Exome Sequencing, Trio

**Indications for Ordering**

Determine the etiology of a patient’s symptoms when an unknown Mendelian genetic condition is suspected

**Test Description**

- Short tandem repeat markers are used to confirm familial relationships
- Liquid RNA- or DNA-based probes capture exons and intron/exon junctions of the known protein-coding RefSeq genes followed by massively parallel sequencing
- Sequences are aligned with the human reference sequence (Hg19) to identify variants
- Variants related to patient’s phenotype are confirmed by Sanger sequencing as needed
- Exome sequencing is performed for the patient, his/her parents, and other informative family members
- At ARUP’s discretion, additional testing, such as X-chromosome inactivation or mRNA studies, is performed to aid variant interpretation
- 4-8 weeks may be required for test results

**Information required for testing**

- Completed **Patient History for Exome Sequencing** form for proband and familial controls
- Completed Informed Consent for Exome Sequencing form for patient and controls
- Three-generation medical pedigree
- Results of genomic microarray and any other previous testing
- Summary notes from genetic and specialist consultations

**Tests to Consider**

**Primary tests**

- **Exome Sequencing, Trio 2006332**
- Preferred test to determine etiology of a patient’s symptoms if Mendelian genetic condition is suspected
- Parental specimens are required to identify de novo variants and interpret patient’s results (order test 2006340 for parental or other family controls)

- **Exome Sequencing, Familial Control 2006340**
- Order for exome sequencing for family members of proband to aid in the interpretation of proband test results
  - A consent form must be completed for family members desiring a report of American College of Medical Genetics and Genomics (ACMG) secondary findings

**Related tests**

- **Exome Sequencing, Proband 2006336**
  - Determine etiology of a patient’s symptoms if Mendelian genetic condition is suspected, and specimens from both parents are not available
  - Obtaining specimens from parents or family members significantly increases the chance of determining a cause for the patient’s condition
  - If familial control specimens are available, order test 2007820

- **Exome Control, Targeted Sequencing 3001114**
  - Familial control specimens are critical for variant interpretation of exome sequencing test results for proband
  - Order for available family members
  - Only targeted testing of selected gene variants is performed
  - ACMG gene variants are not analyzed

**Clinical Background**

**Diagnostic/prognostic issues**

- The exome accounts for 1-2% of the human genome but harbors ~85% of pathogenic variants
- Exome sequencing decodes and analyzes the majority of human genes and their intron/exon boundaries
- The function of only ~4,500 genes is currently known
- Exome sequencing may or may not
  - Determine etiology of medical condition
  - Predict prognosis or severity
  - Guide medical management

**Test Interpretation**

**Clinical sensitivity**

- Unpublished internal data
  - ~20% when only the proband is sequenced and one or both parental samples are unavailable
  - ~35% when only performing targeted sequencing of parental samples based on variants identified in the proband exome
  - ~45% when the proband and both parents undergo exome sequencing
Variants
- Tens of thousands of genetic variants will be detected
  - May be
    - Pathogenic
    - Benign
    - Of unknown clinical significance
- Variants reported
  - Those predicted to be related to the patient’s symptoms
  - De novo and rare compound heterozygous variants in genes of unknown function, if a causative variant is not identified
  - Pathogenic variants in genes recommended by ACMG for analysis, unless declined on consent form
    - See table
    - ACMG variants not associated with the patient’s symptoms are considered “incidental findings”

Results
- Positive – pathogenic gene variant(s) were identified that are predicted to be associated with the patient’s condition
- Negative – no pathogenic variants were identified that are predicted to provide an explanation for the patient’s condition
  - Does not exclude a genetic cause
- Uncertain – one or more gene variants were detected that may be related to the patient’s condition
- Incidental findings – family members with a completed consent form who undergo exome sequencing will receive separate reports indicating whether pathogenic ACMG recommended variants were identified, unless declined on the consent form

Reporting and interpretation
- Accurate knowledge of biological relationships between family members is imperative for correct test interpretation
- Test interpretation is based on information available at the time of testing and may change in the future
- Exome sequencing data will be stored for a minimum of 5 years, in compliance with ARUP’s data retention policy
- Reanalysis of data is available upon request up to 12 months after the original report was issued
- Reanalysis will only be performed two times upon request, and may be associated with additional charges
- Deidentified information about genetic variants and clinical symptoms may be published in international databases unless declined on consent form
- Raw exome sequencing data may be requested by the ordering healthcare provider

Limitations
- Not all genes are analyzed, as they may not be identified or amenable to capture
- Only variants related to the proband phenotype and identified pathogenic ACMG gene variants are reported
- Testing may fail to identify secondary findings in some ACMG genes
- Variants that may not be detectable include
  - Those located in genes with corresponding pseudogenes
  - Those in repetitive or high GC-rich regions
  - Those outside coding regions
  - Those within the mitochondrial genome
  - Large deletions/duplications/rearrangements
  - Some small deletions/duplications
  - Small insertions/deletions (indels)
  - Mosaic variants
- Chromosomal phase of identified variants may not be determined without parental specimens
- Rare variants in probe hybridization sites may compromise analytical sensitivity
- Mode of inheritance, reduced penetrance, and genetic heterogeneity could reduce clinical sensitivity

References
American College of Medical Genetics and Genomics (ACMG) (Kalia, 2016) recommends reporting secondary findings for these genes.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Associated genes</th>
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<td><strong>Tumors/cancer syndromes</strong></td>
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<tr>
<td>Familial adenomatous polyposis</td>
<td>APC</td>
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<tr>
<td>Familial medullary thyroid cancer</td>
<td>RET</td>
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<tr>
<td>Multiple endocrine neoplasia type 2</td>
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<tr>
<td>Hereditary breast and ovarian cancer</td>
<td>BRCA1, BRCA2</td>
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<td>Hereditary paraganglioma/pheochromocytoma syndrome</td>
<td>SDHD, SDHAF2, SDHC, SDHB</td>
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<td>Juvenile polyposis</td>
<td>BMP1A, SMAD4</td>
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<td>Li-Fraumeni syndrome</td>
<td>TP53</td>
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<tr>
<td>Lynch syndrome</td>
<td>MLH1, MSH2, MSH6, PMS2</td>
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<tr>
<td>Multiple endocrine neoplasia type 1</td>
<td>MEN1</td>
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<tr>
<td>MUTYH-associated polyposis</td>
<td>MUTYH</td>
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<tr>
<td>Neurofibromatosis type 2</td>
<td>NF2</td>
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<td>Peutz-Jeghers syndrome</td>
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<td>PTEN hamartoma tumor syndrome</td>
<td>PTEN</td>
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<td>Retinoblastoma</td>
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<td>Tuberous sclerosis complex</td>
<td>TSC1, TSC2</td>
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<td>Von Hippel-Lindau syndrome</td>
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<td>WT1-related Wilms tumor</td>
<td>WT1</td>
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<td><strong>Cardiovascular conditions/syndromes</strong></td>
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<tr>
<td>Arrhythmogenic right-ventricular cardiomyopathy</td>
<td>PKP2, DSP, DSC2, TMEM43, DSG2</td>
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<td>Brugada syndrome</td>
<td>KCNQ1, KCNH2, SCN5A</td>
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<td>Romano-Ward long QT syndrome types 1, 2, and 3</td>
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<tr>
<td>Catecholaminergic polymorphic ventricular tachycardia</td>
<td>RYR2</td>
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<td>Ehlers-Danlos syndrome, vascular type</td>
<td>COL3A1</td>
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<tr>
<td>Familial hypercholesterolemia</td>
<td>LDLR, APOB, PCSK9</td>
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<tr>
<td>Familial thoracic aortic aneurysms and dissections</td>
<td>SMAD3, ACTA2, MYLK, MYH11</td>
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<td>Hypertrophic cardiomyopathy, dilated cardiomyopathy</td>
<td>MYBPC3, MYH7, TNNT2, TNNI3, TPM1, MYL3, ACTC1, PRKAG2, GLA, MYL2, LMNA</td>
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<td>Loes-Dietz syndromes</td>
<td>TGFBR1, TGFBR2</td>
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<td>Von Hippel-Lindau syndrome</td>
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<td><strong>Other conditions</strong></td>
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<tr>
<td>Malignant hyperthermia susceptibility</td>
<td>RYR1, CACNA1S</td>
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<tr>
<td>Ornithine transcarbamylase deficiency</td>
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<td>Wilson disease</td>
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