Original Study



# Next Generation Sequencing Reveals Novel Mutations in Mismatch Repair Genes and Other Cancer Predisposition Genes in Asian Patients with Suspected Lynch Syndrome

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## Abstract

**The genetic spectrum of Asian patients with Lynch Syndrome (LS) is not well understood. This study from an Asian cancer center studied multigene panel testing in patients with clinically suspected LS and identified novel mutations in both LS and non-LS genes, pointing to alternative culprit cancer predisposition genes that may not have been suspected using traditional clinical criteria.**

**Background:** Although at least 5 genes are implicated in Lynch Syndrome (LS), up to 50% of suspected cases are owing to undefined genes. We utilized next generation sequencing (NGS) to characterize the mutation profile of patients with cancer (CA) suspected to have LS. **Patients and Methods:** We enrolled 174 Asian patients with CA from our CA Genetics Clinic from 2000 to 2014 suspected to have LS, and obtained germline DNA for NGS using TruSight Cancer. Frameshift, nonsense, and known deleterious mutations were considered pathogenic. Polymorphisms $\leq$ 1% frequency in 1000 Genomes (Asian) were classified using established databases. **Results:** Of the 174 probands, 80.5% were Chinese, the median age at CA diagnosis was 45 years (range, 18-82 years), and 84.5% and 8.6% had colon and LS-like CA, respectively. Forty-seven of 100 evaluable colon CA probands had LS-like histopathologic features. Nineteen of 174 had family history fulfilling Amsterdam I/II Criteria, whereas the rest fulfilled Bethesda Guidelines. Thirty-one of 174 harbored pathogenic mutations with 10 in LS genes only, 20 in non-LS genes only, and 1 in both. Of the 11 with LS gene mutations, *MLH1* was most commonly involved (n = 7), followed by *MSH2*, *MSH6*, and *PMS2*. Nine of 174 had pathogenic mutations diagnostic of alternative hereditary syndromes including 2 each in *CDH1*, *APC*, and *BRCA1*, and 1 each in *BRCA2, SMAD4*, and *MUTYH*. Ten unique mutations were detected in low-to-moderate penetrance genes: 6 individuals had a recurring novel *KIT:c.2836C>T* nonsense mutation (n = 3) or *ERCC4:c.2169C>A* nonsense mutation (n = 3) without LS gene mutation, which is of clinical interest. **Conclusions:** In this Asian study, NGS proved to be feasible in screening for causative mutations in patients with CA suspected to have LS.

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**Keywords:** Familial cancer, Genetic testing, Genetics, Germ-line mutation, Hereditary non-polyposis colorectal cancer

## Introduction

Lynch Syndrome (LS), or hereditary non-polyposis colorectal cancer, is the most common hereditary colorectal cancer syndrome, and accounts for up to 4% of colorectal cancers.[1] LS is owing to an underlying germline defect in DNA mismatch repair (MMR) genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*) and results in an increased lifetime risk of colorectal and extra-colonic cancers.[2] Guidelines have been established for the use of enhanced

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surveillance and prophylactic surgery to reduce morbidity and mortality in patients and families diagnosed with LS, but clinching the correct diagnosis is not an easy task.[3]

Diagnosis of LS is traditionally achieved in a time-consuming stepwise fashion, through clinico-pathologic risk assessment using the Amsterdam I/II criteria (AC) and revised Bethesda guidelines (BG), demonstration of tumoral MMR deficiency, and finally, confirmation of germline DNA defect in an MMR gene via gene sequencing.[4,5] However, this process is not fool-proof, as up to 28% of LS cases can be missed with clinical criteria.[1] In addition, uptake for germline genetic testing has been low in Asia and worldwide, with cost being a major patient barrier.[6,7] The cost of testing is approximately USD$600 per gene (or USD$3000 for 5 MMR genes) using traditional Sanger sequencing, which is prohibitive for many patients.[8] In addition, not uncommonly, high-risk individuals may have personal and/or family cancer histories suggestive of more than 1 possible hereditary cancer syndrome, such that multiple genes have to be tested sequentially or simultaneously, further increasing the test cost.[9,10] This has hampered our ability to accurately diagnose patients with LS in the clinic.

The advent of next generation sequencing (NGS) technology and multi-gene panels has enabled massive parallel sequencing of multiple genes at a fraction of the cost of traditional Sanger sequencing.[11-13] NGS has been increasingly utilized in the clinical and research setting to aid in diagnosis and explore implication of other genes in patients with hereditary colon cancer, but data is lacking in the Asian population.[9] This is an important gap, as present literature on deleterious mutations associated with hereditary cancer syndromes have been derived mainly from Western studies. Limited preliminary data show that novel mutations and differences in clinical characteristics do exist in the Asian high-risk cancer population.[14-16] We hope to increase the understanding of causative genes and mutations specific in Asians with suspected LS through the use of NGS.

## Materials and Methods

### *Familial DNA Bank*

Patients were recruited through the Clinical Cancer Genetics Service at the National University Cancer Institute, Singapore from January 2001 to December 2014.[17] Patients with cancer who were deemed to have increased risk for hereditary cancer after comprehensive assessment in the clinic were invited to join the Familial DNA Bank study for genetics research. All participants provided written informed consent as per the institutional ethics guidelines and were given the option to be notified of their test result. Each subject provided information on their cancer history and 3-generation family history (when available) and donated 10 mL of blood for genotyping. Of 364 unrelated cancer probands, 174 were identified to have suspected LS based on AC or revised BG.

### *Next Generation Sequencing*

Blood samples were screened for germline variants in 94 cancer predisposition genes of interest (see Supplemental Table 1 in the online version) including the 5 relevant MMR genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*), *APC*, *BRCA1/2*, etc, using the TruSight Enrichment Kit together with TruSight Cancer content and sequenced on the Illumina MiSeq platform according to manufacturer's standard protocol (Illumina Inc, San Diego, CA). In brief, 50 ng of genomic DNA was fragmented and tagged followed by the addition of sequencing adaptors and indices by polymerase chain reaction. Sample libraries (each containing genetic material from 12 individuals) were pooled and denatured into single-stranded DNA, before being hybridized to biotin-labeled probes specific to the targeted region. The pool was further enriched by adding streptavidin beads that bind to the biotinylated probes. Biotinylated DNA fragments bound to the streptavidin beads were subsequently magnetically pulled down from the solution, eluted from the beads, and hybridized for a second enrichment reaction followed by polymerase chain reaction amplification. After amplification, the targeted library was loaded onto the MiSeq platform for cluster generation and subsequent sequencing.

### *Bioinformatics Analysis*

The primary and secondary data analysis as well as quality and coverage information about each sample were performed using the onboard MiSeq Reporter software (Illumina Inc). The mean sequencing coverage for the regions targeted by the Trusight panel was 366.2×. The fraction of targeted region with $\geq$ 30× coverage was on average 97.6% across all samples. Correspondingly, 89.1% of all regions targeted by the Trusight panel had $\geq$ 100× coverage. Further information is provided in Supplemental Table 2 (in the online version).

Sequencing data was aligned using Novoalign. Genetic variants were identified using SAMtools and were subjected to further analysis if the genotype quality calculated was $\geq$ 99, and if the site was identified as a heterozygous site or a homozygous variant site. Detected variants were subsequently annotated using ANNOVAR. Polymorphisms at $>$ 1% frequency were removed using 1000 Genomes (Asian), and the remaining rare non-synonymous variants were classified with reference from established databases such as InSiGHT, ClinVar, and Human Genome Mutation Database.[18-20] Variants classified as pathogenic or novel (not previously reported at the point of analysis) were confirmed on Sanger sequencing.

### *Variant Characterization*

*LS Genes.* Mutations in classical MMR genes (*MLH1*, *MSH2*, *MSH6*, *PMS2*, *EPCAM*) identified via NGS were classified into 2 main categories: (1) pathogenic mutations that were defined as nonsense or frameshift mutations in coding regions, or mutations previously reported in literature to be pathogenic, and (2) variants of unknown significance (VUS), which were identified through established databases (eg, InSiGHT) or novel non-synonymous mutations in coding regions. *PMS2* variants were manually inspected using BLAT in a 100-bp window, to ensure that the variant was present on the functional gene.

*Non-LS Genes.* Non-MMR genes included gastrointestinal cancer-related predisposition genes, which are strongly linked to colorectal and other gastrointestinal cancers (*APC, BMPR1A, CDH1, MUTYH, SMAD4, STK11*) and other candidate genes screened using TruSight Cancer (eg, *BRCA1/2*, known to be associated with increased risk of cancer). Pathogenic mutations were defined as those previously reported to be associated with hereditary

cancer syndromes in literature, or if there were novel nonsense or frameshift mutations in coding regions. Germline mutations in *MUTYH* had to be biallelic to be considered pathogenic.

*Recurring Mutations.* For mutations occurring in > 2 probands that have not been previously reported, the frequency of these mutations in the Asian Chinese population was interrogated in germline samples from 100 unmatched cancer-free blood donors (51% female; median age, 31 years; range, 19-68 years), from whom a 3-generation family cancer history was obtained and deemed not to be suspicious for hereditary cancer syndrome (see Supplemental Table 3 in the online version). Mutations seen in ≥ 1% of the cancer-free controls were considered commonly occurring polymorphisms.

## Results

### Patient Characteristics

In this cohort of 174 probands, the majority were male (55.7%), of Chinese ethnicity (80.5%), and had a personal history of LS-associated cancer (93.1%) (Table 1). The median age of cancer onset was 45 years (range, 18-82 years), with 68.4% diagnosed before age 50. The most common cancer among probands was colorectal cancer (147/174; 84.5%), followed by cancer of the ovary (10/174; 5.7%), breast (9/174; 5.2%), and endometrium (8/174; 4.6%). Of 100 individuals with detailed tumor information available, 47% had histopathologic features suggestive of LS (Table 1). In terms of clinical risk criteria, 10.9% fulfilled AC, whereas the remaining 89.1% fulfilled revised BG.

**Table 1 Patient Characteristics of Study Population**

| Demographics (N = 174) | N (%) |
|---|---|
| Gender | |
| Male | 97 (55.7) |
| Female | 77 (44.3) |
| Race | |
| Chinese | 140 (80.5) |
| Malay | 22 (12.6) |
| Indians/others | 12 (6.9) |
| Median age of CA diagnosis, y (range) | 45 (18-82) |
| No. with young onset CA < 50 y | 119 (68.4) |
| Personal history of colon CA | 147 (84.5) |
| Histopathologic features[a] suggestive of LS[b] | 47 (47) |
| Personal history of non-CRC LS-like CA[c] | 15 (8.6) |
| Risk criteria | |
| Amsterdam I/II | 19 (10.9) |
| Bethesda Criterion 5 | 27 (15.5) |
| Bethesda Criterion 4 | 19 (10.9) |
| Bethesda Criterion 3 | 31 (17.8) |
| Bethesda Criterion 2 | 22 (12.6) |
| Bethesda Criterion 1 | 56 (32.2) |

Abbreviation: CA = cancer.
[a]Microsatellite instability-high tumors or presence of tumor-infiltrating lymphocytes, Crohn's-like lymphocytic reaction, mucinous/signet-ring differentiation, or medullary growth pattern.
[b]Information available for n = 100.
[c]Cancers of the biliary tract, brain, endometrium, pancreas, ovary, small bowel, stomach, ureter and renal pelvis, sebaceous gland adenomas, and keratocanthomas.

### Pathogenic Variants

Of the 174 probands screened, 31 (17.8%) were found to harbor pathogenic mutations in LS or non-LS genes. Among the 31 mutation carriers, 10 (32.3%) had mutations in LS genes alone, 20 (64.5%) had mutations in non-LS genes only, whereas 1 individual had both LS and non-LS mutations. There was no significant difference in the distribution of pathogenic mutations between the AC and BG risk groups ($P = .158$) (Figure 1). A total of 11 probands were found to have mutations in LS genes, for which *MLH1* was most commonly involved (7 cases; 63.5%), followed by *MSH2* (2; 18.2%), *MSH6* (1; 9.1%), and *PMS2* (1; 9.1%). There were a total of 11 unique mutations in LS genes, with 4 novel mutations not previously reported in literature (Figure 2 and Table 2).

### High Penetrance Genes with Alternative Hereditary Cancer Syndromes

Nine (5.2%) of 174 of the probands were found to have pathogenic mutations in high penetrance cancer predisposition genes, which imply an alternative hereditary cancer syndrome that would impact on genetic counselling and subsequent management. Two had mutations in *CDH1* (hereditary diffuse gastric cancer syndrome), 2 in *APC* (familial adenomatous polyposis), 2 in *BRCA1* (hereditary breast-ovarian cancer), 1 in *BRCA2* (hereditary breast-ovarian cancer), 1 in *SMAD4* (juvenile polyposis syndrome), and 1 had biallelic mutation in *MUTYH* (MUTYH-associated polyposis) (Table 2). The frameshift deletion *APC:c.1006_1009del,* frameshift deletion *APC:c. .4972_4974delAGA*, frameshift insertion *BRCA2:c.6302_6303insA*, and frameshift insertion *BRCA1:-c.2722_2723insA* seen in 4 individuals were novel mutations.

Ten unique mutations (7 nonsense, 3 frameshift) in low-to-moderate penetrance non-LS genes that are not linked to any major hereditary cancer syndromes were seen in 13 probands (Table 2). Of note, 6 individuals with strong cancer risk had a recurring novel *KIT*:c.2836C>T nonsense mutation (n = 3) or *ERCC4*:c.2169C>A nonsense mutation (n = 3) in the absence of MMR gene mutation, which may be of clinical interest.

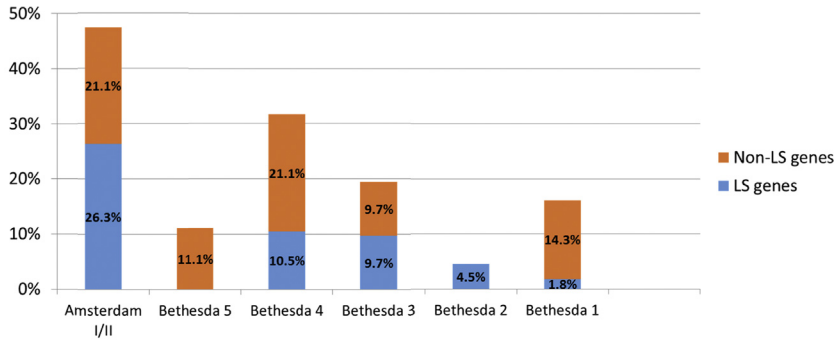
### VUS

A total of 39 VUS in LS genes were seen in 37 individuals (21 unique mutations, 8 novel) in this analysis. (see Supplemental Table 4 in the online version). The most common LS gene in which VUS were identified was *EPCAM* (n = 10), followed by *MSH6* (n = 6), *MSH2* (n = 5), *MLH1* (n = 4), and *PMS2* (n = 4).

### Testing of Recurring Mutations in Chinese Cancer-free Controls

We were particularly interested in the discovery of recurring mutations in *MSH6* (novel c.4071_4072insGATT frameshift insertion mutation seen in 10 individuals, 9 of which were Chinese), as well as in *KIT* (c.2836C>T nonsense mutation in 3 Chinese individuals) and *ERCC4* (c.2169C>A nonsense mutation in 3 Chinese individuals), which are genes not typically associated with LS, and sought to determine if they were commonly occurring

**Figure 1** Likelihood of Pathogenic Mutations by Clinical Risk




| Bethesda Guideline | Clinical History |
|---|---|
| Bethesda 5 | Colorectal CA (cancer) diagnosed in a patient with ≥2 first- or second-degree relatives with LS-related CA, regardless of age |
| Bethesda 4 | Colorectal CA diagnosed in a patient with ≥1 first-degree relatives with an LS-related CA, with ≥1 CA being diagnosed at age<50y |
| Bethesda 3 | Colorectal CA with the LS-like histology diagnosed in a patient <60y |
| Bethesda 2 | Presence of synchronous, metachronous colorectal, or other LS-like CA |
| Bethesda 1 | Colorectal CA diagnosed in a patient <50y |

Abbreviations: CA = cancer; LS = Lynch Syndrome.

genetic polymorphisms in the Chinese population. Sanger sequencing for these 3 genetic mutations was performed on germline samples of 100 Chinese cancer-free controls from our local population. The *KIT*:c.2836C>T and *ERCC4*:c.2169C>A nonsense mutations were not detected in any of the controls tested. We looked further into large population genetic databases and noted that they have been reported to exhibit low allele frequencies (<0.1%) in the East Asian subpopulation. (see Supplemental Table 4 in the online version) On the other hand, 7 of 100 of the cancer-free controls were found to have the *MSH6*:

**Figure 2** Distribution of Pathogenic Mutations in Lynch Syndrome Genes (N = 11)

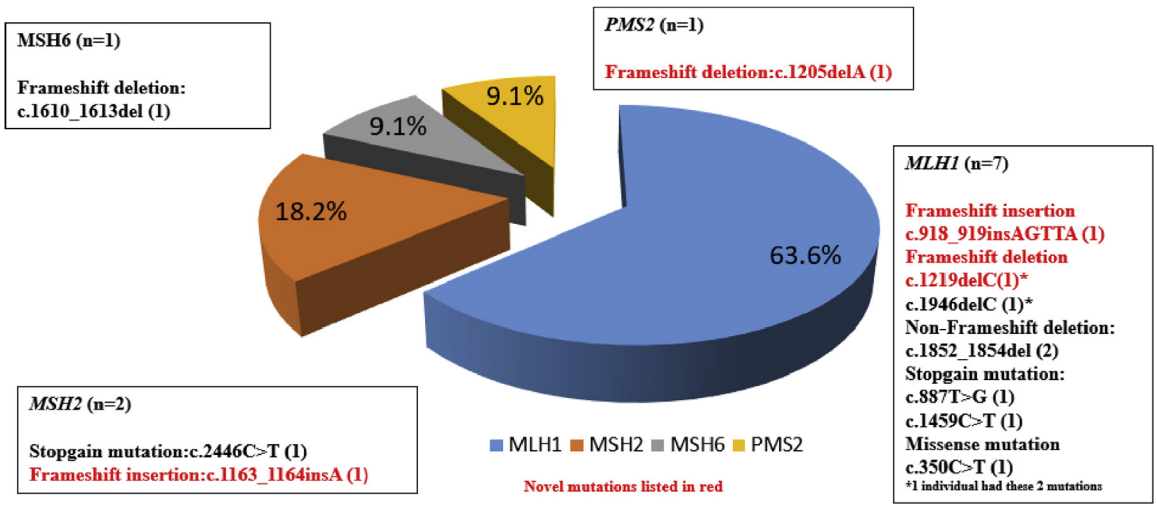


**Table 2** Pathogenic Mutations in LS and Non-LS Genes Detected and Associated Pedigree Information

| ID | Race | Gender | Cancer (CA) Primary | Age at CA Diagnosis | Presence of LS-like Histopathologic Features | Family CA History | $PREMM_5$ Score[a] | Clinical Criteria[b] | Gene Affected | Nucleotide Change | Amino-acid Change | Type of Mutation | Population Frequencies[c,d] (Asian) | Population Frequencies (European) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I0213 | Chinese | M | Colon (×3) | 30, 46, 46 | MSI-H | Mother, endometrium (50's); Mat aunt, colon; Mat aunt, colon (40's); Mat aunt, unknown (30-40's); Mat uncle, lung (50's); Mat aunt, colon; Mat cousin, colon (30's) | ≥50% | Amsterdam II | *MLH1* | c.918_919insAGTTA (Novel) | p.L306fs | Frameshift insertion | NR | NR |
| I0204 | Chinese | M | Colon | 45 | MSI-H | Sister, ovarian CA (36); Father, colon (51, 71); Pat uncle, colon (60's); Pat cousin, colon & ovary; Pat cousin, colon (50's) | 16% | Amsterdam I | *MLH1* | c.1946delC | p.P649Lfs | Frameshift deletion | NR | NR |
| I0020 | Chinese | F | Colon | 30 | | Sister, colon (40); Mother, colon (66); Mat uncle, colon (45); Mat aunt, colon (44) & ovarian (45); Mat uncle, colon (57) & kidney (67) | ≥50% | Amsterdam I | *MLH1* | c.1852_1854delAAG | p.K618del | Non-frameshift deletion | NR | NR |
| I0201 | Chinese | F | Ampulla of Vater, endometrium, colon | 38, 48, 54 | MSI-H | Niece, NPC (30's); Father, stomach (D60's) | 30.4% | Bethesda | *MLH1* | c.1852_1854delAAG | p.K618del | Non-frameshift deletion | NR | NR |
| I0051 | Chinese | M | Colon | 49 | MSI-H | Father, colon (55); Pat uncle, intestine (D30); Mother, brain (D39); Pat aunt, ?CA; Mat cousin, ?CA | 26.1% | Amsterdam II | *MLH1* | c.887T>G | p.L296X | Stopgain SNV | NR | NR |
| I0174 | Chinese | M | Colon (×3) | 40's, 67, 70's | MSI-H | Daughter, colon (34); Brother, blood CA (30's); Mother, stomach (95) | 25.6% | Bethesda | *MLH1* | c.1459C>T | p.R487X | Stopgain SNV | NR | NR |
| I0156 | Indian | M | Colon | 32 | MSI-H | Brother, lung; Sister, colon (39); Father, stomach (40's) | ≥50% | Bethesda | *MLH1* | c.350C>T | p.T117M | Non-synonymous SNV | NR | NR |

**Table 2** Continued

| ID | Race | Gender | Cancer (CA) Primary | Age at CA Diagnosis | Presence of LS-like Histopathologic Features | Family CA History | $PREMM_5$ Score[a] | Clinical Criteria[b] | Gene Affected | Nucleotide Change | Amino-acid Change | Type of Mutation | Population Frequencies[c,d] (Asian) | Population Frequencies (European) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I0204 | Chinese | M | Colon | 45 | MSI-H | Sister, ovarian CA (36); Father, colon (51, 71); Pat uncle, colon (60's); Pat cousin, colon & ovary; Pat cousin, colon (50's) | 16% | Amsterdam I | *MLH1* | c.1219delC (Novel) | p.P407fs | Frameshift deletion | NR | NR |
| I0119 | Malay | M | Colon | 25 | MSI-H | Pat uncle, colon (45); Mat uncle, small Intestine | 43% | Bethesda | *MSH2* | c.2446C>T | p.Q816X | Stopgain SNV | NR | NR |
| I0225 | Chinese | M | Colon | 59 | Poorly-differentiated, right-sided mucinous with lymphocytic infiltrate associated with 2 polyps of low-grade dysplasia | Brother, brain (50's); Nephew, brain (38) | 4.5% | Bethesda | *MSH2* | c.1163_1164insA (Novel) | p.N388fs | Frameshift insertion | NR | NR |
| I0211 | Chinese | F | Colon, ovary, endometrium | 52 | | Mother, colon (66) | 22.5% | Bethesda | *MSH6* | c.1610_1613delAGTA (Novel) | p.K537Ifs | Frameshift deletion | NR | NR |
| I0115 | Chinese | M | Colon | 43 | Poorly-differentiated tumor, MSI-H | Pat uncle, colon (48); Father, liver (62); Mat uncle, lung | 10.0% | Bethesda | *PMS2* | c.1205delA (Novel) | p.Q402fs | Frameshift deletion | NR | NR |
| I0092 | Chinese | M | Colon | 29 | Moderately differentiated mucinous tumor, MSI-H | Nil | 17.8% | Bethesda | *APC* | APC:c.4972_4974delAGA (Novel) | p.R1676del | Non-frameshift deletion | NR | NR |
| I0011 | Chinese | F | Colon | 43 | Associated with 8 polyps of mild dysplasia | Nil | 1.2% | Bethesda | *APC* | APC:c.1006_1009delGCTA (Novel) | p.L334del | Frameshift deletion | NR | NR |
| I0236 | Indian | M | Colon | 57 | Associated with 10 polyps | Nil | 3.5% | Bethesda | *MUTYH* | c.1240C>T (bi-allelic) | p.Q414X | Stopgain | NR | NR |
| I0219 | Chinese | F | Breast, colon | 37, 49 | Right-sided, mucinous tumor associated with 1 polyp of severe dysplasia | Brother, stomach CA (41); Mother, colon (62) | 4.6% | Bethesda | *SMAD4* | c.1236C>G | p.Y412X | Stopgain | NR | NR |
| I0129 | Chinese | M | Colon | 42 | | Mother, NPC; Father, unknown | 8.3% | Bethesda | *CDH1* | c.1018A>G | p.T340A | Non-synonymous SNV | 1000Genomes Asian: 0.0017 ExAC East Asian: 0.003582 | ExAC European (non-Finnish): 1.498e-05 |

**Table 2 Continued**

| ID | Race | Gender | Cancer (CA) Primary | Age at CA Diagnosis | Presence of LS-like Histopathologic Features | Family CA History | PREMM$_5$ Score[a] | Clinical Criteria[b] | Gene Affected | Nucleotide Change | Amino-acid Change | Type of Mutation | Population Frequencies[c,d] (Asian) | Population Frequencies (European) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I0204 | Chinese | M | Colon | 45 | MSI-H | Sister, ovarian CA (36); Father, colon (51, 71); Pat uncle, colon (60's); Pat cousin, colon & ovary; Pat cousin, colon (50's) | 16% | Bethesda | *CDH1* | c.1018A>G | p.T340A | Non-synonymous SNV | 1000Genomes Asian: 0.0017 ExAC East Asian: 0.003582 | ExAC European (non-Finnish): 1.498e-05 |
| I0153 | Malay | F | Colon | 48 | | Sister, breast (38) | 3.0% | Bethesda | *BRCA2* | c.6302_6303insA (Novel) | p.N2101fs | Frameshift insertion | NR | NR |
| I0222 | Malay | F | PPC, colon | 68, 69 | | Daughter, ovarian (41); Sister, cervix (50's) | 1.4% | Bethesda | *BRCA1* | c.2722_2723insA (Novel) | p.E908fs | Frameshift insertion | NR | NR |
| A0602 | Malay | F | Ovary | 43 | | Sister, ovary (43); Sister, ovary (41); Mother, colon (60's); Mat aunt, cervix (40-50's) | 3.6% | Bethesda | *BRCA1* | c.2722_2723insA (Novel) | p.E908fs | Frameshift insertion | NR | NR |
| A0042 | Chinese | F | Lung | 36 | | Mother, ovary (38); Mat aunt, colon (55); Mat uncle, colon (61); Mat grandfather, cecum (71); Mat grandmother, cervix (71) | 1.8% | Bethesda | *ATM* | c.2413C>T | p.R805X | Stopgain SNV | NR | NR |
| I0182 | Chinese | M | Colon | 37 | | Nil | 11.5% | Bethesda | *DIS3L2* | c.2170C>T (Novel) | p.R724X | Stopgain SNV | NR | NR |
| I0120 | Chinese | F | Colon | 43 | | Pat uncle, colon (50); Father, bone (D60's) | 4.6% | Bethesda | *FAM157B* | FAM157B:c.297G>A (Novel) | p.W99X | Stopgain SNV | NR | NR |
| I0128 | Malay | F | Colon | 45 | | Nil | 3.4% | Bethesda | *JPH3* | c.489C>A (Novel) | p.C163X | Stopgain SNV | NR | NR |
| I0063 | Chinese | F | Colon | 42 | | Sister, breast (42); Brother, colon (29); Father, colon (79) | 47.1% | Amsterdam I | *XPA* | c.631C>T (Novel) | p.R211X | Stopgain SNV | NR | NR |
| I0222 | Malay | F | PPC, colon | 68, 69 | | Daughter, ovarian (41), Sister, cervix (50's) | 1.4% | Bethesda | *MPL* | c.235_236delCT (Novel) | p.L79Qfs | Frameshift deletion | NR | NR |
| I0090 | Chinese | F | Colon | 38 | | Nil | 5.1% | Bethesda | *KCNK12* | c.27_28insCCCCCCCC (Novel) | p.P10fs | Frameshift insertion | NR | NR |
| I0001 | Malay | M | Colon | 82 | Associated with 21 tubular adenoma, MSI-S | Brother, colon (82); Father, colon (50's); Daughter, breast (32) | 3.2% | Bethesda | *MFSD3* | c.1010_1011delCA (Novel) | p.T337del | Frameshift deletion | NR | NR |

**Table 2** Continued

| ID | Race | Gender | Cancer (CA) Primary | Age at CA Diagnosis | Presence of LS-like Histopathologic Features | Family CA History | $PREMM_5$ Score[a] | Clinical Criteria[b] | Gene Affected | Nucleotide Change | Amino-acid Change | Type of Mutation | Population Frequencies[c,d] (Asian) | Population Frequencies (European) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A0040 | Chinese | F | Breast | 46 | | Pat uncle, NPC (42); Pat uncle, stomach (56) & bladder (59); Pat cousin, breast (36, 47); Pat cousin, colon (28, 43) | 0.8% | Bethesda | *ERCC4* | c.2169C>A | p.C723X | Stopgain SNV | ExAC East Asian: 0.000578 | NR |
| I0184 | Chinese | M | Colon | 36 | MSI-S | Sister, colon (22); Sister, lymphoma (39); Father, colon (50); Pat grandfather, gastroesophageal | ≥50% | Amsterdam I | *ERCC4* | c.2169C>A | p.C723X | Stopgain SNV | ExAC East Asian: 0.000578 | NR |
| I0025 | Chinese | M | Colon | 56 | Mucinous tumor | Nil | 3.7% | Bethesda | *ERCC4* | c.2169C>A | p.C723X | Stopgain SNV | ExAC East Asian: 0.000578 | NR |
| A0042 | Chinese | F | Lung | 36 | | Mother, ovary (38); Mat aunt, colon (55); Mat uncle, colon (61); Mat grandfather, cecum (71); Mat grandmother, cervix (71) | 1.8% | Bethesda | *KIT* | c.2836C>T | p.R946X | Stopgain SNV | ExAC East Asian: 0.0002313 | ExAC European (Non-Finnish): 2.997e-05 |
| I0122 | Chinese | F | Colon | 64 | MSI-H | Sister, ovary (36); Brother, colon (61); Niece, lung (36); Father, colon (71); Mother, cervix (72) | 4.9% | Bethesda | *KIT* | c.2836C>T | p.R946X | Stopgain SNV | ExAC East Asian: 0.0002313 | ExAC European (Non-Finnish): 2.997e-05 |
| I0073 | Chinese | F | Colon | 30 | MSI-H | Mat aunt, throat (33); Mat grandfather, stomach (39); Mat grand uncle, prostate (80); Mat second cousin, leukemia (40) | 9.6% | Bethesda | *KIT* | c.2836C>T | p.R946X | Stopgain SNV | ExAC East Asian: 0.0002313 | ExAC European (Non-Finnish): 2.997e-05 |

Abbreviations: CA = cancer; ExAC = Exome Aggregation Consortium; LS = Lynch Syndrome; Mat = maternal; MSI-H = microsatellite instability-high; NPC = nasopharyngeal cancer; NR = not reported; Pat = paternal; SNV = single nucleotide variant.
[a]PREdiction Model for gene Mutations 5. Kastrinos F, Uno H, Ukaegbu C, et al. Development and Validation of the PREMM5 Model for Comprehensive Risk Assessment of Lynch Syndrome. *J Clin Oncol* 2017; 35:2165-72.
[b]Amsterdam I/II or Revised Bethesda guidelines.
[c]Derived from the 1000 Genomes Project. Auton A, Brooks LD, Garrison EP, et al. A global reference for human genetic variation. *Nature* 2015; 526:68-74.
[d]Derived from Exome Aggregation Consortium (ExAC). Lek M. Karczewski KJ, Minikel EV, et al. Analysis of protein-coding genetic variation in 60,706 humans. *Nature* 2016; 536:285-91.

c.4071_4072insGATT frameshift insertion mutation, suggesting this is commonly occurring and unlikely pathogenic in our local Chinese population, which may not be well-represented in the available population genetic databases.

## Discussion

To the best of our knowledge, our study is one of the first to study panel-based NGS testing in a cohort of Asian high-risk patients with cancer with suspected LS and yielded interesting results. Eleven (6.3%) of 174 probands tested were found to have a pathogenic mutation in an MMR gene, which is similar to panel-based NGS studies done in the Western population.[9,10] We identified 4 novel frameshift indel mutations in MMR genes in this cohort − 2 in *MLH1*, 1 each in *MSH2* and *PMS2*.

As anticipated, VUS in LS genes not previously reported in literature were also discovered via NGS in our study. The paucity of mutational data from Asian LS cases in the international cancer databases makes it difficult to assess if these variants are pathogenic. In the clinic setting, VUS are non-informative in risk assessment, but as more studies are performed, some of these novel variants may be re-categorized and be of clinical relevance. This is clearly evident in our study, where 10 unrelated patients with cancer with a significant family cancer history harbored a novel *MSH6* frameshift insertion mutation (*c.4071_4072insGATT*) that was initially thought to be pathogenic. However, the same mutation was seen in 7 of 100 cancer-free Chinese individuals, suggesting that this is unlikely cancer-causing. We also reported the presence of 2 novel distinct mutations in *MLH1:c.1219delC* and *c.1946delC* in a single individual, which were confirmed on Sanger sequencing. We believe that the presence of either pathogenic mutation in *MLH1* would have resulted in cancer predisposition in this proband with young-onset colorectal cancer with a strong family history of cancers. In this case the co-existence of both mutations in a single individual is unique, but would not change the clinical management given the confirmation of LS. The results from this study serve to enrich the current repository of genetic variants in Asian patients with cancer and describe the spectrum of mutations seen in LS.

Broad-based NGS screening of multiple genes has proven useful in this study as 21 (12.1%) of 174 probands had a pathogenic mutation in a non-LS gene. This is particularly informative in 9 individuals, all of whom fulfilled AC or revised BG for LS, but who were ultimately found to be carriers of mutations in genes associated with other known hereditary cancer syndromes with screening and/or preventive guidelines. Of note, the novel *APC c.1006_1009del* frameshift deletion mutation was seen in an individual with relatively young-onset colon cancer who had 8 adenomatous polyps, which fulfills revised BG criterion 1 for LS, but was not found to have any pathogenic germline mutations in MMR genes. Although this proband may not yet show the classical phenotype of familial adenomatous polyposis (FAP), the presence of an *APC* mutation is supportive of attenuated FAP which would not have been suspected otherwise. The implications of a different diagnosis are significant, as the counseling and recommendations to the proband and family members for FAP are vastly different from that of LS.

Multi-gene testing is also efficient in the context of family cancer histories that fit multiple cancer syndromes. The pedigrees of the 3 probands found to have *BRCA1/2* mutations were suspicious for both LS and hereditary breast-ovarian cancer, and testing with NGS allows both differentials to be tested concurrently for a diagnosis to be made. This overlap between *BRCA* genes and LS-phenotype was also seen in a large study of 1260 individuals with suspected LS, of which 8.2% (15/185) of mutation carriers were found to carry *BRCA1/2* mutations using a 25-gene NGS panel.[10] This is not surprising, as lifetime risk of ovarian cancer (which is an indication for *BRCA* testing), is approximately 8% in LS carriers.[21] Thus, clinicians need to have a heightened index of suspicion of both LS and hereditary breast-ovarian cancer when assessing pedigrees for hereditary cancer risk. Testing multiple genes with traditional Sanger sequencing (eg, MMR and *BRCA1/2* concurrently or sequentially) can be extremely costly and time-consuming, which can be a further deterrent in Asia where genetic testing cost is largely out-of-pocket. Indeed, cost has been reported to be an important barrier to genetic testing by our group.[6] Furthermore, the psychological distress of waiting for a series of genetic tests may mount in an individual who already has to grapple with a diagnosis of cancer. Hence, panel-based NGS is a practical and economical option to the traditional paradigm of phenotype-directed testing.

However, the advantage of NGS may not be as evident in cases where the identified pathogenic mutation is not congruent with the clinical phenotype (eg, 2 probands were found to have pathogenic *CDH1* mutations known to be associated with hereditary diffuse gastric cancer, but there were no cases of gastric cancer or lobular breast cancer in the personal or family cancer history to support the syndromic diagnosis), which adds to the challenge in interpreting the clinical significance of these mutations and the appropriateness of a drastic intervention such as prophylactic gastrectomy. Clearly, this is a result of "blanket" screening of patients without the correct clinical context, which has been seen in a similar study, but it also reveals the spectrum of non-LS gene mutations in patients who otherwise fulfill traditional screening criteria for LS, and provides information on the potential pathogenicity of these mutations in such individuals.[10]

Our study utilized TruSight Cancer, which screens 94 cancer predisposition genes, many of which are low-to-moderate penetrance genes. As expected, a subset in the cohort were found to have nonsense or frameshift mutations in low-to-moderate penetrance genes for which management guidelines are not well-established (13/174 [7.4%] in 10 genes). This genetic information may not be as useful in the clinical setting, but can be helpful in cancer research to explore the effects of germline mutations in a multitude of these cancer predisposition genes in the absence of MMR gene defects. This is especially so as LS gene mutations are only found in 50% of individuals who meet AC, with no attributable genetic mutations seen in the remaining probands with Familial Colon Cancer Type X Syndrome. In this study, we identified a recurring *KIT:c.2836C>T* nonsense mutation (n = 3) and *ERCC4:c.2169C>A* nonsense mutation (n = 3) in 6 Chinese probands with strong family history of cancer, in the absence of MMR gene mutation. These 2 mutations were not detected in a limited sample of 100 Chinese cancer-free controls, suggesting that they are not commonly occurring benign polymorphisms, and may well be pathogenic. The *KIT:c.2836C>T* mutation has previously been reported as a somatic variant in 1 individual with colorectal cancer and 2 with esophageal cancers.[22,23] *KIT* is an oncogene and

would theoretically result in cancer very early on in life if there is a gain-of-function mutation. However, this nonsense mutation is noted to be in the C-terminus, and may result in regulatory effects causing an increased cancer risk in adulthood. The *ERCC4*:c.2169C>A nonsense mutation has been previously reported on germline testing in 2 Southeast Asian Chinese probands (1 with alveolar rhabdomyosarcoma, 1 with giant cell tumor of the bone) although the personal and family history was not suggestive of LS or other hereditary syndromes.[24] This nonsense mutation is within the nuclease catalytic site of *ERCC4*, and is likely to be pathogenic, but the cancer risk associated remains unclear, and warrants further investigations.

We recognize that our study had limitations, one of which is the low numbers of patients who had tumor tissue samples tested for microsatellite instability (MSI). MSI has recently been reported to be contributory to the diagnostic pick-up of patients with LS based on a recent study, even in non-colorectal or endometrial cancers.[25] However, although universal tumor MSI testing has been advocated by professional organizations, the ultimate goal remains confirmatory diagnosis with genetic testing, and germline testing based on clinical criteria is an acceptable option.[3] This single institution study is relatively small compared with other NGS studies, but it represents one of the largest cohorts of Asian patients with suspected LS, and would be of interest to cancer geneticists and medical oncologists who manage high-risk patients with colon cancer of Asian ethnicity. Lastly, our panel of 94 cancer predisposition genes did not include newer candidate genes that have been associated with familial colorectal cancer (eg, *POLE*/*POLD1*), which has been recently described to result in an LS-mimic, polymerase proofreading associated polyposis.[26] A study looking at whole exome sequencing in families with strong colorectal cancer aggregation without mutations in known hereditary cancer genes listed mutations in *CDKN1B*, *XRCC4*, *EPHX1*, *NFKB12*, *SMARCA4*, and *BARD1* as promising causative variants, which supports expanding the list of candidate genes considered for research in patients with suspected hereditary colorectal cancer.[27]

In spite of these limitations, our study managed to reveal a spectrum of novel mutations in both LS and non-LS genes in a cohort of Asian high-risk patients with cancer with suspected LS. This will add much lacking Asian-specific genetic information to the growing international database of variants seen in suspected LS probands. We also showed that panel-based NGS is feasible and practical in identifying underlying causative genetic mutations in patients with atypical phenotypes or multiple syndromic differentials. Our Clinical Cancer Genetics Service has already started offering NGS-based multi-gene panels to high-risk patients with cancer who warrant clinical genetic testing and will continue to utilize this tool to understand the genetic profile unique to our patient population.

### *Clinical Practice Points*

- LS is the most common hereditary colorectal cancer syndrome.
- LS can be attributed to germline mutations in 5 MMR genes.
- The spectrum of genetic mutations in Asian patients suspected to have LS is not well-understood.
- Targeted germline testing of MMR genes in suspected LS is laborious and may miss underlying mutations in other cancer predisposition genes.
- We enrolled 174 Asian probands with suspected LS for multigene panel testing.
- Pathogenic mutations were seen in 17.8% of the tested probands, of which LS genes were implicated in 35.5% of mutation carriers.
- Pathogenic mutations in non-LS genes were discovered in 67.7% of mutation carriers, with 29% of carriers having an alternative hereditary cancer syndrome.
- Novel mutations in both LS and non-LS genes were identified in our study.
- Multigene panel testing is feasible, reveals mutations in non-LS genes in Asian patients with suspected LS, and impacts on subsequent management.

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## Disclosure

The authors have stated that they have no conflicts of interest.

## Supplemental Data

Supplemental data accompanying this article can be found in the online version at https://doi.org/10.1016/j.clcc.2019.05.007.

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