Heterogeneous breakpoints in patients with acute lymphoblastic leukemia and the dic(9;20)(p11~13;q11) show recurrent involvement of genes at 20q11.21

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ABSTRACT

The dic(9;20)(p11~13;q11) is a recurrent chromosomal abnormality in patients with acute lymphoblastic leukemia. Although it results in loss of material from 9p and 20q, the molecular targets on both chromosomes have not been fully elucidated. From an initial cohort of 58 with acute lymphoblastic leukemia patients with this translocation, breakpoint mapping with fluorescence *in situ* hybridization on 26 of them revealed breakpoint heterogeneity of both chromosomes. *PAX5* has been proposed to be the target gene on 9p, while for 20q, FISH analysis implicated the involvement of the *ASXL1* gene, either by a breakpoint within (n=4) or centromeric (deletion, n=12) of the gene. Molecular copy-number counting, long-distance inverse PCR and direct sequence analysis identified six dic(9;20) breakpoint sequences. In addition to the three previously reported: *PAX5-ASXL1*, *PAX5-C20ORF112* and *PAX5-KIF3B*; we identified three new ones in this study: sequences 3' of *PAX5* disrupting *ASXL1*, and *ZCCHC7* disrupted by sequences 3' of *FRG1B* and *LOC1499503*. This study provides insight into the breakpoint complexity underlying dicentric chromosomal formation in acute lymphoblastic leukemia and highlights putative target gene loci.

Key words: acute lymphoblastic leukemia, dic(9;20), fusion genes, genetic target.

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Introduction

Chromosomes 7, 9, 12 and 20 are frequently involved in the formation of dicentric chromosomes in patients with B-cell precursor acute lymphoblastic leukemia (BCP ALL), constituting the dic(7;9)(p11;p11),[1] dic(9;12)(p11~13;p13)[2] and dic(9;20)(p11~13;q11).[3] The dic(9;20) occurs in ~2% and ~0.5% of childhood and adult precursor-B ALL (BCP ALL), respectively.[4] Although the cytogenetic and clinical associations of this translocation have been widely reported over the last decade, its molecular consequences remain unclear. FISH has shown that dic(9;20) contains the centromeres of both chromosomes 9 and 20, resulting in the loss of 9p and 20q material.[3,5] Array-based comparative genomic hybridization (aCGH), with BAC clones at tiling path resolution, showed clustering of breakpoints within 9p13.2 (genomic position 37.1-38.7Mb) and 20q11.2 (29.2-30.8Mb).[6] The 9p breakpoints precluded the involvement of *PAX5* as a fusion gene. Notably, these data implicated the involvement of the hemopoietic cell kinase gene (*HCK*) at 20q11~12 in a single case.[6]

Our previous FISH mapping studies of dicentric chromosomal abnormalities showed partial or complete deletion of *PAX5* on the short arm of chromosome 9, while molecular analysis identified five novel sequence partners of *PAX5*, three of which were found in patients with dic(9;20): *ASXL1* (20q11.21), *C20ORF112* (20q11.21) and *KIF3B* (20q11.21). With targeted expression analysis, this study highlighted disruption of *PAX5*, regardless of the heterogeneous breakpoints on 9p.[7] The aim of this present study was to further unravel the breakpoint complexity in these patients and identify potential genetic targets on chromosome 20 in dic(9;20) patients.

The online version of this article contains a supplementary appendix.

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Design and Methods

Samples were received from 58 patients with BCP ALL and dic(9;20)(p11~13;q11) entered onto a Medical Research Council (MRC/NCRI) United Kingdom Childhood or Adult ALL treatment trial. Local Ethical Committee approval was obtained by treating centers, and informed consent was given by parents and/or patients. Diagnostic cytogenetic and FISH data is shown in Table 1. The presence of dic(9;20) was confirmed according to previous studies.[5] FISH mapping was performed on 26 cases with dic(9;20) in order to precisely define the breakpoints on 9p and 20q. FISH clones and their genomic positions are listed in *Online Supplementary Table S1*.

Molecular copy number counting (MCC) was carried out on 2 cases as previously described.[8] MCC allows the progressive delineation of unbalanced copy number breakpoints to within a few hundred base-pairs and facilitates rapid sequence analysis. With the use of subgenome quantities of DNA distributed into a 96-well plate, the frequency of PCR well positivity for any given primer pair is a direct reflection of the genomic copy number at that site. Based on the FISH data, genomic locations (markers) in *PAX5*, the *PAX5* 3' downstream region and *ZCCHC7* were chosen for MCC. The data were analyzed as previously described.[9] Long distance inverse-PCR (LDI-PCR) was carried out on 3 cases, as previously described with modifications.[7] The MCC primers, restriction enzymes and the sequences of LDI-PCR primers are shown in *Online Supplementary Tables S2 and S3*. Protein sequences were predicted using the online programme GENSCAN (*http://genes.mit.edu/GENSCAN*).

Results and Discussion

Since the first reports in 1995,[3,10] several studies of dic(9;20) have characterized this abnormality at both the cytogenetic and molecular levels, revealing breakpoint heterogeneity on both chromosomes.[5,6,11,12] In our series, dic(9;20) was the sole visible cytogenetic change in 21/58 patients. Confirming previous observations, we identified recurrent gain of chromosomes X (n=5) and 21 (n=21) (Table 1).[11] Diagnostic FISH analysis performed in 39/58 (67.2%) cases did not confirm the pre-

Table 1. Clinical and cytogenetic data on patients with dic(9;20), with the molecular investigation performed on different cases.

Patient ID	Diagnostic FISH	Gender[a]	Age	Karyotype
198	nd	F	2	45,XX,dic(9;20)(p11~13;q11)[25]
468	nd	F	2	46,XX,dic(9;20)(p11~13;q11),+21[8]
508	nd	F	2	45,XX,dic(9;20)(p11~13;q11)[11]
745	nd	M	2	45,XY,dic(9;20)(p11~13;q11)[11]
753	nd	F	2	45,XX,inv(7)(p11q22),dic(9;20)(p11~13;q11)[21]
1127	nd	F	2	45,XX,dic(9;20)(p11~13;q11)[cp4]
1534	nd	F	1	45,XX,dic(9;20)(p11~13;q11)[9]/45,idem,inv(9)(p22q12)c[3]
1553[b]	nd	M	1	45,XY,dic(9;20)(p11~13;q11)[5]
1616	nd	F	2	46,XX,del(9)(p22)[2]/49,idem,+X,dic(9;20)(p11~13;q11),+10,+21,+21[15]/ 49,idem,+X,+10,del(17)(p13),-20,+21,+21[2]
1729	nd	M	5	45,XY,dic(9;20)(p11~13;q11)[5]/46,idem,+X[2]
1846	nd	F	6	45,XX,dic(9;20)(p11~13;q11),del(11)(q14q23)[5]
1920	nd	F	2	45,XX,dic(9;20)(p11~13;q11)[3]
2208	nd	M	17	45,XY,dic(9;20)(p11~13;q11)[5]/46,idem,+mar[6]
2384[b]	nd	F	5	46,XX,del(9)(p21)[2]/46,idem,del(6)(q15q21)[4]/45,idem,dic(9;20)(p11~13;q11)[3]
2443	nd	F	2	46,XX,dic(9;20)(p11~13;q11),+mar[3]/47,idem,+2mar[3]
2763	N	F	8	46,XX,dic(9;20)(p11~13;q11),+21[4]
2857	nd	M	23	45,XY,dic(9;20)(p11~13;q11)[6]
2894[b]	N	M	5	46,XY,dic(9;20)(p11~13;q11),+21[7]
3087[b]	N	M	14	47,XY,add(1)(q2?),dic(9;20)(p11~13;q11),del(17)(p11.2),+18,+21
3101	nd	M	13	45,XY,dic(9;20)(p11~13;q11)[3]
3155	nd	F	13	46,XX,dic(9;20)(p11~13;q11),+21[8]/46,idem,i(8)(q10)[8]/46,idem,add(15)(p10)[6]
3275	N	F	11	45,XX,dic(9;20)(p11~13;q11)[5]/46,idem,+20[3]/ 52,XX,idem,+6,+8,+9,+12, +18,+20,+22[15]/52,idem,+6,+8,+dic(9;20),+12,+18,+20,+22[2]
3363	N	F	32	46,XX,dic(9;20)(p11~13;q11),+21[10]
3450	N	M	2	45,XY,dic(9;20)(p11~13;q11)[13]
3507[d]	N	F	4	47,XX,+X,dic(9;20)(p11~13;q11),t(9;14)(p21;q11),+21[12]/ 47,idem,del(5)(q22q34)[3]
3599	nd	M	1	46,XY,t(5;12)(q33;p13),del(9)(p13)[2]/45,idem,-del(9)(p13),dic(9;20)(p11~13;q11)[3]
3916	N	M	24	45,XY,dic(9;20)(p11~13;q11)[7]

To be continued on next page.

The normal clone has been omitted from the karyotypes. [a]Male (M), Female (F). N: normal diagnostic FISH results for BCR-ABL1, ETV6-RUNX1, and rearrangements of MLL; E, ETV6-RUNX1 fusion identified by diagnostic FISH. nd, FISH test not done. [b]analyzed with FISH mapping; [c]analyzed with FISH mapping, molecular copy-number counting and breakpoint cloning. [d]Case previously reported by aCGH.[18]

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4039	N	M	7	45,XY,dic(9;20)(p11~13;q11)[5]/46,idem,+18[5]
4141	N	M	11	45,XY,dic(9;20)(p11~13;q11)[6]/46,idem,+15[2]
4377[b]	N	F	15	45,XX,dic(9;20)(p11~13;q11)[21]
4451[c]	N	F	2	45,XX,dic(9;20)(p11~13;q11),del(16)(q22)
4485	N	M	6	46,XY,del(1)(p1?p3?1),dic(9;20)(p11~13;q11),+21[9]
4506[b]	N	M	6	46,XY,+8,dic(9;20)(p11~13;q11)[6]
4510[b]	N	F	2	45,XX,dic(9;20)(p11~13;q11)[8]/46,idem,+21[2]
4549[b]	N	M	51	46,XY,dic(9;20)(p11~13;q11),+21[9]/46,idem,+20,r(20)(p?q?),-21[6]/ 45,idem,add(20)(q13),-21[4]
4718[b]	N	F	2	45,XX,t(5;9)(?q13;?p22),dic(9;20)(p11~13;q11)[11]/45,idem,del(6)(q?)[7]
4887[b]	N	F	8	44,XX,-7,dic(9;20)(p11~13;q11)[14]
5037	N	F	1	45,XX,dic(9;20)(p11~13;q11)
5618[c]	N	F	2	45,XX,dic(9;20)(p11~13;q11)[11]
5824[b]	N	F	3	46,XX,dic(9;20)(p11~13;q11),+21[5]/47,idem,+X[2]/48,idem,+X,+9[2]
6789[b,d]	N	F	4	46-47,XX,del(6)(q1q2),+7,dic(9;20)(p11~13;q11),+mar[cp9]
6897[c]	N	M	1	45,XX,dic(9;20)(p11~13;q11)[10]
7063[c]	N	F	7	46,XX,del(6)(q16q24.1),dic(9;20)(p11~13;q11),+21[10]
7167[b]	N	F	1	45,XX,t(7;15)(p1;q1),dic(9;20)(p11~13;q11)[8]
7201[b]	N	M	16	45,XY,der(2)t(2;9)(q3;p?2),der(9)del(9)(p21p21)t(2;9)(q3;p?2),- 7,dic(9;20)(p11~13;q11),+20[10]
7209[b]	N	F	2	45,XX,dic(9;20)(p11~13;q11)[6]
7339	N	F	2	45,XX, dic(9;20)(p11~13;q11)[6]
7550[c]	N	M	8	46,XY,dic(9;20)(p11~13;q11),+mar[8]
8330	N	M	4	45,XY,dic(9;20)(p11~13;q11)[6]
8901[c]	N	M	3	45,XY,dic(9;20)(p11~13;q11),del(9)(q2?q3?),del(16)(q22)[9]/45,XY,-9,del(16)(q22), der(20)t(9;20)(q22;q11)[3]/46,XY, -9,del(16)(q22),der(20)t(9;20)(q22;q11),+21[5]
9491	N	M	4	46,XY,dic(9;20)(p11~13;q11),+21
10061[b]	N	M	4	47,XY,dic(9;20)(p11~13;q11),+21,+mar[7]
10219[b]	N	M	48	45,XY,dic(9;20)(p11~13;q11)[10]
10401[b]	N	F	1	45,XX,dic(9;20)(p11~13;q11)[6]
10537[b]	E	F	7	46,XX,dic(9;20)(p11~13;q11),+mar[2]
10862	N	F	4	46,XX,dic(9;20)(p11~13;q11),+21[11]
10868[b]	N	F	2	48,XX,+X,del(9)(p2?),+10,del(15)(q2?2),dic(9;20)(p11~13;q11),+21[7]
11906	N	M	8	45,XY,dic(9;20)(p11~13;q11)

The normal clone has been omitted from the karyotypes. [a]Male (M), Female (F). N: normal diagnostic FISH results for BCR-ABL1, ETV6-RUNX1, and rearrangements of MLL; E, ETV6-RUNX1 fusion identified by diagnostic FISH. nd, FISH test not done. [b]analyzed with FISH mapping; [c]analyzed with FISH mapping, molecular copy-number counting and breakpoint cloning. [d]Case previously reported by aCGH.[18]

vious association with the t(9;22)(q34;q11) (11), but did identify a single patient positive for the *ETV6-RUNX1* fusion (10537; Table 1).

We have previously shown that breakpoints on dicentric chromosomes involving chromosome 9 target the *PAX5* locus.[7] However, the genetic targets on 20q in dic9;20 remain unclear. FISH mapping performed in this current study revealed considerable breakpoint heterogeneity on both 9p and 20q (Figure 1A). A total of 24/52 (46.2%) breakpoints were located within the centromeric regions of both chromosomes 9 and 20, either centromeric to RP11-113O24 (38.261-38.427Mb) on 9p or RP5-854E16 (29.267-29.338Mb) on 20q. These regions could not be further defined due to the repetitive nature of the DNA sequences within the centromeres. The remaining 28 (53.8%) breakpoints were positioned within euchromatic regions of chromosomes 9 and 20 and, with the exception of case 6789, had fixed cells available for further study. On 9p, FISH analysis indicated the involvement of *PAX5* (4451, 5618 and 7550) and *ZCCHC7* (1153, 2894, 7063 and 8901) in 3 and 4 cases, respectively. A further 2 cases showed breakpoints in the region 3' of *PAX5* (6897 and 10401). In their 7 dic(9;20) cases, Schoumans *et al.*, reported that the 9p breakpoints mapped centromeric of *PAX5*, resulting in total deletion of the gene in all cases[6] (Figure 1B). Furthermore, a recent study revealed intronic *PAX5* breakpoints in 5/11 (45%) dic(9;20) cases.[13]

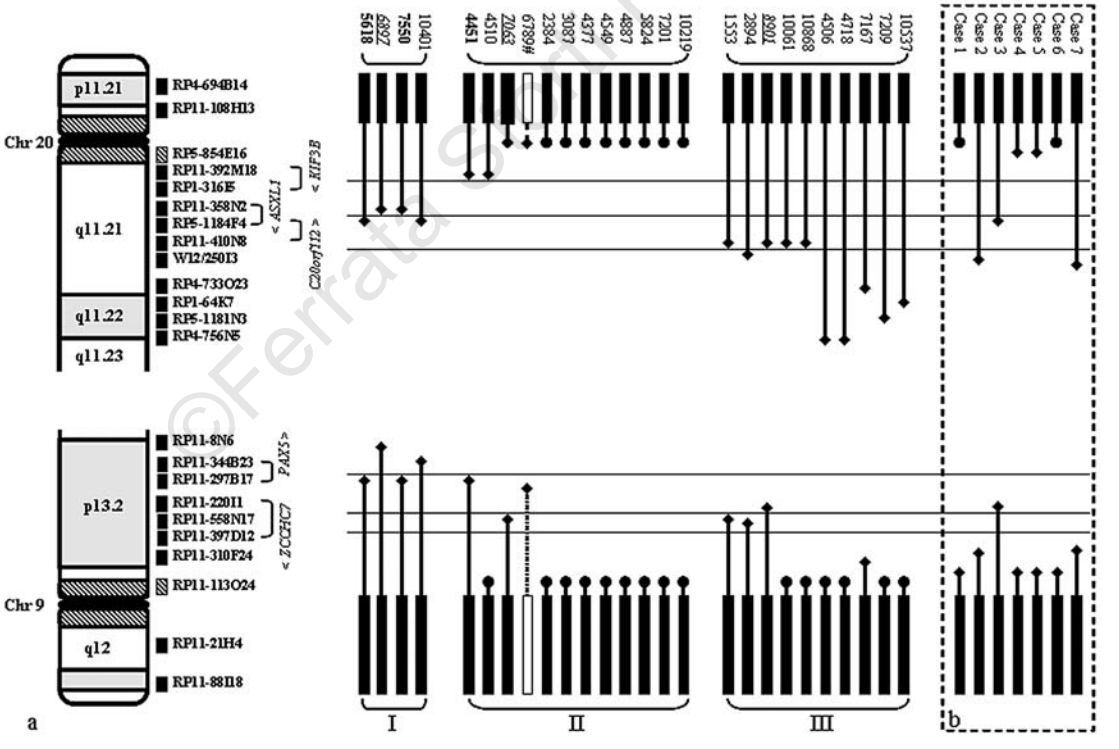
In our study, breakpoints on 20q determined by FISH mapping, revealed three breakpoint clusters as detailed in *Online Supplementary Table S4*: Type I- 4 cases with breakpoints directly involving *ASXL1/C20ORF112* (5618, 6897, 7550 and 10401), Type II- 12 cases with breakpoints proximal of *ASXL1*, resulting in deletion of this gene, and Type III- 10 cases with breakpoints distal to *ASXL1*, 5 of which were clustered between clones RP11-410N8 and WI2-250I3 (1553, 2894, 8901, 10061 and 10868) (Figure 1A). There are no known genes within this region. In total, ~61.5% (16/26) of the breakpoints on 20q resulted in disruption of the *ASXL1* gene.

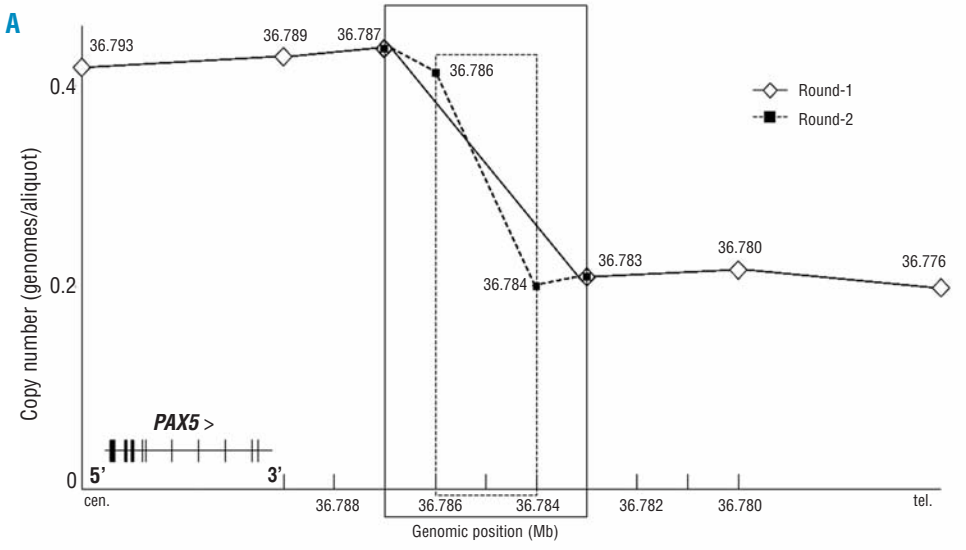
MCC was employed to refine, while LDI-PCR was used to amplify the translocation breakpoints for direct

sequence analysis in 3 dic9;20 cases with material available (6897, 7063, 8901) (Figure 2). Each of the breakpoints identified by LDI-PCR was confirmed using standard PCR approaches. In case 6897, 1st and 2nd round MCC analyses mapped the breakpoint to a ~3.2kb (36.783-36.787Mb) and a ~1.4kb (36.784-36.786Mb) region 3' of *PAX5* (resulting in deletion of the entire *PAX5* gene), respectively (Figure 2A). Sequence analysis showed that a region 3' of *PAX5* on 9p13.2 was juxtaposed with exons 1-4 of the *ASXL1* gene on 20q11.21. The representative MCC data from case 6897 are illustrated in Figure 2A.

MCC markers (markers 39-44) were designed to cover a region of ~7kb (37.240-37.247Mb) within intron 2 of the *ZCCHC7* gene. When applied to case 7063, they showed evidence of a copy number change between markers 43 and 44, indicating the location of the breakpoint to be within a ~1.2kb region (37.245-37.247Mb). As both cases displayed the same FISH pattern on 9p, the predicted breakpoint sequence of case 8901 was amplified directly by LDI-PCR, based on the MCC results of case 7063. LDI-PCR and sequencing analyses showed *ZCCHC7* to be the partner of sequence 3' of *FRG1B* (20q11.1) and *LOC149950* (20q11.21) in patients 7063 and 8901, respectively.

In 3 previously published cases, 4451, 5618 and 7550, we showed that the *PAX5* gene was juxtaposed to *KIF3B* (30.379Mb), *C20ORF112* (30.609Mb) and *ASXL1* (30.465Mb) sequences, respectively.[7] Taken together with the dic(9;20) breakpoint sequences identified in this study, certain conclusions can be made: there is recurrent involvement, by a breakpoint within or a deletion of *ZCCHC7* at 9p13.2 and *ASXL1* at 20q11.21 in cases with dic(9;20). The cases with breakpoints within either *ZCCHC7* or *ASXL1* displayed common breakpoints within intron 2 (retained exons 1 and 2) and 4 (retained exons 1-4), respectively. Due to lack of material, we were unable to perform further genomic or expression studies of these patients. The analysis of DNA sequence flanking the breakpoints provided no further insight into the mechanism by which these rearrangements occurred. *ZCCHC7* has been previously shown to juxtapose *MYC* in B-cell lymphoma.[14] *ASXL1* (30.410-30.491Mb) encodes a protein product of 170 kDa, which is a mammalian homolog of *Drosophila ASX* (additional sex combs). It has been shown to act as a novel ligand-dependent coactivator of the retinoic acid (RA) receptor.[15] The study of Schoumans *et al.* supports the involvement of *ASXL1*, as their cases show a similar pattern, as indicated in Figure 1B.[6] Further studies should include investigation of the





B

Patient ID	9p fusion gene /region (location)	20q fusion gene /region (location)	Breakpoint sequence
4451	*PAX5* (36899347)	*KIF3B* (30378673)	chr 9 GCGAGTGCAAAGTTCTCAAGAATGTGCAGCAACACAGCGTTTCATCCCAGTGCAATGACATCCGCTCTCTCCAGACTCAA dic (9;20) GCGAGTGCAAAGTTCTCAAGAATGTGCAGCAACACAGCGTGTTTCTATTGTCGAGACTTAACGGGGAATGCACTGGATCG chr 20 GTACTCTTAGGAAGAGTTGTGACACCCAGTCTTACTGTGTGTTTCTATTGTCGAGACTTAACGGGGAATGCACTGGATCG
5618	*PAX5* (36903071)	*C20orf112* (30608872)	chr 9 AATGTTTGGAGTCTCAATACCCAAAACTTGGCAGCCAAAGAAAGTCCACCCTCTTCTGGCTTTTAATCTGAGCAACAGCC dic (9;20) AATGTTTGGAGTCTCAATACCCAAAACTTGGCAGCCAAAGTTATAGGTCGTGCGAAAAAAGGGCGAGGGCCCAGGGATGT chr 20 GCCTCCCACTGCCTGGTTTGTCTGCGTGCCTGTCCCCATTTTATAGGTCGTGCGAAAAAAGGGCGAGGGCCCAGGGATGT
7550	*PAX5* (36995786)	*ASXL1* (30464846)	chr 9 ATGTGTCTATTTAAGGTGTATTATTTCTCCACTTGGGATACAGGAAACACCGCTGGTTAGCTGGGGGAAAAGTTATTGTA dic (9;20) ATGTGTCTATTTAAGGTGTATTATTTCTCCACTTGGGATATAGTTTTGATATATTTTCCAATATGAGTCTCATTCACTGT chr 20 TAGATGTATAACTTAATACACTTTATATTGTACTAATGTTTAGTTTTGATATATTTTCCAATATGAGTCTCATTCACTGT
6897	*PAX5 3' region* (36784201)	*ASXL1* (30472078)	chr 9 TGCCACTGGCCTCAAAGTTCAGCTCCAAGGCCCCCTCCTTTAAAAAGCCTTCTGTGACACCCCTGCTGGCCAGGATCCCC dic (9;20) TGCCACTGGCCTCAAAGTTCAGCTCCAAGGCCCCCTCCTTATGTTTCTTCTGAACGACATATGAAAAATGAGTGCAAGTA chr 20 ACGTTTTCGAAAAGTAAGATGAAAACGTTCTCATTTTGTCATGTTTCTTCTGAACGACATATGAAAAATGAGTGCAAGTA
7063	*ZCCHC7* (37245498)	*FRGIB 3' region* (28250500)	chr 9 ATATACTTTTAAGGTCTTTATTTGTTAAGAATTCAAAATTTGACGTGCGGTAAGACTCATCGTACTACTTTAGAACGTGA dic (9;20) ATATACTTTTAAGGTCTTTATTTGTTAAGAATTCAAAATTTAATAGTTTTAAAAGTCTCCTTGTTTTGTTTTAGATCAAA chr 20 TCTTTTTTTTAGTTTGTTTTATGTTGTTTATGTTGTTTTTTAATAGTTTTAAAAGTCTCCTTGTTTTGTTTTAGATCAAA
8901	*ZCCHC7* (37217433)	*LOC149950 3' region* (30660774)	chr 9 ACTGTAAGAGGTATATAAACTTTACCAACTAGAAAAGTTAATACTGGATAAGATCACCTGTTCTCGTCATAGAGTAACAC dic (9;20) ACTGTAAGAGGTATATAAACTTTACCAACTAGAAAAGTTATATTCATCTCGCACCGACTCAGGGTCCCACGTGTCTCTCC chr 20 CCGAGGACTGGGTCGCCCCGGGGGTGGACCGAATCGATAATTATTCATCTCGCACCGACTCAGGGTCCCACGTGTCTCTCC

Figure 2. MCC and sequence analyses of 6 ALL cases with dic(9;20) abnormality. (A) Representative MCC graph of case 6897. The genomic position of each MCC marker is shown above/beside the marker; the breakpoint region is indicated in boxes (solid line for round-1 and dotted line for round-2). MCC refines the position of the breakpoint in *PAX5* 3' downstream region, from 3.2kb (round-1) to 1.4kb (round-2). (B) The dic(9;20) breakpoint sequence and the genomic location on 9p and 20q; alignment of the dic(9;20) breakpoint sequence (middle line) against the normal sequences of chromosome 9 (top line) and 20 (bottom line).

mutation and methylation status of *ASXL1* and its expression in dic(9;20)-positive ALL patients.

Due to the breakpoint heterogeneity of 20q, and considering the location of the type III breakpoints clustering distal to *ASXL1*, the involvement of alternative genes telomeric to *ASXL1* cannot be excluded. A recent array-based study identified 3 dic(9;20) cases with deletion of 9p and 20q. The molecular consequence of the 3 cases was a *PAX5-C20ORF112* fusion, which specifically repressed the transcriptional activity of *PAX5* in a dominant-negative fashion.[13] Our current study identified a single case with the *PAX5-C20ORF112* fusion (1/26, 3.8%), providing a frequency much lower than reported by Kawamata *et al*. of 27% (3/11).[13] Although the involvement of *ASXL1* is implicated in this study, the loss of other tumor suppressor genes from the region, such as *DIDO1* (60.979-61.040Mb) and *L3MBTL* (41.576-41.604Mb), may represent important additional events.[16,17]

This study provides further evidence to support our hypothesis that specific gene loci can be the target of heterogeneous translocation breakpoints in human cancer, acting through a variety of mechanisms. The involvement of *ASXL1* at 20q11.21 is highlighted both in our dataset and information published by others.[6] Further evidence will likely emerge from the use of molecular analysis of larger patient cohorts. Future work should focus on expanding this type of approach to other chromosomal abnormalities, with the aims of identifying novel cancer-associated genes, improving the understanding of carcinogenesis and improving the management of cancer patients.

Authorship and Disclosures

QA performed the experiments, analyzed the data and wrote the manuscript. SLW and HP performed the experiments and analyzed the data. AVM, MG, FMR, TD, CJH analyzed data and reviewed the manuscript. JCS supervised the research and wrote the manuscript.

The authors reported no potential conflicts of interest.

References

1. Pan J, Xue Y, Wu Y, Wang Y, Shen J. Dicentric (7;9)(p11;p11) is a rare but recurrent abnormality in acute lymphoblastic leukemia: a study of 7 cases. Cancer Genet Cytogenet 2006; 169:159-63.
2. Behrendt H, Charrin C, Gibbons B, Harrison CJ, Hawkins JM, Heerema NA, et al. Dicentric (9;12) in acute lymphocytic leukemia and other hematological malignancies: report from a dic(9;12) study group. Leukemia 1995;9:102-6.
3. Rieder H, Schnittger S, Bodenstein H, Schwonzen M, Wormann B, Berkovic D, et al. dic(9;20): a new recurrent chromosome abnormality in adult acute lymphoblastic leukemia. Genes Chromosomes Cancer 1995;13:54-61.
4. Mitelman F, Mertens F, Johansson B. A breakpoint map of recurrent chromosomal rearrangements in human neoplasia. Nat Genet 1997;15:417-74.
5. Clark R, Byatt SA, Bennett CF, Brama M, Martineau M, Moorman AV, et al. Monosomy 20 as a pointer to dicentric (9;20) in acute lymphoblastic leukemia. Leukemia 2000;14:241-6.
6. Schoumans J, Johansson B, Corcoran M, Kuchinskaya E, Golovleva I, Grander D, et al. Characterisation of dic(9;20)(p11-13;q11) in childhood B-cell precursor acute lymphoblastic leukaemia by tiling resolution array-based comparative genomic hybridisation reveals clustered breakpoints at 9p13.2 and 20q11.2. Br J Haematol 2006;135:492-9.
7. An Q, Wright SL, Konn ZJ, Matheson E, Minto L, Moorman AV, et al. Variable breakpoints target PAX5 in patients with dicentric chromosomes: A model for the basis of unbalanced translocations in cancer. Proc Natl Acad Sci USA 2008; 105:17050-4.
8. Strefford JC, Worley H, Barber K, Wright S, Stewart ARM, Robinson HR, et al. Genome complexity in acute lymphoblastic leukaemia is revealed by array-based comparative genomic hybridization. Oncogene 2007;26:4306-18.
9. Daser A, Thangavelu M, Pannell R, Forster A, Sparrow L, Chung G, et al. Interrogation of genomes by molecular copy-number counting (MCC). Nat Methods 2006;3:447-53.
10. Jalali GR, An Q, Konn ZJ, Worley H, Wright SL, Harrison CJ, et al. Disruption of ETV6 in intron 2 results in upregulatory and insertional events in childhood acute lymphoblastic leukaemia. Leukemia 2008;22:114-23.
11. Slater R, Smit E, Kroes W, Bellomo MJ, Muhlematter D, Harbott J, et al. A non-random chromosome abnormality found in precursor-B lineage acute lymphoblastic leukaemia: dic(9;20)(p1?3;q11). Leukemia 1995; 9:1613-9.
12. Forestier E, Gauffin F, Andersen MK, Autio K, Borgström G, Golovleva I, et al. Clinical and cytogenetic features of pediatric dic(9;20) (p13.2;q11.2)-positive B-cell precursor acute lymphoblastic leukemias: a Nordic series of 24 cases and review of the literature. Genes Chromosomes Cancer 2008; 47:149-58.
13. Heerema NA, Maben KD, Bernstein J, Breitfeld PP, Neiman RS, Vance GH. Dicentric (9;20)(p11;q11) identified by fluorescence in situ hybridization in four pediatric acute lymphoblastic leukemia patients. Cancer Genet Cytogenet 1996;92: 111-5.
14. Kawamata N, Ogawa S, Zimmermann M, Niebuhr B, Stocking C, Sanada M, et al. Cloning of genes involved in chromosomal translocations by high-resolution single nucleotide polymorphism genomic microarray. Proc Natl Acad Sci USA 2008;105:11921-6.
15. Bertrand P, Bastard C, Maingonnat C, Jardin F, Maisonneuve C, Courel MN, et al. Mapping of MYC breakpoints in 8q24 rearrangements involving non-immunoglobulin partners in B-cell lymphomas. Leukemia 2007;21:515-23.
16. Cho YS, Kin EJ, Park UH, Sin HS, Um SJ. Additional sex comb-like 1 (ASXL1), in cooperation with SRC-1, acts as a ligand-dependent coactivator for retinoic acid receptor. J Biol Chem 2006;281:17588-98.
17. Fütterer A, Campanero MR, Leonardo E, Criado LM, Flores JM, Hernández JM, et al. Dido gene expression alterations are implicated in the induction of hematological myeloid neoplasms. J Clin Invest 2005;115:2351-62.
18. Bench AJ, Li J, Huntly BJ, Delabesse E, Fourouclas N, Hunt AR, et al. Characterization of the imprinted polycomb gene L3MBTL, a candidate 20q tumour suppressor gene, in patients with myeloid malignancies. Br J Haematol 2004;127:509-18.