Supporting Information

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SI Materials and Methods

Patients. All patients had a confirmed diagnosis of B-cell precursor acute lymphoblastic leukaemia (BCP-ALL) and were entered onto a NCRI Childhood or Adult ALL Working Party treatment trial and registered on the Leukaemia Research United Kingdom Cancer Cytogenetics Group (UKCCG) Karyotype Database in Acute Leukaemia (1). Diagnostic cytogenetic and fluorescence in situ hybridization (FISH) analysis of bone marrow or peripheral blood was carried out in the U.K. regional genetic laboratories and described according to ISCN (2). The presence of a dicentric chromosome was confirmed with chromosome painting and locus-specific probes according to previous studies (3). In total, 110 patients harboured a dicentric involving the short arm of chromosome 9 involving 3 different partner chromosomes; dic(7;9)(p11;p11~13) (n = 13), dic(9;12)(p11~13;p13) (n = 38) and dic(9;20)(p11~13;q11) (n = 59).

FISH. The position of deletion breakpoints were investigated with clones from the National Center for Biotechnology Information map for chromosomes 9, 12 and 20. DNA clones were extracted, labeled, hybridized and visualized using standard methodologies. Probes on the long arm of each chromosome were used as controls. The involvement of *PAX5* was determined with a dual-color break-apart probe constructed from clones position proximal (RP11–297B17) and distal (RP11–344B23) to the gene.

Molecular Copy-Number Counting (MCC). MCC was carried out as described in refs. 4 and 5 with slight modifications. Human male genomic DNA (Promega) was used in preliminary experiments to determine the working concentration of DNA for MCC. A series of dilutions were made to give ≈0.25–8 genomes of DNA per aliquot. The copy-number change of genomic loci (i.e., markers) in the *PAX5* gene (located on chromosome 9p; 2 copies per genome) and in the *MGC39900* gene (located on chromosome Xq; 1 copy per genome) was analyzed by MCC using 96-well plates, as described in refs 4 and 5. A clear copy-number change was observed at the concentration of 0.03 genomes/μl (0.09 pg/μl) of genomic DNA that was used as the working concentration for MCC analyses of the patient (data not shown).

Markers covering *PAX5* exon 2, 3, 4, 5, 7, 8 and 9 were chosen to confirm FISH results of dicentric chromosomal ALLs with breakpoints in the PAX5 gene on chromosome 9p13. Markers in *PAX5* intron 4 and 7 were analyzed subsequently to further refine the breakpoints. Three PCR primers (external forward, internal forward and common reverse) were designed to amplify each marker. The genomic locations of the markers and sequences of the primers are shown in Table S3.

A seminested PCR assay was performed for MCC. For the first PCR, a master mix was prepared that contained 1 × GoTaq Flexi buffer (Promega), 1.5–2.0 mM $MgCl_2$, 200 μM each dNTP, the external forward and common reverse PCR primers of all markers to be analyzed (0.15 μM each oligo), 0.05 u/μl GoTaq Flexi DNA polymerase (Promega) and ≈0.09 pg/μl of genomic DNA. An aliquot of 10 μL of the master mix was dispensed into each of 88 wells of a 96-well plate, and 10 μL of a similar mix lacking DNA was aliquoted into the remaining 8 wells as negative controls. PCR was performed using an MJ PTC-225 Thermal Cycler (MJ Research) with hot start at 95 °C for 2 min, then 30 cycles of 30 sec at 95 °C, 30 sec at 52–56 °C and 1 min at 72 °C, followed by a terminal extension at 72 °C for 7 min. After first PCR, each reaction was diluted to 200 μL with water, and 2 μL of the diluted first PCR product was used as template in each second PCR, containing 0.5 μM the relevant internal-forward and common reverse primers for each specific marker (same concentrations of other reagents as in first PCR). The same thermocycling was carried out for second PCR.

After second PCR, 3 μL of each reaction was mixed with 2 μL of formamide dye mix (98% formamide, 10 mM EDTA/pH 8.0, 0.015% xylene cyanol FF), and loaded on 192-well horizontal 7.5% polyacrylamide gel (MadgeBio Ltd, Grantham, U.K.). The amplified second PCR products were separated by electrophoresis for 15 min at 200 V, then the gel was stained in 1 × SYBR Gold (Invitrogen) for 10 min and scanned using Typhoon Trio Imager (Amersham). The presence or absence of PCR product in each well was scored using MADGE-specific gel image analysis software (Phoretix), and MCC data were analyzed as described in refs. 4 and 5.

Long-distance Inverse PCR (LDI-PCR) Cloning. LDI-PCR was carried out as described in refs. 5 and 6 with modifications. Briefly, 1 μg of genomic DNA was digested with 10 units of the relevant restriction enzyme (New England BioLabs) overnight. The digested DNA was purified using QIAquick PCR Purification Kit (Qiagen), and 0.4 μg of purified DNA was ligated at 4 °C overnight in a total volume of 200 μL with 5 units of T4 DNA ligase (Promega). The ligated DNA was then purified using the same kit and eluted in a final volume of 30 μL. For the first PCR, 10 μL of the ligated DNA was used in a 25 μL of reaction mixture, containing 1 × GoTaq Flexi buffer (Promega), 1.5 mM $MgCl_2$, 200 μM each dNTP, 0.2 μM first-forward and reverse PCR primers, and 0.08 u/μl GoTaq Flexi DNA polymerase (Promega). PCR was carried out with hot start at 95°C for 2 min, then 35 cycles of 40 sec at 95 °C, 40 sec at 55 °C and 5 min at 72 °C, followed by a final extension at 72°C for 10 min. The second PCR mixture contained 5 μL of the first PCR product, 0.2 μM second-forward and the same reverse primers in a final volume of 50 μL (same concentrations of other reagents and same PCR conditions as before). The PCR product was separated on 0.8% agarose gel and purified for direct sequencing. The second-forward and reverse PCR primers were used as sequencing primers. The sequences of LDI-PCR primers are shown in Table S4.

Genomic Breakpoint Cloning with PCR. A seminested PCR was carried out to amplify the fusion sequences in 5 dic(9;12) cases where good quality genomic DNA was available (cases 2616, 3742, 4662, 8952, and 10630). Two oligos spanning *PAX5* exon 4 and the beginning of intron 4 were chosen as the common first-(AGCCACCCAACCAACCAG) and second-forward (GTCACAGCATAGGTAAGAGG) primers. A set of 4 scattering oligos in *ETV6* intron 2 (base pairs 11833587–11859330) were chosen as reverse primers. The sequences of reverse primers are as follows: GAGGAGAGTGAGGCAGG (base pairs 11833587–11833603), CTTACAGGAATCTTTATGG (base pairs 11835721–11835739), GCACCCTCCATACCTAAG (base pairs 11854626–11854643) and CACTAAGTCCTAAGTAGG (base pairs 11859313–11859330). PCR was performed using GoTaq Flexi DNA polymerase (Promega), according to the manufacturer's recommendation. The PCR product was purified for direct sequencing as described above.

Fusion Protein Sequences Prediction. Sequences of chimeric proteins were predicted using the online program GENSCAN (http://genes.mit.edu/GENSCAN).

Quantitative Analysis of PAX5 and Target Gene Expression. Real-Time quantitative RT-PCR (qRT-PCR) was carried out to assess the expression of *PAX5* (exon boundaries 1/2 and 4/5) and *PAX5*-target genes; *EBF1*, *FLT3*, *ALDH1A1* and *ATP9A* genes, using the Taqman Gene Expression Assays (Applied Biosystems) according to the manufacturer's instructions. Seven patients with dicentric chromosomes were compared with 6 cases with a high-hyperdiploidy karyotype (where chromosome 9 was diploid). The comparative *C*t method was used for quantitation of relative gene expression. The average *C*t value of the endogenous control gene, *GAPDH*, was subtracted from the average experimental gene *C*t value to give the Δ*C*t value. Differences between control and test were carried out by using ΔΔ*C*t. Differences in gene expression between the 2 groups was performed using a standard *t* test.

Mutation Analysis of *PAX5*. *PAX5* exons, previously identified to house mutations in childhood ALL, were amplified by PCR using genomic or whole genome amplified DNA from dicentric cases. The amplicons were then screened for mutations by DHPLC using a Transgenomic Wave machine (Crewe). Primer sequences were those previously cited (7) and the DHPLC parameters used are available on request. For those amplicons with chromatographic profiles that differed from wild type, direct sequencing was performed by purifying 100 μL of PCR product using a Qiaquick PCR purification kit (Qiagen) with a final elution volume of 30 μL and then sequenced using both forward and reverse primers with the ABI Version 3 BigDye terminator cycle sequencing kit and analyzed on an ABI prism DNA sequencer (Applied Biosystems). Sequence alignments were carried out using DS gene software (Accelrys).

***PAX5* Copy Number Analysis with Quantitative Genomic PCR.** This assay was performed as described in ref. 7 but using *ATP10A* as the control gene. Briefly, 5-point standard curves ranging from 150 ng to 1 ng/reaction were constructed using normal human genomic DNA (Roche) and amplified for the 3 target *PAX5* exons (exons 3, 6, and 8) and control *ATP10* gene. Assays were performed in duplicate on 2 separate occasions with 50 ng of sample DNA per 20 μL reaction using a Taqman 7500 Real-Time PCR System (Applied Biosystems). *PAX5* gene dosage for each exon were calculated by dividing the value obtained for *PAX5* by the corresponding value for *ATP10A*. The primer sequences are shown in Table S5.

1. Harrison CJ, Martineau M, Secker-Walker LM (2001) The Leukaemia Research Fund/United Kingdom Cancer Cytogenetics Group Karyotype Database in acute lymphoblastic leukaemia: A valuable resource for patient management. *British J Haematol* 113:3–10.
2. ISCN, ed (2005) *An International System for Human Cytogenetic Nomenclature* (S. Karger, Basel).
3. Clark R, *et al.* (2000) Monosomy 20 as a pointer to dicentric (9;20) in acute lymphoblastic leukemia. *Leukemia* 14:241–246.
4. Daser A, *et al.* (2006) Interrogation of genomes by molecular copy-number counting (MCC). *Nat Methods* 3:447–453.
5. Jalali GR, *et al.* (2007) Disruption of ETV6 in intron 2 results in upregulatory and insertional events in childhood acute lymphoblastic leukaemia. *Leukemia* 22:114–123.
6. Willis TG, *et al.* (1997) Rapid molecular cloning of rearrangements of the IGHJ locus using long-distance inverse polmerase chain reaction. *Blood* 90:2456–2464.
7. Mullighan CG, *et al.* (2007) Genome-wide analysis of genetic alterations in acute lymphoblastic leukaemia. *Nature* 446:758–764.

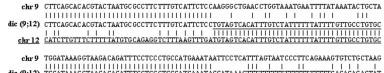
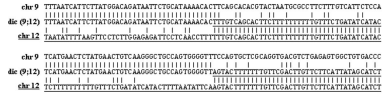
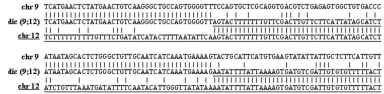
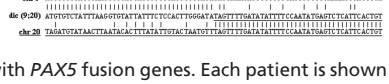
Patient ID	PAX5 breakpoint (location)	*PAX5* fusion Partner breakpoint (location)	Breakpoint sequence
07-07106	Intron 4 (36996026)	*LOC392027*-intron 1 (53037497)	
12-02398	Intron 4 (36996113)	*ETV6*-intron 1 (11715825)	
12-02616	Intron 4 (36995807)	*ETV6*-intron 2 (11853406)	
12-03742	Intron 4 (36995231)	*ETV6*-intron 2 (11854069)	
12-04443	Intron 4 (36996330)	*SLCO1B3*-intron 1 (20895867)	
12-04662	Intron 4 (36995392)	*ETV6*-intron 2 (11834873)	
12-08726	Intron 4 (36996369)	*ETV6*-intron 2 (11834288)	
12-08952	Intron 4 (36995945)	*ETV6*-intron 2 (11834340)	
12-09119	Intron 4 (3693968)	*ETV6*-intron 2 (11831646)	
12-10630	Intron 4 (36994978)	*ETV6*-intron 2 (11854226)	
20-04551	Intron 7 (36899347)	*KIF3B*-intron 6 (30378673)	
20-05618	Intron 7 (36903071)	*C20orf112*-intron 3 (30608872)	
20-07550	Intron 4 (36995786)	*ASXL1*-intron 4 (30464846)	

Fig. S1. Total breakpoint sequencing data from 13 BCP-ALL patients with *PAX5* fusion genes. Each patient is shown horizontally, followed by the location of the *PAX5* breakpoint, the partner gene and the location of the breakpoint within the partner gene. The fusion sequences are show between normal sequence for chromosome 9 and the partner chromosome, where the partner chromosome sequence are underlined.

Table S1. The clinical and cytogenetic data on patients with dicentric chromosomes, with the molecular investigation performed on each case

Patient ID	Gender	Age	Karyotype	Diagnostic FISH	Analysis	*PAX5*
07- 978	F	3	45,XX,dic(7;9)(p13;p11),?t(9;22;21)(q34; q11;q22),add(20)(p)[10]/ 49,idem,+2,+5,+dic(7;9)(p13;p11), +18[12]/ 46,XX[15]	*BCR—ABL*	—	—
07- 1483	F	5	46,XX,dic(7;9)(q36;p12),del(14)(q24q32), +21c[13]/ 47,XX,+21c[2]	—	—	—
07- 2378	F	34	45,XX,dic(7;9)(q10;q10)[12]	Normal	—	—
07- 2521	M	17	45,XY,dic(7;9)(p11;p11)[14]/ 46,XY[1]	—	—	—
07- 4462	F	13	45,XX,dic(7;9)(p11;p11)[7]/ 46,XX[3]	—	—	—
07- 5679	M	18	45,XY,dic(7;9)(p11;p11)[6]/ 46,XY[5]	Normal	—	—
07- 5779	F	46	45,XX,dic(7;9)(p11;p11)[18]/ 46,XX[2]	Normal	BD	Del
07- 5861	F	44	45,XX,dic(7;9)(p11;p1?)[8]/ 46,XX[2]	Normal	BD	Del
07- 7106	M	6	45,XY,dic(7;9)(p11;p11),t(9;22)(q34; q11)[1]/ 46,XY[49]	*BCR—ABL*	BD, MCC, BPC, RT	Par del
07- 7777	M	42	45,XY,dic(7;9)(p1;p1)[6]/ 46,XY[5]	Normal	—	—
07- 9465	M	2	45~46,XY,del(6)(q1q2),dic(7;9)(p1;p1),inc 6/ 46,XY[7]	Normal	BD	Del
07- 10194	M	11	45,XY,dic(7;9)(p1?;p1?),t(9;22)(q34; q11)[9]/ 46,XY[2]	*BCR—ABL*	—	—
07- 11161	M	51	45,XY,dic(7;9)(p11;p11)[10]	Normal	—	—
12- 446	M	6	93?<4n>,XXYY,dic(9;12)(p1?;p1?), add(11)(q23),+mar1,+mar2,+r,inc9	—	—	—
12- 774	M	3	45,XY,dic(9;12)(p11;p12)[3]/ 46,XY[18]	—	BD	Del
12- 1380	M	4	45,XY,del(6)(q?13q?15),dic(9;12)(p11;p11), t(12;13)(p13;q14)[11]/ 46,XY[1]	—	—	—
12- 1441	M	5	46,XY,del(2)(q?23q?31),+8,dic(9;12)(p11~p12; p11~p12),add(10)(q?22),del(17)(p13)[8]/ 45,XY,dic(9;12)(p11~p12;p11~p12), der(18)t(8;18)(q13;q11)[2]/ 46,XY[3]	—	—	—
12- 1620	M	9	47,XY,del(12)(p12),+21[9]/ 46,idem,dic(9;12)(p11;p11)[5]/ 46,XY[6]	*ETV6—RUNX1*[1]	—	—
12- 2392	M	3	45,XY,dic(9;12)(p11;p11)[14]/ 46,XY[6].ish dic(9;12)(wcp9+,wcp12+)	—	BD	Del
12- 2398	M	5	45,XY,dic(9;12)(p11;p11)[5]/ 49,idem,+5,+8,+16,+21[3]/ 46,XY[1]	—	BD, MCC, BPC	Par del
12- 2400	M	2	46,XY,dic(9;12)(p?21;p11.1),+mar[4]/ 46,XY[3]	*ETV6—RUNX1*	BD	Del
12- 2616	M	14	44,XY,der(4;17)(p10;q10),dic(9;12)(p11; p11)[4]/ 45,idem,+der(17;21)(q10; q10)[8]/ 46,XY[2]	Normal	BD, BPC	Par del
12- 3070	F	27	45,XX,dic(9;12)(p1;p1),der(19)t(1;19)(q23; p13)[3]/ 46,XX[4]	Normal	—	—
12- 3255	M	14	45,XY,dic(9;12)(p11;p11),del(13)(q?3), add(20)(q13)[5]/ 46,idem,+8[3]/ 45,idem,-4,-del(13)(q?3),+mar1, +mar2[2]/ 45,XY,-1,dic(9;12)(p11;p11),-10, del(13)(q?3),add(20)(q13),+mar3, +mar4[1]/ 46,XY[20]	Normal	BD	Par del
12- 3278	M	14	46,XY,dic(9;12)(p12;p12),+12[19]/ 46,XY[1]	Normal	BD	Del
12- 3295	M	11	46–47,XY,del(2)(p16),+8,dic(9;12)(p1;p1), +r,+mar11	—	—	—
12- 3742	M	8	45,XY,dic(9;12)(p11–12;p11–12)[22]/ 46,idem,+mar[3]/ 46,XY[8]	Normal	BD, BPC	Par del
12- 3790	M	3	46,XY,dic(9;12)(p1?;p1?),del(11)(q?)[6]/ 46,XY[6]	*ETV6—RUNX1*	BD	Del
12- 3979	M	12	46,XY,+8,add(9)(p),+add(9)(p),dic(9; 12)(p11;p11),-10,del(17)(p11),+22[11]/ 80–86,idemx2,-2,-7,-8,-8,-add(9)(p),- add(9)(p),-dic(9;12)(p11;p11),- del(17)(p11),-18,-20,-?22,-?22,+mar1x25/ 46,XY[3]	Normal	BD, RT	Par del

Patient ID	Gender	Age	Karyotype	Diagnostic FISH	Analysis	*PAX5*
12-4049	F	28	45,XX,dic(9;12)(p1?;p1?), del(17)(p11p13)[20]	Normal	BD	Par del
12-4443	F	1	45,XX,dic(9;12)(p?;p?)[9]/ 46,XX[4]	Normal	BD, MCC, BPC	Par del
12-4512	M	2	45,XY,dic(9;12)(p1?;p1?)[5]/ 46,XY[1]	*ETV6—RUNX1*	—	—
12-4519	F	11	45,XX,dic(9;12)(p?1;p?1)[8]/ 46,XX[2]	Normal	—	—
12-4554	M	2	45,XY,del(12)(p)[3]/ 45,XY,dic(9;12)(p1;p1)[4]/ 46,XY[5]	*ETV6—RUNX1*	—	—
12-4662	M	13	45,XY,dic(9;12)(p11;p11)[22]/ 46,XY,+8,dic(9;12)(p11;p11)[3]/ 46,XY[5]	Normal	BD, BPC	Par del
12-4698	M	4	46,XY[1]/ 45,XY,dic(9;12)(?)[1]	*ETV6—RUNX1*	BD, RT	Del
12-4936	M	4	46,XY,dic(9;12)(p1?3;p11.2)[7]/ 46,XY[1]	*ETV6—RUNX1*	BD	Par del
12-4946	M	15	46,XY,+8,dic(9;12)(p13;p1?2)[7]/ 47,idem,+8[1]	Normal	BD	Par del
12-5928	M	19	44,XY,del(6)(q13q2?5),-7,dic(9;12)(p1;p1), add(11)(q25),add(18)(q21)[2]/ 46,XY[28]	Normal	BD	Par del
12-5999	M	14	45,XY,dic(9;12)(p1;p1),add(11)(q2),-13, der(19)t(1;19)(q23;p13),+mar[8]/ 47,XY,+X,add(2)(p1),add(11)(q2),-13, der(19)t(1;19)(q23;p13),+mar[2]	*PBX1—TCF3*	BD	Par del
12-6067	F	38	45,XX,dic(9;12)(p11;p11)[8]/ 46,idem,+8[4],46,XX[3]	Normal	BD	Par del
12-7088	M	19	45,XY,dic(9;12)(p13;p13)[5]/ 46,XY[4]	Normal	BD	Par del
12-8726	F	16	44,X,der(X)t(X;3)(p22;q21),-3,dic(9;12)(p11;p11)10/ 46,XX[7]	Normal	BD, BPC	Par del
12-8952	M	21	46,XY,+8,dic(9;12)(p13;p13)[8]/ 46,XY[2]	Normal	BD, BPC	Par del
12-9119	M	12	46,XY,+8,dic(9;12)(p13;p13)[10]	Normal	BD, BPC, RT	Par del
12-9380	F	16	46,XX,ins(3;?)(q?22;?),+8,dic(9;12)(p13;p13)[5]/ 46,XX[4]	Normal	BD	Par del
12-10064	F	46	45,XX,der(8)t(8;8)(p?23;q?13),der(2)t(2;9)(?p?;q?25),dic(9;12)(p13;p13)[6]/ 46,XX[4]	Normal	BD	Par del
12-10630	M	10	45,XY,dic(9;12)(q10;q10)[4]/ 46,XY[2]	Normal	BD, BPC	Par del
12-12349	F	18	45,XX,del(6)(q?13q?27),dic(9;12)(p13;p11), add(10)(p11)[10]/ 46,XX[25]	—	—	—
12-12600	F	14	45,XX,i(8)(q10),dic(9;12)(p11;p11),der(17)t(?X;17)(?q12;?p11)[10]	Normal	—	—
20-198	F	2	45,XX,dic(9;20)(p12;q11)[25]/ 46,XX[8]	—	—	—
20-468	F	2	46,XX,dic(9;20)(p1?2;q11),+21[8]/ 46,XX[12]	—	—	—
20-508	F	2	45,XX,dic(9;20)[11]/ 46,XX[9]	—	—	—
20-745	M	2	45,XY,dic(9;20)(p11∼p12;q11)[11]/ 46,XY[15]	—	—	—
20-753	F	2	45,XX,inv(7)(p11q22),dic(9;20)(p1?2;q11)[21]/ 46,XX[9]	—	—	—
20-1127	F	2	45,XX,dic(9;20)4/ 46,XX[7]	—	—	—
20-1534	F	1	45,XX,dic(9;20)(p12;q11)[9]/ 45,idem,inv(9)(p22q12)[3]/ 46,XX[5]	—	—	—
20-1553	M	1	45,XY,dic(9;20)[5]/ 46,XY[58]	—	BD	Del
20-1616	F	2	46,XX,del(9)(p22)[2]/ 49,idem,+X,dic(9;20)(p12;q11), +10,+21,+21[15]/ 49,idem,+X,+10, del(17)(p13),-20,+21,+21[2]/ 46,XX[3]	—	—	—
20-1729	M	5	45,XY,dic(9;20)(p12;q11)[4]/ 46,idem,+X[2]/ 45,idem,del(11)(q21)[1]/ 46,XY[2]	—	—	—
20-1846	F	6	45,XX,dic(9;20)(p1?2;q11), del(11)(q14q23)[5]	—	—	—
20-1920	F	2	45,XX,dic(9;20)(p1?2;q11)[3]/ 46,XX[4]	—	—	—
20-2208	M	17	45,XY,dic(9;20)(p1?2;q11)[5]/ 46,idem,+mar[6]/ 46,XY[6]	—	—	—
20-2384	F	5	46,XX,del(9)(p21)[2]/ 46,idem,del(6)(q15q21)[4]/ 45,idem,dic(9;20)(p1?2;q11)[3]/ 45,idem,del(6)(q15q21),dic(9;20)(p1?2;q11)[1]/ 46,XX[5]	—	BD	Del

Patient ID	Gender	Age	Karyotype	Diagnostic FISH	Analysis	*PAX5*
20-2443	F	2	46,XX,dic(9;20)(p1?2;q11),+mar[3]/ 48,idem,+2mar[3]/ 46,XX[2]	—	—	—
20-2763	F	8	46,XX,dic(9;20)(p1;q1),+21[4]/ 46,XX[30]	Normal	—	—
20-2857	M	23	45,XY,dic(9;20)[6]/ 46,XY[4]	—	—	—
20-2894	M	5	46,XY,dic(9;20)(p12;q11),+21[7]/ 46,XY[10]	Normal	BD	Del
20-3087	M	14	46–48,XY,add(1)(q2?),dic(9;20),del(17)(p11.2),+18,+21	Normal	BD	Del
20-3101	M	13	45,XY,dic(9;20)(p11;?q11),?16[3]	—	—	—
20-3155	F	13	46,XX,dic(9;20)(p11;q11),+21[8]/ 46,idem,i(8)(q10)[8]/ 46,idem,add(15)(p10)[6]/ 46,XX[6]	—	—	—
20-3275	F	11	45,XX,dic(9;20)(p12;q11)[5]/ 46,XX,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]	Normal	—	—
20-3363	F	32	46,XX,dic(9;20)(p12;q11),+21[10]/ 46,XX[1]	Normal	—	—
20-3450	M	2	45,XY,dic(9;20)(p11∼13;q11)[13]/ 46,XY[7]	Normal	—	—
20-3507	F	4	47,XX,+X,dic(9;20)(p11;p11),t(9;14)(p21;q11),+21[12]/ 47,idem,del(5)(q22q34)[3]/ 46,XX[8]	Normal	—	—
20-3599	M	1	46,XY,t(5;12)(q33;p13),del(9)(p13)[2]/ 46,idem,-del(9)(p13),dic(9;20)(p12–13;q13.3)[3]/46,XY[6]	—	—	—
20-3916	M	24	45,XY,dic(9;20)(p1,q11)[7]/ 46,XY[3]	Normal	—	—
20-4039	M	7	45,XY,dic(9;20)(p11;q11),-20[5]/ 46,idem,+18[5]	Normal	—	—
20-4141	M	11	46,XY,dic(9;20)(p11;q11),+21[6]/ 47,idem,+15[2]/ 46,XY[1]	Normal	—	—
20-4377	F	15	45,XX,dic(9;20)(p1?3;p11)[21]	Normal	BD	Del
20-4451	F	2	45,XX,dic(9;20)(p1;q1),del(16)(q22)	Normal	BD, MCC, BPC	Par del
20-4485	M	6	46,XY,del(1)(p1?p3?1),dic(9;20)(p1;q1),+21[9]/ 46,XY[1]	Normal	—	—
20-4506	M	6	46,XY,+8,dic(9;20)(p13?;q11.2?)[5]/ 47,idem,+16[1]	Normal	BD	Del
20-4510	F	2	45,XX,dic(9;20)(p11;p11)[8]/ 46,idem,+21[2]/ 46,XX[2]	Normal	BD	Del
20-4549	M	51	46,XY,dic(9;20)(p1?;q1?),+21[9]/ 46,XY,dic(9;20),+20,r(20)(p?q?)[6]/ 45,XY,dic(9;20),add(20)(q13)[4]/ 46,XY[1]	Normal	BD	Del
20-4718	F	2	45,XX,t(5;9)(?q13;?p22),dic(9;20)(p11–13;q11)[11]/ 45,idem,?del(6)(q)[7]/ 46,XX[2]	Normal	BD	Del
20-4887	F	8	45,XX,-7,dic(9;20)(p1?;q1?)[14]/ 46,XX[6]	Normal	BD	Del
20-5037	F	1	45,XX,dic(9;20)(p11;q11)	Normal	—	—
20-5618	F	2	45,XX,dic(9;20)(p11;q11)[11]/ 46,XX[4]	Normal	BD, MCC, BPC	Par del
20-5824	F	3	46,XX,dic(9;20)(p11;q11),+21[5]/ 47,idem,+X[2]/ 48,idem,+X,+?9[2]/ 46,XX[2]	Normal	BD	Del
20-6789	F	4	46–47,XX,del(6)(q1q2),+7,dic(9;20)(p11;q11),+mar9/ 46,XX[8]	Normal	BD	Del
20-6897	M	1	45,XX,dic(9;20)(p11;q11)[10]/ 46,XX[2]	Normal	BD	Pres
20-7063	F	7	46,XX,del(6)(q16q24.1),dic(9;20)(p13.2;q1),+21[10]	Normal	BD	Del
20-7167	F	1	45,XX,t(7;15)(p1;q1),dic(9;20)(p13;q11)[8]/ 46,XX[2]	Normal	BD	Del
20-7201	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)(p13;q11),+20[10]	Normal	BD	Del
20-7209	F	2	45,XX,dic(9;20)(p11–13;q11)[6]/ 46,XX[4]	Normal	BD	Del
20-7339	F	2	45,XX,dic(9;20)(p11;q11)[6]/ 46,XX[1]	Normal	—	—
20-7550	M	8	46,XY,dic(9;20)(p1;q11),+mar[8]/ 46,XY[2]	Normal	BD, MCC, BPC	Par del
20-8330	M	4	45,XY,dic(9;20)(p11;q11)[6]/ 46,XY[4]	Normal	—	—

Patient ID	Gender	Age	Karyotype	Diagnostic FISH	Analysis	*PAX5*
20- 8901	M	3	45,XY,dic(9;20)(p13;q11),del(9)(q2q3), 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]/46,XY[3]	Normal	BD	Del
20- 9491	M	4	46,XY,dic(9;20)(p1?1;q11),+21	Normal	—	—
20- 10061	M	4	46,XY,dic(9;20)(p1?1;q11),+21,+mar[7]/ 46,XY[2]	Normal	BD, RT	Del
20- 10219	M	48	45,XY,dic(9;20)(p1;q11)[10]	Normal	BD	Del
20- 10401	F	1	45,XX,dic(9;20)(p13.2;q11.21)[6]/ 46,XX[14]	Normal	BD	Pres
20- 10537	F	7	46,XX,dic(9;20)(p11∼13;q11.22), +mar[2]/ 46,XX[23]	*ETV6—RUNX1*	BD	Del
20- 10862	F	4	46,XX,dic(9;20)(p13;q11),+21[11]/ 46,XX[3]	Normal	—	—
20- 10868	F	2	48,XX,+X,del(9)(p2?),+10,del(15)(q2?2), dic(9;20)(p11∼13;q11.21),+21[7]/ 46,XX[4]	Normal	BD, RT	Del
20- 11906	M	8	45,XY,dic(9;20)(p11–13;q11)	Normal	—	—

Gender, Male (M) and Female (F), Several cases were diagnosed with RT-PCR ([1]), breakpoint delineation with FISH probes (BD), molecular copy number counting (MCC), breakpoint cloning (BPC), quantitative reverse transciptase PCR (RT), no material available for analysis (-), loss of PAX5 (Del), partial deletion (Par Del) and retained (Pres).

Table S2. Quantitative PCR data for *PAX5* and *PAX5* target gene expression in ALL patients with dicentric chromosomal abnormalities (1) and a high-hyperdiploidy chromosome complement (2)

	PAX5 exon 1/2		PAX5 exon 4/5		EBF1		FLT3		ALDH1A1		ATP9A	
Patients ID	$^{\Delta\Delta}C_t$	Fold change	$^{\Delta\Delta}C_t$	Fold change	$^{\Delta\Delta}C_t$	Fold change	$^{\Delta\Delta}C_t$	Fold change	$^{\Delta\Delta}C_t$	Fold change	$^{\Delta\Delta}C_t$	Fold change
12–3979[1]	−6.24	75.58	−4.7	25.99	−1.07	2.1	−4.22	18.64	7.09	0.01	7.61	0.01
12–4698[1]	−4.71	26.17	−4.73	26.54	−1.77	3.41	−5.56	47.18	7.5	0.01	7.52	0.01
12–9119[1]	−6.28	77.71	−4.71	26.17	−3.26	9.58	−5.28	38.85	6.75	0.01	4.41	0.05
12–9380[1]	−6.18	72.5	−4.7	25.99	−1.62	3.07	−4.93	30.48	6.94	0.01	6.9	0.01
20–10061[1]	−4.77	27.28	−4.73	26.54	−2.2	4.59	−6.55	93.70	8.81	0	4.71	0.04
20–10868[1]	−4.67	25.46	−4.71	26.17	−1.79	3.46	−5.85	57.68	7.71	0	4.32	0.05
07–7106[1]	−5.73	53.08	−4.68	25.63	−1.53	2.89	−3.36	10.27	6.91	0.01	4.5	0.04
11384[2]	−6.42	85.63	−6.37	82.71	−6.85	115.36	−1.53	2.89	2.48	0.18	1.37	0.39
11387[2]	−6.96	124.5	−6.9	119.43	−4.9	29.86	−2.42	5.35	2.07	0.24	2.28	0.21
11674[2]	−6.92	121.1	−6.94	122.79	−5.26	38.32	−2.72	6.59	2.72	0.15	1	0.5
11738[2]	−7.35	163.14	−7.41	170.07	−6.15	71.01	5.18	0.03	2.91	0.13	−0.65	1.57
11884[2]	−8.83	455.09	−8.87	467.88	−6.42	85.63	−1.9	3.73	3.02	0.12	1.45	0.37
12202[2]	−6.85	115.36	−6.79	110.66	−5.57	47.50	−1.95	3.86	2.51	0.18	0.09	0.94

1. Harrison CJ, Martineau M, Secker-Walker LM (2001) The Leukaemia Research Fund/United Kingdom Cancer Cytogenetics Group Karyotype Database in acute lymphoblastic leukaemia: a valuable resource for patient management. *British J Haematol* 113:3–10.
2. ISCN, ed (2005) *An International System for Human Cytogenetic Nomenclature* (S. Karger, Basel).

Table S3. Primer sequences for MCC analysis of PAX5

Marker (location in PAX5 in bp)	External forward primer sequence	Internal forward primer sequence	Common reverse primer sequence
1 (37010544–37010810)	GATCTGTTTCAGGACATGG	CATCTCCAGGCAGCTTCG	CTATGATACTGTCATATTGG
2 (37004914–37005184)	GAGACAGGAAGCATCAAG	CACCATGTTTGCCTGGG	CAGGAAAGGCACATGCAG
3 (36996465–36996653)	CTCAGCTCGAACCATGG	CTCTTCTCCTTCCTGATGG	CTTACCTATGCTGTGACTGG
4 (36992704–36992937)	CTCGTGGCTCAGGTGTGG	CCTCACGTGCGGTGACAG	CTGATGGAGTACGACGAG
5 (36913347–36913581)	GACTAACTCAGCTTGGTGG	CAGAGTATTCAGCCATGG	CTCACCTGTCACAATGGG
6 (36871987–36872254)	GTGAGAGCGTGTGAGCG	GTTGCTTTGATTCCTCAGG	CAGTGCAAACTCACCAGG
7 (36836858–36837093)	CACTCCATAATGATGTGG	GAACTGGATTTGACGTTTGG	GGAACCTCCAGGAGTCG
8 (36996229–36996479)	CACAGCATAGGTAAGAGG	CAGCACACGTACTAATGCG	CAATTTTCTGTCTCATGGTG
9 (36995778–36996057)	GCACTTGCAGTCTTGAGTG	GGTGACGTCTGAGAGTGG	GTTTCCTGTATCCCAAGTGG
10 (36995299–36995594)	GAATGGGCAGAGTCCTGG	CTGATCCTCCATGGTTGG	GTCTTCCCTTTCTTATAGG
11 (36994780–36995032)	GAAGAATAATTCTACTGTAGG	GTTCACAAACTTACCGAGG	CAGAGCCCATGACATTCG
12 (36994315–36994566)	CTGAAATGTGAGAAGAAAGG	CTACTCTTAAGAGTCAAAGG	GCTGAATTGCCATTTCTGG
13 (36993761–36994012)	GATAAATAATAGCACTCTGG	CGAAGCAGATTAGTTATAGG	GAAGTCGAGGTTGTAGTG
14 (36993279–36993548)	GCCTCTTATTAGCTGTATG	GATAATGCCTCAATGCCTGG	CAAACTCCAAACCGCAGAG
15 (36909513–36909803)	GTGCAGTGGCATGATCTG	CGGAGTTTCACCATCTTG	GGAAAGAATTCACTATTAAAG
16 (36904834–36905069)	CGCAACATCCATCAATAGG	GTGTCTAAGATATACTTTGAAG	CACACGCACATGCATCTG
17 (36900684–36900945)	CGAATGAATGAATGACATGC	GGAAAGCATAGAGGTCCG	CAACTGAGAACAGCTTTGG
18 (36896175–36896448)	GGACACACAAATTCCTTGG	CTCTTGTGGTTACATTGGG	TCCTAATTCCCAGAACCTG
19 (36891580–36891853)	AGATCCTCCTACCATCTTG	ACAGAGCATCACCATTAGG	TGAGGAAATGGCGTATCAG
20 (36887153–36887432)	TAGAAGTGAGGTTCACTGG	TCCCTGATTCCACAGCG	TCAGTCTGAGCTTCCTCG
21 (36882072–36882337)	ATCCTTTAGAGCACTCAGG	CTCCTGTCTCTGGAGCAG	CGGAACCAGCAACTGTTG
22 (36877094–36877377)	GAGATCCTACCTTCATCG	TTCAGGTGCTGGTATCAC	AACAGGGACTTCAATTCAG
23 (36904035–36904317)	CTTTGCTCCATTTACACGG	CACGTACCTGAATGTTTGG	TAACTGCACCTCCAGAGG
24 (36903173–36903431)	GGTGAAGGACTTAGCTGG	TGTGAGGAGGAGACAGAG	GTCTTTGTGGGTATTTCCG
25 (36902089–36902331)	TCACAGCTTCTGGCAAGG	TGGGCTCTTGTCCAGAG	AGACTCCACCACCTTCTG
26 (36899524–36899761)	GTCTACATTCCCAAGTGG	ACTTACCTTACCTGCTGG	CTTCCTGGCTTGAGAGAG
27 (36898527–36898817)	TCTGCTAATGGTCTCATAG	GCTTGTTCTTGTGGTCAG	CATGAAGGAGGAGTTCAG
28 (36897501–36897760)	TCAGGATGTACACATACAG	CTTCCACCCTGTCTGATG	CCTGTTCACCTCTGTCTG

Table S4. Restriction enzymes and primers for LDI-PCR

Case no.	Restriction enzyme	First Forward primer sequence	Second Forward primer sequence	Reverse primer sequence
9119	Nla III	GAAGTGTGTCTGAGATTGG	CCCAGAAATGGCAATTCAG	CTTTGACTCTTAAGAGTAGG
8726	Bsl I	AGCCACCCAACCAACCAG	GTCACAGCATAGGTAAGAGG	GCTATTTACCATCAGGAAGG
4443	NcoI/ApaII	AGCCACCCAACCAACCAG	GTCACAGCATAGGTAAGAGG	GCTATTTACCATCAGGAAGG
2398	Nco I	CACAGCATAGGTAAGAGG	CAGCACACGTACTAATGCG	GCTATTTACCATCAGGAAGG
7550	Nco I/Bsl I	GCACTTGCAGTCTTGAGTG	GGTGACGTCTGAGAGTGG	ATCTGACATTCTATAGACCC
4451	TaqI	ACTTACCTTACCTGCTGG	CTCTCTCAAGCCAGGAAG	TGGGAATGTAGACCCAAG
5618	PstI/SphI	TGTGAGGAGGAGACAGAG	AGCGGAAATACCCACAAAG	CTGTCCTCTCTGGCAGG
7106	Nco I	CACAGCATAGGTAAGAGG	CAGCACACGTACTAATGCG	GCTATTTACCATCAGGAAGG

Table S5. Oligonucleotide primers and probes used for PAX5 genomic DNA real-time PCR

Primer	Sequence
PAX5 exon 3 Forward	5′-GGTTCCTCATGGCTAAGCTTCTT-3′
PAX5 exon 3 Reverse	5′-CCTCACCTGTTGATGGAACTGA-3′
PAX5 exon 3 Probe	6FAM-CGCCACACCCAAAGTGGTGGAAAAAA-TAMRA
PAX5 exon 6 Forward	5′-TGTCTTTCTTTAGCAACGTGTATAACC-3′
PAX5 exon 6 Reverse	5′-TGGTGGGCGTGCATCAC-3′
PAX5 exon 6 Probe	6FAM-ACGGCCACTCGCTTCCGGG-TAMRA
PAX5 exon 8 Forward	5′-GGGCACATTGCCGTTCA-3′
PAX5 exon 8 Reverse	5′-GACGCCGACAGTGCAAACT-3′
PAX5 exon 8 Probe	6FAM-CCCCGCTGGACAGGGCAGC-TAMRA
ATP10A Forward	5′-AGCCCCATGGTGAGTGTACAG-3′
ATP10A Reverse	5′-GTAGGATTAAATATAGACACCTTCCATGAG-3′
ATP10A Probe	6FAM-CCACGTCTCTTCCCCATTTTCACCATC-TAMRA