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Medi Quest BRS Hospital

A monthly News letter from BRS Hospital

APPROPRIATE USAGE OF GENETIC TESTS AND HOW TO READ A NGS REPORT

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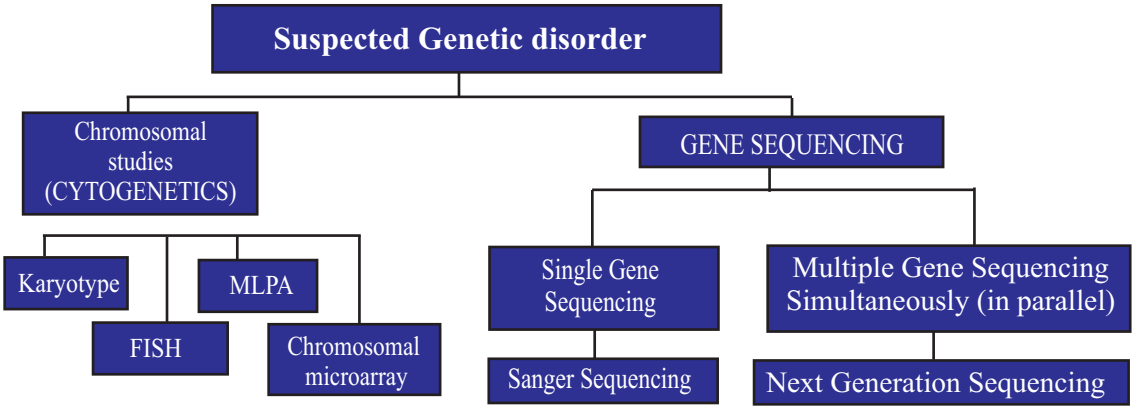
Genetic Tests in Frequent Use



Choosing The Appropriate Genetic Test

Genetic Test	Clinical Conditions - Indication	Advantages	Disadvantages
Karyotyping	1. Trisomy 13, 18, 21 (Aneuploidies) 2. Disorders of sexual differentation. Turner`s syndrome 3. Recurrent pregnancy losses	1.The entire genome is screened 2.Karyotype alone identifies a balanced translocation in Down‘s syndrome. 3.Most economical genetic test	Time consuming, operator dependent, prolonged TAT can identify defects only if defect is above 5Mb (more than 5 million base pairs)
FISH	1.Counting number of chromosome hence detection of Aneuploidies 2. Identifying known deletions and duplication eg. Digeorge syndrome, William`s Syndrome	1.Can be done on interphase cells hence culturing of cell not needed 2. Better sensitivity, specificity and resolution > 2 Mb in length 3. Faster turn over 2 - 4 days vs 2 weeks for karyotype	Can detect only known deletions and duplication. The clinicians must know what they are looking for. It has been superseded by other tests. Chromosomal defects less than 2Mb cannot be identified
Chromosomal microarray (Comparitive genomic Hybridisation)	1) Neuro devleopmental delay of unknown etiology 2) Multiple congenital Anamolies without a known phenotype 3) Autism 4) William`s Syndrome 5) Digeorge syndrome	Better resolution then FISH identifies deletions and duplication > 25-50kb Can detect single as well as contiguous gene syndromes.	Will not identify balanced translocation, Mosaicism < 20% If error lies outside the probe area

Genetic Test	Clinical Conditions - Indication	Advantages	Disadvantages
MLPA (Multiplex Ligation Probe dependant Amplification)	First Tier test in DMD, Spinal muscular atrophy. Can identify carrier state in these conditions.	Detecting CNV`s upto 60 different genomic DNA sequences. Deletions and duplication at Gene level can be detected. MS - MLPA is the test for Prader Willi Syndrome, even a WGS misses it.	Probe dependant. Can detect only known deletions and duplication. The clinicians must know what they are looking for.
Sanger Sequencing (First Generation Sequencing)	I) Disease caused by single or few mutations eg: Achondroplasia, sickle cell disease. ii) The gene is small and can be sequenced with one or few Sanger sequences. Eg. β-thalassemia iii) Genetic mutation for disease is available from previously affected child and prenatal testing is required in future pregnancies or detecting for carrier in the family	Method of choice for DNA sequencing in the cases where there is clear diagnosis	Not suitable in sequencing of large genes, time consuming
Next generation sequencing 1.Targeted panel sequencing 20-200 genes sequence.	Hearing loss, Genetic epilepsy, muscular dystrophy panel, Retinitis Pigmentosa		Pretest diagnosis needed
2. .Whole exome sequencing (20,000 genes sequencing)	Whole exome sequencing used in patients with suspected mendelian disorder where definitive diagnosis not available		Whole exome sequencing miss 1.Deletions and Duplications 2.Intronic errors 3.Methylation Errors 4.Trinucleotide errors 5.Mitochondrial DNA mutation
3..Whole genome sequencing	Whole genome sequencing is used when WES is inconclusive. It is the big daddy of genetic testing, most expensive. It is a single test for SNV, SNP`s.	WGS can identify 1.Deletions and Duplications 2.Intronic errors 3.Trinucleotide errors 4.Mitochondrial DNA mutation An article in European Journal of Human Genetics 2023 has stated that WGS as a single first tier test offers increased conclusive diagnosis in Genetic diseases compared to WES with other tests	WGS will miss Methylation abnormalities

How to read a Next Generation Sequencing Report

The report given below is from a actual patient with recurrent seizures in whom a genetic cause was suspected. The text accompanying it interprets the report .

DNA TEST REPORT

Full Name / Ref No:		Order ID/Sample ID:	
Gender:		Sample Type:	Blood
Date of Birth / Age:		Date of Sample Collection:	
Referring Clinician:	Dr. Ramesh, BRS Hospital Pvt Ltd, Chennai	Date of Sample Receipt:	
		Date of Order Booking:	
		Date of Report:	
Test requested	Whole - Exome Sequencing	WES : The test ordered, sequences all the coding genes, numbering about 22,000 genes - the exome	

CLINICAL DIAGNOSIS / SYMPTOMS / HISTORY

presented with clinical indications of refractory and complex partial seizures (right parietal region). He is suspected to harbour mutations in *KCNT* and *SCN* genes and has been evaluated for pathogenic variations.

The clinical findings / phenotype must be conveyed clearly to the Clinical Geneticist supervising the test

RESULTS

VARIANT OF UNCERTAIN SIGNIFICANCE RELATED TO THE GIVEN PHENOTYPE WAS DETECTED

I. Gene (Transcript)	II. Location	III. Variant	IV. Zygosity	V. Disease	VI Inheritance	VII. Classification	VIII. Copy number variant
PRRT2 **(+)** (ENST00000358758.12)	Exon 2	c.523G>A **(p.Val175Ile)**	Heterozygous	Episodic kinesigenic dyskinesia 1 (OMIM#128200) /Benign familial infantile seizures 2 (OMIM#605751) / Familial infantile convulsions with paroxysmal choreoathetosis (OMIM#602066)	Autosomal dominant	Uncertain Significance (PM2)	No significant CNVs were detected.

I. Gene (Transcript)

I. PRRT2+ Denotes name of the gene which has shown a mutation/variant among the ~ 20,000 genes sequenced. PRRT2is located on chromosome 16p11.2 and consists of four exons

(ENST00000358758.12)This refers to the gene transcript. Transcripts are defined as RNA molecules that are made from a DNA template. Databases like the ones at the National Library of Medicine's NCBI or the European Bioinformatics Institute (EBI) collect these transcript sequences from biologists working on a gene.

It serves as another ID for the gene.

II. Location

Exon 2

It is the second exon in the gene .

III. Variant

c.523G>A (Refers to a change in the gene PRRT2 gene)

c. informs that there is a change in the DNA. The number 523 gives the position in the gene where the nucleotide sequence has changed.

G> A means
G is the base which is normally present at that position.
> Greater than symbol means changed to
A means what the patient has at that position
In this report Guanine has been replaced by Adenine.

(p.Val175Ile)
- p - Refers to the protein

Val 175 refers to the aminoacid that is present normally at position 175, Val refers to Valine.

Ile refers to the variant aminoacid that has replaced Valine in this case Isoleucine .

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IV. Zygosity

Zygosity - Heterozygous

Which means this change is found in one allelic position ,

Heterozygous, as related to genetics, refers to having inherited different versions (alleles) of a genomic marker from each biological parent. Thus, an individual who is heterozygous for a genomic marker has two different versions of that marker. By contrast, an individual who is homozygous for a marker has identical versions of that marker.

Testing of parents would reveal from whom the patient has inherited the allele

V. Disease

Gives two syndromic conditions associated with variants in PRRT2 gene, this does not mean that the patient has these syndromes.

VI Inheritance

The earlier two syndromes have an autosomal dominant penetrance.

VII. Classification

Uncertain significance, more commonly called variant of unknown significance. This means a mutation variant or any other disease has been detected, but it does match known mutations causing the syndromes given in the fifth column.

A benign variant is not known to cause disease whereas a pathologic variant or likely pathogenic causes a genetic disease.

For this patient , this is a uncertain result, if such variants appear in a similar set of patients in the future, then it would be of clinical significance .

VIII Copy Number Variant :

No significant Copy Number Variants for the given clinical indications that warrants to be reported was detected signifies
No significant deletions or duplications for the given clinical condition were detected

Conclusion

It is imperative that the physician must know what genetic test to order for the given phenotype. The following caveats appear in the ever expanding field of Genetic Testing

1. The clinical findings /or the phenotype in genetic parlance must be clearly elucidated and conveyed to the Genetic Lab.

2. Ideally a genetic test should be ordered after consultation with a Clinical geneticist.

3. Pre test counselling of the parents /patient is an absolute must . Keeping in mind the fact that Whole Genome Sequencing gives definitive diagnosis in 40 % of the cases where Mendelian inheritance is suspected or present without a clinical diagnosis. In other words 60% of patients who have spent close to Lakh of rupees or more for WGS ,are still where they started.

4. A positive test need not necessarily mean the person has a genetic disease and negative test does not rule it out either . Sensitivity and specificity issues exist.

5. All Genetic labs are not the same.

6. A genetic diagnosis imposes emotional trauma on the patient and their families. A person with a genetic disease, may not get a job, may not be able to find a spouse, may not get insurance and one should remember genetic tests are expensive.

The field of genetic testing is emerging as a sunshine industry in medicine. It offers mind boggling possibilities in diagnosis and predicting response to treatment. The utopian dream of feeding the blood sample into the machine at one end and getting the diagnosis at other end seems to be a possibility in the near feature.

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