A pooled analysis of karyotypic patterns, breakpoints and imbalances in 783 cytogenetically abnormal multiple myelomas reveals frequently involved chromosome segments as well as significant age- and sex-related differences.

Nilsson, Therese; Höglund, Mattias; Lenhoff, Stig; Rylander, Lars; Turesson, Ingemar; Westin, Jan; Mitelman, Felix; Johansson, Bertil

Published in:
British Journal of Haematology

DOI:
10.1046/j.1365-2141.2003.04221.x

2003

British Journal of Haematology, 2003, **120**, 960–969

A pooled analysis of karyotypic patterns, breakpoints and imbalances in 783 cytogenetically abnormal multiple myelomas reveals frequently involved chromosome segments as well as significant age- and sex-related differences

Thérèse Nilsson,[1] Mattias Höglund,[1] Stig Lenhoff,[2] Lars Rylander,[3] Ingemar Turesson,[4] Jan Westin,[2] Felix Mitelman[1] and Bertil Johansson[1] [1]*Department of Clinical Genetics,* [2]*Department of Haematology, and* [3]*Department of Occupational and Environmental Medicine, Lund University Hospital, and* [4]*Department of Medicine, Section of Haematology and Coagulation, Malmö University Hospital, Sweden*

Received 15 September 2002; accepted for publication 21 November 2002

Summary. The cytogenetic features (ploidy, complexity, breakpoints, imbalances) were ascertained in 783 abnormal multiple myeloma (MM) cases to identify frequently involved chromosomal regions as well as a possible impact of age/sex. The series included MM patients from the Mitelman Database of Chromosome Aberrations in Cancer and from our own laboratory. Hyperdiploidy was most common, followed by hypodiploidy, pseudodiploidy and tri-/tetraploidy. Most cases were complex, with a median of eight changes per patient. The distribution of modal numbers differed between younger and older patients, but was not related to sex. No sex- or age-related differences regarding the number of anomalies were found. The most frequent genomic breakpoints were 14q32, 11q13, 1q10, 8q24, 1p11, 1q21, 22q11, 1p13, 1q11, 19q13, 1p22, 6q21 and 17p11. Breaks in 1p13, 6q21 and 11q13 were more common in the younger age group. The most frequent imbalances were + 9, – 13, + 15, + 19, + 11 and – Y. Trisomy 11 and monosomy 16 were more common among men, while –X was more frequent among women. Loss of Y as the sole change and + 5 were more common in elderly patients, and – 14 was more frequent in the younger age group. The present findings strongly suggest that some karyotypic features of MM are influenced by endogenous and/or exogenous factors.

Keywords: myeloma, chromosomes, karyotype, age, sex.

Cytogenetic investigations of haematological malignancies, particularly acute lymphoblastic leukaemia (ALL), acute myeloid leukaemia (AML) and the myelodysplastic syndromes (MDS), have resulted in a wealth of information of both biological and clinical importance. For example, numerous specific chromosomal abnormalities are now known to correlate closely with several clinical characteristics, such as morphology, immunophenotype and prognosis (Greenberg *et al*, 1997; Grimwade *et al*, 1998; Harrison, 2001), and the identification of leukaemia-associated translocations and inversions has paved the way for the detection and characterization of molecular genetic events that are closely associated with the leukaemogenic process (Look, 1997; Rabbitts, 2001). Furthermore, the cytogenetic features of ALL, AML and MDS have been shown to vary in relation to age, sex, occupational and iatrogenic exposure, and geographical origin, indicating that the development of chromosomal aberrations is influenced by constitutional as well as exogenous factors (Johansson *et al*, 1991; Mertens *et al*, 1993; Pedersen-Bjergaard & Rowley, 1994; Mauritzson *et al*, 1999, 2002; Albin *et al*, 2000; Björk *et al*, 2000, 2001).

Less is known about the karyotypic patterns of multiple myeloma (MM), despite the fact that the incidence of MM is quite similar to that of acute leukaemias (The National Board of Health and Welfare, 2001; Mitelman *et al*, 2003). The reasons for the relative lack of published cytogenetically abnormal MM patients include low mitotic index and poor chromosome morphology (Nilsson *et al*, 2002). In addition, the cytogenetic complexity of MM is, at least compared with ALL, AML and MDS, quite pronounced, which, in the past,

Correspondence: Thérèse Nilsson, Department of Clinical Genetics, University Hospital, SE-221 85 Lund, Sweden. E-mail: therese.nilsson@klingen.lu.se

has hampered the identification of biologically and clinically important chromosomal abnormalities. However, during the last few years, some cytogenetic features have been shown to be associated with certain clinical parameters in MM. For example, loss of chromosome 13 material and hypodiploidy seem to confer a worsened prognosis (Tricot *et al*, 1995; Desikan *et al,* 2000; Smadja *et al,* 2001), and t(11;14)(q13;q32)-positive MM patients are characterized by a lymphoplasmacytic morphology (Fonseca *et al*, 1998; Hoyer *et al,* 2000). Furthermore, molecular genetic investigations have clearly shown that illegitimate recombinations of the immunoglobulin heavy chain (*IGH*) gene, at chromosome band 14q32, occur in the vast majority of MM, resulting in dysregulation of the target genes (Kuehl & Bergsagel, 2002). However, very little is known about the molecular genetic consequences of other chromosomal anomalies in MM. To some extent, this can be explained by the lack of a detailed and genome-wide cytogenetic map of MM that could form the basis for further molecular genetic analyses.

In the present study, karyotypically abnormal MM, including patients from the Mitelman Database of Chromosome Aberrations in Cancer (Mitelman *et al,* 2003) and from our own laboratory, were collected and reviewed cytogenetically. The aim was to construct genomic breakpoint and imbalance maps, delineating regions likely to harbour genes of pathogenetic importance. In addition, a pooled statistical analysis evaluating the possible impact of age and sex on the karyotypic features was performed.

MATERIALS AND METHODS

Patients. The study was based on all cytogenetically abnormal MM analysed in our laboratory between 1978 and 2000; the vast majority were obtained after 1995. Clinical and karyotypic data on all but a few of these patients have been reported previously (Nilsson *et al*, 2002). In addition, the Mitelman Database of Chromosome Aberrations in Cancer (Mitelman *et al*, 2003) was used to ascertain previously published MM with chromosomal aberrations (the search included all patients reported until 2000). Information on sex and age was collected whenever available.

Cytogenetic classification. All patients were subgrouped in relation to ploidy levels, in accordance with the International System for Human Cytogenetic Nomenclature (ISCN, 1995), as follows: hypodiploidy (35–45 chromosomes), pseudodiploidy (46 chromosomes), hyperdiploidy (47–57 chromosomes) and tri-/tetraploidy (58–103 chromosomes). Patients containing clones of different ploidy levels were grouped according to the lowest modal number. The MM patients were also subdivided according to number of anomalies, i.e. one or two abnormalities or complex karyotypes (more than two aberrations). Patients with clones harbouring different numbers of aberrations were grouped in relation to the most simple clone present, and patients who were incompletely karyotyped, i.e. containing 'inc' in the karyotype, were included in the complex group. In the cytogenetic classification and in the breakpoint and imbalance maps (see below), constitutional chromosomal abnormalities were disregarded.

Genomic breakpoint map. For the breakpoint (bp) map, if more than one copy of the same chromosome aberration was found in the same or related clone, the bp involved was plotted only once and, if the same bp was involved in different aberrations, it was plotted once per aberration. Furthermore, rearrangements with an uncertain bp localization, e.g. add(14)(q?32) or del(6)(q13–16), were not included.

Genomic imbalance map. The imbalances were ascertained according to the following criteria: (1) the net results of the aberrations were always registered with regard to the nearest ploidy level; (2) when additional chromosomal changes were acquired during clonal evolution, only novel abnormalities were included, i.e. if the same imbalance was found in more than one related clone, it was recorded only once; (3) when the same chromosome was involved in several aberrations, only the total net imbalances were plotted; and (4) in cases in which a particular chromosome segment was involved in different types of imbalances, only the largest imbalance was registered. Clones with loss of chromosome Y as the sole anomaly were not included in the imbalance map.

Statistical investigations. For comparing independent groups of observation by significance testing, i.e. cytogenetic features in relation to age and sex, we used the chi-square test. Two age groups were defined for the analyses: ≤ 61 years and ≥ 62 years. The *P*-values reported are two-sided and $P < 0{\cdot}05$ was considered to be significant. In an attempt to focus on pathogenetically important changes, only genomic breakpoints and imbalances found in more than 5% and 10%, respectively, of the MM patients were included in the statistical analyses.

RESULTS

A total of 783 cytogenetically abnormal MM cases were retrieved, including 40 patients from our own laboratory and 743 patients from the database. The series comprised 442 men and 341 women, with a median age of 61·5 years (information on age was lacking in 334 patients). The most common modal number was hyperdiploidy (308/783; 39%), followed by hypodiploidy (212/783; 27%), pseudodiploidy (188/783; 24%) and tri-/tetraploidy (75/783; 10%) (Table I). Most patients (567/783; 72%) were karyotypically complex, whereas one or two abnormalities were found in 170 (22%) and 46 (6%) MM patients respectively. The median number of chromosomal abnormalities per MM patient was eight (Fig 1). More than one cytogenetically abnormal clone was identified in 71 patients (9%), of whom 19 (27%) had unrelated clones and 52 (73%) displayed a clonal evolution.

Among the 170 MM patients with sole chromosomal aberrations, 55 (32%) had numerical anomalies, most frequently loss of the Y chromosome (26 patients), and monosomies X, 7, 18 and 21 (three patients each), while 115 (68%) displayed structural rearrangements, with t(11;14)(q13;q32) (12 patients), add(14)(q32) (seven

Table I. Cytogenetic features of 783 multiple myeloma patients in relation to sex and age.

	Sex			Age†		
Cytogenetic features	Male $n = 442$ (%)	Female $n = 341$ (%)	*P*-value*	≤ 61 years $n = 216$ (%)	≥ 62 years $n = 233$ (%)	*P*-value*
Ploidy levels						
hypodiploid	117 (26·5)	95 (27·9)		59 (27·3)	71 (30·5)	
pseudodiploid	102 (23·1)	86 (25·2)	0·59	66 (30·6)	56 (24·0)	**0·02**
hyperdiploid	183 (41·4)	125 (36·7)		62 (28·7)	94 (40·3)	
tri-/tetraploid	40 (9·0)	35 (10·3)		29 (13·4)	12 (5·2)	
Number of anomalies						
1 anomaly	98 (22·2)	72 (21·1)		43 (19·9)	67 (28·8)	
2 anomalies	26 (5·9)	20 (5·9)	0·93	15 (6·9)	11 (4·7)	0·07
≥ 3 anomalies	318 (71·9)	249 (73·0)		158 (73·1)	155 (66·5)	
Genomic breakpoints						
1p22	24 (5·4)	16 (4·7)	0·74	15 (6·9)	10 (4·3)	0·30
1p13	25 (5·7)	22 (6·5)	0·65	22 (10·2)	10 (4·3)	**0·02**
1p11	36 (8·1)	25 (7·3)	0·69	15 (6·9)	23 (9·9)	0·31
1q10	54 (12·2)	56 (16·4)	0·10	25 (11·6)	17 (7·3)	0·14
1q11	26 (5·9)	24 (7·0)	0·56	18 (8·3)	14 (6·0)	0·36
1q21	29 (6·6)	23 (6·7)	1·00	20 (9·2)	12 (5·2)	0·10
6q21	29 (6·6)	14 (4·1)	0·16	18 (8·3)	7 (3·0)	**0·02**
8q24	50 (11·3)	25 (7·3)	0·07	14 (6·5)	23 (9·9)	0·23
11q13	77 (17·4)	52 (15·2)	0·44	63 (29·2)	39 (16·7)	**0·002**
t(11;14)‡	40 (9·0)	28 (8·2)	0·70	35 (16·2)	24 (10·3)	0·07
14q32	133 (30·1)	91 (26·7)	0·30	79 (36·6)	66 (28·3)	0·07
17p11	21 (4·8)	19 (5·6)	0·63	14 (6·5)	7 (3·0)	0·12
19q13	27 (6·1)	17 (5·0)	0·53	15 (6·9)	9 (3·9)	0·21
22q11	33 (7·5)	20 (5·9)	0·39	13 (6·0)	24 (10·3)	0·12
Genomic imbalances						
del(1)(p21–22)	72 (16·3)	61 (17·9)	0·57	44 (20·4)	34 (14·6)	0·13
dup(1)(q10q44)	57 (12·9)	48 (14·1)	0·67	26 (12·0)	22 (9·4)	0·45
+ 3	85 (19·2)	58 (17·0)	0·46	36 (16·7)	44 (18·9)	0·62
+ 5	91 (20·6)	65 (19·1)	0·65	29 (13·4)	55 (23·6)	**0·008**
del(6)(q21–27)	57 (12·9)	31 (9·1)	0·11	27 (12·5)	21 (9·0)	0·29
+ 7	81 (18·3)	64 (18·8)	0·93	32 (14·8)	42 (18·0)	0·38
+ 9	125 (28·3)	87 (25·5)	0·42	51 (23·6)	63 (27·0)	0·45
+ 11	109 (24·7)	51 (15·0)	**< 0·001**	40 (18·5)	56 (24·0)	0·17
− 13	114 (25·8)	96 (28·2)	0·47	59 (27·3)	50 (21·5)	0·15
− 14	58 (13·1)	36 (10·6)	0·32	27 (12·5)	14 (6·0)	**0·02**
+ 15	113 (25·6)	77 (22·6)	0·36	40 (18·5)	50 (21·5)	0·48
− 16	71 (16·1)	35 (10·3)	**0·02**	29 (13·4)	29 (12·4)	0·78
+ 18	40 (9·0)	39 (11·4)	0·28	17 (7·9)	26 (11·2)	0·26
+ 19	105 (23·8)	66 (19·4)	0·16	38 (17·6)	55 (23·6)	0·13
+ 21	69 (15·6)	52 (15·2)	0·84	27 (12·5)	43 (18·5)	0·09
–X	18 (4·1)	113 (33·1)	**< 0·001**	37 (17·1)	28 (12·0)	0·14
–Y	89 (20·1)§	–	–	18 (14·0)§	30 (23·3)§	0·05
–Y (sole)	26 (5·9)§	–	–	1 (0·8)§	17 (13·2)§	**< 0·001**

*Significant P-values are given in bold type.
†Information on age was lacking in 334 of the patients.
‡The t(11;14)(q13;q32) represents a subgroup of the patients with breakpoints in 11q13.
§Based on men only.

patients), del(1)(p11p22) (four patients), and add(2)(p25), del(11)(q13), del(16)(q22), del(17)(p11) and add(17)(p?) (three patients each) being most common.

The cytogenetic features, i.e. ploidy levels, number of anomalies, and the most frequent genomic breakpoints and imbalances, of the 783 MM patients in relation to sex and age are given in Table I. The modal numbers did not vary in relation to sex, whereas their distribution differed between the two age groups ($P = 0·02$) with, in particular, hyperdiploidy being more common in elderly patients. No sex- or age-related differences as regards number of anomalies were discerned.

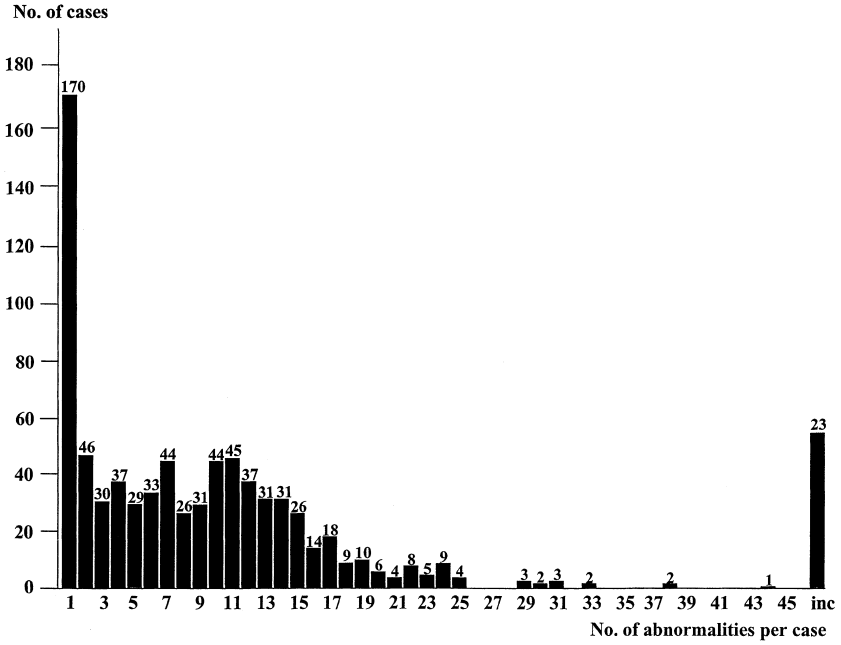

Fig 1. The distribution of the number of chromosomal abnormalities per patient among 783 cytogenetically abnormal MM. The bar denoted 'inc' represents 23 patients with an unknown number of aberrations, i.e. incomplete karyotypes.

A total of 3176 genomic breakpoints were mapped in the 783 MM patients (Fig 2). The most frequently affected chromosome bands were 14q32 (29%), 11q13 (16%), 1q10 (14%), 8q24 (10%), 1p11 (8%), 1q21 (7%), 22q11 (7%), 1p13 (6%), 1q11 (6%), 19q13 (6%), 1p22 (5%), 6q21 (5%) and 17p11 (5%). There were no frequency differences between men and women, but breaks in 1p13, 6q21 and 11q13 were more common in younger patients ($P = 0{\cdot}02$, 0·02 and 0·002 respectively; Table I).

The genomic imbalances identified are depicted in Fig 3, the most common of which were +9 (27%), −13 (27%), +15 (24%), +19 (22%), + 11 (20%) and –Y (20% of 442 men). Both sex- and age-related differences were noted (Table I). Trisomy 11 and monosomy 16 were more common among men ($P < 0{\cdot}001$ and $P = 0{\cdot}02$ respectively), –X was more frequent among women ($P < 0{\cdot}001$), –Y as the sole change and + 5 were more common in elderly patients ($P < 0{\cdot}001$ and $P = 0{\cdot}008$ respectively), and −14 was more frequent in the younger age group ($P = 0{\cdot}02$).

DISCUSSION

Because of the heterogeneous nature of the ascertained MM patients, including samples analysed in various laboratories during different time periods and by different chromosomal banding techniques, karyotypic errors undoubtedly exist in the reviewed material. In order to minimize the effects of these, abnormalities with questionable breakpoint mapping were excluded. Furthermore, only breakpoints and imbalances reported in more than 5% and 10% of the patients, respectively, were included in the statistical analyses. By thus decreasing the impact of cytogenetic uncertainties/mistakes and karyotypic noise, we believe that the abnormalities and abnormality patterns delineated in the present study (Figs 1–3, Table I) should be representative for MM.

The most common modal number was hyperdiploidy, followed by hypodiploidy, pseudodiploidy and tri-/tetraploidy (Table I). This distribution of ploidy levels differed from those seen in monoclonal gammopathy of undetermined significance (MGUS) and in plasma cell leukaemias (PCL) – the vast majority of MGUS with cytogenetic abnormalities reported to date have been pseudodiploid and almost 50% of PCL are hypodiploid, with the remaining being pseudodiploid or hyperdiploid in equal frequencies – but is quite similar to the one observed in Waldenström's macroglobulinaemia (WM), which is hyperdiploid, pseudodiploid or hypodiploid in approximately one third of the patients respectively (Palka *et al*, 1987; Calasanz *et al*, 1997; Avet-Loiseau *et al,* 2001; Mitelman *et al,* 2003). Also the cytogenetic complexity seems to vary among the different plasma cell dyscrasias. The vast majority of PCL and MM are karyotypically complex, with half of the MM patients harbouring more than eight chromosomal aberrations (Fig 1), whereas most MGUS and WM patients have only one or two abnormalities (Mitelman *et al,* 2003). Compared with other haematological malignancies, the karyotypic pattern of MM is similar to those found in follicular lymphomas and diffuse large B-cell lymphomas, but quite different from the ones seen in, for example, B-cell chronic lymphocytic leukaemia and myeloid malignancies, such as AML and MDS (Mauritzson *et al,* 2002; Mitelman *et al,* 2003). Taken together, the basic cytogenetic features, i.e. ploidy levels and degree of complexity, of MM compare well with those characterizing PCL, WM and lymphomas.

Among the 3176 genomic breakpoints in MM ascertained herein, more than a third of them clustered to 1p22, 1p13, 1p11, 1q10, 1q11, 1q21, 6q21, 8q24, 11q13, 14q32, 17p11, 19q13 and 22q11 (Table I and Fig 2). It should be stressed that, in this context, the vast majority of the reviewed karyotypes were based on G-banding alone

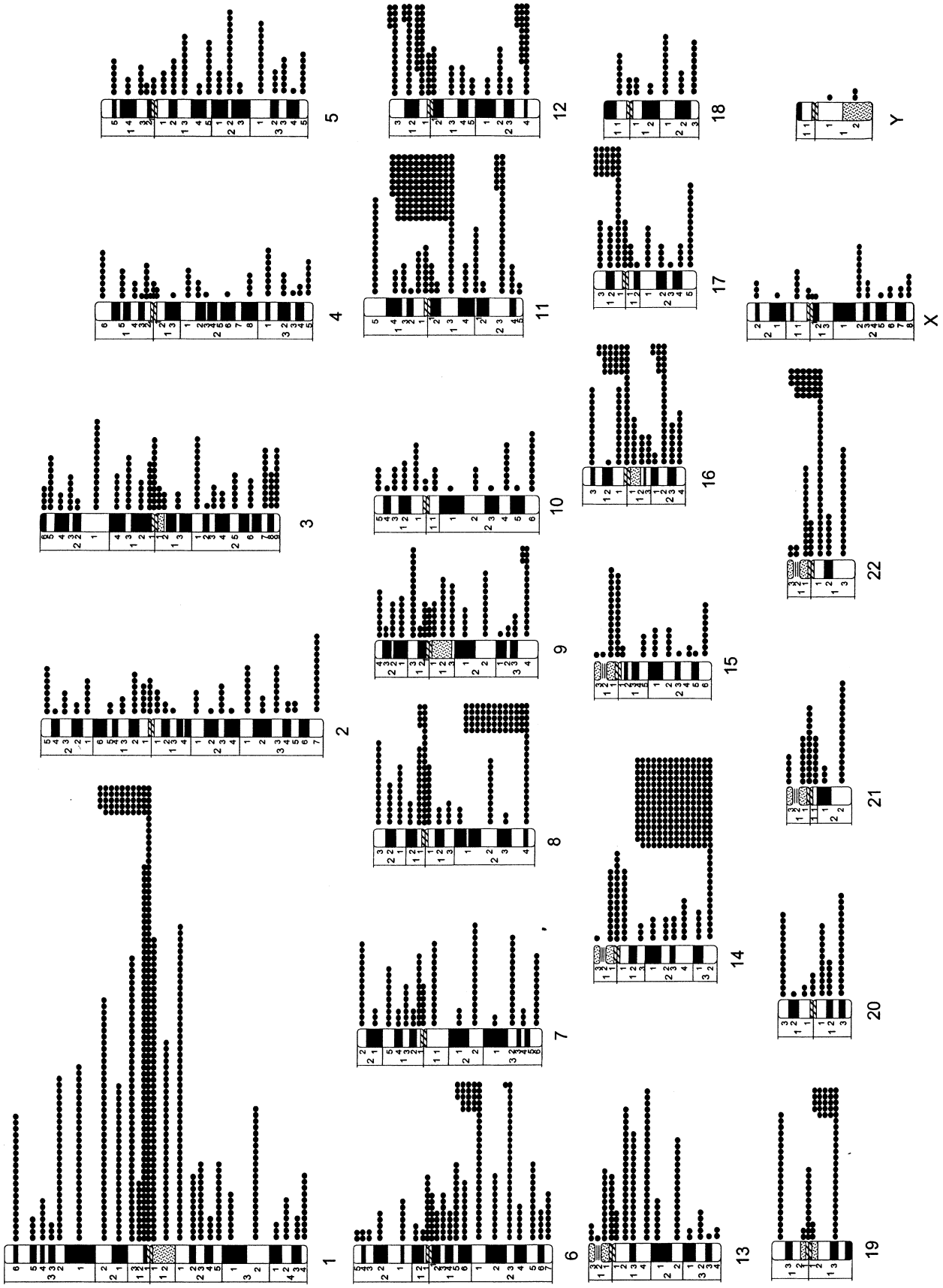

Fig 2. Breakpoint map of 783 cytogenetically abnormal MM. Each black dot represents a breakpoint (plotted once per aberration and case).

and that cryptic translocations, identifiable only by fluorescence *in situ* hybridization (FISH) or pure molecular genetic techniques, generally were not included. Hence, in the present breakpoint map (Fig 2), there is an under-representation of breaks in, e.g. 4p16 and 16q23, known to be affected by the common MM-associated t(4;14)(p16;q32) and t(14;16)(q32;q23) (Kuehl & Bergsagel, 2002). Furthermore, although 3176 breakpoints is an impressive figure, it is probably an underestimation, considering that recent multicolour spectral karyotyping (SKY) analyses of MM have identified several novel recurring sites of breakage that were not identifiable by conventional chromosome banding (Rao *et al*, 1998; Sawyer *et al*, 1998a, 2001; Ng *et al,* 2001). Despite this, the frequently involved chromosome bands identified in this study (Fig 2) probably harbour genes of importance in the development and/or progression of MM. However, apart from the known involvement of the *MYC*, *CCND1*, *IGH* and *IGL* genes, as a result of breaks in 8q24, 11q13, 14q32 and 22q11, respectively (Kuehl & Bergsagel, 2002), the molecular genetic consequences of these rearrangements remain to be elucidated. Although structural aberrations of genes located in the breakpoint regions are a probable result, one cannot exclude the possibility that genomic imbalances are the important outcome. For example, breaks, such as whole-arm translocations and jumping translocations (Keung *et al*, 1998; Sawyer *et al*, 1998b), in the pericentromeric region of chromosome 1 (Fig 2) have been suggested to lead to gene dosage effects rather than to structural aberrations of single genes (Le Baccon *et al,* 2001). In fact, most of the observed breakpoints were involved in unbalanced rearrangements, resulting in loss or gain of genetic material.

The most frequent, i.e. > 10%, genomic imbalances in MM were trisomies and monosomies, in particular + 9, + 11, – 13, + 15, + 19 and –Y, losses of 1p and 6q, and gain of 1q (Table I). Although some, or even several, abnormalities may have escaped detection by conventional chromosome banding analyses – a large proportion (roughly 20%) of MM patients had multiple marker chromosomes – a high incidence of the above-mentioned gains and losses has also been detected by comparative genomic hybridization analyses (Avet-Loiseau *et al*, 1997; Cigudosa *et al*, 1998; Gutiérrez *et al,* 2001). Thus, the present genomic imbalance map of MM (Fig 3) should be valid. However, SKY analyses have revealed that some of the imbalances, mainly those leading to partial losses, are generated through a different mechanism than suggested by G-banding alone. For example, many abnormalities identified as deletions are, in fact, derivative chromosomes of unbalanced translocations (Rao *et al*, 1998; Sawyer *et al*, 1998a, 2001). Because partial gains or losses of chromosomes that often are trisomic or monosomic are notably rare (Fig 3), with the exception of dup(11q) and del(13q), the pathogenetically important effect of these numerical changes is probably not reducible to minimally duplicated or deleted segments, and hence not to the altered expression of only a few genes. Considering that a recent microarray-based gene expression analysis of AML patients with trisomy 8 as a sole anomaly showed that numerous genes on this chromosome were overexpressed (Virtaneva *et al,* 2001), it is also reasonable to suggest a similar gene–dosage effect of the trisomies in MM. However, identification of the relevant genes will be an arduous task, although recently reported (Shaughnessy *et al,* 2001; Claudio *et al,* 2002; Zhan *et al,* 2002) and ongoing microarray analyses of MM may prove fruitful in this respect.

Significant age- and sex-related differences in karyotypic patterns have previously been identified in several haematological malignancies. Well-known examples of the impact of age include: the higher frequencies of t(4;11) in infant ALL; t(8;21), t(15;17) and inv(16) in younger AML age groups; and deletions involving chromosomes 5 and 7 in elderly AML patients (Johansson *et al*, 1998; Mauritzson *et al*, 1999; Moorman *et al,* 2001). The influence of sex is generally less pronounced (Mertens *et al*, 1993). However, there is a preponderance of women with the '5q- syndrome' and with chronic myeloproliferative syndromes with trisomy 8 as the sole change, whereas the der(1;7)(q10;p10) in myeloid malignancies, in particular MDS, is more common in men (Pedersen, 1992; Boultwood *et al*, 1994; Paulsson *et al,* 2001; Mitelman *et al,* 2003). In MM, on the other hand, most studies addressing this issue have only compared the frequencies of normal and abnormal karyotypes, without detecting any significant age- or sex-related differences (Gould *et al,* 1988; Weh *et al*, 1993; Cigudosa *et al*, 1994; Calasanz *et al*, 1997; Nilsson *et al,* 2002). Only a few investigations have focused on specific chromosomal aberrations. Malgeri *et al* (2000) found no significant correlations between the presence of t(4;14)(p16;q32) and age and sex, whereas t(8;22)(q24;q11) is more common among men (Yamamoto *et al*, 1998; Mitelman *et al,* 2003). The available data are somewhat conflicting with regard to the t(11;14)(q13;q32). There were no apparent age- or sex-related differences among the t(11;14)-positive MM patients reviewed by Laï *et al* (1998), whereas Fonseca *et al* (2002a) reported that patients below the age of 40 years appeared ($P = 0{\cdot}08$) to be more likely to have t(11;14). In the present study (Table I), the frequencies of t(11;14) did not differ between men and women, but seemed to be higher in the younger age group (16% *vs* 10%, $P = 0{\cdot}07$). Although this association was not significant, a skewed age distribution of MM patients with all types of abnormalities involving 11q13 was found, with 11q13 rearrangements clearly being less common ($P = 0{\cdot}002$) in elderly patients (Table I). Studies of – 13/13q– have also yielded contradictory results. Zojer *et al* (2000) and the present study found no significant associations with age or sex (Table I), whereas Facon *et al* (2001) and Fonseca *et al* (2002b) reported higher frequencies of such aberrations in women. Facon *et al* (2001) also detected a significant association with age: patients with losses of chromosome 13 material were significantly older. Finally, our pooled analysis revealed additional changes displaying age- or sex-related frequency heterogeneity (Table I). For example, + 5 was more common in the older

Fig 3. Imbalance map of 783 cytogenetically abnormal MM, showing losses to the left and gains to the right. Thin lines represent single patients, whereas thick lines represent several patients (the numbers of which are given above each line).

age group and +11 was more frequent in men. The relatively high frequency of –Y, also as a sole change, among elderly men (Table I) most likely reflects the well known age-related loss of this sex chromosome (Stone & Sandberg, 1995; Herens *et al*, 1999). As regards chromosome X, only three MM patients with –X as a sole anomaly have been reported (Mitelman *et al*, 2003), precluding meaningful statistical analyses of the impact of age.

Finally, although some of the observed frequency differences may be fortuitous, the present findings strongly suggest that some karyotypic features of MM are influenced by endogenous and/or exogenous factors.

ACKNOWLEDGMENTS

This work was supported by grants from the Swedish Cancer Society, the John and Augusta Persson Foundation for Medical Research, and Lund University Medical Faculty.

REFERENCES

Albin, M., Björk, J., Welinder, H., Tinnerberg, H., Mauritzson, N., Johansson, B., Billström, R., Strömberg, U., Mikoczy, Z., Ahlgren, T., Nilsson, P.G., Mitelman, F. & Hagmar, L. (2000) Acute myeloid leukemia and clonal chromosome aberrations in relation to past exposure to organic solvents. *Scandinavian Journal of Work, Environment and Health*, **26**, 482–491.

Avet-Loiseau, H., Andree-Ashley, L.E., Moore, II, D., Mellerin, M.P., Feusner, J., Bataille, R. & Pallavicini, M.G. (1997) Molecular cytogenetic abnormalities in multiple myeloma and plasma cell leukemia measured using comparative genomic hybridization. *Genes, Chromosomes and Cancer*, **19**, 124–133.

Avet-Loiseau, H., Daviet, A., Brigaudeau, C., Callet-Bauchu, E., Terré, C., Lafage-Pochitaloff, M., Désangles, F., Ramond, S., Talmant, P. & Bataille, R. (2001) Cytogenetic, interphase, and multicolor fluorescence in situ hybridization analyses in primary plasma cell leukemia: a study of 40 patients at diagnosis. *Blood*, **97**, 822–825.

Björk, J., Albin, M., Mauritzson, N., Strömberg, U., Johansson, B. & Hagmar, L. (2000) Smoking and myelodysplastic syndromes. *Epidemiology*, **11**, 285–291.

Björk, J., Albin, M., Mauritzson, N., Strömberg, U., Johansson, B. & Hagmar, L. (2001) Smoking and acute myeloid leukemia: associations with morphology and karyotypic patterns and evaluation of dose–response relations. *Leukemia Research*, **25**, 865–872.

Boultwood, J., Lewis, S. & Wainscoat, J.S. (1994) The 5q– syndrome. *Blood*, **84**, 3253–3260.

Calasanz, M.J., Cigudosa, J.C., Odero, M.D., Ferreira, C., Ardanaz, M.T., Fraile, A., Carrasco, J.L., Solé, F., Cuesta, B. & Gullón, A. (1997) Cytogenetic analysis of 280 patients with multiple myeloma and related disorders: primary breakpoints and clinical correlations. *Genes, Chromosomes and Cancer*, **18**, 84–93.

Cigudosa, J.C., Calasanz, M.J., Odero, M.D., Prosper, F. Etxaniz, A., Marin, J., Rifón, J., Gullón, A. & Rocha, E. (1994) Cytogenetic data in 41 patients with multiple myeloma. *Cancer Genetics and Cytogenetics*, **78**, 210–213.

Cigudosa, J.C., Rao, P.H., Calasanz, M.J., Odero, M.D., Michaeli, J., Jhanwar, S.C. & Chaganti, R.S.K. (1998) Characterization of nonrandom chromosomal gains and losses in multiple myeloma by comparative genomic hybridization. *Blood*, **91**, 3007–3010.

Claudio, J.O., Masih-Khan, E., Tang, H., Gonçalves, J., Voralia, M., Li, Z.H., Nadeem, V., Cukerman, E., Francisco-Pabalan, O., Liew, C.C., Woodgett, J.R. & Stewart, A.K. (2002) A molecular compendium of genes expressed in multiple myeloma. *Blood*, **100**, 2175–2186.

Desikan, R., Barlogie, B., Sawyer, J., Ayers, D., Tricot, G., Badros, A., Zangari, M., Munshi, N.C., Anaissie, E., Spoon, D., Siegel, D., Jagannath, S., Vesole, D., Epstein, J., Shaughnessy, J., Fassas, A., Lim, S., Roberson, P. & Crowley, J. (2000) Results of high-dose therapy for 1000 patients with multiple myeloma: durable complete remissions and superior survival in the absence of chromosome 13 abnormalities. *Blood*, **95**, 4008–4010.

Facon, T., Avet-Loiseau, H., Guillerm, G., Moreau, P., Geneviève, F., Zandecki, M., Laï, J.-L., Leleu, X., Jouet, J.-P., Bauters, F., Harousseau, J.-L., Bataille, R. & Mary, J.-Y. (2001) Chromosome 13 abnormalities identified by FISH analysis and serum β2-microglobulin produce a powerful myeloma staging system for patients receiving high-dose therapy. *Blood*, **97**, 1566–1571.

Fonseca, R., Witzig, T.E., Gertz, M.A., Kyle, R.A., Hoyer, J.D., Jalal, S.M. & Greipp, P.R. (1998) Multiple myeloma and the translocation t(11;14)(q13;q32): a report on 13 cases. *British Journal of Haematology*, **101**, 296–301.

Fonseca, R., Blood, E.A., Iken, M.M., Kyle, R.A., Dewald, G.W., Bailey, R.J., Van Wier, S.A., Henderson, K.J., Hoyer, J.D., Harington, D., Kay, N.E., Van Ness, B. & Greipp, P.R. (2002a) Myeloma and the t(11;14)(q13;q32); evidence for a biologically defined unique subset of patients. *Blood*, **99**, 3735–3741.

Fonseca, R., Harrington, D., Oken, M.M., Dewald, G.W., Bailey, R.J., Van Wier, S.A., Henderson, K.J., Blood, E.A., Rajkumar, V., Kay, N.E., Van Ness, B. & Greipp, P.R. (2002b) Biological and prognostic significance of interphase fluorescence in situ hybridization detection of chromosome 13 abnormalities (delta 13) in multiple myeloma: an Eastern Cooperative Oncology Group Study. *Cancer Research*, **62**, 715–720.

Gould, J., Alexanian, R., Goodacre, A., Pathak, S., Hecht, B. & Barlogie, B. (1988) Plasma cell karyotype in multiple myeloma. *Blood*, **71**, 453–456.

Greenberg, P., Cox, C., LeBeau, M.M., Fenaux, P., Morel, P., Sanz, G., Sanz, M., Vallespi, T., Hamblin, T., Oscier, D., Ohyashiki, K., Toyama, K., Aul, C., Mufti, G. & Bennett, J. (1997) International scoring system for evaluating prognosis in myelodysplastic syndromes. *Blood*, **89**, 2079–2088.

Grimwade, D., Walker, H., Oliver, F., Wheatley, K., Harrison, C., Harrison, G., Rees, J., Hann, I., Stevens, R., Burnett, A. & Goldstone, A. (1998) The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. *Blood*, **92**, 2322–2333.

Gutiérrez, N.C., Hernández, J.M., García, J.L., Canizo, M.C., González, M., Hernández, J., Gonzalez, M.B., Garcia-Marcos, M.A. & San Miguel, J.F. (2001) Differences in genetic changes between multiple myeloma and plasma cell leukemia demonstrated by comparative genomic hybridization. *Leukemia*, **15**, 840–845.

Harrison, C.J. (2001) The detection and significance of chromosomal abnormalities in childhood acute lymphoblastic leukaemia. *Blood Reviews*, **15**, 49–59.

Herens, C., Brasseur, E., Jamar, M., Vierset, L., Schoenen, I. & Koulischer, L. (1999) Loss of the Y chromosome in bone marrow cells: results on 1907 consecutive cases of leukaemia and preleukaemia. *Clinical and Laboratory Haematology*, **21**, 17–20.

Hoyer, J.D., Hanson, C.A., Fonseca, R., Greipp, P.R., Dewald, G.W. & Kurtin, P.J. (2000) The t(11;14)(q13;q32) translocation in multiple myeloma. A morphologic and immunohistochemical study. *American Journal of Clinical Pathology*, **113**, 831–837.

ISCN (1995) *An International System for Human Cytogenetic Nomenclature* (ed. by F. Mitelman). S. Karger, Basel.

Johansson, B., Mertens, F. & Mitelman, F. (1991) Geographic heterogeneity of neoplasia-associated chromosome aberrations. *Genes, Chromosomes and Cancer*, **3**, 1–7.

Johansson, B., Moorman, A.V., Haas, O.A., Watmore, A.E., Cheung, K.L., Swanton, S. & Secker-Walker, L.M. (1998) Hematologic malignancies with t(4;11)(q21;q23): a cytogenetic, morphologic, immunophenotypic and clinical study of 183 cases. *Leukemia*, **12**, 779–787.

Keung, Y.-K., Yung, C., Wong, J.W., Shah, F., Cobos, E. & Tonk, V. (1998) Unusual presentation of multiple myeloma with 'jumping translocation' involving 1q21. A case report and review of the literature. *Cancer Genetics and Cytogenetics*, **106**, 135–139.

Kuehl, W.M. & Bergsagel, P.L. (2002) Multiple myeloma: evolving genetic events and host interactions. *Nature Reviews of Cancer*, **2**, 175–187.

Laï, J.L., Michaux, L., Dastugue, N., Vasseur, F., Daudignon, A., Facon, T., Bauters, F. & Zandecki, M. (1998) Cytogenetics in multiple myeloma: a multicenter study of 24 patients with t(11;14)(q13;q32) or its variant. *Cancer Genetics and Cytogenetics*, **104**, 133–138.

Le Baccon, P., Leroux, D., Dascalescu, C., Duley, S., Marais, D., Esmenjaud, E., Sotto, J.J. & Callanan, M. (2001) Novel evidence of a role for chromosome 1 pericentric heterochromatin in the pathogenesis of B-cell lymhoma and multiple myeloma. *Genes, Chromosomes and Cancer*, **32**, 250–264.

Look, A.T. (1997) Oncogenic transcription factors in the human acute leukemias. *Science*, **278**, 1059–1064.

Malgeri, U., Baldini, L., Perfetti, V., Fabris, S., Vignarelli, M.C., Colombo, G., Lotti, C., Compasso, S., Bogni, S., Lombardi, L., Maiolo, A.T. & Neri, A. (2000) Detection of t(4;14)(p16.3;q32) chromosomal translocation in multiple myeloma by reverse transcription-polymerase chain reaction analysis of *IGH–MMSET* fusion transcripts. *Cancer Research*, **60**, 4058–4061.

Mauritzson, N., Johansson, B., Albin, M., Billström, R., Ahlgren, T., Mikoczy, Z., Nilsson, P.G., Hagmar, L. & Mitelman, F. (1999) A single-center population-based consecutive series of 1500 cytogenetically investigated adult hematological malignancies: karyotypic features in relation to morphology, age and gender. *European Journal of Haematology*, **62**, 95–102.

Mauritzson, N., Albin, M., Rylander, L., Billström, R., Ahlgren, T., Mikoczy, Z., Björk, J., Strömberg, U., Nilsson, P.G., Mitelman, F., Hagmar, L. & Johansson, B. (2002) Pooled analysis of clinical and cytogenetic features in treatment-related and *de novo* adult acute myeloid leukemia and myelodysplastic syndromes based on a consecutive series of 761 patients analyzed 1976–1993 and on 5098 unselected cases reported in the literature 1974–2001. *Leukemia*, **16**, 2366–2378.

Mertens, F., Johansson, B. & Mitelman, F. (1993) Age- and gender-related heterogeneity of cancer chromosome aberrations. *Cancer Genetics and Cytogenetics*, **70**, 6–11.

Mitelman, F., Johansson, B. & Mertens, F. (eds) (2003) *Mitelman Database of Chromosome Aberrations in Cancer* WWW document. URL. http://cgap.nci.nih.gov/Chromosomes/Mitelman

Moorman, A.V., Roman, E., Willett, E.V., Dovey, G.J., Cartwright, R.A. & Morgan, G.J. (2001) Karyotype and age in acute myeloid leukemia. Are they linked? *Cancer Genetics and Cytogenetics*, **126**, 155–161.

Ng, M.H.L., Wong, N., Tsang, K.-S., Cheng, S.-H., Chung, Y.-F. & Lo, K.-W. (2001) Recurrent chromosomal rearrangements involving breakpoints 3p21 and 19q13 in Chinese IgD multiple myeloma detected by G-banding and multicolor spectral karyotyping: a review of IgD karyotype literature. *Human Pathology*, **32**, 1016–1020.

Nilsson, T., Lenhoff, S., Turesson, I., Rylander, L., Mitelman, F., Westin, J., Höglund, M. & Johansson, B. (2002) Cytogenetic features of multiple myeloma: impact of gender, age, disease phase, culture time, and cytokine stimulation. *European Journal of Haematology*, **68**, 345–353.

Palka, G., Spadano, A., Geraci, L., Fioritoni, G., Dragani, A., Calabrese, G., Guanciali Franchi, P. & Stuppia, L. (1987) Chromosome changes in 19 patients with Waldenström's macroglobulinemia. *Cancer Genetics and Cytogenetics*, **29**, 261–269.

Paulsson, K., Säll, T., Fioretos, T., Mitelman, F. & Johansson, B. (2001) The incidence of trisomy 8 as a sole chromosomal aberration in myeloid malignancies varies in relation to gender, age, prior iatrogenic genotoxic exposure, and morphology. *Cancer Genetics and Cytogenetics*, **130**, 160–165.

Pedersen, B. (1992) Survival of patients with t(1;7)(p11;p11). Report of two cases and review of the literature. *Cancer Genetics and Cytogenetics*, **60**, 53–59.

Pedersen-Bjergaard, J. & Rowley, J.D. (1994) The balanced and the unbalanced chromosome aberrations of acute myeloid leukemia may develop in different ways and may contribute differently to malignant transformation. *Blood*, **83**, 2780–2786.

Rabbitts, T. (2001) Chromosomal translocation master genes, mouse models and experimental therapeutics. *Oncogene*, **20**, 5763–5777.

Rao, P.H., Cigudosa, J.C., Ning, Y., Calasanz, M.J., Iida, S., Tagawa, S., Michaeli, J., Klein, B., Dalla-Favera, R., Jhanwar, S.C., Ried, T. & Chaganti, R.S.K. (1998) Multicolor spectral karyotyping identifies new recurring breakpoints and translocations in multiple myeloma. *Blood*, **92**, 1743–1748.

Sawyer, J.R., Lukacs, J.L., Munshi, N., Desikan, K.R., Singhal, S., Mehta, J., Siegel, D., Shaughnessy, J. & Barlogie, B. (1998a) Identification of new nonrandom translocations in multiple myeloma with multicolor spectral karyotyping. *Blood*, **92**, 4269–4278.

Sawyer, J.R., Tricot, G., Mattox, S., Jagannath, S. & Barlogie, B. (1998b) Jumping translocations of chromosome 1q in multiple myeloma: evidence for a mechanism involving decondensation of pericentromeric heterochromatin. *Blood*, **91**, 1732–1741.

Sawyer, J.R., Lukacs, J.L., Thomas, E.L., Swanson, C.M., Goosen, L.S., Sammartino, G., Gilliland, J.C., Munshi, N.C., Tricot, G., Shaughnessy, Jr, J.D. & Barlogie, B. (2001) Multicolour spectral karyotyping identifies new translocations and a recurring pathway for chromosome loss in multiple myeloma. *British Journal of Haematology*, **112**, 167–174.

Shaughnessy, Jr, J., Gabrea, A., Qi, Y., Brents, L., Zhan, F., Tian, E., Sawyer, J., Barlogie, B., Bergsagel, P.L. & Kuehl, M. (2001) Cyclin D3 at 6p21 is dysregulated by recurrent chromosomal translocations to immunoglobulin loci in multiple myeloma. *Blood*, **98**, 217–223.

Smadja, N.V., Bastard, C., Brigadeau, C., Leroux, D., Fruchart, C. & Groupe Français de Cytogénétique Hématologique (2001) Hypodiploidy is a major prognostic factor in multiple myeloma. *Blood*, **98**, 2229–2238.

Stone, J.F. & Sandberg, A.A. (1995) Sex chromosome aneuploidy and aging. *Mutation Research*, **338**, 107–113.

The National Board of Health and Welfare (2001) *Cancer incidence in Sweden 1999* WWW document. URL. http://www.sos.se/epc

Tricot, G., Barlogie, B., Jagannath, S., Bracy, D., Mattox, S., Vesole, D.H., Naucke, S. & Sawyer, J.R. (1995) Poor prognosis in multiple

myeloma is associated only with partial or complete deletions of chromosome 13 or abnormalities involving 11q and not with other karyotype abnormalities. *Blood*, **86**, 4250–4256.

Virtaneva, K., Wright, F.A., Tanner, S.M., Yuan, B., Lemon, W.J., Caligiuri, M.A., Bloomfield, C.D., de la Chapelle, A. & Krahe, R. (2001) Expression profiling reveals fundamental biological differences in acute myeloid leukemia with isolated trisomy 8 and normal cytogenetics. *Proceedings of the National Academy of Sciences of the United States of America*, **98**, 1124–1129.

Weh, H.J., Gutensohn, K., Selbach, J., Kruse, R., Wacker-Backhaus, G., Seeger, D., Fiedler, W., Fett, W. & Hossfeld, D.K. (1993) Karyotype in multiple myeloma and plasma cell leukaemia. *European Journal of Cancer*, **29A**, 1269–1273.

Yamamoto, K., Hamaguchi, H., Nagata, K. & Taniwaki, M. (1998) A variant Burkitt-type translocation (8;22)(q24;q11) in multiple myeloma. *Cancer Genetics and Cytogenetics*, **104**, 98–103.

Zhan, F., Hardin, J., Kordsmeier, B., Bumm, K., Zheng, M., Tian, E., Sanderson, R., Yang, Y., Wilson, C., Zangari, M., Anaissie, E., Morris, C., Muwalla, F., van Rhee, F., Fassas, A., Crowley, J., Tricot, G., Barlogie, B. & Shaughnessy, Jr, J. (2002) Global gene expression profiling of multiple myeloma, monoclonal gammopathy of undetermined significance, and normal bone marrow plasma cells. *Blood*, **99**, 1745–1757.

Zojer, N., Königsberg, R., Ackermann, J., Fritz, E., Dallinger, S., Krömer, E., Kaufmann, H., Riedl, L., Gisslinger, H., Schreiber, S., Heinz, R., Ludwig, H., Huber, H. & Drach, J. (2000) Deletion of 13q14 remains an independent adverse prognostic variable in multiple myeloma despite its frequent detection by interphase fluorescence in situ hybridization. *Blood*, **95**, 1925–1930.