Mutation screening of germline TP53 mutations in high-risk Chinese breast cancer patients

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Abstract

Background: Germline TP53 mutations are associated with Li-Fraumeni syndrome, a severe and rare hereditary cancer syndrome. Despite the rarity of germline TP53 mutations, the clinical implication for mutation carriers and their families is significant. The risk management of TP53 germline mutation carriers is more stringent than BRCA carriers, and radiotherapy should be avoided when possible.

Methods: TP53 gene mutation screening was performed in 2538 Chinese breast cancer patients who tested negative for BRCA mutations.

Results: Twenty TP53 mutations were identified with high next-generation sequencing concerning for germline mutations in Chinese breast cancer families. The majorities of the TP53 carriers had early-onset, hormone receptor-positive breast cancer, and had strong family history of cancer. Among all, 11 patients carried a germline mutation and 6 of which were likely de novo germline mutations. In addition, 1 case was suspected to be induced by chemotherapy or radiation, as this patient had no significant family history of cancer and aberrant clonal expansion can commonly include TP53 mutations. Furthermore, we have identified one mosaic LFS case. Two novel mutations (c.524_547dup and c.529_546del) were identified in patients with early-onset.

Conclusions: In view of the high lifetime risk of malignancy, identification of patients with germline TP53 mutations are important for clinicians to aid in accurate risk assessment and offer surveillance for patients and their families.

Keywords: Hereditary breast cancer, TP53 mutation, Chinese, Breast cancer risk

Background

Li-Fraumeni syndrome (LFS) is a rare autosomal genetic disorder which is frequently associated with germline TP53 mutations. Germline TP53 mutations are seen in 70% of families with LFS features. Individual with the mutation commonly present with LFS spectrum tumors (sarcoma, brain tumor, adrenocortical carcinoma, leukemia, germ cell tumor and breast cancer) [1, 2]. The lifetime risk of breast cancer in TP53 mutation carriers is up to 80–90%, which is even higher than those harboring a BRCA1 or BRCA2 (BRCA) mutation: the most commonly identified high penetrance germline gene mutations in hereditary breast cancer [3].

Although rare, germline TP53 mutations are estimated to occur in up to 1% of all breast cancer cases [4]. Very early-onset of breast cancer is a common characteristic of TP53 mutation carriers in which the median age being 27–30 years old [5]. TP53 breast tumors are usually enriched with HER2-positive receptors, and 84% are either...
estrogen and/or progesterone receptor positive [6, 7]. Patients with TP53 mutations have also been shown to have a shorter survival when compared to non-carriers [8].

The National Comprehensive Cancer Network (NCCN) has published testing and management guidelines for TP53 gene mutation carriers. Under the NCCN guidelines, TP53 mutation testing is recommended for early-onset breast cancer patients (age of diagnosis < 31) or those who meet classic LFS or Chompret criteria. It is also recommended that women who are TP53 mutations carriers have breast surveillance similar to that of BRCA mutation carriers, and in addition, receive an annual total body MRI scan and skin surveillance similar to that of BRCA1 cases [9]. TP53 mutations carriers have breast cancers with medullary type histology; (6) patients having at least one relative with BRCA-associated cancer other than breast and ovarian cancer (such as stomach or prostate cancers) or known to be BRCA mutation related family; (7) patients with male breast cancer.

DNA extraction
Blood, hair follicles and/or buccal swab DNA samples were collected from patients. Genomic DNA extraction was performed using QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) or QIAasymporny DNA Mini Kit (Qiagen) according to manufacturer’s instructions. Genomic DNA was quantified using a Qubit dsDNA BR Assay Kit and a Qubit 2.0 Fluorometer (Life Technologies, USA).

Sequencing of TP53 gene
Extracted DNA was applied to the QIAseq Human BRCA1 and BRCA2 Plus Panel DHS-103Z (Qiagen). Sequencing libraries were prepared according the QIAseq™ Targeted DNA Panel protocol (Qiagen). The libraries were pooled and sequenced on MiSeq or NextSeq (Illumina, San Diego, CA) instruments to reach minimum sequencing depth of 50-fold. Median coverage typically ranged between 200-300X.

To confirm germline mutations, Sanger sequencing of specific mutations was carried out on blood, hair follicles, and/or buccal swab DNA.

Bioinformatics analysis
The bioinformatics analysis was performed on a Cray XC30 supercomputer (Cray, Seattle, WA). Paired sequencing reads were mapped to the human reference genome sequence GRCh37/hg19 using BWA-MEM v0.7.7 by default parameters [14]. Post-alignment primer clipping and unique molecular identifier (UMI) extraction were performed using BAMClipper [15]. Samples having at least 75% gene-specific primers with at least 100 detected UMI per primer were considered to pass quality control and subject to variant calling by FreeBayes v1.0.2–15 [16]. Called variants with variant allele fraction (VAF) of at least 5% were annotated by Ensembl Variant Effect Predictor v75 [17]. Variants with minor allele frequency of at least 1% reported by The 1000

Methods
Participants and selection criteria
TP53 gene mutation screening was performed on 2538 Chinese breast cancer patients with no BRCA1 and BRCA2 germline mutations. Patients were recruited from the Hong Kong Hereditary and High Risk Breast Cancer Program (HRBCP) Registry through the Hong Kong Hereditary Breast Cancer Family Registry from March 2007 to August 2019. Patient selection criteria was as follows: (1) patients had at least one first- or second-degree relative with breast and/or ovarian cancer, regardless of age; (2) the age at breast cancer diagnosis was under 45 years; (3) patients with bilateral breast cancer; (4) patients with triple-negative hormone receptors breast cancers, (5) cancers with medullary type histology; (6) patients having at least one relative with BRCA-associated cancer other than breast and ovarian cancer (such as stomach or prostate cancers) or known to be BRCA mutation related family; (7) patients with male breast cancer.

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Genomes Projects [18] were excluded from manual variant curation. Variants in exon and at least 10 bp of the flanking introns were reported and described according to the standardized recommendations of the Human Genome Variation Society (HGVS) nomenclature [19]. Variant descriptions were checked by IARC TP53 database (http://p53.iarc.fr/) and Mутalyzer Name Checker (http://mutalyzer.nl). Variants in this study were interpreted based on classification from ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) with clinical adjustment with reference to the classic for Li-Fraumeni syndrome criteria [2].

Molecular analysis of de novo germline mutations
Mutations from families in which both parents tested negative were presumed as de novo mutations. Haplotype analysis was also performed to confirm de novo cases. In cases where the patient had no first or second-degree relatives with cancer history or positive test result, and the blood samples from the patient's parent were unavailable, the patient was considered as likely de novo.

Statistical analysis
Fisher’s exact test and Wilcoxon rank sum test were used to study the relationship between clinicopathological variables and mutation status. The limit of significance for all analyses was defined as $P$-value of $<0.05$. Data analyses were performed using statistical software R (version 3.4.2) [20].

Results
In a cohort of 2538 breast cancer patients, there were 28 PALB2 and 2 PTEN mutation identified, which were excluded from the study. Among 2508 patients, the mean age at diagnosis was 45.63 years (range 18–95). Of all primary tumors, 1760 (75.41%) were hormone receptor positive, 211 (9.04%) were HER2+, and 327 (14.01%) were triple-negative. A positive family history of breast cancer (first- or second-degree relatives) was seen in 922 (36.76%) of the patients and 473 (18.86%) of primary tumors, 1760 (75.41%) were hormone receptor positive, 211 (9.04%) were HER2+, and 327 (14.01%) were triple-negative. A positive family history of breast cancer (first- or second-degree relatives) was seen among 922 (36.76%) of the patients and 473 (18.86%) of the patients had a family history with ≥3 different types of cancers in their first- and second-degree relatives.

TP53 mutations were infrequent in this cohort. Only 20 different mutations (0.80%) were identified among the 2508 breast cancer patients. The mean age at diagnosis of breast cancer for the mutation carriers and non-carriers were 31.65 years and 45.74 years ($p$-value $<0.001$), respectively. In TP53 mutation carriers, the majority of the tumors were hormone receptor-positive (16/21, 76.19%) (OR compare with non-carriers: 1.04, 95% CI: 0.363–3.661; $p$-value =1). A positive family history of breast cancer (among first- and second-degree relatives) was reported in 5 (25%) TP53 mutation carriers compared to 917 (36.86%) non-carriers (OR 0.571, 95% CI: 0.162–1.660, $p$-value = 0.355). Moreover, there were 8 (40%) mutation carriers with a family history of ≥3 different types of cancers in first- and second-degree relatives compared to 465 (18.69%) of non-carriers (OR: 2.90, 95% CI 1.022–7.764, $p$-value = 0.038). Characteristics of mutation carriers and non-carriers are shown in Table 1.

The majority of the mutations (15/20) identified were missense mutations, followed by 2 nonsense mutations, 2 deletions/insertions and 1 splice site mutation (Tables 2 and 3). By testing ancillary materials, multiple germ layers and/or clinical data to interrogate germline status on the 20 carriers, 11 (55%) patients were confirmed to carry a germline mutation, 2 (10%) patients were confirmed to have de novo germline mutations (Fig. 1), and 4 (20%) were presumed to have de novo germline mutations based on the negative test result of TP53 mutation among multiple family members and/or lack of cancer history in families. Five of the patients were deceased and three of the patients refused further investigation on family studies, some information were no longer traceable. In all, 70% had early-onset of breast cancer (<35 years) and 60% had bilateral breast cancer. Interestingly, we found that 25% (5/20) of the patients had no family history of cancer, 2 patients had bilateral breast cancer, one had bilateral breast cancer and thyroid cancer and one had multiple cancers in breast and brain.

Novel mutations (c.524_547dup and c.529_546del) were seen in 2 patients with both diagnosed breast cancer at age below 40. One of the novel mutation carriers (F19) shows equivocal result in both of her blood, hair follicle and buccal swab DNA with trace amount of TP53 duplication, serving as evidence of mosaicism or ACE (in Table 3). The other novel TP53 carrier (F06) was de novo germline mutation, with significance family history of cancer.

In this cohort, there were 5 suspected germline mutation cases (F03, F05, F08, F11 and F18); these patients had strong family history with cancers and fulfill either Li-Fraumeni-like (LFL) criteria and/or Chompret criteria, however, family cascade testing was not possible due to loss of follow-up or family members refusing testing. In addition, we suspected that one of the cases (F20) was likely ACE induced by chemotherapy or radiation (Table 3); this patient had breast cancers at age > 45, received chemotherapy before the genetic test, and had no other significant personal or family history of cancer and hence is likely not a germline related.

Discussion
Among 2508 Chinese breast cancer patients, we identified 18 germline TP53 and 2 ACE/mosaic TP53 cases. Of 18 germline cases, two of them did not meet the
NCCN guidelines for TP53 genetic testing but the families had LFL syndrome (Table 2). In general, germline TP53 families had at least one member with LFS tumor spectrum i.e. sarcoma, brain tumor, breast cancer, leukemia, bronchoalveolar lung carcinomas, germ cell tumor or adrenocortical carcinoma [1, 2, 21–23]. However, we found that 16.67% (3 of 18) of the patients had no family history of cancer.

Among 374 patients in our study with early-onset breast cancer (age < 35), the detection rate of a TP53 germline mutation was 3.74% which is comparable to other studies in the West (2–7.1%) [24, 25], and among Chinese high risk breast patients (1–5%) [12, 26, 27] (Table 2). A study of French-Canadian cancer families suggested that women with breast cancer before the age of 50 with no family history of cancer still warrant screening for TP53 mutations, even though the mutation frequency (0.5%) is low compare to BRCA mutations (4.8%) [28].

Interestingly, there were 6 (33.3%) de novo or likely de novo cases. Another study on early-onset cancer study suggests that the frequency of de novo TP53 mutations is 7–20% [29]. There were two TP53 mutations, c.490A > G (F04) and c.536A > G (F07) both of the families showed characteristics of classical LFS. Their families have significant family history of sarcoma, although ClinVar has classified them as variant of unknown significance (VUS), we believe that the pathogenicity of these two variants should be further determined based on their family histories. With a significant family

### Table 1 Characteristics of Chinese breast cancer patients screened for TP53 mutations

|                        | Mutation Negative % | TP53+ % | Total % | P-value (Wilcoxon rank sum test/ Fisher Exact Test) |
|------------------------|---------------------|---------|---------|---------------------------------------------------|
| Mean/Median age at Diagnosis | 45.74/44 | 31.65/30 | 45.63/44 | < 0.001                                           |
| Age range              | 18–95              | 18–47   | 18–95   |                                                   |
| Bilateral cases        | 439                | 12      | 451     | 17.98% < 0.001                                   |
| Age at breast cancer diagnosis |           |         |         |                                                   |
| ≤ 29                   | 116                | 9       | 125     | 4.98% < 0.001                                    |
| 30–39                  | 637                | 6       | 643     | 25.64%                                           |
| 40–49                  | 998                | 5       | 1003    | 39.99%                                           |
| ≥ 50                   | 737                | 0       | 737     | 29.39%                                           |
| Family history of breast cancer |         |         |         |                                                   |
| Yes                    | 917                | 5       | 922     | 36.76% 0.355                                     |
| No                     | 1571               | 15      | 1586    | 63.24%                                           |
| Family history of > =3 different types of cancers | |         |         |                                                   |
| Yes                    | 465                | 8       | 473     | 18.86% 0.038                                     |
| No                     | 2023               | 12      | 2035    | 81.14%                                           |
| Histologya              | N = 2927           | N = 32  | N = 2959 |                                                   |
| Ductal                 | 1991               | 17      | 2008    | 71.08% 0.283                                     |
| Lobular                | 94                 | 1       | 95      | 3.36%                                            |
| DCIS                   | 498                | 9       | 507     | 17.95%                                           |
| Others                 | 213                | 2       | 215     | 7.61%                                            |
| Unclassified           | 131                | –       | 134     | –                                                 |
| Molecular subtypesa (excluded in-situ CA) | |         |         |                                                   |
| Hormone receptor +     | 1744               | 16      | 1760    | 75.41% 0.291                                     |
| Hormone receptor -     | 36                 | 0       | 36      | 1.54%                                            |
| HER2+                  | 207                | 4       | 211     | 9.04%                                            |
| TNBC                   | 326                | 1       | 327     | 14.01%                                           |
| Unclassified           | 116                | –       | 118     | –                                                 |

Abbreviation: DCIS ductal carcinoma in situ, HER2 human epidermal growth factor receptor 2, TNBC Triple-negative breast cancer
*aCount for each primary of bilateral cases
| Patient ID | Nucleotide alteration | Clin-Var | Number of cases in IARC TP53 (Asian/All) | VAF (%) | Germline evidence(s) | C/R | Tumor types | Age at Dx | Mutation type | Stage of disease | Vital status | Family history | LFS/ LFL Classification |
|------------|-----------------------|----------|------------------------------------------|---------|----------------------|-----|--------------|----------|---------------|------------------|-------------|----------------|--------------------------|
| F01        | c.96 + 1G > T; Del of exon3 c.75_96del p.Leu26Profs*11 | P/LP     | 0/0                                      | 51.5    | Confirmed germline   | -   | +            | NA       | Y             | Breast CA         | Alive       | Breast CA, Colorectal CA, Brain and CNS Tumors | LFL Chompret  |
| F02        | c.422G > A; p.Cys141Tyr | P        | 5/11                                     | 56.9    | Confirmed Germline; DeNovo | -   | +            | Y        | N             | Breast CA, Thyroid CA | Alive       | Breast CA, Colorectal CA, Lung CA | LFL Chompret  |
| F03        | c.473G > A; p.Arg158His | P/LP     | 2/23                                     | 51.4    | Suspected germline   | -   | NA           | NA       | NA            | Breast CA         | Alive       | Multiple Lung CAs, Colorectal CA, Brain and CNS Tumor | LFL N         |
| F04        | c.490A > G; p.Lys164Glu | VUS      | 0/0                                      | 54.3    | Confirmed germline   | +   | /            | /        | /             | Breast CA         | Alive       | Osteosarcoma, Soft Tissue Tumor, NPC | LFS Chompret  |
| F05        | c.527G > T; p.Cys176Phe | LP       | 0/1                                      | 36.9    | Suspected germline   | NA  | NA           | NA       | NA            | Breast CA, Breast CA | Alive       | Liver, Stomach CA | N Chompret    |
| F06        | c.529_546del; p.Phe177Cys182del | Ni    | Novel                                   | 33.1    | Confirmed Germline; DeNovo | -   | +            | +        | Y             | Breast CA         | Alive       | Multiple Lung CAs, Stomach CA, Laryngeal CA, Prostate CA | LFL Chompret  |
| F07        | c.536A > G; p.His179Arg | VUS      | 2/2                                      | 48.5    | Confirmed germline   | +   | /            | /        | /             | Breast CA, Breast CA | Alive       | Unknown CA | LFS Chompret |
| F08        | c.541C > T; p.Glu177Val | P/LP     | 17/21                                    | 55.1    | Suspected germline   | NA  | NA           | NA       | NA            | Breast CA, Breast CA | Alive       | Lung CA, Testicular CA, Ovarian CA | LFL N         |
| F09        | c.626_627dupGA; p.Asn210Glu211del | Ni    | 0/3                                      | 35.0    | Confirmed Germline; Likely DeNovo | -   | +            | NA       | N             | Breast CA, Thyroid CA | Alive       | Unknown CA | N Chompret |
| F10        | c.638G > A; p.Arg213Gln | P        | 5/16                                     | 53.8    | Confirmed germline   | +   | /            | /        | Y             | Breast CA, Thymus CA, Adenocarcinoma CA | Alive       | Thyroid CA, Lung CA, Brain and CNS Tumors | LFL Chompret  |
| F11        | c.722C > T; p.Ser241Phe | LP       | 3/6                                      | 65.8    | Suspected germline   | -   | NA           | NA       | NA            | Breast CA, Breast CA | Alive       | Colorectal CA, Breast CA | LFL Chompret  |
| F12        | c.743G > A; p.Arg248Gln | P/LP     | 14/64                                    | 50.9    | Confirmed germline   | -   | /            | /        | Y             | Breast CA, Breast CA | Alive       | Sarcoma Multiple Breast CAs, Stomach CA | LFS Chompret  |
| F13        | c.818G > A; p.Arg273His | P/LP     | 23/75                                    | 48.3    | Confirmed germline   | NA  | +            | NA       | N             | Breast CA, Breast CA | Alive       | Unknown CA | N Chompret |
| F14        | c.825T > G; p.Cys275Tyr | LP       | 0/0                                      | 43.7    | Germline; Likely DeNovo | -   | NA           | NA       | NA            | Breast CA, Breast CA | Alive       | Unknown CA | N Chompret |
| Patient ID | Nucleotide alteration | Clinvar Number | Number of cases in IARC TP53 (Asian/All) | VAF (%) | Germline evidence(s) | C/Tumor types | Age at Dx | Stage of disease | Vital status | Family history | LFS/LFL classification |
|------------|------------------------|----------------|------------------------------------------|---------|----------------------|--------------|----------|------------------|-------------|----------------|----------------------|
| F15       | c.844C > T; p.Arg282Trp | P/LP           | 13/52                                    | 51.7    | Germline; Likely DeNovo | Breast CA, Brain and CNS Tumors | 27        | Stage II         | Deceased | N              | Chompret |
| F16       | c.916C > T; p.Arg306*   | P             | 9/33                                     | 44.3    | Confirmed Germline; Likely DeNovo | Breast CA | 32        | Stage I         | Alive      | NA            | LFS Chompret |
| F17       | c.1010G > A; p.Arg337His | P/LP           | 2/112                                    | 42.4    | Confirmed germline   | Breast CA, Lung CA | 42        | Stage II         | Alive      | Colorectal, Breast, Uterus, Breast CA | LFS Chompret |
| F18       | c.1025G > C; p.Arg342Pro | P/LP           | 2/6                                      | 51.0    | Suspected germline   | Breast CA, Breast CA | 23        | Stage I, Stage II | Alive      | Nasopharyngeal, Liver, NS | N Chompret |

LFS (Classic Li-Fraumeni syndrome): an individual diagnosed < 45 with a sarcoma AND a first degree relative diagnosed < 45 with cancer AND an additional first or second degree relative diagnosed < 45 with cancer AND an additional first or second degree relative diagnosed < 45 with breast cancer. OR proband with any childhood cancer < 46 AND at least one first or second degree relative with LFS tumor spectrum at any age, with one first or second degree relative diagnosed < 45 or five or more first or second degree relatives diagnosed < 45, regardless of family history or breast cancer diagnosed < 31 [21].

LFL (Li-Fraumeni like syndrome): an individual with at least one first or second degree relative with all LFS tumors spectrum at any age, or proband with any childhood cancer < 46 AND one first or second degree relative with typical Li-Fraumeni tumor spectrum at any age.

Chompret: Individual with a tumor from LFS tumor spectrum diagnosed < 46 AND at least one first or second degree relative with typical Li-Fraumeni tumor spectrum at any age, regardless of family history of breast cancer diagnosed < 31 [21].

Abbreviations: FM family member(s); HF: Hair follicles; BS: Buccal Swab; PT: Paternity test; C/R: Chemotherapy or radiotherapy; NI: No information; NA: Not available; Dx: Diagnosis; VAF: Variant allele frequency; P: Pathogenic; LP: Likely pathogenic; VUS: Variant of unknown significance.
Table 3 Characteristics of Chinese breast CA patients identified with aberrant clonal expansion TP53 mutation or mosaic TP53 mutation

| Patient ID | Nucleotide alteration | Clinvar | Number of cases in IARC (Asian/All) | VAF (%) | Germline (deNovo)/ACE | C/R | Tumor types | Age at Dx | Mutation type | Stage of disease | Vital status | Family history | LFS/LFL | Classification |
|------------|-----------------------|---------|------------------------------------|---------|-----------------------|-----|--------------|----------|--------------|-----------------|-------------|----------------|--------|----------------|
| F19        | c.524_547dup; p.Cys182Ser183insCys CysProHisHisGluArgCys | Novel   | 17.5 Mosaic                         | L       | N                     | N   | Breast CA   | 40       | Insertion    | Stage II        | Alive / N   | N              | N      | LFS/LFL        |
| F20        | c.775G > A; p.Asp259Asn | Novel   | 58.7 Likely ACE*                    | NA      | NA                   | NA  | Breast CA, Breast CA | 46 48   | Missense Mutation | Stage II Stage I | Alive / N   | N              | N      | LFS/LFL        |

LFS (Classic Li-Fraumeni syndrome): an individual diagnosed age < 45 with a sarcoma AND a first degree relative diagnosed age < 45 with cancer AND an additional first or second degree relative in the same lineage with cancer diagnosed age < 45 or with a sarcoma at any age [1]

LFL (Li-Fraumeni-like Syndrome): an individual with two first- or second degree relatives with LFS tumors spectrum at any age, rather than the three required by the classic criteria [20] OR proband with any childhood cancer or sarcoma, brain tumor, or adrenocortical carcinoma diagnosed under 45 years of age, with one first or second degree relative with typical LFS cancer diagnosed at any age, plus one first or second degree relative in the same lineage with any cancer diagnosed under age 60 [19]

Chompret: Individual with a tumor from LFS tumor spectrum diagnosed < 46 AND at least one first or second degree relative with any of the aforementioned cancers (other than breast cancer if the proband has breast cancer < 56 OR individual with multiple primaries at any age OR individual with multiple tumors (excluding multiple breast tumors), two of which belong to LFS tumors spectrum with the initial cancer occurring < 46 OR individual with adrenocortical carcinoma or choroid plexus carcinoma or rhabdomyosarcoma of embryonal anaplastic subtype at any age, regardless of family history or breast cancer diagnosed < 31 [21].

Abbreviations: FM family member(s); HF hair follicle; BS buccal swab; PT paternity test; C/R chemotherapy or radiotherapy; N/A no information; NA not available; Dx diagnosis; VAF variant allele frequency; ACE aberrant clonal expansion; P pathogenic; LP likely pathogenic; VUS variant of unknown significance; L low VAF

*Likely ACE: an individual diagnosed age < 35 AND with no family history of cancer(s) AND received chemotherapy or radiotherapy before genetic test
The mutation $TP53$, $c.1010G > A$, has been previously reported as founder mutation in Southern Brazilian [30]. Interestingly, it was detected in one of the Chinese families who had breast and lung cancer and multiple family cancers. The mutation $TP53$, $c.529_546_{del}$, has been identified somatically in thyroid cancer [31], small cell lung cancer [32] and breast cancer [24, 33]. We detected this mutation in one of the families who had breast cancer at age 30 and a family history of multiple cancers. The variant allele fraction (VAF) was at 33% by NGS, which was lower than the average range of $TP53$ VAFs identified in our study. Further analysis was performed on a buccal swab by Sanger sequencing in which the VAF was ~50% and therefore the mutation was confirmed to be germline.

In another family (F19), we detected a 17.5% VAF by NGS, which was much lower than the normal germline range of 40–60% VAF. Further analysis on hair follicles and buccal swab by both NGS and Sanger sequencing showed trace amounts of the mutation, therefore testing on tumor tissue would be able to confirm somatic mosaicism or ACE [10], however, the patient received neoadjuvant chemotherapy before surgery and there was no tissue available for further testing.

Radiation induced genomic instability causing aberrant hematopoietic stem/progenitor cells mobilized into the peripheral blood circulation result in ACE sometimes involving $TP53$ [10, 34]. In one of the patients who was only tested after chemotherapy has been administered, breast cancer was diagnosed at old age (> 35) and there was no cancer history in their families, suggesting the variant was more likely due to ACE rather than LFS.

Increased risk of secondary malignancies in $TP53$ mutation carriers with radiation exposure has been reported [35]. In a preclinical study of 6 germline $TP53$ mutated breast cancer patients who received adjuvant radiotherapy, 3 later developed ipsilateral breast recurrences, 4 developed contralateral breast cancers, 2 developed radiotherapy-induced cancers, and 2 developed new primaries (1 of which was an ipsilateral chest wall angiosarcoma and the other was a grade 2 ethmoidal leiomyosarcoma) [36].

**Conclusion**

Overall, our study shows the spectrum of $TP53$ germline mutations in a Chinese cohort and also clinical characteristics of Chinese $TP53$ carriers and their families which may help clinicians identify patients for $TP53$ mutation screening. Young aged (even without a cancer family history) women with breast cancer is a major...
association and should be considered for TP53 genetic testing. Identification of a TP53 mutation may also affect the treatment options for these patients, i.e. potentially minimizing the use of radiation to prevent radiation-related malignancies [37]. Moreover, our findings may aid in the development of new guidelines for TP53 screening in breast cancer patients with Chinese ethnicity.

Supplementary information
Supplementary information accompanies this paper at https://doi.org/10.1186/s12885-020-07476-y.

Additional file 1.

Abbreviations
ACE: Aberrant clonal expansion; HRBCP: Hong Kong Hereditary and High Risk Breast Cancer Program; HGVS: Human Genome Variation Society; LFS: Li-Fraumeni syndrome; NCCN: The National Comprehensive Cancer Network; UMI: Unique molecular identifier; VAF: Variant allelic fraction

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Authors’ contributions
The study was designed by AK, EM, JW, TC and TS. CH and CA performed the experiments. CA performed the bioinformatics analysis and interpreted the results. CH and VS collected data and drafted the manuscript. AK, EM, JW, TC and TS reviewed the manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics approval and consent to participate
This study was approved by the Institutional Review Board of the University of Hong Kong/Hospital Authority West Cluster and other contributing hospitals in Hong Kong (UW06–274 T1299) and was conducted in accordance with the Declaration of Helsinki. All recruited participants provided written informed consent for sample and data collection in this study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflicts of interest.

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