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Using next-generation sequencing (NGS) platform to diagnose pathogenic germline *BRCA1/2* mutations from archival tumor specimens

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HIGHLIGHTS

- Archival tumor testing using an NGS platform can detect about 70% of germline *BRCA1/2* pathogenic variants.
- No false positive findings were identified for germline BRCA1/2 pathogenic variants.
- Tumor testing can potentially diagnose *BRCA1/2* mutation to facilitate predictive testing in cancer-free family members.
- False positive results were noted in 4 genes with the exception of *ATM*, being highest in *TP53* followed by *PTEN*.
- Tumor testing will not be a reliable test to diagnose Cowden syndrome or Li-Fraumeni syndrome with current technology.

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ABSTRACT

Objective: Clinical genetic testing to diagnose germline mutations often requires blood sample or saliva smear from a cancer-affected individual. This rules out testing in families when cancer-affected individuals are deceased. We explored the use of a next-generation sequencing (NGS) platform to diagnose germline pathogenic mutations from tumors.

Methods: Archival tumors (ovarian = 26, breast = 25, others = 9) were retrieved from 60 cancer patients who have undergone multi-gene panel blood testing. Genomic DNA was extracted and sequenced for *BRCA1/2* using a NGS platform. 41/60 specimens were sequenced for 5 other genes (*APC*, *ATM*, *PALB2*, *PTEN*, *TP53*). Tumor testing and results interpretation were performed blinded to the blood test result.

Results: All 38 patients with no *BRCA1/2* mutations on blood testing were correctly tested negative on tumor. Tumor testing correctly diagnosed *BRCA1/2* pathogenic mutations in 15/22 (68%) patients while in 7/22 (32%) patients, the mutation was either detected but incorrectly classified as VUS ($n = 3$) or not detected at all ($n = 4$). Overall concordance rate for tumor and blood testing for *BRCA1/2* mutations was 88%, with 0% false positive and 32% false negative rate for pathogenic mutations. Tumor testing correctly diagnosed 1/2 pathogenic germline *ATM* mutation, 1/1 pathogenic germline *PALB2* mutation and 2/2 pathogenic germline *TP53* mutations. False positive germline mutations were diagnosed in 4 genes at a rate of 2.4%–10.3% (*APC* = 2.4%, *PALB2* = 2.4%, *PTEN* = 4.9%, *TP53* = 10.3%).

Conclusion: Tumor testing for *BRCA1/2* germline mutations using an NGS platform is fairly reliable with no false positive findings, and correctly diagnosed more than two-thirds of pathogenic germline *BRCA1/2* mutations. However, it is not reliable to diagnose pathogenic germline mutations in genes frequently mutated in sporadic cancers, such as *PTEN* and *TP53*.

1. Introduction

About half of hereditary breast and ovarian cancer syndromes are attributed to germline mutations in the *BRCA1/2* genes [1]. Women with germline *BRCA1/2* mutations have 50–85% lifetime risk of breast cancer and up to 40% lifetime risk of ovarian cancer [1,2]. The

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conventional indication for diagnosing *BRCA1/2* hereditary breast and ovarian cancer is to identify high-risk individuals for early cancer screening and preventive strategies and to facilitate predictive testing for family members. Blood sample, saliva or buccal smear of a cancer-affected individual are common sources of DNA utilized for clinical genetic testing to diagnose hereditary cancer syndromes. However, this traditional method of genetic testing can only be performed in living patients. Cancer-free individuals with strong family history of cancer suspicious of hereditary syndrome but whose cancer-affected family members are deceased makes up about 10% of patients we see in our cancer genetics clinics. Genetic testing of these cancer-free individuals as index patients without reference to the mutation status of cancer-affected family members is generally not recommended as the failure to identify a pathogenic mutation is uninformative and does not reassure the healthy relative that he/she is at low risk of developing cancers. On the other hand, if testing of archival tumor can reliably detect a germline pathogenic mutation in a deceased cancer-affected family members, the information can potentially be used to facilitate predictive (cascade) testing in healthy relatives, which will then be informative. However, most clinical laboratories do not offer archival tumor testing due to technical challenges. In this study, we explore the use of a next-generation sequencing (NGS) platform to diagnose *BRCA1/2* germline mutations from archival tumor specimens.

2. Materials and methods

2.1. Sample selection

Archival formalin fixed paraffin-embedded (FFPE) tumor specimens (breast = 25, ovarian = 26, others = 9) from 60 cancer patients were selected. All patients provided written informed consent for the study, and the study was approved by the institutional ethics review board. The patients were clinically suspected to have hereditary cancer syndromes, underwent genetic counselling at the cancer genetics clinic at the National University Cancer Institute, Singapore (NCIS), followed by clinical germline testing using a blood sample with comprehensive sequencing and deletion/duplication analysis of a panel of genes ranging from 29 to 82 genes. Patients were found to carry germline pathogenic mutations, variants of uncertain significance (VUS) or no pathogenic mutations/VUS in moderate- to high-penetrance breast cancer predisposition genes including *BRCA1*, *BRCA2*, *ATM*, *PALB2*, *PTEN* and *TP53*. Twenty-two patients have *BRCA1/2* pathogenic mutations and 38 patients are without. Five patients have mutations in 3 other breast cancer predisposition genes (*ATM* = 2; *PALB2* = 1; *TP53* = 2). Tumor specimens of all 60 patients were sequenced for *BRCA1/2* genes and 41 specimens were sequenced for 5 additional genes (*APC*, *ATM*, *PALB2*, *PTEN*, *TP53*) with an NGS platform. Tumor testing and results interpretation were performed blinded to the clinical germline test results.

2.2. Sample preparation

Genomic DNA was extracted from 10-uM thick archival FFPE tissue samples using the RecoverAll™ Total Nucleic Acid Isolation Kit (Qiagen, Hilden, Germany). The concentration and integrity of the purified DNA were examined using the Qubit-iT™ dsDNA HS Assay Kit (Invitrogen, Carlsbad, CA, USA) and a Fragment Analyzer (Advanced Analytical Technologies, Ankeny, IA, USA), respectively.

2.3. Targeted massively parallel sequencing and bioinformatics analyses

Next-generation sequencing was performed by ACT Genomics Co., LTD. using the ACTBRCA™ and ACTRepair™ targeted gene panel. In brief, 40 ng of genomic DNA was PCR amplified to enrich the coding exons of *BRCA1*, *BRCA2*, *APC*, *ATM*, *PALB2*, *PTEN*, and *TP53* gene. The exonic regions of the gene of interest were covered 100%, including the intronic regions within 20-basepairs of a splicing junction. The mean sequencing depth for the tumor samples was >1000× with a mean uniformity of 92%.

Raw sequence reads were mapped to the human reference genome (hg19) using The Torrent Suite Server (v. 5.2) and base calling and variant calling were performed with the Torrent Suite Variant Caller plug-in version 5.2. Variants with a read count <25 and a variant frequency < 5% were not analyzed further. Common single nucleotide polymorphisms (SNPs) were identified using the 1000 Genome (Phase 3 data). Variant with 30–70% mutant allele frequency is interpreted as a germline mutation [3]. Previously reported *BRCA1/2* mutations were identified and classified with the BIC (Breast Cancer Information Core, http://research.nhgri.nih.gov/bic/), ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/), LOVD (Leiden Open Variation Database, http://www.lovd.nl/3.0/home), ARUP (http://arup.utah.edu/database/BRCA/), and BRCA Share (http://www.umd.be/BRCA1/) data sets. A variant classified as "likely benign" or "likely pathogenic" by the ClinVar was considered as a variant of uncertain significance (VUS). Variants were classified as pathogenic if 1) they were labeled as such in any of the datasets used for the study or 2) they were frameshift or stop mutations. Variants were considered as benign if they were unequivocally classified as such (i.e., without a concurrent classification either as VUS or pathogenic within the same database) in the consulted data sets. All of the previously unidentified variants that were not clearly benign or pathogenic were regarded as VUS. A pathogenic variant was considered as novel if it was absent in the above-mentioned data sets as well as in the COSMIC database (version 81, http://cancer.sanger.ac.uk/cosmic). SIFT (http://sift.jcvi.org/), PolyPhen2 (http://genetics.bwh.harvard.edu/pph2/index.shtml), and Grantham (http://asia.ensembl.org/info/genome/variation/predicted_data.html) were used to predict the functional impact of the detected variants. Variants classified as "probably damaging" in PolyPhen2 were considered as pathogenic, whereas "possibly damaging" variants were classified as VUS. SNP data of 997 healthy subjects of the population-based project in Taiwan were downloaded for comparison (https://taiwanview.twbiobank.org.tw/index). In line with previous methodology [4], loss of heterozygosity (LOH) was determined by analyzing the allele frequency of the patient's SNPs within the mutated *BRCA* gene using the Aberration Detection in Tumor Exome (ADTEx) tool.

2.4. Correlative analysis between tumor results and direct germline results with blood testing

Direct germline results from blood testing were initially blinded to the tumor test. A tumor variant was independently assessed as pathogenic or non-pathogenic and germline or somatic using the above described algorithms. Thereafter, direct germline test results from blood were unblinded and used as the reference and correlated with the tumor results to calculate concordance, false negative and false positive rates.

3. Results

3.1. Overall concordance rate for germline variants between tumor and blood

Of 60 patients, 27 (45%) were clinically tested to carry pathogenic germline mutations, 21 (35%) had variants of uncertain significance, and 12 (20%) were tested negative with no pathogenic mutations or variants of uncertain significance identified in blood. Tumor testing achieved an overall concordance rate of 80% (48/60) with blood testing for all 5 genes and 88% (15/17) for *BRCA1/2*. Concordance rates were 71.4% (19/27), 81% (17/21) and 100% (12/

12) for pathogenic mutations, variants of uncertain significance and no mutations/VUS, respectively.

3.2. Tumor: blood concordance rate of germline pathogenic versus no pathogenic variants for each gene tested (Fig. 1)

3.2.1. BRCA1/2

13/60 (21.7%) and 9/60 (15%) patients were clinically tested to carry *BRCA1* and *BRCA2* germline pathogenic mutations respectively on blood testing. Tumor testing correctly diagnosed all 38 patients with no pathogenic germline *BRCA1/2* mutations as negative, with no false positive results. Of the 13 patients with pathogenic germline *BRCA1* mutations, 8/13 (61.5%) were correctly diagnosed on tumor testing, while 5/13 (38.5%) were discordant due to technical errors ($n = 3$) and interpretation errors ($n = 2$). Of the 9 patients with pathogenic germline *BRCA2* mutations, tumor testing correctly diagnosed 7/9 (77.8%) patients, while 2/9 (22.2%) were discordant due to technical errors ($n = 1$) and interpretation errors ($n = 1$). In total, tumor testing identified 17 *BRCA1/2* pathogenic mutations, of which 15/17 (88%) were classified as germline (concordant with blood results) and 2/17 (12%) were classified as somatic. Overall false negative rate with tumor testing for *BRCA1/2* germline pathogenic mutation was 32%. 2/13 (15.4%) and 5/9 (55.6%) false negative results were identified in Chinese versus non-Chinese patients ($p = 0.047$), while 4/9 (44.4%) and 3/13 (23.1%) false negative results were identified in patients with cancer diagnosis below age 50 versus age 50 and above ($p = 0.290$).

3.2.2. ATM, TP53, PALB2, PTEN and APC

Of 41 patients whose tumor specimens were tested for 5 additional genes, 2 (4.9%), 2 (4.9%), 1 (2.4%), 0 (0%) and 0 (0%) had pathogenic germline mutations in *ATM*, *TP53*, *PALB2*, *PTEN* and *APC* respectively. Tumor testing correctly diagnosed 1/2 *ATM*, 2/2 *TP53* and 1/1 *PALB2* germline pathogenic mutations. However, in contrast to tumor testing for *BRCA1/2*, false positive results were noted in 4 genes with the exception of *ATM*, being highest in *TP53* (4/39, 10.3%), followed by *PTEN* (2/41, 4.9%), *PALB2* (1/40, 2.5%) and *APC* (1/41, 2.4%). No significant correlation was found between false positive results in these 5 genes and patient ethnicity (Chinese versus non-Chinese, $p = 0.571$) or age at cancer diagnosis (<50 versus ≥50, $p = 0.244$).

3.3. Tumor: blood concordance rate with respect to type of mutations

Of the 27 specimens with pathogenic germline mutations identified from direct blood testing in the 60 patients, 1 (3.6%) was a large duplication in *BRCA1*, 14 (53.6%) were frameshift (*ATM* = 1, *BRCA1* = 7, *BRCA2* = 6), 4 (14.3%) were missense (*BRCA1* = 2, *TP53* = 2), and 8 (28.6%) were nonsense mutations (*ATM* = 1, *BRCA1* = 3, *BRCA2* = 3, *PALB2* = 1). Tumor testing correctly diagnosed 7/8 (87.5%) germline nonsense mutations, 11/15 (73.3%) germline frameshift mutations, 2/4 (50%) germline missense mutations, and 0/1 large duplication (*BRCA1* = 1). Of the 8 pathogenic germline mutations that were not correctly diagnosed on tumor testing, both missense mutations and one frameshift mutation were missed due to interpretation error (i.e., mutation detected in the tumor but wrongly classified as VUS), while the remaining of the other mutation types were missed due to technical errors (i.e., mutation was not detected in the tumor; Table 1).

3.4. Tumor: blood concordance rate with respect to age of archival tumor specimens

Age of the archival tumor specimens was calculated based on the number of days between the date of surgery and date of tumor analysis. The median age of the archival tumor specimens was

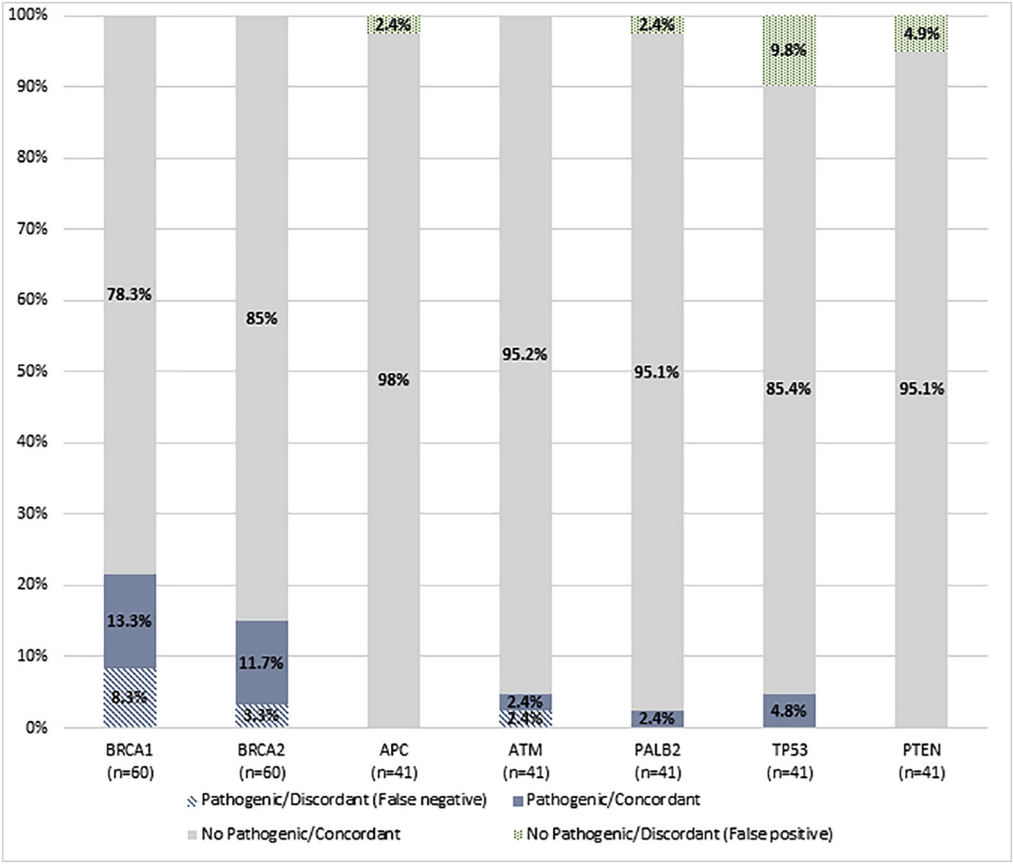

Fig. 1. Concordance rates for pathogenic mutations versus no pathogenic mutations identified for each gene.

Table 1
Germline and somatic pathogenic mutations identified/not identified on tumor testing.

Pathogenic germline mutations detected in tumor						
S/N	Specimen ID	Genes	Allele freq.	cDNA change	Status	Tumor: blood concordance
1	NB12-17302	BRCA1	74.1%	c.2866_2870delTCTCA	Pathogenic	Yes
2	NB13-21756	BRCA1	84.0%	c.5525delT	Pathogenic	Yes
3	NB15-21981	BRCA1	69.7%	c.4065_4068delTCAA	Pathogenic	Yes
4	NB14-13090	BRCA2	51.5%	c.8585_8586delTA	Pathogenic	Yes
5	NB14-14991	BRCA2	83.8%	c.9376C > T	Pathogenic	Yes
6	NB15-07689	BRCA1	75.2%	c.4201C > T	Pathogenic	Yes
7	NB16-20579	BRCA2	64.8%	c.1763_1766delATAA	Pathogenic	Yes
8	NB16-11140	BRCA2	81.3%	c.6405_6409delCTTAA	Pathogenic	Yes
9	NB16-07110	BRCA1	45.0%	c.2726dupA	Pathogenic	Yes
10	NB16-04847-1	BRCA2	66.5%	c.5722_5723delCT	Pathogenic	Yes
11	NB15-10122	ATM	87.3%	c.785T > A	Pathogenic	Yes
12	NB15-10105	BRCA2	85.1%	c.262_263delCT	Pathogenic	Yes
13	NB10-01881	BRCA1	80.6%	c.3214delC	Pathogenic	Yes
		TP53	58.0%	c.1024delC	Pathogenic	No (false positive)
14	NB15-08130	PTEN	47.6%	c.126dupT	Pathogenic	No (false positive)
15	NB14-04415	TP53	57.0%	c.636delT	Pathogenic	No (false positive)
16	NB13-03774	PTEN	29.5%	c.955_958delACTT	Pathogenic	No (false positive)
17	NB13-01189	TP53	52.4%	c.817C > T	Likely pathogenic	No (false positive)
18	NB17-21889	PALB2	80.7%	c.3523C > T	Likely pathogenic	Yes
19	NB11-16540	APC	63.8%	c.3151A > T	Likely pathogenic	No (false positive)
		TP53	78.5%	c.733G > A	Likely pathogenic	Yes
20	NB17-07790	TP53	61.0%	c.329G > T	Likely pathogenic	Yes
21	NB13-11829	BRCA1	61.7%	c.3661G > T	Pathogenic	Yes
22	NB15-07067	BRCA2	51.5%	c.3109C > T	Pathogenic	Yes
23	NB17-18766	BRCA1	58.1%	c.4508C > A	Pathogenic	Yes
24	NB16-14600	PALB2	40.7%	c.149A > C	Likely pathogenic	No (false positive)
		TP53	48.3%	c.1015G > T	Pathogenic	No (false positive)

Pathogenic germline mutations not identified on tumor testing						
S/N	Specimen ID	Genes	Allele freq.	cDNA change	Status	Technical/interpretation errors
1	NB13-09226	BRCA2	NA	c.9027T > G (p.Tyr3009*)	NA	Technical
2	NB14-22350	BRCA1	NA	Duplication of exon 12	NA	Technical
3	NB13-07796	BRCA1	NA	c.2726dupA (p.Asn909fs)	NA	Technical
4	NB12-11992	BRCA1	NA	c.329dupA (p.Glu111Glyfs*3)	NA	Technical
5	NB13-01189	ATM	NA	c.8435_8436delCT(p.Ser2812Phefs*2)	NA	Technical
6	NB15-09166	BRCA2	83.6%	c.2094_2095delAC	VUS	Interpretation
7	NB14-04364	BRCA1	79.7%	c.5072C > A	VUS	Interpretation
8	NB13-06871	BRCA1	77.6%	c.5165C > T	VUS	Interpretation

Other tumor pathogenic mutations identified that were classified as somatic					
S/N	Specimen ID	Genes	Allele freq.	cDNA change	Status
1	NB14-13090	BRCA2	32.7%	c.196C > T	Pathogenic
2	NB13-06871	BRCA1	21.0%	c.824G > A	Likely pathogenic
3	NB15-08130	PTEN	24.6%	c.388C > G	Likely pathogenic
4	NB12-11992	TP53	10.9%	c.772G > T	Pathogenic
5	NB16-05223-2	TP53	24.0%	c.638G > A	Likely pathogenic
6	NB13-11829	TP53	25.7%	c.380C > T	Likely pathogenic
7	NB17-18766	TP53	12.4%	c.818G > A	Likely pathogenic
8	NB17-12562	TP53	22.9%	c.437_458delGGGTTGATTCCACACCCCCGCC	Likely pathogenic

894.5 days or 2.45 years (range 178–2795 days [0.5–7.7 years]). Tumor specimens that are <1 year old achieved a 100% concordance rate with germline testing while tumor specimens of 1 to 3 years achieved a slightly lower concordance rate of 91.2%. Concordance rates for older tumor specimens aged 3 to 5 years and >5 years were much lower at 67% and 75% respectively.

3.5. Tumor: blood concordance rate with respect to tumor type

The tumor origin of the 60 specimens tested were ovarian ($n = 26$), breast ($n = 25$), colon (n = 2), peritoneal (n = 2), paraganglioma ($n = 1$), adrenal (n = 1), prostate (n = 1), stomach (n = 1) and thyroid (n = 1). Tumor testing correctly diagnosed germline pathogenic mutations in 19/26 (73.1%) ovarian tumor specimens and 20/25 (80%) breast tumor specimens. Concordance rate for the other 7 tumor types ($n = 9$) was 100%.

4. Discussion

Next-generation sequencing has been available for the last decade. Due to its ability to sequence multiple genes simultaneously and its cost effectiveness, it is now widely used in the clinic to diagnose hereditary cancer syndromes in cancer patients using blood sample, buccal swab or saliva smear. Besides being used to identify germline mutations, NGS has increasingly been used to detect somatic mutations in tumor to guide targeted therapies [5]. To our knowledge, this is the first study to prospectively assess the ability of tumor NGS testing to diagnose germline mutations for hereditary cancer.

In our study, we observed that tumor testing was able to diagnose more than two thirds of patients with germline *BRCA1/2* pathogenic mutations. Notably, no false positive results were identified in *BRCA1/2* genes. Of the 17 *BRCA1/2* pathogenic mutations detected in the tumor, the majority were germline mutations, with only 12% (2/17) being somatic in nature, which is consistent

with the observation from another study that most tumor pathogenic *BRCA1/2* are germline in nature [6]. However, approximately 30%of pathogenic germline *BRCA1/2* mutations were not diagnosed on tumor testing, due to either technical or interpretation errors. Specifically, three *BRCA1/2* germline mutations were identified on tumor testing but were wrongly classified as variants of uncertain significance (interpretation errors) based on the existing bioinformatics pipelines used in our study. To improve the interpretation of the pathogenicity of an identified variant, we propose to include ExAC population database into our existing bioinformatics pipelines (i.e. CLINVAR, BIC, BRCA share databases, which we believe will reduce the interpretative error). Four *BRCA1/2* germline mutations which were not identified at all can all be recovered in the variant calling bioinformatics pipelines once we adjust the threshold and filtering of the algorithm. Thus, we believe that the accuracy of tumor testing to diagnose *BRCA1/2* germline mutations can be further improved by refining the bioinformatics algorithm.

In contrast to *BRCA1/2*, false positive rates of ~2–10% were reported in 4 other moderate- to high-penetrance cancer susceptibility genes tested, and was particularly high for *TP53* (~10%) and *PTEN* (~5%). Of note, the 2 false positive *PTEN* variants identified on tumor testing had an allele frequency of 29.5% and 47.6% while the 4 false positive *TP53* variants identified on tumor testing had an allele frequency ranging from 48.3% to 58%, overlapping with the allele frequency expected for a germline variant (30–70%). *TP53* and *PTEN* are two genes in which somatic mutations are common [7,8], indicating that using tumor to diagnose germline mutations in genes that are commonly mutated in tumors, such as *TP53* and *PTEN*, is unreliable with current testing technology and algorithms. Given the high false positive rate of *TP53* and *PTEN* germline pathogenic mutations detected in tumor testing, we do not recommend using archival tumor specimens from deceased cancer patients as the surrogate biological materials for detection of germline *TP53* or *PTEN* mutation.

We found that tumor specimens that are older than 3 years have lower concordance rate with germline (blood) test results compared to newer tumor specimens, and this may be due to poorer DNA quality from older tumor specimens. In this study, approximately 18.2% of pathogenic germline *BRCA1/2* mutations were missed due to technical errors, which may in part be contributed by poor tumor DNA quality. Tumor testing also failed to identify a *BRCA1* large duplication. Although NGS can be used to identify germline variants in blood samples, there are still pitfalls in the use of an NGS platform to diagnose germline variants in archival tumors. However, its reliability may potentially be improved in the future with further advancements in sequencing technology and variant calling algorithms.

How should we interpret a tumor test that is negative for *BRCA1/2* pathogenic mutations? If the tumor test is negative in the context of a deceased index patient, the result is considered uninformative, and further testing in cancer-free family members will not be recommended, and cancer screening and preventive recommendations will be provided based on the family cancer history. On the other hand, in the context of a living index patient who underwent tumor NGS testing in search for actionable mutations for therapeutic indications and no pathogenic *BRCA1/2* mutation is detected in the tumor, direct germline testing with blood or saliva may still be warranted if the personal and/or family cancer history fulfils traditional criteria for genetic testing, as tumor testing does not exclude all identifiable germline mutations. In particular, false negative rates are likely to be higher with older archival tumor specimens and when the *BRCA1/2* mutations are large deletions, duplications or rearrangements.

5. Conclusions

In conclusion, archival tumor testing using an NGS platform can detect ~70% of germline *BRCA1/2* pathogenic variants, although it will not diagnose all mutations owing to either technical or interpretative errors. Nonetheless, in the context of a high-risk family with no living affected index patient, tumor testing can potentially diagnose germline *BRCA1/2* mutation in the family to facilitate cascade testing in cancer-free family members for early cancer screening and prevention. However, this is best done in specialized clinics by trained personnel and the test results interpreted cautiously. On the other hand, due to the high false positive rates observed in *TP53* and *PTEN*, tumor testing will not be a reliable test to diagnose Cowden syndrome or Li-Fraumeni syndrome with current technology and is not recommended.

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Declaration of competing interest

Song Ling Poon, Kien Thiam Tan, Shu-Jen Chen and Chien-Hong Chen are employed by ACT Genomics Co., Ltd. Tumor NGS analysis for the study was supported by ACT Genomics Co., Ltd.

Author contribution

Soo Chin Lee has conceived and designed the study. Soo Chin Lee and Pei Yi Ong contributed to the writing of the manuscript, analysis and interpretation of the results. Thomas Choudary Putti has contributed in the preparation of the tumor sample slides. Samuel Guan Wei Ow provided critical feedback of the manuscript. Shu-Jen Chen and Chien Hong Chen contributed to the bioinformatics analysis. Kien Thiam Tan and Song Ling Poon contributed to the medical informatics analysis. Kien Thiam Tan is also the Principal Investigator guiding the compilation of all analysis. All authors provided critical feedback and helped shape the research, analysis and manuscript. All authors have approved the final article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ygyno.2019.08.027.

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