Article

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Study on the Application of MLPA Detection for Large Fragment Loss of Mismatch Repair Genes in Chinese HNPCC Families

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Background: Hereditary nonpolyposis colorectal cancer (HNPCC) is an autosomal dominant disease caused by germline mutations of human DNA mismatch repair (*MMR*) genes. A significant proportion of HNPCC cases are attributed to large genomic rearrangements of *MMR* genes, but this finding has been less frequently reported in Chinese populations.

Methods: Array-based multiplex ligation-dependent probe amplification (array-MLPA) was employed in this study to detect genomic rearrangements of 82 probands of Chinese HNPCC families.

Results: According to the results, 18 probands harbored germline genomic deletions of MutL homolog 1 (*MLH1*) and MutS homolog 2 (*MSH2*) genes, accounting for approximately 22% (18/82) of the total subjects. Meanwhile, *MSH6* gene deletion occurred only in about 2.4% of the probands (2/82). The deletions of *MLH1*, *MSH2* and *MSH6* genes were confirmed by classic MLPA analysis, with a concordance rate of 95.5% (21/22).

Conclusion: Array-MLPA is a highly efficient and precise method for clinical screening and diagnosis of HNPCC. By using this method, we found that the HNPCC families carry deletions of *MLH1* and *MSH2* genes, which are the major germline genomic aberrations in the studied probands. Nevertheless, the deletion of the *MSH6* gene is considered a rare occurrence in Chinese HNPCC families, according to our researche. Despite that, it is of clinical significance to screen and diagnose the HNPCC at the early phase by detecting the germline genomic large aberrations in *MSH2/MLH1* genes.

Keywords: MLPA; mutation; HNPCC; hereditary nonpolyposis colorectal cancer; microarray

Introduction

The hereditary nonpolyposis colorectal cancer (HNPCC; MIM#114500) is an autosomal dominant disease featuring early onset of colorectal cancer and other related cancers [1,2], mainly caused by the inactivating mutations in the genes responsible for DNA mismatch repair (*MMR*). Presently, five *MMR* genes, MutL homolog 1 (*MLH1*), MutS homolog 2 (*MSH2*), *MSH6*, *MSH3* and Postmeiotic Segregation Increased 2 (*PMS2*), are reported to be associated with human *MMR* function [3]. Moreover, it has been found that most of the germline mutations in hereditary nonpolyposis colorectal cancer (HNPCC) families occur in *MLH1* (MIM#120436; GDB: 249617) and *MSH2* (MIM#120435; GDB: 203983) genes [4].

By utilizing traditional detection techniques such as single-stranded conformation polymorphism (SSCP) analysis, denaturing high-performance liquid chromatography (DHPLC) and heterozygous double-stranded analysis, researchers have detected numerous lineage mutations, mainly including small deletions or insertions related to frameshift and missense mutations [5–7]. However, these methods are incapable of detecting large-scale genomic aberrations and exon deletions, which are the driver genomic aberrations causing HNPCC; therefore, this underscores the need to deploy appropriate techniques for detecting large-scale genomic mutations [8,9].

Large genomic deletions or duplications can be detected by using methods such as Southern blotting, real-time polymerase chain reaction (PCR), comparative genomic hybridization (CGH), and semiquantitative multiplex PCR array [10–12]. However, these methods are of low sample throughput and thus not suitable for detection of large genomic deletions or duplications. Recently, Schouten *et al*. [13] proposed a multiplex ligation-dependent probe amplification (MLPA), which can quantitate up to 50 different PCR amplicons in one reaction containing a minute amount of sample DNA. Thus, MLPA is suitable for identifying deletions and duplications of genes. However, one prominent limitation of the MLPA method is

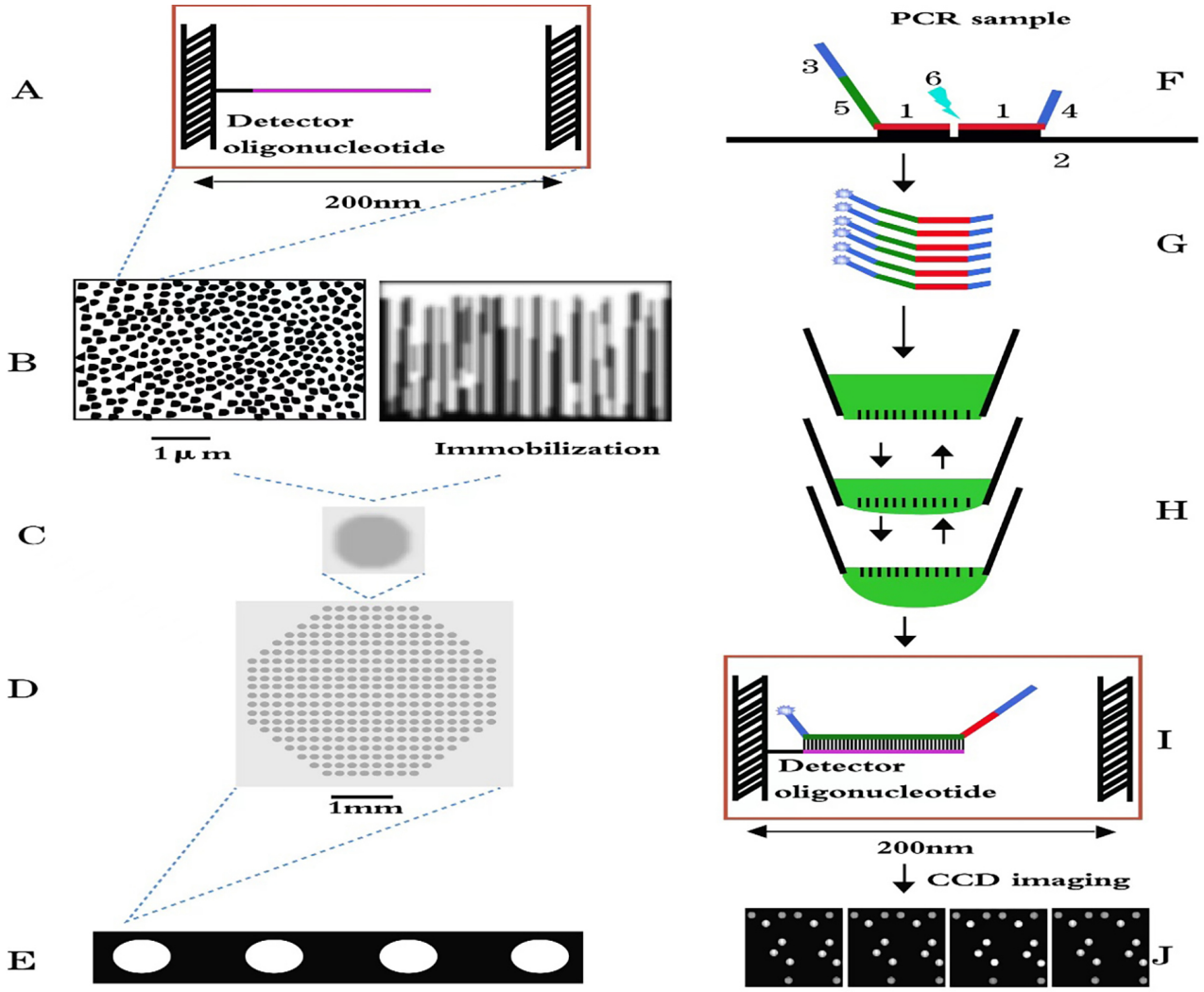

Fig. 1. The array-based MLPA platform. (A) Detection of oligonucleotide fixation on alumina substrate. (B) The porous structure of alumina substrate (indicated by bars on the scale): the left panel shows the top view of the substrate. The diameter of a single pore is approximately 200 nm. The right panel shows a partial cross-section of the substrate. The substrate thickness is 60 μm, with capillary pores extending from top to bottom. (C) Single array light spot. (D) An array. (E) A microtiter plate with four arrays. (F) Design of ligation-mediated probes: (1) target-specific binding sequence; (2) genomic DNA sequences with target sites; (3) PCR forward primers; (4) PCR reverse primers; (5) tag sequences complementary to array probes; and (6) ligation site. (G) Fluorescent-labeled PCR samples. (H) Fluorescent-labeled PCR products flowing through the incubator. (I) Hybridization of the PCR sample with detection of oligonucleotides fixed on the array. (J) Raw images acquired on 4 arrays in a microtiter plate. Schematic diagram drawn using Adobe Illustrator software 2024 (the version number is 28.5.0. This software is developed by Adobe Systems in the San Jose, CA, USA). MLPA, multiplex ligation-dependent probe amplification; PCR, polymerase chain reaction.

the necessity to employ the length differentiation results of the connecting products for analysis. This limits the number of probes that can be used in a group, thus affecting detection efficiency [14]. Besides, the quantitative PCR detection entails a very complex process. Adding to the list of limitations is the drop in detection accuracy when the fragments to be detected are of small sizes. To effectively avoid these defects, it is necessary to develop new detection techniques in which length-based differentiation is not mandatory for product amplification.

This paper introduces an array-based readout system in this context, which can efficiently quantitate MLPA amplification products. In order to improve measurement efficiency and automate data processing, the array form of porous alumina matrix was chosen (Fig. 1) [15,16]. Through this method, the sample will pass through a porous structure during the hybridization process, and the hybridization process will take 5–30 minutes. This method has proven applicable for detecting gene deletions and duplications in probands of Chinese HNPCC family patients. In this study, we successfully detected the deletion or du-

Table 1. Demographic details of the probands in this study.

Proband	Sex	Age of onset	Tumor location	MSI status	Clinical criteria
H3	M	46	Sigmoid colon cancer	MSI-H	AC I
H8	M	47	Transverse colon cancer	MSI-H	BG
H9	M	38	Cancer of the ileocecal region	MSI-H	AC II
H21	M	33	Elevated colon cancer	MSI-H	AC I
H22	M	45	Sigmoid colon cancer	MSI-H	AC II
H28	F	35	Elevated colon cancer	MSI-H	AC I
H36	M	32	Elevated colon cancer	MSI-H	JC
H45	M	37	Sigmoid colon cancer	MSI-H	AC I
H51	F	32	Rectal cancer	MSI-H	BG
H57	F	53	Elevated colon cancer	MSI-H	AC I
H63	F	53	Elevated colon cancer	MSI-H	AC I
H64	M	42	Transverse colon cancer	MSI-H	JC
H83	M	37	Elevated colon cancer	MSI-H	AC I
H85	M	38	Elevated colon cancer	MSI-H	AC I
H86	M	41	Rectal cancer	MSI-H	AC I
H89	M	43	Colonic splenic flexure carcinoma	MSI-H	JC
H100	M	42	Rectal cancer	MSI-H	AC I
H102	M	57	Elevated colon cancer	MSI-H	BG
H126	M	47	Rectal and gastric cancer	MSI-H	BG
H130	F	52	Transverse colon cancer	MSI-H	AC I
H131	M	29	Sigmoid colon cancer	MSI-H	JC

Abbreviations: AC I, Amsterdam Criteria I; AC II, Amsterdam Criteria II; BG, Bethesda Guidelines; F, female; JC, Japanese Criteria; M, male; MSI-H, microsatellite instability-high.

plication in *MSH2*, *MLH1* and *MSH6* genes by using array-based MLPA to investigate the frequency of large genomic aberrations of *MMR* genes in a group of families that had been clinically diagnosed with HNPCC. Moreover, our research also shows many large fragment abnormalities of *MMR* genes were detected in probands from Chinese HNPCC families. Therefore, it may have significant implications for detecting large segment abnormalities of the *MMR* gene in HNPCC families.

Materials and Methods

Patients

DNA samples were obtained from the Department of Pathology and the Department of Abdominal Surgery in Xinhua Hospital, Shanghai Jiaotong University School of Medicine from January 1998 to October 2020 after obtaining their official approval. The samples were selected based on the clinical profile of source patients, that is meeting the diagnosis criteria including Amsterdam Criteria I (AC I) [17], Amsterdam Criteria II (AC II) [2], Japanese Criteria [18], and Bethesda Guidelines (BG) (Table 1) [19]. The age of tumor onset of the 21 probands of HNPCC families was relatively young (average about 40.5 years), with a significant overrepresentation of male subjects than female ones (male:female = 16:5). Most of the tumors were right-sided colon cancer (9 cases), along with 4 cases of sigmoid colon cancer, 4 cases of rectal cancer, 3 cases of transverse colon cancer and 1 case of splenic flexure colon cancer. Among them, the clinical profiles of 13 families were concordant with the Amsterdam Criteria, 4 families fulfilled the Japanese Criteria, and the rest conformed to the Bethesda Guidelines. All tumor tissues exhibited high microsatellite instability. The research was performed in accordance with the Declaration of Helsinki and approved by the ethics committee of Xinhua Hospital Affiliated with Shanghai Jiaotong University School of Medicine (Approval No. XHEC-D-2024-201). The acquisition and application of all clinical data have obtained informed consent from all participants.

Materials

The extraction of genomic DNA from blood samples was performed using the QIAGEN QIAamp DNA extraction kit (P003, P008, QIAGEN, Hilden, Germany). Briefly, 1 mL of whole blood sample was added to a 5 mL sterile centrifuge tube. Then, 1 volume of ice-cold cell lysate and 3 volumes of ice-cold sterile water were added to the tube, which was then inverted and mixed until the suspension became semi-transparent. The tube was kept on an ice bath for 10 minutes, centrifuged at 6000 rpm and 4 °C for 5 minutes. After discarding the supernatant, 250 µL of cold cell lysate was combined with 750 µL of cold sterile water, followed by shaking and resuspension. The cell suspension was centrifuged at 6000 rpm and 4 °C for 5 minutes, and the supernatant was discarded afterward. A total of 220 µL of Acid tissue lysis (ATL) solution was added, prior to mixing with

Table 2. Sample screening in the *MSH2, MLH1* and *MSH6* genes.

Sample	Sex	First array-MLPA		Second array-MLPA		Confirmation
		Gene deletion		Gene deletion		Classic MLPA
H3	M	*MSH2*	Del e9	*MSH2*	Del e9	Del e9
H8	M	*MLH1*	Del e10	*MLH1*	Del e10	Del e10
H9	M	*MLH1*	Del e11	*MLH1*	Del e11	Del e11
H21	M	*MHS2*	Del e13	*MHS2*	Del e13	Del e13
H22	M	*MSH2*	Del e11	*MSH2*	Del e11	Del e11
H28	F	*MLH1*	Del e7	*MLH1*	Del e7	Del e7
H36	M	*MSH2*	Del e11-13	*MSH2*	Del e11-13	Del e11-13
H45	M	*MSH6*	Del e1	*MSH6*	Del e1	Del e1
H51	F	*MLH1*	Del e14	*MLH1*	Del e14	Del e14
H57	F	*MSH2*	Del e12	-	-	Del e12
H63	F	*MLH1*	Del e3	*MLH1*	Del e3	Del e3
H64	M	-	-	*MSH2*	Del e13	Del e13
H83	M	*MSH2*	Del e9	*MSH2*	Del e9	Del e9
H85	M	*MLH1*	Del e8-15	*MLH1*	Del e8-15	Del e8-15
H86	M	*MSH6*	Del e5	*MSH6*	Del e5	Del e5
H89	M	*MSH2*	Del e9	*MSH2*	Del e9	Del e9
H100	M	*MLH1*	Del e16-17	*MLH1*	Del e16-17	Del e16-17
H102	M	*MSH2*	Del e9	*MSH2*	Del e9	Del e9
H126	M	-	-	*MHS6*	Del e5-6	No aberration
H130	F	*MLH1*	Del e7	*MLH1*	Del e7	Del e7
H131	M	*MLH1*	Del e6	*MLH1*	Del e6	Del e6
		MSH2	Del e4	*MSH2*	Del e4	Del e4

Aberrations: *MSH2*, MutS homolog 2; *MLH1*, MutL homolog 1; Del, deletion mutations.

a vortex mixer at the maximum speed for 30 seconds until the cell nuclei were completely released, leaving behind no lumps of sediment. The suspension was gently mixed following the addition of 18 µL of proteinase K, and then incubated at 60 °C for 60 minutes. Acid lysis (AL) solution (250 µL) was added and the mixture was vortex-mixed thoroughly, prior to incubation at 70 °C for 10 minutes. The mixture was then added with 250 µL of anhydrous ethanol and mixed well. The solution (including precipitate) obtained in the previous step was transferred into the centrifugal adsorption column. After centrifuging at 10,000 rpm for 1 minute, the column solution was discarded. Subsequently, 500 µL of pre-bleaching solution was added and centrifuged at 10,000 rpm for 1 minute; the column solution was discarded. Rinse solution (600 µL) was added and centrifuged at 10,000 rpm for 1 minute; the column solution was discarded afterward. Next, the adsorption column was placed into another clean centrifuge tube. After centrifugation at 10,000 rpm for 3 minutes, the column liquid and collection tube were discarded. The centrifugal adsorption column was then placed into a sterilized 1.5 mL Eppendorf tube, followed by the addition of 100 µL of eluent preheated at 50 °C and subsequent incubation at 50 °C for 3 minutes. The column was then centrifuged at 13,000 rpm for 1 minute. The formula for calculating DNA concentration is as follows:

DNA concentration (µg/µL) = A_{260} × dilution factor × 0.05 µg/µL

The purity of DNA was determined in terms of the ratio of A_{260}/A_{280}: the DNA is considered of better quality when the ratio of A_{260}/A_{280} lies in the range of 1.7 to 1.9 (A, Absorbance). Array based-MLPA reagents (a gift from Prof. Ying Wu, PamGene Corp, Amsterdam, the Netherlands) were applied to detect large deletions or duplications in *MSH2*, *MLH1* and *MSH6* genes. The probe contained 16 exon-probe pairs for *MSH2*, 19 for *MLH1*, 10 for *MSH6* and 10 control-probe pairs. Genomic DNA was isolated from the peripheral blood. The reagents of MLPA Kit (P003) and MLPA Kit (P008) were obtained from MRC, Amsterdam, the Netherlands. The corresponding probe information can be obtained from the website (https://www.mrcholland.com/technology/mlpa).

MLPA Method

The MLPA was conducted according to the previously reported protocol [14]. About 100 ng of genomic DNA dissolved in 5 µL of DNA dissolution buffer was heated at 98 °C for 5 minutes. After cooling to room temperature, 1.5 µL of probe mixture and MLPA buffer was added to each sample, denatured at 95 °C for 5 minutes, hybridized at 55 °C for 4 hours, and incubated at 98 °C for another 5 minutes. PCR amplification was performed using 200 nM Premier

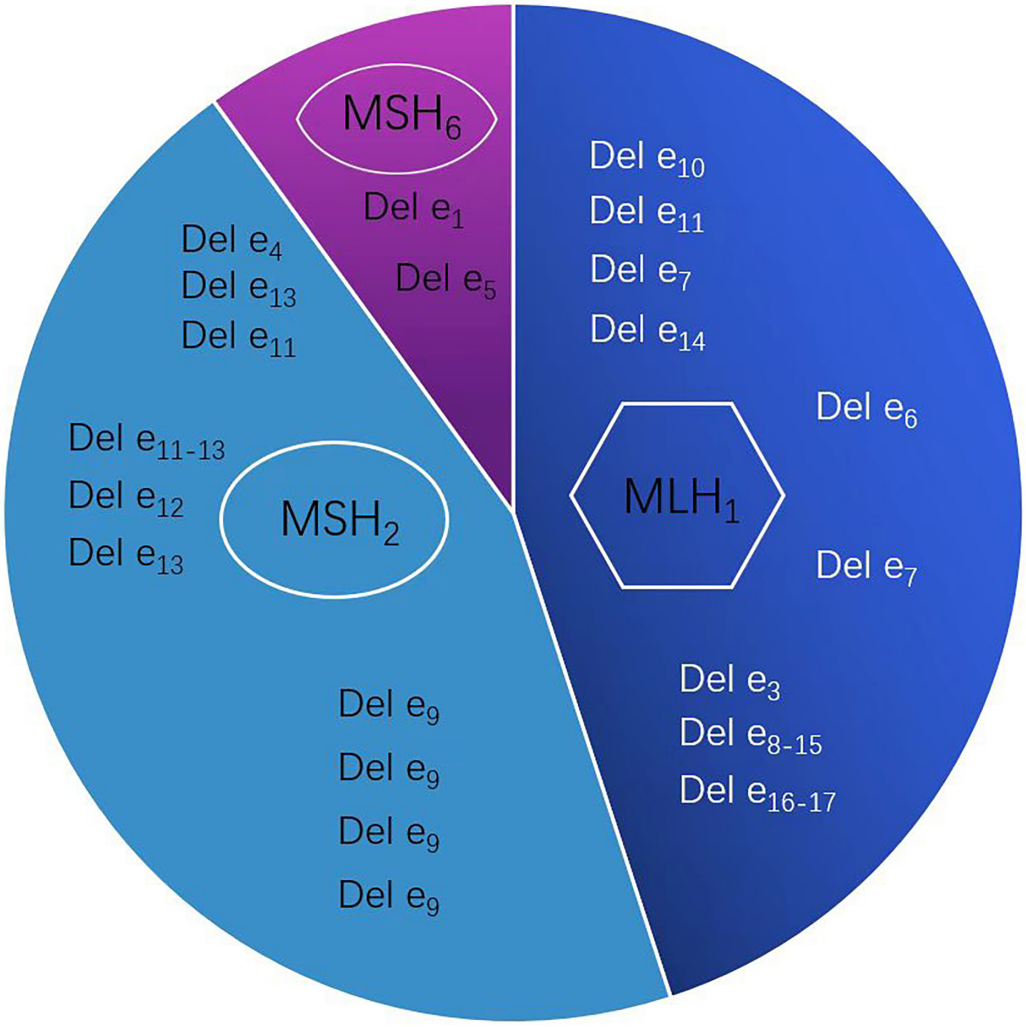

Fig. 2. Distribution map of the 21 detected deletion mutations of *MSH2*, *MLH1* and *MSH6* genes, encompassing 10 in *MSH2*, 9 in *MLH1*, and 2 in *MSH6*.

Accessory Mix-forward (*PAM*-F) as well as Premier Accessory Mix-reverse (*PAM*-R) primers labeled with Cy5. The amplification conditions are as follows: denaturation at 95 °C for 5 minutes, 33 cycles of 95 °C for 20 seconds each, 50 °C for 30 seconds, 72 °C for 1 minute, and extension at 72 °C for 20 minutes.

Array Hybridization and Detection

Flow-through microarrays can be conducted in systems of different formats: 4-, 12- and 96-well systems (PamGene Corp, Amsterdam, the Netherlands). The fluidics and epi-fluorescent Charge-Coupled Device (CCD) images were integrated into the 4-well system, which was applied to perform screening on patients. Every individual MLPA sample (10 µL) that was tagged with Cy5 was mixed with 5 µL of 5× hybridization buffer, 1 µL of blocker and 9 µL of water. The samples were denatured at 95 °C for 5 minutes and hybridized at 45 °C for 32 minutes. Then, the samples were pumped through the porous substrate at a rate of 3 cycles per minute. After washing with 1× hybridization buffer (0.1% N-lauroylsarcosine, pH = 7.0) prewarmed at 50 °C, images at 1000 ms and 3000 ms were captured with a Cy5 filter set.

Data Analysis

After preprocessing, the collected images were input into BioNavigator v6.2 (PamGene Corp, Amsterdam, the Netherlands) for conversion into point intensity values, determination of the median signal strength of each point, and subtraction of the local background signal. If the signal strength is three times higher than the background standard deviation (SD), it is considered a positive signal. The corrected signal median was input into GeneSpring GX 7.3 (Agilent Technologies, Santa Clara, CA, USA) and the copy number after correction was calculated as required. The average signal of repeated points on every single array was normalized. The copy number was determined by dividing the standardized signals of each *MSH2/MLH1/MSH6* exon by the average exon signal of 10 controls. The obtained results were then input into Microsoft Excel (Mi-

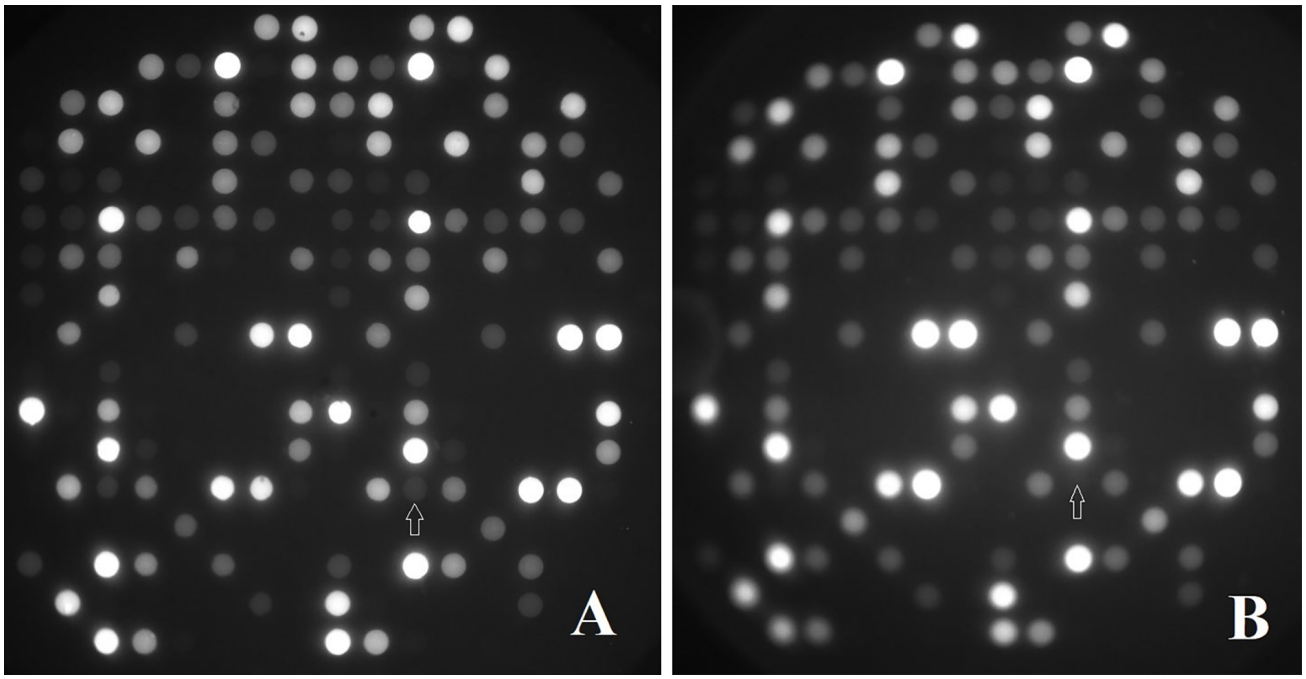

Fig. 3. Representative raw images of control and test samples. (A) Raw image of an array for a healthy control: the amplification of exon 9 is marked by the arrow. (B) Raw image of an array for a proband (H102) with a deletion of exon 9 in *MSH2* gene; the deletion of exon 9 is marked by the arrow.

crosoft Corporation, Redmond, WA, USA) for calculating the median value, standard deviation, and coefficient of variation (CV) of those corresponding exons. A difference in copy number that is three times greater than that of the control exon SD is considered a significant change. A copy number of the proband of less than 0.75 implies that the *MSH2/MLH1/MSH6* exon has been deleted, whereas surpassing 1.5 is indicative of duplication. If the average CV of the sample is greater than 20%, it is considered that the reliability requirements are not met.

Confirmation of Genomic Deletion or Duplication

All the detected copy number variants were screened using the traditional MLPA method to confirm gene deletion or duplication in the probands of Chinese HNPCC families. The GeneScan model (Applied Biosystems, Carlsbad, CA, USA) was applied to analyze the fragments detected using a Beckman CEQ-8800 Avant sequencer (Foster City, CA, USA).

Results

Sample Screening by Array-MLPA

After screening 82 samples, we detected 22 deletions of exon in *MSH2*, *MLH1* and *MSH6* genes in the probands of Chinese HNPCC families. Ten out of the 22 aberrations were deletions in the *MSH2* gene, nine out of 22 were deletions in the *MLH1* gene, and the remaining three were deletions in the *MSH6* gene. However, the deletion of exons 5 to 6 in the *MSH6* gene was excluded by following the confirmation analysis of traditional MLPA. Therefore, 21 deletions of exon in *MSH2*, *MLH1* and *MSH6* genes were detected in 82 probands of Chinese HNPCC families. Particularly, a deletion in the *MSH2* gene and a deletion in the *MLH1* gene occurred in the same proband (H131). The 10 deletion mutations of *MSH2* are mainly distributed in exons 4,9, and 11–13. A total of four deletion mutations of exon 9 were detected, accounting for 40% (4/10) of all *MSH2* gene deletions. All the details are shown in Table 2 and Fig. 2. Surprisingly, we also observed one large deletion in *MLH1* gene, as well as deletions from exons 8 to 15 in the same proband (H85) of the HNPCC kindreds. The large genomic aberrations frequency of *MSH2* and *MLH1* genes accounted for 90.5% (19/21) of all the detected aberrations of *MMR* genes and constituted 24.4% (20/82) of the total probands of HNPCC families. The primary images obtained from the arrays for health control and a proband of the HNPCC family are shown in Fig. 3. These image results can aid in the subsequent research, clarify the mutation-related situation of this kind of gene in HNPCC patients, and provide support for disease analysis.

Validation of Genomic Deletion or Duplication

Such validation was conducted with classic MLPA. The fragment and data were analyzed by Coffalyser. NET software (MRC Holland, Amsterdam, the Netherlands. http s://www.mlpa.com/coffalyser). Except for one deletion of exon 5–6 in the *MSH6* gene that was examined using array-

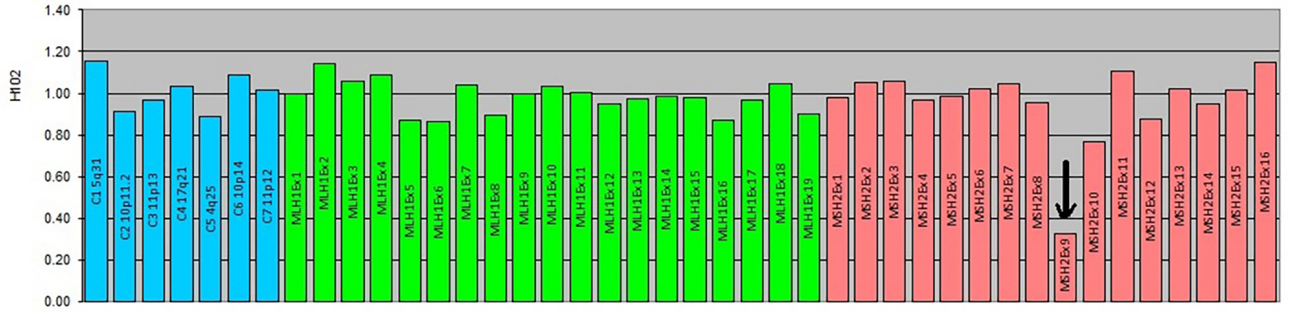

Fig. 4. Detection of the germline genomic aberration in the proband (H102) using classic MLPA. The blue, green and pink bars represent the control, *MLH1* gene and *MSH2* gene, respectively. Arrow indicates the deletion site of *MSH2* gene, with the resulting value approximating to 0.4 but far less than the value of 0.5.

MLPA, the confirmation for the remaining deletions was conducted using classic MLPA analysis, which achieved 100% concordance (Table 2). In our studies, the resulting values were about 1.0 for every normal individual, indicating two copies in each sample. A drop of the value to 0.5 signifies heterozygous deletion of the sequence, while a value exceeding 1.5 indicates a duplication. In all of the probands, we found numerous deletions of exon in different *MMR* genes but did not detect duplication in every proband (Fig. 4).

Discussion

Changes in DNA sequence copy number (CNV), deletions, and duplications are the main contributors to a plethora of genetic diseases [20]. In hereditary ovarian cancer, for instance, the types of CNVs present a wide spectrum, ranging from whole chromosome duplication to small single exon deletions [21]. Some scholars have found a close relationship between CNV and DNA mutations in healthy individuals [22–25]. Therefore, it is of huge clinical significance to be able to detect CNV accurately, generating findings that aid in pathological research and optimization of treatment methods for related diseases. Related experimental studies have found that the main cause of HNPCC is the deletion and duplication of *MSH2* and *MLH1* genes [8,9,26]. CNV testing has been recommended as a necessary component of routine HNPCC mutation screening. However, traditional mutation detection techniques such as heterozygous double-stranded analysis, denaturing gradient gel electrophoresis (DGGE), SSCP analysis, *etc.*, present significant practical limitations and cannot detect mutations at the genomic level. Technological and molecular biology advancements have led to the development of numerous new detection methods with satisfactory efficacy, effectively addressing current challenges [27].

In this research, a new flow-type porous microarray technology that is capable of efficiently performing CNV detection was introduced [28]. This method needs to be applied to porous alumina substrates during detection. Compared with two-dimensional geometric shapes, the reaction surface in three-dimensional substrates increases significantly, thereby improving detection efficiency. When the sample passes through the porous structure under a certain pressure, it can combine and react with the captured molecules on the surface. Continuously replenishing the solution consumed near the spots can reduce diffusion distance and improve the dynamic performance of the system. In this study, a 4-array system capable of performing synchronous detection on 4 samples [29] was applied. When the sample enters the lower side of the substrate, corresponding images are captured to enable efficient real-time detection of hybridization reactions.

Array-MLPA technology has significant advantages in detecting *MSH2/MLH1/MSH6* gene deletions or duplications in HNPCC patients, including ease of operation, high efficiency, and high throughput. In some cases, detection can be achieved within six hours after collecting fresh blood samples, underlining the potential of this method to meet the real-time requirements in clinical treatment interventions. This technology is also equipped with the necessary software for performing automatic measurement and data analysis—an inherent feature that can significantly reduce human biasedness in analysis and interpretation. This method has been applied in gene expression profiling [16] and related studies for quantitating peripheral myelin protein 22 (*PMP22*) gene copy numbers [30].

In this study, we screened a total of 82 probands from HNPCC families based on different clinical criteria. Twenty-two germline genomic large aberrations were investigated in three genes by using array-MLPA; all the aberrations were found to be deletions. Then, the obtained results were compared and analyzed to determine the incidence of mutations in these genes, yielding results that can aid in analyzing the disease etiologies for these patients and form a reference for future research. To confirm the detected germline genomic large aberrations, we applied the classic MLPA for analyzing the same *MMR* genes in the same probands. The results revealed that 21 deletions were detected, except for one deletion of the *MSH6* gene.

Confirmation of detection results using classic MLPA analysis had a concordance rate of 95.5% (21/22). Among all 21 detected aberrations, the deletion rate of *MLH1* and *MSH2* genes accounted for 90.5% (19/21). Thus, we may conclude that array-MLPA is a reliable method to detect the germline genomic large aberrations in the *MMR* genes in Chinese HNPCC families. Compared with traditional methods, this detection method, coupled with bioinformatics tools, possesses obvious advantages, such as increased result accuracy and reliability, fast detection speed, low cost, and lower expertise-knowledge requirements expected from technician personnel. According to our results, we also found that the major aberrations of MMR were *MLH1* and *MSH2*, accounting for about 22% (18/82) of the whole HNPCC probands. Several studies showed that the large genomic aberrations in the *MLH1* and *MSH2* genes account for 30% of the total aberrations found in the western HNPCC families [8,9]. This reported aberration frequency was a little higher than our results. Our study also showed that the aberration of the *MSH6* gene only accounted for 2.4% (2/82). This highlights that the germline genomic aberrations of *MSH6* are rare mutations among the Chinese HNPCC families. Therefore, these findings reaffirm that the etiology testing package for HNPCC in Chinese patients does not generally need to incorporate a test panel that detects mutations in this gene, in a bid to reduce the testing cost.

In summary, array-MLPA is a rapid, precise, highly efficient method for clinical screening and diagnosis of HNPCC. The deletions of *MLH1* and *MSH2* genes are the major germline genomic aberrations in the HNPCC families, while the deletion of *MSH6* is considered a rare mutation in the Chinese HNPCC families. Therefore, the detection of large fragment abnormalities in *MSH2* and *MLH1* genes is key to screening and diagnosing HNPCC in Chinese patients. Of course, large-scale validation is still required prior to the widespread adoption of this technology.

A number of shortcomings in this study need to be highlighted and improved through further investigations. For example, the correlation between large fragment abnormalities of *MSH2/MLH1/MSH6* genes and the clinical phenotype and pathological characteristics of HNPCC was not investigated in the present study, adding uncertainties to whether they can be used as molecular diagnostic markers for special clinical phenotypes, *etc.*

Conclusion

The array-MLPA technique can be used as a screening tool for large deletions of *MMR* genes in HNPCC families, among which the large deletions of *MSH2* and *MLH1* genes should be primarily targeted for early screening and diagnosis of HNPCC.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding authors upon reasonable request.

Author Contributions

YD and SY designed the research study. MH and SY performed the research. YZ and HL provided help and advice on the experiments, acquisition of data and interpretation of data. SY analyzed the data. All authors were involved in drafting the manuscript. All authors contributed significantly to editorial changes of important content. All authors have read and approved the final manuscript. All authors also have participated in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The research was performed in accordance with the Declaration of Helsinki and approved by the ethics committee of Xinhua Hospital Affiliated with Shanghai Jiaotong University School of Medicine (Approval No. XHEC-D-2024-201). The acquisition and application of all clinical data have obtained informed consent from all participants.

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Conflict of Interest

The authors declare no conflict of interest.

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