

Exome-to-Genome Reflex: clinical exome interpretation and report with automatic reflex to genome interpretation and report*†

*Reflex testing is only activated if the exome interpretation does not fully explain the phenotype

†Reflex to genome interpretation includes concurrent mitochondrial genome sequencing and deletion testing

DESCRIPTION

The Exome-to-Genome Reflex test uses genome sequencing data to provide a report of the exome interpretation, including variants that are present within the coding regions of the genome that are most likely to explain the proband's (e.g. patient's) clinical presentation. If the exome interpretation does not sufficiently account for the proband's phenotype, then reflex to a genome interpretation is activated, enabling a second report that includes additional variants present in the non-coding genome regions and select repeat expansions (see below for list of genes screened for repeat expansions). If the genome interpretation is activated, then concurrent mitochondrial genome sequencing and deletion analysis is included, which will be issued as a dedicated mitochondrial report.

Exome sequencing identifies a causal variant in 33–38% of cases (PMID: 30002876, 34368901, 37191093), while genome sequencing has been shown to identify a causal variant in 30–57% of probands (PMID: 25937001, 31246743, 34930662, 30002876, 35947384, 37231492, 22863877), with higher diagnostic yields observed when both biological parents are included and there is strict clinical inclusion criteria. The diagnostic benefit of genome sequencing compared with exome sequencing varies across studies; genome sequencing yields an approximate 8% increase in diagnoses (PMID: 38838312, 40183601, 35970915, 37334785). Exome and genome sequencing can be used to identify the underlying molecular basis of a genetic disorder in an affected individual with:

- One or more congenital anomalies (PMID: 34211152)
- Neurodevelopmental disorders including developmental delay, intellectual disability, and autism spectrum disorders (PMID: 34211152, 31182824, 40545261)
- Unexplained epilepsy (PMID: 36281494, 37596007)
- A phenotype suggestive of a genetic etiology that does not correspond to a specific condition for which genetic testing is available (PMID: 22863877, 25937001, 31246743, 34930662, 30002876, 35947384, 37231492)
- A suspected genetic condition that has a high degree of genetic heterogeneity (PMID: 22863877)
- A suspected genetic condition for which other available genetic testing options did not identify a diagnosis (PMID: 22863877)

The exome interpretation and report will include variants identified in the protein-coding regions (exons) of the genome, including the immediate surrounding intronic sequence 13 bases upstream and 6 bases downstream of each exon. If this does not yield a diagnostic answer for the proband's clinical features, further testing is automatically activated to report known disease causing variants in the non-coding and deep intronic regions of the nuclear genome along with select repeat expansions and the mitochondrial genome. The protein-coding regions represent ~20,000 genes (PMID: 21946919). The non-coding regions include promoter, intronic, and untranslated regions. While much of the data generated from sequencing

the genome is not well understood at this time, genome sequencing may provide more reliable coverage of the exonic regions (PMID: 25973577, 25827230).

For this test, an individual's nuclear genome sequence is filtered against published reference sequences, and compared to other sequenced biological family members, population databases, control sequences, and the GeneDx internal database to reveal potentially reportable variants. The proband's sequence is then evaluated using gene-phenotype associations, in conjunction with multiple resources including gnomAD, HGMD, OMIM, PubMed, and ClinVar (PMID: 26633542). Sequence changes of interest are interpreted according to the American College of Medical Genetics and Genomics (ACMG) guidelines (PMID: 25741868). A report of the exome interpretation is issued with clinically relevant variants that are associated with the proband's reported phenotype, which may include sequencing nucleotide variants (SNV), copy number variants (CNV) greater than 1 kb, uniparental disomy (when parents are submitted), and homozygous loss of exon 8 in the *SMN1* gene. If the variants included in the exome report do not fully explain the proband's phenotype, the genome interpretation is automatically activated, which includes concurrent mitochondrial testing. A separate report for the genome interpretation will be issued and include variants reported in the initial exome-focused report and/or new sequencing nucleotide variants (SNV), copy number variants (CNV) greater than 1 kb, uniparental disomy (when parents are submitted), homozygous loss of exon 8 in the *SMN1* gene, and repeat expansions in select genes that are associated with the proband's reported phenotype. Results of the mitochondrial testing will be issued in a separate report.

For this test, an affected individual's clinical records and prior genetic testing results submitted at the time of test activation will be reviewed prior to analysis. The proband's analysis includes evaluation of variants that are identified to be *de novo* (when both biological parents submitted), compound heterozygous (when both biological parents submitted), homozygous, heterozygous and X-linked. In addition, analysis takes into consideration family structure, reported phenotype, and provided clinical and/or differential diagnosis. This is a comprehensive analysis of a very large number of genes; therefore, reported results are focused on pathogenic and likely pathogenic variants, along with clinically relevant variants of uncertain significance, in genes related to the provided clinical information. Rarely, pathogenic, likely pathogenic, and molecularly strong variants of uncertain significance in genes associated with pediatric morbidity or mortality may be reported.

RESULTS REPORTING

Result reports will be issued only for the proband. A report will be issued for the exome interpretation, and, if necessary, separate reports will be issued for results of the genome interpretation and mitochondrial analysis. A separate report will not be issued for biological parents or other biological family members who may also have submitted a specimen for the purpose of allowing better interpretation of the results for the affected individual. If additional reports are requested for other family members, additional fees will apply.

The report(s) issued for the affected proband may contain variations in genes previously implicated in a human disease similar to the clinical presentation of the affected individual or in genes hypothesized to be related to the cause of the disease (candidate genes or genes of unknown significance). Variants in candidate genes may be reported based upon limited published evidence (including case report(s)) and/or internal data, such as observations of previous cases with similar phenotypes and types of variants in the same gene. In addition, other evidence such as gene function, tissue of expression, and phenotype of

model organisms with alterations in the gene may also be used as evidence to support reporting a variant in a candidate gene.

ACMG Secondary Findings

ACMG recommends that secondary findings identified in a specific subset of genes associated with medically actionable, inherited disorders be reported for all probands undergoing exome or genome sequencing. Please refer to the latest version of the ACMG recommendations for reporting of secondary findings in clinical exome and genome sequencing for complete details of the genes and associated genetic disorders. Secondary findings will be included for all exome or genome sequencing reports, unless a family opts-out of receiving this information on the Informed Consent as part of the test requisition form. If present, secondary findings are not included in preliminary reports. The status of any secondary finding(s) reported for the affected individual will be provided for all relatives included as part of the proband's test; GeneDx does not conduct an independent evaluation of secondary findings in relatives. Relatives have the ability to opt-out of receiving secondary findings. Secondary findings will be confirmed by an alternate test method, as indicated.

Sequence Analysis and Deletion Testing of the Mitochondrial Genome

The mitochondrial genome sequencing and deletion test results are issued in a separate report; these results will only be provided if the exome report does not provide a diagnosis for the proband's clinical features and the genome interpretation test is activated. For more information on the mitochondrial genome sequencing and deletion testing, please visit our "Mito Genome Sequencing & Deletion Testing" page on our website.

TEST METHODS

Genomic DNA from the submitted specimen is sequenced with paired-end reads on an Illumina platform. Average mean sequencing coverage for the proband is at least 40x across the genome, with a minimum threshold of 30x for any individual sample. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19.

Data from next-generation sequencing with CNV calling (NGS-CNV) are filtered and analyzed. The exome interpretation and report will identify sequence variants, homozygous loss of *SMN1* exon 8 (Spinal Muscular Atrophy), and most deletions and duplications greater than 1 kb in size within the coding regions of the genome. If the exome report is non-diagnostic, the genome interpretation and report will be activated and will include variants reported in the initial exome report and/or new sequence variants, repeat expansions of select genes, homozygous loss of *SMN1* exon 8 (Spinal Muscular Atrophy), and most deletions and duplications greater than 1 kb in size. As part of the genome interpretation, screening for disease associated repeat expansions is included for the following genes: *AR*, *ARX* (PA1 only), *ATNI*, *ATXNI*, *ATXN2*, *ATXN3*, *ATXN7*, *ATXN8/ATXN8OS*, *C9orf72*, *CACNA1A*, *CNBP*, *DMPK*, *FMRI* (with methylation status for alleles with >150 repeats), *FXN*, *HOXD13*, *PABPN1*, and *PHOX2B* (see table below for more details). Unless otherwise reported, the proband is considered screen negative for these non-sequencing variants; however, if a high clinical suspicion of any of these disorders is present, gene/disease specific diagnostic testing should be considered.

Reportable variants include pathogenic, likely pathogenic, and variants of uncertain significance; likely benign and benign variants, if present, are not routinely reported. Carrier status for the proband is not reported unless associated with the provided phenotype. Reported variants are confirmed by an appropriate orthogonal method in the proband and, if submitted, in selected relatives as necessary.

Sequence variants are reported according to the Human Genome Variation Society (HGVS) guidelines. Copy number variants are reported based on the probe coordinates, the coordinates of the exons involved, or precise breakpoints when known. Repeat expansions are reported with an accuracy of ± 2 repeats for alleles that contain up to 150 total nucleotides (e.g., 50 triplet repeats or 25 hexanucleotide repeats) and ± 5 repeats for alleles that contain >150 nucleotides. The exact number of repeats cannot be determined for very large alleles, which will be reported as "greater than" a gene-specific threshold. Available evidence for variant classification may change over time and reported variant(s) may be reclassified according to the ACMG/AMP Standards and Guidelines (PMID: 25741868), which may lead to issuing a revised report.

LIMITATIONS

Each component of this test attempts to evaluate the complete coding and non-coding regions of the genome. However, it is not technically possible to uniquely resolve and align the entire genome at present due to homology and other structural complexities in some regions. This test is limited in the types of variants that are detected and reported including nucleotide repeat expansion (with the exception of those noted in this document), and some structural variants. It is anticipated that approximately 97% of the coding region of an affected individual's genome (i.e., the exome) will be assessed at 15x coverage.

The available scientific knowledge about the function of all genes in the human genome is incomplete at this time. It is possible that these tests may identify the presence of a genetic variant in the genome sequence of an affected individual, but it will not be recognized as causative for the affected individual's disorder due to insufficient knowledge about the variant or the gene and its function. Reanalysis of the nuclear genome data is available upon request by the healthcare provider to incorporate updated clinical information and/or newly emerging gene and variant information. Updates to the classification of a sequence variant may be accessed through ClinVar (www.clinvar.com). Even if this test identifies the underlying genetic cause of a disorder in an affected individual, it is possible that such a diagnosis will not permit an accurate prediction of the prognosis or severity of the disease. While there is a possibility that identifying the genetic cause may help direct management and treatment of the disease, it is also possible that this knowledge will not change management or treatment.

Genes Screened for Repeat Expansions by Exome-to-Genome Reflex (Genome Interpretation)[†]:

Reference Ranges of Repeat Expansions for Genes Evaluated by Exome-to-Genome Reflex (Genome Interpretation) [†]						
Not evaluated on exome interpretation						
Gene	Positive	Incomplete Penetrance	Premutation	Intermediate	Uncertain Significance	Normal
AR*	38 or greater	35-37				34 or less
ARX (PAI)	23 or greater				19-22	18 or less
ATN1*	48 or greater	36-47	21-35			20 or less
ATXN1*	47 or greater				36-46	35 or less
ATXN2*	35 or greater (AD)	33-34			31-32	30 or less
ATXN3*	60 or greater				45-59	44 or less
ATXN7*	37 or greater	34-36	28-33		20-27	19 or less
ATXN8/ATXN8OS*	80 or greater				51-79	50 or less
CACNA1A*	20 or greater		19			18 or less
C9orf72*	61 or greater			25-60		24 or less
CNBP*	75 or greater			27-74		26 or less
DMPK	50 or greater		35-49			34 or less
FMR1	201 or greater		55-200	45-54		44 or less
FXN	66 or greater	44-65	34-43			33 or less
HOXD13	22 or greater				16-21	9-15
PABPN1*	12 or greater (AD) 11 (AR)					10
PHOX2B	26 or greater (AD)	24-25			21-33	20 or less

AD=autosomal dominant

AR=autosomal recessive

*=typically adult onset

Not Applicable

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