The Frequency and Management of TP53 Mutation Carriers in Turkish Patients with BRCA-Negative Breast Cancer Under 50 Years of Age

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OBJECTIVE

Germline mutations in the *TP53* gene cause Li-Fraumeni Syndrome (LFS). Breast cancer (BC) is the most common cancer that is seen in young women with LFS. The majority of BC in LFS occurs between 15 and 44 age of years. The present study aims to determine the frequency of *TP53* gene germline mutation carriers in Turkish patients with BRCA-negative BC under 50 years of age as the first study from Turkey, to our knowledge, and to emphasize the importance of management in *TP53* gene mutation carriers.

METHODS

One hundred patients with BRCA-negative BC younger than 50 years old were evaluated concerning mutations in the *TP53* gene between 2016 and 2017 years. Sequencing analysis using targeted next-generation sequencing (NGS) and deletion/duplication analysis using multiplex ligation-dependent probe amplification (MLPA) method were performed in *TP53* gene in all patients.

RESULTS

Five variants were identified in five of 100 patients (5%) in this study. Four of them were evaluated as known as pathogenic/likely pathogenic (4%; 4/100). One variant was evaluated as a variant of uncertain clinical significance (VUS).

CONCLUSION

The patients with BRCA-negative BC younger than 50 years old should be evaluated concerning *TP53* gene mutations because of increased lifetime risk of various developing cancer. Appropriate genetic counseling should be given to patients with *TP53* gene mutations, and the follow-up of these patients should be provided multidisciplinary.

Keywords: BRCA-negative breast cancer; early-onset; Li-Fraumeni syndrome; molecular analysis; *TP53* gene.

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Introduction

Approximately 5-10% of all breast cancer (BC) in women is hereditary BC. Hereditary breast-ovarian cancer (HBOC) syndrome and Li-Fraumeni syndrome (LFS) are the most common cause of inherited BC. HBOC arises from germline mutations in BRCA1 and BRCA2 genes that are responsible for approximately half of all cases with hereditary BC. TP53, PTEN and CDH1 genes are the other high-penetrance genes that play an important role in the genetic etiology of BC.[1]

TP53 gene is a tumor suppressor gene (also known as the guardian of the genome). The TP53 protein plays a major role in response to cell damage. Mutations in the *TP53* gene lead to LFS that has a predisposition to a wide range of cancers, including BC and childhood cancers (sarcomas, leukemia, brain tumor, adrenocortical carcinoma). Women with LSF are at high risk for developing BC (up to 85% by age 60 years).[2] However, radiotherapy in BC patients with a mutation in *TP53* gene could be a risk factor for developing a second primary cancer.[3]

The most common feature of inherited BC is an early-onset. Several studies conducted on a mutation screening in the *TP53* gene in patients with BRCA negative BC, but there are differences in the threshold for age.[2,4,5] However, patients with LFS have a 25-fold increased risk of developing cancer under 50 years of age compared with the general population. For these reasons, we evaluated 100 patients with BRCA negative BC under 50 years of age concerning mutation in the *TP53* gene.

In this study, we emphasize the importance of diagnosing LFS in patients with BRCA negative BC under 50 years of age for patient management, a mutation screening of asymptomatic kindred and for giving accurate and reliable genetic counseling.

Materials and Methods

Patients

The files of patients with BC who were tested for a mutation in BRCA genes between 2016 and 2017 years were reviewed in the Genetic Diagnostic Center. One hundred patients with BRCA negative BC diagnosed at age 50 years or younger were selected regardless of family history. All patients underwent genetic counseling. It was decided to perform molecular tests in the *TP53* gene.

Ethics committee approval was received for this study as a retrospective study and informed consent was obtained from all patients studied.

Isolation of Genomic DNA

Genomic DNA was obtained from all patients by using the MagPurix Blood DNA Extraction Kit (Zinexts Life Science Corp., New Taipei City, TAIWAN) according to the manufacturer's specifications.

Targeted Next-generation Sequencing (NGS)

NEXTflex® TP53 Amplicon Panel (Bioo Scientific Corp., Austin, TX, USA) was used for the enrichment of the coding regions and the intronic regions (up to the area covered by the kit) of *TP53* gene. Targeted NGS was performed on Illumina MiSeq NGS System (Illumina Inc., San Diego, CA, USA) using MiSeq Reagent Kit v2 (500-cycles) (Catalog No: MS-102-2003. Illumina Inc., San Diego, CA, USA).

NGS Data Analysis

Firstly, 'SEQ software' (Genomize, İstanbul, TURKEY) was used for analyzing the raw data according to the reference genome of GRCh37. The minimum coverage-depth was 100X in all target regions. In addition, Integrative Genomics Viewer (IGV) software was used for evaluating the reads.[6,7]

Secondly, variants were detected based on minimum 5X coverage-depth per allele. Then, they were filtered by the following criteria:

1. Variants that had all submissions as Benign (B)/ Likely Benign (LB) in ClinVar database were excluded, and
2. Variants that had allele frequency >5% in any population databases (1000Genomes, ExAC, ESP) were excluded, and
3. Variants that were in the coding and intronic regions were included.

Finally, filtered variants were interpreted based on ACMG Standards and Guidelines recommendations. [8] Ensembl, dbSNP, ClinVar, PubMed, International Agency for Research on Cancer (IARC) TP53 database,[9] LOVD (Leiden Open Variation Database), HGMD® Professional 2017.3 (Human Gene Mutation Database) and ExAC, ESP, 1000Genomes population databases, and in silico prediction tools [10-13] were used for interpreting variants.

Confirmation Analysis

The pathogenic variants revealed by the NGS analysis were confirmed by performing Sanger sequencing on

Table 1   Classifying the identified variants (n=5)

Patient	Identified variants	Evaluated as	ClinVarID	dbSNP
1	TP53:NM_000546:c.469G>T(p.Val157Phe)(Exon5) Heterozygous	Likely pathogenic (known)	12353	rs121912654
2	TP53:NM_000546:c.638G>A(p.Arg213Gln)(Exon6) Heterozygous	Pathogenic (known)	135359	rs587778720
3	TP53:NM_000546:c.332T>C(p.Leu111Pro)(Exon4) Heterozygous	Likely pathogenic (known)	376630	rs1057519997
4	TP53:NM_000546:c.817C>T(p.Arg273Cys)(Exon8) Heterozygous	Pathogenic (known)	43594	rs121913343
5	TP53:NM_000546:c.1078G>A(p.Gly360Arg)(Exon10) Heterozygous	VUS	186887	rs786203298

VUS: Variant of uncertain clinical significance

Table 2   Number of the variants according to age distribution

Age at diagnosis (between)	Case number	Variant identified
24-29	5	2 (LP, LP)
30-39	43	2 (P, P)
40-48	52	1 (VUS)
Total	100	5

LP: Likely pathogenic; P: Pathogenic; VUS: Variant of uncertain clinical significance

ABI PRISM 3500 DNA analyzer (Applied Biosystems, Foster City, CA, USA).

Multiplex Ligation-dependent Probe Amplification Analysis (MLPA)

SALSA® MLPA® P056 TP53 probemix (MRC-Holland, Amsterdam, the Netherlands) was used for MLPA analysis in patients who had no pathogenic variants. The Coffalyser software (MRC-Holland, Amsterdam, the Netherlands) was used for interpreting the MLPA data.

Results

Five variants were detected in five of 100 (5%) patients (Table 1). Four of five variants were assessed as known as pathogenic/likely pathogenic. These variants were confirmed by Sanger sequencing. One of five variant was identified as VUS. All of five variants were found to be heterozygous. No deletion or duplication was detected in the *TP53* gene in patients who had no pathogenic/likely pathogenic variant.

The mean age at diagnosis for all patients was 39.1 years (range 24-48 years) (Table 2). The mean age of four patients who had pathogenic/likely pathogenic variants at diagnosis was 30.2, with a range of 24-38 years.

Discussion

BC is the most common malignancy among women worldwide. Although most cases of BC are sporadic, approximately 5-10% of BC cases have a hereditary BC. The majority of inherited BC in women arises from mutations in BRCA1 and BRCA2 genes. Other rare causes of hereditary BC include mutations in the high-penetrance genes, such as TP53, PTEN, CDH1, STK11, PALB2, and mismatch repair (MMR) genes.

LFS, also known as SBLA (Sarcoma, Breast, Leukemia and Adrenal Gland) cancer syndrome, is a cancer predisposition disease with an autosomal dominant inheritance. LFS occurs at an early age. LFS arises from a mutation in the *TP53* gene. *TP53* gene is activated when DNA is damaged. Cell cycle progression is delayed, and DNA is repaired. If TP53 protein is not activated by mutations, cells with damaged DNA can survive and proliferate to malignant transformation. More than 300 germline variants have been reported in the *TP53* gene.[14] The majority of variants are missense variants. Most of them are detected in the DNA-binding region of the gene (exons 4 to 8).

The clinical spectrum and age in LFS may vary in populations.[15] Patients with *TP53* gene mutation have a high risk of developing a second malignancy. [16] Women with LFS have a high risk of developing BC with an early age onset. The majority of BC in LFS occurs between 15 and 44 age of years.[17] The lifetime risk of cancer for women is estimated to be about 90% by 60 years of age.[18] Germline molecular testing (sequencing/MLPA) of *TP53* gene is required for definitive diagnosis of LFS. There are several different criteria (such as classical LFS, Li-Fraumeni-like (LFL) syndrome, Chompret criteria) to determine patients for molecular testing in the *TP53* gene. However, not all patients with *TP53* gene mutation meet these clinical criteria.[15]

We identified two pathogenic and two likely pathogenic variants that were located in the DNA-binding
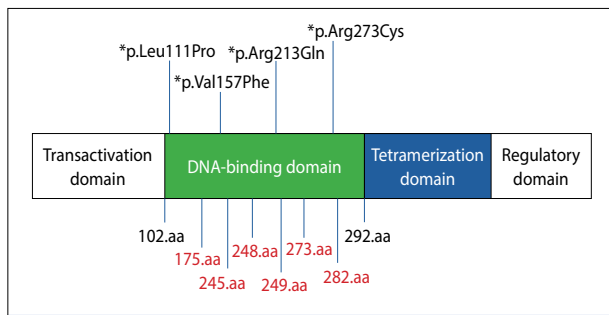
Fig. 1. Illustration of the *TP53* gene showing domains of the protein together with the location of the hotspot mutations (shown in red).
*Indicates P/LP variants identified in this study; aa: Amino acid; P: Pathogenic; LP: Likely pathogenic.

domain in this study (Fig. 1). All of them had been reported in IARC TP53 [9] and ClinVar databases. One of the pathogenic variants was found as c.638G>A (p.Arg213Gln) (Exon6) in patient 2 (Fig. 2). She was 38 years old and diagnosed at 38 years old. Her mother died at 60 years old because of spinal cord cancer. Her uncle (maternal) had prostate cancer and died at 65 years old. Her daughter was healthy (18 years old). The pathogenic variant was also found in her daughter. The second pathogenic variant was detected in patient 4. It was one of the hotspot mutations as c.817C>T(p. Arg273Cys) (Exon8) (Fig. 3). She was 42 years old. The first primary tumor was detected on the right breast at 32 years old, and the second primary tumor was oc-
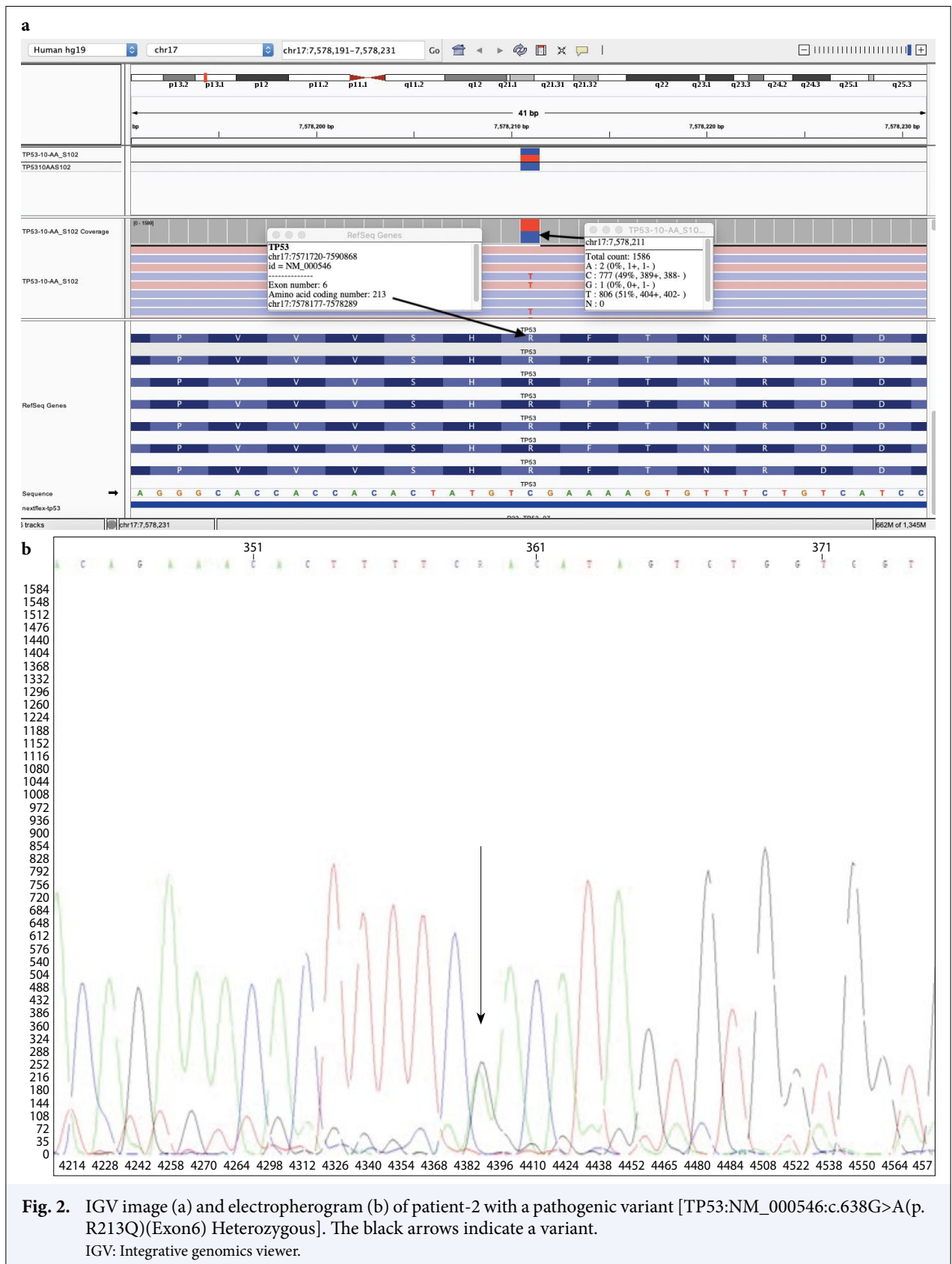
curred on the left breast at 42 years old. Her father diagnosed with gastric cancer at 64 years old and died 65 years old. One of the likely pathogenic variants was revealed in patient 1 as c.469G>T(p.Val157Phe) (Exon5) (Fig. 4). She was 24 years old and diagnosed at 24 years old. Her father died because of a brain tumor. She had three healthy sisters. Her uncle's (paternal) daughter had a BC. The second likely pathogenic variant was found in patient 3 as c.332T>C(p.Leu111Pro) (Exon4) (Fig. 5). She was 37 years old. She diagnosed with BC at 27 years old. Her father had a brain tumor and died at 40 years old. Her grandmother (paternal) died at 40 years old because of BC. We also identified a variant [c.1078G>A(p.Gly360Arg) (Exon10)] as VUS in patient 5, which was reported in ClinVar and IARC databases. She was 41 years old and diagnosed at 40 years old. She had not a family history.
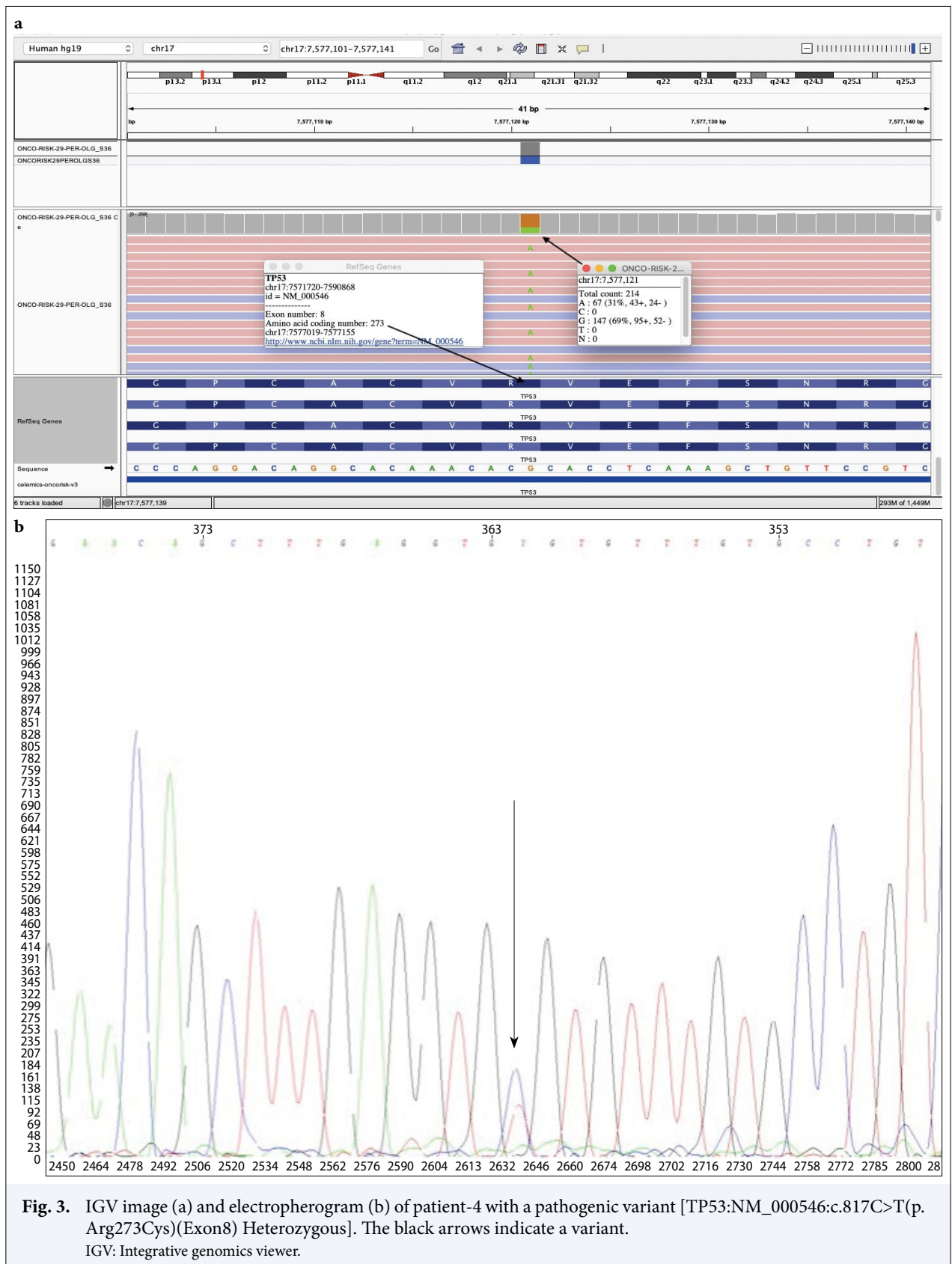
There are several studies that reported the frequency of TP53 mutations in patients with BRCA negative early-onset BC. These studies were conducted in different ethnic groups with different age thresholds (Table 3). For example, Lalloo et al.[19] carried out a mutation analysis of the *TP53* gene by Sanger sequencing in 82 English BC patients diagnosed at ≤30 age of years. They identified four variants (4.9%; 4/82). In another study, Bougeard et al.[20] revealed four variants in 45 French BC patients (0.7%; 4/45) diagnosed at <33 years. The other study was performed by Ginsburg et al.[4] in 95 BC patients diagnosed at <30 age of years from different ethnicities. They did not find any muta-
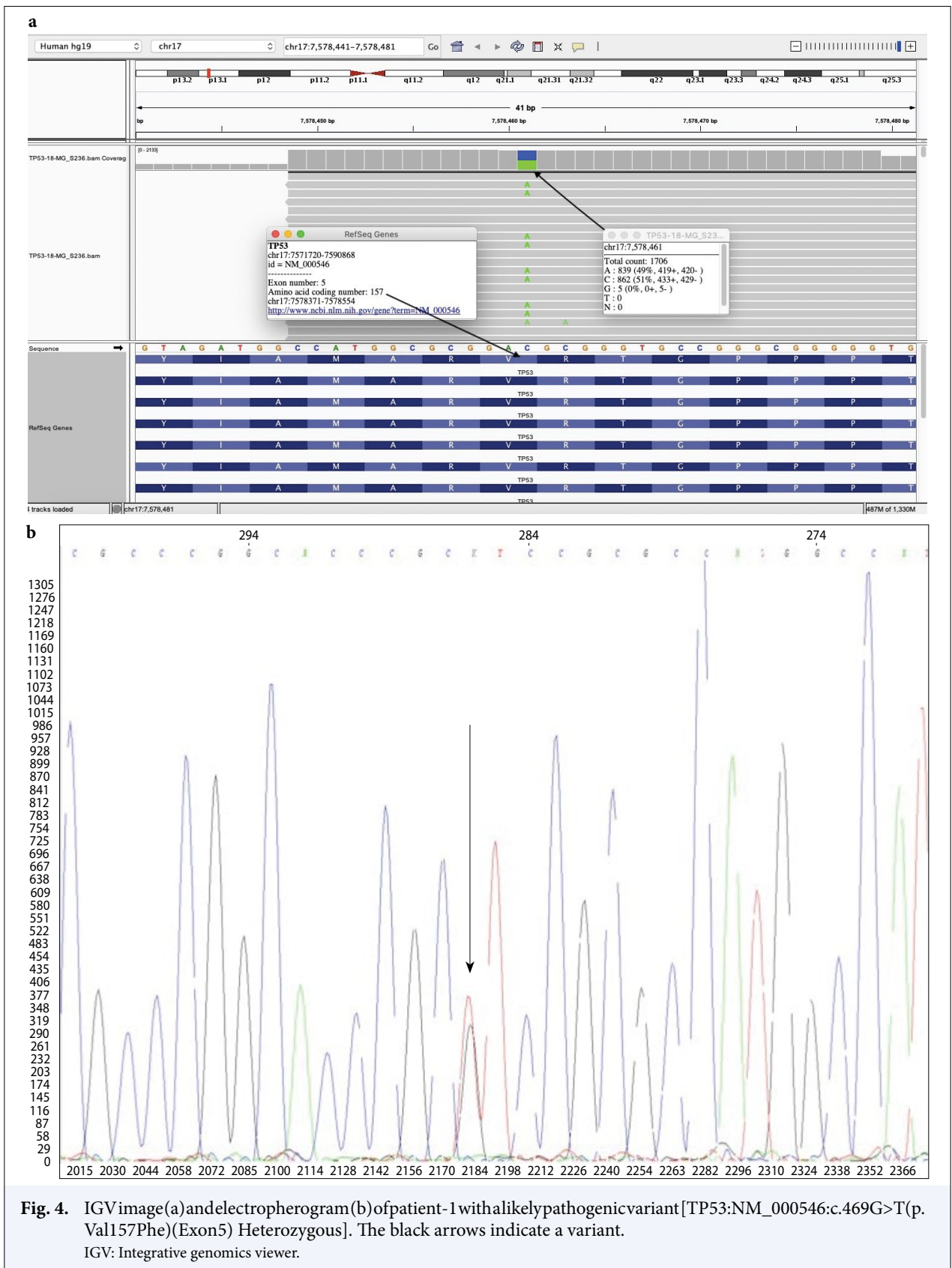
Table 3 Summary of the studies of mutation analysis of the *TP53* gene in patients with BRCA negative early-onset BC
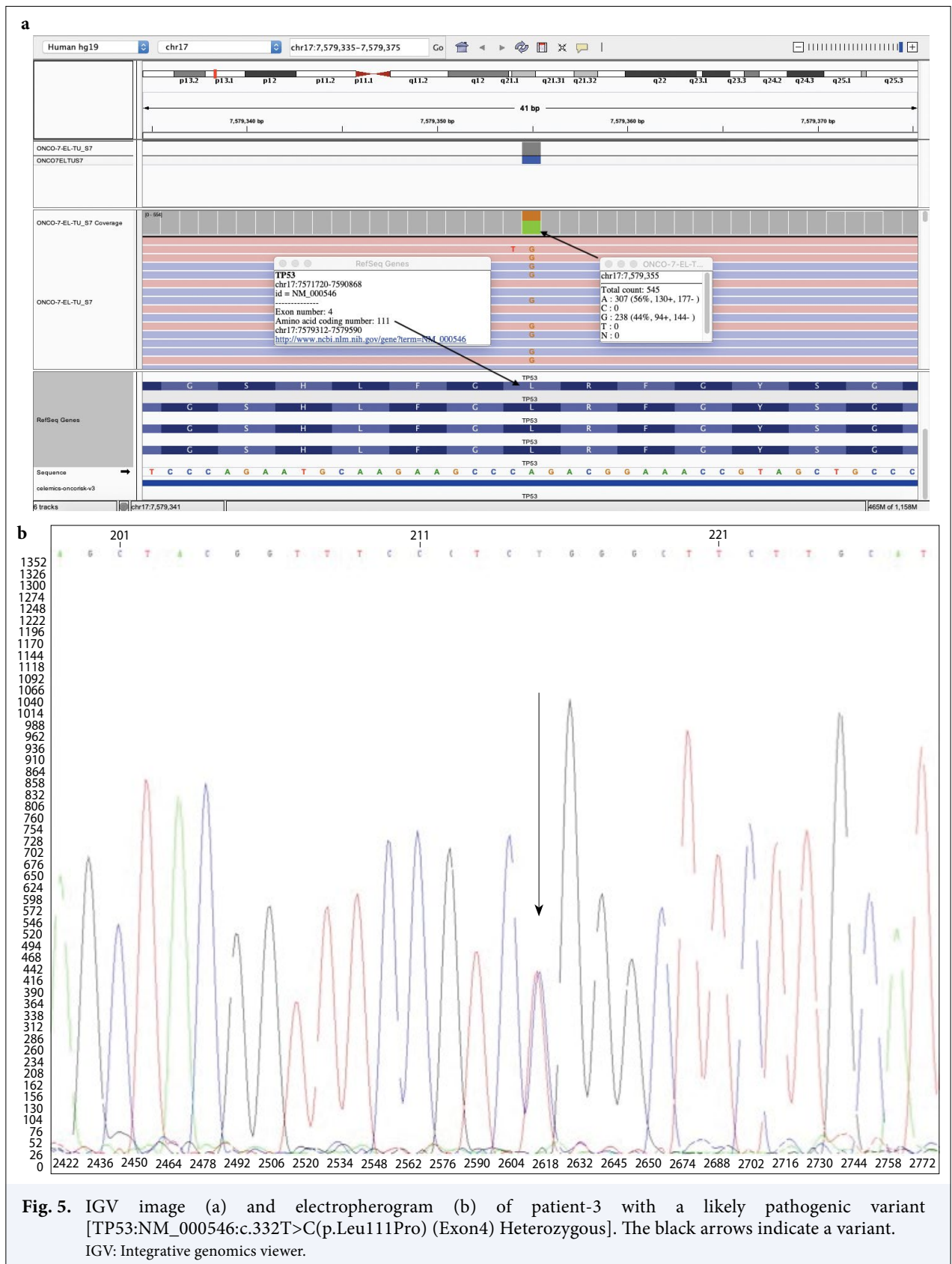
Year	Study [ref]	Age at diagnosis	Case number	Variant identified	Methods	Ethnicity
2006	Lalloo et al. [19]	≤30	82	4 (4.9%)	Sanger sequencing	English
2008	Bougeard et al. [20]	<33	45	3 (0.7%)	Sanger sequencing	French
2009	Ginsburg et al. [4]	<30	95	0	Sanger sequencing	Multi-ethnic
2009	Gonzalez et al. [21]	<30	14	1 (7%)	Sanger sequencing	American
		30-49	15	0		
2010	Mouchawar et al. [17]	<30	41	2 (4.9%)	Sanger sequencing and MLPA	Australian
2012	Lee et al. [5]	≤35	83	5 (6%)	Sanger sequencing	Asian (multi-ethnic)
2012	Rashid et al. [22]	≤40	105	1 (1.5%)	DHPLC and Sanger sequencing	Pakistani
2013	Carraro et al. [23]	≤35	43	1 (2.3%)	Sanger sequencing	Brazilian
2015	Yang et al. [1]	≤30	20	2 (10%)	NGS	Chinese
2019	Gallardo-Alvarado et al. [24]	≤36	53	5 (9.4%)	NGS	Mexican
		37-45	25	0		
In this study	Özdemir et al.	24-29	5	2 (40%)	NGS and MLPA	Turkish
		30-39	43	2 (4.6%)		
		40-48	52	0		

MLPA: Multiplex ligation-dependent probe amplification; DHPLC: Denaturing high-performance liquid chromatography; NGS: Next-generation sequencing

Fig. 2. IGV image (a) and electropherogram (b) of patient-2 with a pathogenic variant [TP53:NM_000546:c.638G>A(p. R213Q)(Exon6) Heterozygous]. The black arrows indicate a variant.

IGV: Integrative genomics viewer.

Fig. 3. IGV image (a) and electropherogram (b) of patient-4 with a pathogenic variant [TP53:NM_000546:c.817C>T(p. Arg273Cys)(Exon8) Heterozygous]. The black arrows indicate a variant.

IGV: Integrative genomics viewer.

Fig. 4. IGV image (a) and electropherogram (b) of patient-1 with a likely pathogenic variant [TP53:NM_000546:c.469G>T(p. Val157Phe)(Exon5) Heterozygous]. The black arrows indicate a variant.

IGV: Integrative genomics viewer.

Fig. 5. IGV image (a) and electropherogram (b) of patient-3 with a likely pathogenic variant [TP53:NM_000546:c.332T>C(p.Leu111Pro) (Exon4) Heterozygous]. The black arrows indicate a variant.

IGV: Integrative genomics viewer.

tion in the *TP53* gene. Gonzalez et al.[21] revealed one variant in 14 American patients (7%; 1/14) with BC diagnosed at <30 years. All of the patients had no family history. In the same study, no mutation was found in 15 BC patients diagnosed between ages 30 and 49. However, in our study, we detected two variants in five BC patients diagnosed at <30 years and two variants in 95 BC patients diagnosed between 30-48 years (Table 2). In another study, Mouchawar et al.[17] performed mutation analyses by Sanger sequencing and by MLPA method in 41 Australian patients with BC regardless of family history and diagnosed at <30 years. Two variants were identified (4.9%; 2/41). One of them was [(p.Arg175His) (Exon6)] found in a patient who diagnosed at 24 years old. The other variant was found as a large deletion encompassing exon 2-4. She was diagnosed at 26 years old. Lee et al.[5] identified five variants in multi-ethnic 83 Asian patients (6%; 5/83) with BC diagnosed at ≤35 years of age. The median age of onset of BC was 31 years. In the other study, Rashid et al.[22] detected the frequency of TP53 mutations in 105 Pakistani patients with BC. Of 67 patients diagnosed at ≤30 years of age had no family history. The remaining 38 patients diagnosed at ≤40 years of age had a family history. Only one variant was revealed in this study (1%; 1/105). It was a frameshift mutation (c.499_500delCA) in exon 5. It was found in a 28-year-old patient with no family history. Carraro et al.[23] identified only one variant in 43 Brazilian patients (2.3%; 1/43) with BC diagnosed ≤35 years of age. It was found as c.427G>A (p.Val143Met) in a 24 years old patient who had no family history. Yang et al.[1] performed a mutation analysis of 152 genes associated with hereditary cancers using NGS in 99 Chinese patients with BC. They identified three variants in the *TP53* gene (3%; 3/99). Of two variants were found in patients with BC diagnosed ≤30 years of age. They accounted for 10% (2/20) of all patients with BC diagnosed at ≤30 years in their study. In another study was performed in the Mexican population by Gallardo-Alvarado et al.[24] They tested 78 Mexican patients with BC diagnosed at <45 years of age. They determined the frequency of TP53 germline mutation using NGS as 6.4% (5/78). All five patients with BC diagnosed before the age of 36 (9.4%; 5/53).

BC patients with TP53 mutation and their relatives who have the same mutation can benefit from surveillance programs for various cancers to aim at early tumor detection.[25] The surveillance program for BC includes breast self-examination, clinical examination and imaging. In general, monthly breast self-examination starting from the age of 18 years is recommended. Clinical breast examination is recommended every 6-12 months, starting from the age of 20-25 years. Breast MRI annually is recommended at 20-75 years. Mammography is controversial because there are several reports suggesting that cancers induced by radiation are more common in patients with LFS.[26,27] While some of the guidelines suggest annual breast MRI and mammography, some guidelines recommend annual breast MRI screening without mammography.[2] Therefore, patients should be informed about the risks of malignancy associated with radiotherapy. Risk-reducing mastectomy should be evaluated in BC patients with TP53 mutation because of the high contralateral BC risk. Moreover, the option for risk-reducing bilateral mastectomy should be considered in women without cancer with TP53 mutation.[2] Recommendations for other cancer risks include comprehensive physical examinations every 6-12 months, colonoscopy and upper endoscopy starting at 25 age of years every 2-5 years,[2] dermatological examination especially for melanoma annually starting at 18 years of age.[25] Whole-body MRI, especially for sarcomas, is recommended once a year.[25,28]

Brain MRI is recommended once a year for brain tumors (can be performed as part of whole-body MRI or as a separate examination).[25]

Conclusion

In conclusion, *TP53* gene mutation analyses should be performed in BRCA-negative BC patients under 50 years of age although differences in the threshold for age because there is an increased lifetime risk for various cancers in LFS. *TP53* gene mutation analysis can be performed as a single test or as a part of a multigene panel using NGS because NGS technology provides simultaneous analysis of multiple genes in a single test at a comparable cost to Sanger sequencing.

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