through a blood sample analysis -- DNAs are extracted from leukocytes of patients and pulsed-field gel electrophoresis is employed to isolate large segments of DNA encompassing CMTIA duplication-specific junction fragments which are then detected by hybridization with aCMTIA duplication-specific probe (CMTIA-REP). This probe identifies the homologous regions that flank the CMTIA duplication monomer unit.

A positive CMTIA DNA test means the presence of a 500 kilobases CMTIA duplication specific junction fragment, and is diagnostic for CMT Type IA. A negative CMT Type IA means the absence of the CMTIA duplication specific junction fragment, and does not rule out a diagnosis of CMT disease. This is because patients with CMT Type IA represent approximately 50 % of all CMT cases. The value of this molecular test in family planning is questionable because of its relatively low detection rate and its inability to predict the severity of the disease. Moreover, it is likely that there are undiscovered CMTI genes since there are addition, other investigators have reported X-linked forms of CMTI at the region of Xq13-21, and Xq26.

Since CMT is not life-threatening, rarely severely disabling, and has no specific treatment, it is unclear how the results of this CMT Type I DNA test, which can not predict the severity of the disease, would affect family planning. Moreover, because of its low detection rate, the CMT Type I DNA test appears to be inferior to the conventional means of diagnosis through physical examination, family history, electromyography and nerve conduction velocity studies. Thus, the sole value of genetic testing for CMTIA is to establish the diagnosis and to distinguish this from other causes of neuropathy.

Familial amyotrophic lateral sclerosis (SOD1 Mutation)

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease involving both the upper motor neurons (UMN) and lower motor neurons (LMN). UMN signs include hyperreflexia, extensor plantar response, increased muscle tone, and weakness in a topographical representation. LMN signs include weakness, muscle wasting, hyperreflexia, muscle cramps, and fasciculations. In the early stage of the disease, the clinical aspects of ALS can vary. Affected individuals typically present with asymmetric focal weakness of the extremities (stumbling or poor handgrip) or bulbar findings (dysarthria, dysphagia). Other findings include muscle fasciculations, muscle cramps, and lability of affect but not necessarily mood. Regardless of initial symptoms, atrophy and weakness eventually affect other muscles. Approximately 5,000 people in the U.S. are diagnosed with ALS each year.

Most people with ALS have a form of the condition that is described as sporadic or non-inherited. The cause of sporadic ALS is largely unknown but probably involves a combination of genetic and environmental factors. About 10 % of people with ALS have a familial form of the condition, which is caused by an inherited genetic mutation, usually as an autosomal dominant trait. The mean age of onset of ALS in individuals with no known family history is 56 years and in familial ALS it is 46 years.

The diagnosis of ALS is based on clinical features, electrodiagnostic testing (EMG), and exclusion of other health conditions with related symptoms. At present, genetic testing in ALS has no value in making the diagnosis. The only genetic test currently available detects the SOD1 mutation. Since only 20 % of familial ALS patients will test positively for an SOD1 mutation, this test has limited value in genetic counseling.

Migrainous vertigo

Migrainous vertigo is a term used to describe episodic vertigo in patients with a history of migraines or with other clinical features of migraine. Approximately 20 to 33 % of migraine patients experience episodic vertigo. The underlying cause of migrainous vertigo is not very well understood. There are no confirmatory diagnostic tests or susceptible genes associated with migrainous vertigo. Other conditions, specifically Meniere's disease and structural and vascular brainstem disease, must be excluded (Black, 2006).

Prostate cancer

At this time, there are no susceptibility genes that have been unequivocally associated with prostate cancer predisposition. Genetic testing for prostate cancer is currently available only within the context of a research study. A special report on prostate cancer genetics by the BlueCross BlueShield Association Technology Evaluation Center (BCBSA, 2008) stated that single-
nucleotide polymorphisms (SNPs) do not predict certainty of disease, nor do they clearly predict aggressive versus indolent disease. The report noted that, while the monitoring of high-risk men may improve outcomes, it is also possible that these could be offset by the harms of identifying and treating additional indolent disease.

Type 2 diabetes

Available evidence has shown that screening for a panel of gene variants associated with type 2 diabetes does not substantially improve prediction of risk for the disease than an assessment based on traditional risk factors. Available evidence suggests that both genetic and environmental factors play a role in the development of type 2 diabetes. Recent genetic studies have identified 18 gene variants that appear to increase the risk for type 2 diabetes.

A study reported in the New England Journal of Medicine evaluated the potential utility of genetic screening in predicting future risk of type 2 diabetes (Meigs et al, 2008). The investigators analyzed records from the Framingham Offspring Study, which follows a group of adult children of participants of the original Framingham Heart study, to evaluate risk factors for the development of cardiovascular disease, including diabetes. Full genotype results for the 18 gene variants as well as clinical outcomes were available for 2,377 participants, 255 of whom developed type 2 diabetes during 28 years of follow-up. Each participant was assigned a genotype score, based on the number of risk-associated gene copies inherited. The investigators compared the predictive value of the genotype score to that of family history alone or of physiological risk factors. Overall, the genetic score was 17.7 among those who developed diabetes and 17.1 among those who did not. The investigators found that, while the genetic score did help predict who would develop diabetes, once other known risk factors were taken into consideration, it offered little additional predictive power. The investigators concluded that: "[t]he genotype score resulted in the appropriate risk reclassification of, at most, 4% of the subjects, compared with risk estimates based on age, sex, blood lipids, body mass index, family history, and other standard risk factors." The investigators reported that "[o]ur findings underscore the view that identification of adverse phenotypic characteristics remains the cornerstone of approaches to predicting the risk of type 2 diabetes," the authors said.

A similar study among Swedish and Finnish patients, published in the same issue of the New England Journal of Medicine, also found only a small improvement in risk estimates when genetic factors were added to traditional risk factors (Lyssenko et al, 2008).

The OncoVue breast cancer risk test

The OncoVue breast cancer risk test (Intergenetics, Inc., Oklahoma City, OK) is a genetic-based breast cancer risk test that incorporates both individualized genetic-based single nucleotide polymorphisms (SNPs) and personal history measures to arrive at an estimate of a woman’s breast cancer risk at various stages in her life.

Cells that are collected from the inside of the cheek are analyzed using thousands of proprietary (Intergenetic, Inc.) combinations of multiple genes. The genetic information and the data from the medical history are combined to assign a numeric value that tells a woman’s lifetime risk of developing breast cancer. Her OncoVue risk test will tell her if she is standard, moderate or high risk for developing breast cancer during each stage of her life.

OncoVue is based on an unpublished case-controlled associative study that examined common genetic polymorphisms and medical history variables. Currently, 117 common polymorphisms (mostly SNPs) located in over 87 genes believed to alter breast cancer risk are examined. Most result in amino acid changes in the proteins encoded by the genes in which they occur. The medical history variables include answers to questions concerning women’s reproductive histories, family histories of cancer and a few other questions related to general health.

There are no published controlled studies on the OncoVue breast cancer risk test in the peer-reviewed medical literature.

Gail (2009) evaluated the value of adding SNP genotypes to a breast cancer risk model. Criteria that are based on 4 clinical or public health applications were used to compare the National Cancer Institute's Breast Cancer Risk Assessment Tool (BCRAT) with BCRATplus7, which includes 7 SNPs previously associated with breast cancer. Criteria included number of expected life-threatening events for the decision to take tamoxifen, expected decision losses (in units of the loss from giving a mammogram to a woman without detectable breast cancer) for the decision to have a mammogram, rates of risk re-classification, and number of lives saved by risk-based allocation of screening mammography. For all calculations, the following assumptions were made: Hardy-Weinberg equilibrium, linkage equilibrium across SNPs, additive effects of alleles at each locus, no interactions on the logistic scale among SNPs or with factors in BCRAT, and independence of SNPs from factors in BCRAT. Improvements in expected numbers of life-threatening events were only 0.07 % and 0.81 % for deciding whether to take tamoxifen to prevent breast cancer for women aged 50 to 59 and 40 to 49 years, respectively. For deciding whether to recommend screening mammograms to women aged 50 to 54 years, the reduction in expected losses was 0.86 % if the ideal breast cancer prevalence threshold for recommending mammography was that of women aged 50 to 54 years. Cross-classification of risks indicated that some women classified by BCRAT would have different classifications with BCRATplus7, which might be useful if BCRATplus7 was well calibrated. Improvements from BCRATplus7 were small for risk-based allocation of mammograms under costs constraints. The author reported that the gains from BCRATplus7 were small in the applications examined and that models with SNPs, such as BCRATplus7, have not been validated for calibration in independent cohort data. The author concluded that additional studies are needed to validate a model with SNPs and justify its use.

There is insufficient evidence on the effectiveness of the OncoVue breast cancer risk test in determining a woman’s breast cancer risk at various stages in her life.

The phosphatase and tensin homolog (PTEN) gene test
Phosphatase and tensin homolog (PTEN) hamartoma tumor syndrome is an autosomal dominant group of disorders with significant clinical overlap, most notably predisposition to hamartomatous polyposis of the gastro-intestinal tract. Laurent-Puig et al. (2009) stated that the occurrence of KRAS mutation is predictive of non-response and shorter survival in patients treated by anti-epidermal growth factor receptor (anti-EGFR) antibody for metastatic colorectal cancer (mCRC), leading the European Medicine Agency to limit its use to patients with wild-type KRAS tumors. However, only 50% of these patients will benefit from treatment, suggesting the need to identify additional biomarkers for cetuximab-based treatment efficacy. These investigators retrospectively collected tumors from 173 patients with mCRC. All but 1 patient received a cetuximab-based regimen as second-line or greater therapy. KRAS and BRAF status were assessed by allelic discrimination. EGFR amplification was assessed by chromogenic in situ hybridization and fluorescent in situ hybridization, and the expression of PTEN was assessed by immunochrometry. In patients with KRAS wild-type tumors (n = 116), BRAF mutations (n = 5) were weakly associated with lack of response (p = 0.063) but were strongly associated with shorter progression-free survival (p < 0.001) and shorter overall survival (OS; p < 0.001). A high EGFR polysomy or an EGFR amplification was found in 17.7% of the patients and was associated with response (p = 0.015). PTEN null expression was found in 19.9% of the patients and was associated with shorter OS (p = 0.013). In multi-variate analysis, BRAF mutation and PTEN expression status were associated with OS. The authors concluded that BRAF status, EGFR amplification, and cytoplasmic expression of PTEN were associated with outcome measures in KRAS wild-type patients treated with a cetuximab-based regimen. They stated that more studies in clinical trial cohorts are needed to confirm the clinical utility of these markers.

Siena et al. (2009) noted that the monoclonal antibodies panitumumab and cetuximab that target the EGFR have expanded the range of treatment options for mCRC. Initial evaluation of these agents as monotherapy in patients with EGFR-expressing chemotherapy-refractory tumors yielded response rates of approximately 10%. The realization that detection of positive EGFR expression by immunostaining does not reliably predict clinical outcome of EGFR-targeted treatment has led to an intense search for alternative predictive biomarkers. Oncogenic activation of signaling pathways downstream of the EGFR, such as mutation of KRAS, BRAF, or PIK3CA oncoproteins, or inactivation of the PTEN tumor suppressor gene is central to the progression of colorectal cancer. Tumor KRAS mutations, which may be present in 35% to 45% of patients with colorectal cancer, have emerged as an important predictive marker of resistance to panitumumab or cetuximab treatment. In addition, among colorectal tumors carrying wild-type KRAS, mutation of BRAF or PIK3CA or loss of PTEN expression may be associated with resistance to EGFR-targeted monoclonal antibody treatment, although these additional biomarkers require further validation before incorporation into clinical practice. Additional knowledge of the molecular basis for sensitivity or resistance to EGFR-targeted monoclonal antibodies will allow the development of new treatment algorithms to identify patients who are most likely to respond to treatment and could also provide rationale for combining therapies to overcome primary resistance. The use of KRAS mutations as a selection biomarker for anti-EGFR monoclonal antibody (e.g., panitumumab or cetuximab) treatment is the first major step toward individualized treatment for patients with mCRC.

**Epsilon-sarcoglycan gene (SCGE) deletion analysis**

Myoclonus-dystonia (M-D), an autosomal dominant inherited movement disorder, has been associated with mutations in the epsilon-sarcoglycan gene (SCGE) on 7q21. Raymond et al. (2008) noted that M-D due to SGCE mutations is characterized by early onset myoclonic jerks, often associated with dystonia. Penetrance is influenced by parental sex, but other sex effects have not been established. In 42 affected individuals from 11 families with identified mutations, these researchers found that sex was highly associated with age at onset regardless of mutation type; the median age onset for girls was 5 years versus 8 years for boys (p < 0.0097). Moreover, the authors found no association between mutation type and phenotype.

Ritz et al. (2009) stated that various mutations within the SGCE gene have been associated with M-D, but mutations are detected in only about 30% of patients. The lack of stringent clinical inclusion criteria and limitations of mutation screens by direct sequencing might explain this observation. Eighty-six M-D index patients from the Dutch national referral center for M-D underwent neurological examination and were classified according to previously published criteria into definite, probable and possible M-D. Sequence analysis of the SGCE gene and screening for copy number variations were performed. In addition, screening was carried out for the 3 bp deletion in exon 5 of the DYT1 gene. Based on clinical examination, 24 definite, 23 probable and 39 possible M-D patients were detected. Thirteen of the 86 M-D index patients carried a SGCE mutation: 7 in the definite M-D group. However, in 50% of definite M-D cases, no mutation could be identified.

**Home genetic tests**

Walker (2010) stated that according to an undercover investigation by the Government Accountability Office (GAO), home genetic tests often provide incomplete or misleading information to consumers. For the GAO investigation, investigators purchased 10 tests each from 4 different direct-to-consumer genetic tests companies: 23andMe, deCode Genetics, Navigenics, and Pathway Genomics. Five saliva donors each sent 2 DNA samples to each company. In one sample, the donor used his or her real personal and medical information, and for the second sample, they developed faux identifying and medical information. The results, according to the GAO, were far from precise. For example, a donor was told by a company that he had a "below average" risk of developing hypertension, but a second company rated his risk as "average", while a third company, using DNA from the same donor, said the sample revealed an "above average" risk for hypertension. In some cases, the results conflicted with the donor's real medical condition. None of the genetic tests currently offered to consumers has undergone FDA pre-market review.

_Familial Cold Autoinflammatory Syndrome_
Familial cold autoinflammatory syndrome (FCAS), also known as familial cold urticaria (FCU), is an autosomal dominant condition characterized by rash, conjunctivitis, fever/chills and arthralgias elicited by exposure to cold – sometimes temperatures below 22° C (72° F). It is rare and is estimated as having a prevalence of 1 per million people and mainly affects Americans and Europeans. Familial cold autoinflammatory syndrome is one of the cryopyrin-associated periodic syndromes (CAPS) caused by mutations in the CIAS1/NALP3 (also known as NLPR3) gene at location 1q44. Familial cold autoinflammatory syndrome shares symptoms, and should not be confused, with acquired cold urticaria, a more common condition mediated by different mechanisms that usually develop later in life and are rarely inherited. There is insufficient evidence to support the use of genetic testing in the management of patients with FCAS/FCU. UpToDate reviews on “Cold urticaria” (Maurer, 2011) and “Cryopyrin-associated periodic syndromes and related disorders” (Nigrovic, 2011) do not mention the use of genetic testing.

Santome Collazo et al (2010) noted that congenital adrenal hyperplasia (CAH) is not an infrequent genetic disorder for which mutation-based analysis for CYP21A2 gene is a useful tool. An UpToDate review on “Diagnosis of classic congenital adrenal hyperplasia due to 21-hydroxylase deficiency” (Merke, 2011) states that “[g]enetic testing also can be used to evaluate borderline cases. Genetic testing detects approximately 95 percent of mutant alleles”. Furthermore, the Endocrine Society’s clinical practice guideline on congenital adrenal hyperplasia (Speiser et al, 2010) suggested genotyping only when results of the adrenocortical profile following cosyntropin stimulation test are equivocal or for purposes of genetic counseling. The Task Force recommends that genetic counseling be given to parents at birth of a CAH child, and to adolescents at the transition to adult care.

Malignant Hyperthermia

Wappler (2010) stated that malignant hyperthermia (MH)-susceptible patients have an increased risk during anesthesia. The aim of this review was to present current knowledge about pathophysiology and triggers of MH as well as concepts for safe anesthesiological management of these patients. Trigger substances and mechanisms have been well-defined to date. Anesthesia can be safely performed with i.v. anesthetics, nitrous oxide, non-depolarizing muscle relaxants, local anesthetics as well as xenon. Attention must be directed to the preparation of the anesthetic machine because modern work-stations need longer cleansing times than their predecessors. Alternatively, activated charcoal might be beneficial for elimination of volatile anesthetics. Day case surgery can be performed in MH-susceptible patients, if all safety aspects are regarded. Whether there is an association between MH susceptibility and other disorders is still a matter of debate. The authors concluded that the incidence of MH is low, but the prevalence can be estimated as up to 1:3,000. Because MH is potentially lethal, it is relevant to establish management concepts for peri-operative care in susceptible patients. This includes pre-operative genetic and in-vitro muscle contracture test (IVCT), preparation of the anesthetic work-station, use of non-triggering anesthetics, adequate monitoring, availability of sufficient quantities of dantrolene and appropriate post-operative care. Taking these items into account, anesthesia can be safely performed in susceptible patients.

Moreover, an UpToDate review on “Susceptibility to malignant hyperthermia” (Litman, 2011) states that “the contracture test is performed at specific centers around the world (four in the United States). Following testing, the referring physician receives a report indicating whether testing was positive, negative, or equivocal. Positive or equivocal results should be followed-up with genetic testing. Referral information can be found on the Malignant Hyperthermia Association of the United States (MHAUS) website”. Genetic testing for MH is indicated in the following groups:

- Patients with a positive or equivocal contracture test to determine the presence of a specific mutation.
- Individuals with a positive genetic test for MH in a family member.
- Patients with a clinical history suspicious for MH (acute MH episode, masseter muscle rigidity, post-operative myoglobinuria, heat or exercise induce rhabdomyolysis) who are unable or unwilling to undergo contracture testing.

Sleep-Walking

Licis et al (2011) stated that sleep-walking is a common and highly heritable sleep disorder. However, inheritance patterns of sleep-walking are poorly understood and there have been no prior reports of genes or chromosomal localization of genes responsible for this disorder. These researchers described the inheritance pattern of sleep-walking in a 4-generation family and identified the chromosomal location of a gene responsible for sleep-walking in this family. A total of 9 affected and 13 unaffected family members of a single large family were interviewed and DNA samples collected. Parametric linkage analysis was performed. Sleep-walking was inherited as an autosomal dominant disorder with reduced penetrance in this family. Genome-wide multi-point parametric linkage analysis for sleep-walking revealed a maximum logarithm of the odds score of 3.44 at chromosome 20q12-q13.12 between 55.6 and 61.4 cM. The authors described the first genetic locus for sleep-walking at chromosome 20q12-q13.12; and concluded that sleep-walking may be transmitted as an autosomal dominant trait with reduced penetrance.

In an editorial that accompanied the aforementioned study, Dogu and Pressman (2011) noted that “[a]ccording to currently accepted evidence-based theories, the occurrence of sleepwalking requires genetic predisposition, priming factors such as severe sleep deprivation or stress, and, in addition, a proximal trigger factor such as noise or touch. These factors form the background for a “perfect storm,” all of which must occur before a sleepwalking episode will occur. Hereditary factors likely play an important role, with recessive and multifactorial inheritance patterns having been reported. A recent genetic study has shown that the HLADQB1*05 Ser74 variant is a major susceptibility factor for sleepwalking in familial cases, but this finding has yet to be replicated. Another study attempted to find a causal relationship between sleepwalking and sleep-disordered breathing in cosegregated families of both disorders. However, this study was limited by the absence of molecular data .... The current diagnosis of sleepwalking is based almost entirely on clinical history. There are no objective, independent means of confirming the diagnosis. Additionally, treatment of sleepwalking is symptomatic, aimed at suppressing arousal or reducing
deep sleep. Identification of causative genes may eventually permit development of an independent test and treatments aimed at the underlying causes of this disorder.

**Choroidal Neovascularization**

RetnaGene AMD (Sequenom Center for Molecular Medicine) is a laboratory developed genetic test to assess the risk of developing choroidal neovascularization (CNV), the wet form of age-related macular degeneration (AMD), a common eye disorder of the elderly that can lead to blindness. The test identifies at-risk Caucasians, age 60 and older. A report of the American Academy of Ophthalmology (Stone, et al., 2012) recommends avoidance of routine genetic testing for genetically complex disorders like age-related macular degeneration and late-onset primary open-angle glaucoma until specific treatment or surveillance strategies have been shown in one or more published clinical trials to be of benefit to individuals with specific disease-associated genotypes. The report recommends that, in the meantime, genotyping of such patients should be confined to research studies. The report stated that complex disorders (e.g., age-related macular degeneration and glaucoma) tend to be more common in the population than monogenic diseases, and the presence of any one of the disease-associated variants is not highly predictive of the development of disease. The report stated that, in many cases, standard clinical diagnostic methods like biomicroscopy, ophthalmoscopy, tonography, and perimetry will be more accurate for assessing a patient’s risk of vision loss from a complex disease than the assessment of a small number of genetic loci. The report said that genetic testing for complex diseases will become relevant to the routine practice of medicine as soon as clinical trials can demonstrate that patients with specific genotypes benefit from specific types of therapy or surveillance. The report concluded that, until such benefit can be demonstrated, the routine genetic testing of patients with complex eye diseases, or unaffected patients with a family history of such diseases, is not warranted.

**Malignant Hyperthermia**

Central core disease (CCD) also known as central core myopathy and Shy-Magee syndrome, is an inherited neuromuscular disorder characterized by central cores on muscle biopsy and clinical features of a congenital myopathy. Prevalence is unknown but the condition is probably more common than other congenital myopathies. CCD typically presents in infancy with hypotonia and motor developmental delay and is characterized by predominantly proximal weakness pronounced in the hip girdle; orthopedic complications are common and malignant hyperthermia susceptibility (MHS) is a frequent complication.

Malignant hyperthermia (MH) or malignant hyperpyrexia is a rare but severe pharmacogenetic disorder that occurs when agents that may trigger MH are desflurane, enflurane, halothane, isoflurane, sevoflurane, and suxamethonium chloride. MH usually occurs in the operating theater, but can occur at anytime during anesthesia and up to an hour after discontinuation.

CCD and MHS are allelic conditions both due to (predominantly dominant) mutations in the skeletal muscle ryanodine receptor (RYR1) gene, encoding the principal skeletal muscle sarcoplasmic reticulum calcium release channel (RyR1). Altered excitability and/or changes in calcium homeostasis within muscle cells due to mutation-induced conformational changes of the RyR protein are considered the main pathogenetic mechanisms(s).

The diagnosis of CCD is based on the presence of suggestive clinical features and central cores on muscle biopsy; muscle MRI may show a characteristic pattern of selective muscle involvement and aid the diagnosis in cases with equivocal histopathological findings. Mutational analysis of the RYR1 gene may provide genetic confirmation of the diagnosis. Further evaluation of the underlying molecular mechanisms may provide the basis for future rational pharmacological treatment.

The reference standard test for establishing a clinical diagnosis of MHS is the caffeine halothane contracture test (CHCT) in the United States, and the in vitro contracture test (IVCT) in Europe and Australasia. The CHCT and IVCT are similar and measure the muscle contracture in the presence of the anesthetic halothane and caffeine. Both tests categorize patients as being MHS, MH equivocal (MHE), or MH negative (MHN). These tests are invasive and must be performed using a skeletal muscle biopsy that is < 5 hours old. Sequence variants in the ryanodine receptor 1 (skeletal) (RYR1) gene have been shown to be associated with MH susceptibility (MHS) and are found in up to 80% of patients with confirmed MH, usually with an autosomal dominant pattern of inheritance. Although additional genetic loci have been associated with MH, the contribution of these other loci to MH is low.

Genetic testing for RYR1 sequence variants from commercial providers is performed by polymerase chain reaction (PCR) followed by direct sequencing. Genetic tests for RYR1 sequence variants can be performed to either identify sequence variants in genetic hot spots of the RYR1 gene that cover all exons on which causative MH variants can be found, or for screening of sequence variants across the entire 106 exons of the RYR1 gene.

Examples of commercially available tests are: Malignant Hyperthermia/Central Core Disease (570-572) RYR1 Sequencing (Prevention Genetics); Malignant hyperthermia (RYR1 gene sequenced analysis, partial) (University of Pittsburgh Medical Center, Division of Molecular Diagnostics [UPMC Molecular Diagnostics]).

**Hereditary Hemorrhagic Telangiectasia**

Hereditary hemorrhagic telangiectasia (HHT), also called Osler-Weber-Rendu syndrome, is an autosomal dominant trait disorder that results in the development of multiple abnormalities in the blood vessels. Some arterial vessels flow directly into veins rather than into the capillaries resulting in arteriovenous malformations. When they occur in vessels near the surface of the skin, where they are visible as red markings, they are known as telangiectases (the singular is telangiectasia). Nosebleeds are very common in people with HHT, and more serious problems may arise from hemorrhages in the brain, liver, lungs, or other organs. Forms of HHT include type 1, type 2, type 3, and juvenile polyposis/hereditary hemorrhagic telangiectasia syndrome. People with type 1 tend to develop symptoms earlier than those with type 2, and are more likely to have blood vessel malformations in the lungs and brain. Type 2 and type 3 may be associated with a higher risk of liver involvement. Women are
more likely than men to develop blood vessel malformations in the lungs with type 1, and are also at higher risk of liver involvement with both type 1 and type 2. Individuals with any form of hereditary hemorrhagic telangiectasia, however, can have any of these problems.

Genetic testing utilizes a blood test to determine whether or not an at risk individual carries the genes responsible for the development of disease. Mutations in two genes, endoglin and ALK-1, have been shown to be responsible for pure HHT, with the disease subtypes designated HHT1 and HHT2. Mutations in Smad4 result in a juvenile polyposis-HHT overlap syndrome.

In 2010, Shah and group wrote that hereditary hemorrhagic telangiectasia (HHT) is an autosomal dominant disorder with age-dependent penetrance characterized by recurrent epistaxis, mucocutaneous telangiectasias, and visceral arteriovenous malformations (AVMs). AVMs can occur in multiple organs, including brain, liver, and lungs, and are associated with a large portion of disease morbidity. Pulmonary AVMs (PAVMs) can be asymptomatic or manifest as dyspnea and hypoxemia secondary to shunting. The presence of untreated PAVMs can also lead to transient ischemic attacks, stroke, hemothorax, and systemic infection, including cerebral abscesses. Definitive diagnosis is made when three or more clinical findings are present, which include the features mentioned above and a first-degree relative diagnosed with HHT. Diagnosis is suspected when two findings are present. Genetic testing can help confirm diagnosis. Mutations in three genes are known to cause disease: ENG, ACVRL1, and SMAD4. Genetic testing involves sequence and duplication/deletion analysis and identifies a mutation in roughly 80% of patients with clinical disease.

The textbook Flint: Cummings Otolaryngology: Head & Neck Surgery (2010) states that genetic testing is available for prenatal diagnosis of hereditary hemorrhagic telangiectasia. This is important, because catastrophic hemorrhage can occur in children with clinically silent disease, thus screening imaging for cerebral and pulmonary arteriovenous malformations is indicated in children who have a family history.

According to the textbook of Feldman: Sleisenger and Fordtran's Gastrointestinal and Liver Disease (2010), genetic testing to detect mutations in the ENG, ALK-1, or MAHD4 genes may be helpful in selected cases. Patients suspected of having HHT should be screened for cerebral and pulmonary arteriovenous malformations (AVMs), and family members of the patient should consider genetic testing.

The textbook Cassidy: Management of Genetic Syndromes (2005), reports that, to date, mutation testing has not been widely used in the diagnosis of HHT. However, mutations in either ALK1 or endoglin have been demonstrated in over 70% of unrelated, affected individuals tested using direct gene sequencing of genomic DNA. Genetic testing for HHT will have an important role in both the testing of individuals for whom the diagnosis is uncertain and in presymptomatic testing of young adults at risk of HHT.

In 2006, Bossler and group describe the results of mutation analysis on a consecutive series of 200 individuals undergoing clinical genetic testing for HHT. The observed sensitivity of mutation detection was similar to that in other series with strict ascertainment criteria. A total of 127 probands were found, with sequence changes consisting of 103 unique alterations, 68 of which were novel. In addition, eight intragenic rearrangements in the ENG gene and two in the ACVRL1 gene were identified in a subset of coding sequence mutation-negative individuals. Most individuals tested could be categorized by the number of HHT diagnostic criteria present. Surprisingly, almost 50% of the cases with a single symptom were found to have a significant sequence alteration; three of these reported only nosebleeds. The authors concluded, "genetic testing can confirm the clinical diagnosis in individuals and identify presymptomatic mutation carriers. As many of the complications of HHT disease can be prevented, a confirmed molecular diagnosis provides an opportunity for early detection of AVMs and management of the disease."

Spinal Muscular Atrophy

Spinal muscular atrophy (SMA), which has an estimated prevalence of 1 in 10,000, is characterized by proximal muscle weakness resulting from the degeneration of anterior horn cells in the spinal cord. SMA type I is typically diagnosed at birth or within the first 3 to 6 months of life; affected children are unable to sit unassisted and usually die from respiratory failure within 2 years. Those with SMA type II, which is diagnosed before 18 months of age, are unable to stand or walk unaided, although they may be able to sit and may survive beyond age 4. The clinical features of SMA types III and IV are milder and manifest after 18 months of age or in adulthood, respectively. SMA is inherited in an autosomal recessive manner and is caused by alterations in the survival motor neuron 1 (SMN1) gene located on chromosome 5 at band q12.2 to q13.3. Approximately 95% of SMA patients have the condition as a result of a homozygous deletion involving at least exon 7 of SMN1. Approximately 5% are compound heterozygotes, with a deletion in 1 allele of SMN1 and a subtle intragenic variation in the other. SMN2, a gene nearly identical in sequence to SMN1, is located in the same highly repetitive region on chromosome 5. Although it does not cause SMA, it has been shown to modify the phenotype of the condition; those with the milder SMA types II or III tend to have more copies of SMN2 than those with the severe type I.

SMN1 deletions are detected by polymerase chain reaction (PCR) amplification of exon 7 of the SMN genes, followed by restriction fragment length polymorphism (RFLP) analysis. Following amplification, exon 7 of SMN2 will be cut with the restriction enzyme DraI, while exon 7 of SMN1 will remain intact. SMA patients with homozygous SMN1 deletions will show an absence of the uncut SMN1 exon 7 PCR products. To detect heterozygous SMN1 deletions in SMA carriers or compound heterozygotes, quantitative PCR (qPCR) is performed. To identify subtle intragenic variations in SMA patients found to have only 1 copy of the deletion, the SMN1 gene is typically sequenced. Candidates for diagnostic testing include infants, children, and adults with generalized hypotonia and proximal muscle weakness of unknown etiology. Carrier testing may be offered to couples considering pregnancy, including those with a family history of SMA, and prenatal diagnosis should be made available to all identified carriers.

Heterotaxy
Genetic Dysfunction. To investigate this in more detail, 8 patients (6 women, 2 men; mean age of 22.3 years) were studied. All completed an intelligence test (Wechsler Adult Intelligence Scale; WAIS), and 4 were also given memory tests and a comprehensive neuropsychological test battery. Patients with MSCAE showed significant cognitive dysfunction. Mean Verbal IQ (84.3) was significantly better than Performance IQ (71.8) (t = 5.23, p = 0.001), but memory testing and neuropsychological transactivation analyses. The temporo-spatial expression pattern of Zic3-B overlaps with Zic3-A in-vivo, and both isoforms are localized to the nucleus in-vitro. Both isoforms can transcriptionally activate a Gli binding site reporter, but only Zic3-A synergistically activates upon co-transfection with Gli3, suggesting that the isoforms are functionally distinct. The authors concluded that screening 109 familial and sporadic male heterotaxy cases did not identify pathogenic mutations in the newly identified fourth exon and larger studies are necessary to establish the importance of the novel isoform in human disease.

Tarig et al (2011) heterotaxy-spectrum cardiovascular disorders are challenging for traditional genetic analyses because of clinical and genetic heterogeneity, variable expressivity, and non-penetrance. In this study, high-resolution single nucleotide polymorphisms (SNPs) genotyping and exon-targeted array comparative genomic hybridization (CGH) platforms were coupled to whole-exome sequencing to identify a novel disease candidate gene. SNP genotyping identified absence-of-heterozygosity regions in the heterotaxy proband on chromosomes 1, 4, 7, 13, 15, 18, consistent with parental consanguinity. Subsequently, whole-exome sequencing of the proband identified 26,065 coding variants, including 18 non-synonymous homozygous changes not present in dbsNP132 or 1000 Genomes. Of these 18, only 4 -- 1 each in CXXCL2, SHROOM3, CTSO, RFXP1 -- were mapped to the absence-of-heterozygosity regions, each of which was flanked by more than 50 homozygous SNPs, confirming recessive segregation of mutant alleles. Sanger sequencing confirmed the SHROOM3 homozygous missense mutation and it was predicted as pathogenic by 4 bio-informatic tools. SHROOM3 has been identified as a central regulator of morphogenetic cell shape changes necessary for organogenesis and can physically bind ROCK2, a rho kinase protein required for left-right patterning. Screening 96 sporadic heterotaxy patients identified 4 additional patients with rare variants in SHROOM3. The authors concluded that using whole exome sequencing, the authors identify a recessive missense mutation in SHROOM3 associated with heterotaxy syndrome and identify rare variants in subsequent screening of a heterotaxy cohort, suggesting SHROOM3 as a novel target for the control of left-right patterning. This study revealed the value of SNP genotyping coupled with high-throughput sequencing for identification of high yield candidates for rare disorders with genetic and phenotypic heterogeneity. Also, UpToDate reviews on “Clinical manifestations, pathophysiology, and diagnosis of atrioventricular (AV) canal defects” (Fleishman and Tugertimur, 2013) and “Congenital heart disease (CHD) in the newborn: Presentation and screening for critical CHD” (Altman, 2013) do not mention the use of genetic testing as a management tool.

Mitochondrial Recessive Ataxia Syndrome

Lee et al (2007) stated that spino-cerebellar ataxia (SCA) is a heterogeneous group of neurodegenerative disorders with common features of adult-onset cerebellar ataxia. Many patients with clinically suspected SCA are subsequently diagnosed with common SCA gene mutations. Previous reports suggested some common mitochondrial DNA (mtDNA) point mutations and mitochondrial DNA polymerase gene POLG1 mutations might be additional underlying genetic causes of cerebellar ataxia. These researchers tested whether mtDNA point mutations A3243G, A8344G, T8993G, and T8993C, or POLG1 mutations W748S and A467T are found in patients with adult-onset ataxia who did not have common SCA mutations. A total of 476 unrelated patients with suspected SCA underwent genetic testing for SCA 1, 2, 3, 6, 7, 8, 10, 12, 17, and DRPLA gene mutations. After excluding these SCA mutations and patients with paternal transmission history, 265 patients were tested for mtDNA mutations A3243G, A8344G, T8993G, T8993C, and POLG1 W748S and A467T mutations. No mtDNA A3243G, A8344G, T8993G, T8993C, or POLG1 W748S and A467T mutation was detected in any of the 265 ataxia patients, suggesting that the upper limit of the 95 % confidence interval (CI) for the prevalence of these mitochondrial mutations in Chinese patients with adult-onset non-SCA ataxia is no higher than 1.1 %. The authors concluded that the mtDNA mutations A3243G, A8344G, T8993G, T8993C, or POLG1 W748S and A467T are very rare causes of adult-onset ataxia in Taiwan. Routine screening for these mutations in ataxia patients with Chinese origin is of limited clinical value.

Gramstad et al (2009) noted that mitochondrial polymers are gamma (POLG1) produce a wide variety of neurological disorders including a progressive ataxic syndrome with epilepsy: mitochondrial SCA and epilepsy (MSECA). The authors’ earlier studies of patients with this syndrome raised the possibility of more prominent right than left hemisphere dysfunction. To investigate this in more detail, 8 patients (6 women, 2 men; mean age of 22.3 years) were studied. All completed an intelligence test (Wechsler Adult Intelligence Scale; WAIS), and 4 were also given memory tests and a comprehensive neuropsychological test battery. Patients with MSECA showed significant cognitive dysfunction. Measur...
known epilepsy syndrome, epileptic encephalopathy, encephalhepatopathy, or neuropathologically verified Alpers syndrome. A total of 7 patients had POLG1 mutations, and all of them had severe encephalopathy with intractable epilepsy. Four patients had died after exposure to sodium valproate. Brain MRI showed parieto-occipital or thalamic hyper-intense lesions, white matter abnormality, and atrophy. Muscle histology and mitochondrial biochemistry results were normal in all. The authors concluded that POLG1 analysis should belong to the first-line DNA diagnostic tests for children with an encephalitis-like presentation evolving into epileptic encephalopathy with liver involvement (Alpers syndrome), even if brain MRI and morphology, respiratory chain activities, and the amount of mitochondrial DNA in the skeletal muscle are normal. POLG1 analysis should precede valproate therapy in pediatric patients with a typical phenotype. However, POLG1 is not a common cause of isolated epilepsy or ataxia in childhood.

Tang et al (2012) determined the prevalence of MNGIE-like phenotype in patients with recessive POLG1 mutations. Mutations in the POLG1 gene, which encodes for the catalytic subunit of the mitochondrial DNA polymerase gamma essential for mitochondrial DNA replication, cause a wide spectrum of mitochondrial disorders. Common phenotypes associated with POLG1 mutations include Alpers syndrome, ataxia-neuropathy syndrome, and progressive external ophthalmoplegia (PEO). Mitochondrial neuro-gastro-intestinal encephalomyopathy (MNGIE) is an autosomal recessive disorder characterized by severe gastrointestinal dystomyotility, cachexia, PEO and/or ptosis, peripheral neuropathy, and leuкоencephalopathy. MNGIE is caused by TYMP mutations. Rare cases of MNGIE-like phenotype have been linked to RRM2B mutations. Recently, POLG1 mutations were identified in a family with clinical features of MNGIE but no leuкоencephalopathy. The coding regions and exon-intron boundaries of POLG1 were sequence analyzed in patients suspected of POLG1-related disorders. Clinical features of 92 unrelated patients with 2 pathogenic POLG1 alleles were carefully reviewed. Three patients, accounting for 3.3 % of all patients with 2 pathogenic POLG1 mutations, were found to have clinical features consistent with MNGIE but no leuкоencephalopathy. Patient 1 carries p.W748S and p.R953C; patient 2 is homozygous for p.W748S, and patient 3 is homozygous for p.A467T. In addition, patient 2 has a similarly affected sibling with the same POLG1 genotype. POLG1 mutations may cause MNGIE-like syndrome, but the lack of leuкоencephalopathy and the normal plasma thymidine favor POLG1 mutations as responsible molecular defect.

Furthermore, UpToDate reviews on “Overview of the hereditary ataxias” (Opal and Zoghbi, 2013a) and “The spinocerebellar ataxias” (Opal and Zoghbi, 2013b) do not mention the use of POLG1 genetic testing.

**Myelodysplastic Syndromes**

National Comprehensive Cancer Network’s clinical practice guideline on “Myelodysplastic syndromes” (2014) stated that further evaluations are necessary to establish the role of these genetic lesions on risk stratification systems in myelodysplastic syndrome. The guidelines stated that mutations in TET2 are among the most common mutations reported in patients with myelodysplastic syndromes (about 20 % of cases). Mutations in SF3B1 are one of several common molecular abnormalities involving the RNA splicing machinery, occurring in 14.5 to 16.0 % of MDS cases.

**Exome Sequencing**

A special report on “Exome sequencing for clinical diagnosis of patients with suspected genetic disorders” by the BCBSA's Technology Evaluation Center (2013) stated that “Exome sequencing has the capacity to determine in a single assay an individual’s exomic variation profile, limited to most of the protein coding sequence of an individual (approximately 85 %), composed of about 20,000 genes, 180,000 exons (protein-coding segments of a gene), and constituting approximately 1 % of the whole genome. It is believed that the exome contains about 85 % of heritable disease-causing mutations … Exome sequencing, relying on next-generation sequencing technologies, is not without challenges and limitations … Detailed guidance from regulatory or professional organizations is under development, and the variability contributed by the different platforms and procedures used by clinical laboratories offering exome sequencing as a clinical service is unknown … Currently, the diagnostic yield for single-gene disorders appears to be no greater than 50 % and possibly less, depending on the patient population and provider expertise. Medical management options may be available for only a subset of those diagnosed”.

**May-Hegglin Anomaly**

Strasser et al (2012) stated that Alport syndrome (ATS) is a type-IV collagen inherited disorder, caused by mutations in COL4A3 and COL4A4 (autosomal recessive) or COL4A5 (X-linked). Clinical symptoms include progressive renal disease, eye abnormalities and high-tone sensori-neural deafness. A renal histology very similar to ATS is observed in a subset of patients affected by mutations in MYH9, encoding non-muscle-myosin Type IIa – a cytoskeletal contractile protein. MYH9-associated disorders (May-Hegglin anomaly, Epstein and Fechtner syndrome, and others) are inherited in an autosomal dominant manner and characterized by defects in different organs (including eyes, ears, kidneys and thrombocytes). These researchers described here a 6-year old girl with hematuria, proteinuria, and early sensori-neural hearing loss. The father of the patient is affected by ATS, the mother by isolated inner ear deafness. Genetic testing revealed a pathogenic mutation in COL4A5 (c.2605G>A) in the girl and her father and a heterozygous mutation in MYH9 (c.4952T>G) in the girl and her mother. The paternal COL4A5 mutation seems to account for the complete phenotype of ATS in the father and the maternal mutation in MYH9 for the inner ear deafness in the mother. It has been discussed that the interaction of both mutations could be responsible for both the unexpected severity of ATS symptoms and the very early onset of inner ear deafness in the girl.

An UpToDate review on “Congenital and acquired disorders of platelet function” (Coutre, 2013) states that “Giant platelet disorders -- Inherited platelet disorders with giant platelets are quite rare (picture 2 and algorithm 1 and table 4). These include platelet glycoprotein abnormalities (e.g., Bernard-Soulier syndrome), deficiency of platelet alpha granules (e.g., gray platelet syndrome), the May-Hegglin anomaly, which also involves the presence of abnormal neutrophil inclusions (i.e., Dohle-like bodies), and some kindreds with type 2B von Willebrand disease (the Montreal platelet syndrome)”. This review does not mention the use of genetic testing as a management tool for giant platelet disorders.
UpToDate reviews on “Inborn errors of metabolism: Epidemiology, pathogenesis, and clinical features” (Sutton, 2013a) and “Inborn errors of metabolism: Classification” (Sutton, 2013b) do not mention the use of genetic testing as a management tool.

Very Long Chain AcylCoA Dehydrogenase Deficiency (VLCAADD)

An UpToDate review on “Newborn screening” (Sietki, 2013) states that “MS-MS [tandem mass spectrometry] detects more cases of inborn errors of metabolism than clinical diagnosis. In a study from New South Wales and the Australian Capital Territory, Australia, the prevalence of 31 inborn errors of metabolism affecting the urea cycle, amino acids (excluding PKU), organic acids, and fatty acid oxidation detected by MS-MS in 1998 to 2002 was 15.7 per 100,000 births, compared to 8.6 to 9.5 per 100,000 births in the four four-year cohorts preceding expanded screening. The increased rate of diagnosis was most apparent for the medium-chain and short-chain acyl-Co-A dehydrogenase deficiencies. Whether all children with disorders detected by MS-MS would have become symptomatic is uncertain .... The American Academy of Pediatrics has developed newborn screening fact sheets for 12 disorders, biotinidase deficiency, congenital adrenal hyperplasia, congenital hearing loss, congenital hypothyroidism, cystic fibrosis, galactosemia, homocystinuria, maple syrup urine disease, medium-chain acyl-coenzyme A dehydrogenase deficiency, PKU, sickle cell disease and other hemoglobinopathies, and tyrosinemia .... With the use of tandem mass spectrometry (MS-MS), the prevalence of a confirmed metabolic disorder detected by newborn screening is 1:4000 live births (about 12,500 diagnoses each year) in the United States. The most commonly diagnosed conditions are hearing loss, primary congenital hypothyroidism, cystic fibrosis, sickle cell disease, and medium-chain acyl-CoA dehydrogenase deficiency”. This review does not mention very long chain acylCoA dehydrogenase deficiency.

Congenital Stationary Night Blindness

According to Orphanet (a portal for rare diseases and orphan drugs), congenital stationary night blindness (CSNB) is an inherited retinal disorder that predominates on rods. It is a rare disease, and 3 types of transmission can be found: (i) autosomal dominant, (ii) recessive, and (iii) X-linked recessive. The affection is heterogeneous. The only symptom is hemeralopia with a moderate loss of visual acuity. Both the funduscopy and visual field are normal. In recessive forms, the “b” wave on the electroretinogram/electrooculography (ERG) is not found in the scotoscopic study, while the “a” wave is normal and increases with light intensity. In dominant forms, the “b” wave is seen. Levels of rhodopsine are normal and regenerate normally. Signal transmission may be affected. There is no specific treatment for CSNB. http://www.orpha.net/consor/cgi-bin/OC_Exp.php?lng=EN&Expert=215.

According to Genetic Home Reference, X-linked CSNB is a disorder of the retina. People with this condition typically have difficulty seeing in low light (night blindness). They also have other vision problems, including reduced acuity, high myopia, nystagmus, and strabismus. Color vision is typically not affected by this disorder. The visual problems associated with this condition are congenital. They tend to remain stable (stationary) over time. Researchers have identified 2 major types of X-linked CSNB: (i) the complete form, and (ii) the incomplete form. The types have very similar signs and symptoms. However, everyone with the complete form has night blindness, while not all people with the incomplete form have night blindness. The types are distinguished by their genetic cause and by the results of ERG. http://ghr.nlm.nih.gov/condition/x-linked-congenital-stationary-night-blindness.

In general, the diagnosis of X-linked CSNB can be made by ophthalmologic examination (including ERG) and family history consistent with X-linked inheritance (Boycott et al, 2012) http://www.ncbi.nlm.nih.gov/books/NBK1245/.

According to a Medscape review on “The Genetics of Hereditary Retinopathies and Optic Neuropathies” (Iannaccone, 2005), CSNB can be inherited according to all Mendelian inheritance patterns; 2 X-linked and 2 autosomal dominant genes have been cloned. In all types of CSNB, night vision is congenitally but non-progressively impaired and the retinal examination is normal. Most CSNB patients also have congenital nystagmus as the presenting sign, which can create a differential diagnostic challenge with Leber congenital amaurosis. Typically, patients with complete X-linked CSNB are also moderate-to-high myopes. The X-linked CSNB forms, which are the most common ones, all share an electonegative electroretinogram response similar to that seen in X-linked retinoschisis, and are distinguished in CSNB type 1 (also known as complete CSNB) and CSNB type 2 (incomplete CSNB) based on additional electroretinogram features, a distinction that has been confirmed at the genetic level”. http://www.medscape.com/viewarticle/501761_6.

Price et al (1988) reported that 7 of 8 patients presented initially or were followed for decreased acuity and nystagmus without complaints of night blindness. The diagnosis of CSNB was established with ERG and dark adaptation testing. They stated that careful electrodiagnostic testing is needed to provide accurate genetic counseling. Two patients showed pupillary constriction to darkness, which is a sign of retinal disease in young patients.

Lorenz et al (1996) presented the clinical data of 2 families with X-linked incomplete CSNB previously undiagnosed; ERG recordings in both families were suggestive of CSNB. The ERG of the obligate carrier was normal. In an attempt to distinguish between the complete and the incomplete type, and to identify further carrier signs, scotopic perimetry and dark adaptation were performed in both affected males and carriers. Scotopic perimetry tested the rod-mediated visual pathway in its spatial distribution. In affected males with non-recordable ERGs, scotopic perimetry and dark adaptation disclosed residual rod function indicating an incomplete type. In carriers, there was a sensitivity loss at 600 nm, which may be a new carrier sign. The authors concluded that correct diagnosis of the different forms of CSNB together with the identification of carriers is important for (i) genetic counseling, and (ii) linkage studies to identify the gene(s) for CSNB.

Kim et al (2012) evaluated the frequency of negative waveform ERGs in a tertiary referral center. All patients who had an ERG performed at the electrophysiology clinic at Emory University from January 1999 through March 2008 were included in the study. Patients with b-wave amplitude less than or equal to a-wave amplitude during the dark-adapted bright flash recording, in at least 1 eye, were identified as having a “negative ERG”. Clinical information, such as age, gender, symptoms, best corrected visual acuity, and diagnoses were recorded for these patients when available. A total of 1,837 patients underwent ERG testing.
during the study period. Of those, 73 patients had a negative ERG, for a frequency of 4.0 %. Within the adult (greater than or equal to 18 years of age) and pediatric populations, the frequencies of a negative ERG were 2.5 and 7.2 %, respectively. Among the 73 cases, negative ERGs were more common among male than female patients, 6.7 % versus 1.8 % (p < 0.0001). Negative ERGs were most common among male children and least common among female adults, 9.6 % versus 1.1 %, respectively. (p < 0.0001). Overall in this group of patients, the most common diagnoses associated with a negative ERG were CSNB (n = 29) and X-linked retinoschisis (XLRS, n = 7). The authors concluded that the overall frequency of negative ERGs in this large retrospective review was 4.0 %. Negative ERGs were most common among male children and least common among female adults. Despite the growing number of new diagnoses associated with negative ERGs, CSNB, and XLRS appear to be the most likely diagnoses for a pediatric patient who presents with a negative ERG.

It is also interesting to note that in a recently completed clinical trial (last verified June 2012) of "Treatment of Congenital Stationary Night Blindness with an Alga Containing High Dose of Beta Carotene", the selection criteria for participants of this trial do not include genetic testing. They included the following: http://www.clinicaltrials.gov/ct2/show/NCT00569023.

Isolated rod response markedly reduced (less than 20 % of normal) after 20 mins dark adaptation and improved by 50 % after 2 hrs
Negative maximal response ("a" wave to "b" wave ratio less than 2)
Retinal mid-peripheral white dots (more than 3,000 dots).

Kumar et al (2009) noted that many independent prognostic markers have been identified for predicting survival and helping in the management of lung cancer cases. p53 protein over-expression and mutation have been the topic of numerous such publications. However, little is known about the role of anti-p53 antibodies as a prognostic marker in lung cancer. These investigators searched the MEDLINE database and the bibliographies of the retrieved manuscripts and reviews. The retrieved studies are grouped according to the cohort studied. Out of 179 citations retrieved, 17 met selection criteria. A total of 7 studies used only non-small-cell lung cancer (NSCLC); 4 studies used only small-cell lung cancer; and 6 studies used the mixed cohort of both types of lung cancer. The studies varied in the concept design, cohort studied and the methodology. The diagnostic potential of anti-p53 antibodies in lung cancer remained contradictory and as some studies showed an association with poor prognosis, others showed a favorable association and still others showing no association what so ever. The frequency of detection of anti-p53 antibody was very low, highly specific with result being independent of the cohort studied. The authors concluded that adequate clinical trials, with optimized cohort, antigen and assay validation, are needed to address patients and physician's concerns regarding these associations.

Ciancio et al (2011) stated that over-expression of the tumor suppressor gene p53 and the marker for cellular proliferation Ki67 in open lung biopsies are indicated as predictor factors of survival of patients with lung cancer. However, the prognostic value of p53 and Ki67 in fiberoptic bronchial biopsies (FBB) has not been fully investigated. These researchers evaluated p53 and Ki67 immunostaining in FBB from 19 with NSCLC (12 adenocarcinomas, 5 squamous cell carcinomas and 2 NSCLC-NOS). Fiberoptic bronchial biopsy specimens were fixed in formalin, embedded in paraffin, and immunostained using anti-p53 and anti-Ki67 antibodies. Slides were reviewed by 2 independent observers and classified as positive (+ve) when the number of cells with stained nuclei exceeded 15 % for p53 or when greater than 25 % positive cells were observed throughout each section for Ki67. Positive (+ve) immunostaining was found in 9 patients for p53 (47.37 %) and 8 patients for Ki67 (42.10 %). These investigators examined overall survival (OS) curves of the patients with Mantel's log-rank test, both p53 -ve and Ki67 -ve patients had significantly higher survival rates than p53 + ve (p < 0.005) and Ki67 + ve (p < 0.0001), respectively. The authors concluded that the findings of this study suggested that negative immunostaining of fiberoptic bronchial biopsies for p53 and Ki67 could represent a better prognostic factor for patients with NSCLC.

Mattioni et al (2013) noted that TPS3 gene mutations can lead to the expression of a dysfunctional protein that in turn may enable genetically unstable cells to survive and change into malignant cells. Mutant p53 accumulates early in cells and can precociously induce circulating anti-p53 antibodies (p53Abs); in fact, p53 over-expression has been observed in pre-neoplastic lesions, such as bronchial dysplasia, and p53Abs have been found in patients with chronic obstructive pulmonary disease, before the diagnosis of lung and other tobacco-related tumors. These researchers performed a large prospective study, enrolling non-smokers, ex-smokers and smokers with or without the impairment of lung function, to analyze the incidence of serum p53Abs and the correlation with clinico-pathologic features, in particular smoking habits and impairment of lung function, in order to investigate their possible role as early markers of the onset of lung cancer or other cancers. The p53Abs levels were evaluated by a specific ELISA in 675 subjects. Data showed that significant levels of serum p53Abs were present in 35 subjects (5.2 %); no difference was observed in the presence of p53Abs with regard to age and gender, while p53Abs correlated with the number of cigarettes smoked per day and packs-year. Furthermore, serum p53Abs were associated with the worst lung function impairment. The median p53Abs level in positive subjects was 3.5 units/ml (range of 1.2 to 65.3 units/ml). Only 15 positive subjects participated in the follow-up, again resulting positive for serum p53Abs, and no evidence of cancer was found in these patients. The authors concluded that the presence of serum p53Abs was found to be associated with smoking level and lung function impairment, both risk factors of cancer development. However, in this study these researchers did not observe the occurrence of lung cancer or other cancers in the follow-up of positive subjects, therefore they cannot directly correlate the presence of serum p53Abs with cancer risk.

Lei et al (2013) stated that the diagnosis of lung cancer remains a clinical challenge. Many studies have assessed the diagnostic potential of anti-p53 antibody in lung cancer patients but with controversial results. These researchers summarized the overall diagnostic performance of anti-p53 antibody in lung cancer. Based on a comprehensive search of the PubMed and Embase, these investigators identified outcome data from all articles estimating diagnostic accuracy of anti-p53 antibody for lung cancer. A summary estimation for sensitivity, specificity, and other diagnostic indexes were pooled using a bivariate model. The overall measure of accuracy was calculated using summary receiver operating characteristic curve and the area under curve (AUC) was calculated. According to the inclusion criteria, a total of 16 studies with 4,414 subjects (2,249 lung
cancers, 2,165 controls) were included. The summary estimates were: sensitivity 0.20 (95% confidence interval [CI]: 0.15 to 0.27), specificity 0.97 (95% CI: 0.95 to 0.98), positive likelihood ratio 6.64 (95% CI: 4.34 to 10.17), negative likelihood ratio 0.83 (95% CI: 0.77 to 0.89), diagnostic odds ratio 8.04 (95% CI: 5.05 to 12.79), the AUC was 0.84. Subgroup analysis suggested that anti-p53 antibody had a better diagnostic performance for small cell lung cancer than non-small cell lung cancer. The authors concluded that anti-p53 antibody can be an assistant marker in diagnosing lung cancer, but the low sensitivity limits its use as a screening tool for lung cancer. Moreover, they stated that further studies should be performed to confirm these findings.

UpToDate reviews on “Overview of the initial evaluation, treatment and prognosis of lung cancer” (Midhun, 2014a) and “Overview of the risk factors, pathology, and clinical manifestations of lung cancer” (Midhun, 2014b) do not mention anti-p53 and anti-MAPKAPK3 as biomarkers.

Also, an UpToDate review on “Screening for lung cancer” (DeFechbach and Humphrey, 2014) does not mention anti-MAPKAPK3 as a biomarker. Moreover, it lists “Immunostaining or molecular analysis of sputum for tumor markers. As examples, p16 ink4a promoter hypermethylation and p53 mutations have been shown to occur in chronic smokers before there is clinical evidence of neoplasia” as one of the technologies under investigation.

Furthermore, National Comprehensive Cancer Network’s clinical practice guideline on “Non-small cell lung cancer” (Version 4.2014) does not mention anti-p53 and anti-MAPKAPK3 as biomarkers.

Talameh and Kitzmiller (2014) noted that statins are the most commonly prescribed drugs in the United States and are extremely effective in reducing major cardiovascular events in the millions of Americans with hyperlipidemia. However, many patients (up to 25%) cannot tolerate or discontinue statin therapy due to statin-induced myopathy (SIM). Patients will continue to experience SIM at unacceptably high rates or experience unnecessary cardiovascular events (as a result of discontinuing or decreasing their statin therapy) until strategies for predicting or mitigating SIM are identified. A promising strategy for predicting or mitigating SIM is pharmacogenetic testing, particularly of pharmacokinetic genetic variants as SIM is related to statin exposure. Data are emerging on pharmacokinetic genetic variants and SIM. A current, critical evaluation of the literature on pharmacokinetic genetic variants and SIM for potential translation to clinical practice is lacking. This review focused specifically on pharmacokinetic genetic variants and their association with SIM clinical outcomes. These investigators also discussed future directions, specific to the research on pharmacokinetic genetic variants, which could speed the translation into clinical practice. For simvastatin, these researchers did not find sufficient evidence to support the clinical translation of pharmacokinetic genetic variants other than SLCO1B1. However, SLCO1B1 may also be clinically relevant for pravastatin- and pitavastatin-induced myopathy, but additional studies assessing SIM clinical outcome are needed. CYP2D6*4 may be clinically relevant for atorvastatin-induced myopathy, but mechanistic studies are needed. The authors concluded that future research efforts need to incorporate statin-specific analyses, multi-variant analyses, and a standard definition of SIM. As the use of statins is extremely common and SIM continues to occur in a significant number of patients, future research investments in pharmacokinetic genetic variants have the potential to make a profound impact on public health.

Kuhnenbaum and colleagues (2014) provided a comprehensive meta-analysis and review of the clinical and molecular genetics of essential tremor (ET). Studies were reviewed from the literature. Linkage studies were analyzed applying criteria used for monogenic disorders. For association studies, allele counts were extracted and allelic association calculated whenever possible. A meta-analysis was performed for genetic markers investigated in more than 3 studies. Linkage studies have shown conclusive results in a single family only for the locus ETM2 (essential tremor monogenic locus 2, logarithm of odds score [lod] greater than 3.3). None of the 3 ETM loci had been confirmed independently with a lod score greater than 2.0 in a single family. A mutation in the FUS gene (fused in sarcoma) was found in one ET family by exome sequencing. Two genome-wide association studies demonstrated association between variants in the LINGO1 gene (leucine-rich repeat and Ig domain containing 1) and the SLCA2 gene (solute carrier family 1 member 2) and ET, respectively. This meta-analysis confirmed the association of rs9652490 in LINGO1 with ET. Candidate gene mutation analysis and association studies have not identified reproducible associations. The authors concluded that problems of genetic studies of ET are caused by the lack of stringent diagnostic criteria, small sample sizes, lack of biomarkers, a high phenocopy rate, evidence for non-Mendelian inheritance, and high locus heterogeneity in presumably monogenic ET. They stated that these issues could be resolved by better worldwide cooperation and the use of novel genetic techniques.

Taylor et al (2014) noted that mitochondrial disorders have emerged as a common cause of inherited disease, but their diagnosis remains challenging. Multiple respiratory chain complex defects are particularly difficult to diagnose at the molecular level because of the massive number of nuclear genes potentially involved in intra-mitochondrial protein synthesis, with many not yet linked to human disease. These researchers determine the molecular basis of multiple respiratory chain complex deficiencies. They studied 33 patients referred to 2 national centers in the United Kingdom and Germany between 2005 and 2012. All had biochemical evidence of multiple respiratory chain complex defects but no primary pathogenic mitochondrial DNA mutation. Whole-exome sequencing was performed using 62-Mb exome enrichment, followed by variant prioritization using bioinformatic prediction tools, variant validation by Sanger sequencing, and segregation of the variant with the disease phenotype in the family. Presumptive causal variants were identified in 28 patients (53%; 95% CI: 39% to 67%), and possible causal variants were identified in 4 (9%; 95% CI: 2% to 18%). Together these accounted for 32 patients (60% 95% CI: 46% to 74%) and involved 18 different genes. These included recurrent mutations in RMND1, AARS2, and MTO1, each on a haplotype background consistent with a shared founder allele, and potential novel mutations in 4 possible mitochondrial disease genes (VARS2, GARS, FLAD1, and PTCD1). Distinguishing clinical features included deafness and renal involvement associated with RMND1 and cardiomyopathy with AARS2 and MTO1. However, atypical clinical features were present in some patients, including normal liver function and Leigh syndrome (subacute necrotizing encephalomyelopathy) seen in association with TRMU mutations and no cardiomyopathy with founder SCO2 mutations. It was not possible to confidently identify the underlying genetic basis in 21 patients (40%; 95% CI: 26% to 54%). The authors concluded that exome sequencing enhanced the ability to identify potential nuclear gene mutations in patients with biochemically defined defects affecting multiple
mitochondrial respiratory chain complexes. Moreover, they stated that additional study is needed in independent patient populations to determine the utility of this approach in comparison with traditional diagnostic methods.

UpToDate reviews on “Diagnostic evaluation of women with suspected breast cancer” (Esserman and Joe, 2014a), “Clinical features, diagnosis, and staging of newly diagnosed breast cancer” (Esserman and Joe, 2014b), and “Clinical manifestations and diagnosis of a palpable breast mass” (Sabel, 2014) do not mention RAD51C gene testing.

Furthermore, NCCN’s clinical practice guidelines on “Breast cancer” (Version 3.2014) and “Ovarian cancer including fallopian tube cancer and primary peritoneal cancer” (Version 3.2014) do not mention RAD51C gene testing.

Yang et al. (2013) stated that osteoporosis is characterized by low bone mineral density (BMD), a highly heritable trait that is determined, in part, by the actions and interactions of multiple genes. Although an increasing number of genes have been identified to have independent effects on BMD, few studies have been performed to identify genes that interact with one another to affect BMD.

Kim et al. (2013) noted that BMD loci were reported in Caucasian genome-wide association studies (GWAS). These researchers investigated the association between 59 known BMD loci (200 suggestive SNPs) and DXA-derived BMD in East Asian population with respect to sex and site specificity. They also identified 4 novel BMD candidate loci from the suggestive SNPs. A total of 2,729 unrelated Korean individuals from a population-based cohort were analyzed. The authors selected 747 single-nucleotide polymorphisms (SNPs). These markers included 547 SNPs from 59 loci with genome-wide significance (GWS, p value less than 5×10⁻⁸) levels and 200 suggestive SNPs that showed weaker BMD association with p value less than 5×10⁻⁵. After quality control, 535 GWS SNPs and 182 suggestive SNPs were included in the replication analysis. Of the 535 GWS SNPs, 276 from 25 loci were replicated (p<0.05) in the Korean population with 51.6 % replication rate. Of the 182 suggestive variants, 16 were replicated (p<0.05, 8.8 % of replication rate), and 5 reached a significant combined p value (less than 7.0×10⁻⁵, 0.05/17 SNPs, corrected for multiple testing). Two markers (rs11711157, rs3732477) are for the same signal near the gene CPN2 (carboxypeptidase N, polypeptide 2). The other variants, rs6436440 and rs2291296, were located in the genes AP1S3 (adaptor-related protein complex 1, sigma 3 subunit) and RARB (retinoic acid receptor, beta). The authors concluded that these results illustrated ethnic differences in BMD susceptibility genes and underscored the need for further genetic studies in each ethnic group. The authors were also able to replicate some SNPs with suggestive associations. These SNPs may be BMD-related genetic markers and should be further investigated.

The Institute for Clinical Systems Improvement’s clinical guideline on “Diagnosis and treatment of osteoporosis” (Florence et al., 2013) did not mention the use of genetic testing.

Furthermore, an UpToDate review on “Pathogenesis of osteoporosis” (Manolagas, 2014) states that “Genetics -- A portion of the variation in BMD among humans has a genetic basis. Genome-wide association studies have so far identified approximately 80 genetic loci that influence BMD. A remarkable number of these loci are involved in some aspect of Wnt/β-catenin signaling, the receptor activator of nuclear factor kappa-B (RANK)/RANK ligand (RANKL)/osteoprotegerin (OPG) axis, or in mesenchymal cell differentiation. The contribution of individual genetic variants, however, is small, and of the total variance in BMD only a small percentage is explained by variants of genes identified. To date, there are no genome-wide association studies on fracture or BMD loss. Therefore, it remains unclear whether the same genes that determine BMD also affect the rate of bone loss with advancing age or the risk of fractures”.

Thoracic Aortic Aneurysms and Dissections

A number of conditions are associated with aortic dysfunction and dilation, including Marfan syndrome, Loeys Dietz syndrome, Ehler Danlos syndrome type IV, Turner syndrome, and arterial tortuosity syndrome.

Ehlers-Danlos syndrome type IV (EDS type IV) is characterized by thin, translucent skin; easy bruising; characteristic facial appearance; and arterial, intestinal, and/or uterine fragility (Pepin & Byers, 2011). The diagnosis of EDS type IV is based on clinical findings and confirmed by identification of a causative mutation in COL3A1. EDS type IV is inherited in an autosomal dominant manner.

Arterial tortuosity syndrome (ATS) is characterized by severe and widespread arterial tortuosity of the aorta and middle-sized arteries (with an increased risk of aneurysm and dissection) and focal and widespread stenosis which can involve the aorta and/or pulmonary arteries (Callewaert, et al., 2014). The diagnosis of ATS is established in a proband with generalized arterial tortuosity and biallelic (homozygous or compound heterozygous) pathogenic variants in SLC2A10. ATS is inherited in an autosomal recessive manner.

Loeys-Dietz syndrome (LDS) is characterized by vascular findings (cerebral, thoracic, and abdominal arterial aneurysms and/or dissections) and skeletal manifestations (pectus excavatum or pectus carinatum, scoliosis, joint laxity, arachnodactyly, talipes equinovarus) (Loeys & Dietz, 2014). The diagnosis of LDS is based on characteristic clinical findings in the proband and family members and molecular genetic testing of TGFB1, TGFB2, SMAD3, and TGFB2. LDS is inherited in an autosomal dominant manner.
Marfan syndrome is a systemic disorder of connective tissue with a high degree of clinical variability (Dietz, 2014). Cardinal manifestations involve the ocular, skeletal, and cardiovascular systems. Cardiovascular manifestations include dilatation of the aorta at the level of the sinuses of Valsalva, a predisposition for aortic tear and rupture, mitral valve prolapse with or without regurgitation, tricuspid valve prolapse, and enlargement of the proximal pulmonary artery. Marfan syndrome is a clinical diagnosis based on family history and the observation of characteristic findings in multiple organ systems. Marfan syndrome is caused by mutation of FBN1. The sensitivity of molecular genetic testing of FBN1 is substantial yet incomplete for unknown reasons; it may be explained by atypical location or character of FBN1 pathogenic variants in some individuals (e.g., large deletions or promoter mutations) or to locus heterogeneity. Marfan syndrome is inherited in an autosomal dominant manner.

Guidelines from the American College of Cardiology (Hiratzka, et al., 2010) state, if a mutant gene (FBN1, TGFBR1, TGFBR2, COL3A1, ACTA2, MYH11) associated with aortic aneurysm and/or dissection is identified in a patient, first-degree relatives should undergo counseling and testing. Then, only the relatives with the genetic mutation should undergo aortic imaging.

Clinical laboratories may offer a multi-gene Marfan syndrome/Loeys-Dietz syndrome/familial thoracic aortic aneurysms and dissections panel that includes FBN1 as well as a number of other genes associated with disorders that include aortic aneurysms and dissections (Dietz, 2014). These panels vary by methods used and genes included; thus, the ability of a panel to detect a pathogenic variant or pathogenic variants in any given individual also varies. In most circumstances a comprehensive clinical evaluation and imaging studies will point to a specific diagnosis (or subset of diagnoses) that has the highest probability, and thus should be pursued first for molecular confirmation. In the absence of such hypothesis-driven testing, there is an increased risk of erroneous interpretation of variants of uncertain significance when multi-gene panels are applied, especially if the physician requesting testing is not familiar with the specific diagnoses and/or genes under consideration.

Mitochondrial Genome Sequencing

Mitochondrial diseases are a clinically heterogeneous group of disorders that arise as a result of dysfunction of the mitochondrial respiratory chain (Chinnery, 2014). They can be caused by mutation of genes encoded by either nuclear DNA or mitochondrial DNA (mtDNA). Mitochondrial disorders may be caused by mutation of an mtDNA gene or mutation of a nuclear gene. Mitochondrial DNA variants are transmitted by maternal inheritance (mitochondrial inheritance). Nuclear gene variants may be inherited in an autosomal recessive, autosomal dominant, or X-linked manner.

Establishing a molecular genetic diagnosis may have important implications for the counseling of individuals with mitochondrial disease. Molecular genetic testing may be carried out on genomic DNA extracted from blood (suspected nuclear DNA mutations and some mtDNA mutations) or on genomic DNA extracted from muscle (suspected mtDNA mutations) (Chinnery, 2014). Studies for mtDNA mutations are usually carried out on skeletal muscle DNA because a pathogenic mtDNA variant may not be detected in DNA extracted from blood.

Approaches to molecular genetic testing of a proband to consider are serial testing of single genes, multi-gene panel testing (simultaneous testing of multiple genes), and genomic testing (e.g., sequencing the entire mitochondrial genome; whole-exome sequencing or whole-genome sequencing to identify mutation of a nuclear gene) (Chinnery, 2014).

In contrast to genomic testing, serial testing of single genes and multi-gene panel testing rely on the clinician developing a hypothesis about which specific gene or set of genes to test (Chinnery, 2014). Hypotheses may be based on (1) mode of inheritance, (2) distinguishing clinical features, and/or (3) other discriminating features.

The potential role of genomic testing is where single-gene testing (and/or use of a multi-gene panel) has not confirmed a diagnosis in an individual with features of a mitochondrial disorder. Such testing includes whole-exome sequencing, whole-genome sequencing, and whole mitochondrial sequencing.

False negative rates vary by genomic region; therefore, genomic testing may not be as accurate as targeted single gene testing or multi-gene molecular genetic testing panels (Chinnery, 2014). Most laboratories confirm positive results using a second, well-established method. Certain DNA variants may not be detectable through genomic testing, such as large deletions or duplications (>8-10 bp in length), triplet repeat expansions, and epigenetic alterations.

Exome sequencing has shown promise in defining the genetic basis of mitochondrial disorders caused by mutation of nuclear genes. To determine the molecular basis of multiple respiratory chain complex deficiencies, Taylor, et al. (2014) studied 93 patients referred to 2 national centers in the United Kingdom and Germany between 2005 and 2012. All subjects had evidence of histochemical and/or biochemical diagnosis of mitochondrial disease in a clinically affected tissue (skeletal muscle, liver, or heart) confirming decreased activities of multiple respiratory chain complexes based on published criteria. Subjects had no large-scale mtDNA rearrangements, mtDNA depletion, and mtDNA point mutations, in persons in whom decreased levels of mtDNA were confirmed in muscle (mtDNA depletion). In those with congenital structural abnormalities, major nuclear gene rearrangements were excluded by comparative genomic hybridization arrays. Whole-exome sequencing was performed using 62-Mb exome enrichment, followed by variant prioritization using bioinformatics prediction tools, variant validation by Sanger sequencing, and segregation of the variant with the disease phenotype in the family. Presumptive causal variants were identified in 28 patients (53%; 95% CI, 39%-67%) and possible causal variants were identified in 4 (8%; 95% CI, 2%-18%). Together these accounted for 32 patients (60% 95% CI, 46%-74%) and involved 18 different genes. These included recurrent mutations in RMND1, AARS2, and MTO1, each on a haplotype background consistent with a shared founder allele, and potential novel mutations in 4 possible mitochondrial disease genes (VARS2, GARS, FLAD1, and PTCD1). Distinguishing clinical features
Genetic Testing

Genetic Test Panels for Nonsyndromic Hereditary Hearing Loss

There is limited published evidence for the clinical validity and clinical utility of specific genetic test panels for nonsyndromic hearing loss. A number of test panels are currently available commercially (e.g., OtoScope, OtoGenome, OtoSeq). The genes included in these test panels differ significantly, and there is limited published information on their clinical utility and clinical validity.

Hearing loss may be classified as either syndromic or nonsyndromic. Nonsyndromic hearing loss is defined by the absence of malformations of the external ear or other medical problems in the affected individual. With the syndromic hearing loss, malformations of the external ear and/or other medical problems are present. Approximately 50% of nonsyndromic hearing loss can be attributed to a genetic cause, and may be inherited in an autosomal recessive (70% of patients), autosomal dominant (20% of patients), with mitochondrial, X-linked and other genetic causes making up the remainder of patients.

Sequence variants in approximately 60 genes and some micro-RNAs have been associated with causing nonsyndromic hearing loss. Micro-RNAs are post-transcriptional regulators that consist of 20-25 nucleotides. Usher and Pendred syndromes are the most common forms of the approximately 400 forms of syndromic hearing loss. Both have autosomal recessive inheritance. Usher syndrome is characterized by sensorineural hearing loss and later development of retinitis pigmentosa. Usher syndrome has three forms that vary by the profundity of hearing loss and whether vestibular dysfunction is present. The three types of Usher syndrome have been associated with sequence variants in 9 different genes. Pendred syndrome is characterized by congenital hearing loss and euthyroid goiter that develops in the second or third decade of life. Pendred syndrome is associated with sequence variants in the SLC26A4 gene. Some of the genes associated with Usher and Pendred syndromes may also be associated with nonsyndromic hearing loss.

The OtoSCOPE test has been developed to make use of next generation sequencing capabilities, to simultaneously test for sequence variants in 66 genes associated with nonsyndromic hearing loss as well as both Usher and Pendred syndromes. The claimed advantage of the OtoScope test is that simultaneous analysis of the 66 genes included in the test may reduce the time and cost compared with genetic testing of individual genes. The OtoSCOPE genetic testing for hereditary hearing loss is considered investigational/experimental because there is inadequate evidence in the peer-reviewed published clinical literature regarding its effectiveness.

The OtoGenome Test is a next-generation sequencing (NGS) assay that covers all 73 known genes for non-syndromic hearing loss. There is insufficient evidence of the performance and clinical utility of this test panel.

Published evidence for the OtoSeq test panel includes an epidemiological study of the use of a component of the OtoSeq panel in identifying certain hearing loss genes in 54 Pakistani families (Shahzad, et al., 2013). In addition, there is a preliminary study of the performance of the OtoSeq in 8 individuals with hearing loss, comparing the results of Next Generation Sequencing with Sanger Sequencing (Sivakumaran, et al., 2013). There is insufficient published information about the performance and clinical utility of this test panel.

X-Linked Intellectual Disability Panels

Intellectual disability (ID, formerly called mental retardation) is a developmental brain disorder commonly defined by an IQ below 70 and limitations in both intellectual functioning and adaptive behavior (Piton, et al., 2013). ID can originate from environmental causes or genetic anomalies, and its incidence in children is estimated to be of 1%–2%.

ID is more common in males than females in the population (the male-to-female ratio is 1.3–1.4 to 1), assumed to be due to mutations on the X chromosome. Impaired mental functioning occurs as an isolated feature or as part of many X-linked syndromes (McKusick, et al., 2010). ID that is not associated with other distinguishing features is referred to as ‘nonspecific’ or ‘nonsyndromic.’

X-linked intellectual disability (XLID) is a genetically heterogeneous disorder with more than 100 genes known to date (Tzchach, et al., 2015). Fragile X syndrome remains the most common XLID gene discovered so far (Raymond, 2006). FMR1 is a target of the unstable expansion mutation responsible for fragile X syndrome and accounts for about 1%–2% of all ID cases.

Half of the known genes carrying mutations responsible for XLID are associated with syndromic forms (i.e., ID associated with defined clinical or metabolic manifestations), which facilitates the identification of causative mutations in the same gene because unrelated probands with comparable phenotypes can be more easily matched. The other half of known genes carrying mutations responsible for XLID appear to be associated with nonsyndromic or paucisyndromic forms.

Next-generation sequencing panels have been developed to identify mutations associated with XLID. However, little has been published on their analytic validity, clinical validity and clinical utility.

Tzchach, et al. (2015) performed targeted enrichment and next-generation sequencing of 107 XLID genes in a cohort of 150 male patients. One hundred patients had sporadic intellectual disability, and 50 patients had a family history suggestive of XLID. The investigators also analyzed a sporadic female patient with severe ID and epilepsy because she had strongly skewed X-inactivation. Target enrichment and high parallel sequencing allowed a diagnostic coverage of >10 reads for approximately 96% of all coding bases of the XLID genes at a mean coverage of 124 reads. The investigators reported finding 18 pathogenic
variants in 13 XLID genes (AP1S2, ATRX, CUL4B, DLG3, IQSEC2, KDM5C, MED12, OPHN1, SLC9A6, SMC1A, UBE2A, UBF3B and ZDHHC9) among the 150 male patients. Thirteen pathogenic variants were present in the group of 50 familial patients (26%), and 5 pathogenic variants among the 100 sporadic patients (5%). Systematic gene dosage analysis for low coverage exons detected one pathogenic hemizygous deletion. An IQSEC2 nonsense variant was detected in the female ID patient, providing further evidence for a role of this gene in encephalopathy in females. The investigators noted that skewed X-inactivation was more frequently observed in mothers with pathogenic variants compared with those without known X-linked defects. The investigators concluded that the mutation rate in the cohort of sporadic patients corroborates previous estimates of 5-10% for X-chromosomal defects in male ID patients.

Piton, et al. (2013) used data from a large-scale sequencing project to question the implication of XLID in several of the genes proposed to be involved in XLID. The authors stated that mutations causing monogenic XLID have now been reported in over 100 genes, most of which are included in XLID diagnostic gene panels. Nonetheless, the boundary between true mutations and rare non-disease-causing variants often remains elusive. The authors stated that sequencing of a large number of control X chromosomes, required for avoiding false-positive results, was not systematically possible in the past. Such information is now available thanks to large-scale sequencing projects such as the National Heart, Lung, and Blood (NHLBI) Exome Sequencing Project, which provides variation information on 10,563 X chromosomes from the general population. The authors used this NHLBI cohort to systematically reassess the implication of 106 genes proposed to be involved in monogenic forms of XLID. Based on this reassessment, the authors particularly questioned the implication in XLID of ten of them (AGTR2, MAGT1, ZNF674, SRPX2, ATP6AP2, ARHGGEF6, NXF5, ZCCHC12, ZNF41, and ZNF81), in which truncating variants or previously published mutations are observed at a relatively high frequency within this cohort. The authors also highlighted 15 other genes (CCDC22, CLIC2, CNKSR2, FRMPD4, HCF1C, IGBP1, KIAA2022, KLF8, MAOA, NAA10, NLGN3, RPL10, SHROOM4, ZDHHC15, and ZNF261) for which replication studies are warranted. The authors proposed that similar reassessment of reported mutations (and genes) with the use of data from large-scale human exome sequencing would be relevant for a wide range of other genetic diseases.

Appendix

Amsterdam II criteria:

At least 3 relatives must have an HNPCC-related cancer*, and all of the following criteria must be present:

- At least 1 of the relatives with cancer associated with HNPC should be diagnosed before age 50 years; and
- At least 2 successive generations must be affected; and
- FAP should be excluded in the colorectal cancer cases (if any); and
- One must be a 1st-degree relative of the other two; and
- Tumors should be verified whenever possible.

Revised Bethesda criteria:

Member must meet 1 or more of the following criteria:

- Colorectal cancer is diagnosed in a member with 1 or more 1st-degree relatives with an HNPCC-related cancer*, with one of the cancers diagnosed under age 50 years; or
- Colorectal cancer is diagnosed in a member with 2 or more 1st- or 2nd-degree relatives with an HNPCC-related cancer*, regardless of age; or
- Member has colorectal cancer diagnosed before age 50 years; or
- Member has colorectal cancer with microsatellite instability-high (MSI-H) histology, where cancer is diagnosed before age 60 years; or
- Member has synchronous or metachronous HNPCC-related cancers*, regardless of age.

* Hereditary nonpolyposis colorectal cancer (HNPCC)-related cancers include colorectal, endometrial, gastric, ovarian, pancreas, uterine and renal pelvis, brain (usually glioblastoma as seen in Turcot syndrome), and small intestinal cancers, as well as sebaceous gland adenomas and keratoacanthomas in Muir-Torre syndrome.

CPT Codes / HCPCS Codes / ICD-9 Codes

CPT codes covered if selection criteria are met:

81161 DMD (dystrophin) (eg, Duchenne/Becker muscular dystrophy) deletion analysis, and duplication analysis, if performed

81200 ASPA (aspartoacylase)(eg, Canavan disease) gene analysis, common variants (eg, E285A, Y231X)

81201 - 81203 APC (adenomatous polyposis coli) (eg, familial adenomatosis polyposis [FAP], attenuated FAP) gene analysis; full gene sequence, known familial variants, duplication/deletion variants

81205 BCKDHB (branched-chain keto acid dehydrogenase E1, beta polypeptide) (eg, Maple syrup urine disease) gene analysis, common variants (eg, R183P, G278S, E422X)

81209 BLM (Bloom syndrome, RecQ helicase-like) (eg, Bloom syndrome) gene analysis, 2281del6ins7 variant
81220  CFTR (cystic fibrosis transmembrane conductance regulator) (eg, cystic fibrosis) gene analysis; common variants (eg, ACMG/ACOG guidelines)
81221  known familial variants
81240  F2 (prothrombin, coagulation factor II) (eg, hereditary hypercoagulability) gene analysis, 20210G>A variant
81242  FANCC (Fanconi anemia, complementation group C) (eg, Fanconi anemia, type C) gene analysis, common variant (eg, IVS4+4A>T)
81243  FMR1 (Fragile X mental retardation 1) (eg, fragile X mental retardation) gene analysis; evaluation to detect abnormal (eg, expanded) alleles
81250  G6PC (glucose-6-phosphatase, catalytic subunit) (eg, Glycogen storage disease, Type 1a, von Gierke disease) gene analysis, common variants (eg, R83C, Q347X)
81251  GBA (glucosidase, beta, acid) (eg, Gaucher disease) gene analysis, common variants (eg, N370S, 84GG, L444P, IVS2+1G>A)
81252 - 81253  GJB2 (gap junction protein, beta 2, 26kDa, connexin 26) (eg, nonsyndromic hearing loss) gene analysis; full gene sequence, known familial variants
81255  HEXA (hexosaminidase A [alpha polypeptide]) (eg, Tay-Sachs disease) gene analysis, common variants (eg, 1278insTATC, 1421+1G>C, G269S)
81256  HFE (hemochromatosis) (eg, hereditary hemochromatosis) gene analysis, common variants (eg, C282Y, H63D)
81257  HBA1/HBA2 (alpha globin 1 and alpha globin 2 (eg, alpha thalassemia, Hb Bart hydrops fetalis syndrome, HbH disease), gene analysis, for common deletions or variant (eg, Southeast Asian, Thai, Filipino, Mediterranean, alpha3.7, alpha4.2, alpha20.5, and Constant Spring)
81258  IKBKAP (inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase complex-associated protein) (eg, familial dysautonomia) gene analysis, common variants (eg, 2507+6T>C, R696P)
81260  Long QT syndrome gene analyses (eg, KCNQ1, KCNH2, SCN5A, KCNE1, KCNE2, KCNJ2, KCN4, AKAP, NTA1, and ANK2); full sequence analysis
81280  known familial sequence variant
duplication/deletion variants
81290  MCOLN1 (mucolipin 1) (eg, Mucolipidosis, type IV) gene analysis, common variants (eg, IVS3-3A>G, del6, 4kb)
81292  MLH1 (mutL homolog 1, colon cancer, nonpolyposis type 2) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis
81293  known familial variants
duplication/deletion variants
81295  MSH2 (mutS homolog 2, colon cancer, nonpolyposis type 1) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis
81296  known familial variants
duplication/deletion variants
81298  MSH6 (mutS homolog 6 [E. Coli]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis
81299  known familial variants
duplication/deletion variants
81301  Microsatellite instability analysis (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) of markers for mismatch repair deficiency (eg, BAT25, BAT26), includes comparison of neoplastic and normal tissue, if performed
81302  MECP2 (methyl CpG binding protein 2) (eg, Rett syndrome) gene analysis; full sequence analysis
81310  NPM1 (nucleophosmin) (eg, acute myeloid leukemia) gene analysis, exon 12 variants
81317  PMS2 (postmeiotic segregation increased 2 [S. cerevisiae]) (eg, hereditary non-polyposis colorectal cancer, Lynch syndrome) gene analysis; full sequence analysis

http://qawww.aetna.com/cpb/medical/data/100_199/0140_draft.html  04/22/2015
known familial variants

duplication/deletion variants

PTEN (phosphatase and tensin homolog) (eg, Cowden syndrome, PTEN hamartoma tumor syndrome) gene analysis; full sequence analysis, known familial variant, duplication/deletion variant

PMP22 (peripheral myelin protein 22) (eg, Charcot-Marie-Tooth, hereditary neuropathy with liability to pressure palsies) gene analysis; duplication/deletion analysis, full sequence analysis, known familial variant

SMPD1 (sphingomyelin phosphodiesterase 1, acid lysosomal) (eg, Niemann-Pick disease, Type A) gene analysis, common variants (eg, R496L, L302P, fsP330)

SNRPN/UBE3A (small nuclear ribonucleoprotein polypeptide N and ubiquitin protein ligase E3A) (eg, Prader-Willi syndrome and/or Angelman syndrome), methylation analysis

SERPINA1 (serpin peptidase inhibitor, clade A, alpha-1 antiproteinase, antitrypsin, member 1) (eg, alpha-1-antitrypsin deficiency), gene analysis, common variants (eg, *S and *Z)

Chromosome analysis

Molecular cytogenetics

Consultation and report on referred material requiring preparation of slides

Immunohistochemistry or immunocytochemistry, per specimen; each additional single antibody stain procedure (List separately in addition to code for primary procedure)

initial single antibody stain procedure

each multiplex antibody stain procedure

Morphometric analysis, tumor immunohistochemistry (eg, Her-2/neu, estrogen receptor/progesterone receptor), quantitative or semiquantitative, each antibody; manual or using computer-assisted technology

CPT codes not covered for indications listed in the CPB:

CFTR (cystic fibrosis transmembrane conductance regulator) (eg, cystic fibrosis) gene analysis; duplication/deletion variants

full gene sequence

HLA Class II typing, low resolution (eg, antigen equivalents); one antigen equivalent, each

HLA Class II typing, low resolution (eg, antigen equivalents); one antigen equivalent, each

Exome (eg, unexplained constitutional or heritable disorder or syndrome); sequence analysis

sequence analysis, each comparator exome (eg, parents, siblings) (List separately in addition to code for primary procedure)

re-evaluation of previously obtained exome sequence (eg, updated knowledge or unrelated condition/syndrome)

Hearing loss (eg, nonsyndromic hearing loss, Usher syndrome, Pendred syndrome); genomic sequence analysis panel, must include sequencing of at least 60 genes, including CDH23, CLRN1, GJB2, GPR98, MTRNR1, MYO7A, MYO15A, PCDH15, OTOF, SLC26A4, TMC1, TPMRSS3, USH1C, USH1G, USH2A, and WFS1

duplication/deletion analysis panel, must include copy number analyses for STRC and DFNB1 deletions in GJB2 and GJB6 genes

Other CPT codes related to the CPB:

Molecular pathology procedures

Medical genetics and genetic counseling services, each 30 minutes face-to-face with patient/family

HCPCS codes covered if selection criteria are met:

DNA analysis for germline mutations of the RET proto-oncogene for susceptibility to multiple endocrine neoplasia type 2

Genetic testing for retinoblastoma
S3842 Genetic testing for von Hippel-Lindau disease
S3844 DNA analysis of the connexin 26 gene (GJB2) for susceptibility to congenital, profound deafness
S3845 Genetic testing for alpha-thalassemia
S3846 Genetic testing for hemoglobin E beta-thalassemia
S3850 Genetic testing for sickle cell anemia
S3852 DNA analysis for APOE epsilon 4 allele for susceptibility to Alzheimer's disease
S3853 Genetic testing for myotonic muscular dystrophy
S3855 Genetic testing for detection of mutations in the presenilin, 1 gene
S3856 Genetic analysis for a specific gene mutation for hypertrophic cardiomyopathy (HCM) in an individual with a known HCM mutation in the family

HCPCS codes not covered for indications listed in the CPB:
S3861 Genetic testing, sodium channel, voltage-gated, type V, alpha subunit (SCN5A) and variants for suspected Brugada syndrome
S3865 Comprehensive gene sequence analysis for hypertrophic cardiomyopathy

Other HCPCS codes related to the CPB:
G0461 Immunohistochemistry or immunocytochemistry, per specimen; first single or multiplex antibody stain
G0462 each additional single or multiplex antibody stain (list separately in addition to code for primary procedure)
S0265 Genetic counseling, under physician supervision, each 15 minutes

ICD-9 codes covered if selection criteria are met:
151.0 - 151.9 Malignant neoplasm of stomach [with 2 HNPPC-related cancers]
152.0 - 152.9 Malignant neoplasm of small intestine, including duodenum [with 2 HNPPC-related cancers]
153.0 - 154.9 Malignant neoplasm of colon [hereditary non-polyposis colorectal cancer (HNPPC) (MLH1, MSH2)]
155.0 - 155.2 Malignant neoplasm of liver and intrahepatic bile ducts [with 2 HNPPC cancers]
162.2 - 162.9 Malignant neoplasm of bronchus or lung
170.0 - 170.9 Malignant neoplasm of bone and articular cartilage [osteosarcoma]
171.0 - 171.9 Malignant neoplasm of connective and other soft tissue [soft tissue sarcoma]
174.0 - 175.9 Malignant neoplasm of breast
182.0 Malignant neoplasm of body of uterus
183.0 Malignant neoplasm of ovary and other uterine adnexa [with 2 HNPPC cancers]
188.0 - 188.9 Malignant neoplasm of bladder [transitional cell for microsatellite instability (MSI) testing and MLH1 and MLH2 sequence analysis for HNPPC]
189.0 Malignant neoplasm of kidney, except pelvis [renal cell cancer syndrome]
189.1 Malignant neoplasm of renal pelvis [transitional cell for microsatellite instability (MSI) testing and MLH1 and MLH2 sequence analysis for HNPPC]
189.2 Malignant neoplasm of ureter [transitional cell for microsatellite instability (MSI) testing and MLH1 and MLH2 sequence analysis for HNPPC]
190.5 Malignant neoplasm of retina [retinoblastoma]
191.0 - 191.9 Malignant neoplasm of brain [except glioblastoma multiforme]
193 Malignant neoplasm of thyroid gland [medullary thyroid carcinoma]
194.0 Malignant neoplasm of adrenal gland [adrenocortical carcinoma]
204.00 - 208.92 Leukemias
211.3 Benign neoplasm of colon [hereditary polyposis coli (APC)]
227.0 Benign neoplasm of adrenal gland [hereditary paraganglioma (SDHS, SDHB)]
237.71 Neurofibromatosis, type 1 [von Recklinghausen's disease] [neurofibromin] [not covered for Legius syndrome]
237.72 Neurofibromatosis, type 2 [acoustic neurofibromatosis] [Merlin]
238.1 Neoplasm of uncertain behavior of connective and other soft tissue [hereditary leiomyomatosis]
250.0x - 250.9x w/5th digit of 0 or 2 Type 2 Diabetes mellitus [covered for maturity-onset diabetes of the young (MODY)]
253.4 Other anterior pituitary disorders [Kallmann syndrome (FGFR1)]
255.2 Adrenogenital disorders [congenital adrenal hyperplasia]
258.01 Multiple endocrine neoplasia [MEN] type I
259.4 Dwarfism, not elsewhere classified [hypochondroplasia (FGFR 3), thanatophoric dysplasia (FGFR3)]
270.1 Phenylketonuria
270.2 Other disturbances of aromatic amino-acid metabolism [albinism]
270.3 Disturbances of branched-chain amino-acid metabolism
271.0 Glycogenosis [McArdle's disease]
271.1 Galactosemia
272.7 Lipidoses [Fabry/Gaucher (acid beta glucosidase)/Niemann-Pick (sphingomyelin phosphodiesterase)]
273.4 Alpha-1-antitrypsin deficiency
275.01 Hereditary hemochromatosis
275.2 Disorders of magnesium metabolism [Gitelman’s syndrome]
275.42 Hypercalcemia (familial hypocalciuric)
277.00 - 277.09 Cystic fibrosis [CTFR]
277.39 Other amyloidosis [hereditary amyloidosis (TTR variants)]
277.5 Mucopolysaccharidosis [type 1 (MPS-1)]
277.85 Disorders of fatty acid oxidation [medium chain acyl coA dehydrogenase deficiency (ACADM)]
277.87 Disorders of mitochondrial metabolism [MELAS (mitochondrial encephalopathy) (MTTL1, tRNAleu)]
282.3 Other hemolytic anemias due to enzyme deficiency [pyruvate kinase deficiency (PKD)]
282.40 - 282.49 Thalassemias [alpha globin/beta globin/hemoglobin E]
282.5 Sickle-cell trait [hemoglobin S]
282.7 Other hemoglobinopathies [hemoglobin C]
286.0 Congenital factor VIII disorder [hemophilia A/VWF]
286.1 Congenital factor IX disorder [hemophilia B]
286.3 Congenital deficiency of other clotting factors [prothrombin factor II, 20210A mutation]
287.33 Congenital and hereditary thrombocytopenia [amegakaryocytic]
288.01 Congenital neutropenia [cyclic]
288.02 Cyclic neutropenia [congenital]
299.00 - 299.01 Autistic disorder, current or active state or residual state
317 - 319 Mental retardation
327.25 Congenital central alveolar hypoventilation syndrome
330.0  Leukodystrophy [Canavan disease (aspartoacylase A)]
330.1  Cerebral lipidoses [Tay-Sachs disease]
330.8  Other specified cerebral degenerations in childhood [Rett syndrome (MECP2)] [Leigh syndrome]
333.4  Huntington's chorea [Huntington's disease]
333.6  Genetic torsion dystonia [primary TOR1A (DYT1)]
334.0  Friedreich's ataxia [frataxin]
334.1  Hereditary spastic paraplegia [hereditary spastic paraplegia 3 (SPG3A) and 4 (SPG4, SPAST)]
334.3  Other cerebellar ataxia [spinocerebellar ataxia (ataxin, CACNA1A)] [SCA types 8, 10, 17 and DRPLA]
334.8  Other spinocerebellar diseases [spinocerebellar ataxia (ataxin, CACNA1A)]
335.10 - 335.19  Spinal muscular atrophy [Kennedy disease (SBMA) (SMN)]
345.10 - 345.11  Generalized convulsive epilepsy [myoclonic epilepsy (MERRF) (MTTK) (tRNAlys)]
356.1  Peroneal muscular atrophy [Charcot-Marie-Tooth disease]
356.2  Hereditary sensory neuropathy [with liability to pressure palsies (HNPP)]
359.0 - 359.1  Congenital hereditary or hereditary progressive muscular dystrophy [Duchene (dystrophin)] [Becker's type] [limb girdle muscular dystrophy (LGMD1, LGMD2)] [not covered for oculopharyngeal muscular dystrophy (OPMD)] [dystrophy [type 1C] [Emery-Dreifuss muscular dystrophy] [fasioscapulohumeral muscular dystrophy]
359.21 - 359.29  Myotonic disorders [myotonic dystrophy (CMPK, ZNF-9)]
359.89  Other myopathies [dysferlin]
362.74  Pigmentary retinal dystrophy [retinitis pigmentosa]
377.39  Other optic neuritis [Leber hereditary optic neuropathy (LHON)]
389.00 - 389.9  Hearing loss [hereditary (Connexin-26, GJB2)]
425.11 - 425.18  Hypertrophic cardiomyopathy
425.4  Other primary cardiomyopathies [arrhythmogenic right ventricular dysplasia/cardiomyopathy (ARVD/C)]
426.82  Long QT syndrome
427.1  Paroxysmal ventricular tachycardia [persons that display exercise- or emotion-induced polymorphic ventricular tachycardia or ventricular fibrillation, occurring in a structurally normal heart]
427.41  Ventricular fibrillation
441.01  Thoracic aneurysm and dissection
448.0  Hereditary hemorrhagic telangiectasia
577.0  Acute pancreatitis [unexplained episode in a child requiring hospitalization with significant concern that hereditary pancreatitis (PRSS1) should be excluded]
577.1  Chronic pancreatitis [unexplained (idiopathic) for hereditary pancreatitis (PRSS1)]
581.0 - 581.9  Nephrotic syndrome [congenital (NPHS1, NPHS2)]
742.2  Reduction deformities of brain [classical lissencephaly]
742.8  Other specified congenital anomalies of nervous system [familial dysautonomia]
755.55  Acrocephalosyndactaly [Pfeiffer syndrome (FGFR1)]
756.0  Anomalies of skull and face bones [Crouzon syndrome (CTFR), Saethre-Chotzen syndrome (TWIST, FGFR2)]
756.4  Chondrodystrophy [achondroplasia]
756.51  Osteogenesis imperfecta
756.83  Ehlers-Danlos syndrome
Other specified anomalies of muscle, tendon, fascia, and connective tissue [Jackson-Weiss syndrome] [Muencke syndrome (FGFR2)]

Other specified congenital anomalies of skin [Bloom syndrome]

Velo-cardio-facial syndrome [22q11 deletion syndrome (CATCH-22)]

Other hamartoses, not elsewhere classified [Von Hippel Lindau syndrome (VHL)]

Prader-Willi syndrome [GABRA, SNRPN]

Marfan syndrome

Fragile X syndrome

Other specified anomalies [Angelman syndrome (GABRA, SNRPN) Smith-Lemli-Opitz syndrome]

Short stature [SHOX-related]

Other nonspecific abnormal serum enzyme levels [hyper-amylasemia]

Malignant hyperthermia

Personal history of malignant neoplasm of large intestine [with HNPCC related cancers]

Personal history of malignant neoplasm of rectum, rectosigmoid junction, and anus

Family history of malignant neoplasm of gastrointestinal tract

Family history of other neurological diseases

Family history of sudden cardiac death (SCD)

Family history of other cardiovascular diseases [children or young adults (less than 40 years of age) with a first degree relative with a clinical diagnosis of CPVT, or a first or second degree relative with a defined CPVT mutation]

Family history of mental retardation

Family history, colonic polyps [members with first-degree relatives (i.e., siblings, parents, and offspring) diagnosed with familial adenomatous polyposis (FAP) or with a documented APC mutation]

Family history of other digestive disorders [pancreatitis]

Family history of genetic disease carrier

Family history of congenital anomalies

Testing of female for genetic disease carrier status

Testing of male for genetic disease carrier status

Special screening for thyroid disorders

Special screening for phenylketonuria

Special screening for galactosemia

Special screening for cystic fibrosis

Special screening for other inborn errors of metabolism

Special screening for ear diseases

Asymptomatic hemophilia A carrier

Symptomatic hemophilia A carrier

Cystic fibrosis gene carrier

Other genetic carrier status

Genetic susceptibility to malignant neoplasm of breast [covered for Li-Fraumeni syndrome testing other than OncoVue]

ICD-9 codes not covered for indications listed in the CPB:

172.0 - 172.9 Malignant melanoma of skin

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185 Malignant neoplasm of prostate
271.3 Intestinal disaccharidase deficiencies and disaccharide malabsorption [lactose intolerance]
272.6 Lipodystrophy [familial partial lipodystrophy]
277.85 Disorders of fatty acid oxidation [very long chain acylCoA dehydrogenase deficiency (VLCADD)]
284.01 Constitutional red blood cell aplasia [Diamond-Blackfan anemia]
286.2 Congenital factor XI deficiency [hemophilia C]
286.4 von Willebrand's disease
288.2 Genetic anomalies of leukocytes [May-Hegglin anomaly]
307.46 Sleep arousal disorder [somnambulation]
331.0 Alzheimer's disease
332.0 - 332.1 Parkinson's disease
333.2 Myoclonus [-dystonia]
335.20 Amyotrophic lateral sclerosis [familial (SOD1 mutation)]
346.30 - 346.33 Hemiplegic migraine
347.00 - 347.11 Cataplexy and narcolepsy
362.16 Retinal neovascularization NOS
362.50 Macular degeneration (senile), unspecified
362.51 Nonexudative senile macular degeneration
362.52 Exudative senile macular degeneration
389.00 - 389.9 Hearing loss
410.00 - 410.92 Acute myocardial infarction
411.0 - 411.89 Other acute and subacute forms of ischemic heart disease
412 Other acute and subacute forms of ischemic heart disease
413.0 - 413.9 Angina pectoris
414.00 - 414.9 Other forms of chronic ischemic heart disease
746.89 Other congenital anomalies of heart [Brugada syndrome]
753.12 - 753.14 Polycystic kidney
756.16 Klippel-Feil syndrome
756.59 Other congenital osteodystrophies with bracketed info [McCune-Albright syndrome]
757.1 Ichthyosis congenita [epidermolytic hyperkeratosis]
759.9 Congenital anomaly, unspecified [heterotaxy]
780.31 - 780.32 Febrile convulsions
780.4 Dizziness and giddiness [migrainous vertigo]
V16.3 Family history of malignant neoplasm, breast
V81.0 Special screening for ischemic heart disease
V81.2 Special screening for other and unspecified cardiovascular conditions

Other ICD-9 codes related to the CPB:
V13.69 Personal history of other congenital malformations
V26.32 Other genetic testing of female
V26.33  Genetic counseling
V26.39  Other genetic testing of male
V28.0   Screening for chromosomal anomalies by amniocentesis
V29.3   Observation for suspected genetic or metabolic condition
V77.91  Screening for lipid disorders
V77.99  Special screening for other and unspecified endocrine, nutritional, metabolic, and immunity disorders
V78.1   Special screening for other and unspecified deficiency anemia
V78.2   Special screening for sickle-cell disease or trait
V78.3   Special screening for other hemoglobinopathies
V78.8   Special screening for other disorders of blood and blood-forming organs
V80.09  Special screening for other neurological conditions
V82.4   Maternal postnatal screening for chromosomal anomalies
V82.71 - V82.79  Genetic screening
V84.09  Genetic susceptibility to other malignant neoplasm
V84.81 - V84.89  Genetic susceptibility to other disease

**Factor V Leiden:**

CPT codes covered if selection criteria are met:

- 81241  F5 (coagulation Factor V) (eg, hereditary hypercoagulability) gene analysis, 20210G>A variant

ICD-9 codes covered if selection criteria are met:

- 410.00 - 412  Myocardial infarction
- 415.11 - 415.19  Pulmonary embolism and infarction
- 671.20 - 671.33  Pregnancy complicated by thrombosis
- V12.51 - V12.52, V12.55  Personal history of thrombosis
- V17.41 - V17.49  Family history of thrombosis

**EpiSEEK:**

No specific code

ICD-9 codes not covered for indications listed in the CPB:

- 345.00 - 345.91  Epilepsy and recurrent seizures

**POLG1:**

No specific code

ICD-9 codes not covered for indications listed in the CPB:

- 334.3  Other cerebellar ataxia

**SF3B1 and TET2:**

No specific code

ICD-9 codes not covered for indications listed in the CPB:

- 238.72 -238.75  Myelodysplastic syndrome

*There are no specific codes for the genetic testing listed:*

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