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Pediatric-Type Indolent B-Cell Lymphomas With Overlapping Clinical, Pathologic, and Genetic Features

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Abstract: Pediatric-type follicular lymphoma (PTFL) and pediatric nodal marginal zone lymphoma (PNMZL) are rare pediatric-type indolent B-cell lymphomas (PedIBCL) that differ clinicopathologically from their adult counterparts. Accurate diagnosis is important to avoid overtreatment but is often challenging. The mutational landscape of PTFL is known and may aid diagnosis, but the genetic features of PNMZL are not well understood. We analyzed 21 cases of PedIBCL according to their clinicopathologic findings and classified them into PTFL (n = 11), PNMZL (n = 2), and "mixed type" tumors (n = 8) showing ambiguous histology. We also analyzed 2 cases of adult B-cell lymphomas showing features of PedIBCL. Targeted sequencing of 121 lymphoma-related genes was performed. The median age of PedIBCL patients was 16 years (range: 3 to 47), and all but 1 PTFL patient were male. All patients presented with limited-stage disease, and only 1 relapsed. There were no significant differences in clinical features among the 3 PedIBCL groups. The most frequently mutated genes were *MAP2K1*, *TNFRSF14*, *KMT2C*, *IRF8*, and *NOTCH2*. The genetic features of all groups were similar to the established mutational landscape of PTFL. The 2 adult B-cell lymphomas cases also had *MAP2K1*, *TNFRSF14*, and *IRF8* mutations, but the clinical features were not typical of PedIBCL. In summary, this study demonstrated that PTFL and PNMZL are similar diseases with overlapping clinical, pathologic, and genetic features; mixed type tumors can also occur. Atypical adult cases with similar histologic features were also observed. Therefore, the disease spectrum of PedIBCL may be much broader than is currently believed.

Key Words: pediatric-type follicular lymphoma, pediatric nodal marginal zone lymphoma, pediatric-type indolent B-cell lymphoma, targeted gene sequencing

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Pediatric-type follicular lymphoma (PTFL) and pediatric nodal marginal zone lymphoma (PNMZL) are rare indolent B-cell lymphomas (IBCL) that differ clinically and pathologically from their adult counterparts.[1] Although they predominantly affect children and young adults, cases have been reported in older adults.[2–4] Because of their distinct clinicopathologic characteristics, PTFL was recognized as a distinct entity in the revised 4th World Health Organization classification of lymphoid neoplasms, while PNMZL was included as a provisional entity.[5]

Histologically, PTFL presents as effacement of the nodal architecture with expansile or serpiginous follicles composed of intermediate-sized to large-sized blastoid cells. The Ki-67 proliferation index is high, and neoplastic

follicles show no or irregular polarization at the periphery of the germinal center.[1] Perifollicular marginal zone differentiation can be seen in some cases.[4] PNMZL, like its adult counterpart, shows expansion of the marginal zone and interfollicular area, with small to medium monocytoid or centrocytoid cells. However, PNMZL can also have a follicular growth pattern because of progressive transformation of germinal center (PTGC)-like changes and hyperplasia of the reactive follicles.[6]

Meanwhile, PTFL and PNMZL share many clinical characteristics; they both predominantly affect young males, present as a limited-stage nodal disease in the head and neck region, and show an indolent clinical course with remission after complete resection in most cases.[1] Because of their indolent behavior and favorable outcomes with minimal treatment,[7,8] accurate diagnosis is important. However, diagnosis is often challenging because their histology can mimic reactive lymphoid hyperplasia and other B-cell lymphomas.

The mutational landscape of PTFL is well known and can aid diagnosis. PTFL lacks the characteristic *BCL2* gene rearrangement detected in more than 80% of its adult counterpart,[3] and recurrent mutations in *TNFRSF14*, *MAP2K1*, and *IRF8* genes have been reported.[9–13] In contrast, little is known about the genetic features of PNMZL. Cytogenetic abnormalities, most commonly trisomy 18 and trisomy 3, have been reported,[14] but recurrent genetic alterations were not detected in another series.[12]

The aim of this study was to investigate the clinicopathologic and genetic characteristics of the two rare pediatric-type indolent B-cell lymphomas (PedIBCL), PTFL and PNMZL, and their diagnostic implications. We performed targeted next-generation sequencing (NGS) using a customized panel of 121 genes involved in the pathogenesis of hematolymphoid neoplasms, and demonstrated that there may be clinical, pathologic, and genetic overlap between the 2 diseases.

MATERIALS AND METHODS

Patients and Samples

Twenty-two cases diagnosed as PedIBCL, 2 diagnosed as low-grade B-cell lymphoma (LGBCL) with histologic or genetic features of PedIBCL, and 3 diagnosed as atypical lymphoid hyperplasia with PedIBCL included in the differential diagnosis were identified at Seoul National University Hospital (SNUH) and Seoul National University Bundang Hospital (SNUBH) between 2013 and 2021 (Supplementary Fig. S1, Supplemental Digital Content 1, http://links.lww.com/PAS/B365). Archival formalin-fixed, paraffin-embedded (FFPE) tissue samples and slides were obtained. One case of atypical marginal zone hyperplasia with no material for review was excluded. All hematoxylin and eosin and immunohistochemistry (IHC) slides and available genetic test results of the 26 cases were reviewed by 3 expert hematopathologists (Y.K.J., J.H.P., and Y.A.K.). Cases classified by the review board as atypical lymphoid hyperplasia and BCL2-negative adult-type follicular lymphoma were excluded. Cases were reclassified histologically as PTFL (n = 11), mixed PTFL and PNMZL (mixed type, n = 8), or PNMZL (n = 2) by consensus among three hematopathologists. PTFL was further classified into cases with (n = 3) and without marginal zone differentiation (n = 8). Two adult cases (ABCL01 and ABCL02) showing partial histologic features of PedIBCL and initially diagnosed as LGBCL with differential diagnosis including PedIBCL were analyzed separately as adult B-cell lymphoma (aBCL), not otherwise specified (NOS) category. Clinical information, including stage, treatment history, and survival, was collected retrospectively from medical records. The study was approved by the Institutional Review Board of SNUH (H-2011-136-1174). Informed consent was waived.

IHC and B-Cell Clonality Test

IHC was performed using antibodies against CD3 (clone 2GV6; Ventana Medical Systems, Tucson, AZ), CD20 (clone L26; DAKO, Carpinteria, CA), BCL2 (clone 124; DAKO), BCL6 (clone LN22; Novocastra, Newcastle, UK), CD10 (clone 56C6; Novocastra), MUM1 (clone Ma695; Novocastra), MYC (clone EP121; Cell Marque, Rocklin, CA), Ki-67 (MIB-1; Ventana Medical Systems), PD-1 (clone MRQ-22; Cell Marque), IgD (clone DRN1C; Novocastra), and FOXP1 (clone SP133; Cell Marque) on representative whole FFPE tissue sections. Epstein-Barr virus in situ hybridization was performed using the Bond Ready-to-Use ISH EBER probe (Leica Biosystems, Newcastle, UK) or the INFORM EBER probe (Ventana Medical Systems). Immunostaining was performed using Ventana Benchmark XT (Ventana Medical Systems) or Bond-Max autostainer (Leica Microsystems, Melbourne, Australia) according to the manufacturer's protocol.

B-cell monoclonality was detected using the IdentiClone *IGH* Gene Clonality Assay (Invivoscribe Technologies Inc., San Diego, CA).

Targeted NGS

Targeted NGS was performed for cases with available FFPE tissue samples using a customized panel of 121 lymphoma-related genes (SNUH FIRST-Lymphoma Panel v1.1; Supplementary Table S1, Supplemental Digital Content 2, http://links.lww.com/PAS/B366). ABCL01 had previous sequencing data, obtained from SNUH FIRST-Lymphoma Panel v1.0.

Genomic DNA was extracted from FFPE tissues using the Maxwell CSC DNA FFPE Kit or Maxwell 16 FFPE Tissue LEV DNA Purification Kit (Promega, Madison, WI). Libraries were developed using the SureSelect XT-HS Target Enrichment System (Agilent Technologies, Santa Clara, CA). Paired-end sequencing was performed using the NextSeq. 550Dx platform (Illumina Inc., San Diego, CA). Sequenced reads were aligned to the reference human genome (GRCh37/hg19) using Burrows-Wheeler Aligner (BWA v0.7.17) and GATK Best Practice (v4.0.2.1). Single-nucleotide variants (SNVs) and small insertions and deletions (indels) were detected by using an in-house developed pipeline, SNVer (v0.5.3) and LoFreq (v2.1.2). Translocations were

detected using Delly and Mantana, and copy number alterations were called using CNVkit. Mutations were annotated using SnpEff (v4.3).

Mutation Calling and Data Analysis

To identify and remove germline variants and recurrent sequencing artifacts, variants included in an in-house Panel of Normals were excluded. Variants with population frequency over 0.1% on the Genome Aggregation Consortium (gnomAD) East Asian database, Korean Variant Archive (KOVA), or Korean Reference Genome Database (KRGDB) were also filtered out. Mutations with variant allele frequency $<2\%$ and fewer than 10 altered reads were called if the same mutation was previously reported in association with hematolymphoid malignancies on public databases, including COSMIC and cBioPortal. Variants with variant allele frequency $<2\%$ were also called if the mutation was predicted to be pathogenic or had any clinical significance for PedIBCL according to literature review.

Statistical Analysis

Fisher exact test was performed to compare categorical variables. The Kruskal-Wallis test or Mann-Whitney *U* test was performed to compare continuous variables. All statistical analyses were performed using SPSS (ver. 25.0; IBM Corp., Armonk, NY). Two-sided *P*-values <0.05 were considered statistically significant.

RESULTS

Classification and Pathologic Features

Eight cases initially diagnosed as PTFL and three initially diagnosed as PNMZL were reclassified as PTFL (Tables 1 and 2; Supplementary Table S2, Supplemental Digital Content 3, http://links.lww.com/PAS/B367). Three of these cases showed focal marginal zone differentiation, whereas the remaining cases did not (Fig. 1). The cases were classified, by consensus, as mixed type if histologic features of both PTFL and PNMZL were observed. These cases showed expansile follicles with effacement of the nodal architecture, marginal zone hyperplasia with occasional PTGC-like features, and diffuse CD20 staining in both follicular and interfollicular areas (Fig. 2). Four cases initially diagnosed as PTFL, 3 initially diagnosed as PNMZL, and 1 initially diagnosed as indeterminate PedIBCL (PTFL vs. PNMZL) were reclassified as mixed type. All cases reclassified as PNMZL had initially been diagnosed as PNMZL (Fig. 3). A total of 21 cases were finally classified as PedIBCL, including 11 PTFL, 8 mixed type, and 2 PNMZL cases (Tables 1 and 2; Supplementary Fig. S1, Supplemental Digital Content 1, http://links.lww.com/PAS/B365).

Clinical Characteristics

There were no significant differences in clinical characteristics, including age, sex, tumor location, stage, treatment modality, and relapse, between the PedIBCL groups (Tables 1 and 2; Supplementary Table S2, Supplemental Digital Content 3, http://links.lww.com/PAS/B367). The

TABLE 1. Clinical Features of the Patients

Patient ID	Consensus Reclassification	Initial Diagnosis	Age (y)	Sex	Location	Stage	LDH	Treatment	Relapse	PFS (m)	OS (m)
PTFL01	PTFL w/o MZ	PTFL	13	Male	Head and neck LN	2B	197	Chemotherapy	No	62	62
PTFL02	PTFL w/o MZ	PTFL	23	Male	Head and neck LN	1A	141	Radiotherapy	No	14	14
PTFL03	PTFL w/o MZ	PTFL	12	Female	Head and neck LN	1A	NA	Watchful wait	No	46	46
PTFL04	PTFL w/o MZ	PTFL	25	Male	Head and neck LN	1A	145	Watchful wait	No	31	31
PTFL05	PTFL w/o MZ	PTFL	17	Male	Head and neck LN	2A	155	Watchful wait	No	38	38
PTFL06	PTFL w/o MZ	PTFL	12	Male	Head and neck LN	2A	184	Chemotherapy	No	48	48
PTFL07	PTFL w/o MZ	PTFL	14	Male	Head and neck LN	1A	NA	Follow-up loss	No	1	1
PTFL08	PTFL w/o MZ	PNMZL	20	Male	Head and neck LN	1A	143	Chemotherapy	No	18	18
PTFL09	PTFL w/ MZ	PTFL	32	Male	Inguinal LN	1A	454	Radiotherapy	No	68	68
PTFL10	PTFL w/ MZ	PNMZL	16	Male	Head and neck LN	1A	178	Watchful wait	Yes	11	30
PTFL11	PTFL w/ MZ	PNMZL	22	Male	Head and neck LN	2A	186	Watchful wait	No	39	39
MIXED01	Mixed	PTFL	20	Male	Head and neck LN	1A	143	Radiotherapy	No	17	17
MIXED02	Mixed	PTFL	8	Male	Head and neck LN	1A	NA	Follow-up loss	No	2	2
MIXED03	Mixed	PTFL	16	Male	Head and neck LN	1A	136	Watchful wait	No	1	1
MIXED04	Mixed	PTFL	47	Male	Head and neck LN	1A	226	Watchful wait	No	2	2
MIXED05	Mixed	Pediatric MZL	3	Male	Tonsil	1A	250	Watchful wait	No	54	54
MIXED06	Mixed	PNMZL	15	Male	Head and neck LN, axillary LN	2A	NA	Follow-up loss	No	1	1
MIXED07	Mixed	PNMZL	24	Male	Head and neck LN	1A	172	Watchful wait	No	26	26
MIXED08	Mixed	PedIBCL	8	Male	Head and neck LN	2A	261	Chemotherapy	No	1	1
PNMZL01	PNMZL	PNMZL	15	Male	Head and neck LN	1A	159	Chemotherapy	No	61	61
PNMZL02	PNMZL	PNMZL	18	Male	Head and neck LN	1A	127	Watchful wait	No	1	1
ABCL01	aBCL, NOS	Other	52	Female	Head and neck LN, axillary LN, BM	4A	152	Chemotherapy	No	14	14
ABCL02	aBCL, NOS	Other	59	Male	Head and neck LN	1A	147	Watchful wait	Yes	10	33

BM indicates bone marrow; LDH, lactate dehydrogenase; LN, lymph node; MZ, marginal zone differentiation; NA, not available; NOS, not otherwise specified; OS, overall survival.

median age was 16 years (range: 3 to 47 y), and most patients were diagnosed as adolescents to young adults. All patients, except 1 PTFL case without marginal zone differentiation (PTFL03), were males. Patients mostly presented with a single mass in the head and neck region regardless of group, and only 1 patient complained of B symptoms. All patients presented with limited-stage disease according to the Ann Arbor staging system, with 15 stage 1A, 5 stage 2A, and 1 stage 2B patients. Serum lactate dehydrogenase levels were below 250 IU/L in 14 of 17 patients.

All 21 patients underwent surgical excision of the involved lymph nodes. Five patients (23.8%) received chemotherapy, and 3 (14.3%) received radiotherapy after excision. Ten patients (47.6%) did not receive any additional therapy after surgery ("watchful waiting"). Only 1 case of PTFL with marginal zone differentiation (PTFL10) relapsed 11 months after complete excision and watchful waiting. The patient remained relapse-free for additional 19 months after re-excision of the involved node.

Mutational Landscape

Targeted NGS was performed in 20 of 21 PedIBCL cases, excluding 1 PTFL without marginal zone differentiation who had no remaining material for NGS study. We detected a total of 52 SNVs or indels in 22 genes, and 4 copy number alterations in 4 genes (Fig. 4). The top 5 most frequently mutated genes were *MAP2K1* (40%, 8/20 cases), *TNFRSF14* (35%, 7/20 cases, including 1 with multiple mutations), *KMT2C* (25%, 5/20 cases, including 1 with multiple mutations), *IRF8* (15%, 3/20 cases, including 2 with multiple mutations), and *NOTCH2* (15%, 3/20 cases, including 1 with multiple mutations). There was no significant difference in the incidence of mutations of these 5 genes among the 3 PedIBCL groups (ie, PTFL, mixed type, and PNMZL) ($P > 0.05$ for all 5 genes according to the Fisher exact test), or between PTFL and mixed type ($P > 0.05$ for all 5 genes according to the Fisher exact test) (Table 2).

KMT2C was the most frequently mutated gene in PTFL. Four of 10 cases (40%) showed *KMT2C* mutation, 3 of which were the same *KMT2C* G908C mutation. Two PTFL cases, 1 with and 1 without marginal zone differentiation,

TABLE 2. Comparison of Clinical Characteristics and Mutations Between Groups

	PTFL, n (%)									
	Without MZ	With MZ	All PTFL	Mixed, n (%)	PNMZL, n (%)	All PedIBCL, n (%)	aBCL, NOS, n (%)	*P**	*P*†	*P*‡
Number of patients	8	3	11	8	2	21	2	—	—	—
Median age, y (range)	15.5 (12-25)	22 (16-32)	17 (12-32)	15.5 (3-47)	16.5 (15-18)	16 (3-47)	55.5 (52-59)	0.766	0.589	0.492
Sex (M:F)	7:1	3:0	10:1	8:0	2:0	20:1	1:1	1.000	1.000	1.000
Location								0.738	0.429	0.678
Head and neck LN	8 (100)	2 (66.7)	10 (90.9)	7 (87.5)§	2 (100)	19 (90.5)	2 (100)‖			
Tonsil	0	0	0	1 (12.5)	0	1 (4.8)	0			
Inguinal LN	0	1 (33.3)	1 (9.1)	0	0	1 (4.8)	0			
Stage at presentation								—	—	—
Limited-stage	8 (100)	3 (100)	11 (100)	8 (100)	2 (100)	21 (100)	1 (50.0)			
Advanced stage	0	0	0	0	0	0	1 (50.0)			
LDH, IU/L								0.682	0.471	0.525
<250	6 (100)	2 (66.7)	8 (88.9)	4 (66.7)	2 (100)	14 (82.4)	2 (100)			
≥ 250	0	1 (33.3)	1 (11.1)	2 (33.3)	0	3 (17.6)	0			
Treatment								1.000	0.853	1.000
Watchful waiting	3 (37.5)	2 (66.7)	5 (45.5)	4 (50.0)	1 (50.0)	10 (47.6)	1 (50.0)			
Chemotherapy	3 (37.5)	0	3 (27.3)	1 (12.5)	1 (50.0)	5 (23.8)	1 (50.0)			
Radiotherapy	1 (12.5)	1 (33.3)	2 (18.2)	1 (12.5)	0	3 (14.3)	0			
Unknown	1 (12.5)	0	1 (9.1)	2 (25.0)	0	3 (14.3)	0			
Relapse	0	1 (33.3)	1 (9.1)	0	0	1 (4.8)	1 (50.0)	1.000	0.238	1.000
Median PFS, m (range)	34.5 (1−62)	39 (11−68)	38 (1−68)	2 (1−54)	31 (1−61)	18 (1-68)	12 (10−14)	—	—	—
Median OS, m (range)	34.5 (1−62)	39 (30−68)	38 (1−68)	2 (1−54)	31 (1−61)	26 (1-68)	23.5 (14−33)	—	—	—
Mutations										
MAP2K1	1 (14.3)	1 (33.3)	2 (20)	5 (62.5)	1 (50.0)	8 (40.0)	2 (100)	0.139	0.285	0.145
TNFRSF14	1 (14.3)	2 (66.7)	3 (30)	3 (37.5)	1 (50.0)	7 (35.0)	2 (100)	1.000	0.375	1.000
IRF8	0	1 (33.3)	1 (10)	2 (25.0)	0	3 (15.0)	2 (100)	0.684	0.435	0.559
KMT2C	2 (28.6)	2 (66.7)	4 (40)	0	1 (50.0)	5 (25.0)	0	0.109	0.058	0.092
NOTCH2	1 (14.3)	0	1 (10)	2 (25.0)	0	3 (15.0)	0	0.684	1.000	0.559

*P-value for comparison among PTFL, mixed type, and PNMZL.
†P-value for comparison among PTFL without MZ, PTFL with MZ, mixed type, and PNMZL.
‡P-value for comparison between PTFL and mixed type.
§Including 1 case (MIXED06) with main lesion in the head and neck LN and additional involvement of axillary LN.
‖Including 1 case (ABCL01) with main lesion in the head and neck LN and additional involvement of axillary LN and bone marrow.
LDH indicates lactate dehydrogenase; LN, lymph node; MZ, marginal zone differentiation; NOS, not otherwise specified; OS, overall survival.

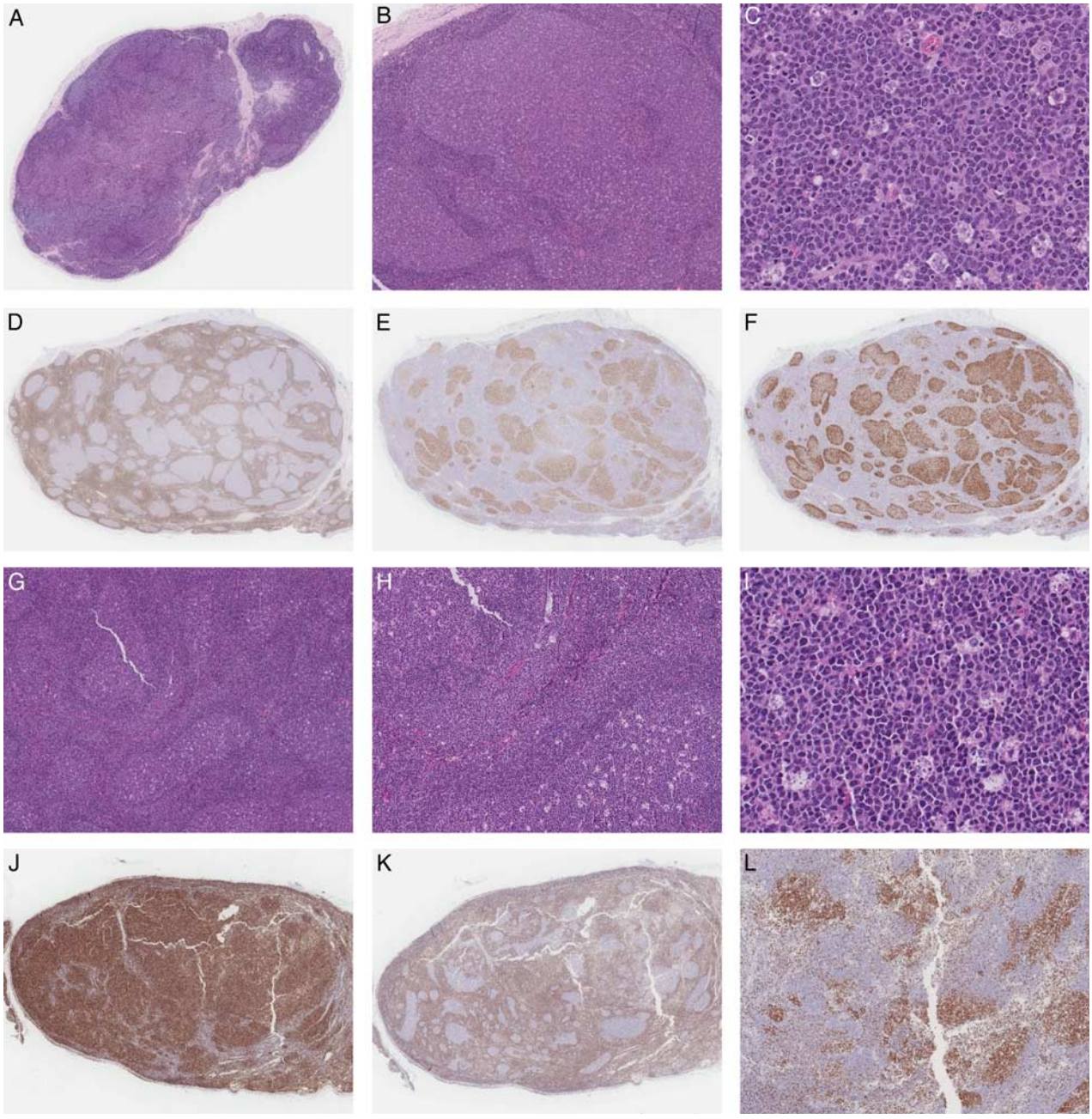

FIGURE 1. Pathologic features of pediatric-type follicular lymphoma. A–F, Representative images of PTFL without marginal zone differentiation (PTFL03). A–C, H&E shows partial effacement of nodal architecture with expansile and serpiginous follicles. Follicles are composed of intermediate to large blastoid cells and have a starry sky appearance. D, BCL2 is negative in expansile follicles. E, CD10 is positive in follicles. F, Ki-67 proliferation index is high in follicles and there is no polarization. G–L, Representative images of PTFL with marginal zone differentiation (PTFL11). G–H, H&E shows effacement of nodal architecture with expansile follicles. Some neoplastic follicles show marginal zone differentiation at the periphery. I, Follicles are composed of intermediate-sized cells with a centrocytic and centroblastic morphology. J, CD20 highlights expansile follicles and the presence of interfollicular B cells. K, BCL2 is negative in follicles. L, Ki-67 proliferation index is high in expansile follicles. Some follicles are showing PTGC-like changes.

shared the same *MAP2K1* Q56P mutation. One PTFL case without marginal zone differentiation showed *TNFRSF14* missense mutation. Two cases with marginal zone differentiation showed *TNFRSF14* mutations, one of which was a start loss mutation; the other was a splicing mutation. Only 1 case of PTFL (with marginal zone differentiation) showed an *IRF8* mutation, which was a missense mutation (Y23H).

The most frequently mutated gene in the mixed type was *MAP2K1*. Five of 8 cases (62.5%) had *MAP2K1* mutations, all of which were missense mutations. Three mixed type cases had

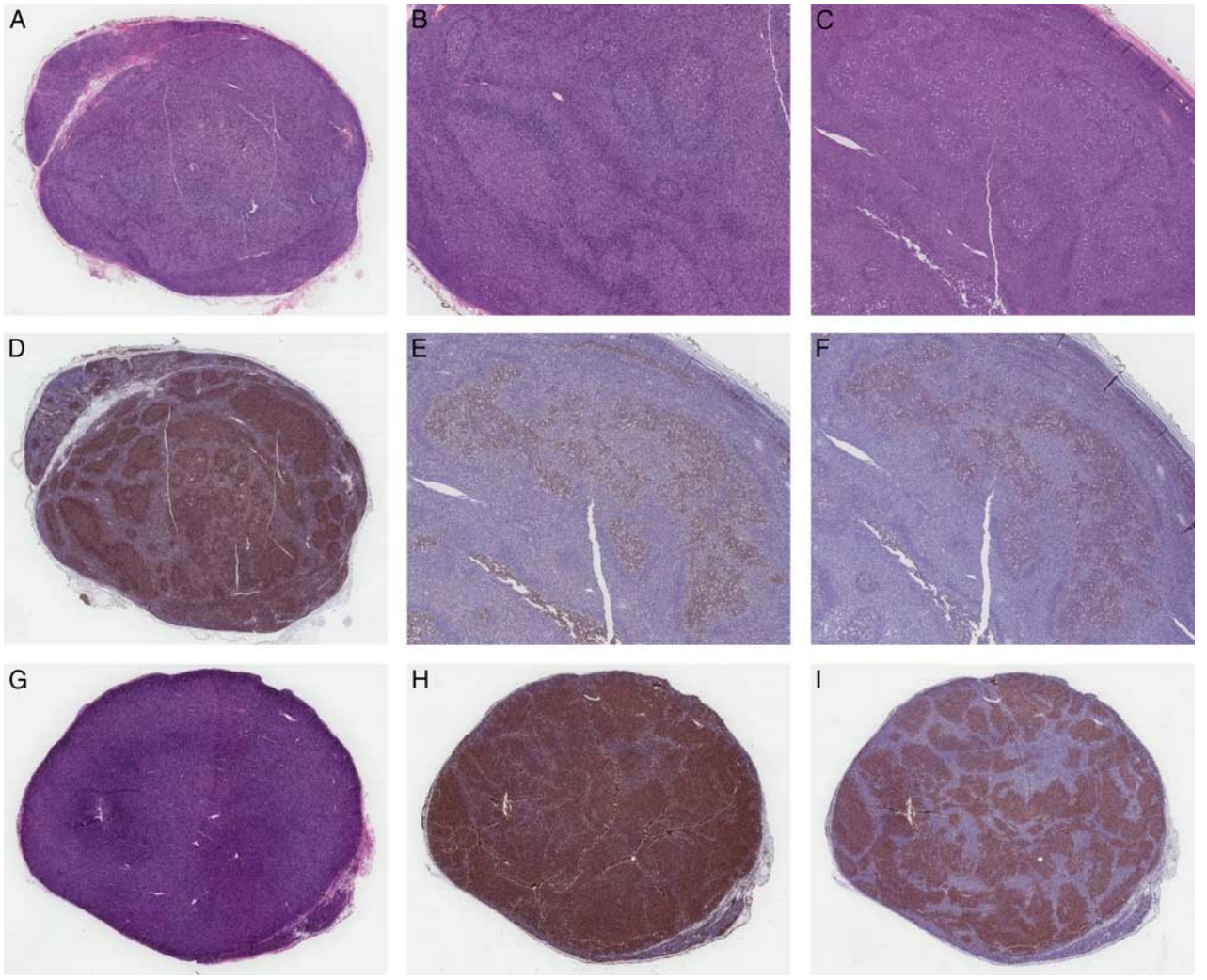

FIGURE 2. Pathologic features of the mixed type (A–F, MIXED07; G–I, MIXED03). A–C and G, H&E shows expansile follicles and surrounding clear cells with marginal zone differentiation. D and H, CD20 highlights expansile follicles and interfollicular B cells. E, Ki-67 proliferation index is high in follicles but low in interfollicular area. Follicles are showing PTGC-like changes. F and I, CD10 highlights PTGC-like changes.

TNFRSF14 mutations. All 3 cases with *TNFRSF14* mutation were originally diagnosed as PTFL. In addition, 2 mixed type cases had multiple missense mutations in the *IRF8* gene (K66R and L82V in MIXED07, and Y23H and F36L in MIXED08). None of the mixed type cases had *KMT2C* mutations.

No mutations were shared between the 2 cases of PNMZL. One case (PNMZL01) showed missense mutations in *MAP2K1* (F53I) and *KMT2C* (G908C) genes, an in-frame deletion mutation of the *EP300* gene, and 1 copy loss of the *ATM*, *BIRC3*, and *CHEK1* genes. The other case (PNMZL02) had a *TNFRSF14* nonsense mutation (Q180*) and *CREBBP* frameshift mutation.

Adult B-Cell Lymphoma, Not Otherwise Specified With Genetic Features of Pediatric-type Indolent B-Cell Lymphomas

The 2 adult cases, separately categorized as aBCL, NOS (ABCL01 and ABCL02), were histologically unusual, with some features of PedIBCL (Fig. 5). ABCL01 showed pure follicular proliferation with a "node within a node" pattern. Neoplastic follicles were composed of intermediate-sized blastoid cells and were negative for BCL2. B-cell monoclonality was detected on *IGH* gene rearrangement study, and it was initially diagnosed as "LGBCL, type undetermined, with differential diagnoses of PTFL and BCL2-negative adult-type follicular lymphoma." The clinical features of ABCL01 were not typical for PTFL in that the patient was a 52-year-old female with stage 4 disease involving cervical and axillary lymph nodes and bone marrow. However, targeted NGS performed for diagnostic purposes revealed the typical PedIBCL mutational pattern (Fig. 4). ABCL01 was therefore diagnosed as "LGBCL, type undetermined with genetic features of PTFL."

In contrast, ABCL02 was a 59-year-old male with a single cervical mass. Histologic examination showed

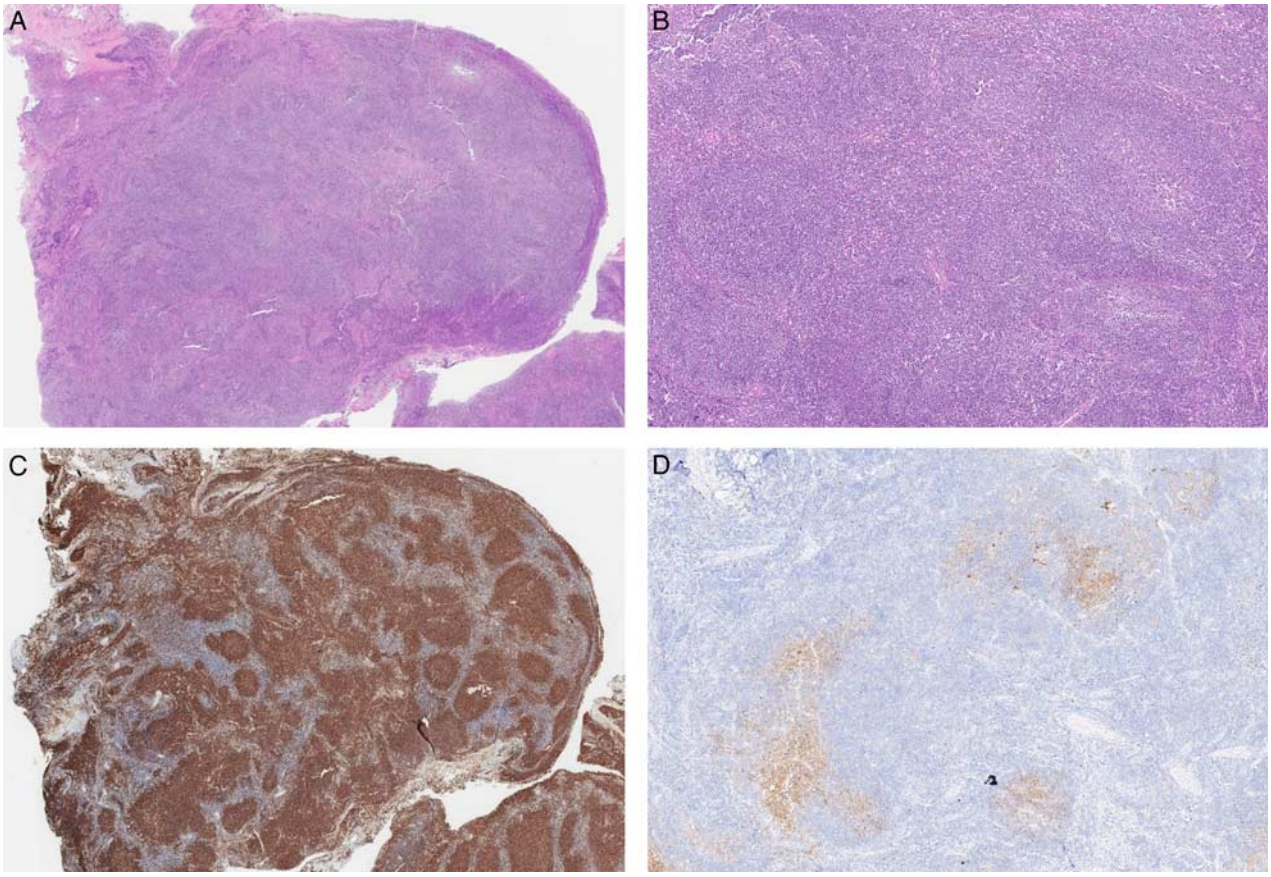

FIGURE 3. Pathologic features of pediatric nodal marginal zone lymphoma (PNMZL01). A and B, H&E shows effacement of the nodal architecture. Lymphoid follicles and clear cells with marginal zone differentiation can be seen. C, CD20 shows nodular architecture with a hyperplastic marginal zone. D, CD10 shows PTGC-like changes.

mixed follicular and interfollicular hyperplasia with scattered PTGC-like changes. B-cell monoclonality was also detected on *IGH* gene rearrangement study, and the patient was diagnosed as "LGBCL, most likely NMZL with features of PNMZL." However, the patient relapsed to stage 3 disease 10 months after excision of the cervical lymph node, which was atypical for PedIBCL (Tables 1 and 2; Supplementary Table S2, Supplemental Digital Content 3, http://links.lww.com/PAS/B367). Targeted NGS was performed in ABCL02, and it also exhibited the typical PedIBCL mutational pattern (Fig. 4).

The 2 aBCL, NOS cases shared the *IRF8* K66R mutation and had missense mutations in the same codon (K57) of the *MAP2K1* gene. They also had *TNFRSF14* mutations, with ABCL01 showing a start loss mutation and ABCL02 showing a missense mutation (C42Y).

DISCUSSION

The 2 conventional PedIBCLs (ie, PTFL and PNMZL), are known to share many clinical characteristics,[1,6,15,16] which was also observed in this study. They also have some histologic similarities, in that PTFL may have marginal zone differentiation at the periphery of neoplastic follicles,[5] while PNMZL may show both interfollicular and follicular proliferation.[6] However, the mixed or gray-zone features of PTFL and PNMZL remain unaddressed. In this study, we performed clinicopathologic analysis of 21 PedIBCL cases, including PTFL and PNMZL. By consensus, the cases were reclassified into 3 groups (PTFL, mixed type, and PNMZL) based on histologic findings. PTFL was subdivided into cases with focal marginal zone differentiation and cases with a pure follicular morphology. In this study, 38.1% (8/21 cases) had an ambiguous mixed type histology with features characteristic of both PTFL and PNMZL. Half of these cases were originally diagnosed as PTFL (4/8 cases), while 37.5% (3/8 cases) were diagnosed as PNMZL. Three of 11 cases (27.3%) reclassified as PTFL were initially diagnosed as PNMZL. These findings suggest histologic overlap between the 2 diseases and highlight the difficulties in diagnosing these histologically ambiguous cases.

The mutational landscape of PTFL has been well described. Unlike its adult counterpart, it lacks *BCL2* gene rearrangement, and mutations in epigenetic modifier genes including *KMT2D*, *CREBBP*, and *EP300* are less common. Mutations in *TNFRSF14*, *MAP2K1*, and *IRF8* are also more frequently detected in PTFL.[3,9–13] To determine the genetic features helpful for differentiating PTFL and

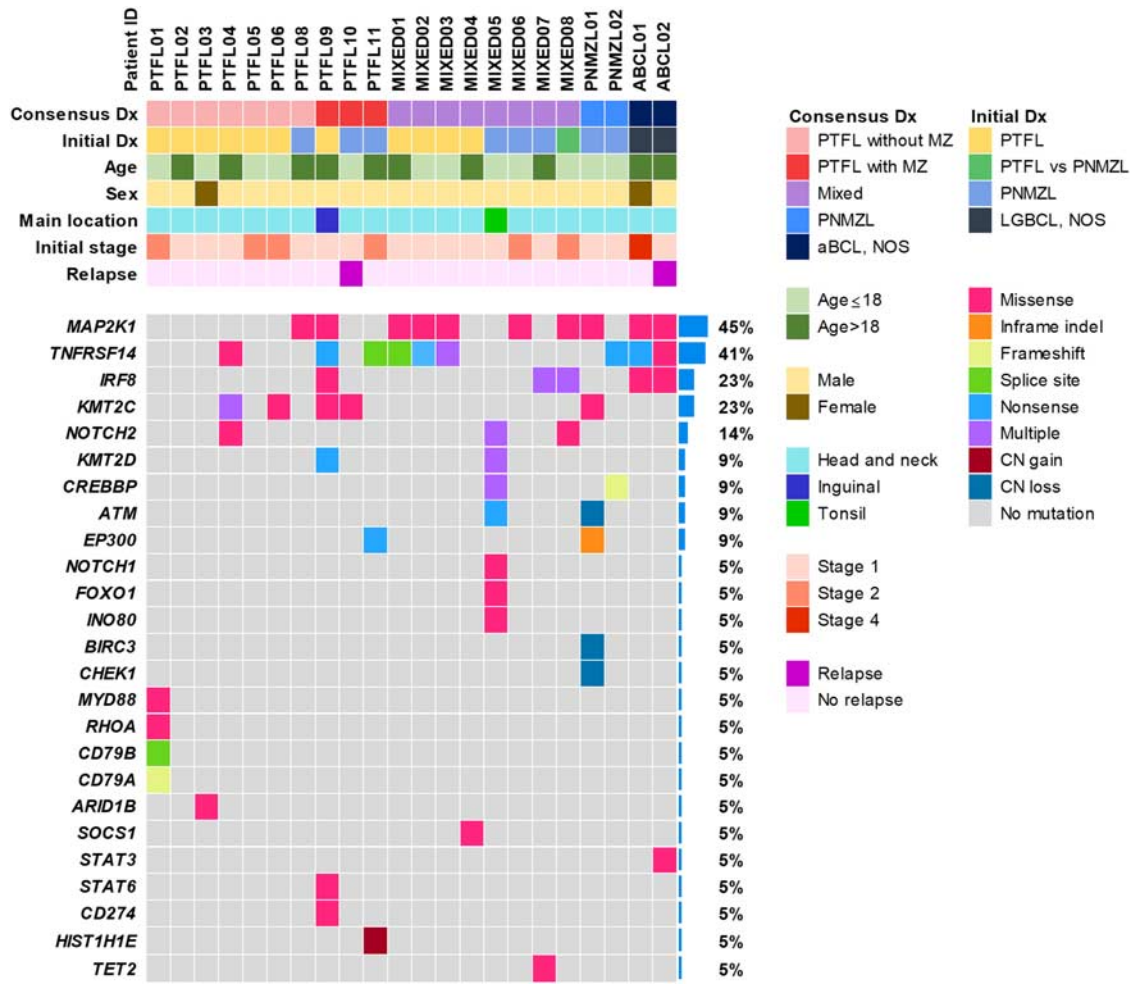

FIGURE 4. Targeted sequencing for 121 lymphoma-related genes. Mutational patterns and the clinicopathologic characteristics are depicted, and no significant differences in genetic alterations were found according to the consensus diagnosis. CN indicates copy number; Dx, diagnosis; MZ, marginal zone differentiation; NOS, not otherwise specified.

PNMZL, we performed targeted NGS of 121 lymphoma-related genes. However, the mutational profiles of the three PedIBCL categories were all similar to the previously known mutational profile of PTFL, and no significant differences were found among the categories. PTFL, mixed type, and PNMZL all had recurrent mutations in *MAP2K1* (2/10, 5/8, and 1/2 cases, respectively) and *TNFRSF14* (3/10, 3/8, and 1/2 cases, respectively) genes. Mutations in *IRF8*, including the known PTFL-specific alteration K66R[9,12] in 1 mixed type case, were also detected in a PTFL case and another mixed type case (1/10 and 2/8 cases, respectively). Although there was a lack of *KMT2C* mutations in mixed type cases, half of the PTFL and PNMZL cases had *KMT2C* mutations and shared the same *KMT2C* G908C mutation. These results suggest overlap in the mutational landscape of PTFL, mixed type, and PNMZL.

Clinically, the mixed type shared features and biological behaviors with PTFL and PNMZL. PTFL, PNMZL, and mixed type were all diagnosed mainly in adolescents and young adult males, and mostly presented as limited-stage disease in the head and neck region. Except for one PTFL case, none of the cases showed progression over the follow-up period of up to 68 months. Because of their indolent course and lack of progression even after the watchful waiting strategy,[7,8,17,18] accurate diagnosis of PedIBCL is important because high-grade lymphomas are more common in the same age group. Recognizing the disease spectra of PTFL and PNMZL, which share clinical, pathologic, and genetic features (as highlighted in this study), will aid appropriate diagnosis and subsequent management of PedIBCL patients.

In this study, we encountered 2 peculiar aBCL cases. These cases were not histologically consistent with any of the conventional B-cell non-Hodgkin lymphomas, and instead had partial PedIBCL features. Consensus review, however, concluded that these cases were not histologically typical of PedIBCL. The clinical course of the two cases was also rather aggressive and atypical of PedIBCL. However, both cases had *MAP2K1*, *TNFRSF14*, and

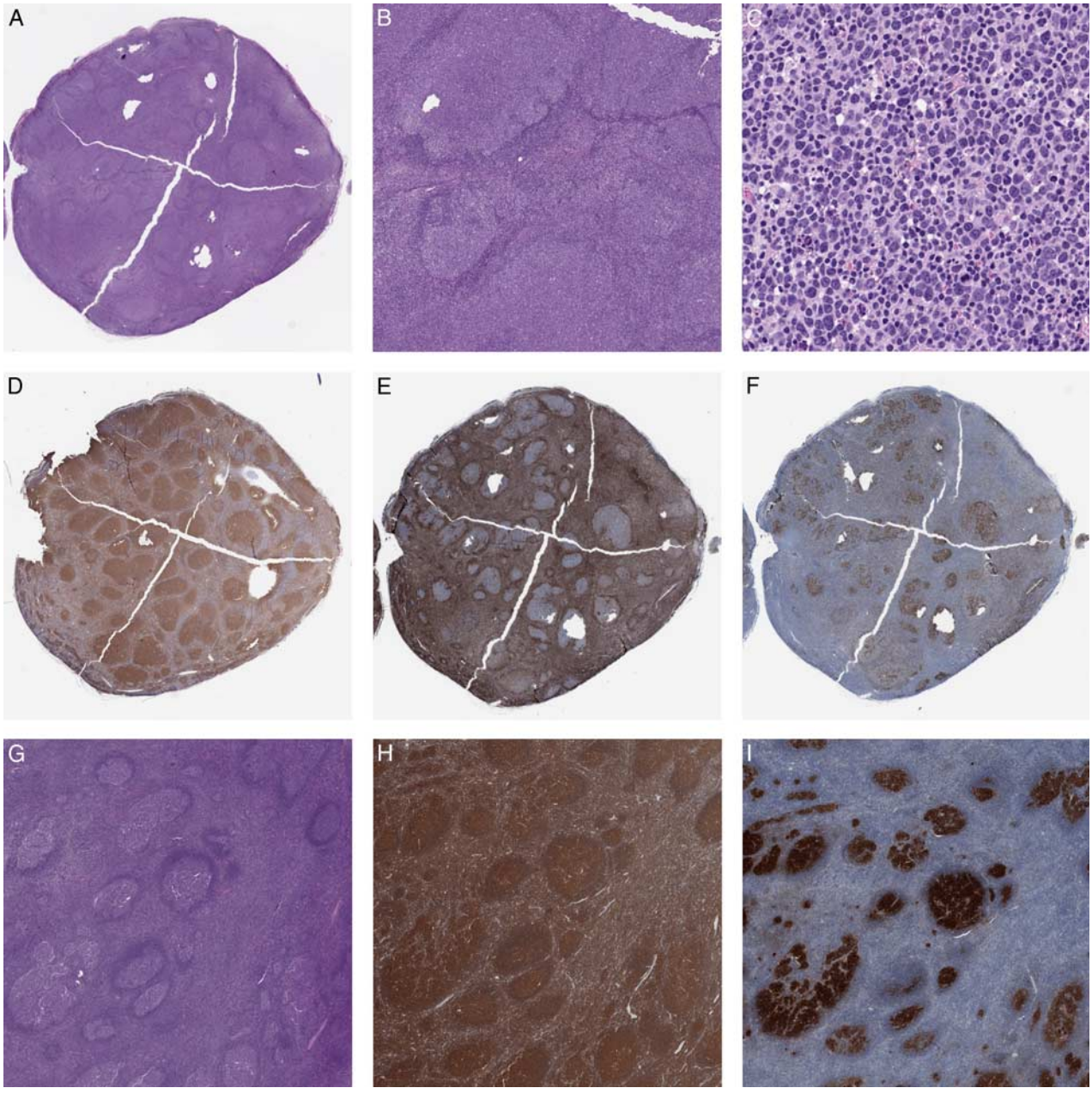

FIGURE 5. Adult B-cell lymphoma, not otherwise specified, with partial features of pediatric-type indolent B-cell lymphoma. A–F, ABCL01, initially diagnosed as LGBCL, type undetermined with a differential diagnosis of PTFL and BCL2-negative adult-type follicular lymphoma. A–C, H&E shows a nodular pattern with follicles composed of intermediate-sized blastoid cells. D, CD20 staining highlights the follicular pattern. E, BCL2 is negative in neoplastic follicles. F, The Ki-67 proliferation index is high in follicles. G–I, ABCL02, initially diagnosed as LGBCL, most likely NMZL with features of PNMZL. G and H, H&E and CD20 staining shows mixed follicular and interfollicular hyperplasia. I, CD10 highlights scattered PTGC-like changes.

IRF8 mutations, which are typical of PedIBCL. In addition, they both had *IRF8* K66R mutations, a known hotspot mutation in PTFL.[9,12] These findings suggest that histologic mimickers of PedIBCL, rarely occurring in adults, may also share its genetic features.

This study raised several questions. Given the shared morphologic and genetic features of PTFL, mixed type, and PNMZL, their diagnostic criteria need to be clarified. Furthermore, whether PTFL and PNMZL are pathogenetically related remains to be clarified. Moreover, marginal zone differentiation in PTFL, even to the level of mixed histology, remains to be investigated. The 3 groups of PedIBCL in this study shared similar clinical, pathologic, and genetic features, and this finding suggests that there may be an overlapping spectrum of disease between PTFL and PNMZL. However, because of small sample

sizes, these questions should be further addressed in larger cohorts.

In conclusion, the 2 PedIBCLs were clinically similar conditions with overlapping pathologic and genetic features. There were also some clinically atypical cases that shared the pathologic and genetic features of PedIBCL. This study may expand the currently recognized disease spectrum of PedIBCL.

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