Specific Extra Chromosomes Occur in a Modal Number Dependent Pattern in Pediatric Acute Lymphoblastic Leukemia

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Children with acute lymphoblastic leukemia (ALL) and high hyperdiploidy (>50 chromosomes) are considered to have a relatively good prognosis. The specific extra chromosomes are not random; extra copies of some chromosomes occur more frequently than those of others. We examined the extra chromosomes present in high hyperdiploid ALL to determine if there were a relation of the specific extra chromosomes and modal number (MN) and if the extra chromosomes present could differentiate high hyperdiploid from near-triploid and near-tetraploid cases. Karyotypes of 2,339 children with ALL and high hyperdiploidy at diagnosis showed a distinct nonrandom sequential pattern of gain for each chromosome as MN increased, with four groups of gain: chromosomes 21, X, 14, 6, 18, 4, 17, and 10 at MN 51–54; chromosomes 8, 5, 11, and 12 at MN 57–60; chromosomes 2, 3, 9,16, and 22 at MN 63–67; chromosomes 1, 7 13, 15, 19, and 20 at MN 68–79, and Y only at MN ≥80. Chromosomes gained at lower MN were retained as the MN increased. High hyperdiploid pediatric ALL results from a single abnormal mitotic division. Our results suggest that the abnormal mitosis involves specific chromosomes dependent on the number of chromosomes aberrantly distributed, raising provocative questions regarding the mitotic mechanism. The patterns of frequencies of tetrasomy of specific chromosomes differs from that of trisomies with the exception of chromosome 21, which is tetrasomic in a high frequency of cases at all MN. These results are consistent with different origins of high hyperdiploidy, near-trisomy, and near-tetrasomy. This article contains Supplementary Material available at http://www.interscience.wiley.com/jpages/1045-2257/suppmat.   © 2007 Wiley-Liss, Inc.

INTRODUCTION

Ploidy in childhood acute lymphoblastic leukemia (ALL) has long been considered a significant prognostic factor (The Third International Workshop on Chromosomes in Leukemia, 1981), and patients with a modal number (MN) of chromosomes greater than 50 (high hyperdiploidy) generally have the best prognosis (The Third International Workshop on Chromosomes in Leukemia, 1981; Trueworthy et al., 1992; Heerema et al., 2000). The specific chromosomes that occur as extra copies also have prognostic significance. The Pediatric Oncology Group (POG) showed that trisomy of chromosomes 4 and 10 is associated with a better prognosis than disomy of these chromosomes (Harris et al., 1992). The Children's Cancer Group (CCG) showed, similarly, that trisomy of chromosomes 10 and 17 confers a superior outcome in high hyperdiploid ALL patients (Heerema et al., 2000). Analysis of United Kingdom data showed a superior prognosis for patients with extra copies of chromosomes 4, 10, and 18 (Moorman et al., 2003). Recently, the Children's Oncology Group (COG) reexamined the POG and CCG data and demonstrated that trisomy of all three chromosomes 4, 10, and 17 confers a better outcome than does trisomy of any two of these three chromosomes (Sutcliffe et al., 2005).

Interestingly, the extra chromosomes that occur in the leukemic cells of high hyperdiploid ALL are not random. Extra copies of chromosomes X, 4, 6, 10, 14, 17, 18, and 21 are observed much more frequently than are extra copies of the remaining chromosomes (Harris et al., 1992; Mertens et al., 1996; Raimondi et al., 1996; Heerema et al., 2000; Moorman et al., 2003). However, the relation between MN and the specific extra chromosomes has not been fully explored.

Near-triploidy (~3n, MN 68–79) and near-tetraploidy (~4n, MN $\geq$80) are infrequent karyotypic findings in childhood ALL, and their relation to high hyperdiploidy with MN 51–67 is not well understood. We examined the specific chromosomes that occurred as trisomies and as tetrasomies at each MN in high hyperdiploid, near-triploid, and near-tetraploid ALL to determine whether there is a pattern of extra chromosomes related to specific MN, and to evaluate the possibility that the pattern of extra chromosomes can differentiate high hyperdiploid karyotypes from those more consistently showing a near-triploid or near-tetraploid karyotype. In addition, we reviewed the mechanisms that might allow us to understand the pattern of gain of chromosomes in high hyperdiploid ALL.

MATERIALS AND METHODS

Karyotypes from children enrolled on CCG ALL studies from 1988 to 1995 and on POG ALL studies from 1986 to 1999 were included in this analysis. All protocols were approved by the National Cancer Institutes and by the Institutional Review Board of each participating institution. Informed consent was obtained from the patients or families prior to enrollment. Cytogenetic evaluation was done at diagnosis in a reference laboratory (early POG studies), approved laboratories (later POG studies), or by institutional laboratories (CCG studies). All karyotypes were reviewed by members of the respective Cytogenetics Review Committees and described according to ISCN (1995). Only cases with complete karyotypic descriptions were included in this analysis. Cases that appeared to be a doubling of a hypodiploid clone were not included. Data were compiled from the POG and CCG cytogenetics databases and combined (Table 1). Initially, the MN of all cases was examined, and all karyotypes with 51 or more chromosomes were further evaluated. A total of 2,339 patients with a satisfactory chromosome analysis and a MN >50 were enrolled on the clinical trials included in this analysis. For each MN, each chromosome present as more than two copies was identified, including cases with two X and cases with two Y chromosomes in males. Thus, the number and percentage of cases with an extra copy of each chromosome, 1–22, X and Y, at each MN from 51 to >80 were determined. To facilitate the evaluation, a chromosome was classified as "extra" regardless of the number of extra copies present and was counted only once for each patient. Extra structurally abnormal chromosomes were included, such as the addition of a deleted chromosome 6; thus, an extra chromosome was defined by the presence of its centromere. Initially, graphs of the specific extra chromosomes present at each MN were prepared and compared. Subsequently, a composite graph showing the frequency of cases with extra copies of each chromosome with increasing MN was constructed and examined. This graph showed a striking pattern of acquisition of extra chromosomes. Tetrasomies for each chromosome were evaluated separately in a similar manner. For tetrasomy of the X chromosome, only females were included. Chromosome Y was not included in the tetrasomy analyses. Graphs of chromosomes present as tetrasomies at each modal number also were constructed. Comparison of both trisomies and tetrasomies showed that both triploidy and tetraploidy are different from high hyperdiploidy, with a single

TABLE I. Numbers and Frequencies of Individual Extra Chromosomes at Each MN

(A) Numbers of cases with extra chromosomes

MN	No. PTS	XX	XY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
51	75	17	58	0	0	0	22	7	21	5	9	11	14	2	0	5	39	1	3	10	14	3	4	61	3	58	5
52	152	61	91	0	5	0	71	2	97	2	13	9	21	3	1	6	117	4	2	59	59	5	0	148	2	137	7
53	239	101	138	2	2	4	141	10	178	7	24	27	90	4	6	5	182	12	2	138	150	7	2	232	14	212	11
54	322	125	197	3	4	5	231	17	264	5	56	31	178	5	6	9	277	14	9	218	242	7	3	315	18	289	21
55	427	192	235	0	9	14	354	28	372	4	108	45	337	21	8	9	370	27	0	308	367	3	12	423	32	408	34
56	360	169	191	4	0	15	307	56	315	14	140	68	314	18	13	14	320	20	9	277	311	6	11	351	31	331	46
57	233	102	131	5	4	4	214	44	214	24	117	61	213	32	12	11	209	24	8	189	211	11	4	228	32	220	39
58	144	61	83	2	11	8	125	58	137	22	82	30	128	24	19	12	128	13	21	115	123	7	7	140	30	127	30
59	90	39	51	3	6	4	85	50	82	18	62	22	84	26	24	3	84	10	17	81	85	3	3	89	28	84	15
60	64	28	36	0	7	5	61	41	59	19	51	15	57	35	34	4	58	5	10	57	56	6	7	62	23	58	7
61	53	24	29	3	4	4	51	40	46	12	49	15	50	30	35	5	50	11	12	50	45	3	5	50	28	49	9
62	44	17	27	3	15	8	40	41	41	6	39	9	39	32	34	7	40	7	13	36	40	2	5	38	19	39	5
63	32	12	20	2	15	2	30	28	28	3	27	5	30	25	25	3	31	5	17	29	32	3	2	31	18	27	7
64	22	5	17	1	8	9	20	19	18	8	21	4	21	19	17	0	20	3	12	20	16	0	1	17	6	20	4
65	19	4	15	3	9	7	15	18	19	3	18	9	18	19	16	4	18	3	11	15	15	3	0	13	6	19	6
66	5	1	4	1	2	1	5	5	5	2	5	2	5	5	5	0	5	1	3	5	5	1	2	4	4	5	1
67	12	5	7	2	10	8	11	11	12	4	12	7	12	10	10	4	11	2	6	8	12	3	5	12	11	11	2
68–79	23	9	14	20	20	17	23	22	23	16	20	14	21	19	20	17	22	17	16	17	23	19	14	23	17	18	6
≥80	23	10	13	17	18	16	18	19	18	15	19	16	18	17	18	16	19	13	19	15	19	18	17	19	18	17	10
Totals	2339	982	1357	71	150	131	1824	516	1949	189	872	400	1650	346	303	134	2000	192	190	1647	1825	110	104	2256	340	2129	265

(B) Frequencies of cases with extra chromosomes

MN	No. PTS	XX	XY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	Y
51	75	23%	77%	0%	1%	0%	29%	9%	28%	7%	12%	15%	19%	3%	0%	7%	52%	1%	4%	13%	19%	4%	5%	81%	4%	77%	9%
52	152	40%	60%	0%	3%	0%	47%	1%	64%	1%	9%	6%	14%	2%	1%	4%	77%	3%	1%	39%	39%	3%	0%	97%	1%	90%	8%
53	239	42%	58%	1%	1%	2%	59%	4%	74%	3%	10%	11%	38%	2%	3%	2%	76%	5%	1%	58%	63%	3%	1%	97%	6%	89%	8%
54	322	39%	61%	1%	1%	2%	72%	5%	82%	2%	17%	10%	55%	2%	2%	3%	86%	4%	3%	68%	75%	2%	1%	98%	6%	90%	11%
55	427	45%	55%	0%	2%	3%	83%	7%	87%	1%	25%	11%	79%	5%	2%	2%	87%	6%	0%	72%	86%	1%	3%	99%	7%	96%	14%
56	360	47%	53%	1%	0%	4%	85%	16%	88%	4%	39%	19%	87%	5%	4%	4%	89%	6%	3%	77%	86%	2%	3%	98%	9%	92%	24%
57	233	44%	56%	2%	2%	2%	92%	19%	92%	10%	50%	26%	91%	14%	5%	5%	90%	10%	3%	81%	91%	5%	2%	98%	14%	94%	30%
58	144	42%	58%	1%	8%	6%	87%	40%	95%	15%	57%	21%	89%	17%	13%	8%	89%	9%	15%	80%	85%	5%	5%	97%	21%	88%	36%
59	90	43%	57%	3%	7%	4%	94%	56%	91%	20%	69%	24%	93%	29%	27%	3%	93%	11%	19%	90%	94%	3%	3%	99%	31%	93%	29%
60	64	44%	56%	0%	11%	8%	95%	64%	92%	30%	80%	23%	89%	55%	53%	6%	91%	8%	16%	89%	88%	9%	11%	97%	36%	91%	19%
61	53	45%	55%	6%	8%	8%	96%	75%	87%	23%	92%	28%	94%	57%	66%	9%	94%	21%	23%	94%	85%	6%	9%	94%	43%	92%	31%
62	44	39%	61%	7%	34%	18%	91%	93%	93%	14%	89%	20%	89%	73%	77%	16%	91%	16%	30%	82%	91%	5%	11%	86%	43%	89%	19%
63	32	38%	63%	6%	47%	6%	94%	88%	88%	9%	84%	16%	94%	78%	78%	9%	97%	16%	53%	91%	100%	9%	6%	97%	56%	84%	35%
64	22	23%	79%	5%	36%	41%	91%	86%	82%	36%	95%	18%	95%	86%	77%	0%	91%	14%	55%	91%	73%	0%	5%	77%	27%	91%	24%
65	19	21%	79%	16%	47%	37%	79%	95%	100%	16%	95%	47%	95%	100%	84%	21%	95%	16%	58%	79%	79%	16%	0%	68%	32%	100%	40%
66	5	20%	80%	20%	40%	20%	100%	100%	100%	40%	100%	40%	100%	100%	100%	0%	100%	20%	60%	100%	100%	20%	40%	80%	80%	100%	25%
67	12	42%	58%	17%	83%	67%	92%	92%	100%	33%	100%	58%	100%	83%	83%	33%	92%	17%	50%	67%	100%	25%	42%	100%	92%	100%	29%
68–79	23	39%	61%	87%	87%	74%	100%	96%	78%	70%	87%	61%	91%	83%	87%	74%	96%	74%	70%	74%	100%	83%	61%	100%	74%	78%	43%
≥80	23	43%	57%	74%	78%	70%	78%	83%	78%	65%	83%	70%	78%	74%	78%	70%	83%	57%	83%	65%	83%	78%	74%	83%	78%	74%	77%
Totals	2339	42%	58%	3%	6%	6%	78%	22%	83%	8%	37%	17%	71%	15%	13%	6%	86%	8%	8%	70%	78%	5%	4%	96%	15%	91%	20%

(A): Numbers of patients (No. PTS) with extra copies of each of chromosome at each MN. (B):Percentages of patients with extra copies of each chromosome at each MN. Percentages of cases with an extra Y chromosome are based on male patients only.
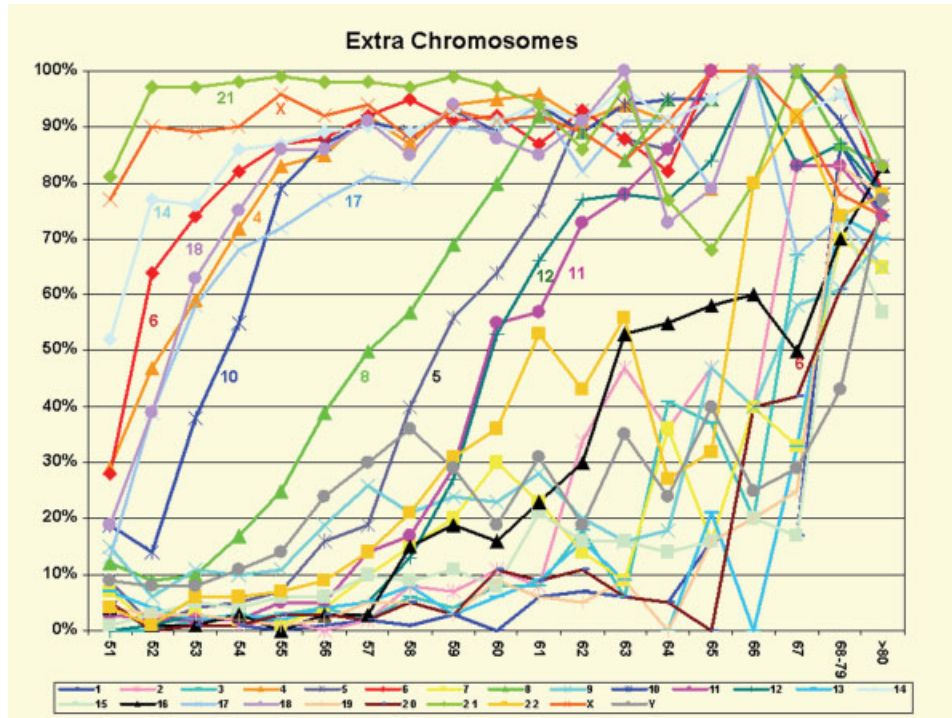
Figure 1. Composite graph showing pattern of gain of extra chromosomes across all modal numbers obtained from 2339 pediatric high hyperdiploid ALL cases. The four groups described are clearly evident, as is the rapid rise in frequency of cases with extra copies of chromosomes 21, X, 14, 6, 18, 4, 17, and 10 at MN 51–54 (group I) and chromosomes 8, 5, 12, and 11 at MN 57–60 (group II). Chromosomes 2, 3, 9, 16, and 22 occur at high frequencies at MN 63–67 (group III). There is not a consistently high frequency of cases with extra copies of chromosomes 1, 7, 13, 15, 19, and 20 until MN are near triploid ($\geq$68; group IV). Chromosome Y frequencies are based on male patients only; it is not frequently disomic until MN $\geq$80. Colors indicating each chromosome are shown at the bottom of the figure. Chromosomes in groups I and II are identified on the graph.

extra copy of most chromosomes in near-triploid cases and two extra copies in near-tetraploid cases.

Statistical Analyses

Cluster analysis was used to analyze the data. This analysis is based on the assumption that each chromosome is equally likely to have extra copies at any modal number.

RESULTS

The number and frequency of 2,339 childhood ALL cases with extra copies ($\geq$3, including two X or Y chromosomes in males) of each chromosome for each MN >50 are shown in Table 1. Bar graphs showing the patterns of extra chromosomes for each MN 51–67 were prepared and compared (Supplemental Fig. 1; Supplementary material for this article can be found at http://www.intersciencе.wiley.com/jpages/1045-2257/suppmat). Because the numbers of cases with MN 68–79 ($\sim$3n, 23 cases) and MN $\geq$80 ($\sim$4n, 23 cases) were small, such cases were combined.

These data are summarized in Figure 1, which demonstrates the frequencies of cases with gain of each chromosome at each modal number and shows a striking pattern of gain. Extra copies of X and 21 were seen in most cases; gain of an X chromosome was equally frequent in males and females. The frequencies of extra copies of chromosomes 14, 6, 18, 4, 17, and 10 rapidly increased to 55–98% of cases at MN 54. Cluster analysis confirmed that extra copies of these chromosomes occur at MN 52–53, although there are extra copies of X and 21 at MN 51 (Fig. 2). We designated these chromosomes as group I. As the MN increased further, the high frequency of cases with extra copies of these chromosomes was maintained.

Above MN 54, no other chromosome had extra copies in at least 50% of cases until MN 57, at which extra copies of chromosome 8 occurred in 50% of cases. At MN 59, all of the group I chromosomes were present in extra copies in 90% or more of cases, chromosome 8 in 69%, and chromosome 5 in 56% of cases. These extra chromosomes were retained at MN 60, when extra copies of chromosomes 11 and 12 were also present in >50% of cases. Cluster analysis confirmed that these chro-

Mean percentage (y-axis) of chromosome groups present in patients with various chromosome numbers (x-axis)
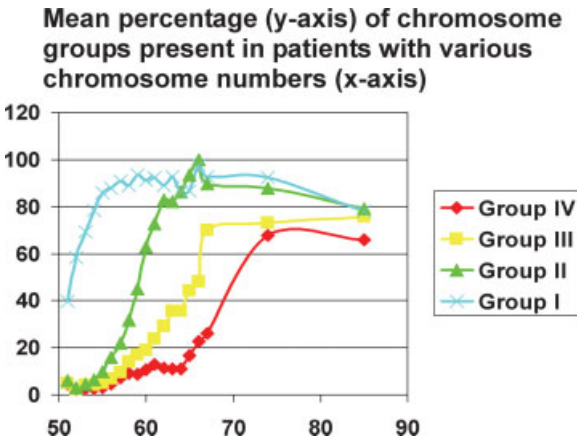

Figure 2. Groups of chromosomes as identified by cluster analysis. Gain of group I chromosomes (4, 6, 10, 14, 17, 18, 21, and X) occurs at MN 52–53, with the exception of chromosomes 21 and X, which are gained already at MN 51. Frequency of patients with gain of group II chromosomes (5, 8, 11, and 12) stays low until approximately MN 60. Frequency of patients with gain of group III chromosomes (2, 3, 9, 16, and 22) stays low until approximately MN 66. And frequency of patients with gain of group IV chromosomes (1, 7, 13, 15, 19, and 20) stays low until MN 68–79. Chromosome Y is not frequently disomic in males until MN ≥80 (not shown).

mosomes represent a second pattern of gain, and extra copies of these chromosomes occur in a low frequency of cases (<50%) until MN is about 60. We designated chromosomes 8, 5, 12, and 11 as a second group (group II) of chromosomes gaining extra copies in at least 50% of cases at MN 57–60. Again, these chromosomes retained extra copies in most cases as the MN further increased.

No additional chromosomes appeared to gain extra copies at MN 61–66, with the exception of chromosome 16, which gradually had extra copies in more cases at MN ≥63. Chromosome 22 had extra copies in 53% of cases at MN 61, but its pattern of gain was different from those of the aforementioned chromosomes (groups I and II): extra copies of chromosome 22 did not rapidly emerge in a high frequency of cases, but fluctuated in frequency at MN 61–65. At MN 67 chromosomes 2, 3, and 9 showed a rapid increase in the frequency of cases with additional copies, as was typical for the chromosomes in groups I and II at lower MN. Cluster analyses also identified these chromosomes, 2, 3, 9, 16, and 22, as a third group, with extra copies in a low frequency of cases until MN is about 66.

At MN 67 only chromosomes 1, 7, 13, 15, 19, 20, and Y did not have extra copies in at least 50% of cases. The number of cases with MN ≥68 was too small for individual MN analysis; therefore, they were combined into groups with MN 68–79 (23 cases) and MN ≥80 (23 cases). At MN 68–79, all

chromosomes except Y were present as extra copies in at least 61% of cases. Chromosomes 1, 7, 13, 15, 19, and 20, which were gained at MN 68–79, were identified by cluster analyses as a fourth group, group IV. At MN ≥80, all chromosomes had extra copies in ≥70% of cases, with chromosome Y disomic in 77% of males. These results show that the extra chromosomes that are present at any MN can be predicted.

We also examined the frequencies of tetrasomies of each chromosome at each MN (Table 2; Fig. 3). Frequencies of cases with tetrasomic chromosomes were consistently high for chromosome 21 (55–96%) at all MN. Tetrasomy X (females only) increased to ∼30% of cases at MN 56, and remained tetrasomic in ∼30% of cases until MN 64, when a higher frequency of cases had tetrasomy X. Frequencies of cases with tetrasomies 14, 18, 4, and 10 gradually increased as MN increased, but none of these occurred in >50% of cases until MN ≥80, when all chromosomes except 9, 11, and 13 were tetrasomic in >50% of cases, although the frequency of cases with tetrasomy 9, 11, and 13 dramatically increased at MN ≥80 compared to cases with MN 68–79. As with trisomies, tetrasomies of chromosomes at lower MN were retained at higher MN, although at much lower frequencies. An exception is MN 66, which consisted of only 5 patients. Thus, tetrasomy for specific chromosomes also can be predicted based on MN.

DISCUSSION

We have shown that the acquisition of extra copies of individual chromosomes in pediatric high hyperdiploid (MN > 50) ALL occurs in a non-random pattern. Chromosomes that are over-represented at lower high hyperdiploid MN are also over-represented at all higher MN. As the MN increases, extra copies of each chromosome occur in a predictable pattern, and the likelihood that a specific chromosome is present in one or more extra copies depends on the number of chromosomes gained (i.e., the MN). Therefore, at any MN, the extra chromosomes that are present can be predicted. At MN ≥68 nearly all chromosomes have extra copies. Interestingly, the pattern of acquisition of extra chromosomes in this large series of reviewed ALL cases is similar to the pattern of over-representation of chromosomes (21, X, 4, 6, 10, 14, 17, and 18) reported in a large series of ALL cases obtained from a literature review (Mertens et al., 1996).

TABLE 2. Numbers and Frequencies of Individual Tetrasomies at Each MN

MN	No. PTS	XX	XY	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
(A) Numbers of cases with tetrasomic chromosomes																										
51	75	17	58	0	0	0	1	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0	0	45	0	2
52	152	61	91	0	0	0	5	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	92	0	5
53	239	101	138	0	0	0	2	0	0	0	0	0	0	0	0	0	6	0	0	0	2	0	0	145	0	10
54	322	125	197	0	0	0	4	0	2	0	1	1	8	0	0	0	13	0	0	0	8	0	0	225	0	18
55	427	192	235	0	0	0	8	0	1	0	4	0	5	0	0	1	27	1	0	0	19	0	0	341	0	33
56	360	169	191	0	0	0	3	2	2	0	4	1	14	0	0	0	43	0	0	4	25	0	0	298	0	49
57	233	102	131	0	0	0	4	0	0	0	5	0	16	1	0	0	37	0	0	2	32	0	0	199	2	28
58	144	61	83	0	0	0	8	1	1	0	4	0	12	1	0	0	33	1	0	1	24	0	0	122	3	19
59	90	39	51	0	0	0	8	0	0	0	4	1	4	2	0	0	22	0	0	1	17	0	0	74	0	11
60	64	28	36	1	0	0	7	0	0	0	4	0	9	0	0	0	20	1	0	2	10	1	1	54	1	7
61	53	24	29	0	0	0	4	2	1	0	4	0	5	1	0	0	16	0	0	1	11	1	0	39	1	7
62	44	17	27	1	0	0	13	0	0	0	7	0	5	0	0	0	11	0	0	2	13	0	0	34	1	2
63	32	12	20	0	0	0	13	0	1	0	9	0	4	0	0	0	12	0	0	1	8	1	0	23	3	4
64	22	5	17	0	0	0	7	0	1	0	2	0	7	1	0	0	10	0	0	0	6	0	0	12	0	2
65	19	4	15	0	0	0	9	2	2	0	3	0	7	1	0	0	9	0	0	0	4	0	0	11	0	3
66	5	1	4	0	0	1	1	0	2	0	4	0	0	1	1	0	4	0	1	0	1	0	0	3	1	0
67	12	5	7	0	1	0	8	0	2	0	7	0	4	3	1	0	3	0	1	5	5	0	1	10	0	2
68–79	23	9	14	5	0	1	8	7	6	2	0	0	10	3	0	0	8	0	0	5	5	5	3	18	6	6
≥80	23	10	13	19	19	16	12	18	20	17	15	11	19	11	14	9	16	13	20	15	20	21	15	22	20	6
Totals	2339	982	1357	29	20	18	118	34	41	19	83	14	129	22	18	12	293	16	23	32	212	31	20	1767	40	216
(B) Frequencies of cases with tetrasomic chromosomes																										
51	75	23%	77%	0%	0%	0%	1%	0%	0%	0%	1%	0%	0%	0%	0%	0%	3%	0%	0%	0%	0%	0%	0%	60%	0%	12%
52	152	40%	60%	0%	0%	0%	3%	0%	0%	0%	1%	0%	0%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	61%	0%	8%
53	239	42%	58%	0%	0%	0%	1%	0%	0%	0%	0%	0%	0%	0%	0%	0%	3%	0%	0%	0%	1%	0%	0%	61%	0%	10%
54	322	39%	61%	0%	0%	0%	1%	0%	1%	0%	0%	0%	2%	0%	0%	0%	4%	0%	0%	0%	2%	0%	0%	70%	0%	14%
55	427	45%	55%	0%	0%	0%	2%	0%	0%	0%	1%	0%	1%	0%	0%	0%	6%	0%	0%	0%	4%	0%	0%	80%	0%	17%
56	360	47%	53%	0%	0%	0%	1%	1%	1%	0%	1%	0%	4%	0%	0%	0%	12%	0%	0%	1%	7%	0%	0%	83%	0%	29%
57	233	44%	56%	0%	0%	0%	2%	0%	1%	0%	2%	0%	7%	0%	0%	0%	16%	0%	0%	1%	14%	0%	0%	85%	1%	27%
58	144	42%	58%	0%	0%	0%	6%	0%	1%	0%	3%	0%	8%	1%	0%	0%	23%	0%	0%	1%	17%	0%	0%	85%	2%	31%
59	90	43%	57%	0%	0%	0%	9%	0%	0%	0%	4%	0%	4%	2%	0%	0%	24%	0%	0%	1%	19%	0%	0%	82%	2%	28%
60	64	44%	56%	0%	0%	0%	11%	0%	0%	0%	6%	2%	14%	0%	0%	0%	31%	0%	0%	2%	16%	2%	2%	84%	2%	25%
61	53	45%	55%	2%	0%	0%	8%	4%	2%	0%	8%	0%	9%	2%	0%	0%	30%	0%	0%	4%	21%	2%	0%	74%	2%	29%
62	44	39%	61%	0%	0%	0%	30%	2%	0%	0%	9%	0%	11%	0%	0%	0%	25%	0%	0%	2%	30%	2%	0%	77%	7%	12%
63	32	38%	63%	3%	0%	0%	41%	0%	3%	0%	22%	0%	13%	0%	5%	0%	38%	0%	0%	0%	25%	3%	0%	72%	0%	33%
64	22	23%	79%	0%	0%	0%	32%	0%	5%	0%	41%	0%	32%	5%	5%	0%	45%	0%	0%	0%	27%	0%	0%	55%	1%	40%
65	19	21%	79%	11%	0%	5%	47%	11%	11%	0%	11%	0%	37%	5%	5%	0%	47%	0%	5%	0%	21%	5%	0%	58%	2%	75%
66	5	20%	80%	0%	0%	0%	20%	0%	40%	0%	60%	0%	0%	20%	0%	0%	80%	0%	0%	0%	20%	0%	0%	60%	0%	0%
67	12	42%	58%	8%	8%	0%	8%	8%	17%	0%	33%	0%	33%	8%	8%	8%	25%	0%	8%	22%	42%	8%	8%	83%	25%	40%
68–79	23	39%	61%	22%	4%	4%	35%	30%	26%	9%	30%	0%	43%	13%	4%	4%	35%	4%	4%	22%	30%	22%	13%	78%	26%	67%
≥80	23	43%	57%	83%	83%	70%	52%	78%	87%	74%	65%	48%	83%	48%	61%	39%	70%	57%	87%	65%	87%	91%	65%	96%	87%	60%
Totals	2339	42%	58%	1%	1%	5%	5%	1%	2%	1%	4%	1%	6%	1%	1%	1%	13%	1%	1%	1%	9%	1%	1%	76%	2%	25%

(A): Numbers of patients (No. PTS) with tetrasomies of each of chromosome at each MN. (B) Percentages of patients with tetrasomies of each chromosome at each MN. Frequencies of tetrasomies of chromosome X are based on female patients only. Chromosome Y was not included in these analyses.
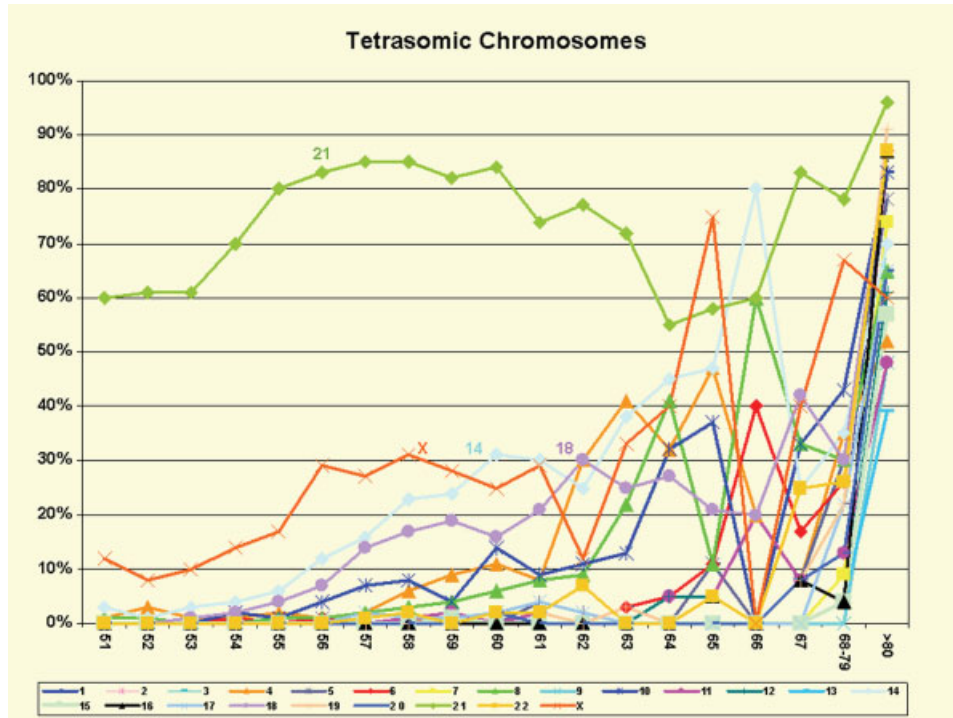
Figure 3.   Composite graph showing frequencies of tetrasomic chromosomes at each MN. Chromosome 21 is tetrasomic in a high frequency of cases at all MN. No other chromosome is consistently tetrasomic in a high frequency of cases. Chromosome X is tetrasomic in ∼30% of cases at MN 56–64. Chromosomes 14, 18, 4, and 10 are tetrasomic in >10% of cases beginning at MN 56–60, chromosome 8 at MN 63, and chromosome 6 at MN 65. All chromosomes except 9, 11, and 13 are tetrasomic in >50% of cases with MN ≥80.

Extra copies of chromosomes X and 21 nearly always are present in high hyperdiploid ALL, with tetrasomy 21 very common. They are also trisomic in ALL with 47–50 chromosomes, albeit at lower frequencies (X, 18%; 21, 40%) (Raimondi et al., 1992). An extra copy of the X chromosome occurs as frequently in males as in females. The Y chromosome is disomic relatively infrequently in high hyperdiploid ALL, and the frequency of disomy Y exceeds 50% in males only when MN is ≥80. Clearly, gain of a sex chromosome favors the X chromosome. Interestingly, expression profiling of pediatric ALL showed that almost 70% of genes that defined high hyperdiploid ALL were on chromosomes X or 21 (Yeoh et al., 2002), consistent with the nearly ubiquitous extra copies of these chromosomes in high hyperdiploid ALL.

High hyperdiploidy in ALL most frequently occurs as the result of one massively abnormal mitosis, although sequential gain of chromosomes or loss from a tetraploid cell may occasionally occur (Onodera et al., 1992; Paulsson et al., 2003, 2005). Hyperdiploidy is an early event in leukemogenesis, and it occurs prenatally in some cases (Panzer-Grumayer et al., 2002; Paulsson et al., 2003, 2005;

Maia, et al., 2004). We have shown that the gain of chromosomes in this abnormal mitosis follows a predictable pattern, depending on MN, with specific chromosomes gained at each MN. We also noted that the karyotypes of individual patients are fairly stable, consistent with the observation that only minor copy number variation is observed when using centromeric probes to evaluate aneuploidy by fluorescence in situ hybridization [unpublished COG data], indicating that there is minimal chromosome instability in these cells. Thus, the mechanism of generation of abnormal karyotypes in pediatric high hyperdiploid ALL is a single abnormal cell division, after which normal mitosis recurs.

The mechanism by which an abnormal mitosis or abnormal segregation occurs and results in specific extra chromosomes is unclear. Chromosomes are not randomly distributed in the cell's nucleus, but occupy chromosome territories (Nagele et al., 1998; Cremer and Cremer, 2001; Cremer et al., 2003; Bell, 2005). This has been shown in tumor nuclei as well as normal nuclei (Cremer et al., 2003). These territories are transmitted through mitosis (Nagele et al., 1998; Gerlich et al., 2003),

with centromere order (perpendicular to the spindle axis) preserved on the metaphase plate, and the original overall pattern of chromosome territories is restored during anaphase and telophase (Gerlich et al., 2003). This process lends itself to the existence of a specific order of chromosomes on the metaphase plate. Additionally, the separation of chromatids from the metaphase plate follows a pattern in which chromosomes destined for a more poleward position separate before those that remain closer to the cleavage furrow (Gerlich et al., 2003; Bell, 2005). We hypothesize that both mechanisms (the propagation of chromosome territories and the sequence of separation of chromatids) could result in the preferential non-disjunction of specific chromosomes seen in high hyperdiploid ALL with MN 51–67.

Classic non-disjunction occurs when a chromosome fails to attach appropriately to a spindle and lags. This occurs only if there is an error in the anaphase promoting complex/cyclosome (APC/C), which is required for normal mitosis. The APC/C prevents premature separation of the chromatids by maintaining metaphase until all kinetochores have a bipolar attachment to the spindle. For APC/C failure to cause high hyperdiploidy with the pattern of gain of chromosomes we observed, specific chromosomes would fail to make a bipolar attachment to the spindle in a particular sequence, as the number of unattached kinetochores increased, and the unattached chromosomes would progress to the same pole. Missing chromosomes would be a consequence if some unattached chromosomes progressed to the opposite pole, and missing chromosomes are extremely rare in high hyperdiploid ALL cells.

The centrosome, which organizes the spindle, is important for maintenance of normal cell division. Extra centrosomes result in multipolar mitoses and aneuploidy. The position of the cleavage plane in multipolar mitosis is determined by the centrosomes, and cytokinesis results in only two daughter cells. Most daughter cells resulting from a multipolar mitosis are eliminated by apoptosis. However, a cell with a specific chromosome complement may occasionally survive. To propagate, the cell must regain mitotic stability either by inactivating the extra centrosomes or by coalescing them into two functional centrosomes, thereby resulting in two spindle poles in subsequent mitoses (Brinkley, 2001). The latter mechanism has been shown to occur in breast cancer cells (Salisbury et al., 1999) and in a murine epithelial $Trp53^{-/-}$ cell line (Chiba et al., 2000). If multipolar spindles give rise to high

hyperdiploid cells in ALL, the pattern of chromosomal gain suggests that certain chromosomes are predictably attached to the spindle such that when cytokinesis occurs, the extent of imbalance determines the specific chromosomes gained. Therefore, if only a few chromosomes are aligned with the extra centrosome, they are most likely to be from group I (X, 21, 14, etc.). The daughter cell with the extra chromosomes regains normal centrosome function, and ensuing divisions are normal. This would be the leukemia initiating event; additional leukemia promoting events would be required for the full malignant phenotype.

Additionally, the kinetochore-spindle interaction is complex and controlled by many proteins. Malfunction of any of these proteins could result in misattachments and lead to aneuploidy (Biggins and Walczak, 2003; Cimini and Degrassi, 2005).

In normal mitosis, cohesin holds the chromatids together until all kinetochores have a bipolar spindle attachment. Cohesins are ubiquitinylated by separin once the APC/C complex is released, thereby allowing mitosis to proceed. Aberrant mitosis results when separin or cohesin is abnormal. If release of cohesin occurs in a specific chromosome order, as suggested by the order of chromosome movement toward the spindle poles (Gerlich et al., 2003), the pattern of extra chromosomes observed in high hyperdiploid ALL may be a consequence of abnormal cohesin or separin. Chromosomes X and 21 would be the last to have cohesin ubiquitinylated, and the chromosomes that maintain cohesin would all have to proceed to the same pole. Chromatid separation is also impaired by improper chromosome condensation resulting, for example, from histone hyperacetylation (Cimini et al., 2003) or histone H3Ser10 dephosphorylation (Wei et al., 1999) during mitosis. This process contributes to non-disjunction and also could result in the pattern of extra chromosomes we observed in high hyperdiploid ALL.

A proliferative or survival advantage for only certain combinations of extra chromosomes in high hyperdiploid ALL is another possible cause of the pattern of extra chromosomes we observed. After an abnormal mitosis, such an advantage might occur only with specific, sequentially added combinations of chromosomes. This theory implies that an abnormal mitosis results in random gain of chromosomes, but that only certain combinations of chromosomes give a cell a proliferative advantage; and therefore, only cells with specific combinations of chromosomes are observed as ALL cells. However, our observation of groups of specific extra

chromosomes acquired at increasing MN makes the proliferative advantage theory seem an improbable explanation for the extra chromosomes observed. For example, extra copies of chromosome 8 appear to have little advantage at MN ≤56, but do have a proliferative advantage at MN ≥57, whereas gain of chromosome 12 has a proliferative advantage only at MN ≥60. According to the proliferative advantage theory, extra copies of chromosome 12 would confer an advantage only when there are also extra copies of chromosome 8. If coexpression of genes was invoked as an explanation for such an advantage, then the acquisition of chromosome 12 would not lag until MN ≥60. Thus, our observation of groups of specific extra chromosomes acquired at increasing MN makes the proliferative advantage theory seem unlikely.

The pattern of gain of chromosomes in high hyperdiploid ALL could result from any of the aforementioned mechanisms. The remarkable specificity of extra chromosomes related to MN and the fact that the reciprocal products (loss of chromosomes) are not observed must be accounted for by any proposed hypothesis.

The pattern of gain of extra chromosomes suggests that the mechanisms resulting in near-triploidy and near-tetraploidy are different from those resulting in hyperdiploidy with 51–67 chromosomes. High hyperdiploid cases infrequently have extra copies of chromosomes 1, 7, 13, 15, 19, 20, and Y (group IV and Y). In most near-triploid cases (MN 68–79), all chromosomes are present in extra copy number, but only chromosomes X and 21 are tetrasomic. In near-tetraploid cases (MN ≥80), nearly all chromosomes are tetrasomic. These patterns of extra chromosomes are consistent with a MN of 51–67 describing high hyperdiploid cases, MN 68–79 for near-triploid cases and MN ≥80 for near-tetraploid cases. These results are consistent with demonstrations that near-triploid and near-tetraploid ALL frequently have an *ETV6/RUNX1* rearrangement (Attarbaschi et al., 2006; Raimondi et al., 2006), whereas such rearrangements are rare in high hyperdiploid ALL (Uckun et al., 2001). *ETV6/RUNX1* analyses were not done in these studies.

A lack of cytokinesis is the most likely cause of near-tetraploid karyotypes, although fusion of two diploid cells has been proposed as an alternative (Trujillo et al., 1971). It is difficult to speculate about the causes of near-triploid karyotypes, although several possibilities have been suggested. These include non-disjunction, duplication of hypodiploid cells (which does occur, but such cases

were classified as hypodiploid in these studies), loss of chromosomes from tetraploid cells, and multipolar mitosis of tetraploid cells (Ohtaki et al., 1985). Regardless, the pattern of gain of chromosomes in these near-triploid cases is clearly different from that in cases with lower MN, which is highly suggestive of a different etiology.

In conclusion, high hyperdiploid ALL cells have a specific chromosome complement that depends on their MN. Furthermore, as the MN increases each chromosome gained at a lower MN is retained. The acquisition of extra copies of each chromosome occurs in a predictable pattern, and the likelihood that a specific chromosome is present in one or more extra copies depends on the number of chromosomes gained (i.e., the MN). The mechanism underlying this phenomenon is unknown. The aberrant mitoses that result in high hyperdiploidy may affect each chromosome differently such that specific additional chromosomes are more likely to be nonrandomly distributed as the aberrant mitoses involve greater numbers of chromosomes. The remarkable specificity of extra chromosomes related to MN and the fact that the reciprocal products (loss of chromosomes) are not observed must be accounted for by any proposed hypothesis.

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