Usefulness of Long Distance Inverse Polymerase Chain Reaction for the molecular detection of the 14q32 translocation in clinical setting

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Running Title: Usefulness of LDI PCR for the 14q32 translocation

Abstract

All mature B-cell leukemias and lymphomas have a clonal Ig gene recombination and a half of them have a reciprocal chromosomal translocation involving the 14q32 locus. The 14q32 translocation partners are variable, such as BCL-2, BCL-1, and BCL-6 accounting for the difficulty in the molecular detection by the current genomic polymerase chain reaction (PCR) method. To efficiently identify B-cell clones with an Ig gene rearrangement and reciprocal inter-chromosomal translocation, we verified the usefulness in a practical laboratory setting of our modified long distance inverse (LDI) PCR method for the detection of IgH gene rearrangements involving inter- and intra-chromosomal segments.

The total run time of this LDI PCR method was 5 and half hours. Using 24 samples of mature B-cell leukemias and lymphomas, the modified LDI PCR gave clonally rearranged amplicons in 83% (20/24) of cases. The direct sequencing results of the amplicons revealed an inter-chromosomal translocations in 5 cases (25%) and intra-chromosomal rearrangements in the remaining 15 cases (75%). The partners of the inter-chromosomal translocation consisted of the 11q13.3 segment containing a partial BCL1 sequence in 3 cases, 18q21.3 segment containing a partial BCL2 sequence in one case, and a segment of 7q11.2 in one case.

We present here an LDI PCR-based methodology for the efficient identification of 14q32 translocations, with modifications to reduce the total run time to within one day.

Key words: PCR, 14q32, IgH gene, B-cell clonality, lymphoma, translocation

Introduction

Most lymphomas are of a B-cell origin, all of which exhibit clonal rearrangement and/or deletion of the *IGH* locus. About 50-70% of mature B-cell leukemias and lymphomas have chromosomal translocations in which other chromosomal segments become juxtaposed with the *IGH* locus, mainly via the J region[1,2]. Such translocations are usually reciprocal between band 14q32, the location of *IGH*, and a gene designated as a "partner". These partners are generally oncogenic and anti-apoptotic, such as *MYC* on 8p24*, BCL2* on 18q21*, BCL6* on 3q27*, BCL1* on 11q13*, PAX5* on 9p13, *BCL3* on 19q13, and *BCL10* on 1p22. The kind of *IGH* translocation is closely associated with the histological subtype of B-cell lymphomas, including t(8;14)(q24;q32) in 95% of Burkitt's lymphoma cases, t(14;18)(q32;q21) in 80% of follicular lymphoma (FL) cases, t(3;14)(q27;q32) in 30% of diffuse large B-cell lymphoma(DLBCL) cases, and t(11;14)(q13;q32) in 90% of mantle cell lymphoma (MCL)[3]cases. Accordingly, it is diagnostically indispensable to detect the clonal *IGH* rearrangement and the 14q32-related translocation.

Although Southern blot and cytogentic analyses are standard for the detection of clonal *IGH* rearrangements and chromosomal translocations, molecular detection by a polymerase chain reaction (PCR)-based technique is now being introduced to carry out diagnosis and monitoring in a clinical setting[4,5]. In order to identify clonal *Ig* gene rearrangements, a consensus *JH* primer in combination with primers designed within conserved regions of the *VH* gene is used[6]. Such PCR is high sensitive, but has a potential disadvantage of amplification failure due to the presence of rearranged *IGH* alleles different from the primers. On the other hand, the molecular detection of proto-oncogene/*IgH* fusion genes by PCR employs *Ig* specific primers in combination with primers specific for the partner gene region. Moreover, the diversity and wide distribution of the breakpoints over several hundred kilo bases, like the *MYC/IGH* and *BCL1/IGH* translocations, are also problematic for standard PCR technology. This compels the setting-up of multiple PCR systems requiring many

primers depending on the number of partner genes and its number in the breakpoint cluster. Accordingly, to efficiently identify the *IGH*-related aberrations, we attempted to apply a long distance inverse (LDI) PCR, which has been developed to amplify unknown sequences adjacent to a known DNA sequence[7,8]. Namely, first of all using *IGH* enhancer and JH primers, LDI-PCR amplifies the fusion DNA sequences involving not only known partner genes, such as *MYC, BCL2, BCL6, BCL1,* and *PAX5*, but also unknown genes. Then, amplicons are directly sequenced and the sequence is referred to the BLAST search system to identify the rearranged status involving the *IGH* locus.

The aim of this study was to establish a relevant methodology combining LDI-PCR with direct sequencing of the amplicons and verify the usefulness of this strategy in a clinical laboratory setting.

Materials and Methods

Samples:

The subjects for this study were 24 samples from 24 patients who were suspected to have mature B-cell leukemia and lymphoma according to the WHO classifications[9]. They consisted of 9 CLL, 3 FL, 3 MCL, 1 MALT, and 8 DLBCL cases (diagnosed at admission). Specimens were 14 from heparinized peripheral blood (PB), 1 from bone marrow (BM), 5 from lymph node (LN), and 3 from others (pleural effusion and tonsil). The samples were fractionated into two tubes for immunophenotyping and genotyping. High molecular weight DNA was extracted using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. As references for germ line and *IGH* translocation, Human Germline, placental (CALBIOCHEM, Cambrige, MA, USA), and cell line CA46 derived from Burkitt's lymphoma were employed.

Methods

Our new paradigm for the molecular detection of *IGH*-related translocations was designed as follows. At first, we conducted LDI PCR, which allows rapid amplification of all recombination events within the *IGH* locus. The positive clonal band(s) was directly sequenced, and the sequence was referred to a BLAST Search.

One μg high molecular weight DNA was digested with a restriction enzyme, *Pst*-Ⅰ, *Bgl*-II, or *Xba*-I (New England Biolabs, Inc. Ipswich, MA, USA), for 3 hours in a 200 μl reaction volume. Residual enzymes were removed using QIAquick spin columns (Qiagen, Valencia, CA, USA). The digestion products were then ligated at 16℃ for 5 min using a 2x Ligation-Convenience Kit (NIPPON GENE Co, Ltd, Tokyo, Japan) according to the manufacturer's instructions. These processing conditions were verified by the completion of the circulized DNA of interest using references of normal, placental, and CA46 DNAs.

LDI PCR was carried out as described previously by Willis et al.[7] with modifications to reduce the total run time to within one day. Our modified LDI PCR was performed in one step and using 3 newly designed primers within the *JH*6 and *IGH* enhancer regions based on the database sequence, NG_001019, as shown in Fig.1. Fig 1 depicts the primer sites for the JH and enhancer regions and the digestion sites of the restriction enzymes, *Pst*-Ⅰ, *Bgl*-II, and *Xba*-I. The two Forward primers were chosen according to the restriction enzymes used.

The PCR reaction, using a Thermal Cycler Gene Amp PCR System 9600 (Perkin Elmer, Ramsey, MN, USA), was performed under the following conditions. The reaction mixture of 50 μl consisted of 40 ng ligated DNA, 1 Unit Phusion High Fidelity DNA Polymerase (Daiichi Pure Chemicals Co Ltd, Tokyo, Japan), 200 μM dNTPs and 0.4 μM primers. The reaction consisted of 30 cycles of denaturation for 8 sec at 98℃ followed by annealing and extension for 2.5 min at 72℃.

The amplified products were separated electrophoretically in 1.5% agarose gels, stained

with Gel Star Nucleic Acid Stain (CAMBREX, Rockland, ME,USA), and then excised from the gel using Dark Reader (BM Equipment Co, Ltd, Tokyo, Japan). The separated amplicons were re-amplified for purification. The final products were sequenced using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an Automated DNA Sequence Analyzer (Model 310, Applied Biosystems). Sequences were compared with the GenBank database at the National Center of Biotechnology Information to identify nucleotide homology.

The clonal rearrangement status of the *IgH* gene was examined by Southern blotting (SB) using the *JH* probe described in detail elsewhere [10)](Uemura *et al*., 2005) and a semi-nested PCR amplification of the *IgH* CDR3 region (CDR3-PCR) using three consensus primers, sense FR3A (V_H gene), anti-sense LJH (J_H segments), and internal VLJH, as described by Brisco *et al*.[11)].

The molecular detection of BCL1/IGH and BCL2/IGH rearrangements by standard PCR was performed according to the methods described previously by Fan H[12)], Horsman DE et al[13)], and Limpens J et al[14)].

Cellular morphologic and flowcytometric analysis:

Morphological characteristics were assessed by using May-Grumwald/Gimsa-stained smears prepared from blood and single-suspension cells from tissues. Cellular surface antigen profiles were immunophenotyped using a flowcytometric analytical method according to the manufacturer's instructions. Commercially available monoclonal antibodies of CD3, CD5, CD4, CD10, CD19, CD23, and CD25 (Becton Dickinson Flowcytometry System, San Jose, CA) and polyclonal antibodies of anti-Ig heavy chains (IgD, IgM, IgA, and IgG) and anti-Ig light chains (κ and λ) were used.

Results

1) Evaluation of LDI PCR

To obtain accurate reproducibility of the LDI PCR results and reduce the total run time of the protocol, the best reagents commercially available were explored. Consequently, using the New England Biolabs's restriction enzymes and NIPPON GENE's ligation kit, a reaction time of 3 hours for digestion and 5 minutes for ligation was sufficient. Then, among 4 kinds of commercially available DNA polymerases for long distance amplification, Phusion High Fidelity DNA Polymerase was chosen because the reaction time was 2 hours and the amplification performance was well. The total run time to present the electrophoretic-band-status of the products amplified by LDI PCR was approximately 5.5 hours.

Using the practical samples from 24 cases, this LDI PCR method gave rise to *IGH* rearranged products in 20 cases (84%), as shown in Table 1 (cases 1 to 20). Fig 2 shows representative results of LDI amplicons and PCR Southern blot analysis. Moreover, all cases were documented to be positive for the clonally rearranged bands using the standard Southern blot analysis. On the other hand, 4 cases, Nos. 4, 12, 14, and 24, were negative for the amplification of clonal rearranged IgH with the CDR3-PCR, as summarized in Table 1.

2) The detection of the 14q32 translocation with LDI PCR:

Twenty-four samples from 24 cases of mature B-cell neoplasms were analyzed. The LDI PCR yielded amplicons showing monoclonal band(s) with sizes of 0.6 to 7.5 kb in 20 samples from 20 cases (case nos.1-20 in Table 1). Twenty-six products excised from these bands (two clonal products in cases 3, 5, 6, 15, 16, and 20) were sequenced. In particular, we sequenced the entire nucleotide sequences of the bands in cases 1 and 7, whereas the remaining products were partially sequenced over lengths of approximately 400 to 800 bp

from the digestion restrictive enzyme site in the direction for the junction with JH. These sequences were subjected to BLAST Search (www. ncbi.nml.nih.gov/BLAST).

BLAST searches revealed that a chromosomal segment of 11q or 18q was juxtaposed with the *IGHJ* region on 14q32 in 4 (20%) out of 20 cases with rearranged band(s), implying interchromosomal translocation between *IGHJH* and chromosomes 11q or 18q. In case 6, in addition to the major intra-chromosomal rearranged band, an inter-chromosomal segment (249 bp) of 7q11.2 was identified. The remaining 15 cases had intrachromosomal rearrangements, as summarized in Table 1.

Fig. 3 shows representative examples of cases 1 and 7, in which the products were sequenced entirely. Scheme-I (case 1) shows *BCL1*/*JH4* junctional sequences; representing the *IGH* sequences from 962411 to 962477bp (accession No. NG_001019), followed by the restriction enzyme site tctaga and insertion of the *BCL1* fragment from 11q, and joined to the *JH4* region 14q32. Scheme-II (case 7) shows *VH*3-30 sequences following the restriction sites, and then joined to the *JH*4 region, implying intrachromosomal rearrangement.

Using the partial sequence alignment of the LDI-PCR products, BLAST searches revealed that the chromosome segment translocated was 11q13.3 in 3 cases, 18q21.3 in one case, and 7q11.2 in one case, respectively. The 11q13.3 products contained a part of the *BCL1* gene. Cases 3 and 6 showed the products of intrachromosomal rearrangement and interchromosomal translocation.

3) Relation between phenotypic features and 14q32 translocation status

14q32 translocation was identified in 5 cases consisting of 3 MCL, 1 FL, and 1 DLBCL case, as shown in Table 2. The respective partner segments were 11q13.3 in MCL, 18q21.3 in FL, and 7q11.2 in DLBCL. Cases with common partners of 11q13.3 and 18q21.3 had typical phenotypic features of surface makers and histopathology for each subtype of lymphoma, as shown in Table 3. On the other hand, case 6 with an uncommon partner of

14q32/7q11.2 showed indeterminate histological and immunophenotypical features equivalent to those of MCL.

Discussion

LDI-PCR is now used for the rapid amplification of translocation breakpoints and gene cloning mainly in research laboratories[15-17]. Recently, molecular detection of 14q32 translocations, such as *BCL2/IGH*, *BCL1/IGH*, and *BCL6/IGH*, is being performed by PCR in clinical laboratories. However, current genomic PCR is unable to detect fully the fusion genes of the molecular counterparts, because the breakpoints and partners of the 14q32 translocations are variable[18]. Accordingly, we attempted to apply a LDI PCR method in a clinical setting to efficiently identify *IGH* translocations. This LDI PCR-based technique possesses the possibility to identify all known and unknown partners of 14q32 in one set of PCR reactions.

We report here the successful amplification of the clonal rearrangement of the *IGH* locus consisting of either intrachromosomal rearrangements or interchromosomal translocations by LDI PCR. Our modified LDI PCR protocol was improved in terms of shortening the total run time, relevant reagents used, only 3 newly designed primers, and amplification in one-step. Consequently, this LDI PCR was validated to efficiently amplify a clonally rearranged *IGH* gene as well as germ-line *IGH* genes using practical clinical samples. The actual detection rate of clonality was equivalent or better than that of the current standard PCR for the CDR3 region of the *IGH* gene[5,11]. In addition, the high detection rate of the 11q13.3 aberration in MCL indicates that the LDI PCR successfully amplifies the fusion genes involving 14q32.

Although the analysis was conducted carefully with the relevant quality assurances, several cases failed to amplify *IGH* rearrangements. This indicates the limitation resulting from the character of PCR methodology, such as primer mismatch, amplification failure,

poor DNA integrity, and *IGH* deletion.

The translocations with the *IGHJ* region were detected in approximately 25% out of Japanese B-CLL and DLBCL samples. Relating to histological subtypes of lymphoma, the translocations were detected in all of 3 MCL (100%) cases, 1 of 2 FL (50%) cases, and 1 out of the other lymphomas. The detection rate of MCL and FL is likely reasonable because the cytogenetic data of t(11;14)(q13;q32) and t(14;18)(q32;q21), corresponding to the *IGH/BCL1* and the *IGH/BCL2*, have been reported to be found in approximately 70-90% of cases [3,6)]

In MCL, the chromosomal partner segments were all 11q13.3, but one case was negative for the *IGH/BCL1* translocation by the standard genomic PCR. In general, about 80 % of the *IGH/BCL1* translocations are clustered at the major translocation cluster (MTC), implying the relevancy of the standard genomic PCR using JH and MTC primers. However, the practical detection rates by standard genomic PCR are reportedly very low, only 30-40% of MCL cases[6)].

The 14q32/18q21 translocation is the most common translocation within lymphoid malignancies. In our small series, the 14q32/18q21 translocation was present in 1 case. Although over-expression of the bcl2 protein is not always concurrent with the translocations, the juxtaposition of such genes with enhancer elements of the *IGH* locus deregulates the genes, leading to oncogenic action for the development of B-cell lymphoma. The tight clustering region (mbr) of the 14q32/18q21 fusion is the target for the standard genomic PCR technique for the detection of t(14;18)(q32;q21)-bearing cells. However, similarly to the case of the *BCL1* translocation, the breakpoints are not all closely clustered. Accordingly, most studies recommend the use of a combination of cytogenetics, SBH, and PCR for the most reliable detection of the *IGH* translocations, such as t(11;14) and t(14;18)[19-21)]. Our LDI PCR with direct sequencing would be expected to be one of a line-up of tests in order to identify IGH translocations in a clinical setting. In particular, this method is valuable for

identifying unknown partner genes. Actually, case no. 6 involved chromosomal segments of 7q11.2, but the BLAST Search did not find any oncogenic genes.

We present here an LDI PCR-based methodology for the efficient identification of 14q32 translocations, and discussed its relevancy and feasibility for use in a clinical setting. Since high-speed sequencers are being developed, this strategy would be expected to be applicable to various gene fusion aberrations at the genomic level.

References

1. Tamura A, Miura I, Iida S, Yokota S, Horiike S, Nishida K, Fujii H, Nakamura S, Seto M, Ueda R, Taniwaki M.Interphase detection of immunoglobulin heavy chain gene translocations with specific oncogene loci in 173 patients with B-cell lymphoma. Cancer Genet Cytogenet 2001;129:1-9.
2. Sonoki T, Willis TG, Oscier DG, Karran EL, Siebert R, Dyer MJS. Rapid amplification of immunoglobulin heavy chain switch (IGHS) translocation breakpoints using long-distance inverse PCR. Leukemia 2004;18:2026-31.
3. Canellos GP, Lister TA, Young B. The Lymphomas, 2nd edition: Philadelphia, SAUNDERS ELSEVIER, USA, 2006.
4. Beishuizen A, Hahlen K, Hagemeijer A, Verhoeven Marie-Anne J, Hooijkaas H, et al. Multiple Rearranged Immunoglobulin Genes in Childhood Acute Lymphoblastic Leukemia of Precursor B-Cell Origin. Leukemia 1991;5:657-67.
5. van Dongen JJ, Langerak AW, Bruggemann M, Evans PA, Hummel M, Lavender FL. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. Leukemia 2003;17:2257-317.
6. Belaud-Roturear MA, Parrens M, Dubus P, Garroste JC, de Mascarel A, Merlio JP. A

comparative analysis of FISH, RT-PCR, and immunohistovhemistry for the diagnosis of mantle cell lymphomas. Mod Pathol 2002;15:517-25.

7. Willis TG, Jadayel DM, Coignet LJA, Abdul-Rauf M, Treleaven JG, Catovsky D, Dyer MJS. Rapid Molecular Cloning of Rearrangements of the IGHJ Locus Using Long-Distance Inverse Polymerase Chain Reaction. Blood 1997;90:2456-64.
8. Basso K, Frascella E, Zanesco L, Rosolen A. Improved Long=Distance Polymerase Chain Reaction for the Detection of t(8;14)(q24;132) in Burkitt's Lymphomas. American Journal of Pathology 1999;155:1479-85.
9. Jaffe ES, Harris NC, Stein H, Vardiman JW: WHO Classification of Tumors. Tumors of Haematopoietic and Lymphoid Tissues. Lyon, IARC Press, 2001.
10. Uemura A, Sugahara K, Nagai H, Murata K, Hasegawa H, Hirakata Y, Tsukasaki K, Yamada Y, Kamihira S. An ATL cell line with an IgH pseudo-rearranged band pattern by southern blotting: a pitfall of genetic diagnosis. Lab Hematol. 2005;11:8-13.
11. Brisco MJ, Tan LW, Orsborn AM, Morley AA. Development of a highly sensitive assay, based on the polymerase chain reaction, for rare B-lymphocyte clones in a polyclonal population. Br J Haematol 1990;75:163-7.
12. Fan H, Gulley ML, Gascoyne RD, Horsman DE, Adomat SA, Cho CG. Molecular methods for detecting t(11;14) translocations in mantle-cell lymphomas. Diagn Mol Pathol 1998;7: 209-14.
13. Limpens J, Stad R, Vos C, de Vlaam C, de Jong D, van Ommen GJ, Schuuring E, Kluin PM. Lymphoma-associated translocation t(14;18) in blood B cells of normal individuals. Blood 1995;85:2528-36.
14. Horsman DE, Gascoyne RD, Coupland RW, Coldman AJ, Adomat SA. Comparison of cytogenetic analysis, southern analysis, and polymerase chain reaction for the detection of t(14;18) in follicular lymphoma. Am J Clin Pathol 1995;103:472-8.
15. Sonoki T, Willis TG, Oscier DG, Karran EL, Siebert R, Dyer MJ. Rapid amplification of

immunoglobulin heavy chain switch (IGHS) translocation breakpoints using long-distance inverse PCR. Leukemia 2004;18:2026-31.

16. Akasaka T, Izidore S Lossos, Ronald Levy. BCL6 gene translocation in follicular lymphoma: a harbinger of eventual transformation to diffuse aggressive lymphoma. Blood 2003;102:1443-8.
17. Akasaka H, AkasakaT, Kurata M, Ueda C, Shimizu A, Uchiyama T, Ohno H. Molecular Anatomy of BCL6 Translocation Revealed by Long-Distance Polymerase Chain Reaction-based Assays. Cancer Research 2000; 60:2335-41.
18. Yonetani N, Ueda C, AkasakaT, Nishikori M, Uchiyama T, Ohno H. Heterogeneous Breakpoints on the Immunoglobulin Genes Are Involved in Fusion with the 5'Region of BCL2 in B-Cll Tumors. Jpn J Cancer Res 2001;92:933-40.
19. Pezzella F, Ralfkiaer E, Gatter KC, Mason DY. The 14;18 translocation in European cases of follicular lymphoma: comparison of Southern blotting and the polymerase chain reaction. Br J Haematol 1990;76:58-64.
20. Ladanyi M, Wang S. Detection of rearrangements of the BCL2 major breakpoint region in follicular lymphomas. Correlation of polymerase chain reaction results with Southern blot analysis. Diagn Mol Pathol 1992:1(1):31-5.
21. Benitez J, Robledo M, Santon A, Santos M, Rivas C, Echezarreta G, Martinez Castro P. Correlation between cytogenetic and molecular analysis of t(14;18) in follicular lymphomas. Cancer Genet Cytogenet 1992;59:68-72.
22. Monni O, Zhu Y, Franssila K, Oinonen R, Hoglund P, Elonen E, et al. Molecular characterization of deletion at 11q22.1-23.3 in mantle cell lymphoma. Brit J Haematol 1999;104:665-71.
23. Glenn H Segal, Aneal S Masih, Canavan Sllyson, Fox C, Jorgensen T, et al. CD5-Expressing B-Cell Non-Hodgkin's Lymphomas with bcl-1 Gene Rearrangement Have a Relatively Homogeneous Immunophenotype and are Associated with an Overall

Poor Prognosis. Blood 1995;85:1570-9.

Legends

Fig 1. Schematic representation of primer sites, restriction enzyme sites, and digestion products of the IGH locus (NG_001019. Homo sapiens immu...[gi:29502084] $^{1)}\rightarrow$; Forward primer site for Bg1-II digestion, $^{2)}\rightarrow$; Forward primer site for Pst-1 and Xba-1, $\leftarrow^{3)}$; Reverse common primer site, 4), 5), and 6); length of germline fragments digested Bg1-II, Pst-1, and Xba-1

Fig.2 (A) The electrophoretic band patterns s of the products amplified by LDI PCR using a restriction enzyme of XbaI

Lanes 1 to 7; cases 1, 2, 4, 5, 6, 14, and 15. Lane 8; normal control,

M; marker

In addition to germ line band (←), clonal band(s) are visible in lanes 1 to 7.

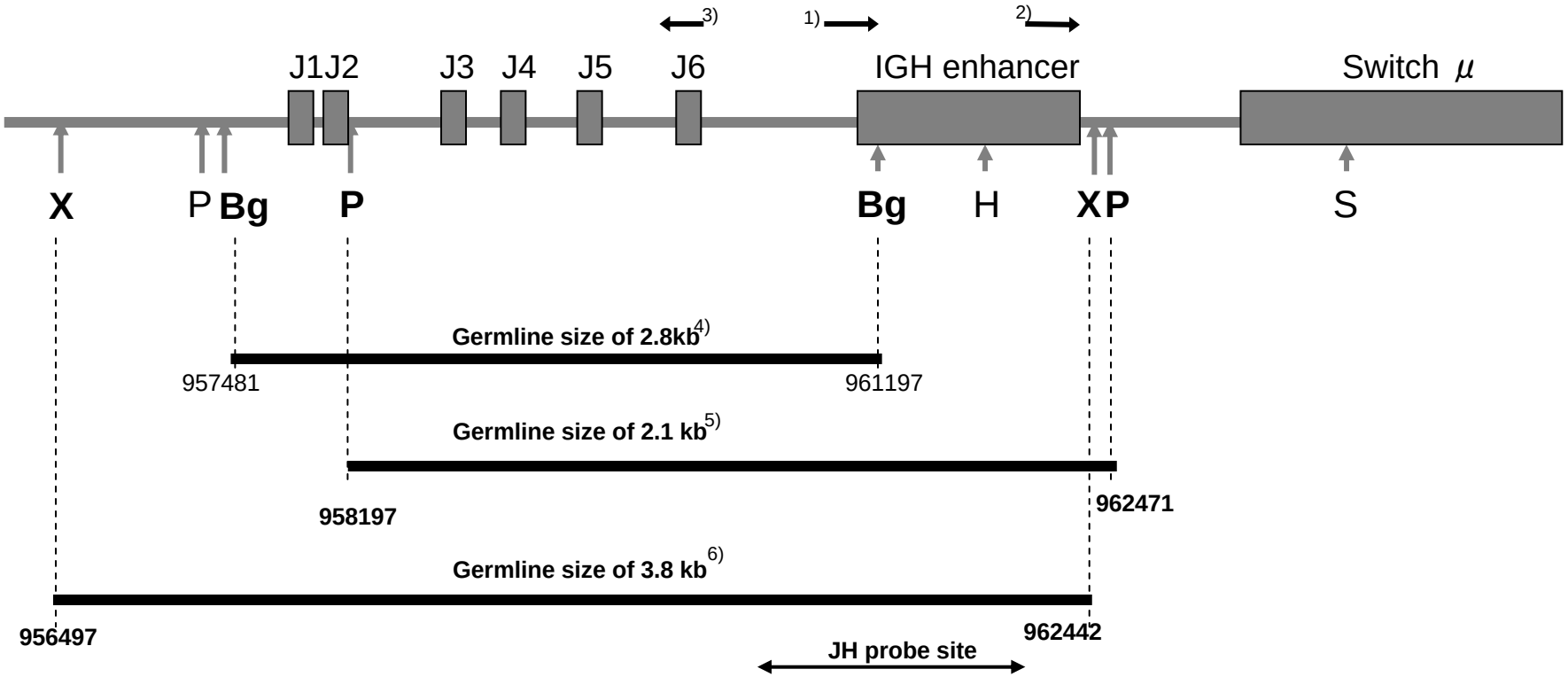
(B) Southern blot analysis for the LDI PCR products using the JH probe.

To sequence chimeric amplicons, DNA was excised from clonal band marked by white arrows (The same blotting sheet was used in A and B).

Fig.3. Nucleotide sequence of the LDI-PCR products in representative cases with inter (Scheme-I)- and intra (Scheme-II)-chromosomal translocation.

In case 1, chimeric DNA representing a fusion of the bcl-1 and JH genes was demonstrated. In case 7, only a segment of the VH gene was identified.

Fig 1.

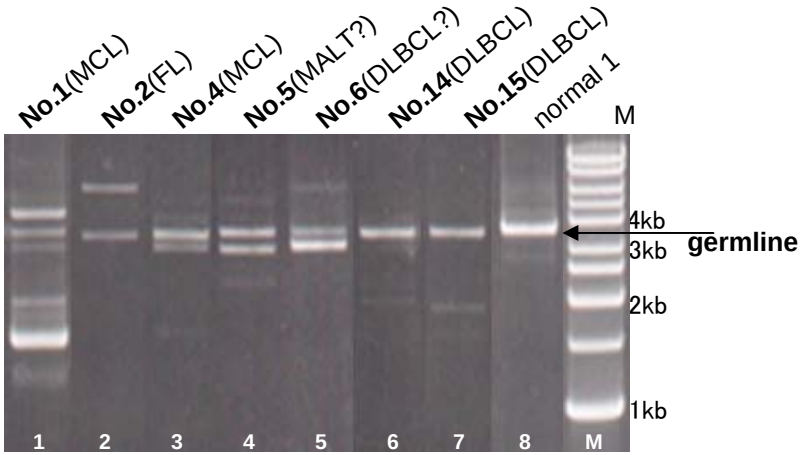


Primers

		Sequence No.（NG_001019）
1) →	**Forward for BglⅡdigestion**	5'-ggcaggaagcaggtcaccgcgagagtct-3' 961095-961122
2) →	**Forward for PstⅠ / XbaⅠ**	5'-atcatcgcacccttgaaagtag-3' 962323-962344
← 3)	**Reverse common primer**	5'-aggagacggtgaccgtggtccctttgcc-3' 960103-960130
↔	**JH probe**	959069 - 961200

Fig.2

A. LDI-PCR



B. PCR Southern blot
(JH probe)

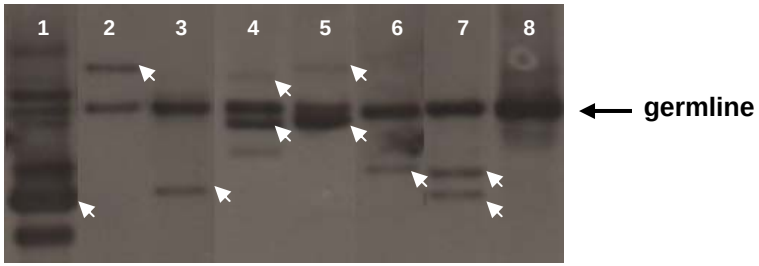
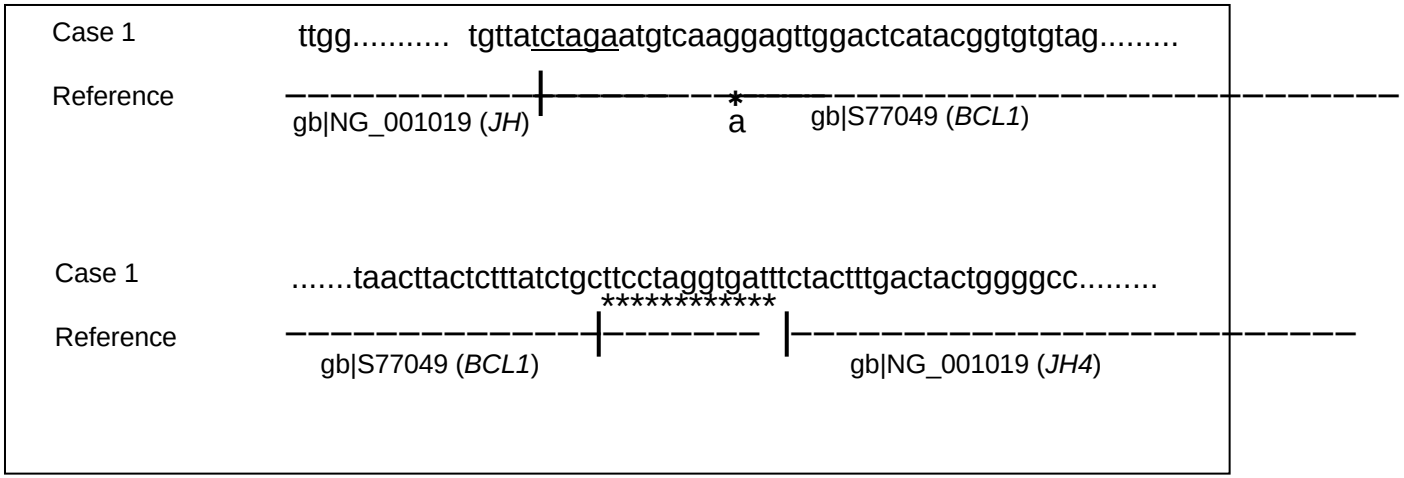


Fig.3.

Scheme-I



Scheme-II

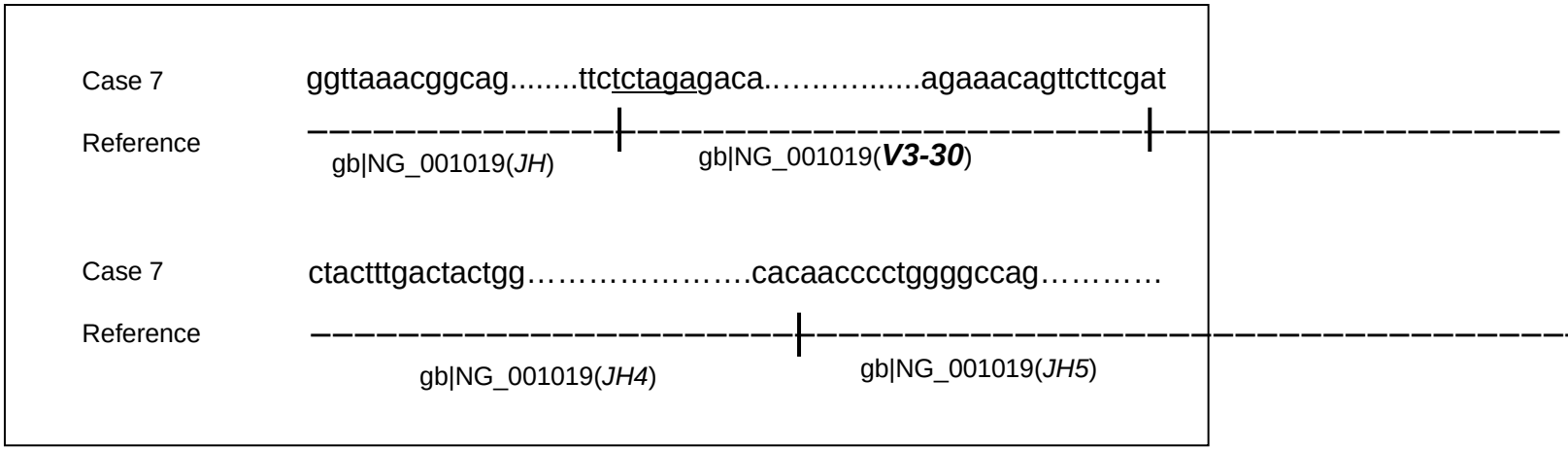


Table 1. Summary of patient characteristics and study results

Case No	Dx	specimen	CDR3 PCR	enzyme	band size	sequence size	BLAST Search hit:Accession	chr map	gene	inter-chromosomal translocation
1	MCL	PB	(+)	Xba I	1.6kb	1.6kb	NC_000011	11q13.3	BCL1	yes
2	FL	LN	(+)	Xba I	7.57kb	0.4kb	NC_000018	18q21.3	BCL2	yes
3	MCL	tonsil	(−)	Bgl II	1.4kb	0.8kb	NC_000011	11q13.3	BCL1	yes
				Bgl II	3.5kb	0.8kb	AB019439	14q32	VH	no
4	MCL	PB	(+)	Xba I	3.5kb	0.6kb	NC_000011	11q13.3	BCL1	yes
5	MALT?	Effusion	(+)	Xba I	3.0kb	0.5kb	AB019439	14q32	VH	no
				Xba I	5.0kb	1.0kb	AB019439	14q32	VH	no
6	DLBCL?	LN	(+)	Xba I	3.2kb	0.7kb	AB019439	14q32	VH	no
				Xba I	6.0kb	1.0kb	NT_007758	7q11.2	?	yes
7	FL	LN	(+)	Bgl II	3.8kb	2.6kb	AB019439	14q32	VH	no
8	CLL	PB	(+)	Xba I	1.3kb	0.7kb	X97051.1	14q32	DH	no
9	CLL	PB	(+)	Xba I	1.8kb	0.4kb	X97051.2	14q32	DH	no
10	CLL	PB	(+)	Xba I	4.0kb	0.5kb	X97051.1	14q32	DH	no
11	CLL	PB	(+)	Xba I	0.6kb	0.5kb	X97051.1	14q32	DH	no
12	CLL	PB	(−)	Xba I	2.2kb	0.8kb	X97051.1	14q32	DH	no
13	CLL	PB	(+)	Xba I	1.1kb	0.7kb	AF466139	14q32	VH	no
14	DLBCL	BM	(−)	Xba I	2.2kb	0.7kb	AB019439.1	14q32	VH	no
15	DLBCL	LN	(+)	Xba I	2.0kb	0.7kb	AB019441.1	14q32	VH	no
				Xba I	1.8kb	0.8kb	X97051.1	14q32	DH	no
16	DLBCL	LN	(+)	Bgl II/Xba	4.5kb	0.6kb	AB019438	14q32	VH	no
					1.6kb	0.5kb	X97051.1	14q32	DH	no
17	DLBCL	PB	(+)	Bgl II	6.0kb	0.5kb	AB019440	14q32	VH	no
18	DLBCL	Effusion	(+)	Bgl II	1.5kb	0.7kb	X97051.1	14q32	DH	no
19	DLBCL	PB	(+)	Bgl II	2.3kb	0.7kb	X97051.1	14q32	DH	no
20	DLBCL	PB	(+)	Bgl II	3.2kb	0.7kb	X97051.1	14q32	DH	no
				Bgl II	2.2kb	0.8kb	AB019439	14q32	VH	no
21	CLL	PB	(+)	Pst/Xba	(−)					
22	FL	LN	(+)	Pst/Xba	(−)					
23	CLL	PB	(+)	Pst/Xba	(−)					
24	CLL	PB	(−)	Pst/Xba	(−)					

Table 2 The 14q32 translocation status and histological subtypes in 20 cases presenting LDI-PCR amplified products

Translocation status	MCL (N=3)	FL (2)	CLL (6)	MALT (1)	DLBCL (8)
11q13.3	3	0	0	0	0
7q11.2	0	0	0	0	1
18q21.3	0	1	0	0	0
total	3	1	0	1	1

Table 3 Immunophenotypic profiles in 6 cases with the 14q32 translocation

Case No	Surface marker							Histology
	CD5	CD10	CD19	CD23	CD25	SmIg		
1	98	1.1	89	20	86	90	(IgD/M λ)	MCL
2	7	76	71	10	0.1	67	(IgM κ)	FL
3	50	0.1	45	35	8	55	(IgD/M λ)	MCL
4	85	0.6	93	10	16	95	(IgD/M λ)	MCL
6	35	2	70	1	9	56	(M κ)	DLBCL(MCL?)