illumina®

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 highly penetrant genetic diseases. The analysis and interpretation are designed to detect and report on single nucleotide variants (SNVs), small insertion/deletion events, copy number variants (CNVs), homozygous loss of *SMN1*, mitochondrial SNVs and short tandem repeat (STR) expansions occurring at sites with associations to genetic disease. Analysis may be family-based or performed on only the proband. 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 when there are a large number of candidate genes to evaluate, the evaluation of the genome may clarify or refine a diagnosis because the presenting set of signs, symptoms, imaging and laboratory tests are inconclusive, or in cases where the phenotype might indicate multiple genetic conditions.

This test is generally not appropriate for conditions which typically have a complex or multifactorial etiology, such as diabetes or some autoimmune disorders. Occasionally, individuals with these types of disorders who have an atypical presentation, such as increased severity, earlier than expected age of onset or unexpected phenotypic complexity, or a family history of multiple affected close relatives may be appropriate candidates for testing. When the suitability of testing is unclear, the referring physician is encouraged to contact the laboratory prior to submitting the samples for testing.

Physicians ordering this test should understand its intended use and performance characteristics. Physicians should provide pre-test 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)[1]. The list of genes included in this analysis is below:

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

Each family member tested through the TruGenome™ Undiagnosed Disease Test has the option to opt-in or opt-out of the analysis. In the instance where a family member opts-out of the secondary findings analysis, please 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 59 genes recommended by the ACMG may still be reported if the finding lies within a large reportable copy number variant (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.

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 "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.
  - Short tandem repeat (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, including variant interpretations according to the ACMG guidelines. Literature references used to support the classifications will be provided.
- A Secondary Findings Report including variants classified as likely pathogenic or pathogenic within the 59 genes recommended by the ACMG for secondary findings.
- A Pharmacogenomics Report including 10 medically actionable genes associated with response to 14 different drugs/drug classes (as specified by the FDA or the Clinical Pharmacogenomics Implementation Consortium (CPIC)).
- Clinical Appendices:
  - A Gene List Appendix including a list of genes generated by searching the Online Mendelian Inheritance In Man (OMIM) database for genes that have been associated with the phenotype. In the case of a proband-only analysis, this gene list is utilized to perform a targeted search for variants in these genes. In the case of a family-based analysis, this list is used to prioritize resulting variants from the family-based analysis and to guide additional analyses of only the patient's genome.
  - An Exon Callability Appendix including a list of all RefSeq genes where at least one exon was less than 90% callable.
- A gVCF file that contains all SNVs and indels identified in the genome.

For family-based testing, technical data files, Secondary Findings Reports and Pharmacogenomics Reports are made available for each family member tested.

Technical data in BAM file format (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. Please contact the laboratory to obtain a release form.

Criteria for the evaluation of gene-disease relationships

We follow the ClinGen framework for evaluating the association of genes with disease[3]. Genetic and experimental data are examined for evidence which supports or contradicts a gene-disease relationship. Genetic evidence may be derived from case-level data and/or case-control studies, and segregation analysis. Experimental evidence can come from a wide range of functional assays but only evidence that supports the role of the gene in disease can be used for scoring. Both the genetic and experimental evidence are assessed qualitatively and semi-quantitatively and used to classify the strength of the gene-disease relationship into one of six categories: definitive, strong, moderate, limited, no known disease relationship or conflicting evidence reported.

Criteria for variant classification of single nucleotide variants (SNVs), small deletions and small insertions, and copy number variants (CNVs)

We follow the ACMG guidelines for variant classification and reporting for SNVs, small deletions and insertions, and CNVs[4,5]. The guidelines for classification of SNVs take into account the variant consequence, location and inheritance, presence or absence of functional data supportive of a damaging effect on the gene or gene product, prevalence of the variant in cases and controls, segregation data, computational evidence, patient phenotype and family history. The guidelines for classifications of CNVs take into account the copy number of the variant, gene content, overlap with established or predicted triplosensitive or haploinsufficient regions of the genome, inheritance and family history. Both variant types are classified into one of five categories: pathogenic, likely pathogenic, variant of uncertain significance, likely benign, or benign.

Test methods, performance characteristics and limitations

Human whole genome sequencing is performed on extracted DNA using sequencing-by-synthesis (SBS) next generation sequencing (NGS). The data are aligned and reported according to build 37.1 of the Human Reference Genome (https://www.ncbi.nlm.nih.gov/grc/human/data?asm=GRCh37). We sequence to a minimum of 40-fold coverage. Over 99% of the genome is covered at 10-fold coverage or more and 97% of the genome is callable (passes all quality filters). Based on the quality filters and through the analysis of an extended, multi-generation family set[6], SNV sensitivity is 99.1% and analytical Positive Predictive Value (PPV), i.e.TP/[TP+FP] is 99.9%. Small insertion and deletion events are detected and reported for this test. Insertions up to 35 bases and deletions up to 28 bases have a sensitivity and analytical PPV of approximately 80-85%. This test has the capability to detect copy number events greater than 10 kb, however sensitivity was only assessed for events greater than 20 kb and was found to be approximately 85%. Boundaries of the CNVs reported cannot be assessed with complete accuracy, and the boundaries are estimated to lie within +/- 1 kb of the event, unless otherwise noted[7]. For SNVs and small insertion and deletion events, interpretation is limited to variant positions that overlap an exon and its 15 base pair flanking sequence. For CNVs, interpretation is limited to events that either overlap an exon or have a boundary 1 kb upstream or downstream of an exon. Mitochondrial SNVs detected at an allele fraction greater than or equal to three percent are assessed for pathogenicity. Heteroplasmy will be reported for clinically significant variants. Mitochondrial CNVs and small insertions and deletions 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. This site is a paralogous sequence variant, c.840C/T, which distinguishes wild-type *SMN1* from the *SMN2* pseudogene. The c.840C>T variant causes alternate splicing of exon 7 of *SMN1* which results in a truncated, unstable protein[8]. An absence of the c.840C allele is consistent with an absence of exon 7 of *SMN1* and in turn absence of wild-type *SMN1* and is reported as a positive result for Spinal Muscular Atrophy (SMA). Over 95% of individuals with SMA have pathogenic variants in *SMN1* which result in a biallelic absence of exon 7[9]. 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. Consequently, these samples will get an outcome of 'undetermined' and a diagnosis of SMA cannot be ruled out in these individuals. Only an outcome of 'SMA positive' will be included on the report. The sensitivity of the SMA pipeline was assessed to be 95.83%, with a specificity >99%. When the test fails to delineate the sample as either 'SMA positive' or 'undetermined', the sample will be retested at the laboratory. If there is insufficient residual sample, a redraw will be requested, particularly if SMA is in the differential diagnosis. However, if a redraw is not possible, the laboratory will inform the ordering physician that SMA testing could not be adequately performed. Please contact the laboratory regarding ability to make calls in other regions of specific interest.

Expansion Hunter (EH) is a method for detecting repeat expansions in clinically relevant STRs. EH estimates the length of the repeat on each allele based on graph-based alignments[10,11]. In clinical validation testing, EH demonstrated an accuracy of >99%, sensitivity of >99% and specificity of >99% in calling an STR as expanded beyond the normal range. 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 conditions in which the premutation range is greater than the fragment length of the sequencing library, i.e. *FMR1, ATXN10, NOP56, CNBP,* and *ATXN8OS*. For genes associated with autosomal recessive repeat expansion disorders, i.e. *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 EH analysis is below:

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

Some regions of the human genome are not covered by this test, including stretches of the human reference genome that have not been completely resolved, or regions where it is difficult to align fragments accurately. Additionally, genes that are associated with regions of high homology are difficult for this test to resolve. These include, but are not limited to, some immunoglobulin (HLA) genes and telomeres. Clinical sensitivity is unknown and may be dependent on the patient's phenotype.

Lab Statement

The TruGenome™ Undiagnosed Disease Test is a Laboratory Developed Test. It is developed and its performance characteristics determined by the Illumina Clinical Services Laboratory (CLIA #05D1092911, CAP #7217613). It has not been cleared or approved by the U.S. Food and Drug Administration. Pursuant to the requirements of CLIA '88, this laboratory test has established and verified the test's accuracy and precision. The laboratory is regulated under CLIA as qualified to perform high-complexity testing. This test is used for clinical purposes. It should not be regarded as investigational or for research.

References

1. Kalia, S.S., 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, 2017 Genet Med. 2017. 19(2): p. 249-255.
2. Li, H., et al., *The Sequence Alignment/Map format and SAMtools.* Bioinformatics, 2009. 25(16): p. 2078-9.
3. Strande N.T., et al., *Evaluating the Clinical Validity of Gene-Disease Associations: An Evidence-Based Framework Developed by the Clinical Genome Resource.* Am J Hum Genet, 2017 Jun 1. 100(6):895-906.
4. Richards, S., et al., *Standards and Guidelines for the Interpretation of Sequence Variants: A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology.* Genet Med, 2015. 17(5): p. 405-24.
5. Riggs E.R., et al., *Technical standards for the interpretation and reporting of constitutional copynumber variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen).* Genet Med, 2020. 22(2): p. 245-257.
6. Eberle, M.A., et al., *A reference data set of 5.4 million phased human variants validated by genetic inheritance from sequencing a three-generation 17-member pedigree.* Genome Res, 2017. 27(1): p. 157-164.
7. Gross, A.M., et al., *Copy-number variants in clinical genome sequencing: deployment and interpretation for rare and undiagnosed disease.* Genet Med, 2018. [Epub ahead of print].
8. Kashima, T. and J.L. Manley, *A negative element in SMN2 exon 7 inhibits splicing in spinal muscular atrophy.* Nat Genet, 2003. 34(4): p. 460-3.
9. Prior, T.W., *Perspectives and diagnostic considerations in spinal muscular atrophy.* Genet Med, 2010. 12(3): p. 145-52.
10. Dolzhenko E. et al., *ExpansionHunter: A sequence-graph based tool to analyze variation in short tandem repeat regions.* Bioinformatics, 2019. [Epub ahead of print]
11. Dolzhenko E. et al., *Detection of long repeat expansions from PCR-free whole-genome sequence data.* Genome Res, 2017. 27(11):1895-1903.