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TruGenome™ Undiagnosed Disease Test

Test description

Test indication

The TruGenome Undiagnosed Disease Test is intended to provide information to physicians to aid in the diagnosis of rare and undiagnosed genetic diseases. The analysis and interpretation are designed to detect and report on single nucleotide variants (SNVs), small insertion/deletion (indels) events, copy number variants (CNVs), homozygous loss of *SMN1*, mitochondrial SNVs, and a set of short tandem repeat (STR) expansions associated with genetic conditions. Proband-only and/or family-based analysis is performed, depending on the availability of samples at the time of testing. Family-based analyses may be comprised of a trio (the proband and their biological parents), a duo (parent and child), or other family structures. Variant characteristics, clinical presentation information, plausible inheritance patterns (based on the reported family history), peer-reviewed literature and information from publicly available datasets are used to contextualize variants identified during analysis.

Reasons for referral

This test is appropriate in cases where there is a suspicion of a rare genetic condition with clinical and genetic heterogeneity and numerous candidate genes to be assessed. The evaluation of the genome may clarify or refine a diagnosis because the presenting set of symptoms, imaging, and laboratory tests (biochemical or molecular) are inconclusive, or in cases where the phenotype might indicate multiple genetic conditions.

This test is not appropriate for certain conditions, including those caused by multiple genes, each with a small effect, gene-environment interactions, and methylation disorders. To assess if a patient's disorder is likely to have a Mendelian etiology, the referring physician should consider other lines of evidence such as increased severity, earlier than expected age of onset, multiple affected close family members, and unexpected phenotypic complexity.

Physicians ordering this test should understand its intended use and performance characteristics. Physicians should provide pretest counseling to their patients and the family members being tested to review the potential benefits, risks, limitations and alternatives to testing. Physicians ordering this test are responsible for obtaining informed consent from the persons being tested.

Optional secondary finding analysis

A secondary findings analysis is available for each individual being tested as part of the TruGenome Undiagnosed Disease Test. This includes a targeted screen of variants that meet the current test definition in genes recommended for reporting of secondary findings by the American College of Medical Genetics and Genomics (ACMG) SF v3.0 list of genes.[1] The list of genes included in this analysis is below:

ACTA2, ACTC1, ACVRL1, APC, APOB, ATP7B, BMPR1A, BRCA1, BRCA2, BTD, CACNA1S, CASQ2, COL3A1, DSC2, DSG2, DSP, ENG, FBN1, FLNC, GAA, GLA, HFE, HNF1A, KCNH2, KCNQ1, LDLR, LMNA, MAX, MEN1, MLH1, MSH2, MSH6, MUTYH, MYBPC3, MYH11, MYH7, MYL2, MYL3, NF2, OTC, PALB2, PCSK9, PKP2, PMS2, PRKAG2, PTEN, RB1, RET, RPE65, RYR1, RYR2, SCN5A, SDHAF2, SDHB, SDHC, SDHD, SMAD3, SMAD4, STK11, TGFBR1, TGFBR2, TMEM127, TMEM43, TNNI3, TNNT2, TP53, TPM1, TRDN, TSC1, TSC2, TTN, VHL, WT1

Each family member tested through the TruGenome Undiagnosed Disease Test has the option to opt-in or opt-out of analysis. In the instance where a family member opts-out of the secondary findings analysis, note the following:

- Opting out of the secondary findings analysis means that a targeted search for variants in the list of genes recommended by the ACMG will not be performed.
- If an individual opts-out of the secondary findings analysis, variants in one of the 73 genes recommended by the ACMG may still be reported if the finding lies within a large reportable CNV that contains multiple genes including those on the ACMG list or if a variant in one of these genes is identified and suspected to contribute to the patient's reported phenotype.
- In the case of a family-based analysis, identification of secondary findings in family members who opt-in for the analysis may inform carrier status of other members of the family, even those who chose to opt-out of the analysis.
- Clinical interpretation is performed for all SNVs, small indels, and CNVs, and only variants classified as pathogenic (P) or likely pathogenic (LP) in a zygotic state consistent with the associated disease are reported. Variants indicating carrier status for recessive conditions are not reported.

Incidental findings

- Incidental findings are defined as clinically significant variants found in genes associated with phenotypes that are unrelated to the patient's primary indication for testing. Unlike secondary findings, these variants are not actively sought, but may be noted during analysis. Variants with the potential to influence medical management, that meet the following criteria, and are deemed reportable by the clinical laboratory director will be returned.
  - The evidence supporting the gene-disease relationship must be classified as "Strong" or "Definitive" per current laboratory protocol.
  - The variant(s) must reach a classification of likely pathogenic or pathogenic and occur in the correct allelic state (or zygosity) for the disease.
  - The finding must influence medical management per the discretion of the laboratory director.
  - STR expansions are not returned as incidental findings.
- The identification of incidental findings is a potential outcome of this clinical test. It is not possible to opt out of incidental findings.
- Incidental findings may be related to pediatric or adult-onset conditions. Reporting of variants in genes related to adult-onset conditions is restricted to conditions in which professional practice guidelines outline condition-specific patient management, surveillance or screening, family management or special circumstances to avoid.
- Families who feel that the potential risk of learning about a medically actionable incidental finding outweighs the potential benefit of receiving that incidental finding or the potential benefit of receiving information related to the patient's clinical indication for testing may choose to not pursue this test.

Deliverables

- A Clinical Report of genomic findings deemed clinically significant based on the patient's reported phenotype will be returned. The Clinical Report, including variant interpretations according to ACMG/Association of Molecular Pathology (AMP) guidelines with some modifications as suggested by the Clinical Genome Resource (ClinGen) Sequence Variant Interpretation Working Group (SVI WG), and/or additional internal laboratory guidelines. Food and Drug Administration (FDA)-approved classifications published by the ClinGen Variant Curation Expert Panels (VCEPs) are used if available. Literature references used to support the classifications will be provided.
- A Secondary Findings Report will be returned. The Secondary Findings Report includes variants classified as likely pathogenic or pathogenic within the 73 genes recommended by the ACMG for secondary findings.
- Clinical Appendices:
  - A Gene List Appendix will be returned. This includes a list of genes generated by searching the Online Mendelian Inheritance In Man (OMIM) and Human Phenotype Ontology (HPO) databases for genes that have been associated with the phenotype. In the case of a proband-only analysis, this gene list is used to perform a targeted search for variants in these genes. In the case of a family-based analysis, this list is used to prioritize variants from the family-based analysis and to guide additional analyses of only the patient's genome in certain cases.
  - An Exon Callability Appendix will be returned. This includes a list of all RefSeq genes where at least one exon was less than 90% callable.
- Technical data in gVCF and BAM file formats (sequence information provided in a standard open source binary format)[2] is available for return to the ordering physician or patient who signs a release. Contact the laboratory to obtain a release form.

Criteria for variant classification and reporting

Variant analysis is based on clinical information submitted by the ordering provider. Not all variants of uncertain significance will meet our criteria for reporting. Variants are filtered and evaluated based on multiple factors including population allele frequency, variant consequence, evolutionary conservation, occurrence in a gene whose gene-disease relationship (GDR) overlaps with the patient's reported phenotype, and inheritance mode, as appropriate. Variant frequency is evaluated using the Genome Aggregation Database (gnomAD).[3] Variants with a population frequency in gnomAD greater than expected, given the prevalence of the disease, are considered to be likely benign or benign and are not reported. Synonymous variants and intronic variants beyond +/-2 are generally not reported unless they are known or suspected to be pathogenic. Single heterozygous variants in genes exclusively associated with autosomal recessive disorders will generally not be reported, except in instances where the variant is classified as likely pathogenic or pathogenic and the GDR is strongly associated with phenotype(s) matching that of the patient and no other explanation for the phenotype under investigation is identified.

Variant nomenclature is based on standardized Human Genome Variation Society (HGVS) convention.[4] CNVs are described using standardized International System for Human Cytogenetic Nomenclature (ISCN) (2016). We follow the ACMG/AMP standards and guidelines for variant interpretation and reporting for SNVs and small indels,[5] with some modifications as suggested by the ClinGen SVI WG, and additional internal laboratory guidelines. FDA-approved classifications published by the ClinGen VCEPs are used if available. We follow the ACMG/AMP guidelines for interpretation of mitochondrial DNA SNVs,[6] and the ACMG/ClinGen standards for the interpretation and reporting of CNVs.[7] We follow the ClinGen framework for evaluating gene-disease relationships.[8]

Methods and limitations

Whole-genome sequencing (WGS) was performed on extracted DNA using Illumina sequencing by synthesis (SBS) next-generation sequencing (NGS) at 2 × 150 bp reads with a minimum mean sequencing depth of 40×. These data were processed using TruSight™ Software Suite on the DRAGEN™ Bio-IT Platform, including read alignment to the GRCh37/hg19 genome assembly, variant calling, variant annotation by Nirvana, and comprehensive variant filtering. At least 98% of the sequenced autosomal genome was callable. Only genomic positions with a minimum coverage of 8× and alternate allele frequency of 25% or higher were assessed for interpretation and reporting. Small indels were detected and reported for this assay. Insertions up to 31 bp and deletions up to 27 bp have a sensitivity and analytical PPV of at least 80%. For SNVs and small indels, interpretation is limited to variant positions that overlap an exon and 8 base pairs of flanking sequence.

This assay has the capability to detect CNVs greater than or equal to 10 kb, however sensitivity was only assessed for events greater than 20 kb and was found to be approximately 85%.[9] CNV boundaries cannot be assessed with complete accuracy. The boundaries are estimated to lie within +/-1 kb of the event, unless otherwise noted. CNV analysis will not detect balanced alterations (reciprocal translocations, Robertsonian translocations, inversions, and balanced indels), triploidy, or genomic imbalances in segmentally duplicated regions.

Mitochondrial DNA is sequenced and analyzed using the same pipeline. Mitochondrial SNVs detected at an allele fraction greater than or equal to 5 percent are assessed.[10] Heteroplasmy will be reported for clinically significant variants. Mitochondrial CNVs and small indels are not reported.

This test is validated to identify the absence of the 'C' allele at GRCh37 Chr5:70247773 (NM_000344.3:c.840C>T) in the *SMN1* gene.[11,12] This test is not validated to detect other variants in the *SMN1* gene, nor quantify the number or phase of *SMN1* and *SMN2* genes. In addition, the test cannot identify individuals who are carriers of SMA. Only an outcome of 'SMA positive' will be included on the report.

ExpansionHunter is used to screen for disorders caused by repeat expansions in clinically relevant STRs.[13-15] Sizes of some repeats can be underestimated due to somatic mosaicism and GC amplification bias. Only the repeats that are 'expanded' in the genes listed below will be included on the clinical report. The specific repeat number will not be reported. This test cannot distinguish between expansions in the premutation range and full mutation range for some conditions. For genes associated with autosomal recessive repeat expansion disorders, ie, *FXN* and *CSTB*, carrier status will not be reported. Orthogonal confirmation of all clinically significant expanded STRs will be performed and results reported in an addended report. The list of genes that are included in the ExpansionHunter analysis is below:

DMPK, CNBP, FXN, FMR1, HTT, JPH3, AR, ATXN1, ATXN2, ATXN3, CACNA1A, ATXN7, ATXN8OS, ATXN10, PPP2R2B, TBP, NOP56, ATN1, CSTB, C9orf72, TCF4

A causal variant(s) explaining the patient's phenotype may not be identified by this test. Reasons for this include limitations of testing and limitations in current scientific and clinical knowledge about certain genetic variants, genes, and the association with human genetic disease. Some regions of the human genome are not covered by this assay, including stretches of the human reference genome that have not been completely resolved, low complexity regions, or regions where it is difficult to align fragments accurately. Additionally, genes that are associated with regions of high homology (pseudogenes) are difficult for this assay to resolve, unless specified to be part of this test. For targeted callers such as Expansion Hunter, the rare instance of a 'no call' will not be reported. This assay is not designed to detect mosaicism, and possible cases of mosaicism may be investigated at the discretion of the laboratory director. This assay will not detect methylation abnormalities. In addition, there are potential sources of error with this test, including, but not limited to, sample misidentification, sample contamination, and misclassification of genetic variants.

Lab statement

Pursuant to the requirements of CLIA '88, this test was developed, and its performance characteristics determined by Illumina Clinical Services Laboratory (CLIA# 05D1092911, CAP #7217613). It has not been cleared or approved by the U.S. FDA. This test is used for clinical purposes.

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