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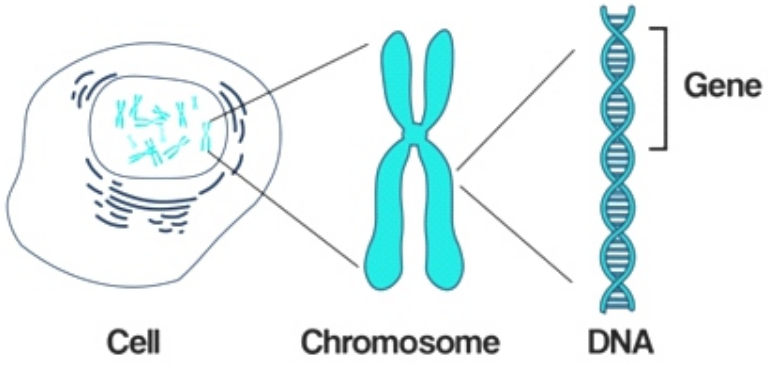
Whole Exome Sequencing - A Guide For Clinicians/Geneticists

We understand that as a Consulting Clinician/Geneticist you have considered genetic testing to evaluate the clinical symptoms of your patient. The genetic test explained in this leaflet is called Exome sequencing (ES) or Whole Exome Sequencing (WES). The terminologies for this test ES and WES are used with the same meaning.

What is an exome; what are variants?

Our body is made up of trillions of cells and each of our cells has information stored and packed in the form of chromosomes. Each cell would have 46 chromosomes, 23 from each parent (except for sex cells/gametes which have only 23 chromosomes). Chromosomes, in turn, are made of DNA that is somewhat similar to information-carrying books; DNA carries this genetic information from one generation to the next.



Genes, the segments of DNA, can be compared to pages of the books; they are made up of 3 letter codons that are made by various combinations of 4 biochemical components called nucleotides - A, T, G, C. People generally have two copies of most genes, one copy obtained from each parent.The Genes ensure optimum body functions via commands to body cells to produce proteins. Each cell has ~19300 genes; they are further organized as exons (protein-coding gene) and introns (the portion of a gene that does not code for proteins).

The Exome, which is the protein-coding region of all the genes, contains the codes for proteins that are essential for the structure and function of our body, and some of these genes also make us the way we are - unique from others! However, Exome accounts for only 1 to 2% of our DNA. Now imagine some of the letters or pages from the book i.e. gene go missing or are changed; any change in the DNA sequence in a given location (for example letter “A” replaced with the letter “T”) that are different from standard human DNA are called mutations (also called 'variants' or 'variations'). Not all variants are pathogenic (disease-causing); several changes are benign and are not associated with human diseases. However, some gene variants could lead to an abnormal protein that may be associated with your patient's clinical symptoms. About 85% of single-gene disorders (monogenic diseases) are caused by changes in the protein-coding region of the DNA. Past research has provided sufficient evidence to indicate that most genetic conditions arise due to mutations in the exonic (protein-coding) region of the gene.

What is whole-exome sequencing (WES)?

The genetic testing that examines the person's DNA and identifies the changes in the 'exome' which result in clinical symptoms is called 'exome sequencing' or 'whole-exome sequencing. Earlier technologies allowed us to test only one gene or a few variants at a time which is both time-consuming and expensive. The WES/Exome Sequencing is an advanced genetic test that captures information of almost ~19,300 genes.WES helps clinicians/geneticists identify the clinical symptoms that may have resulted due to mutations in the exon region of the genes. Oftentimes it is essential to test the biological parents/other family

members to check for carrier family members. Performing exome sequencing has an additional advantage of including recently described genes/diseases, and also has the potential for new gene discovery.

When is WES essential?

- Consider WES when you suspect the patient has a genetic etiology (a single disorder) or when the phenotype may be associated with defects in one of the several genes or in a large gene.
- In conditions where the clinical indications are broad/unclear/unusual, WES provides an unbiased understanding.
- In cases where a targeted single gene/panel test is unavailable, or WES could save time and money for both patients and the healthcare system.
- In cases where past genetic tests haven't provided any information, WES could be beneficial.

At Suma Genomics, we offer two different WES test options. In both the tests, segregation analysis of the selected variants in parents/family members is performed by Sanger sequencing usually, but not always. Both WES tests (WES1 and WES2) have the following options.

- **Singleton:** Patient only
- **Trio:** Patient with father and mother. Using additional WES tests with family members (trio) improves the chance of ascertaining a disease-causing variant in the patient. It also reduces the rate of an inconclusive result. Trio testing will be done only if it has been opted for. Trio ES is always better than singleton ES. In conditions like intellectual disability and epilepsy, where you expect a high proportion of de novo variants, please order trio ES.

WES001	WES002
• WES1 targets all the protein-coding regions (99.77%) along with certain non-coding regions of the human genome. • The enhanced coverage of both coding and non-coding regions facilitates better identification of copy number variants(CNVs). • Covers several intronic variants reported in the HGMD. • Suitable for patients with atypical clinical presentation/no definitive clinical diagnosis/ multiple differential diagnoses e.g., intellectual disability. • Covers the mitochondrial genome. • The Agilent SureSelect Clinical Research Exome V3 capture kit is used in this test.	• WES2 too targets specific genes which are already known to be associated with human genetic diseases. • Suitable for patients who have a definitive (straightforward) clinical diagnosis such as Osteogenesis Imperfecta, Cornelia de Lange syndrome, Ichthyosis, Epidermolysis Bullosa, etc. • A modified TWIST capture kit is used in this test. • In the event where this test fails to provide information, the patient might choose to undergo further testing by trio WES1 or Whole-Genome Sequencing (WGS).

Some laboratories offer to test only 5000-8000 genes which are known to be associated with human diseases (also called 'Mendeliome' or 'targeted exome' or 'focused exome' or incorrectly 'clinical exome'. Suma Genomics does not offer this test)

What are the expected results from the Whole Exome Sequencing test?

- **Positive result:** The WES test has identified a potential genetic change to possibly explain the clinical symptoms in the patient.

- **Negative result:** The WES test did not identify any clinically relevant changes in the genetic material to explain clinical indications in the patient. However, it may not necessarily mean that there is no genetic change. Oftentimes, a reanalysis after a period of 2 to 3 years may provide some information.

- **Inconclusive/variant of uncertain significance (VUS):** The WES test has identified a change in the genetic material but currently has insufficient evidence to prove the pathogenicity of the genetic change that could match with the clinical assessment. In such cases, additional clinical tests/gathering a detailed family history may help clinical decision-making. It is advisable to confirm or rule out the pathogenicity of variants with the help of further studies.

- **Incidental findings/ Secondary findings:** The WES test can sometimes identify a genetic change that is unrelated to your clinical assessment. However, in the event where the incidental deleterious variants are suggestive of important clinical or medical consequences,(e.g, increased risk of cancer or heart disease), it is ideal to discuss the findings with the patient/family members. Unless requested specifically, we do not offer results of incidental findings. Additional charges may be applicable for the same.

Sample requisite

Whole blood (2 ml in EDTA vacutainers) OR 5-10 Dried Blood Spots (DBS) OR extracted DNA Samples.

The importance of testing biological sample from parents, siblings, and other family members

Genetic diseases that follow Mendelian inheritance patterns i.e., dominant and recessive disorders are tested by WES. We have two copies of all genes (except for the male sex chromosomes). Genetic changes in a single copy of the gene (either inherited from one of the parent/ occurred newly in the patient) are enough to cause conditions known as “dominant disease”. On other hand, genetic changes in both the copies (each parent inherits one copy) are needed to cause diseases known as “recessive disease”. So when the WES test identifies the disease-causing variants in the DNA of the patient, we would test the parents and other family members at the same location by targeted sequencing to know the inheritance pattern. This would reduce the rates of variants of uncertain significance and improve the chance of correct diagnosis.

Note: We might decide not to perform segregation in family members if the variant is already known to cause the symptoms/disease in your patient (known pathogenic variants) or if the variant is unrelated to the phenotype observed in your patient (hence, it is important to provide all the clinical information at the outset!). We might also decide not to confirm the variant by Sanger sequencing if the variant is called confidently and it is located in 'difficult to amplify' regions.

Why is Genetic Counseling essential?

Pre-test counseling: A session of genetic counseling with the patient/family members is highly recommended before the WES test. Discuss the following with your patient before undergoing the WES test.

- The likely diagnostic yield of the clinical condition. Sometimes we may not be able to identify any genetic cause.

- Will the WES test result help in the clinical management of the patient? Treatment may not be available for many genetic diseases.

- The implications of the WES test results for the patient/ and your family members.

- Limitations and error rate of the test.

- Turn around time.

- Will insurance cover the test charges?

Post-test counseling: We do not send the test reports to patients or their relatives directly as we believe post-test counseling is essential for genomic tests. The test results will be sent directly to the referral doctor via a secure email or hard copy (if required). The doctor is solely responsible for all the decisions and possible management plans derived based on the test results.

Turnaround time (TAT)

We have a turnaround time of 8 weeks, except in unforeseen or challenging situations but we are usually faster.

What happens to the genomic data?

Suma Genomics would store the data in an anonymized form for its internal research purposes; the data remains safe, and it would not be sold to a third party at any given point in time. Anonymized data relevant to public health may however be revealed to regulated/authorized statutory bodies/researchers for the public good. With the consent of the patient/family member, the doctor may ask for patient data by reimbursing the expenses towards storage and transfer within a year of ordering the test.

Informed Consents

Informed consent is mandatory for all individuals undergoing Whole Exome Sequencing at Suma Genomics. We would accept test request forms from doctors only upon receiving a declaration that genetic counseling for patients/family members has been provided, and on receiving written consent from patient/family member. Direct requests/samples from patients are not encouraged. Sample consent forms and information to the patients are available on our website, which you can use.

Further information

If you would like to read more about next-generation sequencing, chromosomal microarray, and their use in clinical practice, we recommend the following articles (two of them are authored by one of our founders).

1: Narayanan DL, Girisha KM. Understanding Exome Sequencing: Tips for the Pediatrician. Indian Pediatr. 2021 Feb 25:S097475591600295. Epub ahead of print. PMID: 33634792.

2: Narayanan DL, Girisha KM. Genomic Testing for Diagnosis of Genetic Disorders in Children: Chromosomal Microarray and Next-Generation Sequencing. Indian Pediatr. 2020 Jun 15;57(6):549-554. PMID: 32562398.

3: Biesecker LG, Green RC. Diagnostic clinical genome and exome sequencing. N Engl J Med. 2014 Jun 19;370(25):2418-25. doi: 10.1056/NEJMra1312543. PMID:24941179.

For further queries and more information, please contact us at office@sumagenomics.in or visit our website-www.sumagenomics.in