Genes commonly deleted in childhood B-cell precursor acute lymphoblastic leukemia: association with cytogenetics and clinical features

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Online Supplementary Design and Methods

Patients

Patients in this study were diagnosed with BCP-ALL and registered on the UK treatment trials, UKALL97/99 (April 1997-June 2002) for children aged 1-18 years[1] and UKALL2003 (October 2003-July 2011) for children aged 1-18 years, revised in January 2008 to include young adults up to 25 years old.[2] Clinical details, including age, white cell count (WCC) and immunophenotype were collected by the Clinical Trial Service Unit (CTSU), Oxford, UK. All participating centers obtained local ethical committee approval and written informed consent from patients, parents or guardians in accordance with the Declaration of Helsinki. Patients with Down Syndrome (n=81) were excluded as these data are published.[3] Risk was assessed using NCI criteria. Patients aged 1-9 years with a WCC of $< 50 \times 10^9$/L at diagnosis were classified as standard risk. All patients of ≥10 years and any child with a WCC of $> 50 \times 10^9$/L were classified as high risk.

Cytogenetics

Cytogenetic analysis, including fluorescence *in situ* hybridization (FISH) and/or RT-PCR for detection of chromosomal abnormalities of prognostic significance, was carried out in the regional cytogenetic laboratories as part of routine diagnosis and collated by the Leukaemia Research Cytogenetics Group, as previously described.[4] Patients were classified into eight cytogenetic subgroups according to the presence of the following chromosomal abnormalities: 1) t(12;21)(p13;q22)/*ETV6-RUNX1* fusion; 2) high hyperdiploidy (51-65 chromosomes); 3) translocations involving 11q23/MLL rearrangements; 4) t(9;22)(q34;q11)/*BCR-ABL1* fusion; 5) intrachromosomal amplification of chromosome 21(iAMP21);[5] 6) t(1;19)(q23;p13)/TCF3-PBX1; 7) other abnormal (absence of abnormalities in subgroups 1-7 above); and 8) normal karyotype. Patients without abnormalities 1-7 above with 20 or more normal metaphases analyzed were classified as normal, whereas those with less than 20 normal metaphases were regarded as failed, along with patients with no cytogenetic result. The small number (n=26) of patients with near-haploid (24-29 chromosomes) or low hypodiploid (30-39 chromosomes) karyotypes were excluded from the analysis, as from our previous experience, the MLPA results were difficult to interpret due to the cells having a single copy of most of the chromosomes.[6] Patients were classified into good, intermediate, and poor cytogenetic risk groups according to previously-published data.[7]

DNA samples

DNA was obtained from the presentation bone marrow sample of 1199 patients. In 228 patients without available bone marrow cells, DNA was extracted from cell pellets prepared for cytogenetic analysis using the QIAGEN DNeasy tissue and blood kit, according to the manufacturer's instructions. We have previously demonstrated that these samples provide an effective source of DNA for MLPA when used in conjunction with control DNA prepared in the same manner.[6]

Multiplex ligation-dependent probe amplification

The copy number of *IKZF1*, *CDKN2A/B*, *PAX5*, *EBF1*, *ETV6*, *BTG1*, *RB1*, and genes within *PAR1*: *CRLF2*, *CSF2RA*, *IL3RA*, was evaluated using the SALSA MLPA kit P335 IKZF1 (MRC Holland, The Netherlands), as previously described. Data were analyzed using GeneMarker V1.85 analysis software (SoftGenetics, USA). Relative copy number was obtained after normalization of peaks against controls. Values between 0.75 and 1.3 were considered to be within the normal range, equivalent to the normal copy number of 2. Values below 0.75 or above 1.3 indicated loss or gain, respectively. A value below 0.25 indicated biallelic loss. These values correspond to copy numbers of 1, 3+ or 0, respectively.

For patients entered on UKALL2003, deletions of *IKZF1* and *RB1* were confirmed and further characterized by the P202 *IKZF1* and the P047 *RB1* SALSA MLPA kits, respectively. These kits provide increased probe coverage of these genes; full details are available on the MRC Holland website (*www.mlpa.com*). Deletion of single probes was only regarded as positive if confirmed by a different probe(s) in the second kit (*IKZF1* and *RB1*).

Fluorescence *in situ* hybridization

Deletion of genes within the PAR1 region identified by MLPA were confirmed as *P2RY8-CRLF2* or unbalanced *IGH@-CRLF2* translocations by interphase FISH using the following validated commercial and in-house probes: 1) *IGH* dual color break-apart probes (Abbott Diagnostics, UK; Cytocell, UK); 2) break-apart

CRLF2 probe; 3) break-apart *P2RY8* probe. Details of probes 2) and 3) have been previously reported.[8] *ETV6* deletions seen by MLPA were confirmed by the *TEL-AML* ES probe (Abbott Diagnostics, UK) or the ETV6 FISH DNA Probe, Split Signal (Dakocytomation, Denmark).

Statistical analysis

Statistical analysis was carried out using Intercooled STATA v.12.0 (StataCorp, USA), particularly Wilcoxon Rank Sum for non-parametric assays and χ^2 for comparison of categorical variables.

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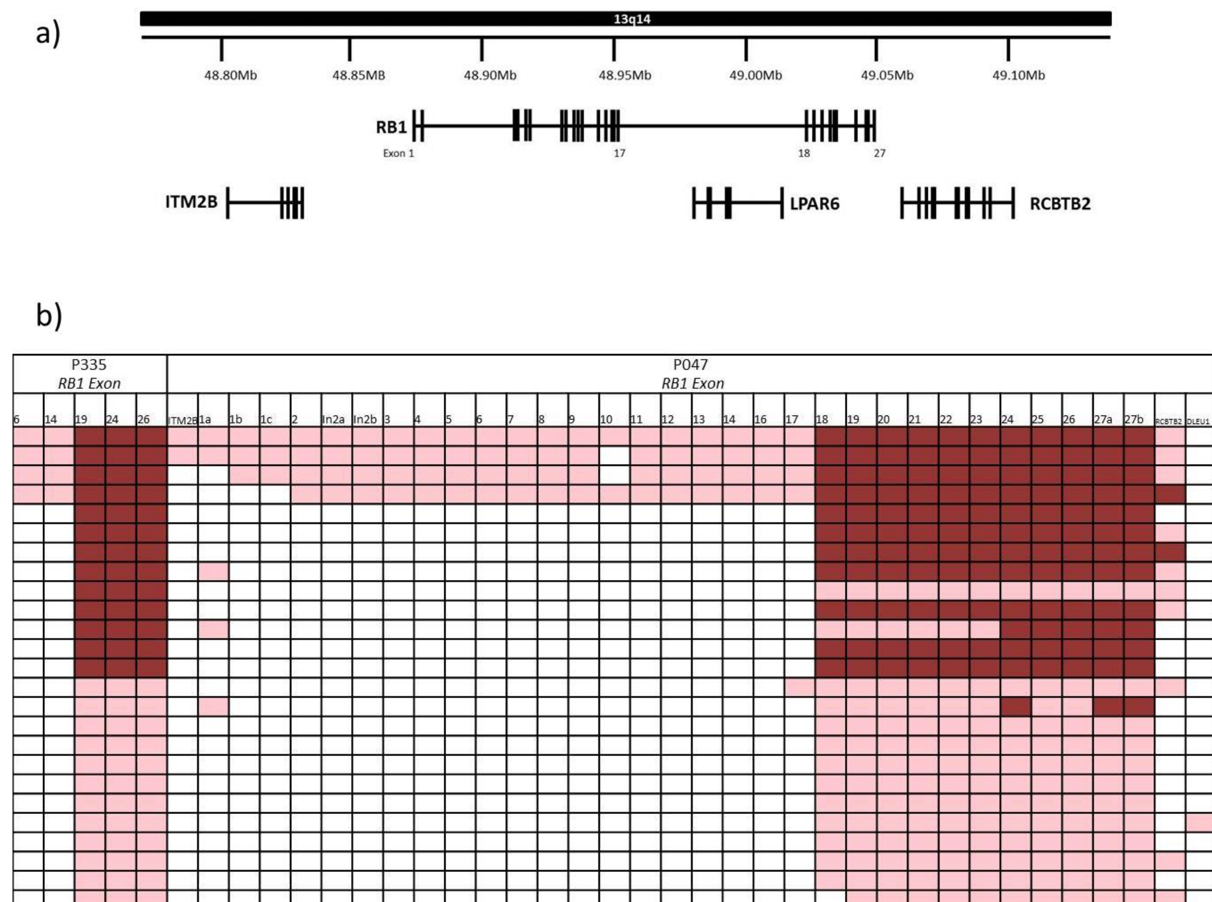
Supplementary Figure 1: Focal *IKZF1* deletions in patients from UKALL2003 analysed with the MLPA kits: P335 and P202.

Deletions of *IKZF1* mapped for the individual patients using the P335-*IKZF1* kit are confirmed and further characterised using the P202-*IKZF1* kit which contains additional probes for *IKZF1*. Monoallelic deletions are shown in pink with biallelic deletion in dark pink. Probes showing borderline deletions are in light pink, which is interpreted as a low percentage of cells having the deletion. This comparison was not carried out on samples from UKALL97/99 patients due to the relative lack of material and the knowledge that 100% concordance was achieved between kits in the testing of UKALL2003.

P335								P202																		
1	2	3	4	5	6	7	8	1a	1b	1c	2a	2b	3a	3b	In3	4a	4b	5a	5b	6a	6b	7a	7b	8a	8b	

Supplementary Figure 2: Focal *RB1* deletions analysed with MLPA kits: P335 and P047



a) The region tested by the P047-*RB1* MLPA kit. The kit includes single probes for 26 of the 27 *RB1* exons. No probe is present for exon 15 which is located in close proximity to the adjacent exons. Exon 1 is covered by three probes and exon 27 by two. Probes are also included for the adjacent genes: *ITM2B*, *RCBTB2* and *DLEU* located 1.6 Mb telomeric of *RB1*. b) Deletions of *RB1* mapped for each patient using the P335-*IKZF1* kit were confirmed and further characterised using the P047-*RB1* kit. Monoallelic deletions are shown in pink with biallelic deletion in dark pink.

Supplementary Table 1: Demographic and clinical features of tested and non-tested patients treated on ALL97/99 and ALL2003.

		Not Tested		Tested		p- value
		Number	Percentage (%)	Number	Percentage (%)	
Total	4941	3514	71%	1427	29%	
Trial	ALL97	1462	42%	507	36%	
	ALL2003	2052	58%	920	64%	<0.001
Sex	Male	1968	56%	762	53%	
	Female	1546	44%	665	47%	0.089
Age	<2	249	7%	101	7%	
	2-4	1528	43%	604	42%	
	5-9	893	25%	385	27%	
	10-14	576	16%	240	17%	0.664
	15-24	268	8%	97	7%	
WCC	<10	1653	47%	633	44%	
	10-49.9	1111	32%	527	37%	0.001
	≥50	750	21%	267	19%	
CNS[1]	Yes	61	2%	17	1%	
	No	3325	98%	1395	99%	0.136
Cytogenetic Subgroups[2]	t(12;21)	732	23%	379	28%	
	HeH	1033	33%	408	30%	
	MLL	68	2%	28	2%	0.004
	t(9;22)	79	3%	33	2%	
	iAMP21	51	2%	33	2%	
	t(1;19)	91	3%	44	3%	
	other	793	25%	324	25%	
	normal	305	10%	102	8%	
NCRI Risk	Standard	2106	60%	882	62%	
	High	1408	40%	545	38%	0.211

[1]data available for 4798 patients (97%)

[2]successful cytogenetics only

HeH, high hyperdiploidy

Supplementary Table 2:

Types of cytogenetic abnormalities associated with *PAX5* and *CDKN2A/B* CNA

Abnormality	*PAX5* CNA and *CDKN2A/B* loss	*CDKN2A/B* loss only
Monosomy 9	4	2
dic(9;20)	17	3
del(9p)*	47	26
der(7;9)	7	0
dic(9;12)	15	1
i(9)(q10)	7	2
Other dicentric	5	1
9p translocation	1	4
9p inversion	0	1
no 9p abnormality	41	171
fail	18	22

**includes some cases reported as add(9p) with associated loss of 9p material