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Heterogeneity of *BCL6* rearrangements in nodular lymphocyte predominant Hodgkin's lymphoma

IWONA WLODARSKA
MICHEL STUL
CHRIS DE WOLF-PEETERS
ANNE HAGEMEIJER

A B S T R A C T

Background and Objectives. Nodular lymphocyte-predominant Hodgkin's lymphoma (NLPHL) showed recurrent rearrangement of the *BCL6* which is gene detected in 48% of cases analyzed by interphase-fluorescent *in situ* hybridization (FISH). These findings point to a critical role for *BCL6* in the development of this distinct Hodgkin's lymphoma. We present our results of metaphase-FISH analyses aimed at identifying and characterizing *BCL6*-related chromosomal translocations in NLPHL.

Design and Methods. Four NLPHL cases with available metaphase spreads obtained either at the time of diagnosis or during progression to diffuse large B-cell lymphoma (DLBCL) were collected. Extensive metaphase-FISH analysis was performed to identify the affected partner chromosomes and reciprocal breakpoints.

Results. Each of the analyzed NLPHL cases showed a different type of *BCL6* rearrangement that included the t(3;22)(q27;q11) targeting immunoglobulin (*IG*) λ chain locus, complex t(3;7;3;1) involving the 7p12/Ikaros gene region, t(3;9)(q27;p13) affecting an unknown gene in vicinity of *PAX5*, and t(3;4)(q27;q32) showing the alternative 3q27 breakpoint outside *BCL6* and possibly, an internal deletion of *BCL6*. Retrospective interphase-FISH analysis of 2 cases with subsequent DLBCL showed the same type of *BCL6* translocation as in NLPHL samples.

Interpretation and Conclusions. The spectrum of *BCL6* aberrations targeting *IG* as well as non-*IG* loci in NLPHL is similar to that found in DLBCL. These findings further support the hypothesis of a germinal center B-cell-derived origin of NLPHL and of a relationship between these two lymphoma entities. This latter issue is additionally illustrated in two NLPHL patients who subsequently developed DLBCL and showed the same type of *BCL6* rearrangements in both tumors.

Key words: *BCL6*, NLPHL, FISH, Hodgkin's lymphoma.

From the Center for Human Genetics, Katholieke Universiteit Leuven, Leuven, Belgium (IW, MS, AH), Division of Morphology and Molecular Pathology, Katholieke Universiteit Leuven, Leuven, Belgium (CDW-P).

Correspondence:
Dr. Anne Hagemeijer
Center for Human Genetics,
Katholieke Universiteit Leuven,
Leuven, Herestraat 49, B-3000
Leuven, Belgium. E-mail:
anne.hagemeijer@med.kuleu-
ven.ac.be

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Nodular lymphocyte-predominant Hodgkin's lymphoma (NLPHL) is a rare indolent disorder recognized as a distinct clinico-pathologic entity of Hodgkin lymphoma in the recently published World Health Organization (WHO) classification of hematologic malignancies.¹ NLPHL is characterized by a minority of diagnostic large atypical cells called *lymphocytic and/or histiocytic* (L&H) cells or popcorn cells, scattered in the nodular infiltrates composed of small lymphoid and epitheloid cells. Immunophenotypic studies supported by recent molecular investigations of isolated L&H cells unequivocally showed that NLPHL harbors a monoclonal population of B cells showing clonal rearrangements of immunoglobulin (*IG*) genes and presence of somatic mutations of the variable region of the *IG* heavy chain genes cluster.²⁻⁶ These findings, together

with the observation that popcorn cells express *BCL6* but not syndecan-1 (CD138),^{7,8} provide evidence for a germinal center (GC) B-cell origin of NLPHL.

The cytogenetic data on NLPHL are scant due to technical difficulties in obtaining representative metaphase cells. To overcome these difficulties and to get insight into genetic features of NLPHL we recently investigated this lymphoma using molecular cytogenetic techniques including comparative genomic hybridization⁹ and interphase fluorescent *in situ* hybridization (FISH).¹⁰ This latter study led to the detection of *BCL6* gene rearrangements in 48% of analyzed NLPHL cases (11/23) but not in the studied cases of classical Hodgkin's lymphoma (0/40). These findings point to a critical role for *BCL6* in the development of NLPHL, provide further evidence for a germinal center (GC) B-cell-derived origin of NLPHL and sup-

Table 1. Relevant clinical and cytogenetic data of 4 NLPHL cases.

Case	Sex/ Age ^a	Clinical history year diagnosis	Lymph node karyotype (N. of analyzed cells)	Chromosomal aberrations revised by FISH and M-FISH
1	M/25	1995 NLPHL	43,X,-Y,i(1)(q10),t(3;22)(q27;q11), -4,add(7)(q36), -10,-17,+ mar [4]/46,XY [6]	t(3;22)(q27;q11)
2	M/27	1992 NLPHL 2000 relapsed NLPHL	43,-X,-Y,+del(2)(q33),der(3)t(3;?)(q27;?;q21), add(4)(p16),del(7)(p12),-11,-21 [1]/46,XY [12] 92,XX,YY,+X,-1,-2,del(2)(q33),der(3)t(3;?)(q27;?;q21),add(4)(p16),-5,+6,del(6)(q13), del(7)(p12),-10,-10,-12,-13,-13,-14,-16, +17, -18,-18,-19,-21,+mar [1]/46,XY [19]	92,XX,YY,+del(Xq26),del(2)(q33), der(3)(3pter_cen_3q27::7p12_7pter:: 3q27q27::7p12p12::1q21_1qter:: der(4)t(4;5)(p16;q31),-5,-5,del(6)(q13), der(7)t(3;7)(q27;p12),del(10)(q21), del(12)(q22),del(18)(q12),-20,-21
3	F/71	1991 stomach DLBCL 1994 NLPHL 2000 nodal DLBCL	27-45,X,-X,add(1)(p36),add(3)(q27), del(6)(q21)[2],-7,del(9)(p13),+13[1],+4mar[1] [cp3] 46,XX[11] 41-48,XX,add(3)(q27),?del(6)(q21),-8, del(9)(p13),+16, +1-2mar [inc8]	t(3;9)(q27;p13)
4	M/69	1992 NLPHL 1997 DLBCL	no mitosis 48,XY,del(2)(p24), add(3)(q27), + add(3)(q27), del(4)(q32),-6, dup(12)(q13q15),add(18)(q23), +19,+mar [cp15]	t(3;4)(q27;q32), +der(3)t(3;4)(q27;q32)

^aAt time of the NLPHL diagnosis.

port the concept of distinct, entity-related mechanisms underlying the pathogenesis of NLPHL and classical Hodgkin's lymphoma.

The *BCL6* gene, located at 3q27, encodes a *POZ*/zinc-finger protein that functions as a DNA-binding transcription repressor regulating expression of at least 14 putative target genes.¹¹ The *BCL6* gene is frequently activated by chromosomal translocations, which have been mainly found in diffuse large B-cell lymphoma (DLBCL) and less frequently in follicular lymphoma.¹¹⁻¹⁴ The particular feature of these translocations is that they can affect not only any one of three *IG* loci but also a variety of non-*IG* partners. It was shown that *BCL6*-associated translocations lead to upregulation of *BCL6* transcription by a mechanism of promoter exchange,^{15,16} probably preventing the downregulation of this gene that occurs upon plasmacytic differentiation. Other rearrangements of the 3q27 region recurrently observed in malignant lymphomas include somatic mutations affecting the 5' non-coding regulatory region of *BCL6*,¹⁷ internal deletions of *BCL6*¹⁸ and chromosomal translocations targeting the alternative breakpoint region telomeric to the gene.¹⁹ Molecular consequences of these latter aberrations are still poorly understood.

We present here our results of FISH studies aimed at characterizing chromosomal aberrations underlying rearrangements of *BCL6* detected in 4 NLPHL cases with available cytogenetic specimens. These 3q27 abnormalities analyzed on metaphase and interphase

cell levels displayed a similar spectrum of *BCL6* rearrangements as that reported in DLBCL.

Design and Methods

Patients

Four cases histologically diagnosed at the Department of Pathology of the University of Leuven as having NLPHL were collected for this study. The clinical history of two of these cases was complicated by subsequent development of DLBCL (Table 1). All four cases were included in previously published series.^{9,10}

G-banding analysis

Cytogenetic analysis was performed after culturing lymph node specimens overnight according to standard methods. Three to 20 G-banded metaphases were analyzed. Chromosomal aberrations are presented in accordance with the International System for Human Cytogenetic Nomenclature.²⁰

Fluorescence in situ hybridization (FISH)

FISH studies were performed on cytogenetic specimens stored at -20°C. The *BCL6* gene was analyzed using the Locus Specific Identifiers (LSI) *BCL6* dual color, Break Apart Rearrangement Probe (Vysis, Downers Grove, IL, USA), and occasionally with a cosmid B5-2 covering the 5' end of *BCL6*.²¹ The first assay uses a mixture of probes separated by a 42 kb gap that contains

the entire *BCL6* gene, including the *BCL6* breakpoint region. Other probes used in the present study are listed in Table 2. The BAC clones (<http://www.ensemble.org>) were obtained from the Roswell Park Cancer Institute libraries (<http://www.chori.org/BACPAC>). The XCyte-mFISH probe kit (MetaSystems, Altlußheim, Germany) was used for MFISH analysis. A human BAC clone for the Ikaros gene, kindly provided by Y. Hosokawa (Division of Molecular Medicine Aichi Cancer Center Research Institute Nagoya, Japan) was isolated from the GenomeSystem BAC library and was found to include the 3' end of the Ikaros gene, but to lack its 5'-non-coding portion.^{21,22} N11E6, the most centromeric cosmid for the variable region of the λ locus *IGVL*^{21,23} and cos607/22 containing sequences of the constant region of the *IGL* region^{21,24} were kindly provided by H. McDermid (University of Alberta, Edmonton, Alberta, Canada) and H.G. Klobeck (Ludwig-Maximilians-Universität, München, Germany), respectively.

Commercial probes were used according to their manufacturers' instructions. Standard FISH experiments were performed as described previously.²⁵ The FISH data were collected on a Leica DMRB (Leica, Wetzlar, Germany) fluorescence microscope equipped with a cooled black and white charged couple device camera (Photometrics, Tuscon, AZ, USA) run by Quips SmartCapture™ FISH Imaging Software (Vysis, Bergisch-Gladbach, Germany). The metaphase FISH results were evaluated on an Axioplan 2 fluorescence microscope equipped with a charge-coupled device Axio-phot 2 camera (Carl Zeiss Microscopy, Jena, Germany) and a MetaSystems' isis/mFISH imaging system (MetaSystems, Altlußheim, Germany).

Southern blot analysis

Southern blot analysis was performed as described previously²⁶ using the restriction endonucleases BamHI, BglII, PstI, EcoRI and HindIII, and a *BCL6* probe. This probe, a 4 kb SacI fragment, (kindly provided by R. Dalla-Favera, University Medical Center, New York, NY, USA) in combination with the restriction enzymes used, explores a region of 15.2 kb containing the 5' portion of the *BCL6* gene (first exon, 7.5 kb of the first intron and 7.4 kb of 5' flanking sequences).²⁷

Results

Relevant clinical and cytogenetic findings of the 4 NLPHL cases with rearrangement of *BCL6* are shown in Table 1. Metaphase FISH studies of cases 1 and 2 were performed on lymph node samples obtained at the time of diagnosing NLPHL and during progression of the disease, respectively. As only single metaphase spreads were found in these samples, only one abnor-

mal cell per FISH experiment could be analyzed. The results obtained were confirmed on interphase cells. Cytogenetic analysis of the remaining 2 NLPHL cases showed a normal karyotype or no mitoses. A few years later, however, both patients developed DLBCL characterized by karyotypic aberrations including 3q27/*BCL6* rearrangements. The latter samples were, therefore, used for an initial FISH characterization of the 3q27-related translocations and critical probes were further applied for retrospective interphase-FISH analysis of the original NLPHL samples. The results of the FISH investigations are summarized in Table 2.

Briefly, in case 1 *BCL6* and *IGL* were involved in the t(3;22)(q27;q11), as demonstrated using LSI *BCL6* and 2 differentially labeled *IGL* cosmids [N11E6 (*IGVL*) and 607/22 (*IGVC*)]. As shown in Figure 1A/B, both assays displayed split hybridization signals between the der(3) and der(22), confirming rearrangements of both genes.

Case 2 showed complex aberrations of chromosome 3 originally described as der(3)t(3;7;1)(q27;?;q21). This aberration was first analyzed by FISH using chromosome 3 paint (Figure 1C, left insert), cosmid B5-2, *BCL6* and Xcyte-mFISH probes (Figures 1C/D). These experiments identified 7p12 as a reciprocal partner of t(*BCL6*) and an associated duplication of the 3' end of rearranged *BCL6* gene on the der(3). The 7p12 breakpoint was further analyzed using a BAC clone containing the 3' end of Ikaros, a known partner gene of *BCL6*.²² The finding of two signals from this clone which co-localized with two B5-2 signals on the der(3) (Figure 1C, right insert) confirmed translocation of the 7p12 sequences in the vicinity of *BCL6* and duplication of the t(3;7) breakpoint region. Considering, however, the likely 7pter-5' Ikaros-3'-cen orientation of the gene, its predicted rearrangement should be associated with a break within the 5' end promoter region and manifested by hybridization of the Ikaros BAC to the der(7). We, therefore, assume that the 7p12 breakpoint of the present translocation targeted another unknown gene located just proximally to Ikaros.

The third patient was diagnosed with a diffuse large B-cell lymphoma of stomach in 1991, presented with NLPHL in 1994 and nodal DLBCL in 2000 (Table 1). Although no abnormal metaphase cells could be found at the time the patient had NLPHL, we had previously detected rearrangement of *BCL6* in atypical large cells from that sample using an interphase-FISH approach.¹⁰ To characterize the 3q27 aberration we initially applied LSI *BCL6* on available metaphase spreads from a nodal DLBCL sample with a documented 3q27/*BCL6* rearrangement (Figure 2). This analysis led to identification of a t(3;9)(q27;p13) (Figure 1G). The 9p13 breakpoint of this translocation was further investigated using a set of probes from the *PAX5* gene region.²⁸ The BAC clone (405L18) covering the breakpoint was suc-

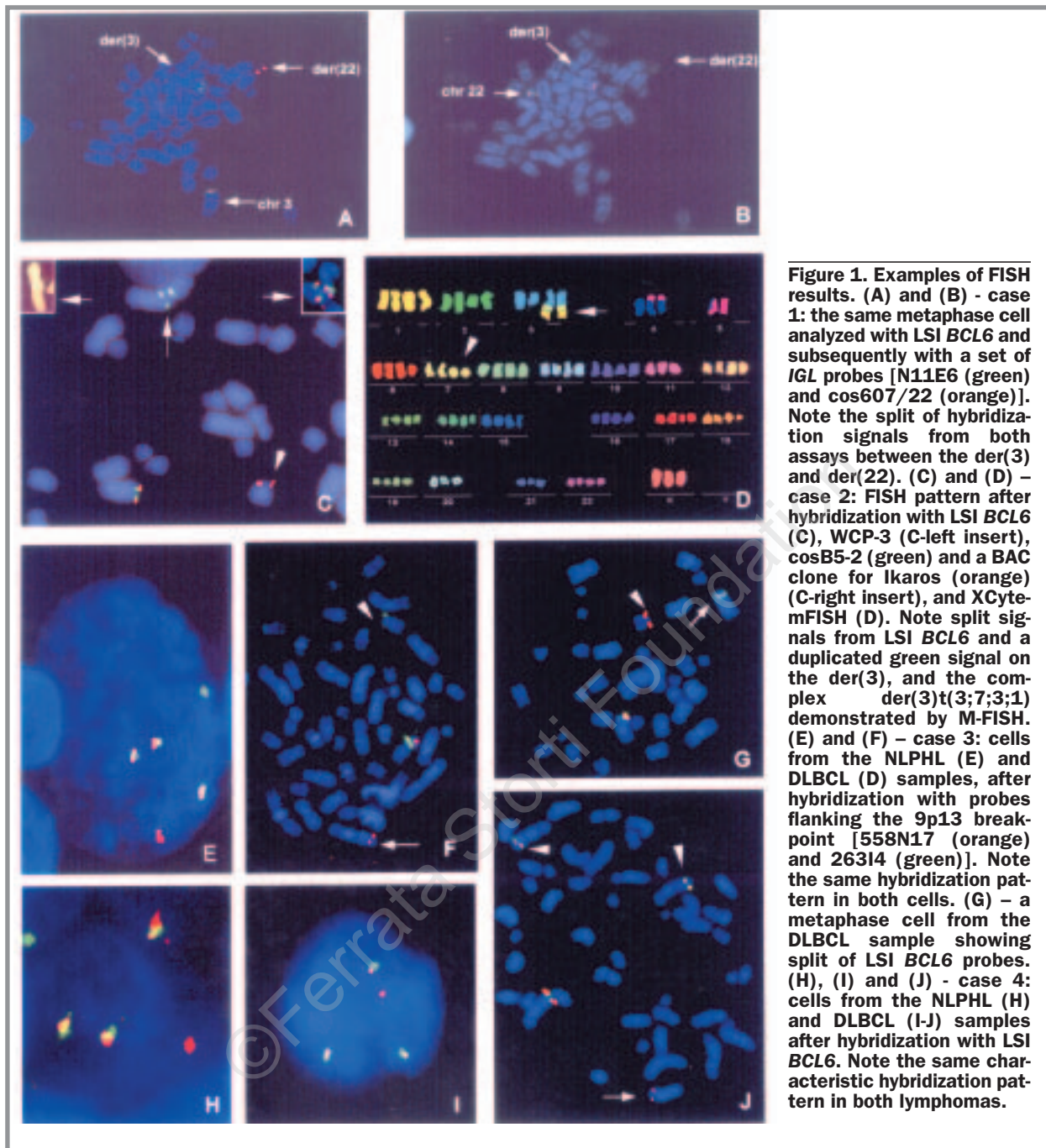


Figure 1. Examples of FISH results. (A) and (B) - case 1: the same metaphase cell analyzed with LSI *BCL6* and subsequently with a set of *IGL* probes [N11E6 (green) and cos607/22 (orange)]. Note the split of hybridization signals from both assays between the der(3) and der(22). (C) and (D) - case 2: FISH pattern after hybridization with LSI *BCL6* (C), WCP-3 (C-left insert), cosB5-2 (green) and a BAC clone for Ikaros (orange) (C-right insert), and XCyte-mFISH (D). Note split signals from LSI *BCL6* and a duplicated green signal on the der(3), and the complex der(3)t(3;7;3;1) demonstrated by M-FISH. (E) and (F) - case 3: cells from the NLPHL (E) and DLBCL (F) samples, after hybridization with probes flanking the 9p13 breakpoint [558N17 (orange) and 263I4 (green)]. Note the same hybridization pattern in both cells. (G) - a metaphase cell from the DLBCL sample showing split of LSI *BCL6* probes. (H), (I) and (J) - case 4: cells from the NLPHL (H) and DLBCL (I-J) samples after hybridization with LSI *BCL6*. Note the same characteristic hybridization pattern in both lymphomas.

cessfully identified (Figure 1F) and clones (558N17 and 263I4) flanking this breakpoint were eventually used for retrospective analysis of the NLPHL sample from 1994. The presence of 3 fused signals (3xchr9) and separated green (G) and orange (O) signals [der(3) and der(9)] in sporadic huge atypical nuclei in that sample (Figure 1E) indicated the same 9p13 breakpoint as during the nodal DLBCL. Altogether, these and the results of LSI *BCL6* analysis indicated the occurrence of t(3;9)(q27;p13) in atypical NLPHL cells. Moreover, based on cytogenetic findings of stomach DLBCL [add(3)(q27), del(9)(p13)], there is a suggestion of the

t(3;9) already in the first diagnostic sample (1991).

The fourth patient was diagnosed in 1992 with NLPHL and developed DLBCL 5 years later (Table 1). Interphase-FISH analysis with the LSI *BCL6* assay performed on both samples led to identification of a similar aberrant signal pattern (2-4GO/10 and 3GO/10, respectively) (Figure 1H/I) in rare huge atypical cells from the NLPHL biopsy and in 45% of interphase nuclei from the DLBCL sample. In 6 analyzed metaphase cells from this latter specimen the fused green/orange (GO) signals were found on normal chromosome 3 and two add(3)(q27), while the orange sig-

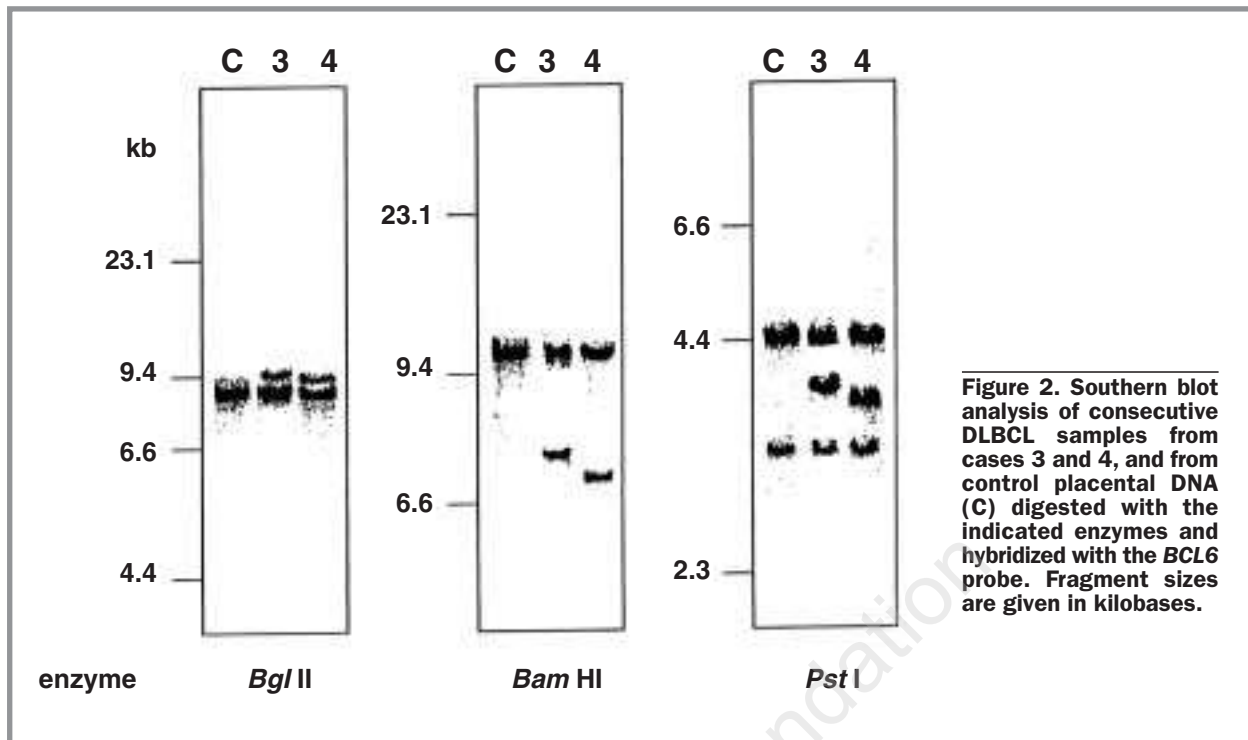


Figure 2. Southern blot analysis of consecutive DLBCL samples from cases 3 and 4, and from control placental DNA (C) digested with the indicated enzymes and hybridized with the *BCL6* probe. Fragment sizes are given in kilobases.

nal was detected on the del(4)(q32) (Figure 1J). Further analysis with the 4q subtelomeric probe (cT55) that hybridized to chromosome 4 and to both add(3)(q27) documented the reciprocal t(3;4)(q27;q32) associated with a duplication of der(3). As signals from the applied cosmid B5-2 were exclusively found on chromosome 3 and both der(3), we presumed that the 3q27 breakpoint occurred in the region covered by a SpectrumOrange-labeled LSI *BCL6* probe that is located telomerically to *BCL6*. Southern blot analysis performed on that sample revealed, however, rearrangement of *BCL6* in the major translocation cluster (MTC) (Figure 2). The reciprocal breakpoint of this translocation was further analyzed with a set of 4q31-q33 BAC clones (Table 2) and was finally narrowed down to the 4q32 region of approximately 1.5 Mb flanked by clones 336N6 and 440L13. These differentially labeled probes were applied for interphase-FISH analysis of the original NLPHL sample and showed the analogous 1G0/1-30/1G FISH pattern in a few identified huge interphase cells. This and LSI *BCL6* hybridization patterns possibly reflect presence of the t(3;4)(q27;q32) accompanied by 1 or 2 extra copies of the der(3) in pathological cells of NLPHL.

Discussion

We present here the results of molecular cytogenetic analysis of *BCL6* aberrations detected in 4 cases of NLPHL. Despite intrinsic problems in obtaining

metaphase chromosomes in NLPHL, our combined metaphase and interphase FISH study led to identification of partner chromosomes involved in all four 3q27-associated translocations and characterization of reciprocal breakpoints mapping either *IG-* or non-*IG* loci.

The first case was characterized by the known t(3;22)(q27;q11)/*IGL-BCL6* aberration recurrently occurring in DLBCL.^{21,29} The second identified 3q27 translocation targeted 7p12 and was cytogenetically masked by complex chromosomal aberrations including duplication of the rearranged 3' end of *BCL6* and translocation of 1q (Figure 1C-D). Interestingly, an analogous t(3;7)(q27;p12) was previously reported in two DLBCL cases,³⁰ of which at least one was characterized by the Ikaros-*BCL6* rearrangement.²² Further FISH analysis carried out in the present NLPHL case led to mapping of the 7p12 breakpoint in the Ikaros region, but did not provide evidence of Ikaros being involved. Unfortunately, the incomplete molecular characterization of this genomic region in genome databases currently prevents further molecular cytogenetic investigation of this translocation.

The *BCL6* translocation detected in case 3 involved 9p13. FISH studies showed that the reciprocal breakpoint of this translocation is covered by 405L18, a BAC clone mapped approximately 600kb proximal to *PAX5* (<http://www.ensembl.org>/<http://www.ensembl.org>). A number of genes have been mapped in this region. Considering, however, that the breakpoint of this

Table 2. Results of FISH analysis of lymph node cells obtained at various disease time-points.

Case	Diagnosis (status) ^b	Partial karyotype: 3q27 aberration	Probe/ fluorochrome ^c	Localization gene	FISH results ^d	Conclusion
1	NLPHL (D)	t(3;22)(q27;q11)	LSI <i>BCL6</i> ^{CO} cN11E6 ^C c607/22 ^O	3q27 - <i>BCL6</i> flanking probes 22q11 - <i>IGLV</i> 22q11 - <i>IGLC</i>	chr3 ^{CO} , der(3) ^C , der(22) ^O chr22 ^{CO} , der(22) ^C , der(3) ^O	<i>BCL6</i> rearrangement <i>IGL</i> rearrangement → <i>IGL-BCL6</i>
2 ^a	NLPHL	der(3)t(3;?;1)(q27;?;q21)	LSI <i>BCL6</i> ^{CO} XCyte-mFISH cosB5-2 ^C BAC Ikaros ^O	3q27- <i>BCL6</i> flanking probes all human chromosomes 3q27 - <i>BCL6</i> 7p12 - Ikaros	chr3 ^{CO} , der(3) ^{CO} , der(7)(p12) ^O der(3)_der(3)t(3;7;3;1) chr3 ^C , der(3) ^{CO} , chr7 ^O , der(7) ^C	<i>BCL6</i> (P) rearrangement and duplication of the 3'end of <i>BCL6</i> on the der(3) complex t(3;7) the 7p12 breakpoint proximal to Ikaros; der(3)_der(3) (3pter_cen_3q27::7p 12_7pter::3q27q27:: 7p12p12::1q21_1qter)
3 ^a	DLBCL (P)	add(3)(q27)	LSI <i>BCL6</i> ^{CO} y788C3 ^C region ³⁴ RP11-344B23 ^C RP11-220I4 ^O RP11-558N17 ^C RP11-263I4 ^O RP11-405L18 ^C	3q27 - <i>BCL6</i> flanking probes 9p13 - <i>PAX5</i> 9p13 - <i>PAX5</i> flanking probes 9p13 - proximal to <i>PAX5</i> 9p13 - proximal to <i>PAX5</i>	chr3 ^{CO} , der(3) ^C , der(9)(p13) ^O chr9 ^C , der(3) ^C , der(9) ^C chr9 ^{CO} , der(3) ^{CO} chr9 ^{CO} , der(3) ^C , der(9) ^O chr9 ^C , der(3) ^C (small signal), der(9) ^C	<i>BCL6</i> rearrangement due to the t(3;9)(q27;p13) the 9p13 breakpoint in the region of <i>PAX5</i> the 9p13 breakpoint proximal to <i>PAX5</i> the 9p13 breakpoint in the region covered by B405L18
NLPHL (D)	no mitosis		LSI <i>BCL6</i> ^{CO} RP11-558N17 ^C RP11-263I4 ^O	3q27 - <i>BCL6</i> flanking probes 9p13 - probes flanking the breakpoint	1GO/1G/1O ^C 2GO/2G/2O 3GO/1G/1O ^C	<i>BCL6</i> rearrangement likely t(3;9)(q27;p13) and 3 copies of chromosome 9
4 ^a	DLBCL (P)	add(3)(q27), +add(3)(q27)	LSI <i>BCL6</i> ^{CO} cosB5-2 ^C cT55 ^C RP11-218F10 ^C RP11-336N6 ^O RP11-440L13 ^C RP11-213L8 ^O	3q27 - <i>BCL6</i> flanking probes 3q27 - <i>BCL6</i> 4q subtel ³⁵ 4q31-q33 4q32	chr3 ^{CO} , add(3) ^{CO} , add(3) ^{CO} , der(4) ^O chr3 ^C , add(3) ^C , add(3) ^C chr4 ^C , add(3) ^C , add(3) ^C chr4 ^{CO} , der(4) ^{CO} chr4 ^{CO} , add(3) ^{CO} , add(3) ^{CO}	likely a t(3;4)(q27;q31) and extra der(3)t(3;4); the 3q27 breakpoint distal to <i>BCL6</i> t(3;4) confirmed breakpoint occurred in the 4q32.3 region flanked by RP11-336N6 and RP11-440L13
NLPHL (D)	no mitosis		LSI <i>BCL6</i> ^{CO} RP11-336N6 ^C RP11-440L13 ^D	3q27 - <i>BCL6</i> flanking probes 4q32	2-4GO/1O ^C 1GO/1O/1-3G ^C	likely a t(3;4)(q27;q31) with the breakpoint distal to <i>BCL6</i> and extra copies of the der(3)

^adel(7) in case 2, del(9) in case 3 and del(4) in case 4 are referred to as der(7), der(9) and der(4), respectively; ^bD, diagnosis; P, progression; ^c(^C), green signal; (^O), orange signal; ^dchromosomal localization of green (^C) and orange (^O) signals; ^eresults of interphase FISH analysis.

translocation likely occurred in the distal region of 405L18 [smaller signal on the der(3) than on the der(9)], the candidate partner genes affected by the t(3;9) are *GRHPR* (glyoxylate reductase/hydroxypyruvate reductase) and *KIAA0354*, defined as leukemia/lymphoma related factor cLRF (<http://cds.celera.com>). Cytogenetic and FISH findings in this case suggest that the t(3;9) was already present in the primary stomach DLBCL (1991) which likely relapsed 9 years later as a nodal DLBCL. Interestingly, the same translocation appeared as a karyotypic feature of NLPHL diagnosed 3 years after the clinical manifestation of stomach DLBCL. Altogether, it seems that both disorders originated from the same precursor B cell targeted by the *BCL6* rearrangement. Further acquisition of distinct molecular hits possibly led to development of either aggressive DLBCL or indolent NLPHL. A similar scenario of shared and distinct pathological pathways in the development of classical Hodgkin's lymphoma and subsequent or concurrent B-NHL were suggested by Bräuninger *et al.*,³¹ who provided evidence of a common as well as lymphoma-specific somatic mutations of *IGH/L* variable-region genes in two such cases.

The most puzzling results were obtained in case 4. Metaphase FISH analysis performed on a nodal DLBCL sample from this patient led to identification of the t(3;4)(q27;q32) with the breakpoint telomeric to *BCL6*. Southern blot analysis of this sample, however, demonstrated rearrangement of *BCL6* within the MTC region. These contradictory FISH and molecular findings might be explained by the occurrence of two distinct molecular events operating on the 3q27 region namely, translocation with the breakpoint outside *BCL6*, and an independent internal deletion of *BCL6* affecting the MTC. The 4q32 breakpoint of this translocation was narrowed down to the approximately 1.5 Mb region (166.775–168.304 Mb) encoding at least 7 known genes (<http://www.ensembl.org>). Findings of a similar LSI *BCL6* and 4q32 (336N6 and 440L13) signal pattern in interphase cells from DLBCL and NLPHL samples from that patient suggest that the aforesaid 3q27 events occurred in the precursor NLPHL B cell, which during clonal proliferation subsequently acquired additional molecular hits and evolved to an aggressive DLBCL. Analogous 3q27 rearrangements clustering in the alternative translocation breakpoint region (ABR) 5' to *BCL6* have been recently identified as a recurrent event in DLBCL.^{19,31} Molecular consequences of these translocations are not known but might result in deregulation of *BCL6* by its juxtaposition to distantly acting, heterologous transcriptional regulatory elements.¹⁹ The ABR was

mapped 245–285 kb upstream of the first exon of *BCL6*, and is thus probably covered by the 5' LSI *BCL6* probe (300kb), found to be split in the present case. Also internal *BCL6* deletions have been recurrently found in DLBCL and these rearrangements occur in the same region as t(3q27)-associated breakpoints and the somatic hypermutations cluster.¹⁸ On Southern blotting using a single DNA probe, these molecular alterations might be indistinguishable from a rearrangement caused by chromosomal translocations.³² Whether internal deletion of *BCL6* and t(3;4) targeted the same or two different *BCL6* alleles in the present case remains unknown.

The molecular mechanisms by which genomic alterations of *BCL6* lead to lymphomagenesis are poorly understood but it is believed that the rearranged *BCL6* gene might promote development of lymphoma by prolonging the exposure of targeted germinal center B cells to a mutagenic environment, allowing secondary oncogenic hits to accumulate.³³ This concept is fully supported by our finding of frequent *BCL6* rearrangements in NLPHL,¹⁰ which behaves clinically as an indolent disorder but shows complex genomic aberrations at a cytogenetic level.⁹

In summary, using FISH we characterized 3q27-associated rearrangements in 4 cases of NLPHL and found that the spectrum of these aberrations is similar to that reported in DLBCL. These findings together with previous observations of a similar frequency of *BCL6* rearrangement (>40%) in NLPHL and DLBCL provide further evidence for a germinal center B-cell-derived origin of NLPHL and a biological relationship between these two entities of lymphomas. This relationship was additionally illustrated in two of our NLPHL patients who subsequently developed DLBCL. Identification of the same type of *BCL6* rearrangement in both lymphomas suggests their common origin from the same GC-derived precursor B cell.

All the authors contributed to the manuscript. IW was the main investigator who designed the study, critically analyzed the data and wrote the paper; MS performed molecular analyses; CDW-P provided patients' material and diagnosis; AH was involved in the design of the study, revised the manuscript and gave the final approval for its submission. Primary responsibility for the paper, figures and tables: IW and MS. The authors are grateful to Ursula Pluys for skillful technical assistance in FISH analyses, Magda Dehean and Kathleen Doms for cytogenetic analyses and Rita Logist for editorial help.

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