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ORIGINAL ARTICLE

Pathogenic germline variants in cancer predisposition genes in patients with multiple primary cancers in an Asian population and the role of extended panel genetic testing

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Background: Multiple primary cancers (MPC) are an indicator of potential hereditary cancer predisposition syndrome. There remains insufficient data on genetic testing outcomes and the optimal testing panel for MPC. We evaluated the prevalence of MPC, the spectrum of pathogenic germline variants (PGVs) and the role of extended panel testing in MPC.

Methods: Cancer patients seen in a cancer genetics clinic in a tertiary cancer centre in Singapore from 2000 to 2023 were included. Clinical characteristics, PGV and patterns of cancer were analysed. Most patients were tested with 49 genes, but in a selected 156 patients with MPC, extended testing with 216 genes was carried out.

Results: Of 3514 cancer patients (male = 17.9%, female = 82.1%), 668 (19%) had MPC (2 primaries, $n = 570$; 3 primaries, $n = 81$; $\geq$4 primaries, $n = 17$). The most common tumour pairs were breast—breast (33.2%), breast—ovary (8.9%), breast—endometrial (4.6%) and endometrial—ovary (4.6%). Patients with MPC had a younger median age of first cancer. Of the MPC patients, 29.4% tested positive for at least one PGV, with PGVs detected in *BRCA1/2* (39.9%), other homologous recombination repair (*HRR*) genes (18.9%), mismatch repair (*MMR*) genes (11.2%) and *TP53* (7%) genes. *HRR* genes included *ATM*, *BARD1*, *BRIP1*, *CHEK2*, *PALB2*, *FANCL*, *RAD51C* and *RAD51D*, while *MMR* genes included *MLH1*, *MSH2*, *MSH6* and *PMS2*. MPC patients were more likely to have PGVs in *TP53* and *BARD1* compared with patients with single primary cancer. Extended testing detected more PGVs in MPC despite initial noninformative testing. It increased the number of PGVs detected in less established cancer predisposition genes, which include *CFTR*, *SPINK1*, *TNFRSF13B*, *TET2*, *ADA*, *CDKN1C*, *CTNNA1*, *DDX41*, *HAX1*, *RECQL4* and *MBD4*.

Conclusion: Patients with MPC were more likely to harbour a PGV. Extended testing improved PGV detection rates, particularly for less well-known cancer predisposition genes.

Key words: multiple primary cancers, pathogenic germline variants, germline genetic testing, extended testing, hereditary cancer predisposition syndrome

INTRODUCTION

Multiple primary cancers (MPC) are defined as two or more histologically distinct malignancies within one individual that are not caused by metastasis, recurrence or local progression.[1] Worldwide, the frequency of MPC is reported to be in the range of 2%-17%.[1] The wide range is likely due to differences in cancer registration practices, case definitions and follow-up duration.[2,3]

Various factors have been implicated in the occurrence of MPC. With better cancer therapeutics, improved survival has led to an increase in the diagnosis of MPC.[4,5] Additionally, patients may develop MPC due to heritable cancer predisposition genes, environmental exposures and/or late effects of therapies.[1,6] Longer survival, indolent primary cancer and positive family history are all reported to be associated with greater incidence of MPC.[7,8]

It is widely recognized that cancer predisposition syndromes can increase the risk of MPC.[9-11] Identification of this population can facilitate enhanced cancer surveillance in cancer-free individuals and personalized therapies in cancer patients. As genetic testing is becoming more common, there are opportunities to study the landscape of heritable mutations in MPC. In this report, we describe the prevalence and

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clinical characteristics of MPC, cancers associated with MPC and the genetic profile of our MPC patients.

Genetic testing plays a vital role in cancer care, yet the optimal approach for MPC patients remains uncertain. Standard multigene panels may miss PGVs in less established cancer predisposition genes. This study explored the potential of broader panel genetic testing using a 216-gene extended panel to capture the genetic diversity of MPC, with a focus on lesser-known genes such as *CFTR* and *SPINK1*. The findings aim to enhance PGV detection, refine testing strategies and shed light on the unique genetic landscape of MPC populations.

MATERIALS AND METHODS

Study population and methods

This single-centre retrospective study was conducted at the National University Cancer Institute (NCIS), Singapore between 2000 and 2023. Cancer patients with personal/family history suspicious of hereditary cancer syndrome and patients testing for therapeutic decisions were included in this analysis. Clinical criteria for suspected hereditary cancer syndrome included young onset of cancer, MPC and/or familial clustering of related cancers. Cancer-free individuals who underwent genetic testing as a result of cascade testing were excluded.

The primary objectives of the study were to evaluate the spectrum of PGV and the role of extended panel testing in MPC. The secondary objectives were to assess the prevalence, clinical characteristics, pattern and genes associated with higher propensity for MPC. Genotype—phenotype associations were also assessed, and concordance was defined as having at least one cancer known to be associated with the PGV. All analyses were carried out using de-identified data. The study was conducted in accordance with the Declaration of Helsinki and approved by the Domain Specific Review Board of Singapore.

Germline genetic testing

Before 2015, genetic testing was carried out using Sanger sequencing with or without deletion/duplication analysis of targeted causative genes of suspected syndromes. From 2015 onwards, next-generation sequencing (NGS) with multigene panel testing (MGPT) was implemented at commercial laboratories that are Clinical Laboratory Improvement Amendments and/or College of American Pathologists certified. MGPT up to 49 genes including full-gene sequencing and deletion/duplication analysis was carried out for most patients. For a subset of MPC patients (n = 156), we carried out an extended MGPT comprising 216 genes (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2025.104495). This group of patients included 107 MPC patients who donated DNA specimens to a pharmacogenetics DNA bank but who were not referred to the cancer genetics clinic and 49 patients who were tested at the cancer genetics clinic but who were either not found with PGV or who had a PGV in a gene that is not completely concordant with the personal and/or family cancer history.

Identification of a pathogenic/likely pathogenic variant was considered a positive result. Variants of unknown significance (VUS) were considered negative results and are not reported in detail in this manuscript. Identified variants were reviewed manually, applying current standards for variant classification by the American College of Medical Genetics and Genomics, to define likely pathogenic/pathogenic variants.[12] PGVs were classified according to high, moderate, low and uncertain penetrance.

Statistical analysis

All statistical analysis were carried out using R packages. Statistical comparisons of categorical variables were undertaken using Fisher's exact test and chi-square test, while comparisons for continuous variables were undertaken with *t*-test or Wilcoxon signed rank test where appropriate. A two-sided P value of <0.05 was considered to be statistically significant. All analyses were conducted using R 4.1.0 with packages 'pheatmap', 'circlize', 'ggplot2' and 'tableone'.

RESULTS

Baseline characteristics and genetic testing outcome of overall population

A total of 3514 patients were seen at the NCIS Cancer Genetics Clinic between 2000 and 2023; of these, 2846 (81.0%) had a single primary cancer (SPC) and 668 (19.0%) had MPC. In the overall population, the median age of first cancer was 51 years (range 6-88 years). Of the patients, 82.1% were female and 68.2% were Chinese. The most common suspected genetic syndromes were hereditary breast ovarian cancer (n = 2377, 67.6%), followed by Lynch syndrome (n = 713, 20.3%) and Li Fraumeni syndrome (n = 86, 2.4%) (Table 1).

A total of 2249 patients (64.0%) underwent genetic testing. The uptake of genetic testing was higher among patients with MPC compared with SPC (72.9% versus 61.9%, $P < 0.001$). The most common gene panel employed was a 49-gene panel (n = 1509; 67.1% of all patients tested). Overall, 510 patients (22.7%) tested positive for any PGV. The most common PGVs were *BRCA1/2* mutations (n = 239, 46.8%), other *HRR* gene mutations (n = 102, 20%) and *MMR* gene mutations (n = 68, 13.3%). *HRR* genes included *ATM*, *BARD1*, *BRIP1*, *CHEK2*, *PALB2*, *FANCL*, *RAD51C* and *RAD51D* while *MMR* genes included *MLH1*, *MSH2*, *MSH6* and *PMS2* (Table 2).

MPC and causative genes

Among the 668 MPC patients, 570 (85.3%) had two primary cancers and 98 (14.7%) had three or more primary cancers. The most common cancer pairs were breast—breast (n = 189, 33.2%), breast—ovary (n = 51, 8.9%), breast—endometrial (n = 26, 4.6%) and endometrial—ovary (n = 26, 4.6%) (Figure 1C; Supplementary Tables S2 and S3, available at https://doi.org/10.1016/j.esmoop.2025.

Table 1. Baseline characteristics of our patients

	All patients ($N = 3514$)	Patients with single primary cancer ($N = 2846$)	Patients with multiple primary cancers ($N = 668$)
Median age of first cancer, years (range)	51 (6-88)	54 (11-88)	45 (6-87)
Sex, *n* (%)			
Male	628 (17.9)	516 (18.1)	112 (16.8)
Female	2886 (82.1)	2330 (81.9)	556 (83.2)
Race, *n* (%)			
Chinese	2397 (68.2)	1894 (66.5)	503 (75.3)
Malay	356 (10.1)	294 (10.3)	62 (9.3)
Indian	205 (5.8)	167 (5.9)	38 (5.7)
Others	556 (15.8)	491 (17.3)	65 (9.7)
Family history, *n* (%)			
Consistent with suspected syndrome	1688 (48)	1410 (49.5)	278 (41.6)
Any family history of cancer	2747 (78.2)	2265 (79.6)	482 (72.2)
No family history of cancer	763 (21.7)	580 (20.4)	183 (27.4)
Information not available	4 (0.1)	1 (0.03)	3 (0.4)
Suspected cancer genetic syndrome, *n* (%)			
Hereditary breast ovarian cancer syndrome	2377 (67.6)	2025 (71.2)	352 (52.7)
Lynch syndrome	713 (20.3)	569 (20)	144 (21.6)
Li Fraumeni syndrome	86 (2.4)	55 (1.9)	31 (4.6)
Cowden syndrome	36 (1)	10 (0.4)	26 (3.9)
Others[a]	302 (8.6)	187 (6.6)	115 (17.2)
Index primary cancer, *n* (%)			
Breast	1871 (53.2)	1531 (53.8)	340 (50.9)
Ovary	400 (11.4)	359 (12.6)	41 (6.1)
Colon	394 (11.2)	317 (11.1)	77 (11.5)
Endometrial	193 (5.5)	152 (5.3)	41 (6.1)
Prostate	166 (4.7)	155 (5.4)	11 (1.6)
Pancreas	93 (2.6)	90 (3.2)	3 (0.4)
Others	397 (11.3)	242 (8.5)	155 (23.2)

[a]Others include less common genetic syndromes and patients who do not fulfil clinical criteria of any known hereditary cancer syndrome.

104495). Patients with MPC had a lower median age of first cancer compared with SPC (45 versus 54 years).

Of the MPC patients, 487 (72.9%) underwent genetic testing and 143 (29.4%) tested positive for any PGV. The most common PGVs were mutations in *BRCA1/2* (39.9%), other *HRR* genes (18.9%), *MMR* gene (11.2%) and *TP53* (7%) (Figure 1B). Most mutations were detected in moderate (18%) to high-penetrance (42%) genes. When compared

Table 2. Uptake of genetic testing and its outcome

	All patients, *n* (%)	Patients with single primary cancer, *n* (%)	Patients with multiple primary cancers, *n* (%)
Underwent genetic testing	($n = 3514$)	($n = 2846$)	($n = 668$)
Yes	2249 (64.0)	1762 (61.9)	487 (72.9)
No	1265 (36.0)	1084 (38.1)	181 (27.1)
Method of testing	($n = 2249$)	($n = 1762$)	($n = 487$)
Single genes testing	273 (12.1)	220 (12.5)	53 (10.9)
NGS testing	1974 (87.8)	1540 (87.4)	434 (89.1)
Information not available	2 (0.1)	2 (0.1)	0
Type of NGS panel	($n = 1974$)	($n = 1540$)	($n = 434$)
<20 genes	31 (1.6)	25 (1.6)	6 (1.4)
21-40 genes	123 (6.2)	110 (7.1)	13 (3.0)
41-60 genes	1642 (83.2)	1387 (90.1)	255 (58.8)
>60 genes	178 (9.0)	18 (1.2)	160 (36.9)
Results	($n = 2249$)	($n = 1762$)	($n = 487$)
Positive for PGV	510 (22.7)	367 (20.8)	143 (29.4)
VUS	840 (37.3)	624 (35.4)	216 (44.4)
Negative for PGV/VUS	892 (39.7)	771 (43.8)	121 (24.8)
Failed testing[a]	7 (0.3)	0	7 (1.4)
PGVs[b]	($n = 510$)	($n = 367$)	($n = 143$)
BRCA1/2	239 (46.8)	182 (49.6)	57 (39.9)
Other *HRR* genes[c]	102 (20.0)	75 (20.4)	27 (18.9)
MMR genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*)	68 (13.3)	52 (14.2)	16 (11.2)
TP53	20 (3.9)	10 (2.7)	10 (7.0)
Others	104 (20.4)	62 (16.9)	42 (29.3)

NGS, next-generation sequencing; PVG, pathogenic germline variant; VUS, variant of unknown significance.
[a]Sample did not meet quality metrics.
[b]The number of mutations is higher than total number of patients with positive germline results as some patients developed two or more PGVs.
[c]Other *HRR* genes include *ATM*, *BARD1*, *BRIP1*, *CHEK2*, *RAD51C*, *PALB2*, *FANCL* and *RAD51D*.

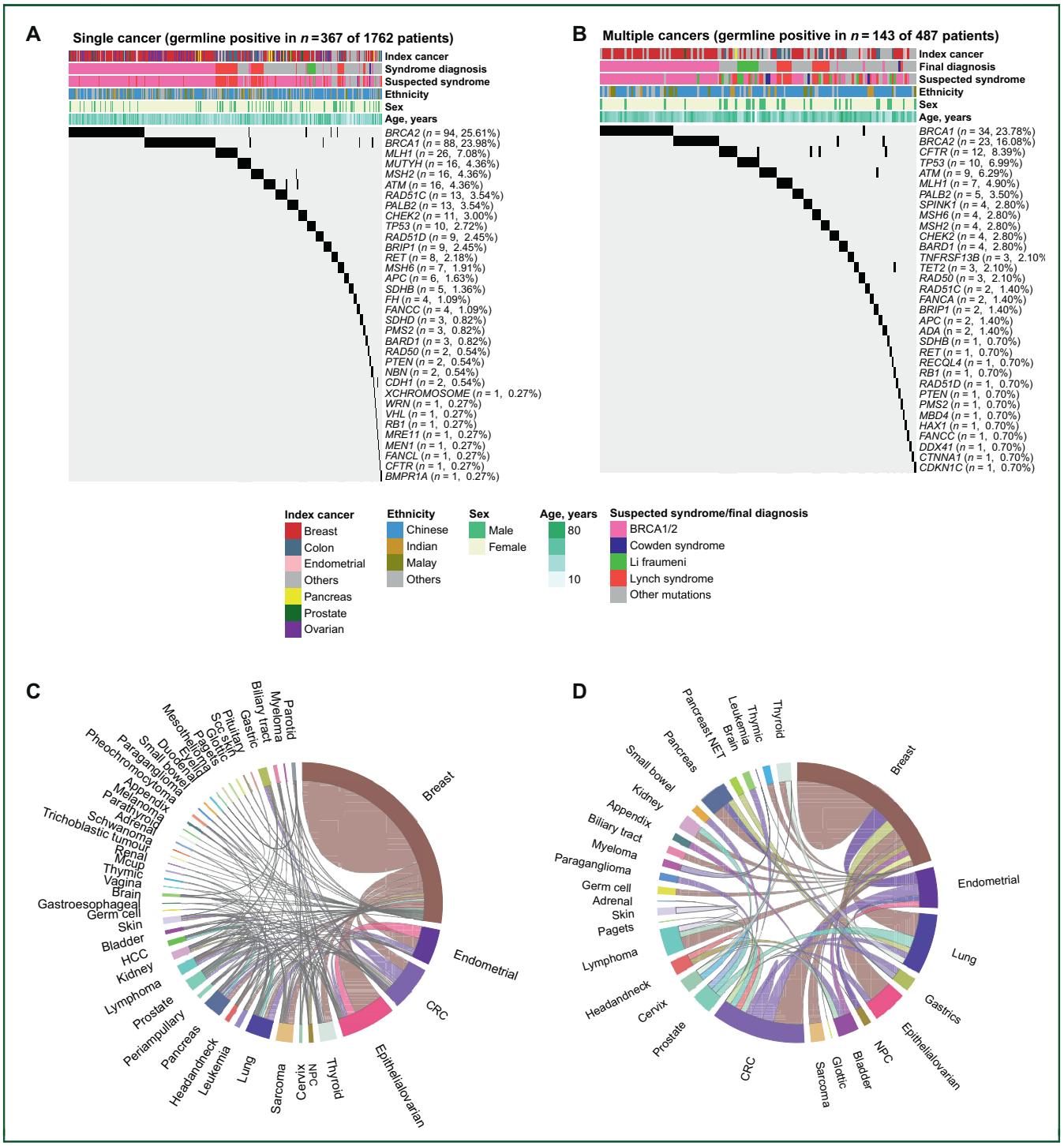

Figure 1. Heatmaps showing mutation pattern observed in patients tested positive for any pathogenic germline variant (PGV) in (A) single primary cancer (SPC; $n = 367$) and (B) multiple primary cancer (MPC; $n = 143$). Each column represents each individual patient and each row represent a gene. Clinical characteristics including index primary cancer, sex, ethnicity and suspected clinical syndrome are shown in colour at the bottom. Percentages reflect the number of patients with the respective mutations among patients with PGV. Among the patients, 14 with SPC and 8 with MPC had more than one mutation. (C) Chord diagram describing the pairs of first and second cancer diagnoses among all patients with MPC. (D) Chord diagram describing the pairs of second and third cancer diagnoses among patients with three or more primary cancers. Each connection in (C) and (D) reflects the cancer pairs, where the colour of the line denotes the preceding cancer. CRC, colorectal cancer; HCC, hepatocellular carcinoma; NET, neuroendocrine tumour; NPC, nasopharyngeal carcinoma.

with SPC, the prevalence of PGV was higher in MPC (29.4% versus 20.8%, $P = 0.04$). Notably, MPC patients had a higher proportion with *TP53* mutations (7.0% versus 2.7%) and *CFTR* mutation (8.4% versus 0.3%). Conversely, SPC patients had a higher proportion of patients with mutations in *BRCA* (49.6% versus 39.9%), other *HRR* (20.5% versus 18.9%), *MMR* (14.2% versus 11.2%) and *MUTYH* genes (4.4% versus 0%) (Table 2; Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2025.104495). The distribution of tumour types in those with an identified well-established

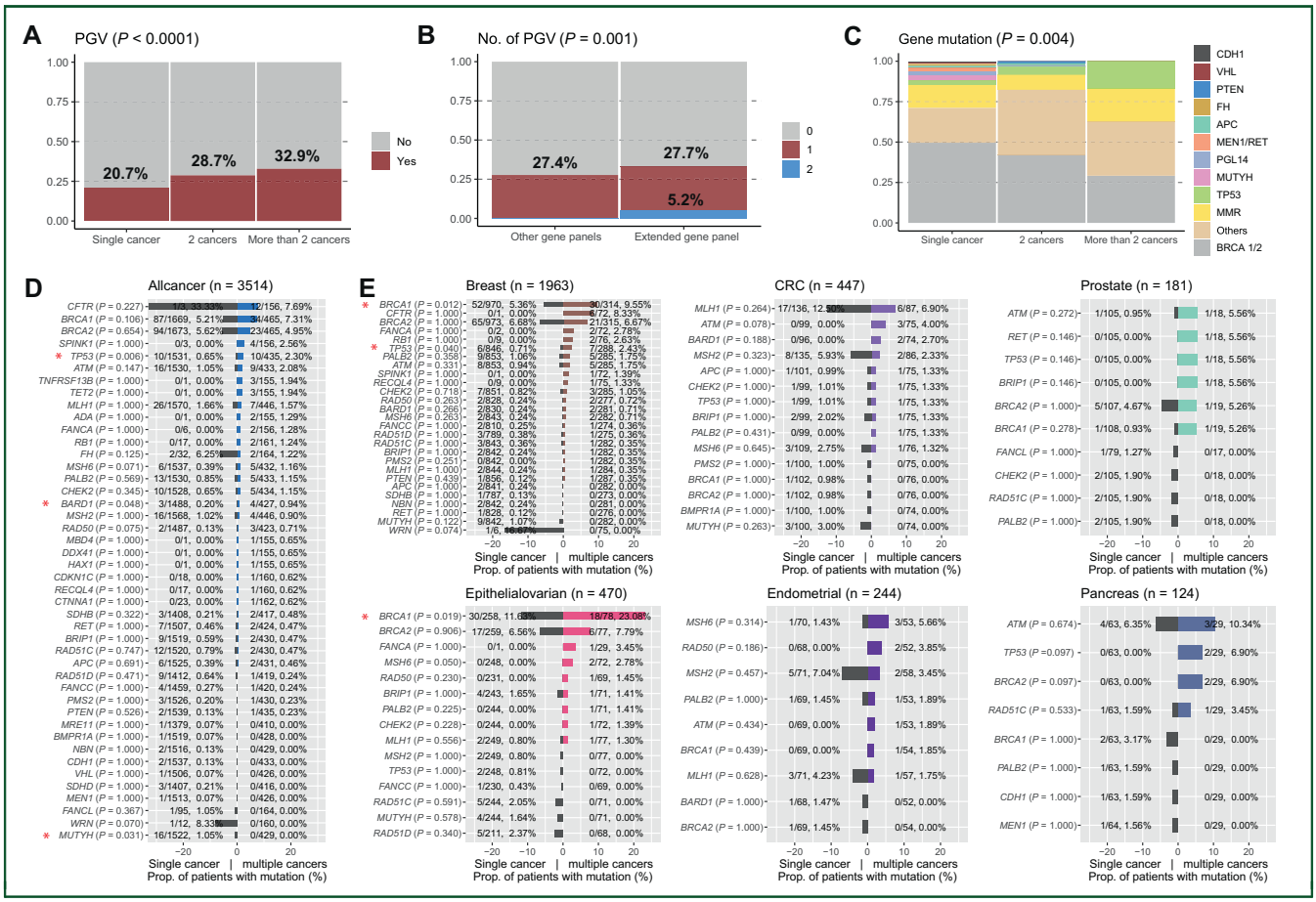

Figure 2. Key comparisons: (A) frequency of pathogenic germline variants (PGVs) among patients stratified by number of cancers; (B) number of PGVs identified per patient stratified by type of gene panels utilized. Other gene panel refers to gene panel other than the 216-gene panel. (C) Distribution of causative genes in patients identified with PGV stratified by number of primaries. (D) Comparisons of PGVs between patients with single versus multiple cancer across all cancer types. Each row denotes percentage of patients tested positive for the PGV (single versus multiple primary cancer). The percentage is calculated based on number of positive patients over total number of patients tested for the PGV. (E) Comparisons of PGVs between patients with single versus multiple cancer by cancer type. CRC, colorectal cancer; MMR, mismatch repair; PGL, paraganglioma—pheochromocytoma syndrome; prop, proportion; VHL, Von Hippel-Lindau.

cancer predisposition gene was consistent with known associations with a high genotype—phenotype concordance rate of 78% (Supplementary Table S5, available at https://doi.org/10.1016/j.esmoop.2025.104495).

We also compared genetic test results from patients with SPC versus MPC to identify genes that were associated with higher propensity for MPC. MPC patients, regardless of tumour types, were significantly more likely to harbour *TP53* and *BARD1* mutations than those with SPC ($P = 0.01$). Specifically, 2.3% (10/435) tested positive for *TP53* compared with 0.65% (10/1531) of SPC patients, while 0.94% (4/427) tested positive for *BARD1* compared with 0.2% (3/1488) of SPC patients. Additionally, MPC patients with ovarian and/or breast malignancies were more likely to carry *BRCA1* mutations ($P < 0.05$) than breast and/or ovarian cancer patients with SPC (Figure 2D and E]. Among breast cancer patients, 9.55% (30/314) of those with MPC tested positive for *BRCA1* compared with 5.36% (52/970) of SPC patients. Similarly, among ovarian cancer patients, 23.1% (18/78) with MPC tested positive for *BRCA1* compared with 11.6% (30/258) of SPC patients.'

Three or more primary cancers

Ninety-eight patients had three or more primary cancers (three cancers, $n = 81$; four cancers, $n = 13$; five cancers, $n = 3$; six cancers, $n = 1$). The median age at cancer diagnosis for this cohort was 51 years (range 10 to 77 years). The most common cancer combinations for this subgroup were breast—breast—breast ($n = 5$), followed by breast—breast—colon ($n = 4$) and breast—breast—ovary ($n = 4$). A total of 73 of the 98 patients (74.5%) underwent genetic testing, with 24 patients (32.9%) testing positive for a PGV. The most common PGVs were detected in *BRCA* ($n = 7$), *MMR* ($n = 5$) and *TP53* genes ($n = 4$) (Figure 3).

Role of extended panel testing in patients with MPC

A subset of 156/487 MPC patients were tested with an extended 216-gene panel. Of these patients, 49 (31.4%) had previously undergone testing with a smaller gene panel at the cancer genetic clinic, yielding noninformative results in 40 patients and positive results in 9 patients for a PGV not fully concordant with their personal and/or family cancer

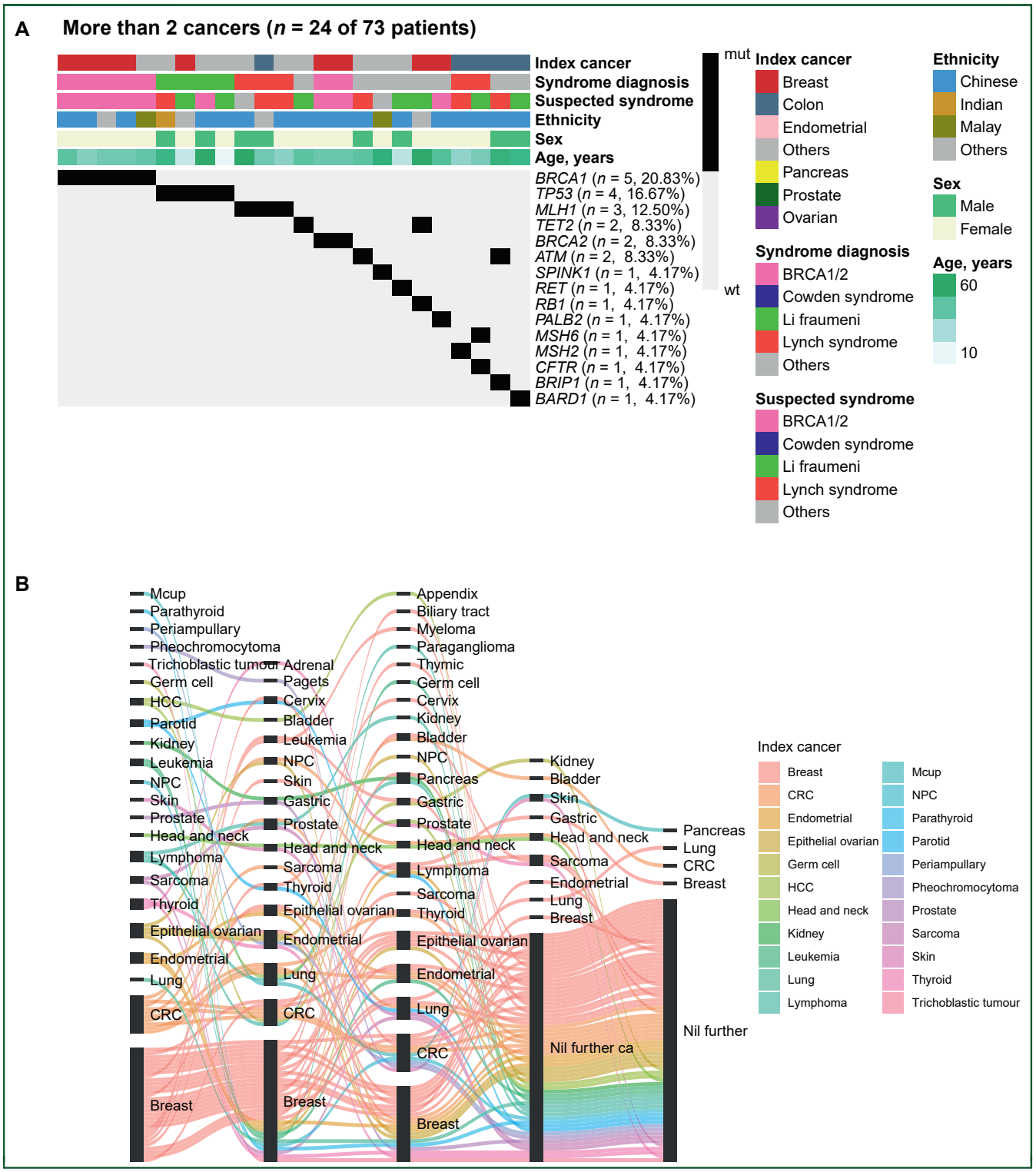

Figure 3. Three or more primary cancers. (A) Heatmap showing mutation patterns observed in 24 patients with three or more primary cancers with at least one pathogenic germline variant (PGV) identified. The plot represents a graphical summary of the distribution of PGVs across patients who underwent germline genetic testing. Each column represents each individual patient and each row represents a gene. Legend for clinical characteristics is shown on the right. Percentages reflect the number of patients with the respective mutations among patients with PGVs. (B) This Sankey plot illustrates the pattern of subsequent cancers for all patients. Each column represents a different cancer at the different timepoint, with nodes labelled by cancer types (e.g. breast, lung, colorectal cancer and others). The leftmost column lists the index primary cancers, followed by the subsequent primary cancers. Flows between nodes indicate transitions from one cancer type to another, with the thickness of each flow proportional to the number of patients making that transition. The final column, labelled 'nil further ca', represents patients who did not develop further additional cancers. The plot illustrates the patterns of multiple primary cancer among our patients.
CRC, colorectal cancer; HCC, hepatocellular carcinoma; mut, mutation; nil further ca, no further cancer; NPC, nasopharyngeal cancer; wt, wild type.

history. The remaining 107 patients (68.6%) were patients who donated a germline DNA sample into a separate DNA bank but were never referred to the cancer genetics clinic; the majority of these patients (65/107, 60.7%) had cancer combinations not suggestive of known hereditary cancer syndromes.

Overall, 32.7% of patients (51/156) tested positive for any PGV on extended panel testing. Seven patients had failed testing. The yield of PGV was 32.7% (35/107) among the 107 patients who were not previously referred to the cancer genetics clinic. Among the 49 patients who were previously tested with a smaller gene panel, 17.5% (7/40) who previously had noninformative testing were found to have a PGV, while 2/9 patients (22.2%) who were already found with a PGV on initial testing were found to carry an additional PGV with extended panel testing ($n = 2$ with *CTFR* PGV).

Extended testing increased the number of detected PGVs ($P = 0.001$). The additional detected PGVs were in lower-level evidence cancer predisposition genes, such as *CTFR* ($n = 12$), *SPINK1* ($n = 4$), *TNFRSF13B* ($n = 3$) and *TET2* ($n = 3$) (Supplementary Table S6, available at https://doi.org/10.1016/j.esmoop.2025.104495). Other uncommon mutations detected included *ADA* ($n = 2$), *CDKN1C*, *CTNNA1*, *DDX41*, *HAX1*, *RECQL4* and *MBD4* (all $n = 1$) (Supplementary Table S7, available at https://doi.org/10.1016/j.esmoop.2025.104495).

Twelve patients had PGV in *CFTR* gene, and four had a co-mutation in another gene (*ADA*, *SPINK1*, *TP53* or *MSH6*). The most common cancer pairs/combinations were breast—GIST ($n = 3$), followed by GIST—rectal, ovary—breast, lymphoma—lung, colon—NPC, lymphoma—HCC, breast—leukaemia, ovary—endometrial, lung—pancreas and colon—sarcoma—breast (all $n = 1$). The most common PGV was *CFTR c.1210-34TG[12]T[5]* which was identified in 12/156 (7.7%) MPC patients. This PGV was only identified in 2/146 (1.4%) healthy cancer-free controls in Singapore. Of 156 MPC patients, 4 (2.6%) had *SPINK1* PGV (*SPINK1 c.101A>G*, $n = 3$; *SPINK1 c.194+2T>C*, $n = 1$). These variants were identified in 0% and 0.7% of the cancer-free population, respectively. The associated cancers in our patients were lung—colon, lymphoma—lung, lymphoma—colon—paraganglioma and breast—thyroid cancers (Supplementary Tables S8 and S9, available at https://doi.org/10.1016/j.esmoop.2025.104495).

DISCUSSION

Several studies have evaluated germline findings in MPC. Chan et al. examined the genetic testing outcome in 1191 Asian patients, of whom 19.4% had MPC, 42% underwent testing and half were tested with MGPT up to 49 genes. PGVs were more prevalent in MPC compared with SPC (35.5% versus 25.6%), with about three-quarters of PGVs identified in *BRCA1/2* (56.4%) and *MMR* genes (23.1%).[13] Another study of 24 241 patients reported that 18% had MPC, and PGVs were detected in 21% of the MPC patients, of which 44% and 20% were in high and moderate penetrance genes, respectively.[14] In our study, 668 patients (19%) had MPC and 85% had dual primary cancers. The prevalence of MPC was consistent with previous reports of 2%-25%.[1,15] Of the MPC patients, 72.9% underwent genetic testing, and 29.4% tested positive for any PGV. PGV was more common in MPC than SPC patients (29.4% versus 20.8%), in line with earlier findings.[13,14] This supports that MPC patients have a likelihood of an underlying cancer genetic syndrome; thus genetic counselling is warranted for MPC patients.

Among MPC patients, 152 PGVs were detected in 33 genes with 39.9% in *BRCA1/2* gene, 18.9% in *HRR* gene, 11.2% in *MMR* gene and 7% in *TP53* gene. The distribution of PGVs was different from that of a previous study from Memorial Sloan Kettering (MSK) in which 20.8% of PGVs were detected in *BRCA1/2* gene, 10.2% in *MMR* gene and 2.8% in *TP53* gene.[14] This could be attributed to different ethnic populations and different testing panels. Our population was predominant Asian whereas patients were predominantly Caucasian in the MSK study. The most common cancer pairs in our study were breast—breast, breast—ovary, breast—endometrial, endometrial—ovary and breast—colon. This reflects the cancer pattern in Singapore.[16] This was in contrast to India, where head and neck, breast, genitourinary and gastrointestinal cancers were common index primary cancers.[17]

PGVs in certain common genes are known to be strongly correlated with MPC. Germline *TP53* mutation is the cause of Li Fraumeni syndrome, and a previous study showed that germline *TP53* PGVs are significantly associated with MPC.[18] Our study further affirmed this association. *BRCA1* is involved in DNA damage repair and maintenance of genomic stability through interaction with *BARD1*.[19] Carriers of *BRCA1* mutation are more likely to develop MPC.[20] Our findings demonstrated a higher rate of MPC among *BRCA1*-mutated breast/ovarian cancer patients and patients with *BARD1* mutations. At present, MGPT is considered the major approach to genetic testing in MPC.[21] Whitworth et al. previously evaluated the role of comprehensive gene testing of 83 genes in MPC patients and found that 15.2% of patients had PGVs despite previous noninformative genetic assessment by targeted gene testing.[22] In our study, 156 MPC patients were tested with an extended 216-gene panel irrespective of their tumour types/combination. Unsurprisingly, extended testing increased the number of detected PGVs, with 17.5% of patients testing positive for a PGV despite initial noninformative testing results with a standard panel.

In cancer, the *CFTR* gene functions as a tumour suppressor gene where mutations lead to carcinogenesis.[23] Several studies have reported potential increased risk of cancers among cystic fibrosis (*CF*) carriers, in particular colorectal and pancreatic cancers.[23-28] The most common PGV in our cohort was *CFTR c.1210-34TG[12]T[5]*. In a large population-based study that screened ~320 000 individuals for *CF* carrier status, the allelic frequency of the variant *c.1210-34TG[12]T[5]* in *CFTR* was 0.04.[29] In Asia, a study in Vietnam found that 4.4% of healthy women carried *CF* PGV, with the most common variant being *c.1210-11T>G*.[30] In our cohort, we detected *CFTR* PGV in 7.7% of

MPC patients with various associated cancers, including three with GIST—breast cancer, while the prevalence of this variant in cancer-free controls in our population was only 1.4%, highlighting *CFTR* gene as a potentially important cancer predisposition gene. Conversely, *SPINK1* induced activation of downstream signalling of epidermal growth factor receptors and modulated carcinogenesis.[31] Emerging evidence clarified a direct/indirect role of *SPINK1* in cancer proliferation, metastasis and drug resistance.[32] In our cohort, 2.6% of MPC patients with lung, colon and breast cancers had *SPINK1* PGV. In contrast, our cancer-free controls had a prevalence of these PGVs of only 0%-0.7%, suggesting that *SPINK1* gene may be a significant cancer predisposition gene.

Our study underscores the value of genetic testing in MPC patients, revealing a higher prevalence of PGVs compared with SPC patients and highlighting associations with genes such as *TP53*, *BRCA1* and *BARD1* with MPC. Extended panel testing in MPC has improved detection rates, particularly for less established genes, although targeted panel testing focusing on high-penetrance and more prevalent genes may be more cost effective in resource-limited settings. In clinical practice, a tiered approach, reserving extended testing for inconclusive cases and integrating clinical data such as early diagnosis and multiple cancers, could optimize resource allocation. These findings emphasize the importance of genetic testing and counselling in underserved regions to guide surveillance, therapies and familial risk assessment. Future studies should explore the cost-effectiveness of such tiered approaches and assess their impact on clinical outcomes.

Our study has several limitations. It was a single-centre retrospective analysis of patients seen in a cancer genetics clinic in a tertiary care hospital, where the majority of the patients were predominantly Asian Chinese and fulfilled suspected clinical criteria for hereditary cancer syndrome. This setting inherently introduces referral bias, as the population studied is likely enriched with individuals with higher pretest probabilities of harbouring PGVs. As a result, this may lead to in overestimation of prevalence of pathogenic mutations in this study compared with a less selected population. In addition, only a subset of 156 patients underwent extended panel testing, which may have introduced selection bias, as this group is enriched with patients with prior non-informative results using a more standard testing panel or atypical cancer patterns. A larger study with more diverse populations and standardized testing approaches is warranted to validate the findings and confirm the relationship between PGVs in less established cancer predisposition genes and the various cancers observed. Further studies are also needed to evaluate the penetrance of these uncommon PGVs, as well as gene—gene, gene—environment interactions and the role of gene modifiers.

CONCLUSION

MPC is not uncommon among cancer patients and is associated with heritable cancer predisposition. Around 19% of cancer patients seen in our study had MPC and 29% of MPC patients tested carried a PGV. Thus, MPC patients will benefit from genetic counselling/testing and cancer prevention strategies. Extended panel testing in MPC improved the detection of PGVs, particularly in less established cancer predisposition genes such as *CFTR*, *SPINK1*, *TNFRSF13B*, *TET2*, *ADA*, *CDKN1C*, *CTNNA1*, *DDX41*, *HAX1*, *RECQL4* and *MBD4*, expanding our understanding of hereditary cancer syndromes and genetic susceptibility. Incorporating extended genetic testing into clinical practice can potentially improve early detection and prevention by identifying at-risk individuals for tailored surveillance and interventions. Moreover, the identification of emerging cancer predisposition genes, such as *CFTR* and *SPINK1*, through extended testing underscores its utility in expanding the scope of genetic testing beyond well-established genes, ultimately improving the precision of cancer risk assessment. Comprehensive genomic interrogation and functional assessment of tumour genomics and its correlation with germline genetic testing will further improve our understanding of the pathogenicity of these less established heritable PGVs.

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