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, Tracking 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	*APC*
	Familial medullary thyroid cancer 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 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*