

blood

1993 81: 136-142

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***FVT-1*, A Novel Human Transcription Unit Affected by Variant Translocation t(2;18)(p11;q21) of Follicular Lymphoma**

By Ruth Rimokh, Mylène Gadoux, Marie-Françoise Berthéas, Françoise Berger, Monique Garoscio, Gilbert Deléage, Daniel Germain, and Jean-Pierre Magaud

Variant t(2;18) and t(18;22) chromosome translocations observed in B-cell chronic lymphocytic leukemias and in follicular lymphomas have been reported to consistently involve the 5' region of the *BCL-2* gene on chromosome 18 and various regions on the Ig light chain loci. We show here that a variant t(2;18)(p11;q21) translocation observed in a case of follicular lymphoma leads to the juxtaposition of a J_{κ} segment to a chromosome 18 transcriptional unit located 10 kpb upstream of the *BCL-2* locus. The cDNA of this new evolutionarily conserved gene, termed *FVT-1* for

follicular-variant-translocation gene, codes for a putatively secreted protein of 36 Kd that is not homologous with any described protein. The *FVT-1* gene is weakly expressed in all the analyzed normal hematopoietic tissues but a very high rate of transcription is observed in some T-cell malignancies and in phytohemagglutinin-stimulated lymphocytes. The proximity of *FVT-1* to the *BCL-2* locus suggests that in the t(14;18) currently observed in follicular lymphomas, both genes would participate in the tumoral process. © 1993 by The American Society of Hematology.

THE t(14;18)(q32;q21) translocation is a consistent feature of a large proportion of non-Hodgkin's B-cell lymphomas, usually of follicular type.¹ As a result of this translocation, the *BCL-2* proto-oncogene, on chromosome 18, is juxtaposed to an Ig JH sequence on chromosome 14.² More recently, variant t(2;18)(p11;q21) and t(18;22)(q21;q11) translocations have been described in B-cell chronic lymphocytic leukemias³ and in follicular lymphomas.^{4,5} Molecular studies of these variant translocations in B-CLL and follicular lymphomas have shown that the chromosomal breakpoints consistently map to the 5' region of the *BCL-2* gene (5' hot spot region or VCR region for variant cluster region) on chromosome 18 and within the κ or λ light chain locus on chromosomes 2 and 22, respectively.^{3,6,7}

In a previously published work⁸ we reported the molecular study of a t(2;18)(p11;q21) translocation that involved the κ light chain locus in a patient with a follicular lymphoma. Although rearrangement of *BCL-2* was not Southern-blot detectable in this lymphoma, mRNA and protein studies showed that *BCL-2* was strongly expressed in this tumor. In the present work we performed the molecular cloning of the t(2;18) chromosomal breakpoints, and showed that a J_{κ} segment was juxtaposed to a chromosome 18 coding region (termed *FVT-1* for follicular lymphoma variant translocation) unrelated to the *BCL-2* gene.

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Submitted April 8, 1992; accepted August 27, 1992.

Supported by grants from ARC (6741), Ligue Contre le Cancer (Comités du Rhône et de l'Yonne), CNRS, and Hospices Civils de Lyon.

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0006-4971/93/8101-0002\$3.00/0

MATERIALS AND METHODS

Cells. Various fresh samples of normal and tumoral human tissues, human cell lines of hematopoietic origin, and fresh samples of murine tissues were used in this study.

Peripheral blood lymphocytes (PBL) were obtained from the blood of normal volunteers by centrifugation on Triosil Ficoll (Seromed, Berlin, Germany). After monocyte depletion by adhesion on plastic Petri dishes, mitogen-stimulated lymphocytes were obtained by incubating PBL at a concentration of 2×10^6 cells/mL in the presence of phytohemagglutinin (PHA; Eurobio, Paris, France) diluted 1:200 in RPMI 1640 medium supplemented with 20% fetal calf serum plus 0.03% L-glutamin, 100 μ g/mL of penicillin G and 100 μ g/mL of streptomycin sulfate (Institut Mérieux, Lyon, France). *FVT-1* RNA level was then assessed at various times after PHA stimulation.

DNA and RNA isolation and analysis. High molecular weight DNA was extracted from fresh cells or frozen material following standard procedures.⁹ After digestion with appropriate endonucleases as recommended by the suppliers (Boehringer Mannheim GmbH, Mannheim, Germany), DNA fragments were electrophoresed on 0.8% agarose gels and transferred onto nylon filters.¹⁰

Total cellular RNA was isolated from cultured cell lines or from frozen samples by the acid guanidium thiocyanate-phenol-chloroform method.¹¹ Poly(A⁺) RNA was selected by chromatography on oligo(dT)-cellulose. For Northern blot analysis, 10 μ g of total RNA, 2 or 5 μ g of poly(A⁺) RNA was size fractionated in formaldehyde-1.2% agarose gels and transferred onto nylon filters.¹²

Preparation and analysis of DNA and cDNA libraries. DNA from the tumoral lymph node (CAM) was partially digested with *Mbo*I restriction endonuclease, DNA fragments averaging 15 to 20 kpb in length were selected by sucrose gradient centrifugation and ligated to *Bam*HI arms of the EMBL 3 phage (Stratagene, La Jolla, CA). Library screenings were performed with probes for Ig κ light chain joining region gene (J_{κ}), for Ig κ light chain constant region gene (C_{κ}), and with probe p140 mentioned in Fig 1. The recombinant positive clones were characterized by restriction endonuclease mapping and subclones were prepared in pUC18, pUC19 or Bluescript vectors.

Poly(A⁺) RNA extracted from the IARC 171 Epstein-Barr virus (EBV)⁺ lymphoblastoid cell line was used to construct an oligo-dT primed human cDNA library in the λ gt11 vector as recommended by the suppliers (Pharmacia, Uppsala, Sweden).

DNA probes and hybridization procedures. J_{κ} probe was a gift from Dr H. Zachau (Munich, Germany), probe C_{κ} was a gift from Dr T.H. Rabbitts (Cambridge, UK), pB16 (*BCL-2*) probe was a gift from Dr Y. Tsujimoto (Philadelphia, PA). Probe α -³²P-labeling, pre-hybridization, hybridization, and washing conditions were performed as previously described.¹²

Sequencing procedures and sequence analysis. Overlapping deletions of DNA and cDNA cloned into Bluescript SK (-) (Stra-

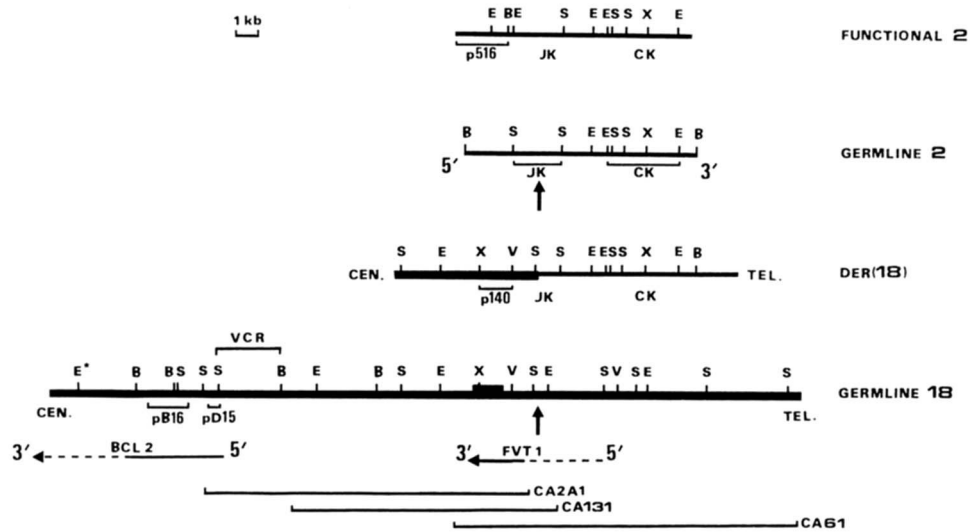


Fig 1. Restriction maps of the germline and functionally rearranged κ light chain alleles, of the rearranged $J_{\kappa}/FVT-1$ allele, and of the normal $FVT-1$ allele. The last exon of $FVT-1$ is indicated by a black box. Thin black bars, chromosome 2 DNA regions; thick black bars, chromosome 18 DNA regions. Arrows indicate the breakpoints on chromosomes 2 and 18. Slided horizontal lines under the maps indicate the limits of the clones originating from germline chromosome 18 (CA2A1, CA131, CA61) and the location of the probes used in this study. VCR, variant cluster region; E, *EcoRI*; B, *BamHI*; S, *SacI*; X, *XbaI*; V, *EcoRV*; E*, polymorphic *EcoRI* restriction site. All *EcoRV* and *XbaI* sites are not shown.

tagene) were obtained by unidirectional exonuclease III digestion method (Erase-a-base system; Promega, Madison, WI). Deletion clones were sized on agarose gels, and the inserts of the selected clones were sequenced using the double-stranded DNA sequencing technique (dideoxy chain termination procedure) with Sequenase II (USB Corp, Cleveland, OH) as described by the manufacturer.

RESULTS

Molecular cloning of the chromosomal translocation t(2;18)(p11;q21) breakpoints and of the normal chromosome 18 counterpart. Previously reported Southern blot analyses have shown that the chromosomal breakpoint on chromosome 2 occurred within or 5' of the J_{κ} segment in a case of follicular lymphoma⁸ (CAM). To analyze this rearrangement, a genomic phage library prepared from CAM cells was screened with a J_{κ} probe. Physical mapping of the bacteriophage recombinant clones allowed their separation into two distinct groups different from one another in their 5' part; the restriction map of their 3' end was identical and matched that of the germline J_{κ} -C κ region. Southern blot analyses of interspecies somatic hybrids DNA using probes derived from the 5' region of these two types of clone (probes p516 and p140) (Fig 1) enabled us to state that one group of recombinant clones originated from the functionally rearranged κ light chain allele and that the others contained sequence originating from the chromosome 18. We were not able to isolate the germline κ light chain allele. The p140 probe was then used to isolate the chromosome 18q21 counterpart from a genomic phage library prepared from normal donor leukocytes, and three overlapping clones (CA2A1, CA131, and CA61) representing a 25-kpb region of chromosome 18 were isolated (Fig 1). Several unique DNA fragments that span this 25-kpb region were used as probes against Southern blots containing normal DNAs digested by *EcoRI*, *HindIII*, and

BamHI restriction endonucleases. One of these fragments, pD15 probe in Fig 1, hybridized with the same bands as did the pB16 probe that maps to the 5' region of the *BCL-2* gene. Furthermore, nucleotide sequence of the pD15 probe perfectly matches that of the *BCL-2* 5' region.¹³ These results clearly showed that, on chromosome 18, the breakpoint

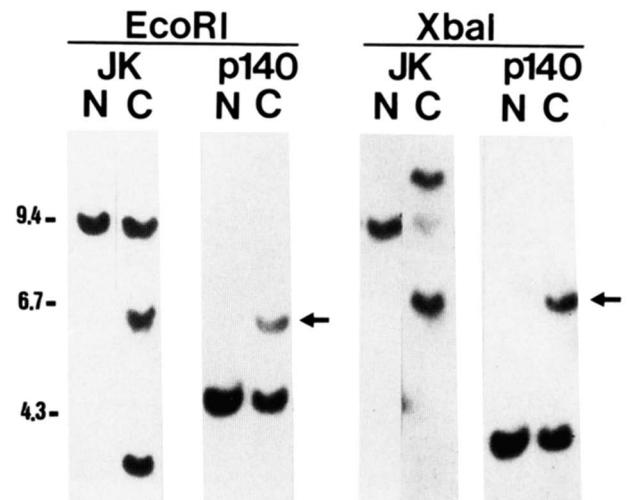


Fig 2. The $t(2;18)(p11;q21)$ results in the juxtaposition of the J_{κ} light chain segment to the $FVT-1$ locus. Ten micrograms of genomic DNA extracted from the tumoral lymph node (C) and from normal peripheral blood lymphocytes (N) was digested with the indicated enzymes, electrophoresed through a 0.8% agarose gel, and blotted onto a nylon membrane. The filters were sequentially assayed for hybridization to the J_{κ} and p140 probes. Arrows indicate the position of the rearranged fragments cohybridizing with both probes. The scale is in kilobases.

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GERM 18  TGGTATCAAGGAGTCATTGAAATGTTTTCATGCAATGATAGTTGCAGTTTAGAAACCACTGGTGTGGAAAATGTGAGGGTACACTCAAATCGAT
DER(18)  TGGTATCAAGGAGTCATTGAAATGTTTTCATGCAATGATAGTTGCCGTTTCCCGGAGGGACCAAGGTGGAGATCAAACGTAAGTGCACCTTTCC
GERM 2   GAGGTTTTTGTGAGGGGAAAGSGTGAGATCCCTCACTGTGGCTCACCTTCGGCGGAGGGACCAAGGTGGAGATCAAACGTAAGTGCACCTTTCC

```

J4

Fig 3. Nucleotide sequence of the t(2;18) translocation and of the corresponding normal chromosomes 2 and 18. Slided-horizontal lines under the sequence indicates the limits of the J4 κ light chain domain; single underlining identifies the heptamer-like sequences on chromosome 18; double underlining identifies the putative N-region on the der(18) chromosome. Slided-horizontal lines above the sequence indicate the location of the heptamer and nonamer consensus on chromosome 2.

was located at about 13 kpb upstream of the first exon of *BCL-2*.

Southern blot analysis using p140 probe detected a single rearranged fragment in addition to the germline band in *EcoRI*- and *XbaI*-cleaved CAM DNA. This rearranged fragment clearly cohybridized one of the two rearranged bands detected by $J\kappa$ probe (Fig 2).

Nucleotide sequence analysis of breakpoints. We next determined the nucleotide sequence spanning the breakpoint on the der(18) chromosome and on the normal chromosome 18 counterpart (Fig 3). The break occurred within the J4 segment of the $J\kappa$ region¹⁴; the existence of eight extranucleotides at the junction (putative N region?) and of two heptamer-like consensus on chromosome 18 in the vicinity of the breakpoint suggests that this translocation might have resulted from an illegitimate chromosome recombination.

Identification of a transcriptional unit on 18q21. To identify transcripts that might arise from 18q21, we identified unique fragments that spanned 18q21 sequences and used them as probes against Northern blots containing RNA from CAM cells and from different human cell lines. The 1.4-kpb p140 probe (Fig 1) of chromosome 18 origin that remained on the der(18) hybridized to a transcript of approximately 2.3 kb present in poly(A⁺) RNA extracted from CAM cells, IARC 171 lymphoblastoid cell line, and normal human

spleen (Fig 4). This transcript can be detected in total RNA extracted from the various normal and tumoral human tissues and cell lines of hematopoietic origin being analyzed (Fig 5). It is noteworthy that the highest expression of the *FVT-1* transcript was observed in some T-cell malignancies and especially in large cell anaplastic lymphoma ("Ki-1 lymphomas") with a T-cell phenotype and genotype (Fig 5). Furthermore, while *FVT-1* transcript is barely detectable in quiescent PBL, PHA stimulation results in an increase of *FVT-1* RNA with a peak at 18 hours (Fig 6).

Once total RNA extracted from follicular lymphomas carrying a t(14;18)(q32;q21) has been hybridized to the *FVT-1* cDNA probe, a weak 2.3-kb signal is observed (Fig 5). To check that expression of *FVT-1* in fresh samples of follicular lymphomas is not caused by the presence of normal T cells, we assessed its expression in poly(A⁺) mRNA extracted from cell lines carrying t(14;18) translocation with a break in the

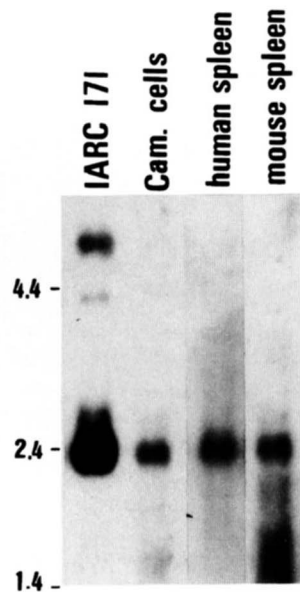


Fig 4. Identification of a transcriptional unit on chromosome 18q21. Five micrograms of poly(A⁺) RNA extracted from the indicated tissues and cell line were loaded in each lane, size-fractionated in formaldehyde-1.2% agarose gel, transferred onto nylon filters, and hybridized to the p140 probe. IARC 171 is a human B-cell EBV⁺ lymphoblastoid cell line. The scale is in kilobases.

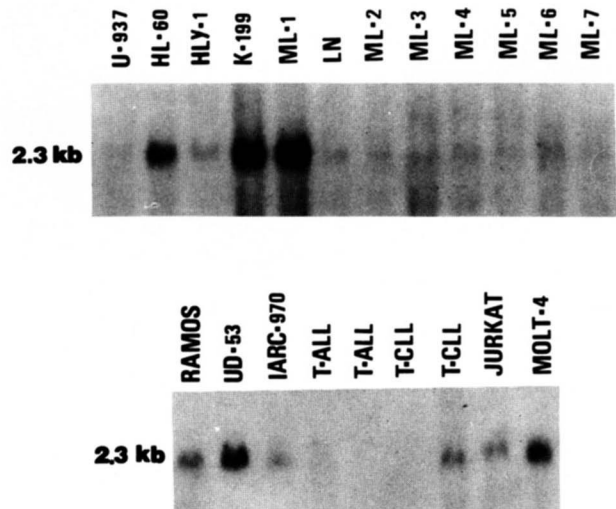
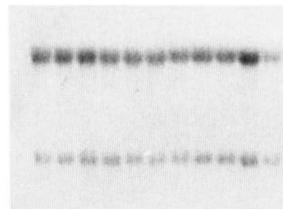


Fig 5. Northern blot analysis of *FVT-1* expression in tissues and cell lines of hematopoietic origin. Ten micrograms of total RNA was loaded in each lane and hybridized to the *FVT-1* cDNA probe. The cell lines used in this study are of B origin (RAMOS, IARC 970, UD 53), of myelomonocytic origin (U937, HL60) and of T origin (JURKAT, MOLT-4). K-199 is a cell line established from a large anaplastic T-cell lymphoma¹⁶ and HLY1,¹² a cell line established from a large anaplastic B-cell lymphoma. Fresh normal or pathologic samples were also used: LN, reactive lymph node; ML-1, large anaplastic T-cell lymphoma; ML-2,3, B-cell non-Hodgkin's lymphomas; ML-4 to ML-7, follicular lymphomas; T-CLL, T-cell chronic lymphocytic leukemias; T-ALL, T-cell acute lymphoid leukemias.

Et. br.
Stain

0 8 12 18 24 30 36 42 48 72 96 hours

2.3 kb



Fig 6. *FVT-1* expression after PHA stimulation of PBL. Total RNA was extracted and processed for Northern blot analysis at the indicated times as described in Materials and Methods. The ethidium bromide staining is shown at the top; for the most part, lanes contain comparable quantities of RNA (10 μ g).

major breakpoint region of the *BCL-2* gene locus. As shown in Fig 7, a normal 2.3-kb *FVT-1* transcript is detected as well. However, we cannot specify if *FVT-1* transcript arises either from the normal or from the translocated allele in these lymphomas and cell lines analyzed.

Cloning and sequencing of the human FVT-1 cDNA. Polyadenylated RNA was used as a template to construct a λ gt11 cDNA library that was screened with the p140 probe mentioned in Fig 1. Nine positive clones were isolated, their restriction mapping and their partial sequences showed that they all coded for the same protein. The longest cDNA clone was sequenced in both strands by generating progressive deletions and by dideoxy sequencing procedure (Fig 8). The sequence exhibits an open reading frame coding for a peptide of 332 amino acids, predicted molecular weight 36 Kd, and a 1,200-nt long 3' untranslated region. The latter region is rich in A/U nucleotides (62.5%) and contains two AUUUA motifs; all elements are implicated in the posttranscriptional regulation of genes with unstable transcripts¹⁶⁻¹⁸ such as certain proto-oncogenes and cytokines. The 3' untranslated region of *FVT-1* is also characterized by the presence of a 80-nt long d(CT)-rich pyrimidine tract including a TCTCTCTCTG tandemly repeated polynucleotide. The 5' part of the cDNA (nucleotide 1 to 307) is characterized by a high G + C level (68%) and corresponds to a CpG island. This CpG island accounts for the existence, 5' of *BCL-2*, of

a second cluster of rare-cutting restriction site as previously observed.¹⁹ There is an in-frame terminator codon (TGA) 87 bases upstream of the initiation codon. None of the *FVT-1* cDNA clones contained a poly(A) tail. However, because the clones were obtained from an oligo-dT primed cDNA library, it is likely that all the cDNA clones were initiated from the numerous poly(A) stretches present within the 3' end of the mRNA. When a genomic clone encompassing the terminal *FVT-1* exon had been sequenced and compared with the longest *FVT-1* cDNA clone, an AATAAA polyadenylation signal was noted 113 nucleotides 3' of the end of the cDNA clone. These data indicate that the 3' end of a full-length *FVT-1* cDNA clone is, in all likelihood, 20 to 30 nucleotides 3' of this polyadenylation signal. This assumption is in agreement with the observed size of the *FVT-1* transcript.

Comparison of the *FVT-1* cDNA sequence with those in data bases (EMBL and GenBank, NBRF and SWISS PROT, February 1992) did not show any significant homologies. The hydrophobic profile of *fv-1* obtained by the Kyte and Doolittle program²⁰ showed a unique region of hydrophobicity (amino acids 1 to 24) at the N terminus of the protein with a potential cleavage site at position 25. *fv-1* is unlikely to be a membrane protein as no further hydrophobic region, which could span the membrane, is found; this suggests that it is secreted.

Comparison of the genomic DNA sequence with the 3' end of the cDNA sequence allowed us to specify the boundaries of the last *FVT-1* exon (Fig 8) and to demonstrate that the break on chromosome 18 occurred within the last intron of *FVT-1* (Fig 1). As a result of the translocation process, the *FVT-1* gene is thus disrupted and the promoting region plus the 5' part of the coding sequence is juxtaposed to the V_{κ} - J_{κ} region of the κ light chain gene in a tail-to-tail configuration. The complete genomic sequence and organization of the *FVT-1* locus have not been determined so far; however, preliminary results show that the gene contains more than four exons extending over about 40 kpb.

The FVT-1 gene is highly conserved in evolution. Zoo-blot analysis using a probe encompassing nucleotides 1 to

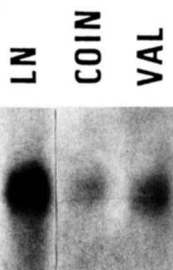


Fig 7. Northern blot analysis of *FVT-1* expression in human t(14;18)(q32;q21) containing cell lines. Two micrograms of poly(A⁺) RNA were loaded in each lane and hybridized to the *FVT-1* cDNA probe. LN, reactive lymph node; COIN and VAL, human cell lines derived from patients with follicular lymphomas.

2.3 kb

	AGGCGCCC	8
	BssHII	EagI
GCCCGCCGCGGTGATTCCTCGCTCGCCGAGCCAGCCCTCGCGCCTTGCCCGCGGCCCGCCCGCCGCTCCGGGCCCTGGCCCGCGGAGCG		107
ATGTGCTGTGCTGGCTGCCGCTTCTCTGCTGGCTTCTGCTGCTGTACATGGTGTCTCCGCTCATCAGCCCCAAGCCCTCGCCCTGCCCGGGCG	SmaI	206
MetLeuLeuLeuA1a1a1a1aPheLeuVal1a1aPheVal1LeuLeuLeuTyrMetVal1SerProLeuI1eSerProLysProLeuA1aLeuProGlyA1a		33
CATGTGGTGGTTACAGGAGGTTCCAGTGGCATCGGGAAGTGCAATTGCTATCGAGTGCTATAACAAGGAGCTTTTATAACTCTGGTGCACGAAATGAG		305
HisVal1Val1ThrGlyGlySerSerGlyI1eGlyLysCysI1eA1a1eGlyCysTyrLysG1nGlyA1aPheI1eThrLeuVal1a1aArgAsnG1u		66
GATAAGCTGCTGCAGGCAAGAAAGAAATGAAATGCACTCTTAATGACAAACAGGTGGTCTTGCATATCAGTTGATGTATCTCAAGACTATAAC	PstI	404
AspLysLeuLeuG1nA1aLysLysG1uI1eGlyMetHisSerI1eAsnAspLysG1nVal1Val1LeuCysI1eSerVal1AspVal1SerG1nAspTyrAsn		99
CAAGTAGAGAAATGTCATAAAACAAGCACAGGAAACTGGGTCCAGTGGACATGCTGGTAAATTTGTCAGGAATGGCAGTGCAGGAAAATTTGAAGAT	BglII	503
G1nVal1G1uAsnVal1I1eLysG1nA1aG1nG1uLysLeuG1yProVal1AspMetLeuVal1AsnCysA1aGlyMetA1aVal1SerGlyLysPheG1uAsp		132
CTTGAAGTTAGTACCTTGAAGGTTAATGAGCATCAATTACCTGGGACGCTGTACCCAGCCGGCCGTGATCACCACCATGAAGGAGCGCCGGGTG		602
LeuG1uVal1SerThrPheG1uArgLeuMetSerI1eAsnTyrLeuGlySerVal1TyrProSerArgA1aVal1I1eThrThrMetLysG1uArgArgVal1		165
GGCAGGATCGTGTGTTGTGCTCCAGGACAGGACAGTTGGGATATTCGGTTTACAGCCTACTCTGCATCCAAGTTGCCATAAGGGGATGGCAGAA	Hfi	701
GlyArgI1eVal1PheVal1SerSerG1nA1aGlyG1nLeuGlyLeuPheGlyPheThrA1aTyrSerA1aSerLysPheA1aI1eArgGlyLeuA1aG1u		198
GCTTTGCAGATGGAGGTGAAGCCATAATGTCTACATCACAGTTGCTTACCACCAGACACAGACACCTGGCTTGGCGAAGAAAACAGAACAAG	ndIII	800
A1aLeuG1nMetG1uVal1LysProTyrAsnVal1TyrI1eThrVal1A1aTyrProProAspThrAspThrProGlyPheA1aG1uG1uAsnArgThrLys		231
CCTTTGGAGACTCGACTATTTTCAGAGACCACATCTGTGTGCAAACAGGAGGCAACAATTGTTAAAGATGCCATACAAGGAAATTTCAAC		899
ProLeuG1uThrArgLeuI1eSerG1uThrThrSerVal1CysLysProG1uG1nVal1A1aLysG1nI1eVal1LysAspA1aI1eG1nGlyAsnPheAsn		264
AGTTCCTTGGCTCAGATGGGTACATGCTCTCGCCCTGACCTGTGGATGGCTCCAGTAACCTCTATTACTGAGGGGCTCCAGCAGGTGGTCAACATG		998
SerSerLeuGlySerAspGlyTyrMetLeuSerA1aLeuThrCysGlyMetA1aProVal1ThrSerI1eThrG1uGlyLeuG1nG1nVal1Val1ThrMet		297
GGCCTTTCCGCACTATTGCTTTGTTTACCTTGGAAAGTTTGACAGCATAGTTCGTGCTGCATGATGCAGAGAGAAAACTGAAAATGCAGACAAA		1097
GlyLeuPheArgThrI1eA1aLeuPheTyrLeuGlySerPheAspSerI1eVal1ArgArgCysMetMetG1nArgG1uLysSerG1uAsnA1aAspLys		330
ACTGCCTAATCTTCTTACCCCTTGAAGAAGACTGTTTCCAATAATTTGAACAGCTGCTGCTAAATGGGACCCAATTTTGGCCTATAGACACTTAT		1196
ThrA1a***		332
GTATTGTTTTCGAATACGTACAGATTGGACAGTGCCTTCAGGAAATGGTGCAGCAAGCAAGGGGCTAGAAGTTCACCTCTGACAGTATTATTAATACT		1295
ATGCAAATAAGGAATAGGAGACCATTTGATTCTAGGCTTTGTGGTAGAGAGGTGAAGGTATGAGAATTAATAGCGTGTGAACAAAGTAAGAACAGG		1394
ATTCAGAAATGATCATAAAATTTGTTTCTATTTATCTTTTTGCCCCCTAGAGATTAAGTCCAGAAAATGACTTTCTGGCACAATAAGAAATCTTGA		1493
GGACTTTGTTTAAACCTTCCATAAAAAACAATTTTCGGTTTCTCGGGTCTCTCTCTCTGTCTCTCTGTCTCTCTGTCTCTCTGTCTCTCT		1592
TCTCTCTCTCTCTCTCTCTCTTTCTTTGTGTATTTTATTCAAGATGAGTTGGACCCATTGCCAGTGAGTCTGAATGTCACTGACAGCCCTGTGTTGTGCTCAG		1691
GACTCACTGCTGCTGGTGGAACTCATGGCTTCTCTCTCTTTGATCCCAAAAGCTACGAGGGGACGGGAGAGGGCAGTGCATGGGAAGTAA		1790
GAGATATTTCCAGTAGGAAAAGCAATGCTTTCTGTCTTTAGACTCAAATGCTTAGGGAACGTTTCATTTCTATTTCATGGGAAAGGCAGCCTCCTT		1889
AAATGTTTTCTGAAGAGCGGTAATACTAGAAGCTTAAGAAATTTACAGTTCCTCAATAACCATGATGACCTGAAGTTACCTATCCCAATTTAGCATC	XbaI HindIII	1988
TACTGTTTTTCCATCTCTCTCTTCCAATTTGCTTACTGCTGTAATATTTTGTAAAAAAGGAAAAAAGACCAGCTAAAAATTT		2087
TCGACTTGACTTTTTAACTTAACATCATGAATTAATAAGCAAATGAAAAAATAAAAAGTGTGACTTTTTCTCGGAGCATATATGTAGCTTTTAGGAA		2186
AGGCTGATGATGGTATAAAGTTGCTCATTAAGAAAAAGACAAGGCTGATTTGAAGAGAGTTGCTTTGAAATAAATGATCA		2272

Fig 8. Nucleotide and predicted amino acid sequence of the *FVT-1* cDNA. Underlining indicates: the in-frame terminator codon (TGA) upstream of the first ATG, the ATTTA motifs and the candidate polyadenylation site (AATAAA). The arrow shows the beginning of the last exon.

320 of the cDNA sequence (Fig 8) showed that sequences homologous to *FVT-1* can be detected in the mouse and chicken genome (Fig 9). It must be also pointed out that a transcript with approximately the same size as the human transcript is observed in murine tissues (Fig 4).

DISCUSSION

It has been shown that in Burkitt's lymphomas the proto-oncogene *MYC* can rearrange at the 5' or 3' regions of the gene with IgH and Ig light chain genes, respectively. However, in some instances these tumors contain variant translocations with breakpoints located more distant from *MYC* in the *PVT* locus that extends from 57 kpb to more than 200 kpb downstream of *MYC*.²¹ A very similar situation is observed in

follicular lymphomas where the t(14;18) translocations consistently involve the 3' end of *BCL-2* and the variant translocations t(2;18) and t(18;22) its 5' end. To make the analogy more complete, we report here that the breakpoint of a variant translocations t(2;18) in a follicular lymphoma fall within a region 13 kpb proximal from *BCL-2* and containing a novel transcriptional unit that we termed *FVT-1*.

The *FVT-1* cDNA encodes for a putatively secreted protein of 332 amino acids that has no homologue in the EMBL and Genbank data bases. This evolutionarily conserved gene is virtually expressed in all the normal and pathologic lymphoid tissues tested with a preferential expression in T-cell malignancies and in PHA-stimulated PBL. The expression profile of *FVT-1* after PHA stimulation, the existence of a potential

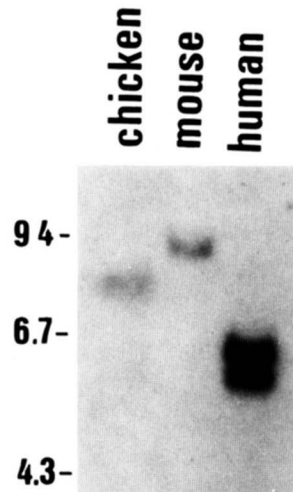


Fig 9. The *FVT-1* locus is conserved in evolution. For Zoo-blot analysis, 10 μ g of *Eco*RI-digested DNA extracted from the indicated species were hybridized under high-stringency conditions to the 0.3-kbp *Pst*I cDNA segment. The scale is in kilobases.

signal peptide in its amino acid sequence, and the presence, in the cDNA, of a 3' end characteristic of genes with unstable transcripts suggest that *FVT-1* could be a new cytokine mainly expressed by activated normal T cells and by some T-cell malignancies. Further experiments are indeed necessary to support this assumption.

We have previously reported that in the follicular lymphoma studied in this work,⁸ the *BCL-2* gene was overexpressed at the mRNA and protein levels, probably as a consequence of the t(2;18) translocation. The present work makes this assumption more likely because we show that, in this translocation, the *BCL-2* gene is juxtaposed to the κ light chain locus in the vicinity of the 5' κ gene enhancer. On the other hand, the *FVT-1* gene seems unlikely to play a role in this lymphoma as the translocation results in the disruption of the *FVT-1* gene and should lead to the constitution of a chimeric gene where the V_{κ} - J_{κ} domains and the 5' region of the *FVT-1* gene are in a tail-to-tail configuration on the der(2) chromosome. However, we were not able to detect any fusion transcript with the J_{κ} probe and cDNA *FVT-1* probes. Because of its proximity to the *BCL-2* gene, the *FVT-1* gene could be involved in the t(14;18) translocation usually observed in follicular lymphomas. Effectively, in the t(14;18) translocation with break at the 3' end of *BCL-2*, the *FVT-1* and *BCL-2* genes are juxtaposed to the Ig heavy chain locus and might both be deregulated as a result of the same mechanisms. The finding that *FVT-1* is expressed in t(14;18) associated lymphomas and cell lines analyzed could be an argument in favor of this hypothesis. Further studies are necessary to specify whether expression of *FVT-1* in t(14;18) containing tumoral cells results from its juxtaposition to *cis*-acting sequence from the IgH locus or not.

With regard to the recombination mechanism involved in this t(2;18) translocation, the existence of two heptamer-like consensus on chromosome 18 in the vicinity of the breakpoint and the presence of a putative N region at the chromosomal junction suggest that this translocation might have resulted from an illegitimate chromosome recombination.²² However, the d(CT)-rich pyrimidine tract located 2 kbp from the breakpoint could also be implicated in the translocation pro-

cess. Such a stretch, which can adopt a triple helical structure,²³ has been involved in recombination, replication, and gene expression; the resulting change in DNA conformation within this region could enhance its accessibility to the recombinational enzymes, leading to the chromosome translocation.

Finally, this work points out that the breakpoints of the t(2;18) variant translocations in follicular lymphomas do not always cluster within the *BCL-2* VCR region. Thus, *FVT-1* probes constitute additional molecular tools in an attempt to widely identify all the breaks occurring on chromosome 18q21 in B-cell chronic lymphocytic leukemias and follicular lymphomas.

ACKNOWLEDGMENT

We gratefully acknowledge Jacques Samarut for helpful discussion, Laurent Duret for his help in nucleotide sequence analysis, Christian Bastard and Dominique Leroux for the gift of cell lines COIN and VAL, and Jean Alban Dohaslum for reading the manuscript. The sequence reported in this paper has been deposited in the EMBL data base (accession number: X63657).

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