# 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


- Farwell KD, Shahmirzadi L, et al. Enhanced utility of family-centered diagnostic exome sequencing with inheritance model-based analysis: results from 500 unselected families with undiagnosed genetic conditions. Genet Med 2015.7;578-586
- Kalia SS, Adelman K, et al. Recommendations for reporting of secondary findings in clinical exome and genome sequencing, 2016 update (ACMG SF v2.0): a policy statement of the American College of Medical Genetics and Genomics. Genet Med. 2017 Feb;19(2):249-255

| American College of Medical Genetics and Genomics (ACMG) (Kalia, 2016) Recommends Reporting Secondary Findings for these Genes | | |
|---|---|---|
| **Conditions** | | **Associated genes** |
| **Tumors/cancer syndromes** | Familial adenomatous polyposis | *APC* |
| | Familial medullary thyroid cancer<br>Multiple endocrine neoplasia type 2 | *RET* |
| | Hereditary breast and ovarian cancer | *BRCA1, BRCA2* |
| | Hereditary paraganglioma/pheochromocytoma syndrome | *SDHD, SDHAF2, SDHC, SDHB* |
| | Juvenile polyposis | *BMPR1A*, *SMAD4* |
| | Li-Fraumeni syndrome | *TP53* |
| | Lynch syndrome | *MLH1, MSH2, MSH6, PMS2* |
| | Multiple endocrine neoplasia type 1 | *MEN1* |
| | *MUTYH*-associated polyposis | *MUTYH* |
| | Neurofibromatosis type 2 | *NF2* |
| | Peutz-Jeghers syndrome | *STK11* |
| | *PTEN* hamartoma tumor syndrome | *PTEN* |
| | Retinoblastoma | *RB1* |
| | Tuberous sclerosis complex | *TSC1, TSC2* |
| | Von Hippel-Lindau syndrome | *VHL* |
| | *WT1*-related Wilms tumor | *WT1* |
| **Cardiovascular conditions/syndromes** | Arrhythmogenic right-ventricular cardiomyopathy | *PKP2, DSP, DSC2, TMEM43, DSG2* |
| | Brugada syndrome<br>Romano-Ward long QT syndrome types 1, 2, and 3 | *KCNQ1, KCNH2, SCN5A* |
| | Catecholaminergic polymorphic ventricular tachycardia | *RYR2* |
| | Ehlers-Danlos syndrome, vascular type | *COL3A1* |
| | Familial hypercholesterolemia | *LDLR, APOB, PCSK9* |
| | Familial thoracic aortic aneurysms and dissections | *SMAD3, ACTA2, MYLK, MYH11* |
| | Hypertrophic cardiomyopathy, dilated cardiomyopathy | *MYBPC3, MYH7, TNNT2, TNNI3, TPM1, MYL3, ACTC1, PRKAG2, GLA, MYL2, LMNA* |
| | Loeys-Dietz syndromes | *TGFBR1*, *TGFBR2* |
| | Marfan syndrome | *FBN1* |
| | Von Hippel-Lindau syndrome | *VHL* |
| **Other conditions** | Malignant hyperthermia susceptibility | *RYR1, CACNA1S* |
| | Ornithine transcarbamylase deficiency | *OTC* |
| | Wilson disease | *ATP7B* |