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Exome Sequencing of Breast Cancer to Develop a New Molecular Target for the Diagnosis and Treatment

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Abstract: With the advent of next generation sequencing (NGS) technology, shifting the paradigm of genomics from single gene to whole genomic landscape, has brought us one step closer to implementation of predictive and individualized medicine. Disease diagnosis, risk stratification, clonal evolution and therapeutic intervention have been proved with application of clinical whole exome sequencing that leads several clinical genomic laboratories start to offer clinical whole exome sequencing with low cost. In this review ,we explore the current state of next generation sequencing that is being used in exome sequencing to study breast cancer to improve women health.

Keywords: Breast Cancer, Next Generation Sequencing, Exome Sequencing, Clinical Whole Genome Sequencing, Pathogenic variants.

1 Introduction

Breast cancer are most common cause of cancer related death in women and It has been estimated that 235,030 breast cancer will be diagnosed end of 2014. Several cancer risk factors have been explored, that include genetic and non genetic factors and they contribute to progression of tumor. Increased cancer incidence with family history indicates a possible genetic contribution to breast cancer risk. Genetic influences can be categorized into germline and acquired mutation. BRCA1 gene and BRCA2 gene deleterious mutation are strong predictors of breast cancer development and they have been involved with increased risks of several additional types of cancer as well. These mutations account for more than 25 % of hereditary breast cancers. Mutations in number of other genes have been also reported with increased risks of breast cancers. Even though these mutation have documented in various ethnic group, function of role of these mutation to breast cancer and ovarian cancer risk depends their prevalence and their penetrance in population. Prevalence of these mutation is influenced by founder mutations in various ethnic group and patient's own genetic and environmental background modify the penetrance [1].

Apart from gremline mutation, tumor acquired mutation have been noted in many cancer that drive heterogeneity of cells within tumours. Tumor suppressor genes are inactivated by acquisition of multiple driver mutations during initiation and progression of cancers. Even though, thousand of variant identified within a genome, not every genetic variant in a tumour translates into differences phenotype. However, they can contribute to certain extent to therapeutic resistance and supportive mutation for other mutation. Therefore, improving our understanding of genetic mechanisms of this cancer might improve clinical management of the disease. In particular, understanding of genetic variants, both germline and somatic mutation in breast cancer may providing accurate risk information to each patient for better management of these cancer. The recent state of the art development in next generation sequencing hold the promise of greater and broader clinical applications understanding these genetic variants that underlie, influence, and cause breast cancer.

2 Whole Exome Sequencing Vs Whole Genome Sequencing

Breakthrough in next generation sequencing technology has the potential to make whole genome sequencing (WGS) available to identify the genetic risk factors for human disease. However, current practices in clinical genomics are not directly applicable to robust genomic analysis because of technical challenge of handling huge data and it is too expensive to perform for large sample sizes. Therefore,

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exome sequencing is proposed as an alternative to whole genome sequencing for diagnosis and this approach reduces sequencing needs by 97%. Protein coding region of genome is called exome and there are approximately 180,000 exons in the human genome, which represents about 2% of the human genome. It is know that more than 85% of known pathogenic variants are in the exome, making whole-exome sequencing a cost-effective alternative to whole-genome sequencing [3].

3 Technology Used In Exome Sequencing

As we know that we target only coding region of the genome in exome sequencing, so various target enrichment technologies are used in exome sequencing. Most common methods are Hybrid Capture or multiplexed PCR. All method starts with the extraction of genomic DNA, after which either DNA is amplified with highly multiplexed PCR or sheared into short fragmented. In multiplexed PCR based method, amplified product is attached with specific adaptor for sequencing. In hybrid capture method, fragmented DNA is ligated to adapters and then hybridized to a pool of biotinylated oligos specific for exons ("baits"). After enriching exome and library preparation, sequences of which are determined with the use of one of various sequencing technologies and then million of sequence reads generated from machine are then aligned to human genome reference sequence . Similarities and differences between the patient's sequence and the reference sequence are called to determine the variant, which includes depth of coverage and the accuracy of the genotype at each position. Finally, variants prediction is used to understand the loss or altered function of a gene, and variants previously reported to cause disease [4]. Overview of exome sequencing is given in the figure 1.

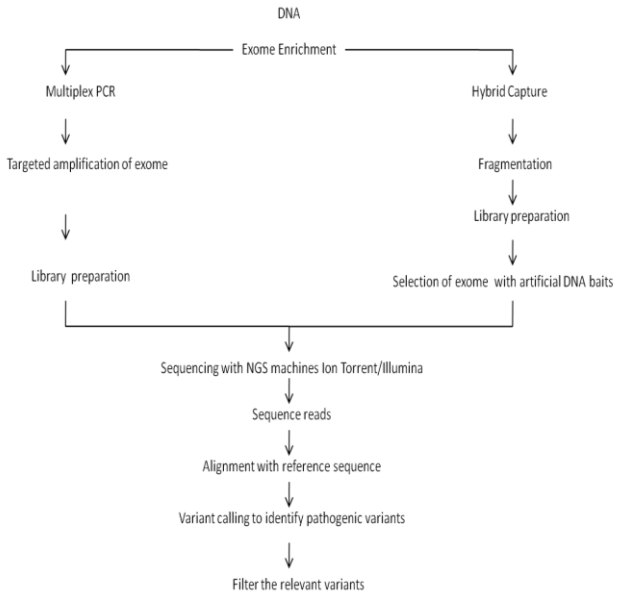

Figure 1: Overview of Exome Sequencing.

4 Role of Exome Sequencing in Identifying Pathogenic Variants in Breast Cancer and Its Importance

In order to identity breast cancer predisposition, various intensive efforts using linkage and candidate gene approaches have been made in the past. However, the genetic etiology breast cancer predisposition is unknown. Indeed, whole-exome sequencing of breast cancer patients have given a strong knowledge about predisposing genes in the recent past (Table 1) . One of the recent studies carried out in University of Helsinki highlights the importance of exome sequencing in breast cancer patient. It is well understood that loss-of-function mutations in *BRCA1*,*BRCA2*, *PALB2*, *CHEK2* known to cause a predisposition to breast cancer. However, Kiiski et al could not find in several families severely affected by breast cancer do not harbor mutations in any of these genes and they performed exome sequencing of 24 high-risk familial BRCA1/2-negative breast cancer patients. They identified FANCM c.5101C>T nonsense mutation associates with breast cancer risk in the Finnish population. However, *FANCM* c.5101C>T is very rare in the 1000 Genomes Project European population (minor allele frequency = 0.005) but it has been observed at a higher frequency in the Finnish population [5]. This exome sequencing study gives importance glimpse of hidden mutation behind the specific population. In overall, this study emphasizes the screening whole landscape of exome instead of targeting single gene that miss the real culprits behind cause of this cancer in any given population. Another study by Park et al used exome sequencing to study breast cancer patient revealed likely pathogenic mutations in RINT1, which were not present in public sequencing databases [6]. It highlight the importance of exome sequencing in identifying novel mutations that are involved with intermediate levels of breast cancer risk.

Table 1: Some of the genomic variants identified by exome sequencing in breast cancer.

1	PALB2, ATM	Cybulski et al
2	FANCM	Kiiski et al
3	RINT1	Park et al
4	TEKT4	Jiang et al
5	GPRC5A	Sokolenko et al
6	P53, PTEN, STK11, PALB2 , ATM	Aznarez et al
7	FANCC , BLM	Thompson et al
8	XRCC2	Park et al

5 Exome Sequencing in Exploring Variants Associated With Breast Cancer Treatment Response

Although various anti-cancer drug are widely used to treat

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breast cancer, their efficacy is still insufficient, as some of the patients do not fully recover even after several treatment trials. Inter-individual genomic differences are thought to contribute to the variability in treatment outcome. Exome sequencing has revealed a potential mechanism of resistance to paclitaxel in breast cancer patients. Comparison of exome of pre and post treat biopsies found two TEKT4 variation enriched in post treatment tumors and further experiments have shown that ectopic expression of variant TEKT4 deregulates the microtubule stability, antagonizes the paclitaxel-induced stabilizing effect of microtubules and increases paclitaxel resistance. Meanwhile, TEKT4 germline variations are associated with reduced disease-free survival and overall survival compared with wild-type TEKT4 in patients [7]. Taken together, this give an insight of how exome sequence may useful to understand the mechanism of resistance to any cancer drugs and address a specific clinical need or guide to a targeted therapy decision. Another whole exome sequencing study revealed that mutant *GATA3* correlated with suppression of proliferation upon aromatase inhibitor treatment [8]. This study illustrates how exome sequencing can be used as treatment predictive tools.

Table 2: Somatic mutation identified by next generation sequencing.

S.No	Tumor subtypes	Variants	References
1	Primary triple-negative breast cancers	p53, PIK3CA, PTEN	Shah et al
2	Estrogen-receptor-positive breast cancer	RUNX1, CBFB, MYH9, MLL3, SF3B1, MAP3K1, TP53, GATA3, MAP2K4, MAP3K1,	Elli et al
3	Diverse subtypes	AKT2, ARID1B, CASP8, CDKN1B, MAP3K1, MAP3K13, NCOR1, SMARCD1 and TBX3	Stephens et al
4	Diverse subtypes	PIK3CA, TP53, AKT1, GATA3, MAP3K1, CBFB,	Banerji et al
5	Breast fibroadenoma	MED12	Lim et al

Genetic changes are acquired during a person's lifetime and are present only in certain cell are called somatic mutations, which are not inherited. Somatic mutations help to initiate and maintain cancer growth. It has been noted that paediatric and haematological cancers have lower mutation rates than melanoma, breast and lung cancer. This observation highlights the environmental insults that influence tumor progression in somatic cells. This observation clearly highlight the essential to understand somatic mutation as it is critical for tumor progression and effective treatments. Somatic mutation have been identified in various breast cancer subtypes, that include, triple-negative breast cancer, ER-positive, negative breast tumours and fibroadenomas. Exome sequencing of fibroadenomas revealed recurrent somatic mutation solely in MED12 and they showed that 59% of breast fibroadenomas contain somatic mutation in exon 2 of MED12. MED12 fibroadenoma mutations are present in stromal but not epithelial mammary cells [9]. This work explain how exome sequencing useful to deepen the conceptual understanding of tumor develop from specific cells.

6 Conclusion

It is important to understand what is written in genomic blueprint of genome and what is acquired over the course of tumor progression. Ultimately, these analyses will lead to individualized breast cancer risk assessment and a reduction in breast cancer incidence. Although they a are promising to be used in clinic to improve human health as address biological questions at genome-wide scale, their expensive costs, ethical issues, user friendly data analysis have to be addressed before to use in clinics.

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