Pathol. Oncol. Res. (2017) 23:225–234
DOI 10.1007/s12253-016-0124-z

REVIEW

Clinical Applications of Next-Generation Sequencing in Cancer Diagnosis

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Received: 1 May 2016 / Accepted: 4 October 2016 / Published online: 8 October 2016

Abstract With the advancement and improvement of new sequencing technology, next-generation sequencing (NGS) has been applied increasingly in cancer genomics research fields. More recently, NGS has been adopted in clinical oncology to advance personalized treatment of cancer. NGS is utilized to novel diagnostic and rare cancer mutations, detection of translocations, inversions, insertions and deletions, detection of copy number variants, detect familial cancer mutation carriers, provide the molecular rationale for appropriate targeted, therapeutic and prognostic. NGS holds many advantages, such as the ability to fully sequence all types of mutations for a large number of genes (hundreds to thousands) and the sensitivity, speed in a single test at a relatively low cost compared to be other sequencing modalities. Here we described the technology, methods and applications that can be immediately considered and some of the challenges that lie ahead.

Keywords NGS · Cancer · Next-generation sequencing · Diagnosis · Clinical practice

Introduction

Malignancy is a disease of genetic alteration. In particular, single nucleotide forms existing as both germline and somatic point mutations are required inducement of tumorigenesis and cellular multiplication in diverse human cancer types. The survey of germline mutations established main gene rolls in cancer participate of single germline allele to the people load of tumor is comparatively down. Identification of tumorigenic procedures has strengthened on somatic mutations. The somatic mutational aspect of cancer has currently extremely been achieved from tiny or targeted procedures, resulting to the examined of genes affected by somatic mutations in many various carcinoma varieties. More comprehensive investigations using Sanger exon sequencing method tender that the mutational outlook will be resolved by relative handfuls of mostly mutated genes and a long tail of scarce somatic mutations in various genes [1]. Cancer is an outstanding public health concern in the all world. However, the estimated some of the malignancy-liked death is decreasing in the United States. Additionally, cancer is the major cause of death in individuals below 85 years [2]. The various alterations known to consist of mutations in oncogenes and tumor suppressor genes (TSG), gene amplifications and deletions, and chromosomal rearrangements; the possessions of these genetic changes lead to prepare a proliferatives and survival gain to cells. Moreover, molecular variations may also take place that let the out coming tumor to invade into surroundings tissue and, finally, metastasize to other organs. Although, the sequencing information was available for many cancer types, the genetic progression of these cancers remained undisclosed. Malignancies launch to expand exclusively from their surroundings normal tissue when a mutation yields proliferative profits on an entity cell [3]. There are multiple obstacles in cancer genomics that obstructed clinical application. One of them would be information reproducibility. They may be due to in section to the experimental biases also model cohort issues. The utilization of different platforms evaluating gene expressions and varied data preparing methods could propagate biased perceiving in every investigation elevating sample size will be one of the keys to disclose appropriate biomarkers,

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overwhelming the reproducibility quandary. Naturally, large-scale specimen assembly provides increased actuarial ability. However, earlier surveys, even with broad sample sizes, have mostly failed to reproduce their detecting in autonomous works [4]. This may be due often to the employment of different protocols and analysis methods. Furthermore, biased sample gathering might also influence the carrying out of diagnostic biomarkers, resulting to pursuant defeat to validate the biomarker in another patient crowd [5].

Current Approaches in Clinical Usage

Cytogenetic analysis other procedure that has been extensively used for the examination of cancer progression is comparative genomic hybridization (CGH). This Diagnostic technique is capable of discover small-scale copy number reformations [6]. Promotion of cancer patterns can be diagnosed from CGH data based on the frequency of these chromosomal insertions or deletions: It should be a specific area be altered in most samples of a tumor; it is derived that genetic incident happened early in the progress of the tumor and was consequently, passed on to its posterity CGH has been used in demonstrating the progress of metastases from the primary place of cancer [7–11].

Other feasible observations in the usage of next generation sequencing to clinical tumor samples consist rotation time, the measure of input DNA needed, the necessity for secondary verification and expense [12].

Present opportunities for cancer diagnostics there are some of the exhilarating opportunities for the performance of NGS in a clinical position and, not wonder, there are a great interest and activity in this background. Some of these styles will easily replace current Sanger sequencing or PCR-based Measurement for genetic examination within genes related to familial cancer syndromes or for diagnosis of mutations in genes of therapeutic significance within cancer cells or tissues [13, 14].

The extension of high-throughput sequencing technologies facilitated research laboratories to appraisal disease mechanisms from the DNA sequence to transcriptional regulation and RNA expression. As complicated diseases are likely secondary to global concerns in cellular and physiologic networks, massive reporting of analyses consisted of DNA sequence types, RNA expression levels, and promoter methylation situation might become progressively related for detections and for prediction of a response to medical care. For the clinical science laboratory, the questions of development into these novel areas of nucleic-acid testing are horrific, and will be the probable need the use of numerous supplementary high-throughput sequencing technologies. In this section, we will momentarily elucidate some of the possible usages of next-generation sequencing technology for clinical diagnosis.

Next-Generation Sequencing Technology

Introduced Next-Generation Sequencing

Recently, much genomic research have noticed that the massive complexity of the cancer genome. Genomic profiling utilizing microarray technology could cover the tumors into identical subgroups, offering new clinical understandings for the progress of diagnosis and therapeutic also systematic insights on the basic mechanisms of tumor development. Moreover, the microarray technologies, detonating progresses on sequencing have been designed lately, which is entitled "next generation sequencing" [15, 16].

Although, due to it are restrictions in throughput and relatively high cost, it was never possible to sequence a large number of genes and samples. In order to overcome this barrier, novel sequencing technologies were invented. NGS technologies sequence thousands of DNA molecules in a parallel shape. It provides high speed and high throughput. It can propagate both quantitative and qualitative sequence data, tantamount to the data from Human Genome Project, in two weeks. At the moment, there are multiple NGS platforms commercially accessible: the Illumina Hiseq and Miseq, the Roche 454 GS and Junior version, the personal genome machine Ion torrent, and the Life Technologies SOLiD. A number of NGS platforms, both Miseq and Ion torrent, are more desirable for clinical utilization because of their more pliable throughput and shorter rotation time [17–22].

Compared to the preceding DNA sequencing of the Sanger procedure employing dideoxynucleoside termination reaction termed as "first-generation" sequencing, NGS brings into service massively parallel sequencing operation generating hundreds of millions of short (~200 bp) DNA reads, which can be the sequence a human genome quickly with sorely lower expense. The previous NGS procedure with the single-end read sequencing naturally generates the short-read difficulties, restricting the fidelity of genome alignment. This could be ameliorated by use a paired-end sequencing procedure, permitting significant progresses in determination not only point mutations but also genomic rearrangements, resembling deletions, amplifications, inversions, translocations, and gene-fusions [15, 16]. The NGS technology is now separated into sections "second generation sequencing" and "third-generation generation sequencing." The second generation sequencing refers to the strategies of short-read alignment, while the quickly being advanced technology of the third-generation sequencing refers to the single DNA molecule based sequencing. The third-generation procedure has a benefit of less rate of DNA input that permits the emerging context of single cell sequencing [23]. Furthermore, there is any stage for PCR amplification, so, the nucleotide incorporation errors can be handled. Anyway, all the platforms of NGS technologies still have limitations in exact base calling and alignment. The errors

seem to be platform-related, which enhances the complexity of the data analysis. So, the cost for conformation analysis, instead of the sequencing itself continues to grow, which is referred to as "the $1,000 genomes, the $100,000 analysis" problem [24].

Variants Platform Options in Next-Generation Sequencing

First-Generation Sequencing

Formerly, Sanger et al. suggested the first DNA sequencing method, which was based on interpolation of little changed nucleotides for chain elongation by DNA polymerase [25]. The Sanger procedure was laborious, time consuming, and fault prone given some of the handy laboratory-based stages needed and hand arrival of resulting sequences. In early 2005, innovative sequencing methods, usually referred to as "next generation sequencing" have revolutionized conventional Sanger sequencing. Contradictory Sanger sequencing; NGS platforms do not need a cloning stage; instead, they employ synthetic DNA fragments (adapters), specially designed for every platform, to augment the DNA library on a solid backing matrix pursued by cyclic sequencing. In additional, whereas various stages of sequencing and signal to trace are sequential in the Sanger procedure, commercial NGS tools are able to implementation these concurrently; therefore, they are mentioned to as parallel sequencing. Eventually, the read length in Sanger sequencing restricted by gel electrophoresis-related issues whereas in NGS it is specified by the signal-to-noise ratio [26]. Discrepancy, in principle and chemistry, the Sanger procedure and NGS are disposed to various types of error and as a consequence, despite higher precision, data from NGS platforms are yet confirmed by the Sanger procedure [27].

Second-Generation Sequencing

Although revealed that the advantages of Second-Generation Sequencing in Cancer usages; even though the outstanding progresses made in discovering how cancers develop by performances such as CGH, LOH, and Sanger sequencing, there are many benefits to be next-generation sequencing technologies that make it desirable to the aforementioned techniques for the examine of cancer progress. Next-generation sequencing overcomes the problem of decreased DNA modality that outcomes from areas of necrosis inside the tumor [16]. In addition to, special evaluations have been developed such that it is easier to discover variations in DNA other than point mutations. To date; next-generation sequencing has been used to the study of chromosomal rearrangements, copy number variations, and RNA sequencing [28–30]. Eventually, it is very infrequent for a cancer sample – be it from a biopsy or surgical resection – to be only cancerous normal genomes attenuate the cancer genomes so that deficient genetic types can be difficult to distinguish with Sanger sequencing. Next-generation sequencing technologies was permission detection of genomes at very low frequencies than Sanger sequencing [16, 31]. Given the many amounts of information produced by next-generation sequencing examinations, the obstacle for investigators has been to specify the developmental importance of the genetic deviations distinguished in these analyses.

As mentioned earlier, an outstanding benefit of second-generation sequencing technology is the potential to prosperously sequence genetic matter from lower-frequency specimens [31]. Investigating the evolution of metastatic ability by Second-Generation Sequencing a main problem that remains in cancer investigation is whether there be present mutations that increase the positive choice of metastatic clones. Whereas the mechanism for normal cells to apportion is generally active and becomes unusual in cancer, a separate set of operation must be deregulated on for a cell to obtain the capability to successfully metastasize. Next-generation sequencing has been applied to identify differences in genetic profiles between early cancers and their matched metastases. By employing paired-end sequencing, a lobular breast cancer and its matched metastasis were compared [32]. Thirty-two total coding mutations were revealed in the metastatic lesion, of which nineteen were not distinguished in the preliminary cancer. Of the residual mutations detected in the metastasis, five were existent in most cells in the early tumor, and six were identified in 1–13 % of cells. These outcomes highlighted two notions: the innate incongruity that extends within cancers as, they evolve, according to genetic changeability the powerfulness of next-generation sequencing to recognize every scarce clone of cells within a crowd. Auxiliary evaluation of matched timely and metastatic pancreatic adenocarcinomas by massively parallel paired-end sequencing patterns [33]. Every patient had specific genomic rearrangements that were current in the early cancer and all of its respective metastases. Whilst some rearrangements were present only a proper subset of wastage or merely in one metastatic tumor. In additional, lung metastases were identified to have progressed more than metastases to ventral organs, according to the number of rearrangements detected in every lesion. These outcomes corroborate that person metastases expand individually within a cancer, an outcome Yachida et al. in a comparable survey utilizing Sanger sequencing [33, 34]. Next-generation sequencing has supplied some remarkable findings, although outcomes in the pressure to identify metastasis-promoting mutations. Sequencing of a matched preliminary breast cancer, xenograft, and brain metastasis displayed that while most mutations that were detected existing in all three lesions, two mutations (in SNED1 and FLNC) existed merely in the metastasis [35]. Furthermore, an assumed lung-specific change in the PARK2gene was diagnosed in a pancreatic cancer patient [33]. Two separate variations were

identified in this gene, one of which was discovered solely in the lung ulcers, whereas the other was only observed in peritoneal, liver, and omental metastases. As previously mentioned, CGH analyses disclosed that definite regions of the genome are continuously obtained or lost squamous-cell lung carcinomas and pancreatic adenocarcinomas metastasize [7, 10] The next-generation sequencing projections explained to in this place in the supply demonstration that mutations, in particular, genes occur in association with metastasis. These commitments support the CGH results, though at much greater resolution. Comparability the real advantages of next-generation sequencing compared to elderly techniques in the chase of metastasis-stimulant genetic variations. However, these outcomes are exciting as they proposal that certain genes might be accompanied with organ-special metastases; it is noteworthy for confirm that these outcomes were gained from only one patient, and a larger cohort must be sequenced in order to investigate assumed metastasis-promoting genes also mechanistic researches of the mutated genes [36].

Third- Generation Sequencing

The foremost interest of this set of sequencing platform is their facility to sequence a single molecule of DNA without the previous clonal amplification of the library [26]. This maintains the library from favorable amplification of specified fragments and artifacts caused by polymerase errors removing the PCR step as well as translate into less handwork and a higher luck of identifying epigenetic conversions in genomic DNA [37]. Commercialized in 2011 by Pacific Biosciences Inc., the PacBio RS IIcombines single molecules real-time sequencing (SMRT) cell with very susceptible fluorescence diagnosis technology. Every cell consists of immobilized polymerases and thousands (approximately75, 000) of zero-mode waveguides (ZMWs) to permit visual perception of DNA polymerase activity as it performs sequencing by synthesis [38]. The small diameter of ZMWs ranges over the polymerase to 20zeptolitres which in spin enhances the signal-to-noise ratio [37]. In additional by enhancing the coating to be 15-equal, the precision of this platform advanced from 83 % to 99 % [39]. Nanopore sequencing is based on change of electric stream or optical signal secondary to the transfer of ingredient bases of a DNA molecule through a pore [37]. DNA strands are loaded onto one face of a lipid player, and a controlled voltage utilized across the player mobilizes the DNA through the pores [23]. Nanoporeis one of the most developed technologies for sequencing DNA and, there are many procedures to this technique. It is useful in utilizing uncorrected strands of DNA and tender investigators with a cheap sample preparation that keeps the DNA sequence effectively. Currently only molecule sequencing is a conceivable option to overcome deficiencies of second generation sequencing; however, their implementation is confined by read length and an intrinsically higher fault amount.

Applications of Next-Generation Sequencing

Next-generation sequencing (NGS) technologies are performing the progressively important role in cancer study. Recently have seen at several of studies researches the mutational perspectives of different cancer subgroups. NGS examinations into prostate [40], breast [35, 41], ovarian [32, 42, 43], pancreatic [33, 34, 42], hematological malignancies [44–48], and others [49, 50] have shown new cancer genes, new understandings into tumors progress, through mutational profiles and discovery of genomic architectures. These researched have constituted NGS experiences as a sorely efficient, impartial procedure to study cancer genomes and perform genome wide somatic mutation finding. In the not far future, large-scale international projects [48, 49] generating wide sequence information reservoirs from hundreds of individual tumors will be perfect. As such there is a major requirement for cancer-focused procedures for robust, through commentary of this information.

Whole Genome Sequencing

Whole genome sequencing implies resequencing the whole genome and mapping the sequence return to the human genome to recognize mutations. The predominant advantage of whole genome sequencing is complete envelopment of the whole genome, containing promoters and regulatory areas. Subsequently, whole genome sequencing is frequently used to recognize new and scarce mutations. In whole exome sequencing, all exons of all known genes are sequenced at a partly deeper depth. Paralleled to whole genome sequencing, the major advantage of exome sequencing is that the expenditure has been decreased substantially. Whole exome sequencing has been utilized to recognize genes related with cancer [50], diabetes [51], immunologic disorders [52], and other circumstances. Transcriptome sequencing includes sequencing cDNA fragments generated by inverse transcription of RNA. Investigators be able to identify an RNA expression and splicing profile based on outcomes from transcriptome sequencing. Epigenetic analysis is and developing NGS usage to describe epigenetics in cancer. The potential Prognosis and diagnostic application of methylation and protein DNA binding profiles have been revealed [53].

NGS in Hereditary Cancer Syndrome Genetic Testing

Approximately lower than 10 % of cancers are family. Genetic examination has been employed for hereditary cancer invalids for over ten years in the US and Europe [54]. Currently, the most extensively utilized technique for genetic examination is Sanger based sequencing, which is considered the gold standard for detecting mutations. However, due to genes relevant to family cancers are many largest and there is no specific mutation hot point, this usual procedure for genetic testing of family cancer has been verified to be time consuming, high expenditure, and Low yields [55]. The progress of NGS supplied very opportunities for genetic examination Walsh et al. utilized purpose region take and NGS to diagnose 21 genes related with hereditary breast and ovarian cancer. This merged method permitted diagnosis of different types of changes, containing single nucleotide substitutions, small insertions and deletions, and large genomic duplications and deletions. In the US and Europe, routine testing for breast cancer 1 (*BRCA1*) and *BRCA2* are based on polymerase chain reaction (PCR) amplification of specific exons and Sanger sequencing of the products. For considerable exonic deletions and duplications, multiplex ligation dependent probe amplification (MLPA) has been supplemented for compatible testing. Nevertheless, MLPA can just be utilized to test known alterations [56]. NGS arranges an excellent method for diagnosis scarce alterations. Due to it applicable testing of numerous genes at once, NGS mostly enhances the alteration detection rate. Most of the patients with hereditary cancer have tested negative for genetic alterations, but with NGS, it is easier to detect causative mutations. Walsh and et al. in a study of 300 high-risk breast cancer families, identified already undetected mutations in 52 probands and the decreased sequencing expenditures and rotation time made the approach even more practical in clinics [57] Ozcelik et al. introduced a procedure that utilized long range PCR plus NGS to diagnosis *BRCA1* and BRCA2 and showed that it was beneficial for *BRCA* examination. For a small sample size, the procedure is combined with the Miseq or Ion torrent platform. Furthermore, this procedure may be more adaptable and profitable than a capture strategy [58]. A comparable procedure has as well as been reported previously by Hernan et al. [59] and De Leeneer et al. [60] The utilization of NGS in genetic testing for hereditary cancer syndromes will be the first and nearest step for its transfer into the clinical study. It is more exciting that whole genome or complete sequencing of malignant tumors has been utilized in numerous clinical trials for personalized therapy.

Complete genome sequencing can prepare a perfect spectrum of the genetic mutations, comprising single nucleotide variants (SNVs), short insertions/deletions (indels), copy number of variations (CNVs), and structure types. Until now, many individual's cancer genomes have been sequenced with success [61–63], and even more are expected in the close future. These usages provide worthwhile sequencing data for individual genomes and make it potential to conduct analysis in a sample-centered way, almost quickening our stages towards personalized detect and therapy.

Detection of Translocations and Inversions

Though many clinical molecular pathology laboratories have personnel with the technical specialty to adapt to doing high-throughput sequencing, the massive quantity of sequence data generated from the single patient specimen provides novel challenges for the laboratory, needing considerable investment in bioinformatics genesis and staff with coding specialty, if the computational examination is to be done in-house. Though every next-generation sequencing platform has an exclusive information-processing pipeline, similar designs are utilized to convert the crude sequence data into a form bowed to commentary. Preliminary as millions of sequencing reactions are an occurrence in parallel; one must be initial analyze universal run performance indexes to ensure that the tool (plate, reagents, etc.) is carrying out within characteristics. To perform this, many of the next-generations instruments consist of within-run standard control sequences. Following, every distinctive read must endure a quality evaluation designed to destination the error processes generally observed with a specific sequencing chemistry. The software algorithms have been advanced to reduce the "dephasing noise" which happens toward the end of Illumina reads [64], and to clarify principles to identify deletion or insertion errors, which happen in homopolymer areas during 454 pyrosequencing [65] High-throughput sequencing procedures can improve MRD discovery by describing genomic changes particular about a given patient's tumor, or through deep sequencing to diagnose small amounts of mutant or clonal DNA without a previous knowledge of the mutant DNA sequence. In an example of the first procedure, Leary et al. [13] utilized mate-pair library sequencing on the SOLiD platform to illustrate patient-particular translocations in solid-organ tumors, and then designed custom digital PCR assays to quantify the number of rearranged DNA molecules circulating in the patient's plasma.

Detection of Copy Number Variants

Copy Number Variation

Although much consideration has been tried to the discovery of SNPs, CNV of DNA fragments consisted of a considerable quantity of the genetic alteration among subjects [66, 67].

CNV has as well as been involved in diseases containing autism and psoriasis [68]. Most of these investigations were performed through the employ of array-based comparative genomic hybridization, whereas array-based procedures can be diagnosed large CNVs (nearly size 1 kb), unfortunately, cannot identified balanced structural alterations such as inversions [69]. High-throughput sequencing can be utilized to detection balanced and unbalanced CNVs through a method termed "paired-end mapping". In this procedure, genomic DNA is sheared to a explained size and ligated at each end as to be compatible oligonucleotides. The adaptors are then ligated to each other to form a circularized segment of DNA. After an extra fragmentation stage, the genomic DNA adjoining to the adaptors is sequenced; and the sequences are mapped to a reference genome. In a demonstration of this procedure utilizing 454 technologies, Korbel et al. identified many genetic alterations such as deletions, inversions, and insertions with a approximately 644 bp [70]. Paired-end mapping has as well as been applied with the Illumina platform to identified somatic gene rearrangements in several malignancies including, lung [28] and breast cancer [71]. Although sequencing-based procedures to diagnose of CNV are presently too costly and grinding for routine clinical detections, longer read lengths and lower reagent expenses may, in the future, capable sequencing methods to replace array genomic hybridization in the clinical investigations. Generally, CNVs are a main source of genomic variability and are particularly considerable in cancer. Until recent years microarray technologies have been employed to identification of CNVs in genomes. Whereas, development in next-generation sequencing technology propose considerable opportunities to decrease copy number straightly from genome sequencing information. Unfortunately cancer genomes vary from normal genomes in several characteristic that make them far less disposed to copy number discovery [72–80]. For example, cancer genomes are mostly aneuploid and a combination of diploid or non-tumor cell fractions. As well as patient-derivative xenograft types can be fraught with mouse pollution that extremely affects exact assignment of copy number. Therefore, there is a requirement to expand analytical instruments that can take into explanation cancer-special criteria for diagnosis of CNVs straightly from genome sequencing information [81]. Previously, have been expanded Wave CNV, a software pack to detection copy number variations by diagnosis breakpoints of CNVs utilizing translation-invariant discrete wavelet transforms and devote digitized copy numbers to any event utilizing next-generation sequencing information. As well as, assigned alleles ascertained the chromosomal proportion pursuing duplication or deletions. In addition, investigated copy number calls assign both microarray and quantitative polymerase chain reaction and found them to be highly coordinated [82].

Detection of Insertions and Deletions

Recently, multiple genomic researches have mentioned the huge convolution of the cancer genome. Genomic profiling applying microarray technology could layer the tumors into homogeneous subtype, offering new clinical visions for the progress of detections and treatments also systematic reviews on the underlying procedures of tumor development. Furthermore to the microarray technologies, eruptive progresses on sequencing technologies have been made newly, which is entitled NGS. This technology utilized massively parallel sequencing procedure generating hundreds of millions of short (~200 bp) DNA reads which can sequence a human genome quickly with much lower expense. This could be recovered by restricting a paired-end sequencing procedure, permitting significant progresses in identifying not only point mutations but as well as genomic rearrangements, such as deletions, amplifications, inversions, translocations, and gene-fusions [15, 16].

Familial Genetic Testing

Genetic analysis for high permeability familial malignancy genes, such as *BRCA1*, *BRCA2*, *APC* and the mismatch repair genes to name a few, is presented to persons presumed to be at high risk arising from their family and clinical history. The beginning of NGS should render to considerable expense savings through concurrent sequencing of numerous goals and numerous specimens. One instant and positive effect of decreased expense sequencing for genetic analysis is that subjects with diseases similar to breast and ovarian cancer, who don't assemble the present accurate criteria for advice genetic analysis, may become qualified for selection, since a main factor in indicating the restriction of such instructions is the expense of genetic analysis and accessible references. It has been descript that about 30–50 % of subjects with a mutation will not have a considerable family history to decision testing [83, 84]. Thus, these subjects would only be examination if other more local instructions are utilized like young age of initiation or triple negative breast tumour pathology. These groups are presumably to profit from a more easily accessible NGS method. There is an analogous status in high-degree serous ovarian cancer in that roughly 50 % of women with serous ovarian cancer who had a *BRCA1* or *BRCA2* mutation did not have a considerable family history [85]. In addition, lower frequency genes not commonly analyzed but involved in familial cancer syndromes could be contained in standard genetic screen if disease risks could be ascribed to mutations in such genes. The choice criteria for analysing could then be based on whether types within a special gene can be utilized to increase risk approximation given by the clinic to the patient instead of resource restrictions. An additional positive impact

would presumably be a decrease in the time period for genetic analysis from months to weeks while simultaneous enhancing the probable throughput for testing. This would enhance the usefulness of *BRCA1* and *BRCA2* assessing in the background of enrolment in trials open to bearers of *BRCA1* or *BRCA2* mutations like Poly (ADP ribose) polymerase (PARP) inhibitor trials and as a assistance to surgical management decisions [86]. There have been multiple recent papers explaining the utilization of NGS for the goal of familial cancer genetic screening [87–90]. A first study by Morgan et al. utilized LRPCR to reinforce and then sequence among all exons of *BRCA1*, *BRCA2* and *TP53* in a series of cell lines and patient samples [87]. Rather small goal size enabled merger of samples for sequencing on a single lane of an Illumina Genome Analyzer (IIx) stream cell. They discovered that all known pathogenic types could be revealed, containing deletions up to16 bp, with zero false positives utilizing both the commercial software NextGene (SoftGenetics) or custom developed software for analysis. A second study by Walsh et al. utilized a hybridization capture procedure to sequence 21 genes known to be associated with aptitude to breast and ovarian cancer [90].

Conclusion

NGS has been used in cancer genomics study and transitioned to be applied in clinical practice. However, one should always keep in mind that although characterization of structural changes in the cancer genomes by NGS will provide important pieces of knowledge, epigenetic changes, contributions from the tumor microenvironment and germline genetic variation will also have to be taken into account to have the full picture of the disease.

Compliance with Ethical Standards

Conflict of Interest Maryam Sabour declares that she has no conflict of interest. Leila Sabour declares that she has no conflict of interest. Saeid Ghorbian declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants performed by any of the authors.

Informed Consent This article is not involved Informed Consent.

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