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Research article

Computer aided analysis of additional chromosome aberrations in Philadelphia chromosome positive acute lymphoblastic leukaemia using a simplified computer readable cytogenetic notation

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Abstract

Background: The analysis of complex cytogenetic databases of distinct leukaemia entities may help to detect rare recurring chromosome aberrations, minimal common regions of gains and losses, and also hot spots of genomic rearrangements. The patterns of the karyotype alterations may provide insights into the genetic pathways of disease progression.

Results: We developed a simplified computer readable cytogenetic notation (SCCN) by which chromosome findings are normalised at a resolution of 400 bands. Lost or gained chromosomes or chromosome segments are specified in detail, and ranges of chromosome breakpoint assignments are recorded. Software modules were written to summarise the recorded chromosome changes with regard to the respective chromosome involvement. To assess the degree of karyotype alterations the ploidy levels and numbers of numerical and structural changes were recorded separately, and summarised in a complex karyotype aberration score (CKAS). The SCCN and CKAS were used to analyse the extend and the spectrum of additional chromosome aberrations in 94 patients with Philadelphia chromosome positive (Ph-positive) acute lymphoblastic leukemia (ALL) and secondary chromosome anomalies. Dosage changes of chromosomal material represented 92.1% of all additional events. Recurring regions of chromosome losses were identified. Structural rearrangements affecting (peri)centromeric chromosome regions were recorded in 24.6% of the cases.

Conclusions: SCCN and CKAS provide unifying elements between karyotypes and computer processable data formats. They proved to be useful in the investigation of additional chromosome aberrations in Ph-positive ALL, and may represent a step towards full automation of the analysis of large and complex karyotype databases.

Background

Chromosome banding analyses have become a fundamental part of the diagnostic panel in haematological neoplasias, and molecular cytogenetic methods have added to more detailed information about the karyotype changes particularly in complexly aberrant karyotypes or even in cryptic chromosome rearrangements [1,2]. Thus, the quantity of cytogenetic data in haematological malignancies has increased not only by additional cases but also by new techniques. Large cytogenetic data pools may provide the basis to identify new rarely occurring primary chromosome aberrations in distinct disease entities. Recurring involvement of the same chromosome band in several different chromosome rearrangements may be detected and, thus, point to new translocation oncogenes which may be relevant for the leukemogenesis [3]. Chromosome banding studies have helped to delineate minimal commonly lost chromosome regions and, thus, to identify tumour suppressor genes, e.g., the *CDKN2A* gene in 9p21 [2,4–7]. Minimal regions of common chromosome gains which may harbour oncogenes important for tumour genesis have as well been narrowed down by the analyses of metaphase chromosomes [8,9].

The degree of karyotype alterations may point to distinct pathways of genetic mechanisms of disease progression [10]. Patients with complex karyotype alterations have been shown to have an inferior prognosis in acute myeloid leukaemia and myelodysplastic syndromes. However, the number of chromosome rearrangements which are used to define a complexly altered karyotype in different studies range from 3–5. Moreover, the number of events which are attributable to a complex chromosome rearrangement has not been defined [11–16]. E.g., unbalanced translocations (see additional file 2: glossary.doc, for explanation), triplications, or inverted duplications may count two events [17]. Therefore, the comparison of the clinical and biological findings in this cytogenetic subgroup is hampered by the different numbers of numerical and/or structural aberrations which have been summarized to determine a complexly aberrant karyotype in the respective studies. Chromosome rearrangements are designated according to the International Standard for Human Cytogenetic Nomenclature, and in the most cases the short karyotype description is used [1,18].

The ISCN 1995 has paid special attention to the accessibility for a computer assisted readability of the karyotypes. Therefore, numerical chromosome changes of whole chromosomes or derived ones, distinct familiar chromosome rearrangements, and chromosomal breakpoints may easily be searched for by using simple text matching in cytogenetic databases which use the short system of the ISCN [1]. However, unbalanced chromosome translocations result in gains and also in losses of chromosomal material. Gain of a derivative chromosome resulting from an unbalanced translocation may lead to gain and also to loss of parts of the same chromosome band.

For instance, in the karyotype

47,XX,der(19)t(1;19)q23;p13),+der(19)t(1;19)

the gain of the derivative chromosome leads to double gain of 1q23->1qter, to loss of a part of chromosome band 19p13->19pter, and also to gain of a part of 19p13. Moreover, complex chromosome rearrangements need to be described in the detailed karyotype notation, from which gains or losses of chromosomal material may only be extracted by comparing the changes described in the single strings with the complete karyotype. Also the type of the rearrangement may be difficult to determine from the detailed karyotype description without the information of the complete karyotype. Thus, the ISCN karyotype may include metainformation about the genomic changes that is not explicitly specified in the ISCN strings and, therefore, not accessible for an automated analysis using simple text matching tools. Extensive programming would be necessary to develop a software, which is able to extract every information included in the ISCN karyotypes. In the past, Hashimoto and Kamada developed computer programs for an automated analysis of large numbers of abnormal karyotypes according to the ISCN 1978 [19,20], but never conformed them to ISCN 1995. In tumour cytogenetics, different levels of chromosome quality are common even within the same disease entity, and the maximum banding resolutions may range from 150 to 800 or even more bands per haploid set (bphs) between different cases. To get access to the metainformation contained in the ISCN karyotypes for a computerized analysis of cytogenetic findings, to join different levels of cytogenetic resolution in a common database, and to address incomplete karyotype descriptions and questionable chromosome or breakpoint assignments we developed a simplified computer readable cytogenetic notation (SCCN). By this approach, the automatic compilation and graphical representation of the chromosome alterations became feasible. Software modules were written to calculate the frequency of gains and losses of chromosome segments, and of types and breakpoint localisations of structural rearrangements. They automatically analyse the SCCN, create proof and error tables, and present the results in separated tables and graphs. To determine the degree of the alterations of a karyotype the chromosome changes were classified according to distinct aberration categories, and a complex karyotype aberration score (CKAS) was calculated. The karyotypes of 94 Ph-positive ALL patients with additional chromosome aberrations were subjected to the computerized analyses of the

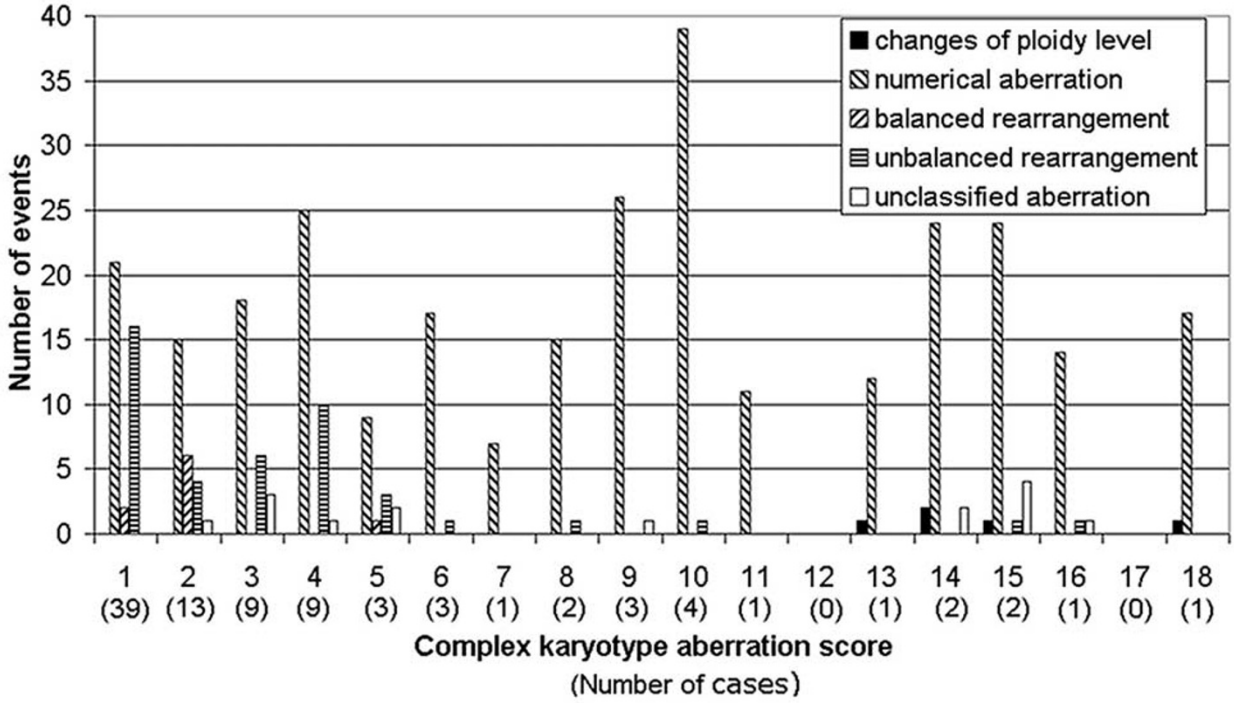

Figure 1

Distribution of the degree of karyotype alterations. Diagram showing the distribution and the number of events according to the aberration categories (columns) in 94 Ph-positive ALL patients with additional chromosome aberrations; the patients were grouped according to the complex karyotype aberration score (CKAS) which was calculated excluding the Ph-translocation.

chromosome findings and the degree of the karyotype alterations was assessed according to the CKAS.

Results

Evaluation of the degree of the karyotype alterations

A complex karyotype aberration score (CKAS) was calculated excluding the Ph-translocation (Figure 1). A total of 367 aberrations was registered. Thirty-nine cases (41.5% of total cases) scored 1, 13 (13.8%) 2, and 42 (44.7%) ≥ 3 additional events. Numerical changes were most frequent (294 events, 80.1%) with hyperdiploid cases with 47–50 chromosomes contributing 66/22.5% (number of events/percent of total numerical changes) events, high-hyperdiploid 51–57 134/45.6% events, and near triploid 67/22.8% events. Unbalanced rearrangements were next most frequent (44/12%; number of events/percent of total events), followed by unclassified aberrations (15/4.1%), balanced rearrangements (9/2.5%), and changes of ploidy level (5/1.4%). Numerical changes contributed most of the karyotype alterations to the CKAS even in the patients with <3 additional aberrations. High-hyperdiploid karyotypes 51–57 chromosomes and near triploid karyotypes (20 cases; 21,3% of total) represented 47.6% of the cases with ≥ 3 additional aberrations. Unbalanced changes contributed 83% of the structural aberration events (44/53 events) and accounted for 30.1% (20/65 events) or 7.9% (24/302 events) of the additional aberrations in the case <3 or ≥ 3 additional aberrations, respectively. Unclassified aberrations accounted for only one (1.9%) event in cases <3 additional aberrations but for 14 (5.3%) in cases ≥ 3 additional aberrations.

Evaluation of quantitative and qualitative chromosome changes

The automated analysis of the chromosome rearrangements revealed quantitative changes of all chromosomes (Figure 2 and 3). Partial gains were most frequently recorded at 9q34 (44 events) and 22q11.2 (47 events). All events at 9q34 were due to additional Ph-chromosomes (41 events in 37 cases) except an isochromosome i(9)(q10), ider(9)t(9;22) or ider(22)t(9;22) in one case each. Gains at 22q11.2 apart from extra Ph-chromo-

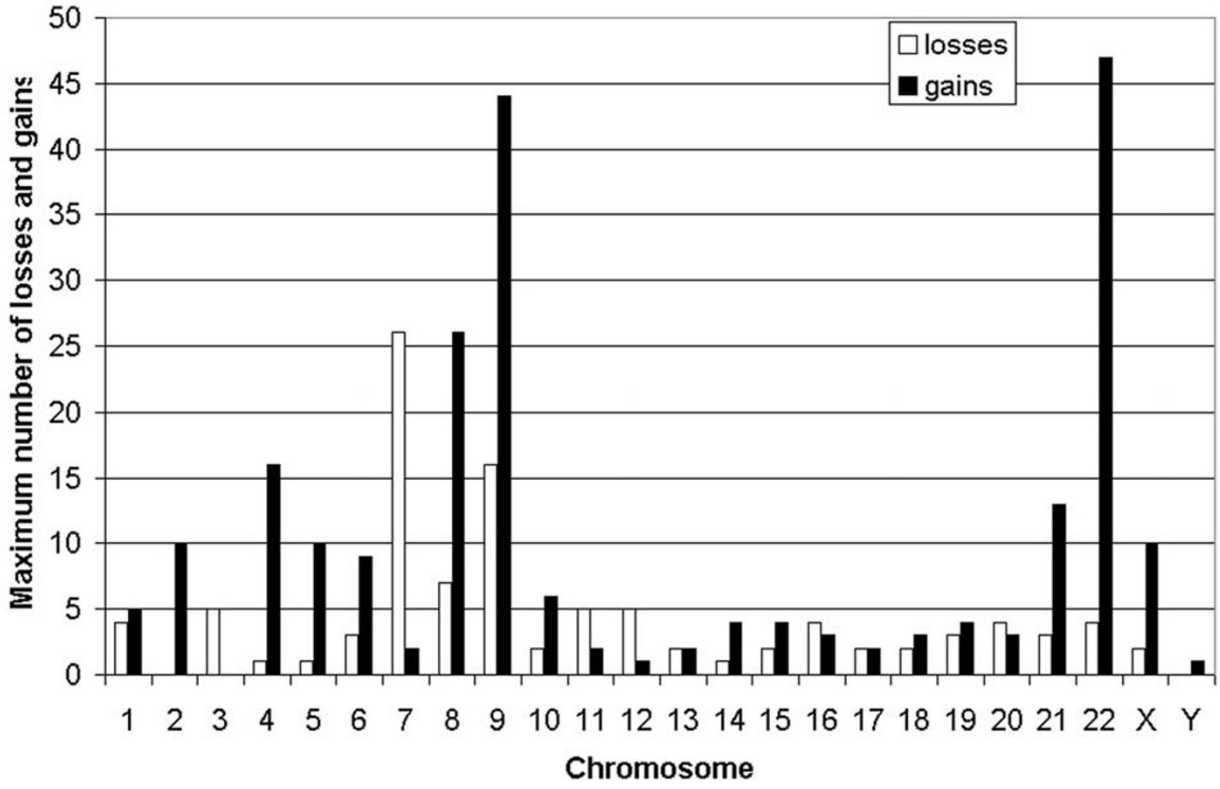

Figure 2
Maximum numbers of quantitative changes per chromosome. Graphical presentation of the results of the automated analysis of the SCCN strings of the karyotypes of 94 Ph positive ALL cases with respect to quantitative chromosome changes. Diagram showing the maximum numbers of gains and losses at each chromosome.

somes, an ider(9)t(9;22) or an ider(22)t(9;22) (1 case each) where caused by an extra chromosome 22 in 4 cases. Multiplication of chromosome 8 was detected next frequently showing a maximum of 26 events along the long arm. A homogeneous distribution of gains involving 3 or more events along the whole chromosomes was present at the chromosomes 4 (16 events), 21 (13 events), X, 2, 5 (10 events each), 6 (9 events), 10 (6 events), 14, 15, 19 (4 events each), 16, 18, and 20 (3 events each). All were due to supernumerary chromosomes which were mainly caused by high-hyperdiploid (>50, <58 chromosomes; n = 15) and near triploid (>57,<81 chromosomes; n = 5) karyotypes. Hyperdiploid cases <51 chromosomes contributed gains at entire chromosomes 4 (3 events), 5 (4 events), and 21 (3 events). Gains at chromosome 1 were increased at 1q21q44 (5 events) which apart from whole chromosome gains (3 events) were due to a duplication dup(1)(q21q44) and a derivative chromosome der(16)t(1;16)(q21q12). Losses of chromosomal material were most frequently observed at chromosome 7 and accumulated at the region 7p15p22 with a maximum of 26 events. Losses of chromosome 9 material peaked at 9p22p24 (16 events), and of chromosome 8 at 8p12p23 (7 events). An overlap of the deleted regions 6q23q27 and 6q15q23 observed in one case each became evident by a peak of loss at chromosome band 6q23 (3 events). The computerized breakpoint analysis detected only few regions with recurrent alterations (Figure 4 and 5). The chromosomal bands most frequently involved in chromosomal rearrangements were 9q34 and 22q11.1q11.2 (94 events each) due to the presence of a Philadelphia translocation in each case. No other breakpoint was recorded more frequently than twice except the pericentromeric region (defined as p11 - q11) of chromosome 8 with a maximum of 4 events at the centromere due to an isochromosome i(8)(q10) (4 cases), a dicentric translocation dic(8;9)(p11;p13), and translocations t(2;8)(p11;p11), t(8;19)(p11;q11), or t(8;21)(q11;p12) (one case each). However, pericentromeric rearrangements were also present in the chromosomes 2

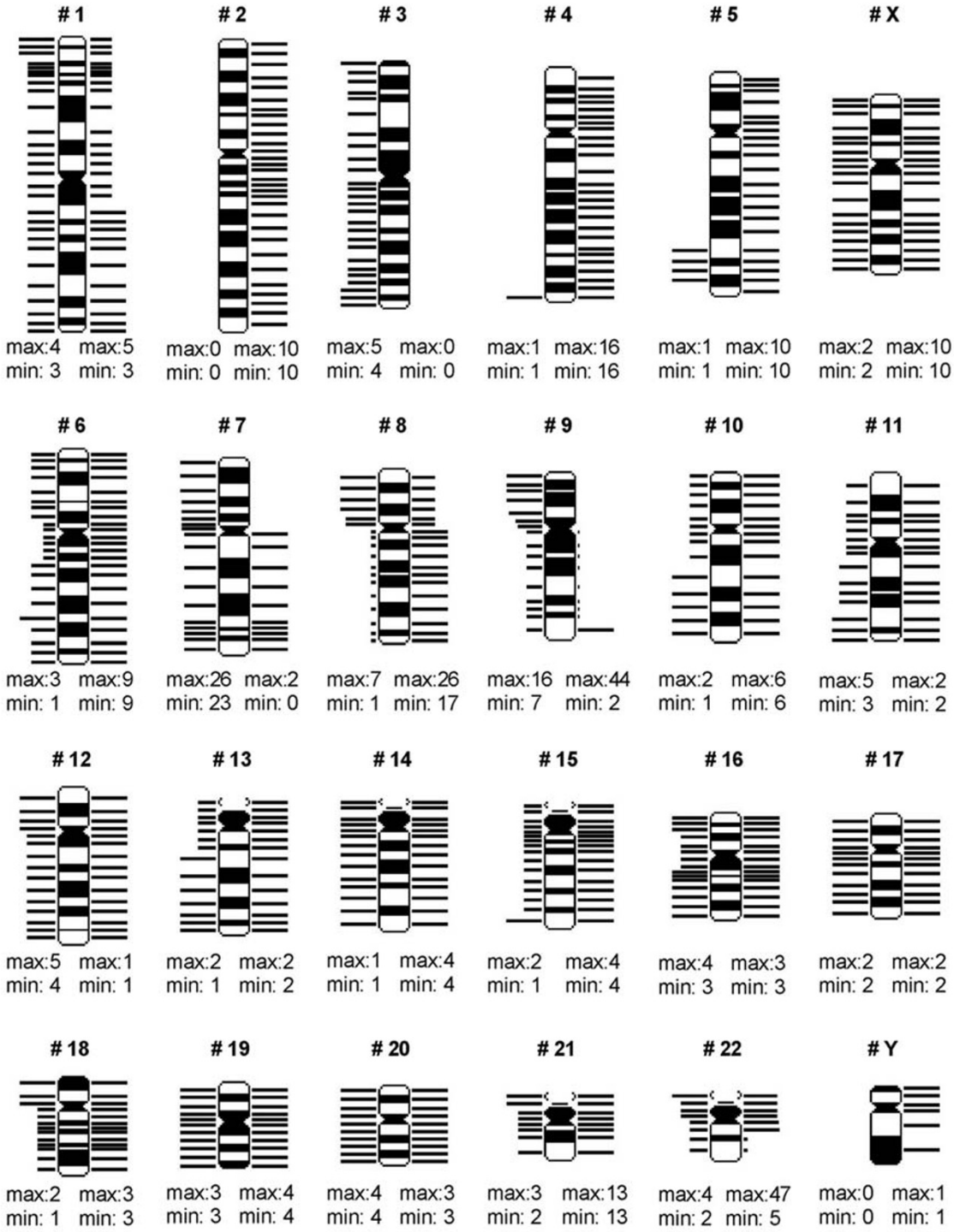

Figure 3

Distribution of the quantitative changes of each single chromosome. Graphical presentation of the results of the automated analysis of the SCCN strings of the karyotypes of 94 Ph positive ALL cases with respect to quantitative chromosome changes. The number of events leading to loss (left side) or gain (right side) of chromosomal material are depicted in bars projecting onto the (sub)bands of the respective chromosome ideogram. The lengths of the bars are relative to the maximum number of losses or gains of the respective chromosome. The minimum and maximum values are given.

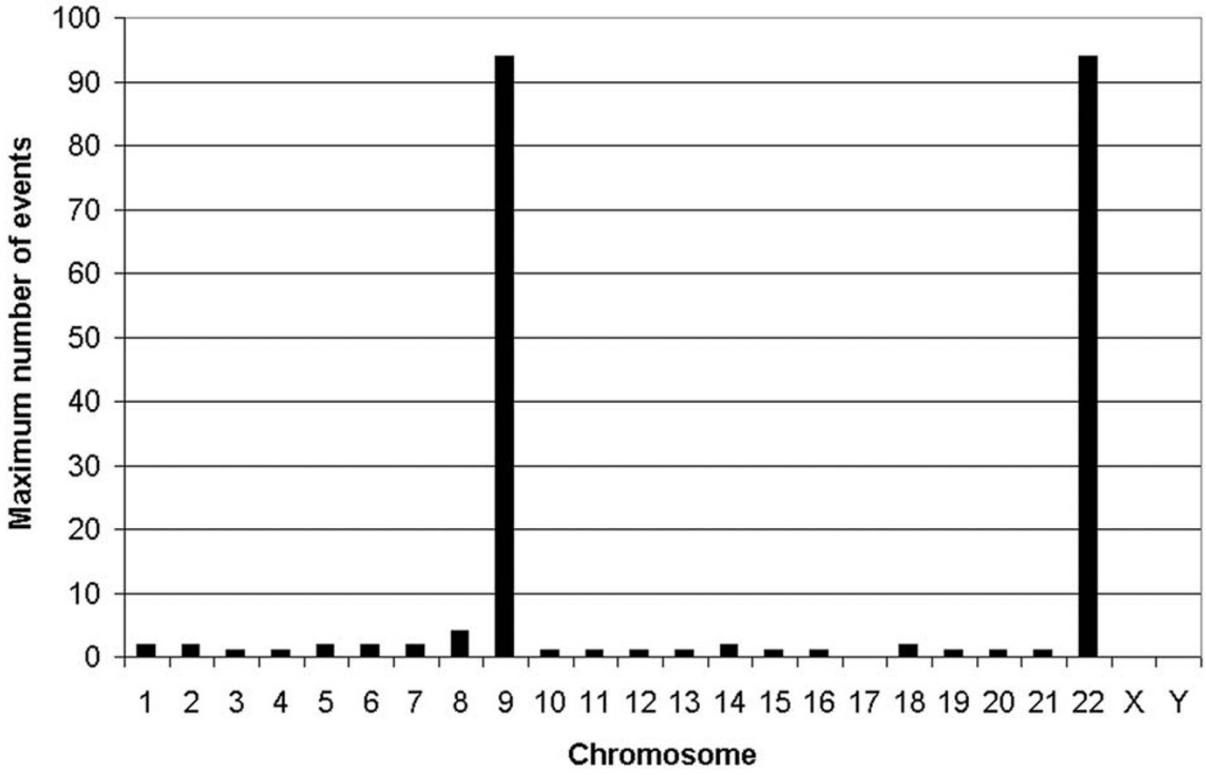

Figure 4
Maximum numbers of qualitative changes per chromosome. Graphical presentation of the results of the automated analysis of the SCCN strings of the karyotypes of 94 Ph positive ALL cases with respect to qualitative chromosome changes. Diagram showing the maximum numbers of events at each chromosome.

(t(2;8)(p11;p11) and t(2;14)(p11;q32); one case each), 7 (i(7)(q10); two cases; dic(7;12)(p11;p11), and del(7)(q11); one case each), 9 (der(9)t(9;22)del(9)(p11); two cases; i(9)(q10), ider(9)(q10), del(9)(p11), and ins(9;?)(p11;?); one case each), 12, 14, 18, 19, 20, 21 and 22 (dic(7;12)(p11;p11), t(14;18)(q11;p11), t(14;20)(q11;q13.3), dic(18;20)(p11;p11), dic(20;22)(q11;p11) and der(21)t(8;21)(q11;p12); one case each). Thus, a total of 23 (24.5%) cases showed rearrangements of pericentromeric chromosome regions which included isochromosomes in 7 (7.4%) and dicentric translocations in 5 (5.3%) cases.

Discussion

Newly developed tools for computer aided analyses were used to investigate the spectrum of additional aberrations in 94 Ph-positive ALL patients. The number of additional aberrations in a patient was determined using different aberration categories and a complex karyotype aberration score (CKAS). Single changes were recorded in only 41.5% of the cases, 13.8% had two, and 44.7% three or more events. The breakdown of the additional chromosome anomalies according to the categories of chromosome changes demonstrated that numerical aberrations contributed to 80.1% of the total events of which 68.4% resulted from chromosome gains or losses in high-hyperdiploid and near triploid karyotypes. Karyotypes >50 chromosomes have been described as additional aberrations in Ph-positive ALL [21–24]. High-hyperdiploid karyotypes in childhood ALL without Ph-translocation have been shown to result from non-disjunction of chromosomes in one single mitosis which seems to occur early in leukemogenesis [25,26]. Thus, in Ph-positive ALL chromosome gains in high-hyperdiploid ranges may also result from one single aberration event instead of multiple numerical changes. However, numerical changes and unbalanced structural rearrangements both accounted for 92.1% of all events. Therefore, by using the proposed categories of the CKAS, changes of the dosage of genetic material were demonstrated to represent the vast majority of

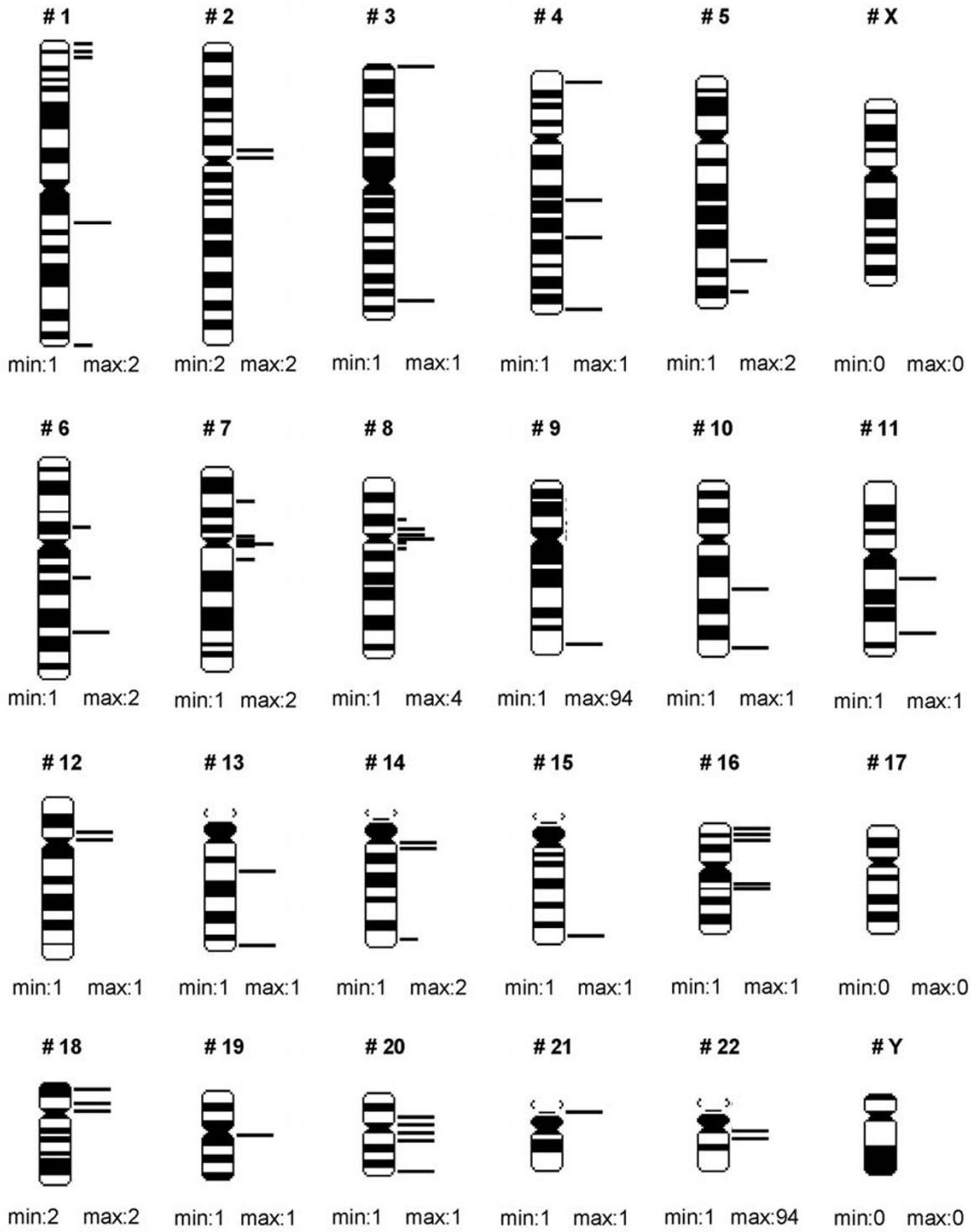

Figure 5

Distribution of the qualitative changes of each single chromosome. Graphical presentation of the results of the automated analysis of the SCCN strings of the karyotypes of 94 Ph positive ALL cases with respect to qualitative chromosome changes. The number of events recorded at a chromosome (sub)band is indicated by a bar projecting onto the respective chromosome ideogram. The lengths of the bars are relative to the maximum number of events at the respective chromosome. The minimum and maximum values are given.

the additional chromosome aberrations in Ph-positive ALL. Recurrent gains were detected at 9q34 and 22q11.2 which were mainly due to the presence of additional Ph-chromosomes which has been observed in up to 26% of Ph-positive ALL-patients [21,22,27]. However, gains of whole chromosomes 22 also contributed to the increased dosage of 22q11.2 which may point to a role of genes of chromosome 22 in addition to the BCR-ABL gene in the development of Ph-positive ALL. Gains at chromosome 1 and 8 accumulating at the entire long arm and also loss of chromosome 7, or of 6q, 8p or 9p confirmed previous findings of recurrent secondary chromosome changes in Ph-positive ALL [22,28–30].

However, losses at chromosome 7 were increased at the short arm which may indicate that 7p is the target of chromosome 7 deficiency in Ph-positive ALL. Moreover, loss at 6q peaked at 6q23. Although this was due to an overlap of the deleted segment in two cases, only, the efficiency of the analysis procedure to detect minimal commonly deleted segments from ISCN karyotypes was demonstrated. The analysis of the distribution of the altered chromosome regions revealed no recurring involvement of particular chromosome bands apart from 9q34 and 22q11. However, pericentromeric rearrangements became evident in 24.5% of the cases including isochromosomes in 7.4% and dicentric translocations in 5.3% of the patients. This suggests that pericentromeric regions are targets for chromosomal rearrangements in Ph-positive ALL. Agents such as mitomycin C, and ionising radiation, for instance, have been shown to induce breaks in the centromeric or heterochromatic parts of chromosomes 1, 9 and 16 [31,32]. Moreover, a constitutional predisposition may be causative for pericentromeric rearrangements as has been demonstrated in patients with ICF (immunodeficiency, centromeric instability and facial abnormalities) syndrome and hypomethylation of satellite II DNA due to DNA methyltransferase deficiency [33,34]. So far, it remains obscure which the causes for pericentromeric rearrangements in Ph-positive ALL may be. However, our procedure of breakpoint analysis may provide new perspectives in the unravelling of the mechanisms of the disease progression in Ph-positive ALL by pointing not only to individual chromosome bands or regions but also by providing an overview over the rearrangements of common chromosome structures of the whole karyotype.

Conclusions

The newly developed scoring system for the assessment of the degree of karyotype alterations (CKAS) and the simplified computer readable cytogenetic notation (SCCN) for karyotype findings, in our hands, appeared to be a helpful tool in the computer aided characterisation of the spectrum of chromosome alterations. The breakdown of the type of chromosome aberrations in the aberration categories of the CKAS may provide the basis for distinct definitions to determine the degree of karyotypic alterations. The SCCN in combination with respective software modules may be suitable for the automated analysis of chromosome findings from complex databases with respect to regions of recurrent chromosome gains, losses, or breakpoints of chromosome rearrangements. Therefore, we believe that the SCCN and CKAS may represent a step towards the development of a completely automated analysis of ISCN karyotypes of large cytogenetic data pools. The program modules for SCCN analyses including the graphical presentation facilities used in this study will be made available to interested readers upon request.

Methods

Patients

The cytogenetic data of 94 patients were chosen from the Leukaemia Cytogenetic Database (LCD) of the German competence net "Acute and Chronic Leukaemias" (see additional file 1: appendix.pdf, for detailed information)". In all patients, the diagnosis had been verified by morphological and immunological analyses. The database contained a case identifier, age, sex, the ISCN karyotypes, SCCN strings, the modal chromosome number, the counts of clones, and the aberration categories.

Computer Software

A commercially available database program (ACCESS 2000 Professional, Microsoft corporation, Wa) was used to program forms which guide the user through the analysing programs by menus. Modules were written using Visual Basic for Applications (VBA, Microsoft Office 2000, Microsoft corporation, Wa) to extract the respective chromosome alterations from the SCCN strings, and to compile the data for the statistical evaluation and graphical presentation of the chromosome changes.

Computerized analysis of chromosome changes

Data source containing the SCCN strings was an ACCESS 2000 database table created by a specific query, selecting data from a complexer ACCESS database. Moreover the import of all common databases, SQL- or Excel-tables was possible. The two main program units for qualitative or quantitative data analysis were editing the incoming data strings following specific algorithms belonging to the possible number of used signs and their combination. (Help-)program modules containing tables with all possible band definitions for every chromosome at a resolution of 400 bphs were stored as well as the according ideograms of all 24 chromosomes and a catalogue of all possible ISCN short terms describing the type of alterations. For the analysis of quantitative chromosome changes, gains or losses were compiled for each chromosome (sub-) band. After selection of a chromosome, the incoming data were compared with the catalogue of help-tables and

Table 1: General rules for the translation of the ISCN karyotypes into a simplified computer readable cytogenetic notation (SCCN) and respective examples.

	Designation patterns	Examples	
No	**Term[a]**	**Karyotype**	**SCCN string**
Quantitative changes			
1	Δ(A),Δ(B),	48,XY,+8,+9	+(8),+(9),
2	(A)Δ(xayb),	46,XX,del(1)(q32)	(1)-(q32q44),
3	(A)Δ(xayb),	46,XY,dup(12)(p11p13)	(12)+(p13p111),
4	(A)Δ(xayb),	46,XX,dup(2)(q11~21q35)	(2)+(q111q35),
5	(A)Δ(xa)x2,	46,XY,add(15)(q26)x2	(15)-(q26)x2,
6	(A)Δ(xa),(B)Δ(xayb),	47,XX,t(9;22)(q34;q11),+der(22)t(9;22)	(9)+(q34),(22)+(p13q112),
7	(A)Δ(xa),Δ(B), (B)Δ(xayb),	47,XY,+22,der(22)t(9;22)(q34;q11)	(9)+(q34),+(22),(22)-(q112q13),
8	(B)Δ(yaxb),	47,XX,der(22)t(9;22)(q34;q11), +der(22)t(9;22)(q34;q11)	(9)+(q34)x2,(22)-(q112q13),(22)+(p13q112),
9	(A)Δ(xayb),(A)Δ(ybxa),	46,XY,idic(16)(q22)	(16)-(q22q24),(16)+(p133q22),
Qualitative changes			
10	∇ (A)(xa),	46,XX,del(1)(q32)	del(1)(q32),
11	∇ (A)(xayb),	46,XX,add(1)(q42~43)	add(1)(q42q43),
Questionable changes			
12	+?(A),	47,XX,+?8	+?(8),
13	(A)Δ(x?ayb),	46,XX,dup(2)(q2?1q35),	(2)+(q2?1q35),

[a]Δ = plus sign (+) or minus sign (-); A, B = chromosome numbers; x, y = chromosome p-, q-arm; a, b = chromosome band or subband; ∇ = ISCN abbreviation of the type of the rearrangement

every fitting string was counted in a new result table referring to the protrusive sign. Single or multiple changes of a chromosome were counted by adding the respective number of gains or losses to each of the (sub-)bands of the respective chromosome. The distribution of the qualitative changes was analysed for each chromosome by recording and counting the involved chromosome (sub)band(s) and the respective type of the rearrangement. Generally, chromosome rearrangements, which lacked a resolution at subband level and as well ranges of possible chromosome breakpoints were recorded by adding one change to each possibly involved chromosome subband. Of each chromosome, the incoming datasets containing the karyotypes and the SCCN translation as well as the processed data containing the extracted SCCN strings and the number of events recorded in each chromosome (sub)band were stored to check the results chromosome by chromosome to identify mistakes at each level of the process. Strings which did not apply to the established SCCN as well as parts of aberration strings containing question marks were excluded from the analysis and stored in error tables for revision. For each chromosome, the results of the analyses were presented as a table with the summed up changes in the order of the respective position on the chromosome. For direct visualisation of the distribution of the alterations within one chromosome, a graph was generated that showed the number of changes of each chromosome band as bars projecting onto the bands of the respective chromosome ideogram relative to the maximum value achieved in this chromosome.

Definition of a simplified computer readable cytogenetic notation

The terms and symbols used for the simplified computer readable cytogenetic notation (SCCN) were taken from the ISCN 1995. All changes were recorded on the basis of a maximum resolution of 400 bphs. The information contained in the karyotypes of the stem line and sub clones of each case was summarized and splitted into the quantitative, e.g., gains and losses, and qualitative elements, e.g., the breakpoints and types of rearrangements of the chromosome changes. For better clearness quantitative and qualitative changes were recorded in separate fields. The manual translation of the chromosome findings into the SCCN followed predefined criteria and designation patterns (Table 1). Generally, subbands were written without period, chromosome numbers were bordered in parenthesis to address them to readability of beginning and end of a chromosome, single terms ended with a comma as terminator to enable the software to recognize the end of a phrase. Whole chromosome changes were recorded by the respective chromosome in parenthesis preceded by the + or - sign for gain or loss, respectively (Table 1, No. 1). Partial chromosome gains or losses with or without involvement of the centromere were described by the respective chromosome in parenthesis, followed by the + or - sign for gain or loss, respectively, and by the altered

Table 2: Examples for the assessment of the degree of karyotype alterations according to different categories of chromosome aberrations and calculation of a complex karyotype aberration score (CKAS).

		Number of aberrations					
No.	**Karyotype(s)**	**ploidy level**	**numerical**	**balanced**	**unbalanced**	**unclassified**	**CKAS**
1	46,XY,t(9;22)(q34;q11),t(14;20)(q11;q13.3)	0	0	2	0	0	**2**
2	46,XY,t(9;22)(q34;q11)/ 49,XY,+5,t(9;22)(q34;q11),+16,+21	0	3	1	0	0	**4**
3	46,XY,t(9;22)(q34;q11)/ 44,XY,t(9;22)(q34;q11),-8?,-11?,-11?,+mar	0	3	1	0	1	**5**
4	59,XXX,-1,-3,-7,-8,-9,t(9;22)(q34;q11),-11,-15,-16,-19,-20,-21, +der(22)t(9;22)(q34;q11)	1	12	1	0	0	**14**
5	48,XX,+5,+8,t(9;22)(q34;q11)/ 48,XX,+5,+8,del(9)(p13),t(9;22)(q34;q11)/ 49,XX,+5,+8,del(9)(p13),t(9;22)(q34;q11),+der(22)t(9;22)(q34;q11)	0	3	1	1	0	**5**
6	46,XX,t(9;22;11)(q34;q11;q13)	0	0	2	0	0	**2**

chromosome segment in parenthesis. Each change was described starting with the topmost and ending with the last chromosome band involved (Table 1, No. 2). If a breakpoint was not assigned to subbands the chromosome subband delimiting the largest chromosome segment involved was recorded (Table 1, No.3). A range of a possible chromosome breakpoint was designated by the breakpoints which delimited the largest possibly involved segment (Table 1, No. 4). Multiple presence of the same aberration was described by a multiplication sign (x), followed by the number of copies and added directly after the last parenthesis (Table 1, No. 5). Molecular and/or molecular cytogenetic information about the chromosomal breakpoints in recurring translocations was integrated in the translation of the chromosome changes into the SCCN. For instance, the BCR gene, which is rearranged by the translocation t(9;22)(q34;q11), is localized in subband 22q11.2. Thus, a duplication of the Philadelphia chromosome was recorded as gain of 9q34 and of 22p13->q11.2 (Table 1, No.6). An unbalanced translocation der(22)t(9;22)(q34;q11) was translated into gain of 9q34 and loss of the segment 22q11.2q13 (Table 1, No. 7). Accordingly, a karyotype with a duplication of an unbalanced translocation chromosome der(22)t(9;22)(q34;q11) was translated into double gain of 9q34, loss of 22q11.2q13, and gain of 22p13->q11.2 (Table 1, No. 8). By this approach, partial gain of 22q11.2 and also partial loss of 22q11.2 were taken into account. The same designation pattern was used for the description of isodicentric chromosomes which resulted in partial gain and partial loss of the chromosome band in which the break occurred (Table 1, No. 9). Each chromosome change instead of net gains and losses was recorded to uncover a partial monosomy of a chromosome region despite duplication of the respective normal chromosome or respective chromosome segments (Table 1, No. 7). Qualitative chromosome changes were recorded starting with the respective ISCN (1995) abbreviation followed by the involved chromosome in parenthesis and the chromosomal region, band or subband in parenthesis (Table 1, No. 10). Chromosome breakpoints were noted according to the level of banding resolution. A range of a chromosome breakpoint was described by the first and the last possibly involved chromosome band or subband (Table 1, No. 11). Questionable identification of a chromosome or a of chromosome structure was indicated by a preceding question mark (Table 1, Nos. 12 and 13). No blanks were allowed in the strings. Each phrase between commas represented one aberration event.

Definition of aberration categories for the assessment of the degree of karyotype alterations

To measure the degree of the karyotype alterations the chromosome changes were recorded according to distinct aberration categories, i.e., the number of changes of the ploidy level, of balanced and of unbalanced aberrations, of numerical anomalies, and of unclassified aberrations. The number of events was recorded and stored separately for each category (Table 2). Balanced rearrangements included reciprocal translocations, insertions, and inversions. For each single event one point was counted (Table 2, No. 1). For each numerical alteration, e.g., gain or loss of a whole chromosome, or gain of derivative chromosome, one point was counted (Table 2, No. 2 – 5). Marker chromosomes were scored as unclassified aberrations and counted as one point for every marker chromosome (Table 2, No. 3). Each gain or loss of a haploid chromosome set counted also as one event (Table 2, No. 4). Unbalanced rearrangements included duplications, deletions, unbalanced translocations, additions of chromosomal

material, isochromosomes, dicentric and isodicentric chromosomes. One point was counted for each event which resulted in an unbalanced change (Table 1, No. 5). If two chromosomes were involved into a balanced or unbalanced rearrangement, one event was counted. If three or more chromosomes were involved, two or more events were recorded (Table 2, No. 6). If the same aberration was found in more than one clone it was counted only once. The complex karyotype aberration score (CKAS) was generated for every case by the sum of all events recorded in the different aberration categories. For present analysis a complex karyotype aberration score (CKAS) was calculated excluding the Ph-translocation

Authors' contributions

H.R. carried out the project design and coordination, the development of the SCCN and CKAS, the provision of cytogenetic data, the validation of results and assisted in manuscript preparation; J.B. carried out the development of the SCCN and CKAS, generation of the results, preparation of the manuscript; H.B. carried out the programming of the database and software modules; C.F., B.H., A.J., C.S. provided cytogenetic findings and assisted in manuscript preparation.

All authors read and approved the final manuscript.

Additional material

Additional File 2

Glossary of cytogenetic terms. List of cytogenetic terms and respective explanation.
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2105-4-4-S2.doc]

Additional File 1

Appendix. ISCN (1995) karyotypes and corresponding SCCN of all 94 investigated ALL patients.
Click here for file
[http://www.biomedcentral.com/content/supplementary/1471-2105-4-4-S1.pdf]

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