

Case report

Usefulness of Next-Generation Sequencing for precision medicine in cancers: Report of three familial cases

Fatima Maarouf^{1*}, Amal Tazzite^{2,3}, Hind Dehbi^{1,2}¹Laboratory of Medical Genetics, Ibn Rochd University Hospital, Casablanca, Morocco²Laboratory of Cellular and Molecular Pathology, Faculty of Medicine and Pharmacy of Casablanca, Hassan II University, Casablanca, Morocco³Higher Institute of Nursing Professions and Health Techniques of Casablanca, Morocco**Abstract**

High-throughput next-generation sequencing has revolutionized cancer molecular diagnosis and research. Indeed, molecular analysis revealed that the same type of tumor may exhibit diverse molecular subgroups. This technology enables the identification of novel specific genes implicated in tumor progression. Moreover, identification of specific mutations and active molecular pathways play a crucial role in identifying new drug targets. The present study highlights the clinical utility of next-generation sequencing in the management of cancer by reporting three patients with family cancer history (Li-Fraumeni syndrome, breast cancer, and colon cancer), observed in the Medical Genetics Department of Ibn Rochd University Hospital in Casablanca, Morocco.

Keywords: Next-generation sequencing, colon, breast, Li-Fraumeni syndrome, cancer, Precision Medicine.

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1. Introduction

Cancer plays a significant role in global mortality [1]. Its complexity arises from a combination of genetic, environmental, and lifestyle factors [2]. Currently, about 350 genes are directly linked to cancers, and 2,000 genes potentially play a role in cancer initiation or progression [3]. Genetic mutations in cancers fall into two categories: "germline" mutations specific to each individual, and "acquired" or "somatic" mutations. Identifying which mutations promote cancer development is crucial for tumor biology understanding and targeted therapy development. Driver mutations drive selective growth promoting cancer; however, passenger mutations lack this effect [4]. Although generally inactive, these passenger mutations can sometimes evolve into driver mutations, contributing to cancer recurrence [5]. The last decade has shown remarkable progress in the development of targeted therapies based on tumor morphological and molecular characteristics. The efficacy of these targeted therapies is closely linked to the activation or inactivation of signaling pathways involved in cancer cells growth and survival, as well as the drug-target affinity. These parameters are influenced by specific genomic alterations [6]. Next-generation sequencing (NGS) has enabled the identification of millions of mutations and epigenetic changes in cancer cells.

*Corresponding author: Dr. Fatima Maarouf, Ibn Rochd University Hospital Center, Casablanca, Morocco.
Email: maaroufcadem@gmail.com

Furthermore, NGS has facilitated the generation of more extensive data and simultaneous analysis of numerous genes, reducing the costs and time associated with these analyses. The present study explores the clinical utility of NGS in cancer diseases, especially in screening, diagnosis, prognosis, determination of therapeutic targets, monitoring, and genetic counseling for cancer patients. We also discuss the current limitations of this technology. For this purpose, we report three patients with different types of cancer (colon cancer, breast cancer, and Li-Fraumeni syndrome) who were observed at the Medical Genetics Department of Ibn Rochd University Hospital in Casablanca, Morocco.

2. Case Presentation

Patient 1

The first case is a 30-year-old woman, married and mother of one child. The patient presented with benign breast adenofibroma and a family history of breast cancer diagnosed in her mother before the age of 30 and her elder sister at the age of 35. Moreover, an undocumented bone neoplasm in her elder brother and a breast neoplasm in her paternal aunt have been reported (Fig. 1). Thereby, hereditary genetic syndrome has been suspected and whole-exome sequencing has been performed in the index case. The results have revealed the presence of a pathogenic heterozygous mutation in the *TP53* gene (NM_000546.5: c.844C>G; p.Arg282Gly) confirming Li Fraumeni syndrome (LFS). In accordance with the "Toronto

Protocol", regular surveillance is recommended for our patient.

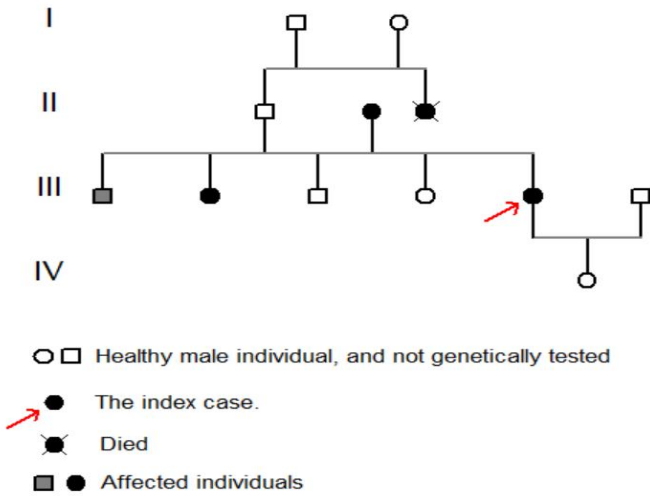


Fig.1. Pedigree of the patient with Li Fraumeni syndrome

Patient 2

The second case is a 41-year-old woman with a family history of breast cancer in her mother and aunt. Indeed, her mother was diagnosed with triple-negative invasive ductal carcinoma and therefore did not undergo hormone therapy or targeted treatment (Fig. 2). The patient has undergone genetic counseling to assess her cancer genetic risk. After obtaining her informed consent, NGS has been conducted. The results revealed a heterozygous pathogenic deletion in the *BRCA2* gene (NM_000059.4: c.3381del; p.Phe1127fs). Subsequently, a familial investigation was initiated, and a genetic testing was prescribed for her daughter to determine whether surveillance should be continued.

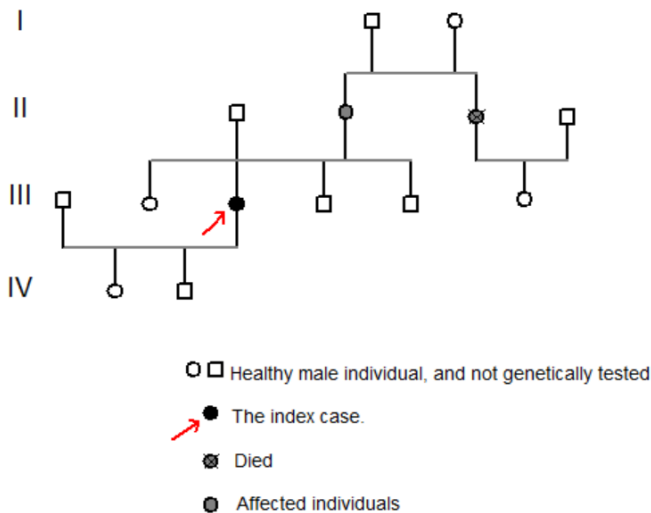


Fig.2. Pedigree of the Patient with hereditary breast cancer

Patient 3

The latest case concerns a 62-year-old man diagnosed with left-sided colorectal adenocarcinoma, who has a family history that includes a sister diagnosed with endometrial

cancer (Fig. 3). He has been referred to our genetic department for personalized treatment and prognostic assessment. The tumor was characterized by elevated tumor markers and the presence of hepatic and bone metastases. Upon obtaining informed consent, NGS of the tumor and whole-exome sequencing of blood were conducted. The tumor analysis revealed the presence of two distinct mutations: K117N (c.351A>T) in *KRAS* gene and H1074R (c.3140A>G) in *PIK3CA* gene. Conversely, the genetic analysis of peripheral blood was normal. The identified *KRAS* mutation is known to be associated with resistance to treatment with epidermal growth factor receptor (EGFR) inhibitors, particularly cetuximab, suggesting potential ineffectiveness of this treatment. However, microsatellite instability in stage 2 and 3 colorectal cancers (CRC) without *BRAF* mutations is associated with a favorable prognosis. These data justify adjuvant chemotherapy recommendation.

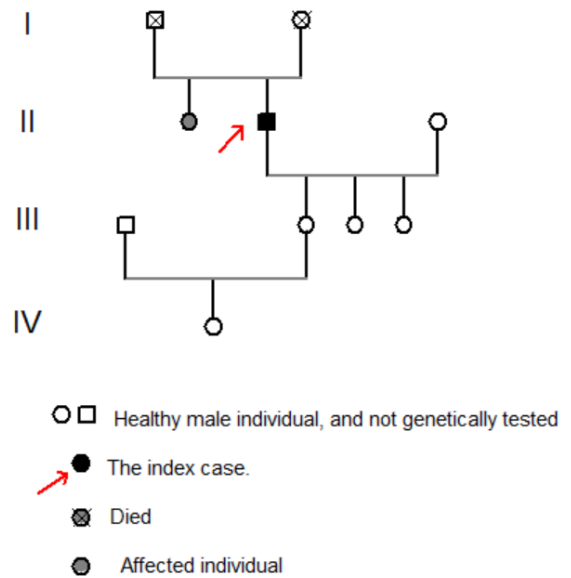


Fig.3. Pedigree of the patient with colorectal cancer

4. Discussion

Cancer is a complex disease resulting from various genetic alterations [7]. To better understand these changes, the NGS technology has been introduced. This latter enables the complete human genome sequencing in a matter of weeks with high sensitivity and specificity. The emergence of NGS has significantly improved medical practice by allowing a more precise diagnosis and personalized treatment development [8]. Moreover, it has enhanced prognostic understanding by identifying genetic markers. Moreover, NGS plays a crucial role in genetic risk prediction for offspring [8,9].

Role of NGS in screening and diagnosis

NGS plays an essential role in the management of cancer patients, particularly in screening and diagnosis. The first case of the present study is an illustrative example (Fig. 1). LFS, a rare autosomal dominant hereditary syndrome, arises from mutations in either *TP53* or *CHEK2* genes, with *TP53* mutations being the most prevalent (70%) [10].

TP53, located on chromosome 17p13.1, serves as a tumor suppressor gene, encoding the p53 protein pivotal in intricate cellular processes like cell cycle arrest, DNA repair, genomic stability, and apoptosis. The loss of p53 functions initiates a series of events culminating in the development of diverse malignant tumors, including premenopausal breast cancers, soft tissue sarcomas, osteosarcomas, brain tumors, and corticosteroidomas [11].

The overall cancer risk exhibits slight gender variation, with women presenting a higher risk at the age of 50 and a tendency to develop cancer at an earlier age. Therefore, vigilant monitoring of cancer onset is important for individuals at risk, including those with known *TP53* mutation. Although the absence of an officially approved or recommended screening program for individuals with LFS, the Toronto Protocol (Table 1) is widely adopted for screening families affected by this syndrome [12].

Role of NGS in genetic counseling

In our second observation, we highlighted the crucial role of NGS in genetic counseling for patients with a family history of breast cancer, the most common form of cancer in women. It is noteworthy that breast cancer in men remains rare. Approximately 5% of breast and ovarian cancer cases result from a hereditary predisposition, transmitted in an autosomal dominant manner and associated with constitutional genetic mutations in the *BRCA1* and *BRCA2* genes [13]. The expressivity of this syndrome varies considerably among individuals and within the same family. In women carrying mutations in the *BRCA1* or *BRCA2* genes, the risk of developing breast cancer is significantly elevated. However, according to a recent meta-analysis, the cumulative risk by the age of 70 is

estimated at 64.6% (95% confidence interval [CI] = 59.5% to 69.4%) for *BRCA1* mutation carriers and 61% (95% CI = 48.1% to 72.5%) for *BRCA2* mutation carriers. In association with breast cancer, ovarian cancer is less frequent and is caused by mutations in the *BRCA2* gene in 15% of cases, and *BRCA1* mutations in 30 to 40% of cases. For ovarian cancer, the cumulative risk by the age of 70 was 48.3% (95% CI = 38.8% to 57.9%) for *BRCA1* mutation carriers and 20% (95% CI = 13.3% to 29%) for *BRCA2* mutation carriers (Table 2) [14].

In the present case, annual radiological surveillance and semi-annual clinical palpation are recommended. Moreover, it is crucial to consider the high risk of ovarian cancer. Thereby, prophylactic surgery, specifically oophorectomy, is recommended at the age of 45 (or from age 40 in the case of *BRCA1* mutation). Additionally, prophylactic mastectomy, with or without reconstruction, may be considered. This preventive intervention also reduces the risk of developing another breast cancer by 50% [16]. In the event that the daughter carries the mutation, a preventive bilateral mastectomy was recommended for both the patient and her daughter. This recommendation is based on the estimated 50% risk of inheriting the mutation for the daughter, considering that the risk of breast cancer in carriers of the *BRCA2* mutation before the age of 50 is approximately 35% [15].

For men carrying this mutation, the risk of breast cancer remains low, but there is a slight increased risk of developing prostate cancer. It is noteworthy that these men have a 50% risk of transmitting the mutation to their offspring. Therefore, appropriate medical management and follow-up are essential to address these potential risks and ensure personalized and preventive monitoring [15,16].

Table 1. Surveillance strategy for individuals with germline *TP53* mutations (Toronto Protocol) [12].

| Tumor Type | Surveillance Strategy |
|--|--|
| Children | |
| <i>Adrenocortical carcinoma</i> | <ul style="list-style-type: none"> • Ultrasound of abdomen and pelvis every 3-4 months • Complete urinalysis every 3-4 months • Bloodwork every 4 months : ESR, LDH, beta-hCG, alphafetoprotein, 17-OH-progesterone, testosterone, DHEA, androstenedione |
| <i>Brain tumor</i> | Annual MRI of the brain |
| <i>Soft tissue and bone sarcoma</i> | Annual rapid total body MRI |
| <i>Leukemia/lymphoma</i> | CBC profile/LDH/ESR every 3-4 months |
| Regular evaluation with family physician with close attention to any medical concerns or complaints | |
| Adults | |
| <i>Breast cancer</i> | <ul style="list-style-type: none"> • Monthly breast self-examination starting at age 18y • Semiannual clinical breast exam starting at age 20-25y, or 5-10y before the earliest known breast cancer in the family • Annual mammogram and breast MRI screening starting at age 20-25y, or individualized based on earliest age of onset in family • Consider risk-reducing bilateral mastectomy |
| <i>Brain tumor</i> | Annual MRI of the brain |
| <i>Soft tissue and bone sarcoma</i> | Annual rapid total body MRI |
| <i>Colon cancer</i> | Biennial colonoscopies beginning at age 40y, or 10y before the earliest known colon cancer in the family |
| <i>Melanoma</i> | Annual dermatology examination |
| <i>Leukemia/ Lymphoma</i> | CBC profile/ LDH/ ESR every 3-4 months |
| <i>Intra-abdominal tumor (eg.Sarcoma)</i> | Annual abdominal ultrasound |
| Regular evaluation with family physician with close attention to any medical concerns or complaints | |

Table 2. Risk of breast and ovarian cancer in *BRCA* mutation carriers according to different studies

| | <i>BRCA1</i> carriers | | | <i>BRCA2</i> carriers | | |
|----------------------------|-----------------------|---------------------------|-----------------------|-----------------------|---------------------------|-----------------------|
| | BCLC [15] | Aissaoui et al. 2017 [15] | Chen et al. 2020 [14] | BCLC [15] | Aissaoui et al. 2017 [15] | Chen et al. 2020 [14] |
| Breast cancer risk | | | | | | |
| 50 years | 73% (49-87) | 38% (30-50) | 39.5% (34.9-44.2) | 28% (9-44) | 16% (11-21) | 31.5 % (24.4-39.5) |
| 70 years | 87% (72-95) | 65% (51-75) | 64.6% (59.5-69.4) | 84% (43-95) | 45% (33-54) | 61% (48.1-72.5) |
| Ovarian cancer risk | | | | | | |
| 50 years | 29% (16-40) | 13% (8-18) | 13.1% (7.8-21.2) | 0.4% (0-1) | 1% (0-3) | 3.7% (2.2-6.1) |
| 70 years | 44% (28-56) | 39% (22-51) | 48.3% (38.8-57.9) | 27% (0-47) | 11% (4-18) | 20% (13.3-29) |

BCLC: Breast cancer linkage consortium. In brackets: 95% confidence interval.

Role of NGS in Prognosis and personalizing oncology treatments

The molecular characterization of tumors before treatment has significantly improved the efficacy of targeted therapies, leading to better management of patients with CRC. The target genes associated with CRC can be divided into two groups: oncogenes such as *KRAS2*, and tumor suppressor genes such as *APC*, *TP53*, and *DCC* [17]. Three major types of instability are observed in CRC: chromosomal instability (LOH), microsatellite instability (MSI), and the CpG island methylation phenotype (CIMP). Each pathway lead to cancer development by modifying a distinct set of pathway-specific genes [18].

Approximately 50% of CRC patients have mutations in the *RAS* gene, resulting in sustained activation of the EGFR signaling pathway and subsequent oncogenic transformation. The majority of these mutations (>90%) are found in the *KRAS* gene, especially in codons 12 and 13 of exon 2 [19]. A recent study has emphasized the importance of extending mutation screening to include exons 3 and 4 of the *KRAS* gene, as well as exons 2, 3, and 4 of the *NRAS* gene, for all patients with metastatic colorectal cancer (mCRC) [20].

According to the literature, it is established that patients with non-mutated *RAS* tumors benefit most from anti-EGFR therapy. The combination of anti-EGFR and chemotherapy has shown substantial improvement in overall survival. Therefore, a thorough screening of these mutations is imperative before initiating anti-EGFR therapy in patients with mCRC, facilitating the prediction of treatment response [21].

The limits of NGS

Despite advancements in NGS, some limitations should be stated. Indeed, short-length sequences present difficulties in analyzing degraded tumor DNA. Pre-analytical variables, including cellular heterogeneity and extraction methods, affect accuracy. Sequencing platforms introduce variability impacting reproducibility, error rates, and sensitivity [22]. Bioinformatics is crucial for data analysis, but the substantial information complicates processing. Interpretation is intricate, and read depth and coverage affect pathogenic variant detection. Storing data on large-capacity hard drives is essential. Cost is a limiting factor, and efficient data analysis remains a bottleneck. The discovery of numerous variants in oncology raises questions about their relevance [22].

The ethical implications of NGS

Overall, the International Bioethics Committee (Comité Consultatif National d'Ethique (CCNE)) has addressed the ethical and legal issues associated with NGS [23]. It highlights the urgent need to regulate the use of NGS in clinical practice NGS and to establish a strict protocol to guide results interpretation. This includes a procedure for dealing with incidental findings of variants considered pathogenic but present in genes that were not initially intended to be explored, taking into account the potential impact of these results on an individual [23].

In conclusion, NGS is designed to sequence long and complex genes and/or multiple genes, with the aim of identifying alterations that can serve as therapeutic targets and therefore facilitate cancer patient management. The current challenge is to understand the biological relevance of the detected genomic aberrations in cancer progression. However, this transition requires active ethical vigilance. Despite current limitations, NGS has the potential to advance personalized medicine in oncology.

Consent of patient

Written informed consent was obtained from the three patients for participation in this study.

Consent for publication

Written informed consent was obtained from the three patients for publication of this case report and any accompanying images.

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None.

Conflict of Interest Disclosures

All authors declare that they have no conflict of interest.

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Authors' contributions

The collection of family data, clinical and paraclinical examination, and the drafting of the initial manuscript were carried out by MF and DH. The analysis and interpretation of data were performed by HD, MF, and TA. Drafting of the manuscript was done by MF. HD coordinated and supervised data collection, and reviewed and revised the manuscript. TA reviewed and revised the manuscript. All authors contributed to the article and approved the submitted version.

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