Case Report

Identification of a Novel Mutation in Hereditary Breast Cancer in a Family with Wide Spectrum of Atypical Malignancies

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Abstract

Due to phenotypic variability, age-related penetrance, and variety of targeted organs, many families with an inherited cancer syndrome will not meet syndrome-specific criteria. We report hereditary breast cancer case in a family with gastric cancer, pancreatic cancer, lung sarcoma and penile cancer, suggesting several alternative hereditary cancer syndromes.

Mutation screening by NGS was performed using three panels with BRCA1, BRCA2, TP53, BMPR1A, SMAD4, CDH1, STK11, PTEN genes and 409 genes from Ion AmpliSeq Comprehensive Cancer Panel.

In maternal lineage we found a novel mutation c.5193_5194del:p.H1731fs in BRCA2 resulted in HBOC. 7 of 8 tested family members (87.5%) were carriers of the mutation; 4 would have been expected from mendelian ratio of 50%. In order to find out whether the paternal lineage could have another hereditary cancer syndrome we tested paternal uncle with penile carcinoma. 409 gene panel revealed no pathogenic mutations. VUS's identified in this study cannot be used to make clinical decisions.

A positive cancer family history in itself is usually not enough to diagnose a cancer syndrome, especially when common type cancers are involved. Chance alone may cause the clustering of cancer (especially in large families) and the number of possible unaffected relatives.

Keywords: Hereditary breast cancer, maternal and paternal lineage, novel mutation

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Over 200 hereditary cancer susceptibility syndromes have been described, they are thought to account for at least 5–10% of all cancers.[1] Hereditary cancer syndromes related to breast cancer include hereditary breast and ovarian cancer syndrome (HBOC), Lynch syndrome, Li–Fraumeni syndrome (LFS), Cowden syndrome, Peutz–Jeghers syndrome, hereditary diffuse gastric cancer (HDGC), ataxia-telangiectasia.[2] Because of phenotypic variability, age-related penetrance, gender-specific cancer risks, and variety of targeted organs, many families with an inherited cancer syndrome will not meet syndrome-specific criteria so definition of syndrome type is often a challenging task. A comprehensive review of the patient's personal and family history is essential to assess the risk for the particular hereditary cancer syndrome. The next step is searching for the affected gene by genetic testing that can aid the patient, family members, and their physicians in making appropriate medical management decisions. Here we present a case of triple negative breast cancer diagnosed in a young woman in a family with different cancer types including gastric cancer, pancreatic cancer, lung sarcoma and penile cancer, suggesting several alternative hereditary cancer syndromes for testing.

Materials and Methods

Mutation screening by NGS was performed using three panels: an Ion AmpliSeq BRCA1 and BRCA2 Panel (Thermo Fisher Scientific), an in-house customized panel comprised of six hereditary cancer related genes, and an Ion AmpliSeq™ Comprehensive Cancer Panel (Thermo Fisher Scientific).

Ion AmpliSeq BRCA1 and BRCA2 Panel covers 100% of the coding regions of BRCA1 and BRCA2 genes, exon-intron boundaries, and 10-20 bases beyond. The panel is comprised of 167 primer pairs in three pools and covers 16,246 bases of human genomic DNA. The Ion AmpliSeq Comprehensive Cancer Panel provides highly multiplexed target selection of 409 genes implicated in cancer research. An in-house customized panel is comprised of BMPR1A, SMAD4, CDH1, TP53, STK11, and PTEN genes. A panel of 218 primer pairs was designed to amplify coding regions, noncoding regions of the terminal exons, and no less than 25 bases of adjacent introns. The panel was designed using the Ion Ampliseq Designer v.3.6, which minimizes the number of oligonucleotide pair pools that are necessary to completely cover the target genomic sequences. The total length of human genome sequences covered is 42,320 bases.

NGS was performed on the Ion Torrent PGM (Personal Genome Machine) platform (Thermo Fisher Scientific). The protocol included preparation of libraries of genomic DNA fragments, clonal amplification, sequencing with a genome analyzer, and bioinformatics analysis of the results. Multiplex PCR (polymerase chain reaction) and subsequent steps of library preparation were carried out using an AmpliSeq Library 2.0 kit as was previously recommended.[3] Aliquots of the libraries were used for clonal amplification on microbeads via emulsion PCR on the Ion OneTouch instrument with the Ion PGM Template OT2 200 Kit (Thermo Fisher Scientific). PCR products (microbeads covered with target amplicons) were purified with an Ion OneTouch ES instrument.

Sequencing was carried out using Ion 316 arrays, and an Ion PGM Sequencing 200 Kit (Thermo Fisher Scientific), according to the manufacturer's recommendations. Sequencing results were analyzed using Torrent Suite software, including Base Caller (a primary analysis of the sequencing results), Torrent Mapping Alignment Program (TMAP; sequence alignment with the NCBI (National Center for Biotechnology Information) reference genome build 37, hg19), and Variant Caller (a nucleotide sequence variation analysis). The functional significance of genetic variations was annotated and known polymorphisms were filtered against gnomAD with ANNOVAR (variant annotation) software. Bioinformatic analyses utilized the ClinVar archive of clinically significant genetic variants. A visual data analysis,

manual filtration of sequencing artifacts, and manual sequence alignment were carried out using Integrative Genomics Viewer.

Verification of mutations detected by NGS-based screening was performed by Sanger sequencing. Individual PCR products were directly sequenced from primers flanking the regions of particular mutations, using a 3500 Genetic Analyzer according to Thermo Fisher Scientific protocols. Sanger sequencing was performed on blood DNA samples from all available family members to determine mutation carriers.

Case Report

A 35-year-old woman presented with a palpable right breast mass in December 2015. She underwent ultrasound imaging, which confirmed a 1.5-cm mass. The mass was biopsied and revealed a high grade, triple negative invasive ductal carcinoma (estrogen receptor 0%, progesterone receptor 0%, and human epidermal growth factor receptor 2 negative, ki-67 85%). She was clinically node negative. Taking into account young age and high ki-67 level the patient received 6 cycles of carboplatin and paclitaxel chemotherapy as neoadjuvant treatment.

Due to the patient's personal and family history, she was offered genetic counseling during chemotherapy period. Her family history was notable for multiple relatives on her paternal side with gastric cancer, sarcoma of lung, penile cancer and low limbs paraplegia of unknown etiology. Additionally, her maternal uncle was diagnosed with pancreatic cancer at 38 years of age (Fig. 1a). So we suggested that the mutation can be inherited both from the mother's and father's sides and suspected three syndromes: HBOC, LFS like syndrome and hereditary cancer-predisposing syndrome (HCPS) not otherwise specified.

Hereditary breast ovarian cancer (HBOC, MIM 113705) syndrome is the most common form of inherited breast cancer and is caused by germline mutations in BRCA1 and BRCA2 genes. Along with early-onset breast and ovarian cancer, other cancers seen more commonly in BRCA mutation carriers include fallopian tube cancer, pancreatic cancer, stomach cancer, and laryngeal cancer.[4,5] A number of founder mutations have been identified in various populations. The most common in Russia are the 8 in BRCA1 (185delAG, 4153delA, 5382insC, 3875delA, 3819del5, C61G, 2080delA, T5286G).[6]

Li–Fraumeni syndrome (LFS, MIM 151623) and Li-Fraumeni-like (LFL) syndrome are characterized by the development of soft-tissue sarcomas, early-onset breast cancer, and other malignancies in young children and adults. The major component cancers of LFS are sarcomas, breast cancer, brain tumours, adrenocortical carcinoma, and acute
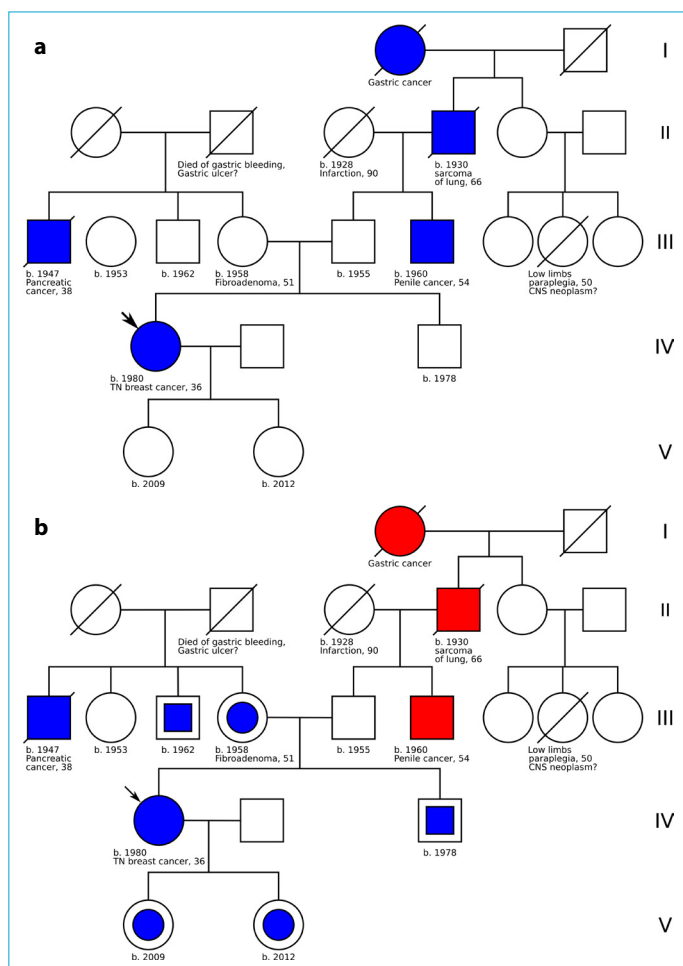
Figure 1. Family tree. **(a)** The arrow indicates the patient. Numbers indicate age (in years) at present, at diagnosis with the cancer type stated or at mortality. Blue color indicates person affected by cancer. **(b)** Family tree after the identification of maternal lineage mutation. Blue color indicates carriers of the BRCA2 mutation. Red color indicates paternal lineage family members without BRCA2 mutation and affected by cancer.

leukemias.[1] Classically, the diagnosis of Li-Fraumeni cancer syndrome requires the occurrence of a sarcoma in a patient younger than 45 years, a first-degree relative younger than 45 years with a cancer (unspecified type), and a first- or second-degree relative with a sarcoma at any age or any cancer diagnosis before the age of 45.[7] Families presenting incomplete features of LFS are referred as having Li-Fraumeni-like syndrome (LFL). Germline mutations of the tumor suppressor gene TP53 account for more than half of the families with classic Li-Fraumeni syndrome. Up to 22% of LFL pedigrees have detectable TP53 mutations.[8] Taking into account paternal grandfather with lung sarcoma at 66, proband with breast cancer at 35 and maternal uncle with pancreatic cancer at 38 we couldn't exclude possibility of LFL of both paternal and maternal lineage and recommend to test TP53 gene mutations.

In addition to hereditary breast and ovarian cancer syndrome and Li-Fraumeni syndrome, combined breast and gastric cancer risk is elevated in Peutz-Jeghers syndrome associated with germline STK11 mutations, and in juvenile polyposis syndrome associated with germline mutations in SMAD4 and BMPR1A genes.[9] Although the family cases fulfilled criteria of above mentioned syndromes partially we decided to test CDH1, BMPR1A, SMAD4, STK11, PTEN together with BRCA1, BRCA2 and TP53.

Results

Patient with breast cancer.

Molecular diagnostics was performed on genomic DNA extracted from a blood sample after informed consent was obtained from the patient. First, screening for BRCA Russian common mutations (185delAG, 4153delA, 5382insC, 3875delA, 3819del5, C61G, 2080delA, T5286G) in BRCA1 was negative for the patient. Next, to screen BRCA1, BRCA2, TP53, BMPR1A, SMAD4, CDH1, STK11 and PTEN genes we performed panel-based next-generation sequencing (NGS). We found a novel heterozygous deletion NM_000059 (BRCA2): c.5193_5194del:p.H1731fs in exon 11 of BRCA2. This deletion results in a frameshift at histidine 1713, truncating the encoded protein. The result was confirmed using Sanger sequencing (Fig. 2). This mutation has not been described in human genome variation databases, including the 1000 Genomes Project, Exome Aggregation Consortium (ExAC) or in disease-causing mutation databases, such as the Human Gene Mutation Database (HGMD) Professional 2021.2 and ClinVar. However the similar mutation located in the same codon NM_000059.3(BRCA2):c.5192_5193del (p.His1731fs) is described as pathogenic by ClinVar database.

Thus, the patient subsequently completed 6 cycles of standard chemotherapy with carboplatin and paclitaxel followed by radical mastectomy with tissue expander reconstruction. Histological examination revealed pathologic complete response. At the latest follow-up point, the patient was 2.5 years after the diagnosis with no evidence of disease. She underwent contralateral prophylactic mastec-
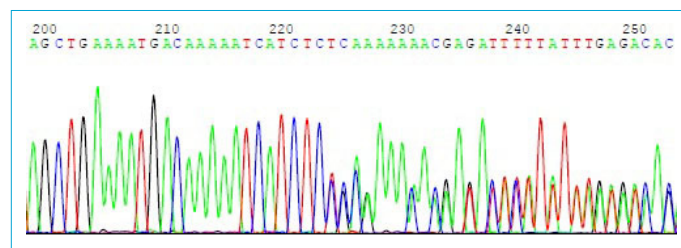

Figure 2. Mutation in BRCA2 observed in the family. Sanger sequencing of c.5193_5194del:p.H1731fs mutation in 11 exon of BRCA2 gene

tomy at age 38. Pathological report revealed no evidence of malignancy. The patient was counseled to undergo risk-reducing bilateral salpingo-oophorectomy. Risk management procedures according to NCCN guidelines were recommended for the patient's mother, uncle and brother who carried mutation. The patient was also educated that her daughters should be managed at age 18. Two years after genetic testing the proband's maternal uncle was diagnosed with prostate cancer at age 56.

Further analysis of her parents' DNA samples revealed that the mother had the same mutation, but the father had wild type of BRCA2. The patient's unaffected uncle, brother and two daughters were heterozygous for this mutation (Fig. 1b). Interestingly, we found that 7 of 8 tested family members (87.5%) were carriers of the mutation; although 4 would have been expected due to mendelian ratio of 50%.

Patient with penile carcinoma.

Thus the patient had the BRCA2 pathogenic mutation inherited from her mother and resulted in HBOC. Still the paternal family history was notable for multiple relatives with penile squamous cell carcinoma, lung sarcoma, gastric cancer and paraplegia of low limbs with unknown etiology (the possibility of central nervous malignancy could not be ruled out) (Fig. 1b). We hypothesized that paternal relatives could have another hereditary cancer-predisposing syndrome. In order to pursue this hypothesis, we chose the paternal patient's uncle with penile carcinoma for the further genetic testing.

A 54-year old male was diagnosed with penile carcinoma. The patient had a 2 cm lesion on the prepuce. Circumcision followed by 2 cycles of adjuvant chemotherapy (cysplatin plus 5- fluorouracil) was performed. Pathological findings revealed moderately differentiated invasive squamous cell carcinoma T1aN0Mx. After treatment, no recurrence was detected during a 4-year observation period. Due to the patient's family history (his father and paternal grandmother were diagnosed with lung carcinoma and gastric cancer, respectively), he was offered genetic counseling. As

the family cases didn't meet any syndrome-specific criteria we decided to use the 409 genes panel covering most frequently cited and frequently mutated oncogenes and tumor suppressor genes.

The NGS results revealed that he carried 8 SNVs with allele frequency <0.0005% in the general population according to the gnomAD database, 5 of which being classified as VUS according to ACMG (Table 1). Unfortunately, testing of affected and unaffected patient's mother and father as well as other affected family members for VUS co-segregation analysis was unavailable (they passed away).

Importantly, no pathogenic mutations were detected and the VUS's identified in this study cannot be used to make clinical decisions. The family members were noticed that they will be informed if these variants may in the future be reclassified as deleterious or benign.

Discussion

In this paper, we report hereditary breast cancer case in a family with different cancer types including gastric cancer, pancreatic cancer, lung sarcoma and penile cancer, suggesting several alternative hereditary cancer syndromes. The wide spectrum of atypical malignancies for hereditary breast cancer was the reason for multi-gene panel testing. We found a novel frameshift heterozygous deletion c.5193_5194del:p.H1731fs in BRCA2 inherited from mother and resulted in HBOC.

In the Breast Cancer Linkage Consortium (BCLC) families, BRCA2 carriers had a breast cancer risk of 84% (95% CI 43–95%), and an ovarian cancer risk of 27% (95% CI, 0–47%).[10] Individuals with a BRCA2 mutation had a high risk for pancreatic cancer in both men and women and prostate cancer in men.[11] BRCA2 mutation identified in our present study has caused breast cancer at age 35, pancreatic cancer at age 38 and prostate cancer at age 56. Two male carriers were affected. To date there have been no ovarian cancer cases in the family.

Table 1. Penile cancer patient NGS results: identified SNV's with frequency <0.0005

Gene	Mutation	GnomAD frequency	Clinical significance	SIFT	Polyphen2_HDIV	Polyphen2_HVAR	LRT	Mutation Taster
ARNT	exon9:c.A887G: p.Y296C	.	VUS	D	D	P	D	D
DAXX	exon2:c.C685T: p.L229F	.	VUS	D	D	D	D	D
MET	exon2:c.G406A: p.V136I	0.0003	VUS	T	P	B	D	D
THBS1	exon17:c.G2646T: p.Q882H	.	VUS	D	D	D	N	D
TGM7	exon3:c.T338C: p.I113T	7.31E-05	VUS	D	D	D	D	D

GnomAD - The Genome Aggregation Database (http://gnomad.broadinstitute.org). Prediction tools: SIFT, Polyphen2 HDIV, Polyphen2 HVAR, LRT, Mutation Taster. D – damaged, T – tolerated, P – probably damaging, B – benign, N – neutral.

The fact of non-random transmission of mutant alleles to offspring in BRCA carriers was discussed earlier.[12-14] Gronwald et al. reported that 61.5% of unaffected female offspring carried the mutant allele in Poland. De la Hoya et al have undertaken a similar study in a Spanish and Dutch cohort and have also observed a higher ratio of carrier daughters (58% in those <30 years of age). However, Evans et al have found no evidence of non-random transmission in an English cohort. Our data is consistent with an excess of carriers over non-carriers.

Still the paternal family history was notable for three cancer cases. In order to find out whether the paternal lineage could have another hereditary cancer syndrome we chose paternal uncle with penile carcinoma for the further genetic testing. 409 gene panel revealed no pathogenic mutations and two VUS's associated with HCPS. The patient's test result did not rule out an inherited risk for cancer for him or his family members. While his multi-gene panel test was inconclusive, the patient and his family members remain at increased risk for the cancers present in close family members. It is possible that he has an inherited mutation in a different cancer risk gene or a mutation in a targeted gene that was not identified. It is also possible that his cancer was due to multifactorial cancer risk, where multiple genetic factors he inherited from one or both sides of the family have combined with environmental factors to increase his risk for cancer. Finally, it could not be ruled out that the patient had sporadic cancer diagnosis. It may be that he, his father or his grandmother developed their cancer sporadically.

NGS panel testing may benefit patients with a personal or family history compatible with more than one recognized inherited syndrome. The use of cancer gene panels, although they can be beneficial in many cases, may also reveal incidental information and inconclusive findings or VUS. The VUS rate increases with the addition of moderate-penetrance and low-penetrance genes.[15] A panel test could show multiple VUS's in different genes.[16] VUS rates are in the range from 9% to 42% in multi-gene panels.[17] There is also the possibility of harm of medical interventions based on erroneous interpretation of VUS.

A positive cancer family history in itself is usually not enough to diagnose a cancer syndrome, especially when common type cancers are involved. Chance alone may cause the clustering of cancer (especially in large families), and the size of the family, the number of possible unaffected relatives, and their place in the pedigree is important.

Disclosures

Informed consent: Written informed consent was obtained from the patient for the publication of the case report and the accompanying images.

Peer-review: Externally peer-reviewed.

Conflict of Interest: None declared.

Authorship Contributions: Concept – T.K.; Design – T.K.; Supervision – V.S.; Materials – A.S.; Data collection &/or processing – A.S.; Analysis and/or interpretation – A.T.; Literature search – T.K.; Writing – T.K.; Critical review – V.S.

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