Analysis of balanced rearrangements of chromosome 6 in acute leukemia: clustered breakpoints in q22-q23 and possible involvement of *c-MYB* in a new recurrent translocation, t(6;7)(q23;q32~36)

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Background and Objectives. Many clinically important oncogenes and tumor suppressor genes have been identified through analysis of recurrent chromosomal rearrangements in acute leukemia. The contribution of sporadic rearrangements to malignancy is less clear and few have been mapped in detail. In this study we investigated the significance of novel translocations and inversions of 6q in acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML).

Design and Methods. Breakpoints of balanced 6q rearrangements were mapped in sequential fluorescent *in situ* hybridization (FISH) experiments with BAC and PAC clones in 11 patients.

Results. Six of seven breakpoints in ALL and two in a single case of AML were localized to within a 10.5 Mb *hotspot* at 6q22-q23 with five analyzed to the level of a single probe. In two cases of childhood T-ALL, both carrying a t(6;7)(q23;q32~36), split FISH signals were produced by adjacent PAC, mapping the breakpoints to within a ~150Kb region containing the genes *c-MYB* and *AHI1*. Five similar rearrangements, four also in pediatric T ALL were identified in the literature. Other 6q22-q23 translocations mapped in detail interrupted regions containing no recognized genes. 6q breakpoints outside the q22-q23 region were widely dispersed and in two were mapped to positions overlapping the cloned fragile sites FRA6E and FRA6F. The involvement of *MLL* was demonstrated in one case with t(6;11)(q15;q23).

Interpretation and Conclusions. We identified a new primary recurrent translocation t(6;7)(q22;q23~q26) in pediatric T-ALL. Other translocations interrupting the 6q22-q23 breakpoint cluster region did not appear to be recurrent and may contribute to leukemogenesis through a novel mechanism.

Key words; chromosome 6, translocation, c-Myb, AHI1.

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Recurrent chromosomal translocations are important diagnostic and prognostic markers contributing to the management of patients with a variety of hematologic malignancies. Moreover, the molecular characterization of breakpoints from such rearrangements has led to the identification of oncogenes and to the design of novel therapeutic approaches and sensitive polymerase chain reaction (PCR)-based systems for monitoring residual disease. In addition to recognized recurrent abnormalities, numerous sporadic translocations have been reported and these may activate known or hitherto unrecognized oncogenes, contribute to leukemia through other mechanisms or be incidental to the malignant process. Among hematologic malignancies, balanced rearrangements of the long arm of chromosome 6 (6q) are most common in acute myeloid leukemia (AML) and frequently involve translocation with 11q23. Two recurrent t(6;11) translocations have been described and these fuse *MLL* (*multi lineage leukemia*) to *AF6* at band 6q27 or *AF6q21* at band 6q2.[1,2] The t(6;8)(q27;p11) that fuses the *FGFR1* (*fibroblast growth factor receptor 1*) gene to *FOP* (*FGFR1 oncogene partner*) is seen in rare cases of myeloproliferative syndrome.[3] Other translocations of 6q have been reported in patients with AML or acute lymphoblastic leukemia (ALL) although none has been cloned and most are currently considered sporadic (*Mitelman Database of Chromosome Aberrations in Cancer;* available at URL *http://cgap.nci.nih.gov/Chromosomes/Mitelman.* By contrast with translocations, cytogenetically visible deletions of 6q are common in lymphoid malignancies, with a reported incidence of 4-13% in ALL, 13-15% in lymphomas and 4.5% in chronic lymphocytic leukemia.[4-8] Both fluorescence *in situ* hybridization (FISH) and microsatellite analysis have been used to define region(s) of minimal deletion (RMD) in ALL and lymphomas.[9-21] Based on these studies several candidate tumor suppressor genes have been proposed but evidence in support of a leukemia protective role for any of these has

as yet been limited.[22-24] With the principle objective of identifying a RMD and candidate tumor suppressor genes we investigated 6q cytogenetic abnormalities in acute leukemia by FISH using PAC and BAC clones. Deletions of 6q defined by this study have been previously reported.[23] Here, we present a detailed map of the 6q breakpoints from translocations and inversions that showed no evidence for loss of chromosome 6.

Design and Methods

Patients' material

Fixed cell suspensions from bone marrow samples of patients with acute leukemia known to have chromosomal abnormalities involving 6q, and prepared for routine cytogenetic analysis were obtained from the Department of Haematology, Royal Free and University College School of Medicine. Further samples from patients with ALL were identified through the Leukaemia Research Fund UK Cancer Cytogenetics Group (UKCCG) Karyotype Database in Acute Leukaemia[25] and provided by the UKCCG laboratories. A single case (#11) was obtained on request from the Department of Hemato-Oncology, Placky University Hospital, Olomouc, Czech Republic. G-banded karyotypes were described according to the International System for Human Cytogenetic Nomenclature (ISCN).[26] Slides were made according to standard techniques and stored at -20°C for future analysis by FISH.

FISH probes and procedures

PAC (from libraries RPCI1, RPCI3 and RPCI5) and BAC (from library RPCI11) clones positioned at known locations on chromosomes 6 and 7 by the Human Genome Mapping Project (HGMP) were obtained on request through the UK HGMP Resource Center, Hinxton, Cambridge, United Kingdom (see *http://www.hgmp.mrc.ac.uk*) and the Children's Hospital Oakland Research Institute, BACPAC resources center (*http://bacpac.chori.org/home.htm*). DNA was prepared from 50 mL overnight cultures of the clones using a standard SDS lysis technique). Probes were prepared, labelled and hybridized as previously described.[27] Visualization of FISH signals was performed on an Axioplan fluorescence microscope (Karl Zeiss, Germany) equipped with appropriate filters (Chroma Technology, VT, USA) and MacProbe (Applied Imaging International, UK) or Quips SmartCapture FISH imaging software (version 2.3.2 Vysis UK). Probes labelled in Spectrum Red from either the 6q centromeric region (*RP1-71H19*) or the 6q sub-telomeric region (*RP1-167A14* or *RP1-57M24*) were hybridized in pairs with other 6q clones labelled with Spectrum Green. A primary panel of 23 Spectrum Green labelled probes, positioned within Sanger institute PAC/BAC chromosome 6 contigs (*http://www.sanger.ac.uk/HGP/Chr6/*), between the centromeric and telomeric clones, was used for initial investigations (Figure 1). Analysis of FISH signals was performed as previously described[23] with reciprocal translocations defined by the presence of red and green signals separated between two different chromosomes in metaphase cells. Breakpoints were located between the separated signals and a translocation defined as clonal if the separation was observed in a minimum of three cells and in not fewer than 20% of cells overall. By performing FISH experiments sequentially with different probes from the primary panel, translocation breakpoints for each patient were accurately located. Additional 6q probes were obtained to map the breakpoints of interest in greater detail.

Reciprocal partner chromosomes of the 6q translocations were investigated with whole chromosome paint (*wcp*) probes and single locus clones. Wcp probes, derived from flow sorted chromosomes and pre-labelled with Spectrum Green or Spectrum Red, were obtained from Cambio Ltd, Cambridge, UK. A two-colour FISH probe (LSI MLL Dual Colour, Break Apart Rearrangement Probe), designed to detect rearrangements within the *MLL* gene, was purchased from Vysis, UK. Hybridization and washing were performed according to the manufacturers' protocols. YAC 14e-e12, containing the coding sequence of *EVI1/MDS1* located to 3q26 was kindly supplied by Dr L. Kearney (MRC Leukaemia Cytogenetics Group, Institute of Cancer Research, London, UK). YAC DNA was prepared and used for FISH analysis as previously described.13 PAC clones, RP1-167F23 and RP5-1200I23, which flank the cluster of *HOXA* genes at 7p15, and PAC clones, *RP5-1121A15* and *RP4-639J15*, positioned proximal and distal to *HLXB9* at 7q36, were used as paired Spectrum Green and Spectrum Red labelled probes as described above.

Results

Primary FISH analysis of 6q rearrangements, revised G-banded karyotypes and clinical details

The chromosome 6q band assignment, nucleotide position and location relative to the translocation or inversion breakpoints of the clones used for primary FISH screening of ten patients found to have balanced 6q rearrangements are presented in Figure 1. Breakpoints fell within a relatively restricted region of 6q between q22 and q23. In five cases of ALL (#2-6) and in both rearrangements from one AML patient (#9). In one ALL (#1) and three AML patients (#7, 8, 10) the

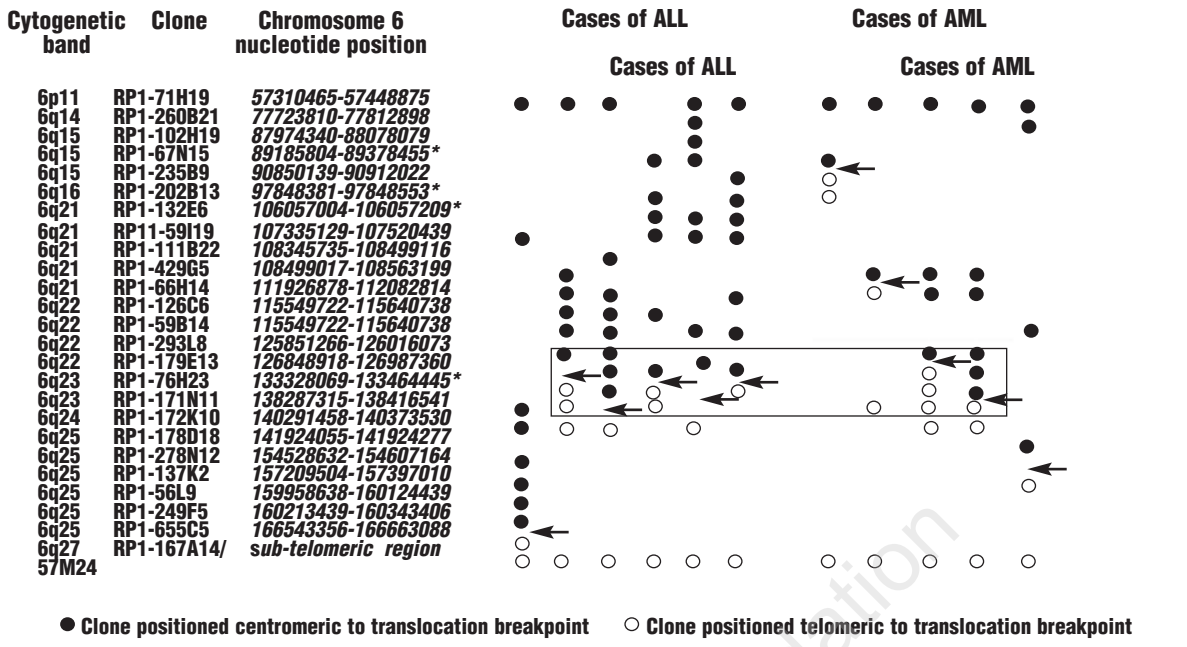


Figure 1. Primary FISH analysis of cases of acute leukemia with balanced rearrangements of 6q. Chromosome 6 nucleotide positions and chromosomal band assignments are as annotated in the ENSEMBL human genome browser. *Clone not fully sequenced, position refers to a corresponding marker or was estimated from the position of overlapping fully sequenced clones. Seven of 11 breakpoints clustered at bands q22 to q23 (highlighted by the box) and these were selected for further analysis.

translocation breakpoints mapped to regions outside 6q22-q23. Patient age, disease type and karyotypes are presented in Table 1. The karyotypes represent the results from G-banded chromosomal analysis with partners and breakpoints of 6q revised following FISH analysis. Among the ALL patients, balanced translocations were evident from cytogenetic analysis in two cases (#3, 6). In both karyotypes FISH redefined the breakpoints from 6q25 to 6q23 (#3) and from 6q21 to 6q23.3 (#6).

The remaining ALL patients, reported to have deletions by G-banded analysis (#1, 2, 4, 5) were shown by FISH to have intact 6q as none of the 6q signals were deleted. Four of the five balanced translocations/inversions of 6q detected by FISH in four cases of AML had been accurately characterized on G-banded analysis. In case #10, the 6q breakpoint, the partner chromosome and the reciprocal nature of the translocation were defined by FISH. The patients showed heterogeneous immunophenotypes and, with the possible exception of cases #9 and 10 (ages unknown), were 16 years old at the time of diagnosis.

Review of published 6q translocations and identification of a new recurrent translocation t(6;7)(q23.3;q32~q36)

We searched the Mitelman database of chromosome abnormalities in cancer (available at URL *http://cgap.nci.nih.gov/Chromosomes/Mitelman*) for balanced rearrangements of 6q occurring in ALL or AML that were similar to those included in our study (Table 1). Eight cases of ALL and seven of AML, with the same partner chromosomes and breakpoints identical or similar to those in our rearrangements, were identified (Table 2).[5,28-41] For the three cases of ALL with partner chromosomes identified, previous reports of each were found. Whilst only one other case each of translocations, t(6;19)(q21;p13) and t(6;9)(q25;q22), had been published, six patients with t(6;7)(q22-24;q32-q36) had been previously described. Five were reported in infant or childhood T ALL, as was case #5 presented here. Fixed cells for FISH analysis were obtained from one of the published cases of pediatric T-ALL with t(6;7) (#11)34. Apart from the inv(6)(p13q22), one or two examples identical or similar to each of the five AML related abnormalities were reported.

Further analysis of 6q translocations with breakpoints at bands 6q22-q23.

A total of 37 additional clones (Fig 2), positioned between RP1-179E13 and RP1-171N11, were used in sequential FISH experiments to map 6q rearrangements down to the resolution of a single BAC/PAC clone in four patients with 6q22-q23 breakpoints, as described below (#5, 6, 9, 11). Further analysis of patients #2 and 4 was also conducted but limited by lack of available material. Although the partner chro-

Table 1. Revised karyotypes, age and immunophenotype of patients with balanced cytogenetic rearrangements of 6q.

Patient Number	*G-banded karyotype*	*Age[4] (years)*	*Immunophenotype*
1	47,XY,add(2)(q2),inv(3)(p2q21),t(6;?)(q21;?),add(6)(q25.3~27)add(7)(q11), -8,t(8;15)(p11;q1), add(12)(p1), add(13)(q3),der(16)t(7;16)(q11;p13),add(17)(p13),der(20),add(20)(q1),-21,+3mar	10	Null ALL[1]/AML[2]
2	45,XY,add(6)(q22.33~23.2),i(9)(q10),dic(12;17)(p11;p11)		ALL
3	51,XY,+X,t(2;18)(p13;q23),t(3;10)(q21;q26),t(3;16)(p25;p13), t(6;9)(q23;q22),inv(12)(p12q21),+14,del(17)(q21;q21),+20,+21,+mar	16	Common ALL
4	46,XY,add(6)(q23.2),add(7)(q3?),del(9)(p1?3),t(11;14)(p13;q11)	10	T-ALL
5	46,XY,t(6;7)(q23.3;q32~36),del(10)(q?),add(20)(p?)	2	T-ALL
6	45,XX,der(18;21)(q10;q10)[4]/87<4n>,XX,-X,-X,-1,-3, add(4)(q31)x2,der(5)t(5;21;15)(q22;q22;q15)t(12;15)(p13;q22)x2,-6,-6,t(6;19)(q23;p13)x2,t(7;22)(q22;q13), +der(7)t(7;22)(q22;q13),del(7)(q22q36)x2,t(8;11)(p?10;q1?4), del(11)(q1?4q23)x2,-16,+der(19)t(6;19)(q?21;p13),der(21)t(6;21)(p?21;q22)x2[5]	10	Common ALL
7	46,XX,t(6;11)(q15;q23)	13	AML (M4)[3]
8	46,X,t(X;22)(q2;q11),t(4;13)(q2;q1),t(6;7)(q21;p15), del(15)(q11q21)	1	AML (M2)[3]
9	47,XY,+6,del(6)(q21q25),inv(6)(p13q22),der(7;17)(p10;q10), t(7;8)(p1;q1),der(15)t(15;17)(q25;q10),+22[3]/47,idem, -del(6)(q21q25),t(8;9)(q22;q32),+mar[5]/46,idem,t(2;6)(q31;q23),inv(5)(q1q22), der(6)t(6;6)(q2;p10),-del(6)(q21q25)[4]		AML
10	46,XX,t(3;6)(q26;q25)		AML

[1]acute lymphoblastic leukemia;[2] acute myeloid leukemia;[3]according to the FAB classification; [4]age at diagnosis.

mosomes were not identified in all cases, FISH demonstrated that there had been no loss of 6q material as a result of these translocations. Limited FISH analysis defined 3.5 Mb and 335 kb breakpoint regions in patients #2 and 4 respectively. With the exception of patients #5 and 11, breakpoints mapped in detail were separated by not less than 400 kb. The larger region defined in patient #2 contained the breakpoints of patients #4 and 6. The 6.7 Mb breakpoint region in patient #3, mapped with the primary panel of probes, also encompassed two of the smaller regions identified in patients #5 and 9.

Patient #9

G-banded chromosomal analysis of patient #9 revealed a complex karyotype including distinct populations with an extra copy and structural rearrangements of chromosome 6. The balanced rearrangements of 6q included a pericentric inversion, inv(6)(p13q22), and a translocation, t(2;6)(q31;q23). Two unbalanced rearrangements, del(6)(q21q25) and der(6)t(6;6)(q2?;p10), were also present. Loss of signals with 6q specific probes was observed in <10% of cells. A large population (~70% cells) was trisomic for chromosome 6, with one homologue involved in the translocation, t(2;6), in the majority of cells. The translocation and inversion co-existed in ~50% cells. In FISH experiments, signals from RP1-187J11 and more centromeric probes occupied the same chromosome arm as RP1-71H19, but were separated from RP1-57M24 by the t(2;6) in approximately 70% of metaphase cells (Figure 3A). In contrast RP11-524A17 and more telomeric probes co-hybridized with RP1-57M24, but were separated from RP1-71H19 in 70% of cells (Figure 3A). In contrast RP11-524A17 and more telomeric probes co-hybridized with RP1-57M24, but were separated from RP1-71H19 in 70% of cells (Figure 3B). This mapped the 6q breakpoint of the t(2;6) to between RP1-187J11 and RP11-524A17, a 24.7 Kb region corresponding to BAC RP11-654K16 (Figure 4A). The inverted chromosome 6 was identified by the occurrence of FISH signals from the 6q sub-telomeric probe (RP1-57M24) on the p arm. RP11-290P3 and more centromeric probes hybridized to the q arm of the inverted chromosome while RP3-372K1 was split, producing a strong signal on the p arm and a second weaker signal on the q arm (Figures 3 C,D). Probes telomeric to *RP3-372K1* hybridized to the p arm only indicating that the inversion breakpoint occurred close to the centromeric end of *RP3-372K1* (Figure 4B).

Table 2. Karyotypes and patients' characteristics of published cases of acute leukemia with rearrangements of 6q similar to those analyzed in the present study.

Rearrangement (present study)	*Published karyotype*[1]	*Age*	*Sex*	*Disease subtype*	*Reference*
t(6;19)(q21;p13)	45,XX,der(6)(q21)t(6;19)(p22;p13)or der(6)t(6;19)(q22;p13)t(6;9;16)(p21;p23;q22),-7,add(9)(p23)or der(9)(p23)	1	F	ALL	(32)
t(6;9)(q25;q22)	47,XY,+X,inv(2)(p?21q31),add(4)(q35),t(4;11),t(6;9)(q25;q22),t(12;19)(q23;p13)	14	M	ALL	(35)
t(6;7)(q23;q32-36)	46,XY,t(6;7)(q24;q34)	1	M	T ALL	(28)
	37,XY,-2,-3,-4,t(6;7)(q21~23;q36),-7,-12,-13,-15,-16,	42	M	B ALL	(30)
	46,XY,t(6;7)(q23;q33)	7	M	T ALL	(31)
	46,XX,t(6;7)(q24;q36)/46,idem,del(6)(q15q21)	ped[2]	F	T ALL	(5)
	46,XX,t(6;7)(q23;q32)/47,idem,del(13)(q14q22),+mar	ped[2]	F	T ALL	(33)
	46,XY,del(1)(p13p31),der(5)del(5)(q33)t(5;7)(q33;q33),t(6;7)(q2??2;q3?3),del(9)(p21p23)x2	8	M	T ALL	(34)
t(6;11)(q15 ;q23)	46,XX,del(5)(q13q15),t(6;11)(q15;q23)	68	F	AML M0	(39)
t(6;7)(q21;p15)	41,XY,der(5)del(5)(q11q13)t(5;17)(q15;q11),der(6)t(6;22)(p25;q13),-7,del(9)(q11),del(12)(p12p13),der(15)t(7;15)(p22;p1?3)t(7;7)(p15;q21),-16,-17,-18,?del(20)(q13q13),dup(21)(q22q22),-22/42,idem,+13/42,idem,-der(6),+der(6)t(6;22)t(6;7)(q21;p22)t(7;7)(p15;q21),+13,der(15),+der(15)t(6;15)(q21;p1?3)	45	M	AML M6	(37)
	45,XY,del(5)(q1?3q3?1),-6,der(7)t(6;7)(q15;p13),-3,+mar/46,idem,+mar	36	F	AML	(40)
t(2;6)(q3?1;q22)	45-46,XY,t(2;6)(q37;q23),-7,del(11)(q11)	30	F	AML	(36)
	46,XY,t(2;6)(q31;q24),del(7)(q31)		M	AML M4	(38)
inv(6)(p13q22)	-	–	–	–	–
t(3;6)(q26;q25)	46,XY,t(3;6)(q26 ;q27)	50	M	AML M2	(39)
	45,XX,t(3;6)(q26;q25),der(7)del(7)(p11)del(7)(q22),der(11)t(7;11) (p?13;p10)dup(11)(q23q12),der(16)t(16;22)(q22;q11),der(19)t(19;22)(q13;q11),i(20)(p10),-22	41	M	AML M1	(41)

[1]*Karyotypes were obtained through a search of the Mitelman database of chromosome abnormalities in cancer (http://cgap.nci.nih.gov/Chromosomes/Mitelman).*
[2]*Patients included in a study of pediatric ALL ages not published.*

Patient #6

A complex karyotype with near-tetraploidy and various structural abnormalities including the translocation, t(6;19)(q21;p13), was described in five of nine cells from this patient. Two copies of this translocation were detected by FISH in ~30% cells. The 6q sub-telomeric probe RP1-57M24 was separated by the translocation from all probes centromeric to BAC *RP11-123H21*, but produced signals on the same chromosome arm as RP5-914N13 and more telomeric probes (Figure 3E). Hybridization of *RP11-123H21* produced a low intensity signal that co-localised with *RP1-57M24* (Figure 3F) and a stronger signal on the derivative chromosome 6 (as marked by the centromeric probe RP1-71H19). This located the t(6;19) breakpoint close to the telomeric end of *RP11-123H21* (Figure 4C).

Patients #5 and 11

A translocation, t(6;7)(q23;q32~36), originally described as a deletion of 6q by cytogenetics alone, was redefined by FISH analysis of patient 5. Probe *RP1-32B1* was split by the translocation, producing signals of approximately equal intensity on different chromosomes, marked by *RP1-57M24* and *RP1-71H19*, in >90% of cells examined (Figures 3G, 3H). Thus the breakpoint mapped within the RP1-32B1 sequence (Figure 4D). In patient #11, *RP1-32B1* hybridized only to the derivative chromosome 6, while the adjacent and telomeric probe *RP3-388E23* produced signals of similar intensity on chromosomes marked by centromeric and sub-telomeric probes (Figures 3I, 3J). The breakpoint in patient 11 was therefore shown to have occurred within the region covered by the *RP3-388E23* clone (Figure 4D). The involvement of chromosome 7

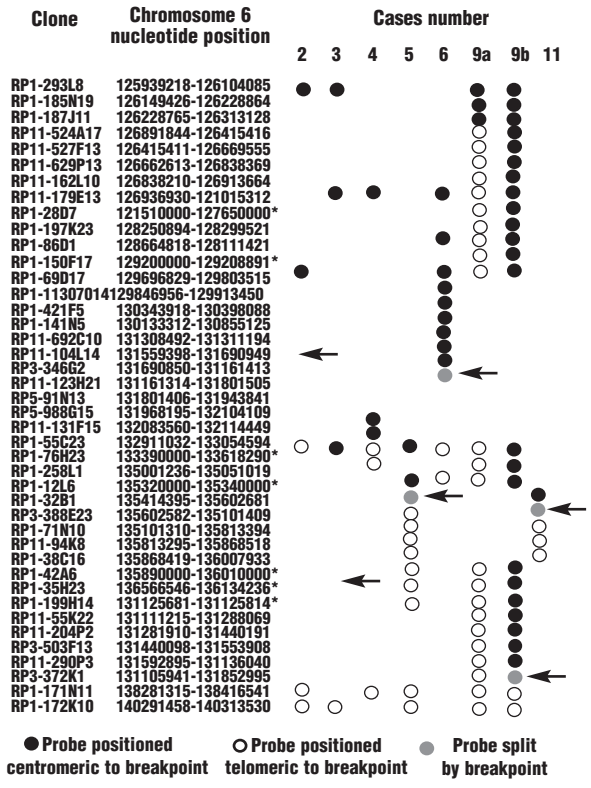


Figure 2. Detailed analysis of cases with rearrangements of bands 6q22-q23. Chromosome 6 nucleotide positions and chromosomal band assignments are as annotated in the ENSEMBL human genome browser. *Clone not fully sequenced, position refers to a corresponding marker or was estimated from the position of overlapping fully sequenced clones.

in both cases was confirmed by wcp and chromosome 7 specific probes. FISH experiments with PACs RP5-112A15 and RP4-639J15 (flanking the *HLXB9* locus), together with 6q centromeric and sub-telomeric probes, RP1-71H19 and *RP1-57M24*, mapped the 7q breakpoints proximal to *HLXB9* at 7q36 (*data not shown*).

Further analysis of 6q translocations with breakpoints outside bands q22-q23

In addition to the 6q specific probes, FISH with wcp and locus specific probes to other chromosomes were used to investigate the translocation partners and the involvement of known oncogenes in three cases (#7, 8, 10) with breakpoints outside 6q22-q23. In patient #7, separation of one paired red/green signal was seen with the *MLL* specific probe, confirming the presence of a translocation involving the *MLL* gene in this patient (previously reported in 42. In patient #8, hybridization of probes *RP1-167F23* (containing *HOXA1-3* and centromeric sequences) labeled in Spectrum Green and RP5-1200I23 (containing sequences telomeric to the *HOXA* cluster) labeled with Spectrum Red resulted in co-localized red/green fusion signals in all cells examined, ruling out involvement of *HOXA* genes in this case. In patient 10, hybridization of wcps 3 and 6 confirmed the presence of a cryptic translocation, t(3;6)(q26;q25), suspected from review of the G-banded karyotype. YAC 14e-e12 co-localized with RP1-167A14, but not with RP1-71H19, demonstrating that the *EVI1/MDS1* locus was proximal to and uninterrupted by the 3q breakpoint.

Discussion

This study demonstrates the power of combining high-resolution FISH with comprehensive analysis of high-throughput sequence data available through HGMP to reveal previously unrecognized cytogenetic abnormalities. Through the systematic mapping of novel balanced 6q rearrangements in acute leukemia, we have obtained clear evidence for a new recurrent primary genetic change. First, analysis of a t(6;7) in a case of childhood T ALL (patient #5) mapped the breakpoint to within a single PAC (RP1-32B1) localizing it within or close to *c-MYB*. Second, a search of the literature identified six similar translocations, five also arising in childhood T ALL, either as a sole cytogenetic abnormality or represented in the major clone. One was available for further analysis and was included in this study. The 6q breakpoint of this patient mapped to within 150kb of the original patient. Five annotated genes have been localized to the region containing the two mapped t(6;7) breakpoints (Figure 4D). While the known coding sequences of two of these, *c-MYB* and *dJ32B1.4* were potentially interrupted by the 6q rearrangement in patient #5, the breakpoint in patient 11 coincided only with the *AHI1* (*Abelson helper integration site 1*) gene. A role for three untranslated genes positioned within the region, *dJ32B1.3*, *dJ32B1.4* and *dJ366E23.2*, should not be discounted because non-coding transcripts may have important regulatory functions and contribute to malignancy.[42]

However though neither has previously been associated with a recurrent translocation in leukaemia, it is likely that one or both of the larger coding genes *c-MYB* and *AHI1* are critical targets of the t(6;7). Of the two, *c-MYB* is known to have oncogenic potential and to play a role in growth, differentiation and survival of immature hemopoietic cells.[44-47] AHI1 is also differentially regulated in haemopoietic tissues with aberrantly high expression reported in some leukemic cell lines and patients.[48,49]

Our analysis shows that breakpoints dispersed within the *c-MYB/AHI1* region arose in human leukemias of identical cytogenetic/immunophenotypic sub-type. Interestingly, the investigation of proviral insertion sites in murine and feline leukemias and lymphomas, has produced parallel observations with integration occur-

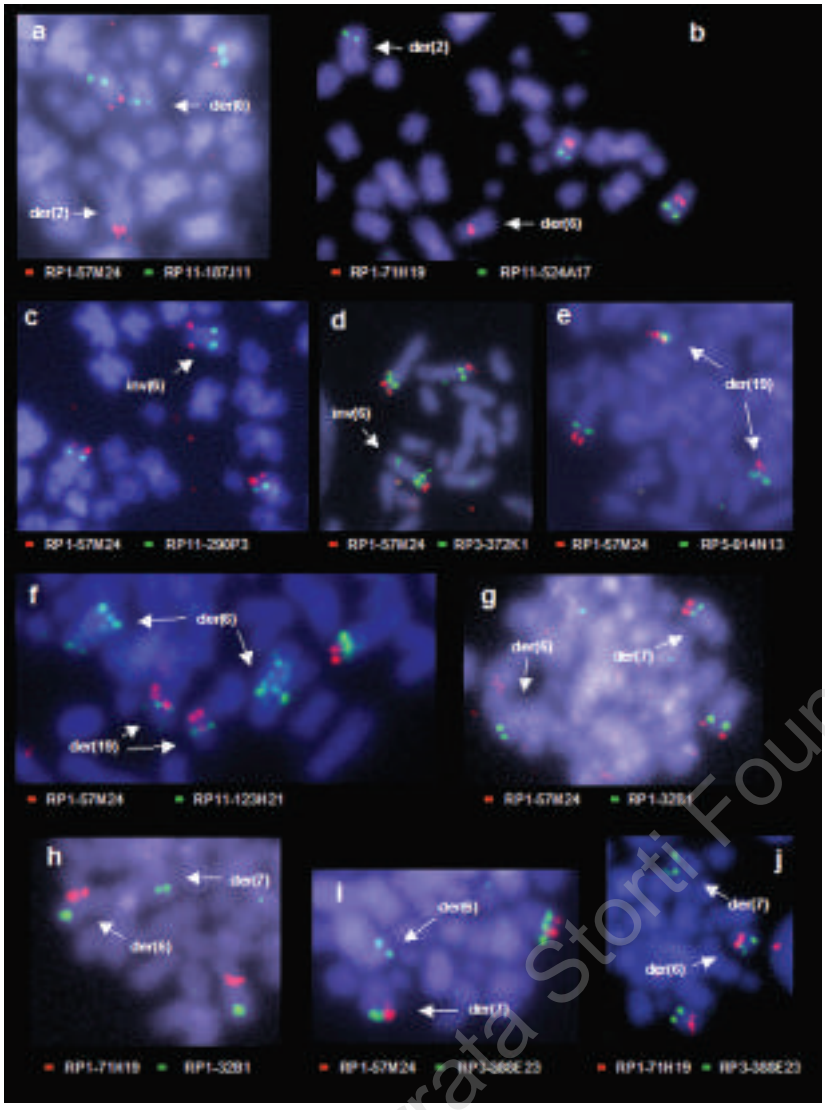
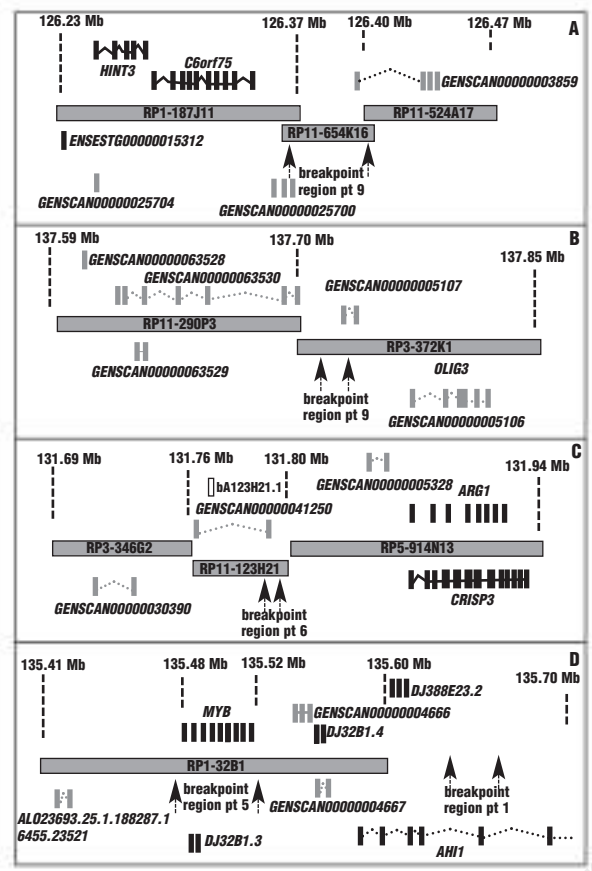


Figure 3. Examples of FISH analysis of cases 9, 6 and 5 and 11. In each case Spectrum Red labeled probes RP1-57M24 (marking the 6q sub-telomeric region) and RP1-71H19 (marking the chromosome 6 centromeric region) appear as red signals, other probes labeled with Spectrum Green mapped to chromosomal bands 6q22, or 6q23 and appear as green signals. *Patient 9*: iAll cells illustrated carry a normal chromosome 6 as well as both the t(2;6) and the inv(6). RP11-187J11 hybridized to a different chromosomal location from RP1-57M24 (a) and RP11-524A17 to a different chromosomal location from 71H19 (b) defining the position of the translocation breakpoint. A wide separation between RP11-187J11 and RP1-57M24 to opposite ends of the inverted chromosome 6 in contrast with the normal chromosome 6 was also seen (a). The breakpoint of the inv(6) was defined in detail by probes RP11-290P3 and RP1-57M24, also showing a wide separation (c) and RP3-372K1 which hybridize to both the p and q arms of the inverted chromosome with a major signal co-localising with RP1-57M24 and smaller signal on the opposite chromosome arm (d). *Patient 6*: in metaphase cells that carried two copies of the t(6;19), RP5-914N13 co-localized with RP1-57M24 on the derivative 19 chromosome (e) and hybridization of RP11-123H21 produced a weak signal on the derivative 19, marked by RP1-57M24, and a brighter signal on the derivative chromosome 6 (f). *Patient 5*: in cells carrying the t(6;7) hybridization of probe RP1-32B1 produced signals of equal intensity on both the derivative chromosomes marked by RP1-57M24 (g) and RP1-71H19 (h). *Patient 11*: in cells carrying the t(6;7) hybridization of probe RP3-388E23 produced signals of equal intensity on both derivative chromosomes marked by RP1-57M24 (i) and RP1-71H19 (j).

ring upstream of *Myb*, within *Myb* or *Ahi1* and also between the two genes.[48,50] In different studies both the generation of abnormal *Ahi1* transcripts and deregulation of c-myb expression were associated with proviral insertion. Therefore in patients carrying the translocation, it will be important to quantify levels of expression of *c-MYB* and *AHI1*, as well as other genes in the region. In addition sequence analysis of the t(6;7) translocation breakpoints may implicate a presently unrecognised oncogene on 7q in the pathogenesis of childhood T ALL.

Including the t(6;7)(q23;q32?q36) cases, all except one of the seven ALL breakpoints mapped to a 3.72-8.51 Mb region within 6q22-q23, defining a *hotspot* in lymphoid malignancy with 6q rearrangements. To our knowledge, only one other balanced rearrangement of 6q, a t(6;8;8) identified in a T-cell leukemia/lymphoma cell line, has previously been mapped in detail in lymphoid malignancy. This rearrangement disrupted the TCBA1 gene, positioned within 6q22, 8 Mb proximal to the breakpoint cluster region we describe here.[51] In AML, the breakpoints were widely dispersed, although in one case with a translocation and an inversion of 6q, both rearrangements involved 6q22-q23.

The mechanism promoting selection of leukemic clones carrying clustered, though non-identical 6q22-q23 breakpoints remains to be elucidated. With the exception of the two t(6;7) translocations, none of the rearrangements mapped in detail interrupted a recognised gene and all where apparently sporadic in nature, making classical oncogene activation unlikely. Some rearrangements might have deregulated a single critical gene on 6q through a long-range position effect. Previously ectopic expression or loss of expression, resulting from translocations positioned up to 500 kb distant from exons of genes such as *EVI1*, *IL3*, *GSH2* and *HACE-1*, has been reported.[52-54] The 6q22-q23 rearrangements span a much greater distance.



Figue 4. Data adapted from the ENSEMBL human genome browser version 20. The position and HUGO nomenclature of recognized genes (in black), pseudogenes (in white), ESTs (in dark gray) and Genescan predicted genes (in light gray) on chromosome 6 clones shown to be in the vicinity of a translocation or inversion breakpoint are given. The positions of breakpoints estimated from FISH data are indicated by arrows and the chromosome 6 map position of each clone end marked in Mb. (A) The t(2;6) chromosome 6 breakpoint in patient #9 was mapped to between BAC RP11-187J11 and RP11-524A17. (B) The 6q inversion breakpoint in patient #9 was mapped to the proximal region of RP3-372K1. (C) The t(6;19) breakpoint in patient 6 was mapped to the distal end of RP11-123H21 (D) The t(6;7) breakpoint in patient #5 was mapped to the central region of clone RP1-32B1 and the t(6;7) breakpoint in patient #11 to the central region of RP3-388E23.

Nevertheless a longer-range position effect, as demonstrated experimentally for the brown eye colour locus, in Drosophila, might have occurred.[55]

Alternatively the 6q22-23 region may be particularly prone to breakage. Of four inducible common fragile sites that have been identified on 6q (FRA6D-G) none map to this region. However *TCBA1* may mark the position of a novel fragile site because in two different lymphoid cell lines it was disrupted by chromosomal rearrangements that had no apparent oncogenic role.[51] Involvement of TCBA1 has been excluded in our cases but a large fragile site might extend distally to include one or more of the breakpoints mapped in the present study.

Of the translocations with breakpoints mapping outside 6q22-q23, none were analysed in detail and any contribution to leukemia of genes positioned within the regions implicated remains speculative. However known oncogenes positioned on partner chromosomes were investigated in three cases and one translocation (patient #7) was shown to involve MLL. The 6q breakpoint of this t(6;11) mapped proximal to AF6 and AF6q21 suggestive for the existence of a third 6q MLL partner gene. Two cloned fragile sites, *FRA6E* and FRA6F overlapped with translocation breakpoint regions in patients 1 and 8, reinforcing the possibility that at least some of the rearrangements mark regions of instability on 6q.

In conclusion, our study suggests that the incidence of balanced rearrangements involving 6q in ALL may be much higher than previously thought, since four of six translocations were originally described as deletions by conventional cytogenetics. This discrepancy, reflecting the indistinct banding pattern of 6q and poor chromosome morphology associated with ALL, could have significant clinical implications. Deletions of 6q have been variously associated with an intermediate or poor outcome, whereas translocations may have different prognostic associations20, 56-58. Further analysis of 6q abnormalities by FISH, in larger studies, should establish the true incidence of the balanced rearrangements and specifically that of the newly described t(6;7)(q23;q31~36) and others involving the 6q22-q23 cluster. It is likely that through such studies, additional as yet unrecognized recurrent rearrangements of 6q will be identified.

PS performed the experiments and collected the data. PS, CH, MJ and LF each contributed to the conception of the study, interpretation of data and preparation and review of the manuscript. L.F. is the author taking primary responsibility for the paper. The authors declare that they have no potential conflict of interest.

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