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Homozygous deletions of *CDKN2A* are present in all dic(9;20)(p13·2;q11·2)-positive B-cell precursor acute lymphoblastic leukaemias and may be important for leukaemic transformation

The dic(9;20)(p13·2;q11·2) is a recurrent chromosomal aberration present in up to 5% of childhood B-cell precursor acute lymphoblastic leukaemia (BCP ALL) patients and associated with worse survival compared with the standard risk aberrations t(12;21)(p13;q22) and high hyperdiploidy (Zachariadis *et al*, 2011). Molecular studies have revealed scattered breakpoints on both chromosomes, however all are clustered within 9p13·2 and 20q11·2 (Schoumans *et al*, 2006; An *et al*, 2009). In some cases, dic(9;20) has been shown to rearrange the *PAX5* gene with various partners at 20q (An *et al*, 2008), but in most instances the functional outcome is unknown and possibly heterogeneous.

CDKN2A deletions at 9p21·3 are among the most frequent aberrations in childhood BCP ALL, and can be detected by single nucleotide polymorphism (SNP array) in 21–34% of cases (Mullighan *et al*, 2007; Sulong *et al*, 2009). In dic(9;20)-positive BCP ALL, one allele of the *CDKN2A* locus is always lost because it is located distally to the 9p13·2 breakpoint (Schoumans *et al*, 2006; An *et al*, 2009). Using interphase fluorescence *in situ* hybridization (FISH) analyses, homozygous *CDKN2A* deletions in dic(9;20) has been reported in about one-third of cases.

To evaluate further the presence of genomic imbalances in this specific subgroup, we analysed diagnostic samples from six dic(9;20)-positive paediatric BCP ALL patients by SNP array (Human610W-Quad v.1·0; Illumina Inc, San Diego, CA, USA), according to the manufacturer's instructions (Infinium HD Assay Super manual, #11322427 rev C). Analysis was done using Illumina Genome Studio (Illumina Inc) and Nexus Copy Number (BioDiscovery Inc, El Segundo, CA, USA). FISH analysis for dic(9;20) was performed as previously described (Zachariadis *et al*, 2011). The study was approved by the research ethics committee at Karolinska Institutet and informed consent was obtained in accordance with the Declaration of Helsinki.

As expected, considering the unbalanced nature of the dic(9;20), the SNP array analysis revealed loss of the p arm of chromosome 9 and the q arm of chromosome 20 in all six patients (Fig 1A). In three cases (Patients 1, 3, and 5), the breakpoints on chromosome 9 were present within the *PAX5* gene, in introns 7, 8, and 5 respectively, whereas Patient 6 had a breakpoint 200 kb closer to the centromere, within *ZCCHC7*, and Patients 2 and 4 had breakpoints an additional 2 Mb away (Table I). On chromosome 20, the three cases with breakpoints within *PAX5* all had breakpoints within *C20orf112* intron 2 (however probe density in this region was low), previously shown to be fused in-frame with *PAX5* in dic(9;20)-positive cases (An *et al*, 2008; Kawamata

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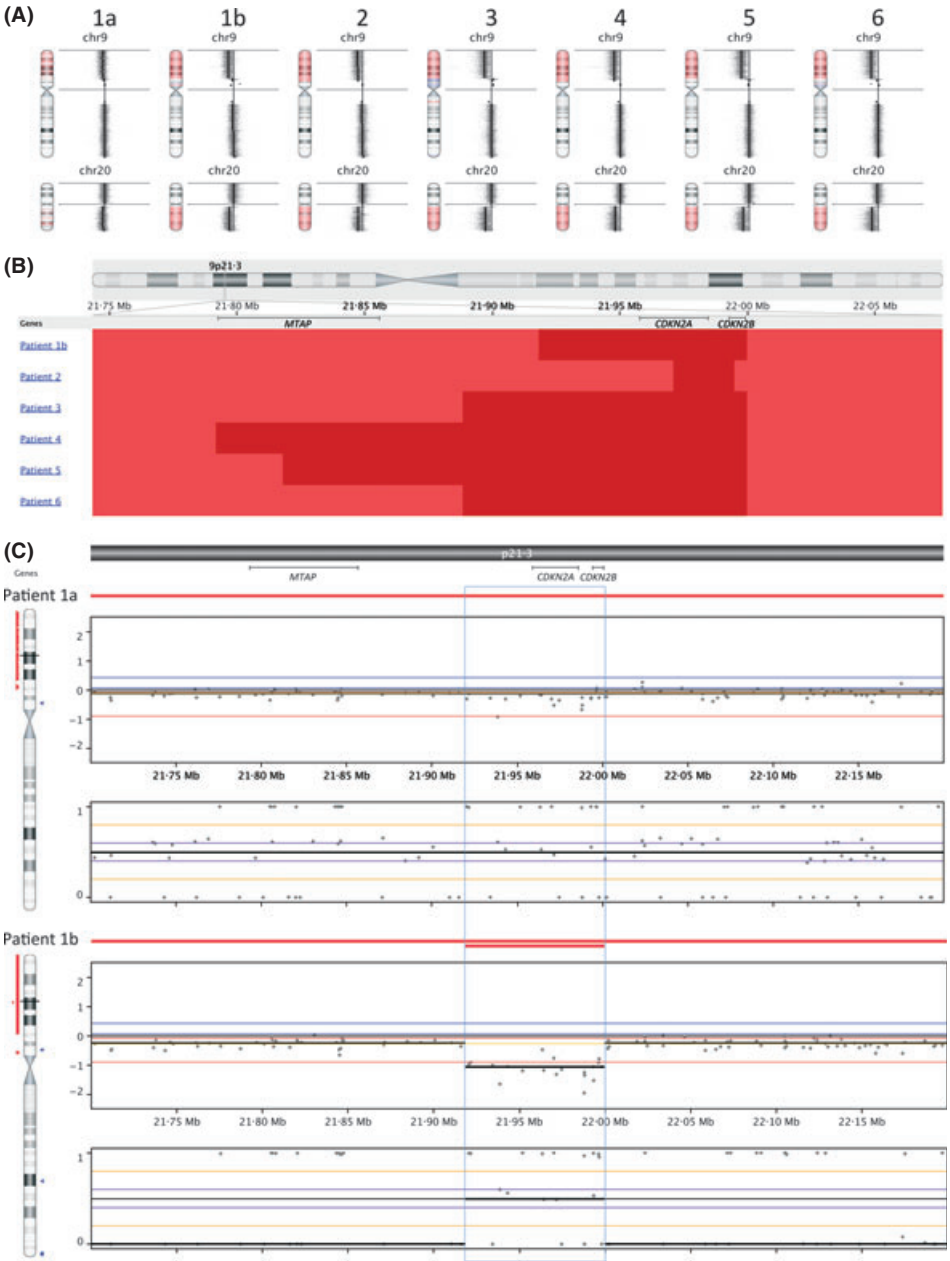

Fig 1. (A) Deletions of chromosome arms 9p and 20q in all six patients analysed by SNP array. Red indicates deletion and blue amplification. Log R ratios (LRR) are shown to the right of respective ideogram; left and right deviations from the midlines indicate deletions and amplifications, respectively. (B) Heatmap of deletions depicting homozygous deletions at 9p21·3 in all six patients. Light red and dark red indicate hemizygous and homozygous deletions, respectively. Review of probe level data suggests an identical breakpoint also for Patient 2, though falling just above the cut-off for homozygous deletion (probe rs2064922, LRR −0·9496). (C) Homozygous loss of *CDKN2A* in the diagnostic (Patient 1b) sample as compared with the hemizygous deletion in the 'pre-leukaemic' sample (Patient 1a). In each sample, the upper box plots LRR and the lower box B allele frequency (BAF). One or two red lines indicate hemizygous or homozygous deletions, respectively, at 9p21·3. Blue rectangle indicates the region of the homozygous deletion detected at diagnosis.

et al, 2008). Apart from homozygous *CDKN2A* deletions (see below) and rearrangements involving the *TRG@*, *HLA* and *IGL@* loci, no other imbalance was recurrent (Table I).

Interestingly, all six patients analysed had homozygous deletions of *CKDN2A*. The deletions displayed a pattern of one large, terminal deletion, as expected in dic(9;20), and one small, interstitial deletion. In contrast, previous interphase FISH analyses of these cases revealed only hemizygous loss of the *CDKN2A* gene (Zachariadis *et al*, 2011). The sizes of the homozygous deletions, as ascertained by the SNP array, ranged from 23 to 201 kb and would thus have escaped detection by FISH analysis (Table I; Fig 1B). It is

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Table I. Clinical, cytogenetic, and SNP array data for six dic(9;20)-positive BCP ALL cases.

Patient	Sex	Age (years)	Karyotype	Breakpoint position*		*CDKN2A* status			Additional aberrations
				Chr 9	Chr 20	Deletion type	Deletion size (kb)	FISH	
1a†	F	1	46,XX[27]	36 884 656	30 512 043	Hemizygous	–	No deletion (60%)/ hemizygous (38%)	None
1b‡	F	1	45,XX,dic(9;20)(p13·2; q11·2)[5]/46,XX[9]	36 884 656	30 512 043	Homozygous	78	Hemizygous	None
2	F	3	46,XX,dic(9;20)(p13·2; q11·2),+21[18]/46,XX[8]	38 781 694	30 116 038	Homozygous	23	Hemizygous	del(12) (q23·3q24·12), +21
3	F	1	46,XX[24].ish. dic(9;20) (p13·2;q11·2)	36 846 268	30 512 043	Homozygous	105	No deletion (68%)/ hemizygous (31%)	del(22) (q13·3q13·31)
4	F	2	46,XX,t(7;22)(p15;q11), dic(9;20)(p13·2;q11·2) [5]/46,XX[6]	38 781 694	29 297 270	Homozygous	201	Hemizygous	None
5	F	1	45,XX,dic(9;20)(p13·2; q11·2) [22]/46,XX[1]	36 975 404	30 512 043	Homozygous	178	Hemizygous	None
6	M	8	46,XY,dic(9;20)(p13·2; q11·2),+8,+21[10]/47, idem,+mar[11]	37 169 379	30 732 585	Homozygous	105	Hemizygous	+8,del(10) (q26·13q26·2),del (16)(q12·1q21), +21

Patients 1b and 2–6 previously published (Schoumans *et al*, 2006; Zachariadis *et al*, 2011).
ish, *in situ* hybridization; FISH, fluorescence *in situ* hybridization.
*Reference build hg18 (http://genome.ucsc.edu/cgi-bin/hgGateway?hgsid=289071031&clade=mammal&org=Human&db=hg18).
†'Pre-leukaemic' sample.
‡Diagnostic sample.

noteworthy that the proximal breakpoints of the *CDKN2A* deletions were identical in all six cases (Illumina probe rs2069422; Fig 1B). Two cases (Patients 3 and 6) also shared distal breakpoints (rs756641), rendering their *CDKN2A* deletions fully synonymous. This strongly indicates a common underlying mechanism, such as illegitimate V(D)J recombination mediated by the recombination-activating gene complex acting on 'ectopic' recombination signal sequences, previously shown to be frequently associated with *CDKN2A* deletions in lymphoid leukaemias (Kitagawa *et al*, 2002).

Patient 1 initially presented with a severe varicella zoster virus infection and neutropenia. Bone marrow (BM) analysis revealed an indeterminate lymphoid clone, and a normal karyotype. The blood parameters improved for 3 weeks, after which the patient was re-admitted due to a grossly increasing white blood cell count, suggesting overt leukaemia. A second BM sample revealed dic(9;20)-positive BCP ALL. FISH analysis on cells from the 'pre-leukaemic' sample confirmed the presence of dic(9;20) in 38% of interphase nuclei but not on metaphases. Most interestingly, the homozygous loss of *CDKN2A* was not present in the 'pre-leukaemic' sample collected 3 weeks prior to overt leukaemia, but was present at the diagnosis of ALL (Patient 1b and 1a respectively; Fig 1C). That the deletion was hemizygous, and not homozygous, in the 'pre-leukaemic' sample was also confirmed using multiplex ligation-dependent probe amplification (SALSA MLPA P355; MRC-Holland, Amsterdam, Netherlands; data not shown). Intriguingly, the homozygous *CDKN2A* deletion in Patient 2 only included the alternative exon 1, thus rendering the cells nullizygous for the transcript variant 4 (P14ARF) while retaining one allele of variant 1 (P16INK4).

It has previously been shown that the frequency of homozygous *CDKN2A* deletions varies significantly among different subgroups of BCP ALL (Sulong *et al*, 2009). Although dic(9;20) was not specifically addressed in the latter study, no other BCP ALL subtype appears to have such a high a frequency (100% in the present study) of homozygous *CDKN2A* deletions as dic(9;20)-positive cases. Lack of *CDKN2A* in ALLs with dic(9;20) is further supported by a recent study on genome-wide RNA expression signatures in ALL – all three dic(9;20)-positive cases included displayed down-regulation of *CDKN2A* (Nordlund *et al*, 2012).

Patient 1 in the present study may provide an important clue regarding the pathogenetic impact of homozygous

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CDKN2A deletions in ALL with dic(9;20). In this patient, BM samples were available both at a 'pre-leukaemic' stage and at the time of overt leukaemia, in both instances having the dic(9;20); a homozygous *CDKN2A* deletion was, however, only found at the latter time point. Thus, there was a progression from a hemizygous to a homozygous deletion of *CDKN2A* during leukaemic transformation of the dic(9;20)-positive clone. In this context it is worthy of note that preferential expansion of *CDKN2A*-deleted clones at relapse has previously been reported (Mullighan *et al*, 2008). However, further studies on the impact of homozygous *CDKN2A* deletions in dic(9;20)-positive BCP ALL and the relationship between ancestral, non-leukaemic dic(9;20)-harbouring clones and *CDKN2A* deletions are warranted.

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VZ performed research, analysed data and wrote the paper. JS, GB, MN analysed data. MH, EF analysed data and provided clinical data. BJ analysed data and wrote the paper. AN, principal investigator, designed research, analysed data and wrote the paper. The authors declare no conflict of interest.

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