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국내 린치증후군 환자에서 발견된 *MLH1, MSH2, MSH6* 유전 변이

Germline Variants in *MLH1, MSH2*, and *MSH6* in Korean Patients with Lynch Syndrome

박경진[1] · 장동경[2] · 김희철[3] · 김종원[1,4]
Kyoung-Jin Park, M.D.[1], Dong Kyung Chang, M.D.[2], Hee Cheol Kim, M.D.[3], Jong-Won Kim, M.D.[4]

양산부산대학교병원 진단검사의학과[1], 삼성서울병원 내과[2], 삼성서울병원 외과[3], 삼성서울병원 진단검사의학과[4]
Department of Laboratory Medicine[1], Pusan National University Yangsan Hospital, Yangsan; Departments of Medicine[2] and Surgery[3] and Laboratory Medicine & Genetics[4], Samsung Medical Center, Sungkyunkwan University School of Medicine, Seoul, Korea

Background: The phenotypic and genetic spectrum of Lynch syndrome (LS) seems to differ according to ethnicity. The aim of this study was to investigate the clinical, pathological, and genetic features of LS in a large sample of Korean patients.
Methods: We enrolled a total of 232 patients who fulfilled the revised Bethesda criteria (81%, 232/286) from 286 individuals who underwent genetic screening for LS (*MLH1, MSH2*, and *MSH6* sequencing) in the Samsung Medical Center in Korea from 2004 to 2015. Histopathologic findings, microsatellite instability data, and clinical information were collected.
Results: We identified 61 different pathogenic or likely pathogenic variants (39 in *MLH1*, 20 in *MSH2*, and 2 in *MSH6*), including 4 novel variants, in 101 unrelated Korean patients (101/232, 44%). When multiple tumor manifestations in a single patient were individually considered, there were 285 cancers recorded from 232 cases. A diverse spectrum of tumors, including colorectal cancer, endometrial cancer, stomach cancer, and ovary cancer, was observed. Patients with genetic alterations were more closely associated with a family history of cancers, double primary cancers, and the development of secondary neoplasms than patients without genetic alterations ($P<0.0001$, $P=0.0052$, and $P=0.0010$, respectively).
Conclusions: We report the distribution of pathogenic variants in *MLH1, MSH2,* and *MSH6*, as well as the tumor spectrum, in a large sample of Korean patients with LS. Genetic testing could be an effective stratification strategy for surveillance of LS. This study sheds light on the genetic features of Asian patients with LS.

Key Words: Korean, Lynch syndrome, Mismatch repair gene, Pathogenic variant

Corresponding author: Jong-Won Kim
Department of Laboratory Medicine & Genetics, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea
Tel: +82-2-3410-2705, Fax: +82-2-3410-2719, E-mail: kimjw@skku.edu

Co-corresponding author: Hee Cheol Kim
Department of Surgery, Samsung Medical Center, Sungkyunkwan University School of Medicine, 81 Irwon-ro, Gangnam-gu, Seoul 06351, Korea
Tel: +82-2-3410-1655, Fax: +82-2-3410-2719, E-mail: hckim@skku.edu

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INTRODUCTION

Lynch syndrome (LS) (MIM# 120435) is caused by a genetic alteration that disrupts the mismatch repair (MMR) pathway. The disease is characterized by an increased risk of colorectal cancer (CRC) and endometrial cancer (EC), as well as cancers of the stomach, small intestine, hepatobiliary tract, bladder, brain, and skin [1-4]. The diagnosis of LS can be made when the clinical information meets the Amsterdam Criteria or the less stringent Bethesda Guideline, or when a genetic alteration is identified in one of the MMR genes [1-3].

The phenotypic and genetic spectrum of LS seems to differ according to ethnicity [5-16]. Data from different ethnic groups contribute to the understanding of the genetic and clinical differences in LS among populations. A recent study based on the data from

the International Society of Gastrointestinal Hereditary Tumors (InSiGHT) reported that variant distribution in MMR genes varies widely between different races [5]. Currently, little genetic data on LS have been reported from Asian patients [8, 15, 17, 18].

Over the past decade, the incidence of CRC has continuously increased in Korea [19, 20]. Although environmental factors and somatic alterations may contribute to carcinogenesis, the incidence change of LS might also be a probable explanation for the trend. According to some reports, LS remains considerably under-diagnosed to date [21, 22]. Furthermore, changes in clinico-pathological features in Korean patients with LS have been reported over the past two decades (1990–2004 vs. 2005–2014) [23]. Currently, little data are available regarding Korean patients with genetic variants in MMR genes. The frequency and spectrum of pathogenic variants in Korean patients with LS have not been updated since 2004 [15].

This is a major update of a previous study [15]. The previous study provided genetic data on 166 patients registered between the years of 1995 and 2004 [15]. The current study describes the clinical, pathological, and genetic features of 286 Korean patients diagnosed during 2004–2015. The aims of this study were to investigate the clinical, pathological, and genetic features of LS in Korea.

MATERIALS AND METHODS

1. Study patients

Genetic screening for LS was performed on a group of 286 individuals from 2004 to 2015. A total of 232 patients fulfilled the revised Bethesda criteria (81%, 232/286). The patients were referred by their treating oncologists or by genetics professionals in the Cancer Genetics Clinic of the Samsung Medical Center in Korea. Cancer cases were not preselected based on the results of microsatellite instability (MSI) or immunohistochemistry (IHC). Clinicopathological data, including age at diagnosis, total tumor history (including double primary cancers and recurrent cancers), last follow-up, sex, family history, and pathologic data were collected. If a second malignant neoplasm (SMN) was identified within 2 months from diagnosis of the first tumor, it was considered a synchronous tumor. In regard to the tumor spectrum, multiple tumor manifestations in a single patient were individually considered. This study was approved by the Institutional Review Board of the Samsung Medical Center in Korea (2015-11-076). Written informed consent was obtained from the patients for genetic testing.

2. Microsatellite instability analyses and immunohistochemistry

The results of MSI and IHC assessments were available for only 187 patients (187/232, 81%) and 166 patients (166/232, 72%), respectively. Both tests were performed using paraffin-embedded tissues. For MSI analyses, five reference markers (BAT25, BAT26, D5S346, D17S250, D2S123) were used on both tumor and normal DNA. MSI status was determined by multiplex PCR analysis (ABI PRISM 310 Genetic analyzer, Applied Biosystems, Foster City, CA, USA) and DNA sequencing. Amplified PCR products were run on an ABI Prism 3130 Genetic Analyzer (Applied Biosystems). Allelic sizes were estimated by Genemapper 4.1 (Applied Biosystems). MSI-high tumor was defined when instability was observed in 2 or more of these five markers, MSI-low was defined as instability in one of the markers, and MSS was defined as instability in none of the markers.

To evaluate MMR protein expression, monoclonal antibodies were used as follows: MLH1 (clone G168-15, 1:200; BD Pharmingen, San Diego, CA, USA), MSH2 (clone FE11, 1:400; Calbiochem, La Jolla, CA, USA), and MSH6 (clone 44, 1:400; BD Transduction Laboratories, San Diego, CA, USA). IHC was performed using a Ventana Bench Mark XT auto-immunostainer (Ventana Medical Systems, Tucson, AZ) after incubation with monoclonal antibodies at 37°C for 30 minutes, followed by standard signal amplification and counterstaining with hematoxylin for 4 minutes. Slides were mounted and examined using light microscopy. Loss of expression of MSH2, MSH6, and MLH1 protein was demonstrated by less than 5 percent of nuclear staining in the tumor.

3. Germline variant analyses

All 232 patients who fulfilled the revised Bethesda criteria were evaluated to identify germline pathogenic variants in *MLH1* (MIM #120436), *MSH2* (MIM #609309), and/or *MSH6* (MIM #600678). Genomic DNA was extracted from EDTA-anticoagulated whole blood using the Wizard® Genomic DNA Purification Kit according to the manufacturer's instructions (Promega, Madison, WI, USA). Entire coding exons and intron-exon junctions were amplified by PCR using primer pairs designed with Primer3 software. The primers are available upon request. Direct sequencing was conducted

using a BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) and an ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

Variant numbers were designated on A of the ATG start codon as +1 in the *MLH1* (NM_000249.2), *MSH2* (NM_000251.1), and *MSH6* (NM_000179.2). In accordance with the American College of Medical Genetics and Genomics (ACMG) guidelines, we selected pathogenic variants and likely pathogenic variants [24]. The criteria were applied as follows: PVS1 (nonsense variants, splicing variants, or frameshift variants with and/or without functional data), PS3 (well-established functional evidence, including splicing/transcript expression, MMR activity, sub-cellular localization, subunit interaction, protein expression, or stability data from the curated data in InSiGHT, available at http://insight-group.org/variants/database/, accessed on January, 2018), PM2 (allele frequency less than 0.01 or absent from the databases, including the 1000 Genomes Project (1000GP, available at http://browser.1000genomes.org/index.html), Exome Sequencing Project (ESP, available at http://evs.gs.washington.edu/EVS/), Exome Aggregation Consortium (ExAC available at http://exac.broadinstitute.org/), or Korean Reference Genome Database (KRGDB, available at http://152.99.75.168/KRGDB/), PP1 (co-segregation data from the curated data in InSiGHT), PP3 (evidence supporting "deleterious" or "damaging" effects was higher than 3 using the *in silico* tools: functional effects of missense variants were predicted by sorting intolerant from tolerant [SIFT], polymorphism phenotyping-2 [PolyPhen], LRT, FATHMM, MutationTaster, MutationAssessor, and Genomic Evolutionary Rate Profiling [GERP] score), PP5 (a "disease-causing mutation" in the human gene mutation database [HGMD professional, updated on March, 2017], a "pathogenic" mutation in ClinVar (available at http://www.ncbi.nlm.nih.gov/clinvar/accessed on January, 2018), a "class 4 or class 5 in InSiGHT and/or Leiden Open Variation Database [LOVD v3.0 Build 19 available at http://www.lovd.nl/3.0/home], or variants reported from previous studies) [18, 24-31]. The status of pathogenic variants was evaluated based on data from InSiGHT, LOVD, HGMD, ClinVar databases, and previous studies.

4. Statistical analyses

Fisher's exact test (or Chi-squared test) and Mann-Whitney *U* test were used to compare the categorical data and continuous data, respectively. Statistical significance was analyzed with MedCalc version 11.5.1.0 (Mariakerke, Belgium) and the R statistical environment version 2.2.3 (http://www.r-project.org/). *P* values less than 0.05 were considered statistically significant.

RESULTS

1. Clinical characteristics of the study patients

The median age of initial cancer diagnosis was 43 years (range 16–82) (Table 1). There were 285 cancers recorded from 232 cases. A diverse spectrum of tumors was observed: CRC (76%, 217/285), EC (9%, 27/285), stomach cancer (5%, 13/285), ovary cancer (2%, 7/285), bladder cancer (1%, 4/285), small intestinal cancer (1%,

Table 1. Baseline characteristics of the study patients

Characteristics	Number/total number (%)
No. of patients	232
Male: Female	116:82
Age at onset of first cancer, median (range)	43 (16–82) year
First tumor manifestations	
Colonic	211/232 (91)
Extracolonic	21/232 (9)
Total tumor history	
Colonic	174/232 (75)
Extracolonic*	15/232 (6)
Both	43/232 (19)
Double primary cancer manifestations	14/232 (6)
Family history of cancers†	
Colonic	69/164 (42)
Extracolonic	37/164 (23)
Both	58/164 (35)
Secondary tumor occurrence	37/232 (16)
Immunohistochemistry‡	
MLH1 loss	92/168 (55)
MSH2 loss	47/168 (28)
MSH6 loss	53/168 (32)
Microsatellite instability‡	
High	162/187 (87)
Stable	25/187 (13)

For the tumor spectrum, multiple tumor manifestations in a single patient were individually considered.
*Endometrial cancer (N=27), stomach cancer (N=13), ovary cancer (N=7), bladder cancer (N=4), small intestinal cancer (N=4), cervical cancer (N=3), breast cancer (N=3), glioblastoma (N=2), lung cancer (N=2), esophageal cancer (N=1), pheochromocytoma (N=1), and skin cancer (N=1); †The tumor spectrum of family members from 164 cases was as follows: CRC (N=240), stomach cancer (N=28), hepatobiliary cancer (N=22), EC (N=18), lung cancer (N=10), pancreatic cancer (N=7), urinary cancer (N=5), ovary cancer (N=5), breast cancer (N=4), skin cancer (N=2), hematologic cancer (N=2), thyroid cancer (N=2), cervical cancer (N=2), brain cancer (N=1), prostate cancer (N=1), small intestinal cancer (N=1), and pharyngeal cancer (N=1); ‡Immunohistochemistry and the microsatellite instability assessments were described as the number/total number available.

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Table 2. Pathogenic or likely pathogenic variants identified in Korean patients with Lynch syndrome

Genes*	NT alterations	AA alterations†	Type	N. of probands	dbSNP†	HGMD	InSiGHT	Clinvar	Status§	ACMG-AMP criteria¶	Pathogenicity
MLH1	c.1758dupC	p.(Met587Hisfs*6)	frameshift	15	NA	DM	Class 5	Pathogenic	Known	PVS1, PM2, PP1, PS3	P
	c.67G>T	p.Glu23*	nonsense	4	rs63750823	DM	Class 5	Pathogenic	Known	PVS1, PS3, PM2, PP1, PP3	P
	c.1918C>T	p.Pro640Ser	missense	3	rs63749792	DM	Class 3	Uncertain significance	Known	PM2, PP3, PP1, PP5	LP
	c.303_304dupTG	p.(Glu102Valfs*7)	frameshift	3	NA	DM	NA	NA	Known	PVS1, PM2, PP5	P
	c.808_811delACTT	p.(Thr270Profs*2)	frameshift	2	rs267607801	DM	Class 5	Pathogenic	Known	PVS1, PM2, PP5	P
	c.440_441insT	p.(Thr148Aspfs*24)	frameshift	2	NA	DM	NA	NA	Known	PVS1, PM2, PP5	P
	c.1975C>T	p.(Arg659*)	nonsense	2	rs63751310	DM	Class 5	Pathogenic	Known	PVS1, PS3, PM2, PP1, PP5	LP
	c.1449_1471dup23	p.(Thr491Lysfs*8)	frameshift	2	NA	DM	NA	NA	Known	PVS1, PM2, PP5	LP
	c.677G>A	p.Gln197Argfs*8	frameshift‖	2	rs63751711	DM	Class 5	Pathogenic	Known	PVS1, PS3, PM2, PP1, PP3, PP5	P
	c.1721T>C	p.(Leu574Pro)	missense	2	rs63751608	DM	Class 4	Likely pathogenic	Known	PM2, PS3, PP1, PP3, PP5	P
	c.884+2dupT	NA	splicing	2	NA	DM	NA	NA	Novel	PVS1, PM2	LP
	c.678-1G>C	p.(Glu227Thrfs*7)	splicing	2	rs267607784	DM	Class 5	Pathogenic	Known	PVS1, PS3, PM2, PP1	P
	c.849T>A	p.(Tyr283*)	nonsense	1	NA	NA	NA	NA	Novel	PVS1, PM2	LP
	c.346dupA	p.(Thr116Asnfs*6)	frameshift	1	rs267607739	DM	Class 5	Pathogenic	Known	PVS1, PM2, PP5	P
	c.1984A>C	p.Thr662Pro	missense	1	NA	DM	Class 4	Likely pathogenic	Known	PM2, PS3, PP1, PP3, PP5	LP
	c.1668-2A>G	NA	splicing	1	NA	DM	NA	NA	Known	PVS1, PM2, PP5	P
	c.2104-2A>G	NA	splicing	1	rs267607889	DM	Class 4	Likely pathogenic	Known	PVS1, PM2, PS3, PP1, PP5	P
	c.887dupT	p.(Leu296Phefs*11)	frameshift	1	rs63751620	DM	Class 5	NA	Known	PVS1, PM2, PP5	P
	c.791-1G>C	p.(His264Leufs*2)	splicing	1	rs267607795	DM	Class 5	Pathogenic	Known	PVS1, PS3, PM2, PP1	P
	c.350C>T	p.Thr117Met	missense	1	rs63750781	DM	Class 5	Pathogenic	Known	PM2, PS3, PP1, PP3, PP5	LP
	c.210_213delAGAA	p.(Glu71Ilefs*20)	frameshift	1	rs267607723	NA	Class 5	Pathogenic	Known	PVS1, PS3, PM2, PP5	P
	c.189C>A	p.(Asp63Glu)	missense	1	NA	DM	Class 5	Pathogenic	Known	PM2, PS3, PP1, PP3, PP5	LP
	c.2041G>A	p.Ala681Thr	missense	1	rs63750217	DM	Class 5	Pathogenic	Known	PM2, PS3, PP1, PP3, PP5	LP
	c.1333C>T	p.(Gln445*)	nonsense	1	NA	NA	NA	NA	Known**	PVS1, PM2	LP
	c.2080G>T	p.(Glu694*)	nonsense	1	rs147542208	DM	NA	NA	Known	PVS1, PM2, PP5	LP
	c.1011dupC	p.Asn338Glnfs*24	frameshift	1	rs63750677	DM	Class 5	NA	Known	PVS1, PS3, PM2, PP1, PP5	P
	c.2142G>A	p.(Trp714*)	nonsense	1	rs63750978	DM	NA	Pathogenic	Known	PVS1, PM2, PP5	LP
	c.1349delA	p.(Asp450Valfs*41)	frameshift	1	NA	DM	NA	NA	Known	PVS1, PM2, PP5	LP
	c.1758delC	p.(Met587Cysfs*4)	frameshift	1	rs63749863	DM	Class 5	NA	Known	PVS1, PM2, PP5	LP
	c.1553_1558+4del10	p.(His518Argfs*48)	frameshift	1	NA	DM	NA	NA	Known	PVS1, PM2, PP5	LP
	c.503delA	p.(Asn168Ilefs*34)	frameshift	1	NA	DM	NA	NA	Known	PVS1, PM2, PP5	LP
	c.1559-1G>A	NA	splicing	1	rs267607837	DM	Class 4	Pathogenic	Known	PVS1, PS3, PM2	LP
	c.1758dupC	p.(Met587Hisfs*6)	frameshift	1	rs367543283	DM	NA	NA	Known	PVS1, PM2, PP5	LP
	c.199G>A	p.Gly67Arg	missense	1	rs63750206	DM	Class 5	Pathogenic	Known	PS3, PM2, PP5, PP1, PP3	LP
	c.1546C>T	p.(Gln516*)	nonsense	1	NA	DM	NA	NA	Known	PVS1, PM2, PP5	LP
	c.2181_2182delCA	p.(Ile728Serfs*4)	frameshift	1	NA	DM	Class 5	NA	Known	PVS1, PM2, PP5	LP
	c.1668-1G>C	NA	splicing	1	NA	DM?	NA	Likely pathogenic	Known	PVS1, PM2, PP5	LP
	c.790+1G>A	p.Glu227_Ser295del	splicing	1	rs267607789	DM	Class 5	Pathogenic	Known	PVS1, PS3, PM2, PP5	P
	c.19delG	p.(Val7Leufs*10)	frameshift	1	NA	DM	NA	NA	Known	PVS1, PM2, PP5	LP

(*Continued to the next page*)

Table 2. Continued

Genes*	NT alterations	AA alterations†	Type	N. of probands	dbSNP†	HGMD	InSiGHT	Clinvar	Status§	ACMG-AMP criteria¶	Pathogenicity
MSH2	c.942+3A>T	p.Val265_Gln314del	splicing	7	rs193922376	DM	Class 5	Pathogenic	Known	PVS1, PS3, PM2, PP1, PP5	P
	c.1024delinsAA	p.(Val342Asnfs*2)	frameshift	3	NA	DM	NA	NA	Known	PVS1, PM2, PP5	P
	c.187delG	p.(Val63*)	nonsense	2	rs63750160	DM	Class 5	Pathogenic	Known	PVS1, PM2, PP5	P
	c.2038C>T	p.Arg680*	nonsense	2	rs63749932	DM	Class 5	Pathogenic	Known	PVS1, PS3, PM2, PP1, PP5	P
	c.2633_2634delAG	p.Glu878Alafs*3	frameshift	2	rs63751618	DM	Class 5	Pathogenic	Known	PVS1, PS3, PM2, PP1	P
	c.387_388delTC	p.(Gln130Valfs*2)	frameshift	2	rs63750924	DM	Class 5	Pathogenic	Known	PVS1, PM2, PP5	P
	c.2089T>C	p.Cys697Arg	missense	1	rs63750961	DM	Class 5	Pathogenic	Known	PS3, PM2, PP1, PP3, PP5	LP
	c.1465G>T	p.(Glu489*)	nonsense	1	NA	DM	NA	NA	Known	PVS1, PM2, PP1	LP
	c.1129C>T	p.(Gln377*)	nonsense	1	rs63750267	DM	Class 5	Pathogenic	Known	PVS1, PM2	LP
	c.1552_1553delCA	p.Gln518Valfs*10	frameshift	1	rs63749930	DM	Class 5	Pathogenic	Known	PVS1, PS3, PM2, PP1, PP5	P
	c.1366dupA	p.(Thr456Asnfs*12)	frameshift	1	NA	NA	NA	Pathogenic	Known	PVS1, PM2	LP
	c.1782_1783delAC	p.(Leu595Glnfs*2)	frameshift	1	NA	NA	NA	NA	Novel	PVS1, PM2	LP
	c.1226_1227delAG	p.Gln409Argfs*7	frameshift	1	rs63750086	DM	Class 5	Pathogenic	Known	PVS1, PS3, PM2, PP1, PP5	P
	c.1861C>T	p.(Arg621*)	nonsense	1	rs63750508	DM	Class 5	Pathogenic	Known	PVS1, PM2, PP5	LP
	c.1127dupT	p.(Leu376Phefs*13)	frameshift	1	NA	DM	NA	NA	Known	PVS1, PM2, PP5	P
	c.2634+1G>A	p.(Gly820Alafs*3)	splicing	1	rs267608019	DM	Class 4	Pathogenic	Known	PVS1, PS3, PM2, PP1	P
	c.881_882del	p.(Phe294*)	frameshift	1	NA	NA	Class 5	NA	Known	PVS1, PM2, PP5	LP
	c.256G>T	p.(Glu86*)	nonsense	1	NA	NA	NA	NA	Novel	PVS1, PM2	LP
	c.2186_2187insAATG	p.(Met729Ilefs*22)	frameshift	1	NA	DM	NA	NA	Known	PVS1, PM2, PP5	LP
	c.1705_1706delGA	p.(Glu569Ilefs*2)	frameshift	1	rs63750393	DM	Class 5	Pathogenic	Known	PVS1, PM2, PP5	P
MSH6	c.3261dupC	p.(Phe1088Leufs*5)	frameshift	1	rs267608087	DM	Class 5	Pathogenic	Known	PVS1, PM2, PP5	LP
	c.873_874delCA	p.(Asn291Lysfs*20)	frameshift	1	rs1060502888	NA	NA	Pathogenic	Known	PVS1, PM2, PP5	LP

Abbreviations: NT, nucleotide; AA, amino acid; dbSNP, database of single nucleotide polymorphisms, v150; HGMD, human gene mutation database (professional version, updated in March 2017); InSiGHT, International Society of Gastrointestinal Hereditary Tumors (updated in August 2017 and accessed on January, 2018); ACMG, American College of Medical Genetics and Genomics; DM, disease-causing mutation; NA, not applicable; P, pathogenic; LP, likely pathogenic.

*_MLH1_ (NM_000249.2), _MSH2_ (NM_000251.1), and _MSH6_ (NM_000179.2); †Alterations at the amino acid level were deduced from nucleotide alterations in the case of experimental evidence and were filled based on predicted protein sequences in parentheses; ‡Allele frequency of SNPs: rs147542208 (0.000200000 from 1000GP, 0.00002486 from ExAC, 0.000909091 from KRGDB databases), rs63749932 (0.000008244 from ExAC), rs267608087 (0.000057680 from ExAC); §The status of variants was evaluated based on the curated data from InSiGHT, HGMD, and ClinVar (accessed in January 2018) databases; ¶The criteria were applied as follows: PVS1 (nonsense variants, splicing variants and frameshift variants), PS3 (functional evidences by splicing/transcript expression, MMR activity, sub-cellular localization, subunit interaction, protein expression, and stability data from the curated data in InSiGHT, available at http://insight-group.org/variants/database/ accessed on January, 2018), PM2 (allele frequency less than 0.01 or absent from the databases including the 1000 Genomes Project, Exome Sequencing Project, Exome Aggregation Consortium, or the Korean Reference Genome Database, available at 1000GP, http://browser.1000genomes.org/index.html, http://evs.gs.washington.edu/EVS/, http://exac.broadinstitute.org/, http://152.99.75.168/KRGDB/, respectively), PP1 (co-segregation data from the curated data in InSiGHT), PP3 (evidence supporting "deleterious" or "damaging" effects was higher than 3 using the _in silico_ tools: functional effects of missense variants were predicted by sorting intolerant from tolerant (SIFT), polymorphism phenotyping-2 (PolyPhen), LRT, FATHMM, MutationTaster, MutationAssesor, and Genomic Evolutionary Rate Profiling (GERP) score), PP5 (a "disease-causing mutation" in the human gene mutation database (HGMD professional, updated on March, 2017, a "pathogenic" mutation in ClinVar (available at http://www.ncbi.nlm.nih.gov/clinvar/ accessed on January, 2018), a "class 4 or class 5 in InSiGHT and Leiden Open Variation Database (LOVD v3.0 Build 19 http://www.lovd.nl/3.0/home), or variants reported from previous studies) [18, 24, 29-31]; ‖This pathogenic variant (c.677G>A) is predicted to be a nucleotide substitution at the coding DNA level. However, protein and RNA-based functional studies have consistently found that this variant results in the skipping of exon 8. This variant was classified as a frameshift variant [26-28]; **This variant (c.1333C>T) was reported in the previous study [35].

4/285), cervical cancer (1%, 3/285), breast cancer (1%, 3/285), glioblastoma (1%, 2/285), lung cancer (1%, 2/285), esophageal cancer (less than 1%, 1/285), pheochromocytoma (less than 1%, 1/285), and skin cancer (less than 1%, 1/285). A summary of baseline characteristics is described in Table 1. A family history of cancers was evident in 71% (164/232) of cases (Table 1). There were 351 cancers recorded from family members from 164 cases: CRC (68%, 240/351), stomach cancer (8%, 28/351), hepatobiliary cancer (6%, 22/351), EC (5% 18/351), lung cancer (3%, 10/351), pancreatic cancer (2%, 7/351), urinary cancer (1%, 5/351), ovary cancer (1%, 5/351), breast cancer (1%, 4/351), skin cancer (1%, 2/351), hematologic cancer (1%, 2/351), thyroid cancer (1%, 2/351), cervical cancer (1%, 2/351), brain cancer (less than 1%, 1/351), prostate cancer (less than 1%, 1/351), small intestinal cancer (less than 1%, /351), and pharyngeal cancer (less than 1%, 1/351).

2. Detection rate and spectrum of pathogenic or likely pathogenic variants

A total of 101 patients were found to have pathogenic or likely pathogenic germline variants, corresponding to an overall detection rate of 101/232, or 44% (Tables 2, 3). Overall, we identified 61 different variants (39 in *MLH1*, 20 in *MSH2*, and 2 in *MSH6*). These include 30 frameshift insertions/deletions (indels), 13 nonsense variants, 10 splicing variants, and 8 missense variants (Table 2). Results of the comprehensive *in silico* analyses of missense variants are described in Table 4.

Four of these pathogenic or likely pathogenic variants were novel; 2 in *MLH1* and 2 in *MSH2* (Table 2). These included 1 frameshift variant (c.1782_1783delAC in *MSH2*), 1 splicing variant (c.884+2dupT in *MLH1*), and 2 nonsense variants (c.849T>A in *MLH1*; c.256G>T in *MSH2*) (Table 2). None of the novel variants were found in control databases, including in WES data from 622 healthy Korean individuals, nor in the 1000GP, ESP6500, and ExAC (Table 2).

Eighteen recurrent pathogenic or likely pathogenic variants were identified: 12 in *MLH1* and 6 in *MSH2* (Table 2). Six of them [c.1758dupC (n=15), c.67G>T (n=4), c.1918C>T (n=3), c.303_304dupTG (n=3) in *MLH1* and c.942+3A>T (n=7) and c.1024delinsAA (n=3) in *MSH2*] have been found in at least three unrelated patients (Table 2).

3. Genotype–phenotype correlation study

The number of patients with variants in *MSH6* was small, there-

Table 3. Detection rate and distribution of the genetic alterations in mismatch repair genes according to ethnicity

Ethnicity	Genetic test	Criteria	Detection of genetic alterations (%)	No. of patients with genetic alterations				References
				MLH1	*MSH2*	*MSH6*	*PMS2*	
Israeli	Sequencing, MLPA	Genetic diagnosis of LS	NA	19/113 (17%)	67/113 (59%)	20/113 (18%)	NA	[14]
Hungarian	Heteroduplex & SSCP, Sequencing, MLPA	Amsterdam I-II (n=20) Pedigree suggestive of LS (n=16)	18/36 (50.0)	9/18 (50%)	9/18 (50%)	NA	NA	[13]
Brazilian	Sequencing, MLPA	Clinical suspicion for LS	45/116 (38.8)	15/45 (33%)	25/45 (56%)	4/45 (9%)	1/45 (2%)	[12]
Dutch	DGGE, SSCP, Sequencing,	Suspected for LS: Amsterdam criteria (n=42)	47/184 (25.5)	28/47 (60%)	19/47 (40%)	NA	NA	[11]
German	DHPLC, Sequencing, Southern blot, MLPA	Amsterdam criteria	281/574 (49.0)	124/281 (44%)	157/281 (56%)	NA	NA	[10]
Cypriot	Sequencing, MLPA	Revised Bethesda guideline, Amsterdam criteria	5/77 (6.5)	4/5 (80%)	1/5 (20%)	0	NA	[9]
Singapore	Sequencing, MLPA	Singapore Polyposis Registry	17/59 (28.8)	11/17 (65%)	6/17 (35%)	0	0	[8]
Korean	PCR-SSCP	Korean Hereditary Tumor Registry	44/164 (26.8)	31/44 (70%)	10/44 (23%)	3/44 (7%)	NA	[15]
Korean	Sequencing	Revised Bethesda guideline	101/232 (44.0)	67/101 (66%)	32/101 (32%)	2/101 (2%)	NA	This study

Abbreviations: MLPA, multiplex ligation-dependent probe amplification; LS, Lynch syndrome; SSCP, single strand conformation polymorphism; DGGE, denaturing gradient gel electrophoresis; DHPLC, denaturing high performance liquid chromatography; NA, not applicable.

Table 4. Allele frequency data and *in silico* analyses of pathogenic or likely pathogenic missense variants

Genes	NT alterations	AA alterations	dbSNP	1000GP	ESP6500	ExAC	KRGDB	SIFT	Polyphen2	LRT	Mutation Taster	Mutation Assessor	FATHMM	GERP
MLH1	c.1918C>T	p.Pro640Ser	rs63749792	A	A	A	A	D	D	D	D	H	D	5.81
	c.1721T>C	p.(Leu574Pro)	rs63751608	A	A	A	A	D	P	D	D	M	D	5.44
	c.1984A>C	p.Thr662Pro	NA	A	A	A	A	T	D	D	D	L	D	5.49
	c.350C>T	p.Thr117Met	rs63750781	A	A	A	A	D	D	D	D	H	T	5.11
	c.189C>A	p.(Asp63Glu)	NA	A	A	A	A	D	D	D	D	H	D	4.98
	c.2041G>A	p.Ala681Thr	rs63750217	A	A	A	A	D	D	D	D	M	D	5.93
	c.199G>A	p.Gly67Arg	rs63750206	A	A	A	A	D	D	D	D	H	D	5.85
MSH2	c.2089T>C	p.Cys697Arg	rs63750961	A	A	A	A	D	D	D	D	H	D	6.08

Abbreviations: NT, nucleotide; AA, amino acid; 1000GP, 1000 Genomes Project; ESP, Exome Sequencing Project; ExAC, Exome Aggregation Consortium; KRGDB, Korean Reference Genome Database; SIFT, sorting intolerant from tolerant; PolyPhen, polymorphism phenotyping-2; GERP, Genomic Evolutionary Rate Profiling; A , Absent; D, damaging (in PolyPhen)/deleterious (in SIFT, LRT, and FATHMM)/disease-causing (in MutationTaster); P, possibly or probably damaging (in PolyPhen); H, high (functional); M, medium (functional); N, neutral (nonfunctional); L, low(nonfunctional); T, tolerated (in SIFT and FATHMM).

fore the comparisons were done in patients with and without genetic alterations in *MLH1* and *MSH2*. The extracolonic tumors, including EC, were more prevalent in patients without genetic alterations compared to patients with genetic alterations, although the most frequent cancer type was CRC in both groups (6% vs. 12%, $P=0.0109$) (Table 5). The patients with genetic alterations had a higher incidence of family history of cancers than patients without genetic alterations (87% vs. 58%, $P<0.0001$) (Table 5). Furthermore, the patients with genetic alterations were more associated with double primary cancers and development of SMNs than patients without genetic alterations (8% vs. 5%, $P=0.0052$ for double primary cancers and 24% vs. 10%, $P=0.0010$ for SMNs) (Table 5).

DISCUSSION

Our study presents the clinical, pathological, and genetic features of LS in a large sample of Korean patients. The incidence of pathogenic variants (44%) during 2004–2015 has increased considerably, compared to the results from the previous study using data registered between the years of 1995 and 2004 (27%) [15]. This suggests that the increase in the genetic diagnosis of LS might be associated with the continued efforts of LS surveillance in Korea. The detection rate of genetic alterations in patients with LS from Korea was lower than in patients from Hungary (50%) and Germany (49%), while it was higher than in patients from Brazil (39%), Holland (26%), and Singapore (29%). Although the criteria for genetic screening and testing methods were different among the previous studies, this suggests that there are ethnic differences in the genetic features underlying LS [8, 10-14].

In this retrospective study, 81% of patients fulfilled the revised Bethesda guidelines. The sensitivity of the revised Bethesda guidelines has been reported to be 82% [32, 33]. Application of the clinical criteria might be impossible in cases in which patients do not know their family history of cancers. Furthermore, pathogenic variants may be identified in patients without a family history of cancers; for instance, in cases with a *de novo* variant. Considering these situations, the detection rate by genetic testing might be an underestimate, because we evaluated patients who fulfilled the revised Bethesda guidelines.

In clinical practice, MSI would be used to investigate the probability of having LS and IHC would be used to select genes that are

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Table 5. Comparisons of the clinicopathologic and molecular characteristics according to the status of the pathogenic variants

Characteristics	Positive for genetic alterations (N=99)		Negative for genetic alterations (N=131)	*P* value	
	MLH1 (N=67)	*MSH2* (N=32)		*Positive (N=99) vs. Negative (N=131)	*MLH1* alterations (N=67) vs. *MSH2* alterations (N=32)
Age at onset, median (range)	43 (24–72)	42 (30–81)	43 (16–82)	0.6110	0.8413
Male: Female, Number	36:31	18:14	70:60	0.8620	1.0000
Tumor spectrum				0.0109	0.0840
Colonic	46/67 (69%)	23/32 (72%)	103/130 (79%)		
Extracolonic	4/67 (6%)	2/32 (6%)	16/130 (12%)		
Both	19/67 (28%)	8/32 (24%)	11/130 (8%)		
Family history of cancers	61/67 (91%)	26/32 (74%)	76/130 (58%)	<0.0001	0.1071
Double primary cancers	7/67 (10%)	1/32 (3%)	6/130 (5%)	0.0052	0.1320
Secondary malignancy	15/67 (22%)	9/32 (24%)	13/130 (10%)	0.0010	0.7524
Immunohistochemistry†					
MLH1 loss	44/48 (92%)	1/26 (4%)	47/92 (51%)	<0.0001	<0.0001
MSH2 loss	0/48 (0%)	24/26 (92%)	23/92 (25%)	0.2096	<0.0001
MSH6 loss	2/48 (4%)	23/26 (88%)	26/92 (28%)	0.4982	<0.0001
Microsatellite instability†				0.0028	0.5534
High	57/61 (93%)	28/30 (93%)	79/99 (80%)		
Stable	4/61 (7%)	2/30 (7%)	20/99 (20%)		
Type of variants			NA	NA	0.1179
Frameshift variants	35/67 (52%)	15/32 (38%)			
Missense variants	11/67 (16%)	1/32 (3%)			
Nonsense variants	11/67 (16%)	9/32 (22%)			
Splicing variants	10/67 (15%)	8/32 (19%)			

Abbreviation: NA, not applicable.
*Positive for genetic alterations vs. negative for genetic alterations; †Immunohistochemistry and the microsatellite instability assessments were described as the number/total number available.

likely to have relevant variants. The sensitivity of IHC and MSI for the prediction of pathogenic variants in MMR genes has been reported to be 92% and 93%, respectively [34]. The exclusive use of MSI or IHC is not sufficient to diagnose LS. Therefore, we did not preselect patients based on the results of MSI or IHC in this study. We demonstrated that the concordance between IHC and pathogenic variants was 94%, while that between MSI and pathogenic variants was 80%. This suggests that the study subjects were considerably appropriate for investigating the genetic spectrum of patients with LS.

We considerably extended the genetic features, as well as the tumor spectrum of LS, by using a large number of Korean patients. Two recurrent pathogenic variants (c.1758dupC in *MLH1* and c.942+3A>T in *MSH2*) that were reported as founder mutations in Korea [15] and Newfoundland [16] accounted for 22% (22/102) of patients with pathogenic variants in this study. In this consecutive series, the gene distribution in Korean patients showed a predominantly high incidence of pathogenic variants in *MLH1*. This distribution is similar to the previous study of Korean patients, while it is different from the data on Israeli, Brazilian, or German patients, in which *MSH2* alterations were more common than *MLH1* alterations [8, 10-15]. In addition, truncation variants, including frameshift variants, were the most frequently observed in both *MLH1* and *MSH2*. This finding is in agreement with the results from the previous study [15]. However, the proportion of missense variants decreased compared to that from the Korean patients registered between the years of 1995 and 2004 (12% vs. 34%) [15]. This could be explained by the possibility that some missense variants were excluded by the stringent ACMG guidelines for the interpretation of pathogenicity.

In terms of the LS-related tumor spectrum, the current study shows that the distribution of cancers in Korean patients was similar to that previously reported in Caucasian populations [4]. Furthermore, we extended the LS-related tumor spectrum to include thyroid cancer, brain cancer, lung cancer, esophageal cancer, and cervical cancer from the MMR gene variant carriers who fulfilled

the revised Bethesda Guideline. These cancers have been recently reported to be associated with LS in rare cases and are not common in the tumor spectrum in LS [4].

We showed that patients with genetic alterations were more closely associated with a positive family history of cancers and development of SMNs than non-carriers. This suggests that there is higher lifetime cancer risk in patients with genetic alterations than in non-carriers. The current study contributes to the recognition that the testing of genetic variants could be a significant indicator for the surveillance of SMNs, as well as a diagnostic test for LS.

This study has some limitations that may influence the detection rate of pathogenic variants. First, some important alterations, including large deletion/duplication, deep intronic variants, and promoter variants, were not considered. Second, we could not comprehensively cover genes belonging to the MMR pathway, including *PMS2*. To date, the sequencing of *PMS2* is limited to a few clinical laboratories, because the high sequence homology of the gene is a technical challenge. Third, this study was not designed to identify *MLH1* promoter methylation and somatic alterations. Another important limitation is that we could not perform functional experiments in 4 novel variants, although comprehensive *in silico* analyses and filtering using population frequency data were done.

In summary, we considerably extended the genetic features, as well as the clinicopathological features, of LS using a large sample of Korean patients. We identified 61 different pathogenic or likely pathogenic variants (39 in *MLH1*, 20 in *MSH2*, and 2 in *MSH6*), including 4 novel variants, in 101 unrelated Korean patients. Furthermore, we found that there were significant differences in the tumor spectrum, family history of cancers, occurrence of SMNs, and MSI status between patients with pathogenic variants and non-carriers. This study sheds light on the genotype-phenotype correlation and genetic features of Asian patients with LS and will provide guidance for genetic counseling for patients with LS. Further studies are warranted to stratify the surveillance program for LS depending on MMR gene variants.

요 약

배경: 린치증후군의 표현형 및 돌연변이 스펙트럼은 인종에 따라 차이를 보이는 것으로 알려져 있다. 본 연구의 목적은 국내 린치증후군 환자의 임상적, 병리학적, 유전적 특성을 규명하는 것이다.

방법: 삼성서울병원에서 2004–2015년 기간 동안 린치증후군에 대해 연속적으로 유전자 검사(*MLH1, MSH2, MSH6* 염기서열분석)를 시행한 286명의 환자 중에서 개정된 베데스다 기준(revised Bethesda criteria)을 충족하는 232명의 환자를 대상으로 분석하였다. 임상 정보, 조직학적 소견 및 미세위성 불안정성(microsatellite instability) 검사 결과를 포함하였다.

결과: 101명(44%)의 린치증후군 환자에서 4개의 새로운 돌연변이(틀이동변이 1개, 짜깁기변이 1개, 무의미변이 2개)를 포함하여 61개(*MLH1* 변이 39개, *MSH2* 변이 20개, *MSH6* 변이 2개)의 다른 돌연변이가 발견되었다. 다양한 스펙트럼의 암종이 발견되었다: 대장암(217명), 자궁내막암(27명), 위암(13명), 난소암(7명), 방광암(4명), 소장암(4명), 자궁경부암(3명), 유방암(3명), 교모세포종(2명), 폐암(2명), 식도암(1명), 갈색세포종(1명), 피부암(1명). 돌연변이 음성 환자에 비해 돌연변이 양성 환자에서 암종에 대한 가족력도 더 높으며($P<0.0001$) 2차 종양이 더 자주 발생하는 것으로($P=0.0010$) 확인되었다.

결론: 본 연구를 통해 국내 대규모 린치증후군 환자군에서 *MLH1, MSH2, MSH6* 돌연변이 분포 및 암종 스펙트럼에 대해 보고하였다. 린치증후군 환자에서 이차 종양의 발생을 감시할 때, 돌연변이의 양성 여부가 효과적인 계층화 전략이 될 수 있음을 규명하였다. 본 연구는 아시아 린치증후군 환자의 유전적 특성을 규명하는 데 기여할 것이다.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

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