Exome Sequencing: Information Sheet

Background

DNA is the basic molecule of life and stores all information about development, growth and functioning of any organism in the simplest form of sequence of 4 nucleotides known as A, T, G, and C. Human beings have 23 pairs of chromosomes which harbour about 25,000 genes coding for various proteins in our body and controlling the functioning. The sequence of these nucleotides is similar in all human beings grossly but there are many variations in each individual, and these are responsible for visible and non-visible differences amongst all. Some of the variations in DNA sequence of genes are damaging and are responsible for more than 5000 genetic disorders known to us.

Identification of disease-causing genetic variations known as mutations is the confirmatory test for genetic disorders and also helps in providing testing of probable carriers of the disease, presymptomatic diagnosis and prevention of recurrence in the family by prenatal diagnosis. However, mutation testing by the traditional method has been a costly and time-consuming task as many diseases with similar clinical features are caused by many genes and many genes are very big in size.

To take care of these problems, a new method of high throughput sequencing has arrived in research and clinical testing. This has a major advantage of sequencing all or multiples genes in one go but also has some limitations and complex issues.

We want you to go through the following information and give your views on the questions following. This is to get opinion of young medical practitioners, as many of these tests are being marketed to physicians without providing adequate information. Your cooperation is requested. Thank you.

Dr Shubha Phadke & Dr Meenakshi Lallar

What is exome Sequencing?

Exome Sequencing is a DNA based test (~ 4-5 ml patient's blood in EDTA) for identifying disease-causing DNA variants within the 1% of the protein coding genome (exons), containing around 25,000 genes. These account for about 85% of known disease-causing variants. Its relatively smaller version is sequencing of exons of about 5000 genes which are known to be associated with known genetic disorders. This is known as Clinical Exome Sequencing and is often used in clinical situations as it covers clinically relevant genes.

What is the exome sequencing technique?

Exome sequencing uses Next Generation Sequencing (NGS) technique. In NGS technique millions or billions of DNA strands are sequenced in parallel and many genes or all genetic material can be sequenced in one go. It is a high throughput technique leading to a short reporting time and the cost is less. The patient DNA is sequenced nucleotide by nucleotide resulting in huge data of at least 8 to 10 GB. The patient's sequence is then compared to the consensus sequences and known variant sequences of the population and the results of these comparisons are interpreted. This needs very high end computational facilities and bioinformatics.

Where is clinical exome sequencing used?

The exome sequencing is used when clinical findings are highly suggestive of a genetic disorder and causative genes are many or very big in size. It is of great use especially when clinical evaluation cannot suggest the diagnosis. Since its inception, it has led to exponential progress in genetic diagnosis of diseases.

How are the results reported?

The DNA variants identified on exome sequencing are classified into one of the five categories (based on American College of Medical Genetics (ACMG) recommendations) –pathogenic, likely pathogenic, benign, likely benign and variant of uncertain significance. This is because many sequence variations in the genome are not yet reported in patients or in normal human beings.

What are the concerns regarding incidental/secondary findings on exome sequencing?

Exome sequencing sequences all genes which include many of the genes which are not necessary

for the diagnosis of the patient. Hence, this test may detect DNA variants associated with other disease condition like mutations for arrhythmia, cardiomyopathy, inherited predisposition to cancers or late onset diseases like Parkinsonism, etc. This can lead to anxiety and dilemmas. For some diseases such 'by chance' presymptomatic diagnosis can help in changing the outcome while for some diseases there may not be any treatment at present. Whether the individual tested will like to know about such incidental findings or not need to be found out before ordering such tests. American College of Medical Genetics had issued guidelines for the reporting of such findings and given a list of a particular group of genes which are considered 'actionable'. However, the debate about whether the laboratory and clinician should report on mutations in these genes or should be left to the decision of the individual in concern continues.

What are advantages/strengths of exome sequencing?

Exome sequencing has an average diagnostic yield of 25 to 30%. Most patients with rare diseases undergo extensive evaluation over an extended period of time. Exome sequencing has often identified the disease in such circumstances and reduced anxiety, time and cost of disease identification and occasionally provides an unsuspected diagnosis of a treatable disorder.

What are limitations of exome sequencing?

One of the main limitations is the high cost ([20B9?] 40,000 to 70000) which sometimes can be considered relatively less if we consider the amount of money spent in the other diagnostic modalities to reach definite diagnosis. Others include- incomplete or low coverage of certain genes, lack of coverage of noncoding regions, and inability at present to detect copy number variants, translocations and inversions (which require other forms of genetic testing). Hence, in some cases the disease-causing mutation may not be detected as that part of gene had not got covered in sequencing. Also, some DNA variants identified may be classified as variants of uncertain significance further adding to anxiety and dilemma.

What are the emerging applications of exome sequencing?

Exome sequencing is being increasingly utilized in clinical situations. In addition to diagnostics, it can be used for preconceptional carrier screening and pharmacogenomic (personalized medicine) testing. Recently in research settings, it has been used in newborn screening and for determining the predisposition to chronic diseases (DM, cancers, hypertension etc.). Sequencing of all genes of the fetus in the womb and in from circulating free fetal DNA obtained from the mother's blood has also become technically possible.

Name [optional]:

Age / Sex:

Qualifications:

Area of specialisation:

QUESTIONS

1. Had you ever heard of exome sequencing before this?
   (a) Yes
   (b) No
2. Have you ordered Next Generation sequencing (NGS) based test? If yes - how many times and did you find it useful?
   (a) Yes
      i. Number of times NGS ordered-
      ii. Was it useful in diagnosis and management?
   (b) No
3. Do you think exome sequencing is a useful genetic diagnostic technique?
   (a) Yes
   (b) No
   (c) Not sure
4. Would you like to get your Exome sequenced? If yes why?
5. Will you like to receive findings suggesting drug effects (pharmacogenomics) from your exome?
   (a) Yes
   (b) No
   (c) Not sure

6. Would you like to receive findings suggesting late onset yet untreatable disorders like Parkinson disease, Alzheimer's etc. from exome sequencing data?

   (a) Yes
   (b) No
   (c) Not sure

7. Would you like to receive information regarding mutations in genes for (kindly encircle):

   (a) Genes for arrhythmias leading to sudden death- Yes/No
   (b) Gene for Marfan syndrome- Yes/No
   (c) Genes for predisposition to cancers- Yes/No
   (d) Genes for predisposition to Age related Macular Degeneration- Yes/No

8. If you are found to harbour a mutation for any of the genes mentioned in question 7; what will be the level of anxiety generated in you on the scale of 1 to 10.

   (No anxiety) 1, 2, 3 ,4 5, 6, 7, 8, 9, 10, (extreme anxiety)

9. Would you like to receive results relating to your risk to chronic diseases, like Diabetes Mellitus, heart disease etc.? Please note that these are multifactorial diseases and genetic variations can only give odds ratio of you getting the disease.

   (a) Yes
   (b) No
   (c) Not sure

10. If the information regarding risk of ischemic heart disease, diabetes can be provided by family history, age, weight and lipid profile; then will you like to get exome sequencing for getting the information on genetic predisposition of these lifestyle diseases?

    (a) Yes
    (b) No
    (c) Not sure

11. Would getting a DNA result stating 'variant of uncertain significance' increase your anxiety?

    (a) Yes

    On a scale of 1 to 10- (No anxiety) 1, 2, 3, 4 5, 6, 7, 8, 9, 10, (extreme anxiety)

    (b) No

12. Single gene defects (familial or sporadic) have an incidence of approximately 1 in 300 neonates, 10% manifest after puberty and 1% after reproductive period. As with most genetic diseases, some of these are associated with high morbidity and mortality.

    Would you consider undergoing exome sequencing to detect your carrier status for single gene defects before planning pregnancy (eg. Cystic fibrosis, neurodegenerative diseases, aplastic anemia, lysosomal storage disorders etc.)?

    (a) Yes
    (b) No
    (c) Not sure

13. In which of the following scenarios would you consider exome sequencing testing in a fetus?

    (a) Regular, antenatal screening
        i. Yes
        ii. No
        iii. Not sure
    (b) Fetus with abnormalities on ultrasound
        i. Yes
        ii. No
        iii. Not sure
    (c) When there is family history of genetic disease
        i. Yes
        ii. No
        iii. Not sure

14. Would you consider exome sequencing for newborn screening?

    (a) Yes
    (b) No
    (c) Not sure

15. In exome sequencing of a newborn, as a part of newborn screening, what findings would you like to know?

    (a) Treatable genetic conditions
        i. Yes
        ii. No
        iii. Not sure

(b) Untreatable genetic conditions
   i. Yes
   ii. No
   iii. Not sure
(c) Late onset genetic conditions
   i. Yes
   ii. No
   iii. Not sure
(d) Carrier status for recessive diseases which can have only reproductive implications
   i. Yes
   ii. No
   iii. Not sure
(e) Predisposition to DM, cancers etc.
   i. Yes
   ii. No
   iii. Not sure

16. Which form of testing by exome sequencing appeals to you the most, besides the established role in patient with suspected genetic disease?
   (a) Preconceptional carrier screening
   (b) Prenatal testing of fetus
   (c) Newborn screening
   (d) None
   (e) All of the above

17. Do you think you will take the same decisions for your patients as you gave answers for yourself?
   (a) Yes
   (b) No
   (c) Not sure

18. Who should decide whether the incidental findings on exome sequencing, which are unrelated to the disease in the individual, should be shared with the individual in concern?
   (a) The individual
   (b) Incharge clinician
   (c) Laboratory
   (d) Some medical body
   (e) Not sure

19. Which of the incidental findings should be shared with the individual in concern?
   (a) All disease causing mutations
   (b) Only for diseases for which the outcome can be improved by presymptomatic diagnosis
   (c) Important for drug toxicity
   (d) As per individual's choice
   (e) None

20. Do you think that you are prepared to deal issues with exome sequencing, when a patient brings a report of exome sequencing?
   (a) Yes
   (b) No
   (c) Not sure

21. Were you aware of the information provided and issues related to exome sequencing before this questionnaire?
   (a) Yes
   (b) No

22. If yes, how much about the information about exome sequencing?
   (a) All
   (b) Some ——— %age
   (c) Very little ——— %age

23. How much information did you have about the issues and controversies related to exome sequencing?
   (a) All
   (b) Some ——— %age
   (c) Very little ——— %age

24. Would you like more information on exome sequencing and issues related to it?
   (a) Yes
   (b) No

25. If you have any question, please write below.

THANK YOU