

Exome Sequencing with Symptom-Guided Analysis

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
- Full 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
- 16 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 with Symptom-Guided Analysis](#) 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 Symptom-Guided Analysis 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's 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 Symptom-Guided Analysis, Patient Only 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

Genomics Patient Control 2007820

- 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 functions of only ~4,500 genes are currently known
- Exome sequencing may or may not
 - Determine etiology of medical condition
 - Predict prognosis or severity
 - Guide medical management

Test Interpretation

Clinical sensitivity

- ~20% when only the proband is sequenced
- ~35% when parental exomes are also sequenced (Farwell, 2015)

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 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 five 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
- Redanalysis may be performed only two times
- De-identified 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's 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*. 2016 (advanced online publishing)

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