Formation of a Hyperdiploid Karyotype in Childhood Acute Lymphoblastic Leukemia

By Norio Onodera, Norah R. McCabe, and Charles M. Rubin

Hyperdiploidy with ≥50 chromosomes is a frequent and distinct karyotypic pattern in the malignant cells of children with acute lymphoblastic leukemia. To understand better the mechanism of formation of the hyperdiploid karyotype, we studied 15 patients using 20 DNA probes that detect restriction fragment length polymorphisms. We first examined disomic chromosomes for loss of heterozygosity. Two patients had widespread loss of heterozygosity on all informative disomic chromosomes, and represent cases of near-haploid leukemia in which the chromosomes doubled. One other patient had loss of heterozygosity limited to chromosome 3; in this patient all of seven other informative disomic chromosomes retained heterozygosity. Loss of heterozygosity was not detected in the remaining 12 patients on a total of 87 informative disomic chromosomes. We then examined tetrasomic chromosomes for parental dosage. Of the 13 patients in whom widespread loss of heterozygosity was not present, 11 patients had tetrasomy 21; 10 of 11 (91%) had an equal dose of maternal and paternal alleles on chromosome 21 and only 1 of 11 (9%) had an unequal dose of parental alleles in a 3:1 ratio. These results suggest that the hyperdiploid karyotype usually arises by simultaneous gain of chromosomes from a diploid karyotype during a single abnormal cell division, and occasionally by doubling of chromosomes from a near-haploid karyotype. The hyperdiploidy in cases without widespread loss of heterozygosity is not caused by stepwise or sequential gains from a diploid karyotype or by losses from a tetraploid karyotype; the former should result in a 3:1 parental dosage for 67% of tetrasomic chromosomes (8% observed) and the latter should result in loss of heterozygosity for 33% of disomic chromosomes (1% observed). Additional studies of the molecular basis for this leukemia subtype are warranted.

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A HYPERDIPLOID karyotype is observed in 23% to 42% of newly diagnosed children with acute lymphoblastic leukemia (ALL).1-6 These patients are generally divided into two subgroups, namely, those with a chromosome number of 47 to 49 and those with ≥ 50 chromosomes. The hyperdiploidy ≥ 50 group has a nonrandom pattern of chromosomal gain. Nearly all of the patients have a chromosome number of 51 to 65 with a peak at 55.7 Frequently gained chromosomes (in more than half of the cases) are chromosomes 4, 6, 10, 14, 17, 18, 20, 21, and X, and rarely gained chromosomes (less than 10% of the cases) are chromosomes 1, 2, 3, 12, and 16.8 Typically, the affected chromosomes are present in three copies (trisomic). However, chromosome 21, the most frequently gained chromosome,2,3,8,9 often is tetrasomic.9

The presence of hyperdiploidy ≥ 50 correlates strongly with good risk features, including age between 3 and 7 years old, white blood cell (WBC) count less than 10 × 10^9/L, French-American-British (FAB) Cooperative Group subtype L1,1 and common ALL antigen (CALLA, CD10) positive early pre-B phenotype.4 Patients with hyperdiploidy ≥ 50 have the longest disease-free survival compared with any other cytogenetic group.10

Recently, we reported two cases of childhood ALL with hyperdiploidy ≥ 50 in whom the hyperdiploid leukemic clone arose from a near-haploid clone through doubling of the chromosomes.11 We verified this mechanism by demonstration of widespread loss of heterozygosity on all disomic chromosomes (chromosomes present in two copies). However, in the majority of cases of hyperdiploid ALL, the mechanism leading to the increased number of chromosomes is unknown. Possibilities include development of tetraploidy with subsequent loss of chromosomes, gains of individual chromosomes in a stepwise or sequential fashion, or simultaneous gain of multiple chromosomes in a single abnormal cell division.

Also, unknown is the biologic significance of hyperdiploidy to the leukemic process.12 The extra chromosomes may result from selection for cells undergoing nondisjunction of a chromosomal homologue containing a specific mutation that provides the cell with a proliferative advantage in a dose-dependent manner. This hypothesis invokes the presence of independent mutations on each of the affected chromosomes. Alternatively, a single mutation or a single carcinogenic event may lead to the hyperdiploid state as a secondary effect. Finally, a gene mutation may not be critical to the development of hyperdiploidy; instead, the gain of certain chromosomes may enhance proliferation of early lymphoid cells through a change in dosage or relative dosage of a set of genes.

Here we report 15 patients with hyperdiploid ALL with ≥ 50 chromosomes including two patients reported previously.11 We used restriction fragment length polymorphisms to address the pathophysiology of the disease. We provide strong evidence to support the view that the hyperdiploid karyotype occurs as a sudden gain of multiple chromosomes.

MATERIALS AND METHODS

Patients. All patients were children with ALL and a hyperdiploid karyotype with ≥50 chromosomes. The patients were se-
lected on the basis of availability of cryopreserved leukemia
marrow containing ≥85% blasts. One patient (patient 6) had only
61% blasts in the leukemic sample. Features of the patients are
shown in Table 1. Twelve patients were studied at diagnosis and
diagnosis and three patients (patients 4, 6, and 10) were studied at relapse. Two
patients (patients 2 and 6) were reported in a previous publica-
tion and are investigated further in this study.

Cytogenetic analysis. Cytogenetic analysis was performed using
a trypsin-Giemsa banding technique. Cells were obtained from
bone marrow and/or peripheral blood before initiation of chemo-
therapy. Metaphase cells from direct preparations and/or short-
term (24 and 48 hours) unstimulated cultures were examined.
Chromosomal abnormalities were described according to the
International System for Human Cytogenetic Nomenclature.

Molecular analysis. DNA was extracted from cryopreserved
buffy coat cells from bone marrow at diagnosis or relapse of
leukemia. In eight patients (patients 1, 2, 5, 6, 11, 12, 14, and 15)
DNA was extracted from fresh mononuclear cells of peripheral
blood or cryopreserved buffy coat cells from bone marrow during
complete remission; this nonleukemic material represented constitu-
tional DNA. Restriction endonuclease digestion, electrophore-
sis, Southern blotting, radiolabeling of probes, and DNA hybridiza-
tion were performed according to standard procedures.

Informative probes were those showing two different alleles. In
patients in whom we did not have a sample of nonleukemic DNA,
we could not distinguish between a noninformative probe and loss
of heterozygosity when only one polymorphic band was observed.

Densitometry was used to quantify the intensity of bands on
exposures of Southern blots. Densitometry was performed by
transmittal of the image to a computer using a CCD camera CX-77
(SONY, Tokyo, Japan) and the program MacVision (Koala Tech-
ologies, Morgan Hill, CA). The image was analyzed further by the
program Densitometry (SONY, Tokyo, Japan) and the program MacVision (Koala Tech-
ologies, Morgan Hill, CA). When quantifying the intensity of bands
for VNTR (variable number of tandem repeats) probes, a mathematical correction
was made. The intensity of lower bands in the autoradiogram were
corrected by the following formula: lower band intensity

\[ \text{Intensity}_{\text{lower}} = \frac{\text{Intensity}_{\text{upper}}}{x} \]

where \( x \) is the upper band intensity.

Results. The 20 probes used to detect DNA restriction fragment
length polymorphisms are described in Tables 2 and 3. Additional
information and references are listed in the report of the 10th
International Workshop on Human Gene Mapping. Probes p2.1,
p21-4U, p95-α1-11a, and pFW228C were gifts from Drs Ellen
Solomon, Gordon D. Stewart, Bradley N. White, and Integrated
Genetics (Framingham, MA), respectively. Probes CRI-R59 and
CRI-L247 were purchased from Collaborative Research Inc (Bed-
ford, MA). The remaining probes were obtained from the Ameri-
can Type Culture Collection (ATCC; Rockville, MD). Probes
pMCT118, cYN9A3, pYNH24, pTBAB5-7, pEFZ64.1, pJCZ67,
PMHZ10, pCMM6, and pMCT15 were deposited at ATCC by Drs Ray
White and Yusuke Nakamura. Probes PCMW-1, p79-2-23,
GSBHs, and 26C and p21.3 were deposited by Drs David Ledbet-
ter, Michael Litt, Gordon D. Stewart, and A. Millington-Ward,
respectively.

RESULTS

Karyotypes. The results of cytogenetic analyses are de-
scribed in Table 1. All 15 patients had one or more
abnormal clones with 50 to 60 chromosomes. Thirteen
patients had typical hyperdiploidy in which most of the
affected chromosomes were present in three copies (tri-
somic), and two patients (patients 2 and 6) were atypical
because nearly all affected chromosomes were tetrasomic.
All 15 patients had extra copies of chromosomes 21 and X.
Extra copies of chromosomes 4, 6, 10, 14, 17, and 18 were
observed in more than 10 of the patients. Thirteen patients
tetrasomy 21 (patients 1 through 13). Some chromo-
somes were not gained in any of the cases including
chromosome 1, 2, 3, 7, 15, and 16; thus, these chromo-
somes were always disomic. Fourteen of 15 patients were
disomic for chromosomes 9, 11, 13, 19, and 20.

Structural abnormalities were observed in five patients.
Two patients (patients 3 and 9) had partial duplication of
the long arm of chromosome 1, one patient (patient 10) had

Table 1. Cytogenetic Studies of 15 Patients With ALL and a Hyperdiploid Karyotype

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>State of Disease</th>
<th>No. of Metaphase Cells Examined</th>
<th>Karotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4</td>
<td>M</td>
<td>Diagnosis</td>
<td>33</td>
<td>46,XY (85%)/56,XY,+X,+4,+5,+6,+14,+17,+18,+21,+22 (15%)</td>
</tr>
<tr>
<td>2*</td>
<td>5</td>
<td>F</td>
<td>Diagnosis</td>
<td>32</td>
<td>46,XX (81%)/50,XX,+18,+21,+21 (19%)</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>F</td>
<td>Diagnosis</td>
<td>33</td>
<td>46,XX (18%)/54,XX,+X,+4,+6,+14,+17,+18,+21,+21 (81%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>55,XX,+X,+4,+6,+14,+17,+18,+19,+21 (12%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>56,XX,+X,+4,+6,+10,+14,+15,+17,+18,+21,+21, dup(1)q21 → q44 (9%)</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>F</td>
<td>Relapse</td>
<td>31</td>
<td>46,XX (3%)/56,XX,+X,+4,+6,+8,+10,+14,+18,+21,+21 (97%)</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
<td>F</td>
<td>Diagnosis</td>
<td>22</td>
<td>46,XX (41%)/54,XX,+X,+4,+12,+14,+17,+18,+21,+21 (55%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>55,XX,+X,+4,+12,+13,+14,+17,+18,+21,+21 (4%)</td>
</tr>
<tr>
<td>6†</td>
<td>18</td>
<td>M</td>
<td>Relapse</td>
<td>21</td>
<td>46,XY (90%)/60,XY,+X,+Y,+5,+6,+8,+9,+9,+14,+14,+19,+20,+21,+21 (10%)</td>
</tr>
<tr>
<td>7</td>
<td>3</td>
<td>M</td>
<td>Diagnosis</td>
<td>12</td>
<td>46,XY (68%)/56,XY,+X,+4,+6,+10,+14,+17,+18,+21,+21 (42%)</td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>F</td>
<td>Diagnosis</td>
<td>22</td>
<td>46,XY (32%)/56,XX,+X,+4,+6,+10,+14,+17,+18,+21,+21,mar(68%)</td>
</tr>
<tr>
<td>9</td>
<td>3</td>
<td>M</td>
<td>Diagnosis</td>
<td>20</td>
<td>46,XY (30%)/55,XY,+X,+4,+6,+10,+14,+17,+18,+21,+21, dup(1)q21 → q32 (40%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>56,XY,+X,+Y,+4,+6,+10,+14,+17,+18,+21,+21, dup(1)q21 → q32 (30%)</td>
</tr>
<tr>
<td>10</td>
<td>9</td>
<td>M</td>
<td>Relapse</td>
<td>21</td>
<td>46,XY (81%)/55,XY,+X,+4,+5,+6,+14,+14,+17,+18,+12,+21,del(12)p11p12,dic(21;21)(p13;p13) (19%)</td>
</tr>
<tr>
<td>11</td>
<td>1</td>
<td>M</td>
<td>Diagnosis</td>
<td>12</td>
<td>46,XY (50%)/54,XX,+X,+6,+13,+14,+17,+18,+21,+21 (50%)</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>M</td>
<td>Diagnosis</td>
<td>13</td>
<td>46,XY (46%)/60,XY,+X,+4,+5,+6,+8,+10,+11,+12,+14,+17,+18,+21,+22 (54%)</td>
</tr>
<tr>
<td>13</td>
<td>3</td>
<td>M</td>
<td>Diagnosis</td>
<td>19</td>
<td>53,XY,+X,+4,+6,+14,+21,+21,mar (100%)</td>
</tr>
<tr>
<td>14</td>
<td>2</td>
<td>M</td>
<td>Diagnosis</td>
<td>11</td>
<td>46,XY (45%)/56,XY,+X,+4,+6,+8,+10,+14,+17,+18,+21,+22 (55%)</td>
</tr>
<tr>
<td>15</td>
<td>2</td>
<td>M</td>
<td>Diagnosis</td>
<td>26</td>
<td>46,XY (88%)/53,XY,+X,+Y,+6,+14,+17,+18,+21 (12%)</td>
</tr>
</tbody>
</table>

*Reported as Patient 1 in reference 11.
†Reported as Patient 2 in reference 11.
Table 2. Analysis of DNA Polymorphisms on Disomic Chromosomes in 15 Patients With ALL and a Hyperdiploid Karyotype

<table>
<thead>
<tr>
<th>Probes</th>
<th>Maintenance or Loss of Heterozygosity in Leukemic DNA</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Map Location</td>
<td>Locus Symbol</td>
<td>Name</td>
</tr>
<tr>
<td>lp</td>
<td>D1S80</td>
<td>pMCT118*</td>
</tr>
<tr>
<td>1q</td>
<td>D1S74</td>
<td>cYNA13*</td>
</tr>
<tr>
<td>2p</td>
<td>D2S47</td>
<td>pTBA85-7*</td>
</tr>
<tr>
<td>2pter-q32.3</td>
<td>D2S44</td>
<td>pYNH24*</td>
</tr>
<tr>
<td>3pter-p21</td>
<td>D3S12</td>
<td>CRI-R59</td>
</tr>
<tr>
<td>3q</td>
<td>D3S42</td>
<td>pEFD64.1*</td>
</tr>
<tr>
<td>7q</td>
<td>D7S396</td>
<td>pJCZ67*</td>
</tr>
<tr>
<td>9q</td>
<td>D9S11</td>
<td>cMHZ10*</td>
</tr>
<tr>
<td>16q24</td>
<td>D16S7</td>
<td>p79.2-23*</td>
</tr>
<tr>
<td>20q</td>
<td>D20S19</td>
<td>pCMN6*</td>
</tr>
</tbody>
</table>

Abbreviations: M, maintenance of heterozygosity; L, loss of heterozygosity; NI, not informative; T, trisomic or tetrasomic, not evaluated; -, unknown because of lack of availability of constitutional nonleukemic DNA or not tested.

*VNTR probe.

Table 3. Analysis of DNA Polymorphisms on Chromosome 21 in 13 Patients With ALL and a Hyperdiploid Karyotype Associated With Tetrasomy 21

<table>
<thead>
<tr>
<th>Probes</th>
<th>Contribution of Parental Alleles</th>
<th>Patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>Map Location</td>
<td>Locus Symbol</td>
<td>Name</td>
</tr>
<tr>
<td>21pter-q21.1</td>
<td>D21S26</td>
<td>26C</td>
</tr>
<tr>
<td>21q11</td>
<td>D21S110</td>
<td>21-4U</td>
</tr>
<tr>
<td>21q11.1-q21.2</td>
<td>D21S1</td>
<td>pPW228C</td>
</tr>
<tr>
<td>21q11.2-q21.2</td>
<td>D21S11</td>
<td>pG94-1-11a</td>
</tr>
<tr>
<td>21q21.1-qter</td>
<td>D21S24</td>
<td>p21.3</td>
</tr>
<tr>
<td>21q22.1-q22.2</td>
<td>D21S17</td>
<td>pGSH8</td>
</tr>
<tr>
<td>21q22.1-qter</td>
<td>D21S113</td>
<td>pMCT15</td>
</tr>
<tr>
<td>21q22.3</td>
<td>D21S112</td>
<td>CRI-L427*</td>
</tr>
</tbody>
</table>

Abbreviations: U, unequal; E, equal; -, unknown because only one band was observed or not tested.

*VNTR probe.

Fig 1. Restriction fragment length polymorphism analysis of chromosome 2 in leukemic cells (lanes a) and nonleukemic cells (lanes b). Lanes are labeled with the patient number. DNA was digested with MspI and hybridized with probe pYNH24, which recognizes a VNTR polymorphism on chromosome 2. The faint bands in the lanes containing DNA from leukemic cells of patients 2 and 6 represent residual normal bone marrow cells present in the samples.
a partial deletion of the short arm of chromosome 12 and a dicentric chromosome 21, and two patients (patients 8 and 13) had marker chromosomes.

Analysis of restriction fragment length polymorphisms. We first examined chromosomes present in two copies for loss of heterozygosity. Two patients (patients 2 and 6) had widespread loss of heterozygosity on all informative disomic chromosomes, and represent cases of near-haploid ALL in which the chromosomes doubled.11 Eight probes from eight chromosomes demonstrated loss of heterozygosity in patient 6. One patient (patient 15) had loss of heterozygosity limited to chromosome 3; this was demonstrated separately with probes for the short and long arms of chromosome 3. Eight loci on seven other disomic chromosomes in patient 15 retained heterozygosity. Loss of heterozygosity was not detected in the remaining 12 patients; a total of 106 informative loci on 87 disomic chromosomes retained both parental alleles (Fig 1 and Table 2).

We then examined the parental dosage of chromosome 21, when it was present in four copies. Eight probes for
chromosome 21 were used; informative results were obtained with three or more probes in all cases. Of the 13 patients without widespread loss of heterozygosity, 11 patients had tetrasomy 21; 10 of 11 (91%) had an equal dose of maternal and paternal alleles on chromosome 21 and 1 of 11 (9%) had an unequal dose in a 3:1 ratio. In all patients the relative parental contribution was consistent at several positions on chromosome 21 (Fig 2 and Table 3).

Results of densitometry for the three autoradiograms in Fig 2 are shown in Table 4. Patient 1 had an approximately 3:1 ratio in the density of the two allelic bands in autoradiograms made with three probes from chromosome 21. Two other patients (patients 3 and 5) and normal controls had an approximately equal ratio in the same experiments.

**DISCUSSION**

The results of this study provide molecular data pertinent to the mechanism of formation of a hyperdiploid karyotype with ≥50 chromosomes in childhood ALL. We have considered four possible routes by which a normal diploid precursor cell might become hyperdiploid: (1) development of near-haploidy followed by doubling of the chromosomes; (2) development of tetraploidy with subsequent loss of chromosomes; (3) gains of individual chromosomes in a sequential fashion through multiple independent nondisjunction events; and (4) simultaneous gain of multiple chromosomes in a single abnormal cell division.

We have demonstrated the occasional occurrence of route 1 in patients 2 and 6.11 There is widespread loss of heterozygosity on all disomic chromosomes. A strong suspicion of this mechanism is raised by the karyotype in these cases. Specifically, all chromosomes are present in either two or four copies in the hyperdiploid clone. These cases probably are classified best with near-haploid cases, which have a relatively unfavorable prognosis.

The evidence produced by our study does not favor route 2. If a diploid cell were to become tetraploid and subsequently lose chromosomes, 33% of disomic chromosomes in the hyperdiploid cell should have loss of heterozygosity. This statement assumes that the chromosomes are lost independent of the parental origin. In fact, we observed loss of heterozygosity in only 1 of 95 (1%) of disomic chromosomes in patients with the typical form of the hyperdiploidy. It is possible that selection against loss of heterozygosity led in part to these results. Nevertheless, our best interpretation of the data is consistent with development of tetraploidy followed by loss of chromosomes.

Route 3 also is not supported by our results. With independent gains of chromosomes by multiple nondisjunction events, it is predicted that 33% of instances of tetrasomy would be characterized by a 2:2 parental dosage and 67% by a 3:1 dosage. Our findings, which were derived from 11 patients with tetrasomy 21, were that 10 of 11 (91%) of the tetrasomies consisted of two maternal and two paternal copies of chromosome 21 and 1 of 11 (9%) consisted of an unbalanced 3:1 parental dosage. These data suggest that the double gain of chromosome 21 occurs in a single cell division as the result of a double nondisjunction; this would consistently result in a 2:2 dosage. We suggest that the chromosomal gains observed in typical cases of hyperdiploid ALL occur in one step during one aberrant cell division (route 4). Supportive of this idea is the cytogenetic16 and DNA content studies17 of cases of hyperdiploid ALL, which show distinct populations of normal and hyperdiploid cells, but do not show a gradation of intermediate cells.

It is difficult to propose a pathophysiologic basis for a sudden gain of chromosomes in a cell. It is possible that it is a reflection of the primary carcinogenic insult to the cell, whether that be an unprovoked error of the mitotic apparatus or an imposed perturbation by an exogenous agent. In either case the insult does not appear to be sustained, because the abnormal karyotype, once formed, is uniform and stable in the malignant cell population.

It remains unclear whether the hyperdiploid karyotype itself contributes directly to the malignant process. Our data do not support the possibility presented in the introduction that all of the extra chromosomes contain mutations that give the cells a proliferative advantage. If this were the case, the tetrasomic chromosomes should have a 3:1 parental dosage. Also, we failed to identify a consistent defective chromosomal region through an extensive search for loss of heterozygosity. In two patients we found widespread loss of heterozygosity and in one patient we found loss of heterozygosity limited to chromosome 3; whether loss of tumor suppressor gene function played a role in these cases is unknown. Additional studies of the molecular basis for this leukemia subtype are needed.

**ACKNOWLEDGMENT**

We thank Drs Michelle M. Le Beau, Janet D. Rowley, Manuel O. Diaz, and Robert Burnett for helpful discussions; Dr Stefan Bohlander for assistance with the densitometry; and Drs Ellen Solomon, Gordon D. Stewart, Bradley N. White, and Integrated Genetics (Framingham, MA) for gifts of probes.

**REFERENCES**


Formation of a hyperdiploid karyotype in childhood acute lymphoblastic leukemia [see comments]

N Onodera, NR McCabe and CM Rubin