Copy number genome alterations are associated with treatment response and outcome in relapsed childhood *ETV6/RUNX1*-positive acute lymphoblastic leukemia

Almut Bokemeyer,[1] Cornelia Eckert,[1] Franziska Meyr,[1] Gabriele Koerner,[1] Arend von Stackelberg,[1] Reinhard Ullmann,[2] Seval Türkmen,[3,4] Günter Henze,[1] and Karl Seeger[1]

[1]Department of Pediatric Oncology/Hematology, Charité – Universitätsmedizin Berlin; [2]Max-Planck Institute for Molecular Genetics, Berlin; [3]Institute of Medical Genetics, Charité – Universitätsmedizin Berlin; and [4]Labor Berlin, Germany

©2014 Ferrata Storti Foundation. This is an open-access paper. *doi:10.3324/haematol.2012.072470*
Manuscript received on September 17, 2012. Manuscript accepted on November 8, 2013.
Correspondence: karl.seeger@charite.de

SUPPLEMENTARY ONLINE INFORMATION

Copy number genome alterations are associated with treatment response and outcome in relapsed childhood *ETV6/RUNX1*-positive acute lymphoblastic leukemia

Short title: Copy number alterations in relapsed *ETV6/RUNX1*-positive ALL

Almut Bokemeyer MD,[1] Cornelia Eckert PhD,[1] Franziska Meyr MD,[1] Gabriele Koerner,[1] Arend von Stackelberg MD,[1] Reinhard Ullmann PhD,[2] Seval Türkmen MD,[3, 4] Prof. Günter Henze MD,[1] Prof. Karl Seeger MD/PhD[1]

[1]Department of Pediatric Oncology/Hematology, Charité – Universitätsmedizin Berlin, Berlin, Germany,
[2]Max-Planck Institute for Molecular Genetics, Berlin, Germany,
[3]Institute of Medical Genetics, Charité – Universitätsmedizin Berlin, Berlin, Germany
[4]Labor Berlin, Berlin, Germany

To whom correspondence should be addressed:

Prof. Dr. Dr. Karl Seeger, MD, PhD (corresponding author)
Department of Pediatric Oncology/Hematology
Charité – Universitätsmedizin Berlin, Campus Virchow Klinikum
Augustenburger Platz 1, 13353 Berlin, Germany
Phone: +49-30-450666163
Fax: +49-30-450566906
E-mail: karl.seeger@charite.de

This study was supported by a grant from the José Carreras Leukämie-Stiftung e.V., Munich, Germany.

Supplementary Tables

Supplementary Table S1. Patients' characteristics and representativity of the study cohort

Clinical feature	Study cohort	(%)	Total ALL-REZ BFM comparison cohort	(%)	P-value (χ^2-Test)
Total	51	(100)	113	(100)	
Gender					0.80
male	31	(61)	71	(63)	
female	20	(39)	42	(37)	
Time of relapse[1]					0.35
very early	0	(0)	4	(4)	
early	9	(18)	16	(14)	
late	42	(82)	93	(82)	
Site of relapse					0.57
BM isolated	40	(78)	84	(74)	
BM combined	11	(22)	29	(26)	
Immunophenotype					0.85
pre-B	13	(25)	27	(24)	
common	38	(75)	85	(76)	
ALL-REZ BFM risk group[2]					0.39
intermediate (S2)	45	(88)	97	(86)	
high (S3)	6	(12)	12	(11)	
ALL-REZ BFM protocol					0.72
90	2	(4)	8	(7)	
95/96	33	(65)	69	(61)	
2002	16	(31)	36	(32)	
PBC per µl					0.69
<1/µl	4	(8)	13	(12)	
≥1 and <10.000/µl	39	(76)	86	(76)	
≥10.000/µl	8	(16)	14	(12)	
MRD[3]					0.95
good response ($\leq 10^{-3}$)	22	(67)	33	(66)	
poor response ($\geq 10^{-3}$)	11	(33)	17	(34)	
NA	18				
Event					0.80
CCR	34	(67)	66	(58)	
subsequent relapse	15	(29)	36	(32)	
induction death	1	(2)	4	(4)	
therapy-related death	1	(2)	2	(2)	

Legend to Supplementary Table S1. Patients' characteristics and representativity of the study cohort (n=51) in comparison with the total of ALL-REZ BFM patients diagnosed with an *ETV6/RUNX1*-positive first relapse with BM involvement over the same period (n=113).

BM: bone marrow; PBC: peripheral blast count; MRD: minimal residual disease; NA: not available.

[1] Time of relapse: *very early*: earlier than 18 months after initial diagnosis and before 6 months after cessation of frontline treatment; *early*: beyond 18 months after initial diagnosis, but before 6 months after cessation of frontline treatment; *late*: beyond 6 months after cessation of frontline treatment.

[2] Risk group stratification according to ALL-REZ BFM trials. Intermediate risk group *S2:* Patients with very early/early isolated extramedullary relapses, with late isolated BM BCP ALL relapses or early/late combined BM BCP ALL relapses. High risk group *S3*: Patients with early isolated BM BCP ALL.

[3]MRD good response: one residual leukemic cell among more than 1000 normal cells after two induction courses.

[3] MRD poor response: more than one residual leukemic cell among 1000 normal cells after two induction courses.

Supplementary Table S2. Detailed clinical characteristics of the 51 patients with *ETV6/RUNX1*-positive ALL relapse

Patient ID	Gender	Age at Relapse Diagnosis [years]	PBC at Relapse Diagnosis [per µl]	Time of Relapse[1]	Site of Relapse	Immuno-phenotype	ALL-REZ BFM risk group[2]	MRD[3]	Outcome[4]	Follow-up time [years]	Frontline Therapy	Relapse therapy ALL-REZ BFM proto-col
P1	female	16.9	1575	late	BM isolated	common	intermediate (S2)	NA	CCR	20.56	ALL-BFM 83 SR	90
P2	male	4.1	192	early	BM isolated	common	high (S3)	NA	second relapse	16.42	ALL-BFM 90 MRG	90
P3	male	4.8	9180	early	BM combined	common	intermediate (S2)	low	CCR	15.92	ALL-BFM 90 MRG	95
P4	female	7.7	230	late	BM isolated	pre-B	intermediate (S2)	low	second relapse	14.02	ALL-BFM 90 SRG	96
P5	male	11.5	0	late	BM combined	pre-B	intermediate (S2)	NA	CCR	13.67	ALL-BFM 90 SRG	96
P6	male	11.5	17990	late	BM combined	common	intermediate (S2)	NA	CCR	13.38	ALL-BFM 86 RG-1	96
P8	female	8.0	2981	late	BM isolated	common	intermediate (S2)	low	second relapse	12.24	ALL-BFM 90 MRG	96
P9	male	5.4	1150	late	BM isolated	common	intermediate (S2)	low	CCR	12.10	COALL-05-92	96
P10	male	10.8	14630	early	BM isolated	common	high (S3)	NA	second relapse	11.76	ALL-BFM 95 MR	96
P11	male	5.6	0	late	BM isolated	pre-B	intermediate (S2)	high	CCR	11.70	ALL-BFM 95 MR	96
P12	female	8.7	5616	late	BM combined	pre-B	intermediate (S2)	low	second relapse	11.36	ALL-BFM 95 MR	96
P13	male	10.3	20520	late	BM isolated	common	intermediate (S2)	NA	CCR	11.31	ALL-BFM 90 MRG	96
P14	male	7.4	594	late	BM isolated	common	intermediate (S2)	NA	CCR	11.29	ALL-BFM 95 MR	96
P15	male	5.7	1070	late	BM isolated	pre-B	intermediate (S2)	NA	second relapse	11.27	ALL-BFM 95 SR	96
P16	female	15.1	320	late	BM isolated	pre-B	intermediate (S2)	high	CCR	11.13	ALL-BFM 95 MR	96
P17	male	11.6	8750	late	BM isolated	common	intermediate (S2)	low	second relapse	11.10	COALL-05-92	96
P18	male	9.0	0	late	BM isolated	common	intermediate (S2)	low	CCR	11.06	ALL-BFM 95 SR	96
P19	female	8.3	371	late	BM isolated	pre-B	intermediate (S2)	high	second relapse	11.06	ALL-BFM 95 HR	96
P20	female	13.6	423	early	BM combined	common	intermediate (S2)	high	second relapse	10.93	ALL-BFM 95 MR	96
P21	female	6.8	248	late	BM isolated	common	intermediate (S2)	low	CCR	10.88	COALL-05-92	96
P22	female	7.6	250	late	BM isolated	common	intermediate (S2)	low	CCR	10.87	ALL-BFM 95 SR	96
P23	male	10.8	4248	late	BM combined	common	intermediate (S2)	low	CCR	10.83	ALL-BFM 95 SR	96
P24	male	12.6	924	late	BM isolated	pre-B	intermediate (S2)	NA	CCR	10.73	COALL-05-92	96
P25	female	11.0	630	late	BM isolated	common	intermediate (S2)	low	CCR	10.64	COALL-05-92	96
P26	female	17.6	680	early	BM isolated	common	high (S3)	NA	second relapse	10.50	ALL-BFM 95 MR	96
P27	male	6.9	3124	late	BM isolated	common	intermediate (S2)	NA	CCR	10.53	ALL-BFM 95 MR	96

P29	male	5.0	7820	late	BM combined	common	intermediate (S2)	low	CCR	10.33	USA	96
P30	female	6.9	88	late	BM isolated	pre-B	intermediate (S2)	low	CCR	10.10	ALL-BFM 95 MR	96
P31	male	9.0	254400	late	BM isolated	common	intermediate (S2)	NA	second relapse	10.05	ALL-BFM 95 MR	96
P32	male	7.3	468	late	BM isolated	common	intermediate (S2)	NA	induction death	10.01	COALL 06-97	96
P33	female	5.4	126	early	BM isolated	common	high (S3)	NA	second relapse	9.93	ALL-BFM 95 SR	96
P34	female	15.0	4150	late	BM isolated	common	intermediate (S2)	low	CCR	9.85	COALL 04-89	96
P35	female	6.7	1428	late	BM isolated	common	intermediate (S2)	low	CCR	9.61	ALL-BFM 95 MR	96
P36	male	15.8	5952	late	BM combined	common	intermediate (S2)	low	CCR	9.55	ALL-BFM 95 MR	96
P37	male	7.1	4416	early	BM combined	common	intermediate (S2)	high	second relapse	9.52	COALL 06-97	96
P38	male	11.6	550	late	BM isolated	pre-B	intermediate (S2)	low	CCR	9.40	ALL-BFM 90 MRG	02
P39	male	5.6	20	early	BM isolated	pre-B	high (S3)	NA	therapy related death	9.32	COALL 06-97	02
P40	male	7.1	154	late	BM isolated	common	intermediate (S2)	low	CCR	9.14	ALL-BFM 95 MR	02
P41	female	13.6	45045	early	BM isolated	common	high (S3)	NA	second relapse	9.13	ALL-BFM 2000 SR	02
P42	female	5.4	57376	late	BM isolated	common	intermediate (S2)	low	CCR	8.97	ALL-BFM 2000 MR	02
P43	male	11.8	3268	late	BM isolated	common	intermediate (S2)	NA	CCR	8.82	ALL-BFM 95 MR	02
P44	female	5.1	294	late	BM isolated	common	intermediate (S2)	high	CCR	8.30	COALL 06-97	02
P45	male	6.6	384	late	BM isolated	pre-B	intermediate (S2)	low	CCR	7.51	ALL-BFM 2000 MR	02
P46	male	9.4	296	late	BM combined	common	intermediate (S2)	high	CCR	7.28	COALL 06-97	02
P47	male	8.9	928	late	BM isolated	common	intermediate (S2)	high	CCR	6.44	ALL-BFM 2000 HR	02
P48	female	9.9	1690	late	BM isolated	common	intermediate (S2)	low	CCR	5.40	ALL-BFM 2000 MR	02
P49	male	8.5	378	late	BM isolated	common	intermediate (S2)	high	CCR	5.21	ALL-BFM 2000 HR	02
P50	male	4.7	228	late	BM isolated	common	intermediate (S2)	high	CCR	5.17	COALL 06-97	02
P51	female	13.9	266	late	BM isolated	common	intermediate (S2)	high	CCR	4.98	ALL-BFM 2000	02
P52	male	10.7	0	late	BM combined	common	intermediate (S2)	low	CCR	4.93	COALL 06-97	02
P53	male	7.1	2088	late	BM isolated	common	intermediate (S2)	NA	second relapse	4.87	COALL	02

PBC: peripheral blast count; BM: bone marrow; MRD: minimal residual disease; NA: not available;

[1]*Early*: >18 months after initial diagnosis but <6 months after cessation of frontline treatment; *late*: > 6 months after cessation of frontline treatment.

[2]Risk group stratification according to the ALL-REZ BFM protocols.

[3]MRD low: one residual leukemic cell among more than 1000 normal cells ($<10^{-3}$) after two induction courses; MRD high: more than one residual leukemic cell among 1000 normal cells ($>10^{-3}$) after two induction courses.

[4]CCR: second continuous complete remission.

Supplementary Table S3.

Whole chromosome aneuploidies in relapsed childhood *ETV6/RUNX1*-positive ALL

Chromosome	WCG[1] (n)	WCL[2] (n)
4	0	1
8	1	0
9	0	1
10	6	0
13	0	1
15	0	2
16	3	0
18	1	0
21	4	0
22	0	0
X	1	9

[1]WCG: whole chromosome gain; [2]WCL: whole chromosome loss.

One *ETV6/RUNX1*-positive ALL relapse with a hyperdiploid karyotype (+10 +16 +18 +21) included.

Supplementary Table S4.

Cox proportional hazard model for the association of pEFS with loss of *CDKN1B*

Parameter		Number of patients	Hazard ratio	95% CI	*P*
Univariate					
CDKN1B loss	negative	32	1		
	positive	19	4.60	1.69 – 12.5	0.003
Multivariate					
CDKN1B loss	negative	32	1		
	positive	19	2.28	0.71 – 7.31	0.162
Immunophenotype	common-ALL	38	1.00		
	pre-B-ALL	13	4.17	1.03 - 16.90	0.046
Time of relapse[1]	early	9	1		
	late	42	0.13	0.04 – 0.41	0.001

[1]Time of relapse: Early: >18 months after initial diagnosis but <6 months after cessation of frontline treatment; late: >6 months after cessation of frontline treatment.

The multivariate Cox proportional hazard model for the association of pEFS with the time point of relapse diagnosis as strongest prognostic variable, the immunophenotype and *CDKN1B* loss which show a weaker evidence of a prognostic impact than in the univariate analysis.

Supplementary Table S5.

Interphase FISH analyses of the 5 relapses with copy number loss of chromosome 5q31.3.

Sample ID	Blast count (%)[1]	Number of cells analyzed	Cells with hemizygous deletion (%)	Cells with homozygous deletion (%)	Cells without deletion (%)	Cells with deletion (%)
P19	81	252	33.8	5.2	58.9	39
P26	79	318	56.3	17.3	22.3	73.6
P41	97	312	42	14.7	39.4	56.7
P44	93	316	37.7	0	53.5	37.7
P46	84	309	54.7	0	42.1	54.7

[1] Blast count as determined prior to further enrichment by Ficoll density separation of mononuclear cells.

Supplementary Table S6.

Interphase FISH analyses of the minimal overlapping region on chromosome 12p13 including *CDKN1B* and *BCL2L14* among other genes.

Sample ID	Blast count (%)[1]	Number of cells analyzed	Cells with hetero-zygous deletion (%)
P10	77	100	65
P17	97	100	99
P18	90	100	98
P20	93	100	76
P23	94	100	98
P26	79	100	91
P31	91	100	99
P32	93	100	95
P35	93	100	95
P36	94	100	64
P39	80	100	68
P53	97	100	98

[1] Blast count as determined prior to further enrichment by Ficoll density separation of mononuclear cells

Supplementary Table S7.

Interphase FISH analyses of chromosome 6q21.

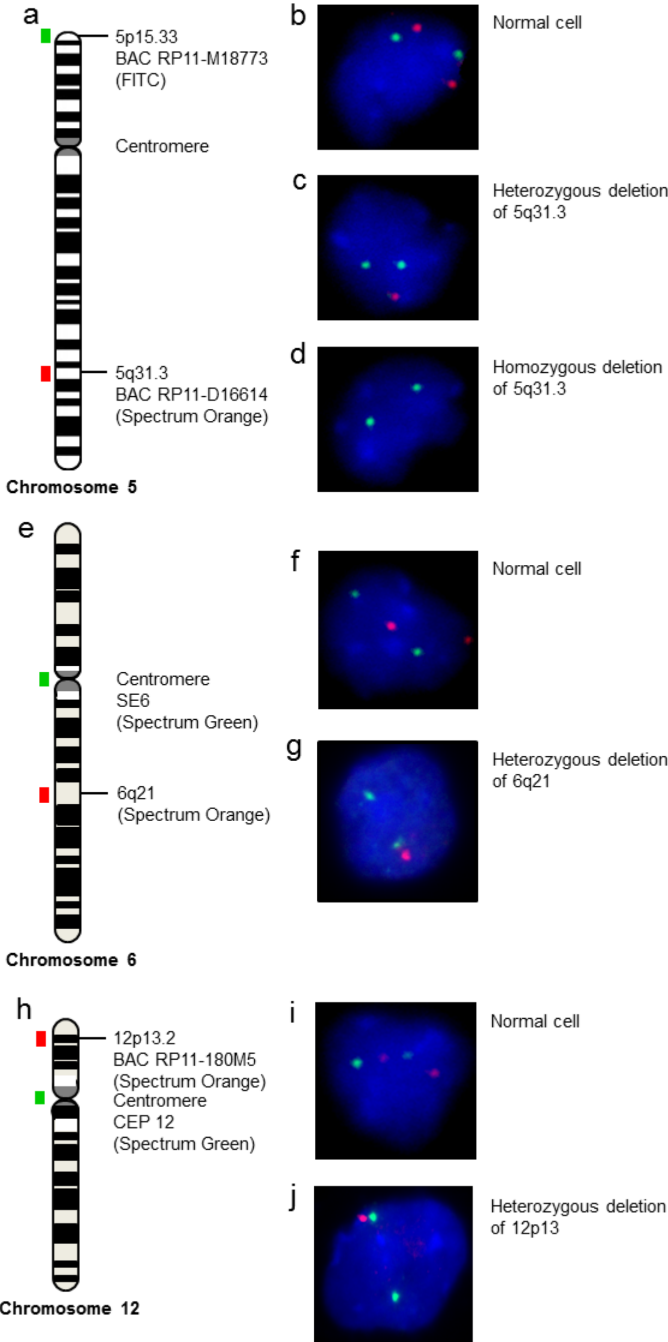
Sample ID	Blast count (%)[1]	Number of cells analyzed	Cells with heterozygous deletion (%)
P17	97	200	90
P18	90	200	95
P20	93	200	61
P24	91	200	74
P30	87	200	26
P33	90	200	68
P45	82	200	65
P48	89	200	90

[1] Blast count as determined prior to further enrichment by Ficoll density separation of mononuclear cells

Supplementary Figures

Supplementary Figure 1.

Interphase FISH validation of the array CGH findings on chromosome 5q31.3, 6q21, and 12p13 (*CDKN1B*).



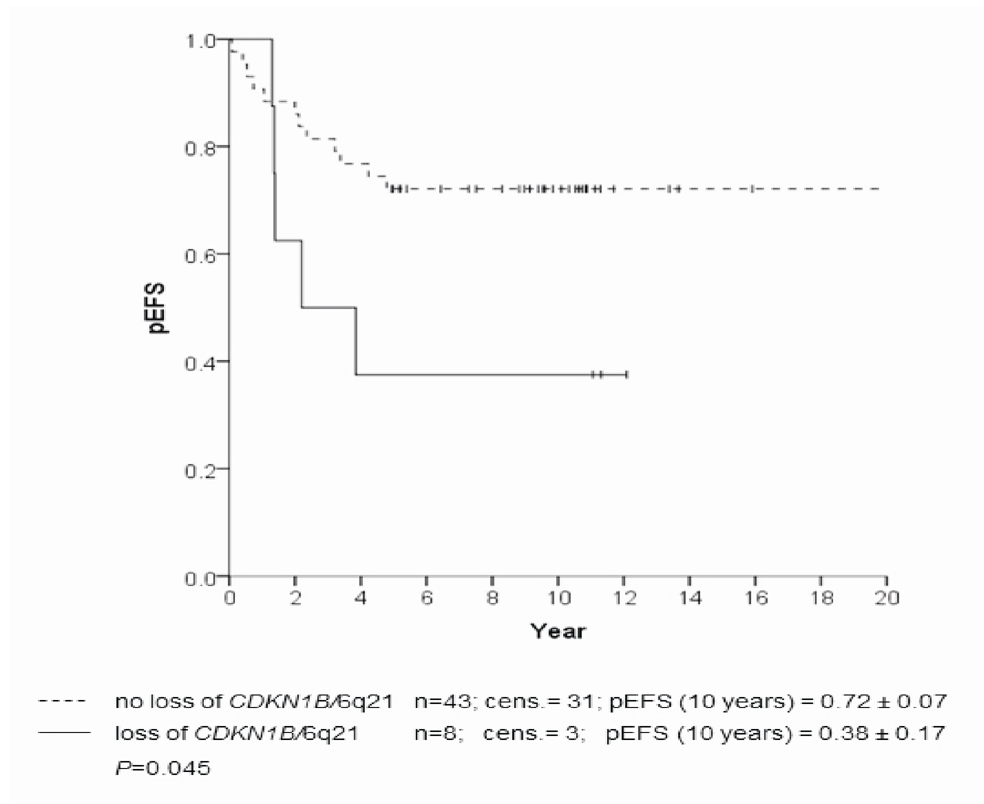
Legend to Supplementary Figure 1. (a) Positions of the two BAC clones used as chromosome 5 FISH-probes. BAC clone RP11-D16614 (chromosome band 5q31.3; genomic position Mb 142.7-142.9) is labeled with Spectrum Orange (red bar in (a), showing red signals in (b) and (c)). BAC clone RP11-M18773 (5p15.33) is used as reference and labeled with FITC (green bar in (a), displaying green signals in (a)-(c)). Shown is a normal interphase nucleus (b) as well as two interphase nuclei of one ALL relapse indicating a heterozygous (c) and a homozygous (d) deletion of 5q31.3.

(e) Positions of the 6q21(SEC63)/SE6 probe. 6q21 is labeled with Spectrum Orange (red bar in (e) showing red signals in (f) and (g)), the SE6 probe is labeled with Spectrum Green (green bar in (e), showing green signals in (f) and (g). Depicted is a normal interphase nucleus (f) and a nucleus of one ALL relapse (g) showing a heterozygous deletion of 6q21.

(h) Position of the 12p13 BAC clone RP11-180M5 (12p13) labeled with Spectrum Orange (red bar in (h), showing red signals in (i) and (j)). The CEP12 probe is used as reference and labeled with Spectrum Green, showing green signals in (i) and (j). Displayed is a normal interphase nucleus (i) and a nucleus of one ALL relapse (j) displaying a heterozygous deletion of 12p13 (*CDKN1B*).

Supplementary Figure 2.

Probability of event-free survival (pEFS ± standard error) according to the concomitant DNA copy number losses of chromosomes 6q21 and 12p13 including *CDKN1B*.



Patients with concomitant losses of the chromosome regions 6q21 and 12p13 including *CDKN1B* showed a tendency to an inferior pEFS. The pEFS is 38% SE±17% in the group of patients with concomitant loss of 6q21 and 12p13 including *CDKN1B* compared to 72% SE±7% in the group without this combination of losses.

Supplementary Methods

Array CGH

Array CGH was performed on a submegabase resolution whole genome tiling-path bacterial artificial chromosome (BAC) DNA array consisting of the Human "32k" BAC Re-array Set (http://bacpac.chori.org/pHuman-MinSet.htm), kindly provided by Pieter de Jong, Children's Hospital Oakland Research Institute,[1, 2] the 1 Mb Sanger Set (kindly provided by Nigel Carter, Wellcome Trust Sanger Centre),[3] and a set of 380 subtelomeric clones (COST B19). CGH arrays were produced at the Max-Planck Institute for Molecular Genetics, Berlin, Germany. Detailed protocols are available at the website http://www.molgen.mpg.de/~abt_rop/molecular_cytogenetics.
In brief, leukemic cell and reference DNA were labeled with Cy3 and Cy5 (Amersham Biosciences, Piscataway, NJ), respectively, using the BioPrime Array CGH labeling kit (Invitrogen, Carlsbad, CA, USA). The array was hybridized overnight at 42°C in a Slidebooster® (Implen, Munich, Germany). Following post-hybridization washes the array was scanned with an Agilent DNA Microarray Scanner BA (Agilent Technologies, Palo Alto, CA, USA) and spot intensities were measured by GenePix Pro 5.0 software (Axon Instruments, Union City, CA, USA). Further analysis and visualization of the array CGH data was performed using CGHPRO software, developed at the Max-Planck Institute for Molecular Genetics.[4] Raw data were normalized by "Subgrid LOWESS". The log2ratio of test to reference was calculated. Copy number gains and losses were determined by a threshold of 0.3 and –0.3, respectively. Aberrant BAC clones were considered as genomic aberrations if two or more neighboring clones showed a log2ratio above or below the threshold, unless they coincided with known copy number variants as listed in the Database of Genomic Variants (http://projects.tcag.ca). For estimation of the content of segmental duplications, each BAC clone was classified into one out of seven categories and colored as described previously.[4]

REFERENCES

1. Krzywinski M, Bosdet I, Smailus D, Chiu R, Mathewson C, Wye N, et al. A set of BAC clones spanning the human genome. Nucleic Acids Res. 2004;32(12):3651-60.
2. Osoegawa K, Mammoser AG, Wu C, Frengen E, Zeng C, Catanese JJ, et al. A bacterial artificial chromosome library for sequencing the complete human genome. Genome Res. 2001;11(3):483-96.
3. Fiegler H, Carr P, Douglas EJ, Burford DC, Hunt S, Scott CE, et al. DNA microarrays for comparative genomic hybridization based on DOP-PCR amplification of BAC and PAC clones. Genes Chromosomes Cancer. 2003;36(4):361-74.
4. Chen W, Erdogan F, Ropers HH, Lenzner S, Ullmann R. CGHPRO -- a comprehensive data analysis tool for array CGH. BMC Bioinformatics. 2005;6:85.