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Loss of chromosomes is the primary event in near-haploid and low hypodiploid acute lymphoblastic leukemia

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Letter to the Editor

Hypodiploidy (<46 chromosomes) is found in approximately 5-8% of acute lymphoblastic leukemia (ALL).[1,2] It may be subdivided into high hypodiploidy (40-45 chromosomes), low hypodiploidy (HoL; 33-39 chromosomes), and near-haploidy (23-29 chromosomes) with distinct genetic and clinical features.[2,3] Only single cases with 30-32 chromosomes have been reported in the literature and it is therefore not known how such ALL should be classified.[4] The majority of patients with hypodiploid ALL have 45 chromosomes; near-haploidy and HoL are very rare, comprising less than 1% of B-cell precursor (BCP) ALL.[2,4] Near-haploid ALL is seen primarily in children and adolescents, although some adult cases have been reported, whereas HoL occurs at all ages.[4] Cases usually have an early pre-B

immunophenotype and a white blood cell (WBC) count of <50 x10^9/l; the sex ratio is close to 1.[2,5,6] In childhood ALL, both near-haploidy and HoL are associated with a dismal prognosis, with a 3-year event-free survival rate of 30%; adult patients with HoL also have extremely poor overall survival rates of only 20-30%.[2,3,7,8] Because of the rarity of near-haploid/HoL ALL, relatively few studies have focused on these genetic subgroups and no SNP array data have been reported to date. Considering the poor prognosis of these cases, further investigations are needed to increase our understanding of the pathogenetic impact of massive chromosomal loss in ALL. In the present study, we performed SNP array analyses of near-haploid and HoL ALL to characterize the chromosomal pattern, identify submicroscopic genetic aberrations, and investigate the mechanism of formation. The study comprised a total of 12 cases of near-haploid (n=8) and HoL (n=4) BCP ALL (Table 1) obtained via an international collaboration between four laboratories in Sweden and the United Kingdom; because of the rarity of these cases, we were not able to perform a population-based study. Diagnostic DNA and in cases 4 and 6 DNA from relapse was extracted according to standard methods from bone marrow and SNP array analysis was performed using the Illumina HumanOmni1-Quad BeadChip platform, containing ~1.1 million markers (Illumina, San Diego, CA), according to the manufacturer's instructions and data analysis was performed using the Genome studio v2011.1 software.

All 12 cases displayed loss of heterozygosity (LOH) resulting from monosomies or uniparental isodisomies (UPIDs) for the majority of the chromosomes (Supplementary Figures 1 and 2). Chromosome 21 was retained in two copies (heterodisomy) in all near-haploid cases, followed by retention of both copies of chromosomes 14 (6/8; 75%), X/Y (5/8; 63%), 18 (3/8 cases each; 38%), and 8 and 10 (2/8 each; 25%) (Table 1), in line with previous studies.[1,2,5] The HoL cases displayed retention of both copies of chromosomes 1, 5, 6, 10, 11, 18, 19, 21, and 22 (4/4 cases each; 100%), X/Y, 8, and 14 (3/4 each; 75%) (Table 1), also

agreeing well with previous investigations.[2,6] Thus, both the near-haploid and the HoL cases displayed a nonrandom retention of both parental copies of chromosomes X/Y, 14, 18, and 21. The reason why some chromosomes are preferentially heterodisomic is unknown. Other tumor types with similar modal numbers also harbor specific heterodisomies; however, the involved chromosomes vary. For example, heterodisomies 18 and 21 are seen also in near-haploid/HoL hematologic malignancies other than ALL, whereas heterodisomies 5, 7, 19, and 21 are common in hyperhaploid chondrosarcomas.[9,10] Chromosome 21, on the other hand, has been found to be retained in two copies in the vast majority of hypodiploid neoplasms.[9] The pathogenetic consequences of near-haploidy and HoL, i.e. why some chromosomes display retained heterozygosity whereas others are monosomic, remain unclear, but it is likely that the loss of chromosomes will affect gene expression. For example, genes that are expressed in a monoallelic manner, either because of random inactivation or imprinting, may be affected. Furthermore, haploinsufficiency of some of the genes on the monosomic chromosomes may also contribute.

In both near-haploid and HoL ALL, a clone constituting a duplication of the stemline, with two and four copies of chromosomes in the doubled clone corresponding to one and two copies in the original near-haploid/HoL clone is frequently seen.[2,6] All cases in the present study harbored complete LOH for chromosomes that were not retained. This could correspond either to the majority of chromosomes being monosomies, i.e., all disomic chromosomes being UPIDs in a duplicated clone. In the SNP array analysis, probes were normalized against the mean copy number and it was therefore not possible to determine whether the analysis targeted the stemline or the duplicated clone in most cases. However, by cytogenetic analysis, the majority harbored a clone with 50-74 chromosomes (Table 1), making it likely that the SNP array results correspond to the duplicated clone, although we cannot exclude that the culturing of cells for banding analysis affected the sizes of the different clones. Taken

together, our findings agree well with the handful of previously reported cases in that the vast majority of gained chromosomes were 2:2 tetrasomies and the remaining chromosomes were UPIDs in the duplicated clone.[11]

The doubled clone in near-haploid and HoL cases may be mistaken for a high hyperdiploid ALL; a separate genetic subgroup with 51-67 chromosomes associated with a favorable outcome in both childhood and adult ALL. Thus, careful examination is important at diagnosis to prevent erroneous classification.[12] In fact, two of the cases in this study (#4 and 6) were initially classified and treated as high hyperdiploid ALL, i.e., as non-high risk ALL since only the duplicated clone was detected by cytogenetic analysis at the time of diagnosis. Both of these patients suffered a relapse, stressing the importance of correct treatment stratification. Notably, the underlying near-haploidy was immediately identified when these cases were investigated with SNP array analysis. Although duplicated near-haploid/HoL clones may be recognized as such by all gained chromosomes being tetrasomies – in contrast to high hyperdiploid ALL, which will mainly harbor trisomies – it should be noted that trisomies sometimes occur also in the duplicated clones of near-haploid ALL, as shown by the trisomy X in case 5 (Table 1). Considering that ALL with near-haploidy/HoL is stratified to high risk groups in most current treatment protocols correct classification is very important. The present study clearly shows the potential of using SNP array analysis in a diagnostic setting. However, it should be noted that this method does not detect balanced aberrations such as translocations; genetic screening with, e.g., G-banding is therefore still needed.

Intrachromosomal copy number abnormalities were detected in 5/12 (42%) cases (Supplementary Table 1). A triplication of a segment in 13q31.3 including *MIR17HG* and *GPC5* was found in case 8 and a duplication in 17q24.3 in case 7 involved *SOX9*. Of these, only *MIR17HG* has been previously associated with tumorigenesis; it is included in the 13q31

amplicon in lymphoma.[13] The only microdeletions identified in this study resulted in loss of *CDKN2A* in four cases and *ETV6* in one case (Supplementary Table 1), corresponding to a mean of 0.42 microdeletions per case. This is a relatively low frequency compared with other subtypes of BCP ALL[14] and could be due to the fact that the leukemic cell has already lost massive amounts of genetic material; additional deletions may therefore not be necessary for leukemogenesis or be incompatible with cell viability. However, it should be noted that a similarly low incidence of microdeletions is seen in high hyperdiploid childhood ALL,[14] which is also highly aneuploid. Thus, one possibility is that different leukemogenic mechanisms are involved in ALL characterized by numerical aberrations compared with those with translocations.

CDKN2A was deleted in 33% of the cases, in line with BCP ALL in general.[14] Interestingly, *CDKN2A* deletions are also very common in chondrosarcomas with 24-34 chromosomes[10] and could hence be a common genetic aberration in tumors characterized by low modal numbers. CDKN2A deficiency has been associated with induction of supernumerary centrosomes, something which could induce aneuploidy.[15] Thus, *CDKN2A* loss could be an initiating step occurring before the chromosomal losses. However, in the present study, case 8 harbored hemizygous *CDKN2A* deletions adjacent to a homozygous deletion, showing that two copies of the chromosome were present, as well as complete LOH, i.e., UPID9 (Figure 1). This strongly indicates that the near-haploidy arose first, followed by a doubling of the chromosomal content where all monosomies became UPIDs, after which two different deletions resulted in homozygous loss of *CDKN2A*. Hence, the deletions occurred after the near-haploidy was established and could not have initiated the chromosomal loss in this case. Although it was not possible to elucidate the order of events in the other cases with 9p deletions, our data nevertheless indicate that *CDKN2A* deletions are not associated with the formation of near-haploidy or HoL in BCP ALL, but rather with clonal evolution.

In conclusion, we confirm that near-haploid/HoL ALL display characteristic patterns of chromosomal gains and show that these cases harbor relatively few microdeletions. Our findings suggest that loss of chromosomes is the primary event, with microdeletions occurring after the near-haploidy/HoL. Since cases may be misclassified as high hyperdiploid, resulting in incorrect treatment stratification, SNP array analysis is a useful complement to cytogenetic analysis in the diagnosis of near-haploid/HoL childhood ALL.

CONFLICT OF INTEREST

The authors declare no competing financial interests.

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Figure 1. Detection of two different types of *CDKN2A* deletion patterns. **(a)** Case 6, displaying a homozygous *CDKN2A* deletion and complete loss of heterozygosity (LOH) for all of chromosome 9. It cannot be concluded whether the deletion occurred before or after the chromosomal loss. **(b)** Case 8, displaying hemizygous *CDKN2A* deletions adjacent to a homozygous deletion, in addition to complete LOH for all of chromosome 9. In this case, monosomy 9 must have occurred first, followed by UPID9, after which both C*DKN2A* deletions occurred independently.

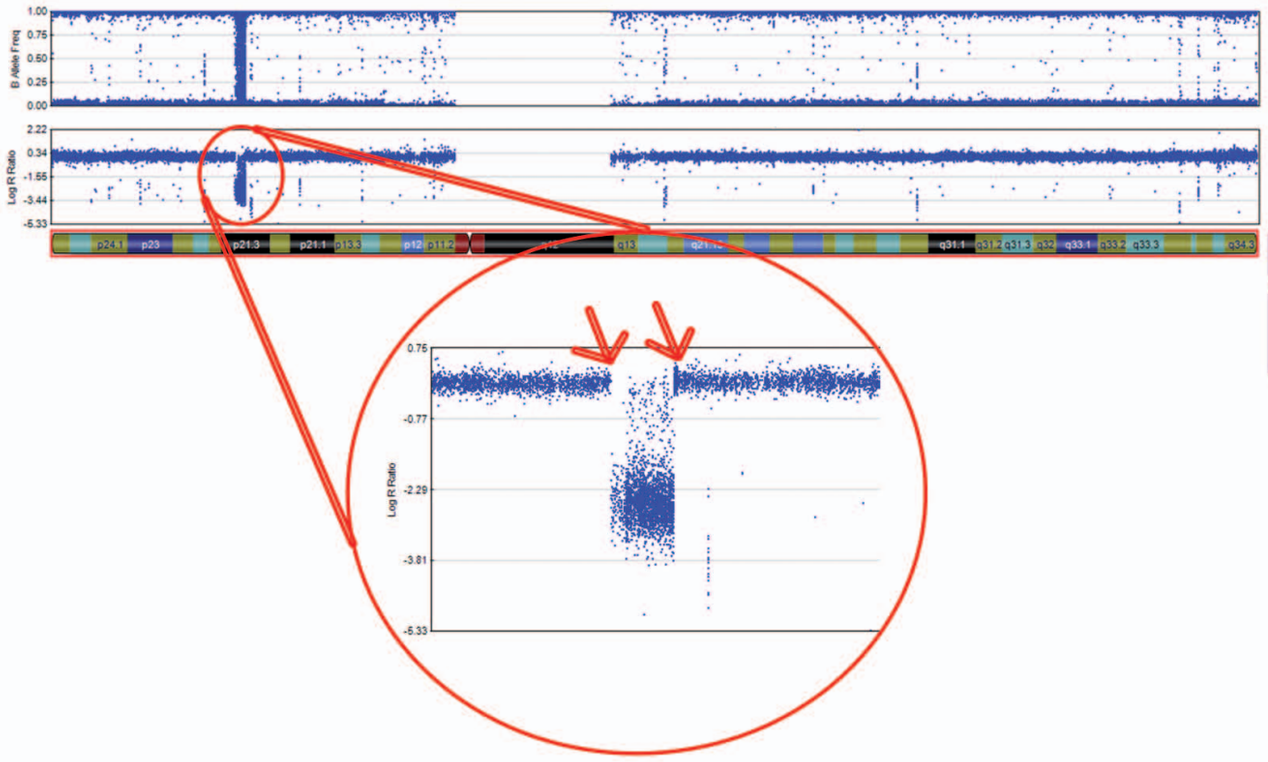
The analysis provides information on genotypes and copy numbers corresponding to B allele frequencies and $\log_2$ ratios. In a diploid segment, the B-allele frequency detects homozygous SNPs (value of 0 or 1) and heterozygous SNPs (value of 0.5). The $\log_2$ ratio detects the average copy number correlating to 0. Near-haploid/HoL samples are normalized to have a $\log_2$ ratio of 0; their respective hyperdiploid/near-triploid mirror clones will also be normalized to have a $\log_2$ ratio of 0. In these particular cases the analysis cannot distinguish between the stemline and duplicated mirror clones.

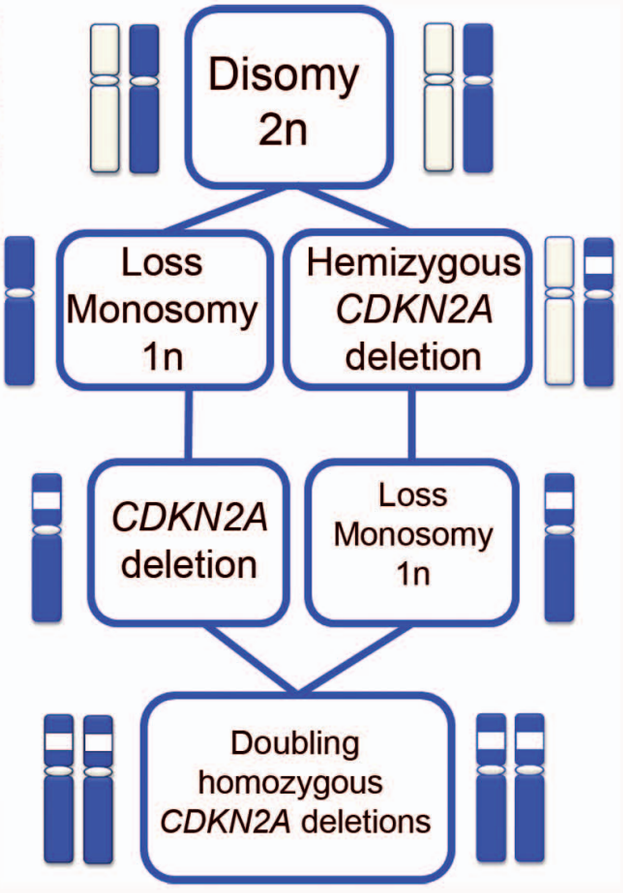
Table 1. Clinical data, cytogenetic features, and SNP array results in 12 cases of near-haploid and low hypodiploid acute lymphoblastic leukemia

Case No.	Sex/ age	Karyotype	Chromosomal copy number changes detected by SNP array analysis[a]
1	M/9	25<1n>,X,+Y,+21[4]/50,XY,idemx2[6][b]	25,X,+Y,+21
2	M/6	26<1n>,X,+Y,+14,+21[8]/52,idemx2[2]	26,X,+Y,+14,+21
3	M/15	26<1n>,X,+14,+18,+21[3]/52,idemx2[8]	26,X,+14,+18,+21
4	F/12	51-52<2n>,XX,+X,+21,inc[3]/46,XX[15]	26,X,+X,+14,+21
	F/15	46,XX[25] [relapse]	26,X,+X,+14,+21
5	F/3	26<1n>,X,+?X,+14,+21[5]/52,idemx2,inc[4]	52,XX,+X,+14,+14,+21,+21
6	F/2	Failure [high hyperdiploidy indicated by FISH]	27,X,+X,+14,+18,+21
	F/4	Failure [near-haploidy indicated by FISH; relapse]	27,X,+X,+14,+18,+21
7	M/4	54<2n>,XY,+X,+8,+8,+10,+10,+18,+21,+21[20]	27,X,+8,+10,+18,+21
8	M/8	27,X,+8,+10,+14,+21[18]/54,idemx2[15]	54,X,+X,-Y,+8,+8,+10,+10,+14,+14,+21,+21
9	M/62	33<1n>,X,+Y,+1,+6,+10,+11,+14,+18,+19,+21,+22,inc[6]/61-65,idemx2,inc[5]	33,X,+Y,+1,+5,+6,+10,+11,+18,+19,+21,+22
10	M/12	33<1n>,X,+1,+5,+6,+8,+11,+14,+18,+19,+21,+22[4]	34,X,+1,+5,+6,+8,+10,+11,+14,+18,+19,+21,+22/33,idem,-10
11	F/13	35-36<1n>,X,+X,inc[11]/68-69,XX,inc[3]	36,X,+X,+1,+5,+6,+8,+10,+11,+14,+15,+18,+19,+21,+22
12	M/14	37<1n>,X,+Y,+1,+5,+6,+8,+10,+11,+14,+18,+19,+20,+21,+22,+mar[5]/74,idemx2[5]	37,X,+Y,+1,+5,+6,+8,+9,+10,+11,+14,+18,+19,+20,+21,+22

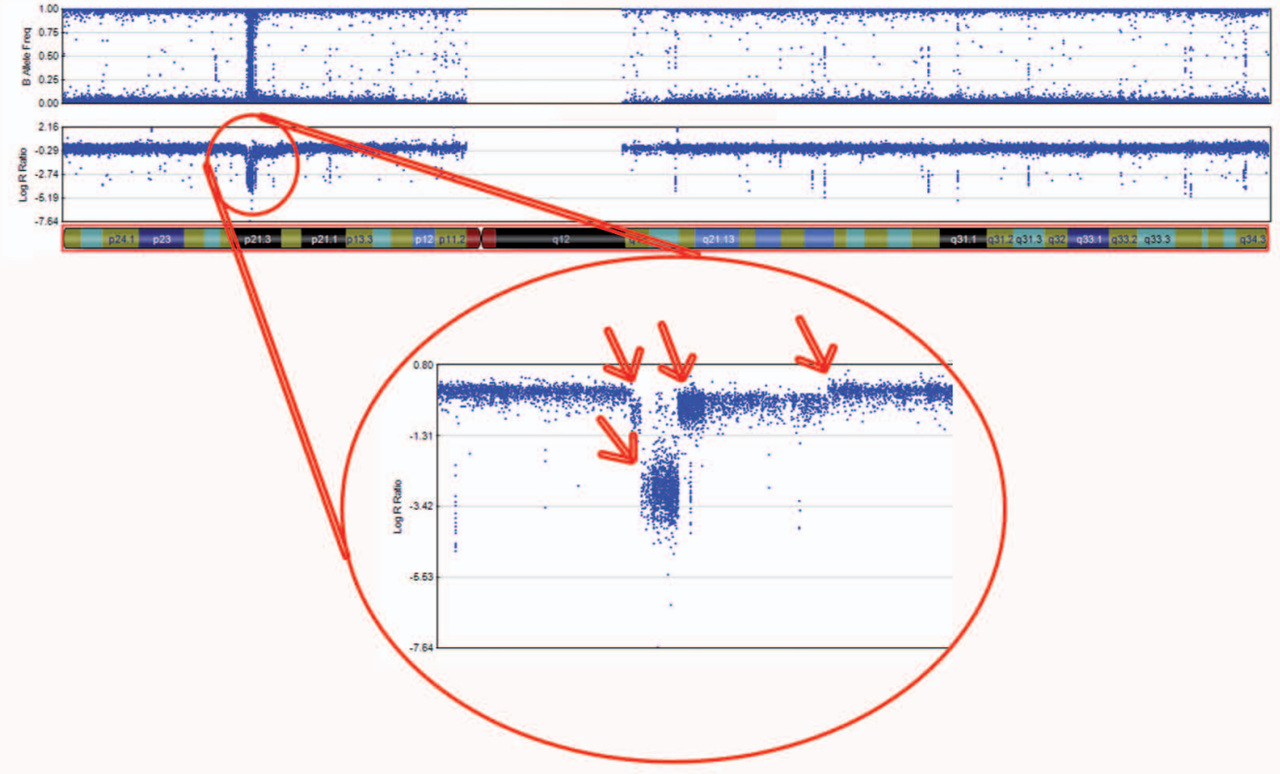
Abbreviations: F, female; M, male; SNP, single nucleotide polymorphism; FISH, fluorescence in situ hybridization. [a]Copy number changes are given in relation to the haploid level except for cases 5 and 8. All chromosomes that were not gained displayed complete loss of heterozygosity. [b]Positive for t(9;22)(q34;q11) and P190 *BCR/ABL1* transcript by FISH and RT-PCR.

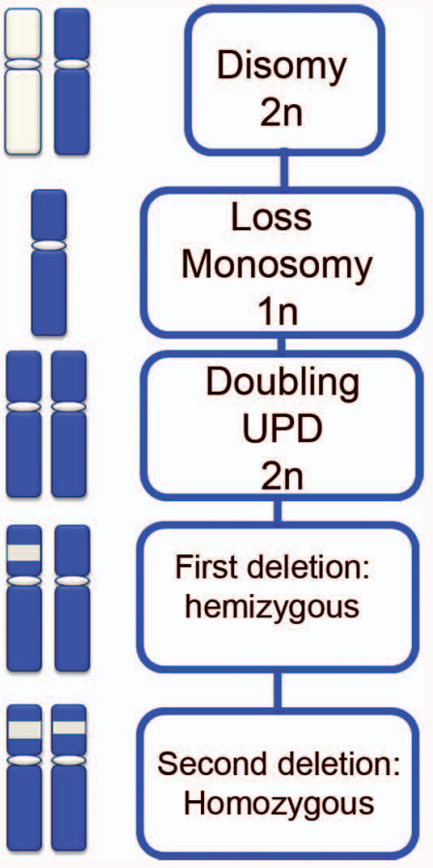
a


B Allele Freq
Log R Ratio


Disomy
2n
Loss
Monosomy
1n
Hemizygous
CDKN2A
deletion
CDKN2A
deletion
Loss
Monosomy
1n
Doubling
homozygous
CDKN2A deletions

b


B Allele Freq
Log R Ratio


Disomy
2n
Loss
Monosomy
1n
Doubling
UPD
2n
First deletion:
hemizygous
Second deletion:
Homozygous