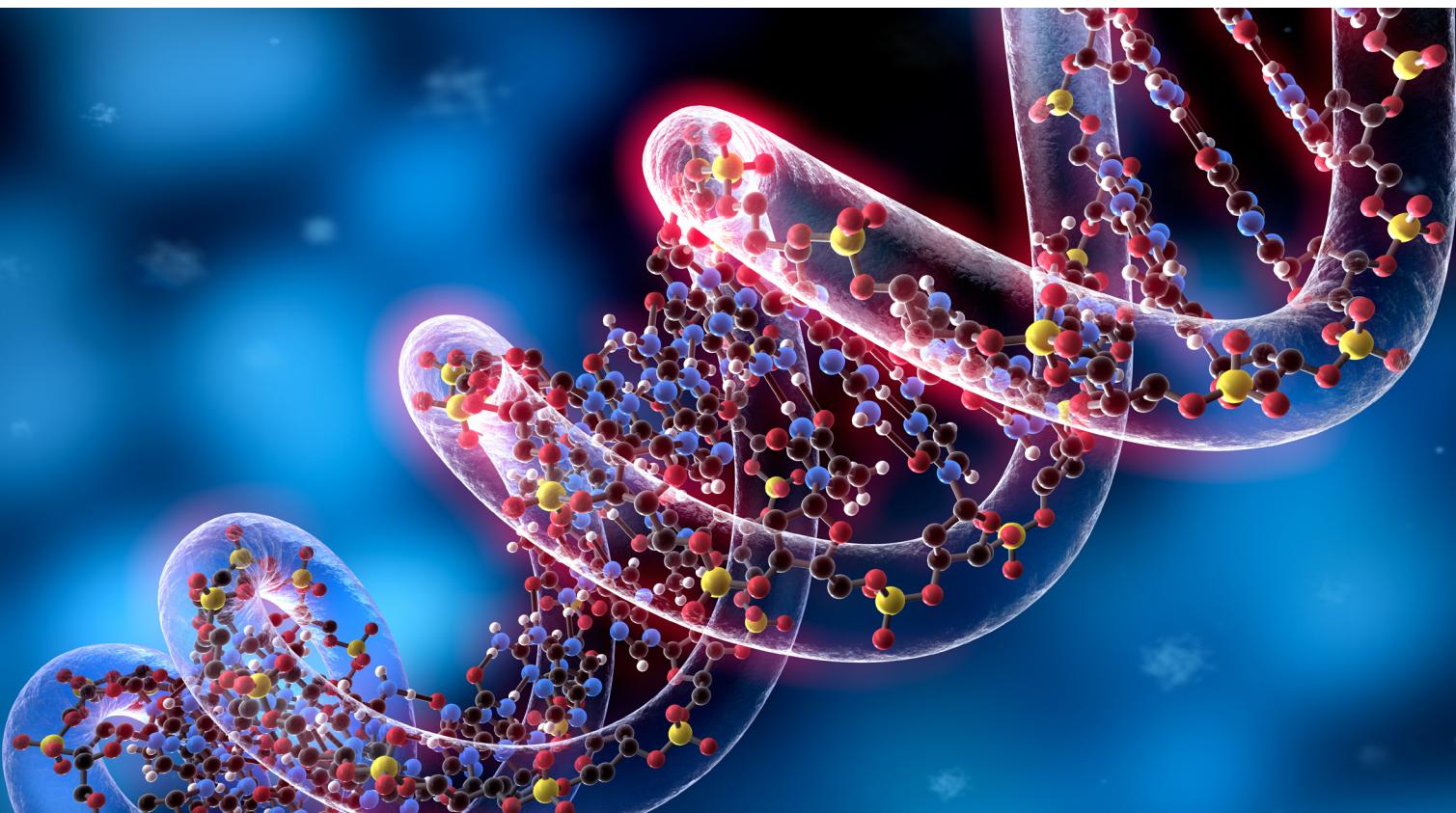


# Medical EmExome – A Strategy for Diagnosing Patients with Genetic Disorders



EMORY  
UNIVERSITY

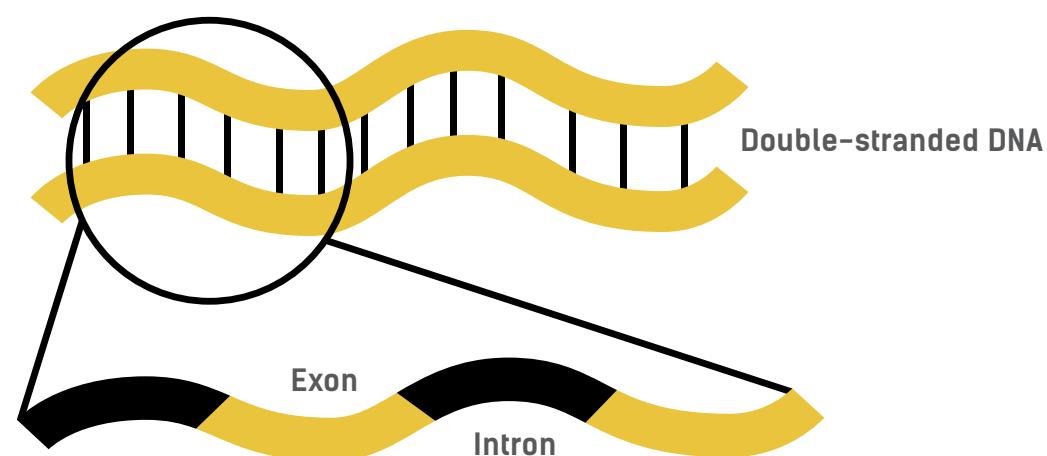
Emory Genetics Laboratory

## Medical Exome Sequencing

Exome sequencing represents a newer testing strategy for diagnosing patients with genetic disorders. The Medical EmExome offered by EGL is the next level of clinical exome sequencing. This guide is meant to answer frequently asked questions about exome sequencing, and the medical EmExome, as well as provide insight into incorporating exome sequencing into clinical practice. But first, what is the human exome?

The exome is the complete coding region of the genome. It instructs the body to make the essential proteins necessary for normal function. The exome is estimated to make up only 1-2% of the genome, yet accounts for approximately 85% of disease-causing gene alterations. The exome consists of roughly 180,000 individual coding regions, also known as exons, arranged in about 22,000 genes. Alterations to these exons may lead to genetic disorders.

Exome sequencing refers to a diagnostic test in which exons are sequenced nucleotide-by-nucleotide to a depth of coverage necessary to determine a patient's genetic sequence. This sequence is compared to the published human genome build UCSC hg19 reference sequence to identify any variation. The variation is then interpreted by board-certified laboratory directors and reported out by board-certified genetic counselors. Test results are related back to the patient's clinical presentation in an effort to discover the cause of the medical condition.



### DID YOU KNOW?

Coverage refers to the average number of reads representing a given nucleotide in the reconstructed sequence. The more coverage, the better.

## Test Indication

While exome sequencing may be an invaluable tool at a clinician's disposal, this type of testing isn't appropriate for all patients with a suspected genetic disorder. Here are some general guidelines to help you determine if exome sequencing is right for your patient:

- Diagnosing the patient requires a long differential, sequential genetic testing is cost-prohibitive, and/or the precise cause for the medical concern is still undetermined.
- A comprehensive test option does not exist for the suspected genetic disorder.
- A novel genetic etiology is suspected to be the cause of the patient's medical concern, as the clinical presentation doesn't match any known genetic disorders.
- The patient has no clear phenotype, atypical clinical features, or presents with overlapping phenotypes, making an accurate diagnosis challenging.
- Multiple candidate genes are suspected, for which a gene panel isn't available.
- Treatment options are not working and an inherited disorder is highly suspected.
- The patient has a heterogeneous disease with a mutation spectrum in which no single gene or group of genes makes up significant portion.



## Curious How the Test Itself Works?

EGL's Medical EmExome features the Agilent V5 Plus capture design, which targets the exome with enhanced coverage of known disease-associated genes. These targeted regions are then sequenced using the Illumina HiSeq 2500 sequencing system, with 150 basepair (bp) paired-end reads (similar to bidirectional Sanger sequencing). Target regions include the exon and 50 bp of flanking intronic regions.



The DNA sequence is analyzed in comparison with the published human genome build UCSC hg19 reference sequence. The targeted coding exons and splice junctions of the known protein-coding RefSeq genes are assessed for the depth of coverage and data quality threshold values.

Exome sequencing technology is subject to false positives. For this reason, all potential positive sequence variants in the proband are confirmed by conventional di-deoxy DNA sequence analysis (Sanger sequencing) using a separate DNA isolation.

EGL's Medical EmExome offers a minimum coverage of 20X, but most genes have coverage levels between 80-120X. [For more information on specific gene coverage, exon-by-exon, use EGL's Exome Coverage Tool located here: <http://geneticslab.emory.edu/exome-coverage/>.](#) Actual coverage may vary slightly from patient-to-patient.

The Medical EmExome has a diagnostic yield of approximately 30%.

### DID YOU KNOW?

The Medical EmExome Trio includes a free next generation sequencing panel related to patient's phenotype.

## Limitations of Exome Sequencing

While exome sequencing can provide a plethora of information about a patient, there are important limitations to be aware of:

- NGS cannot accurately sequence repetitive regions, such as trinucleotide repeats. This means regions such as the fragile X syndrome, Huntington disease, and myotonic dystrophy repeat regions are not analyzed by exome sequencing.
- Results from exome sequencing may indicate that additional testing, such as full gene sequencing or deletion/duplication analysis and additional biochemical testing, is recommended.
- Copy number variation (CNV) is not evaluated in exome sequencing. [EGL offers a separate test called the Medical EmExome Array for deletion/duplication analysis of most medically relevant genes analyzed by exome sequencing.](#)
- Pseudogenes and regions of homology may not be uniquely captured.
- Abnormal methylation will not be detected.



## Sample Report



### MOLECULAR GENETICS LABORATORY

2165 N Decatur Rd  
Decatur GA 30033  
Toll Free: (855) 831-7447  
International: (404) 778-8499  
FAX: (404) 778-8559  
CLIA#: 11D0683478  
CAP#: 7181693  
Page 1 of 3

Patient Information		Final Report					
PATIENT NAME:	LABORATORY #:						
DATE OF BIRTH:	TYPE OF SPECIMEN:	Whole blood (EDTA)					
CROSS REFERENCE #:	DATE COLLECTED:						
REFERRING DIAGNOSIS:	DATE RECEIVED:						
PATIENT ID:	FINAL REPORT:						
Medical EmExome: Clinical Exome Sequencing, Proband Only							
<b>Results: Pathogenic variant detected.</b> One copy of a <i>de novo</i> c.1141T>G (p.Y381D) pathogenic variant in the <i>FGFR2</i> gene was detected in this individual.							
(For non-phenotype related findings, please see report below.)							
Interpretation							
A sample from this individual was referred to our laboratory for exome sequencing. Information provided to us indicates that this individual has a skeletal dysplasia with small chest cavity, developmental delay, dysmorphic features, unstable airway, and respiratory failure. X-rays have identified short limbs, bilateral femur fractures, an abnormal sixth rib, and coarsening of the trabecular pattern of the bones with abnormal metaphyseal lucency. Previous microarray and chromosome analysis were normal.							
Medical EmExome Sequencing Results:							
Diagnostic findings related to phenotype:							
Based on the clinical information provided to the laboratory, the following variants related to or possibly related to this individual's phenotype were detected:							
Gene	MIM#	Disease (Inheritance)	Exon	Variant	Zygosity	Type <sup>1</sup>	Origin
FGFR2	176943	FGFR2-related disorders (AD/AR)	Ex9	c.1141T>G (p.Y381D)	Heterozygous	Pathogenic	de novo
Abbreviations used in this table: AR - autosomal recessive; AD - autosomal dominant							
Comments:							
<b>FGFR2 gene:</b> The c.1141T>G (p.Y381D) variant has not been reported in the general population. <sup>2-5</sup> This variant has been reported as apparently <i>de novo</i> in an individual with bent bone dysplasia syndrome, an autosomal dominant disorder. <sup>6</sup>							
Targeted sequence analysis in this individual's parents did not identify this variant in this individual's mother or this individual's father. This suggests that this variant likely occurred <i>de novo</i> in this individual.							
The detection of a <i>FGFR2</i> gene pathogenic variant is consistent with a diagnosis of <i>FGFR2</i> -related disorder in this individual; however, these results must be interpreted in the context of the individual's clinical and biochemical profile. Genetic counseling is recommended.							
Diagnostic findings not related to phenotype:							
No pathogenic variants in genes that are unrelated to the patient's phenotype were detected in this individual.							

## Reporting of Results

EGL's Medical EmExome reports include mandatory disclosures and optional disclosures. Mandatory disclosures for adults and children include:

1. Diagnostic findings related to the patient's clinical presentation – pathogenic variant(s), likely pathogenic variants(s), and variant(s) of unknown significance in genes interpreted to be responsible for, or contributing to, the patient's clinical presentation.
2. Diagnostic findings not related to the clinical presentation in childhood onset conditions – a single pathogenic or likely pathogenic variant in genes that are known to cause autosomal dominant or X-linked childhood onset conditions, as well as two pathogenic or likely pathogenic variants in genes that are known to cause autosomal recessive childhood onset conditions, even if they are unrelated to the patient's phenotype, will be reported.

In addition, there are several optional disclosures to be discussed with patients:

1. Carrier status for autosomal recessive conditions, such as cystic fibrosis. If a pathogenic variant in a recessive gene that is related to the patient's clinical presentation is found, it will be reported as a diagnostic finding. Further testing may be necessary to look for a second pathogenic variant in that gene not identified by WES. Patients may choose whether or not they want EGL to report carrier status in genes that are not related to their clinical presentation.
2. Adult onset, medically actionable conditions not related to the patient's clinical presentation – EGL will report pathogenic and likely pathogenic variants that are medically actionable such as Lynch syndrome, even though it may not be related to the patient's clinical presentation.
3. Adult onset, not medically actionable conditions that are not related to the patient's clinical presentation – EGL will report pathogenic and likely pathogenic variants for non-medically actionable conditions such as Alzheimer's disease.
4. Pharmacogenetics variants – Pharmacogenetic variants are changes in the DNA that do not cause a disease but may affect how well certain medications, such as chemotherapy drugs, antipyretics, antidepressants, and anticoagulants, will work or if the patient will have side effects.

EGL will report out on all medically relevant genes meeting reporting criteria, even if the gene is patented.

### DID YOU KNOW?

The Medical EmExome is not designed to be a comprehensive carrier test. An individual may be a carrier for a condition in which there was little, or no, coverage.

## Why choose EGL for clinical exome sequencing?

The Medical EmExome is the result of a multi-center collaboration between Emory University, Harvard University, and Children's Healthcare of Philadelphia. With more than 45 years of clinical experience, EGL is truly an expert in clinical genetics diagnostic testing and variant classification.

**The Medical EmExome is the only exome sequencing test that includes a free next generation sequencing panel.** This feature ensures complete coverage of all exons, on all genes related to the patient's phenotype. (not available for proband only testing)

EGL accepts third party payers, though coverage may vary from payer-to-payer and even within plans for the same payer.

When ordering the Medical EmExome, please submit medical records or clinic summary notes, and a signed consent form. Testing will not be initiated until these documents are received. Raw data is available upon request, as are research studies.

Individually, some genetic disorders may be rare. Collectively, however, genetic disorders are common. EGL offers a comprehensive test menu for those affected by rare and common disorders.



## Ordering Exome Sequencing

	Exome Trio	Exome, Proband Only
Test Code	EXOMT	EXOME
Document Requirements	Consent form and test requisition form.	Consent form and test requisition form.
Sample Requirements	Whole blood (or DNA extracted from whole blood) is needed from the proband and biological parents, as whole exome sequencing is being run on all three individuals.	Whole blood (or DNA extracted from whole blood) from the proband only is needed. Saliva may be submitted on additional family members.
Shipping Requirements	Whole blood: Collect 5-10 ml of whole blood and ship at room temperature within 5 business days.  If shipping isolated DNA: Ship 60 ug in a microtainer; refrigerate until time of shipment in 100 ng/ul of TE buffer.	Whole blood: Collect 5-10 ml of whole blood and ship at room temperature within 5 business days.  If shipping isolated DNA: Ship 60 ug in a TE buffer. microtainer; refrigerate until time of shipment in 100 ng/ul of TE buffer.
Secondary Findings Options	Carrier status, pharmacogenetic variants, adult-onset medically actionable, adult-onset not currently medically actionable (adults only). Parents have the option to select carrier status and adult-onset actionable disorders.	Carrier status, pharmacogenetic variants, adult-onset medically actionable, adult-onset not currently medically actionable (adults only).
Turnaround Time	16 weeks	16 weeks
Segregation Studies	Completed during the analysis.	Done as a final step.

Free EGL Specimen Shipping Kits are available by creating a Client Portal Account on the EGL website [www.geneticslab.emory.edu](http://www.geneticslab.emory.edu).



For more information about EGL:

**WEB:**  
[www.geneticslab.emory.edu](http://www.geneticslab.emory.edu)

**CALL:**  
404-778-8499

**EMAIL:**  
[egl.marketing@emory.edu](mailto:egl.marketing@emory.edu)