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 just the proband. Family-based analyses may be composed of a trio (the proband and his or her 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 data sets 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 that 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, 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]. Genes included in this analysis are:

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 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 59 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.
- Incidental findings (variants classified as pathogenic or likely pathogenic in genes that are unrelated to the patient's primary indication for testing and not in the genes listed above) may still be deemed reportable by the clinical laboratory director, if identified.
- The identification of incidental findings is a potential outcome of this clinical test. Reporting of incidental findings is restricted to pathogenic or likely pathogenic variants that appear in a molecular state that corresponds to an expected clinical presentation (eg, biallelic variants in a gene associated with an autosomal recessive disorder). 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 Consortia (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 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 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. Contact the laboratory to obtain a release form.

Criteria for the evaluation of gene-disease associations

We follow the ClinGen framework for evaluating the association of genes with disease[3]. Genetic and experimental data are examined for evidence that supports or contradicts a gene–disease association. Genetic evidence taken into account includes data from both case and case-control studies. Experimental data are weighted according to the evidence type. Both the genetic and experimental evidence are assessed qualitatively and semi-quantitatively and used to classify the strength of the gene–disease association into one of six categories: definitive, strong, moderate, limited, no reported evidence, or conflicting evidence.

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

We follow the ACMG guidelines for variant classification and reporting[4]. The guidelines take into account the variant consequence, location, 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 to classify variants into one of four categories: pathogenic, likely pathogenic, likely benign, or benign. Variants that do not meet the criteria for one of these four categories and variants with conflicting evidence of pathogenicity are classified as variants of uncertain significance.

Criteria for classification of copy number variants (CNVs)

We follow the ACMG guidelines for interpretation and reporting of postnatal copy number variants[5,6].

- Pathogenic: Documented as clinically significant in multiple publications, even if penetrance and expressivity are variable. Includes large CNVs that may not be described in the literature as the same size but overlap with an interval with established clinical significance.
- Uncertain clinical significance–likely pathogenic: CNV described in single case report but with well-defined breakpoints and phenotype that overlaps with the patient, and/or a gene within CNV has a very compelling function relevant to phenotype.
- Uncertain clinical significance: CNV contains genes but unknown if genes are dose sensitive, and/or CNV is described in multiple contradictory publications or databases.
- Uncertain clinical significance–likely benign: CNV has no genes in interval but is identified because of size, and/or CNV is described in small number of cases in databases for the general population but does not represent a common polymorphism.
- Benign: CNV has been reported in publications or curated databases as a benign variant. CNV is documented to represent a common polymorphism.

Test methods, performance characteristics, and limitations

Human whole-genome sequencing is performed on DNA extracted from whole blood using sequencing by synthesis (SBS) next-generation sequencing (NGS) technology. The data are aligned and reported according to build 37.1 of the Human Reference Genome[7]. We sequence to an average of ≥ 30-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, multigeneration family set (Platinum Genomes)[7], SNV sensitivity is 98.7% and analytical Positive Predictive Value (PPV), ie,TP/[TP+FP], is 99.9%. Small insertion and deletion events are detected and reported for this test. Insertions up to 31 bases and deletions up to 31 bases have a sensitivity and analytical PPV ~ 80-85%, determined through Platinum Genomes. This test has the capability to detect copy number events > 10 kb; however, sensitivity was only assessed for events > 20 kb and was found to be ~ 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[9]. 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 wildtype *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[10]. An absence of the c.840C allele is consistent with an absence of exon 7 of *SMN1* and, in turn, absence of wildtype *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[11]. 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 of 100%. 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.

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[12,13]. In clinical validation testing, EH demonstrated an accuracy of 99.1%, sensitivity of 98.2% 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, ie, *FMR1*, *ATXN10*, *NOP56*, *CNBP*, and *ATXN8OS*. 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 EH analysis is below:

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

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. Contact the laboratory regarding ability to make calls in other regions of specific interest.

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 (Clinical Laboratory Improvement Amendments (CLIA) #05D1092911, College of American Pathologists (CAP) #7217613). It has not been cleared or approved by the US 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. We cannot accept orders from the state of New York at this time.

References

1. Kalia SS, Adelman K, Bale SJ, 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;19(2):249-255.
2. Li H, Handsaker B, Wysoker A, et al. The Sequence Alignment/Map format and SAMtools. *Bioinformatics*. 2009;25(16):2078-9.
3. Strande NT, Riggs ER, Buchanan AH, 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;100(6):895-906.
4. Richards S, Aziz N, Bale 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):405-24.
5. Kearney HM, Thorland EC, Brown KK, Quintero-Rivera F, South ST; Working Group of the American College of Medical Genetics Laboratory Quality Assurance Committee. American College of Medical Genetics standards and guidelines for interpretation and reporting of postnatal constitutional copy number variants. *Genet Med*. 2011;13(7):680-5.
6. South ST, Lee C, Lamb AN, Higgins AW, Kearney HM; Working Group for the American College of Medical Genetics and Genomics Laboratory Quality Assurance Committee. ACMG Standards and Guidelines for constitutional cytogenomic microarray analysis, including postnatal and prenatal applications: revision 2013. *Genet Med*. 2013;15(11):901-9.
7. Human Genome Assembly GRCh37. Genome Reference Consortium. www.ncbi.nlm.nih.gov/grc/human/data?asm=GRCh37. Accessed November 27, 2019.
8. Eberle MA, Fritzilas E, Krusche P, 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):157-164.

9. Gross AM, Ajay SS, Rajan V, et al. Copy-number variants in clinical genome sequencing: deployment and interpretation for rare and undiagnosed disease. *Genet Med*. 2019;21(5):1121-1130.
10. Kashima T, Manley JL. A negative element in SMN2 exon 7 inhibits splicing in spinal muscular atrophy. *Nat Genet*. 2003;34(4):460-3.
11. Prior TW. Perspectives and diagnostic considerations in spinal muscular atrophy. *Genet Med*. 2010;12(3):145-52.
12. Dolzhenko E, Deshpande V, Schlesinger F, et al. ExpansionHunter: A sequence-graph based tool to analyze variation in short tandem repeat regions. *Bioinformatics*. 2019;35(22):4754-4756.
13. Dolzhenko E, van Vugt JJFA, Shaw RJ, et al. Detection of long repeat expansions from PCR-free whole-genome sequence data. *Genome Res*. 2017;27(11):1895-1903.

Illumina Clinical Services Laboratory • 1.855.266.6563 toll-free (US) • +1.858.736.8080 tel • www.illumina.com