ARTICLE

Novel recurrent chromosomal aberrations detected in clonal plasma cells of light chain amyloidosis patients show potential adverse prognostic effect: first results from a genome-wide copy number array analysis

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ABSTRACT

Immunoglobulin light chain (AL) amyloidosis is a rare plasma cell dyscrasia characterized by the deposition of abnormal amyloid fibrils in multiple organs, thus impairing their function. In the largest cohort studied up to now of 118 CD138-purified plasma cell samples from previously untreated immunoglobulin light chain amyloidosis patients, we assessed in parallel copy number alterations using high-density copy number arrays and interphase fluorescence *in situ* hybridization (iFISH). We used fluorescence *in situ* hybridization probes for the IgH translocations t(11;14), t(4;14), and t(14;16) or any other IgH rearrangement as well as numerical aberrations of the chromosome loci 1q21, 8p21, 5p15/5q35, 11q22.3 or 11q23, 13q14, 15q22, 17p13, and 19q13. Recurrent gains included chromosomes 1q (36%), 9 (24%), 11q (24%), as well as 19 (15%). Recurrent losses affected chromosome 13 (29% monosomy) and partial losses of 14q (19%), 16q (14%) and 13q (12%), respectively. In 88% of patients with translocation t(11;14), the hallmark chromosomal aberration in AL amyloidosis, a concomitant gain of 11q22.3/11q23 detected by iFISH was part of the unbalanced translocation der(14)t(11;14)(q13;q32) with the breakpoint in the *CCND1/MYEOV* gene region. Partial loss of chromosome regions 14q and 16q were significantly associated to gain 1q. Gain 1q21 detected by iFISH almost always resulted from a gain of the long arm of chromosome 1 and not from trisomy 1, whereas deletions on chromosome 1p were rarely found. Overall and event-free survival analysis found a potential adverse prognostic effect of concomitant gain 1q and deletion 14q as well as of deletion 1p. In conclusion, in the first whole genome report of clonal plasma cells in AL amyloidosis, novel aberrations and hitherto unknown potential adverse prognostic effects were uncovered.

Introduction

AL amyloidosis is characterized by the deposition of abnormal amyloid fibrils in multiple organs, thereby impairing their function. Plasma cells that undergo clonal alterations produce amyloid fibrils emanating from misfolding of the native protein.[1] The panel of iFISH probes in AL amyloidosis originated from the diagnostic management of multiple myeloma (MM). At our center, we use a comprehensive

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probe set for chromosome regions 1q21, 5p15/5q35, 8p21, 9q34, 11q22.3 or 11q23, 13q14, 15q22, 17p13, and 19p13 as well as the IgH translocations t(11;14), t(4;14), and t(14;16) or any other IgH rearrangement. Indeed, the iFISH probe set has shown a very similar aberration pattern in both AL and MM.[2-8]

In analogy to MM patients, the oncogenetic tree model[9] distinguished AL into different subgroups: (i) hyperdiploid (HD), (ii) translocation t(11;14), (iii) non-hyperdiploid (NHD) with deletion of 13q14 / t(4;14), and (iv) IgH translocation with an unknown partner.[6] The only difference was demonstrated for gain of 1q21 showing an association with the hyperdiploid subgroup in AL patients, whereas it was linked to the NHD group with deletion 13q14 / t(4;14) in MM patients. Within the group of patients with gain of 11q23, a dichotomy was observed which split into t(11;14) positive and hyperdiploid karyotypes.

Using different microarray platforms, genome-wide screening for copy number (CN) aberrations has been done in MM[10-16] as well as in monoclonal gammopathy of unknown significance (MGUS)[17] and smoldering MM,[17] the precursors of symptomatic MM. These studies confirmed the aberrations detected by routine iFISH, with the exception of balanced translocations that cannot be identified by CN array. Furthermore, several other aberrant regions were identified, some of which are associated with prognosis or the stage of plasma cell dyscrasia (MGUS, smoldering MM, or MM). Given the overall genomic similarity of AL amyloidosis and MM, it seemed obvious to perform a similar study in this plasma cell disease.

In the study herein, we analyzed 118 CD138-purified plasma cell samples from AL amyloidosis patients by high-density CN array in order to detect novel CN alterations and relate these findings to known molecular entities, in particular to translocation t(11;14), the hallmark molecular alteration of AL amyloidosis.

Methods

Patients

One hundred and eighteen AL amyloidosis patients presenting at the Amyloidosis Center Heidelberg between 2005 and 2014 were included in the study, which was approved by the Ethics Committee (#123/2006) following written informed consent in concordance with the Declaration of Helsinki. iFISH results and clinical correlation of 44 of these patients have been published previously.[5,6] Clinical characteristics of the patients including distribution of age, sex, number of involved organs, underlying clonal plasma cell dyscrasia (AL with less than 10% and AL-MM with 10% or more plasma cells in bone marrow cytology), light chain type, clinical scores, AL-specific serum parameters, and therapy regimen are summarized in Table 1. The distributions of values are typical and representative for AL amyloidosis patients in general.

Interphase FISH diagnostics

For all 118 patients, iFISH was performed on CD138-positive bone marrow plasma cells purified by auto-magnetic-activated cell sorting with anti-CD138 immunobeads as described previously.[18,19] Purity of sorted plasma cells ranged from 75-99% with a median of 90%. Results were available for numerical chromosome aberrations at the loci 1q21, 5p15/5q35, 8p21, 9q34, 11q22.3 or 11q23, 13q14, 15q22, 17p13, and 19p13 as well as the IgH translocations t(11;14)(q13;q32), t(4;14)(p16;q32), t(14;16)(q32;q23), and an IgH break apart probe. Hyperdiploidy was defined according to Wuilleme *et al.*,[20] determining HD-iFISH by gains of at least two of the three iFISH probes for chromosomes 5, 9, and 15. Only patients with a minimum of one aberration detectable by iFISH in at least 60% of cells were included in the study.

Copy number array analysis

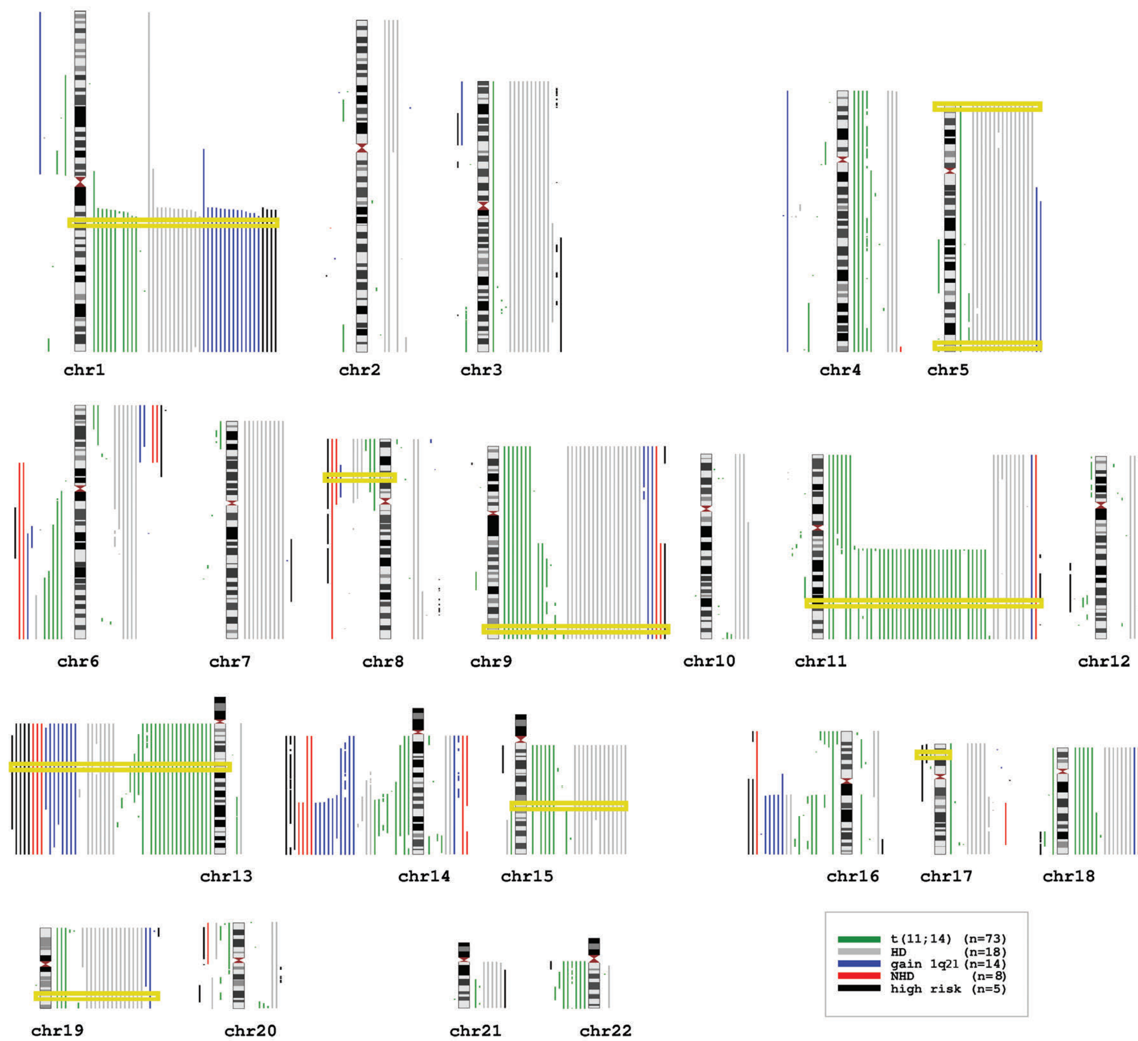
For each patient, 50 ng of DNA from CD138-positive plasma cells (see above) was used. Hybridization to an Affymetrix® CytoScan HD Oligo/SNP-array was performed according to the manufacturer's instructions. Arrays were scanned with the

Table 1. Clinical characteristics of AL amyloidosis patients and iFISH results.

Number of patients	118
Age median (range) in years	65 (41-87)
Sex: female / male	47 / 71 pts
Involved organs: median number (range)	2 (1-6)
Heart	89 pts
Liver	25 pts
Kidney	66 pts
GI tract	38 pts
Soft tissue	57 pts
Peripheral neuropathy	23 pts
Underlying disease[49]	
AL	48 pts
AL-MM	70 pts
Percent PCs median (range)	10 (1-58)
Ig intact present	49 pts
Light chain type: κ / λ	23 pts / 95 pts
Clinical scores	
Karnofsky index in percent: median (range)	80 (50-100)
Mayo score median (range)	2 (1-3)
Mayo I, II, III	24, 43, 48 pts
NYHA classification median (range)	2 (0-4)
Renal stage[51] (median, range)	2 (1-3)
Serum marker median (range)	
NT-BNP ng/l	3075 (22-165677)
Creatinine mg/dl	1.03 (0.06-10.77)
dFLC mg/l	232 (1-12078)
iFISH (No. of pts. (percentage))	
NHD	95 (81)
HD	23 (19)
del 13q14	52 (43)
gain 1q21	43 (36)
del 17p13	6 (5)
t(11;14)	73 (62)
t(4;14)	3 (3)
t(14;16)	2 (2)
Therapy	
None	5
High-dose	24
Len-Mel-Dex	18
Mel-Dex	33
Velcade	38

The table summarizes age, sex, number of involved organs, underlying disease, percentage of plasma cells, heavy and light chain type, Karnofsky index, Mayo score,[50] NYHA classification and several serum markers. AL, stage of clonal disease is amyloidosis; AL- MM, stage of clonal disease is AL+MM. NYHA: New York Heart Association; NT-BNP: N-terminal pro brain natriuretic peptide; AL: immunoglobulin light chain; MM: multiple myeloma; GI: gastrointestinal; pts, patients; FLC: free light chain; Ig: immunoglobulin; PC: plasma cell; HD: hyperdiploid; NHD: Non-hyperdiploid; del: deletion; t: translocation; Len: lenalidomid; Mel: melphalan; Dex: dexamethasone.

A



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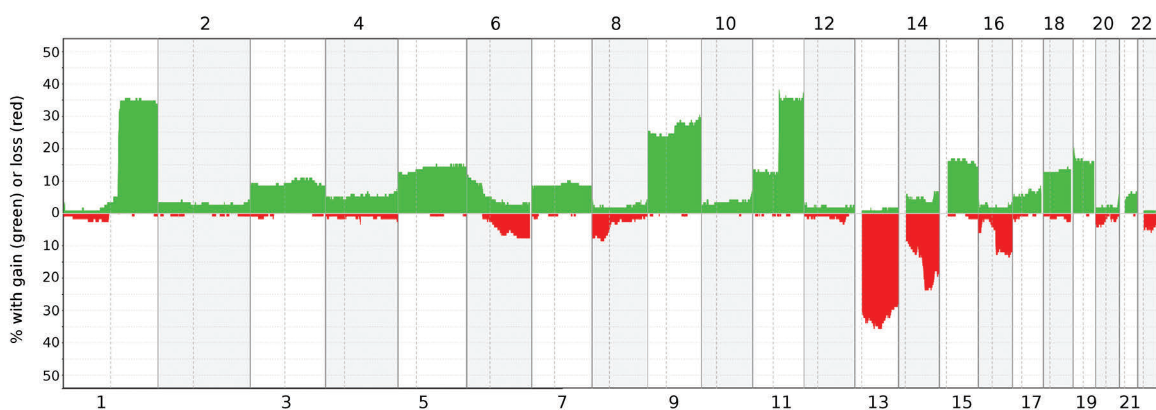

Figure 1. Overview of results from genome-wide copy number array analysis in AL amyloidosis patients. (A) Karyogram depicting each chromosome and each patient sample as gain and loss (right and left of each chromosome, respectively). The karyogram style allows for the clear visualization of gains and losses belonging to each single patient in addition to providing an exact insight of the overall distribution of aberrations and furthermore, trisomies are easily recognizable, which is more difficult to achieve in frequency plots. Different colors indicate the following iFISH defined subgroups: all patients with a translocation t(11;14) (n=73; green) were selected into one group, of the remaining patients, those showing a hyperdiploid karyotype (HD) according to Wuilleme *et al.*,[20] (n=18; gray) were grouped together, again of the remaining patients, those with gain of chromosome region 1q21 (gain 1q; n=14; blue) were put into the respective group. Finally, the still remaining patients were split into those with high-risk aberrations (n=5; black) and a non-hyperdiploid group (NHD; n=8; red). This categorization was chosen in order to visualize the underlying structure according to cytogenetic risk groups. Additionally, the chromosomal location of the iFISH probes is highlighted by yellow rectangles. Genomic Recurrent Event ViEwer was used to visualize aberrations.[52] (B) Frequency plot providing an overview of the distributions of gains (green) and losses (red) as percentages of all 118 patients for chromosomes 1-22.

Affymetrix GeneChip® Scanner 3000 7G and CN analysis was done with Affymetrix Chromosome Analysis Suite software version 2.1.0.16(r6634) and Annotation NetAffx Build 33. Interpretation was based on human reference sequence GRCh37/hg19, February 2009. Data were deposited at the Gene Expression Omnibus (accession GSE89616). The complete data set was visually analyzed. Gains and losses had to meet three criteria to be reported: a minimum of 25 markers per segment, a minimum genome length of 100 kb, and less than 50% overlap with known CN variants from the Database of Genomic Variants[21] and/or in-house data obtained from healthy parents of patients with non-syndromic mental retardation. Copy number aberrations located at 2p11.2 (IgK@), 14q32.33 (IgH@), and 22q11.22 (IgL@), which might stem from rearrangements of the B-cell receptor gene, were excluded from further analysis as well as aberrations on the sex chromosomes. Based on the results from CN analysis, HD-CN was defined by trisomy of at least two chromosomes with respect to all analyzed chromosomes, to compare the difference to the score of Wuilleme *et al.*[20]

Statistical analysis

The χ^2 test was used to analyze correlations of aberrations detected by CN array and the different cytogenetic groups. Differences were considered statistically significant at $P<0.05$. To correlate CN aberrations with categorical clinical and hematological variables we used the χ^2 test, and for the correlation with continuous clinical and hematological variables, we used an analysis of variance model (ANOVA). For all statistical computation, R version 3.2.2 was used with library 'stats' version 3.2.2. The library 'copynumber'[22] (version 1.8.0) was used to prepare the frequency plot in Figure 1B.

Results

Overview of CN aberrations detected in 118 AL amyloidosis patients

In the following analysis, CN aberrations affecting whole chromosomes are not included in counts of smaller alterations, e.g., chromosome arms or regional bands.

In total, the range of aberrant chromosomes per patient varied between zero (11% of patients) and 16 (one patient) with an average and median of five and three aberrant chromosomes, respectively. An overview of the distribution of all CN aberrations is presented in Figure 1. Table 2 comprises - for the sake of clarity - all aberrations detected in at least 5% of patients. The most prevalent gain detected in the study cohort was gain of chromosome

Table 2. Frequency in % of CN aberrations and concordance with iFISH results.

Chr	Trisomy	Gain	Monosomy	Loss	Concordance with iFISH (median: 98%)
1q	–	36	–	–	95.7
3p	9	–	–	–	–
3q		5		–	–
4p	5	–	–	–	–
4q		5		7	–
5p	14	–	–	–	100
5q		–		–	99.2
6p	–	10	–	–	–
6q		5		10	–
7p	8	–	–	–	–
7q		–		–	–
8p	–	–	–	8	98.3
9p	24	–	–	–	–
9q		7		–	94.1
11p	11	–	–	–	–
11q		31		–	95.8
13q	–	–	29	12	92.4
14q	–	5	5	19	–
15q	14	5	–	–	100
16p	–	–	–	5	–
16q		–		14	–
17p	5	–	–	–	97.5
17q		6		–	–
18p	13	–	–	–	–
18q		–		–	–
19p	15	–	–	–	99.2
19q		–		–	–
20p	–	–	–	5	–

Note that the numbers include not only trisomies and aberrations affecting whole chromosome arms but also smaller aberrant regions. Chromosomes with no aberration above 4% are not shown in the table. Chr: chromosome.

1q (36%, including one trisomy 1), followed by trisomy 9 (24%), gain of chromosome arm 11q (24%), and trisomy 19 (15%). Trisomy of chromosomes 15 (14%), 5 (14%), 18 (13%), and 11 (11%) was observed less frequently. The most common deletion affected chromosome 13 in 40% of patients, with 70% of them showing a monosomy and 30% presenting only partial loss of chromosome 13. The most commonly affected chromosomal region was 13q21.32-q21.33 (chr13:67,533,438-70,847,141) in 88% of all patients with deletions affecting chromosome 13. This region includes only two genes, *PCDH9* (Protocadherin 9) and *LINC00550*, a non-coding ribonucleic acid. Deletions affected chromosome 14 in a total of 25% of all patients. In contrast to the aberrations on chromosome 13, mono-

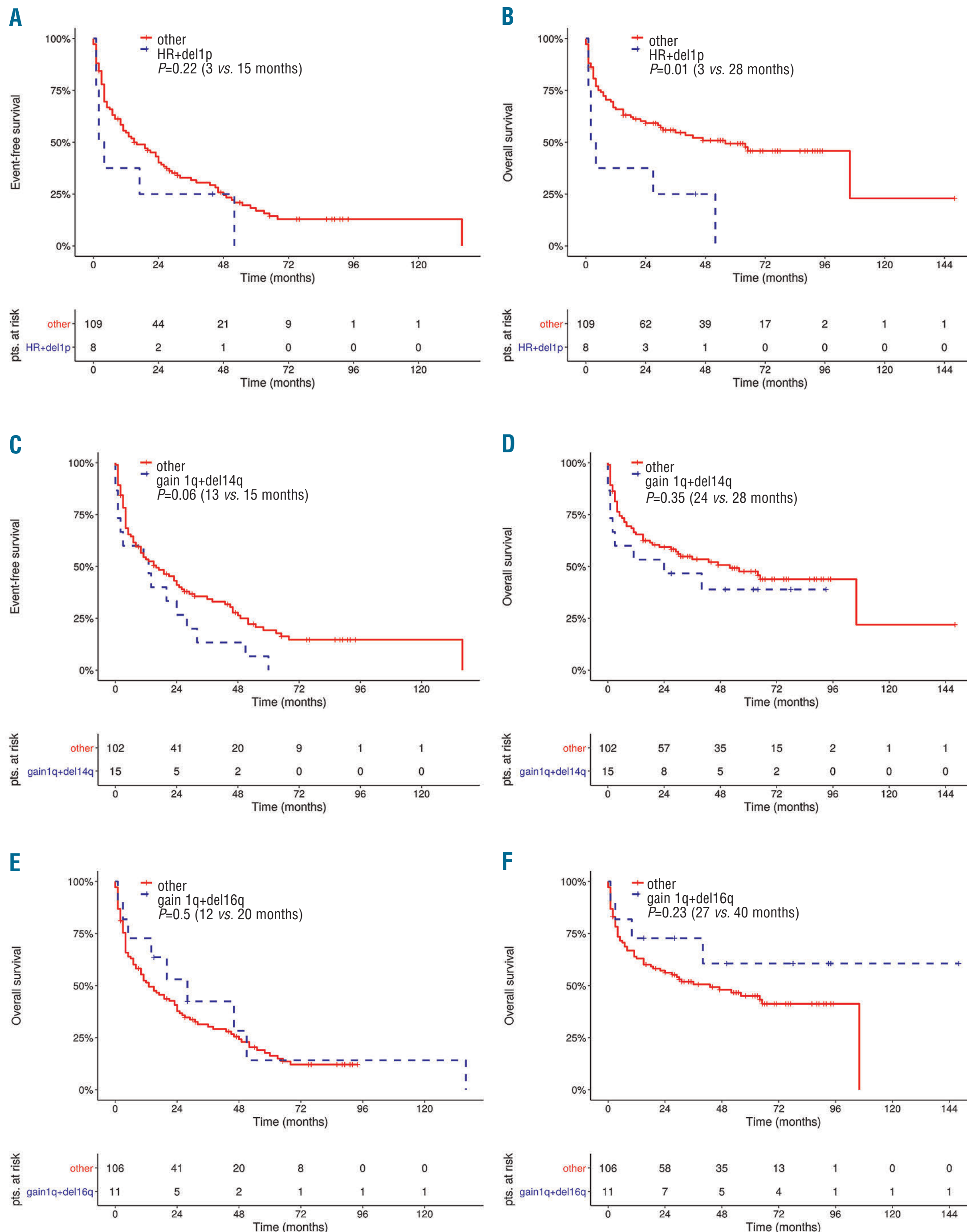

Figure 2. EFS and OS of AL amyloidosis patients. Event-free survival (A, C, E) and overall survival curves (B, D, F) are provided for patients with high-risk aberrations or deletion 1p *vs.* other patients (A, B), patients with gain 1q and concomitant partial deletion 14q *vs.* other patients (C, D), and patients with deletion of chromosome arm 16q *vs.* patients without this aberration (E, F). HR: high-risk aberrations; del1p: deletion 1p; gain1q: gain chromosome 1q; del14q: partial deletion chromosome 14q; del16q: deletion chromosome 16q; pts.: patients.

Table 3. *P*-values (medians) of associations of chromosome aberrations with clinical parameters.

	Percentage of plasma cells	Age	MDRD	dFLC	Intact Ig	Plasma cell dyscrasia = AL+MM
t(11;14) *vs.* no t(11;14)	<0.05 (10 *vs.* 13)	–	–	–	<0.001	–
HD-CN *vs.* NHD	<0.005 (17 *vs.* 10)	<0.01 (73 *vs.* 65)	–	–	<0.005	–
HD-iFISH *vs.* NHD	<0.005 (13.5 *vs.* 10)	<0.01 (73 *vs.* 65)	<0.05 (55 *vs.* 69)	–	<0.005	–
Gain 1q21 *vs.* no gain 1q21	<0.005 (13.5 *vs.* 10)	–	–	–	–	–
Partial deletion 14q *vs.* other	<0.05 (12 *vs.* 10)	–	–	–	–	–
Gain 1q21 & partial loss chr. 14q *vs.* other	<0.05 (21 *vs.* 10)	–	–	<0.05 (488 *vs.* 198)	–	<0.05
Gain 1q21 & partial loss chr. 16q *vs.* other	–	–	–	–	–	–
HR & del 1p *vs.* other	–	–	–	–	–	–
Gain 1q21 & HD-CN *vs.* gain 1q21 & NHD	–	<0.05 (75.5 *vs.* 65)	<0.005 (32.6 *vs.* 69)	–	<0.005	–

t(11;14): Patients with translocation t(11;14); HD-CN: hyperdiploid group determined by CN array; HD-iFISH: hyperdiploid group determined by iFISH; NHD: Non-hyperdiploid group; Gain 1q21: Patients with gain 1q21; Gain 1q21 & partial loss chr. 14: Patients with gain 1q21 and concomitant partial loss of chromosome 14; Gain 1q21 & partial loss chr. 16q: Patients with gain 1q21 and concomitant partial loss of chromosome 16q; Gain 1q21 & HD-CN: Patients with gain 1q21 and concomitant hyperdiploid karyotype determined by CN analysis; MDRD: Modification of Diet in Renal Disease; dFLC: difference between involved FLC and uninvolved FLC; FLC: serum free light chains; HR: high-risk aberrations; del 1p: deletion chromosome 1p; -: not significant.

somy 14 was detected in only 23% of the patients with deletions concerning chromosome 14, with the remaining 77% bearing partial loss. The minimal common region of the partial deletions of chromosome 14q was 14q24.1-q31.1 (chr14: 69,416,523-82,198,988) spanning 14 Mb and containing 122 genes. Seventy-one genes in this region are annotated in the Online Mendelian Inheritance in Man (OMIM) database, including the tumor suppressor genes *JDP2* (Jun dimerization protein 2) and *MLH3* (mutL homolog 3, *E. coli*), which play a role in the apoptosis signaling pathway and DNA mismatch repair mechanism, respectively. Additionally, two oncogenes are located in this region, namely, *FOS* (FBJ murine osteosarcoma viral oncogene homolog) and *ESRRB* (estrogen-related receptor-β). Chromosomal regions less frequently affected by deletions were located on chromosome 16q (14%), 6q (10%), and 8p (8%).

Chromosome aberrations in molecular entities as defined by iFISH

Translocation t(11;14) group: In total, our cohort contained 73 patients (62%) with a translocation involving the genes *CCND1/MYEOV* on chromosomes 11q13 and IgH in 14q32 detected by iFISH. Copy number array detected gain of chromosomal material of 11q in 34 of these 73 patients (47%), in 88% the breakpoint localized within the genes *CCDN1/MYEOV*, the defined breakpoints of the translocation t(11;14) (*data not shown*). Thus, in the majority of patients with a t(11;14), gain of 11q22.3 or 11q23 detected by iFISH was the result of the unbalanced translocation der(14)t(11;14)(q13;q32). This group also included four hyperdiploid patients defined by CN array and 13 patients with gain of chromosome 1q (18%). Monosomy 13 was the most common deletion (16 patients, 22%). There was an association of t(11;14) with a lower total number of aberrations detected in the patients [median of 2 *vs.* 5; *P*<0.001; all aberrations used for the HD score of Wuilleme *et al.*[20] were counted as one (see Bochtler *et al.*[6] for details)].

For every cytogenetic group significant associations of chromosome aberrations with clinical parameters are summarized in Table 3.

Hyperdiploid group: CN array results identified 25% of the patients as HD, in addition, four patients had only one trisomy (all of chromosome 9). Most frequent gains were trisomy of chromosomes 9 (83%), 19 (59%), 15 (55%), 5 (52%), 18 (48%), 11 (45%), 7 (34%), 3 (31%), 17 (21%), 4 (21%), 21 (17%), and 14 (14%). Furthermore, gain of chromosome arm 1q was detected in 52% and partial gain of chromosome arm 6p in 14%. Concerning chromosomal losses, monosomy 13 was the most frequent aberration, found in 31% of patients, followed by deletion of material on chromosome arm 8p (14%). No association of monosomy 13 with the HD group was detected (*P*>0.05). Comparing HD-iFISH to HD-CN, six patients were not classified as HD by iFISH. Five of these patients showed trisomy of chromosomes 11 and 18 together with additional trisomies of either chromosome 4, 9, 14, 17, or 19, and the sixth patient was without a third trisomy. Only four of these HD patients carried a t(11;14). Interestingly, the HD-iFISH group showed a significant association with gain 1q21 (*P*<0.01), which was not the case for the HD-CN group. Clinical associations were similar to the HD-CN group (*cf.* Table 3).

Non-hyperdiploid group: This group included 95 patients (81%) when iFISH was used to distinguish HD and NHD patients. According to CN array results, 75% of our

patient cohort presented a NHD karyotype. The most frequent gains concerned chromosome arm 11q (40%), which included the localization of the breakpoint between genes *CCND1* and *MYEOV* in 36%, followed by gains of chromosome 1q (27%). Deletions affecting chromosome 13 and 14 were detected in 40% and 28% of NHD patients, respectively. This included monosomy 13 (69%) and 14 (28%) as well as partial deletions on 13q (31%) and 14q (72%). Additionally, deletions occurred in this group pertaining to chromosome arms 16q (13%) and 6q (10%). An association of NHD karyotype to translocation t(11;14) was significant as compared to HD patients (P<0.001). In the NHD group, neither an association to IgH translocations with an unknown partner nor monosomy 13 was found (P>0.05).

Patients with gain 1q21: Gain of chromosomal region 1q21 was detected in a total of 45 patients (36%; in one patient the iFISH result for 1q21 was missing where CN array detected gain of chromosome arm 1q), including four patients with subclonal presence of the respective aberration, i.e., here <35% by iFISH analysis that were not detected by CN array. In one patient, gain of chromosome region 1q21 was due to a trisomy 1 in the context of a HD karyotype. According to the number of fluorescence signals, five patients showed four copies of 1q21, indicating a possible additional isochromosome 1q, and in one patient five copies were detected, presumably resulting from unbalanced translocations involving chromosome 1q. Loss of chromosome arm 1p was detected in 3% overall, and only once together with gain 1q. Monosomy of chromosome 13 was the most frequent loss in 41% of these patients. Fifteen of the patients (32%) in this group showed a concomitant partial loss of chromosome 14q. Only three of 45 AL amyloidosis patients with gain 1q21 showed a concomitant gain of 11q.

Focusing on the most commonly affected region of all patients with partial loss of chromosome 14q there was a significant association with gain 1q21 (33% *vs.* 12%, P<0.005). Furthermore, we also detected a significant association between patients with a deletion in this region in combination with gain 1q21 and clinical parameters (Table 3). Two patients in our cohort carried a t(4;14), together with a partial deletion 14q. The other chromosomal region frequently deleted in the gain 1q21 group concerned 16q, which was affected in 11 patients (24%) compared to 1% in the group of patients without gain 1q (P<0.001). Again, there were two patients with translocation t(14;16) in our cohort, none showed a partial deletion of 14q and only one had a deletion 16q. No significant association of this patient group to clinical parameters was observed.

Gain 1q21 in association with HD vs. NHD karyotype: The group of 45 patients with gain 1q21 comprised 16 patients belonging to the HD-CN group (36%) and 29 to the NHD group (64%). These HD-CN patients with gain 1q21 frequently showed trisomy of chromosomes 9 (94%), 19 (69%), 5 (63%), 15 (63%), and 3 (50%). In the NHD group with gain of 1q21, a partial loss of 14q and monosomy 13 was detected in eleven patients each (38%) and loss of 16q in nine patients (31%).

High-risk patients: Defined high-risk aberrations deletion 17p13, t(4;14), and t(14;16) were detected in six, three, and two patients, respectively. Three patients with deletion 17p13 also carried a translocation t(4;14) and two concomitantly showed a t(14;16). This group showed associations to gain 1q and monosomy 13 in four and seven of the eight patients, respectively, whereas monosomy or partial loss of chromosome 14 was detected in three patients.

Potential prognostic role of the new CN array findings

We analyzed the following genetic aberrations in terms of hematologic remission, event-free survival (EFS) and overall survival (OS): loss of 1p (together with high-risk aberrations), gain of 1q with partial deletion 14q, and deletion 16q (Figure 2). We could not find any associations with hematologic remission defined as very good partial response or better after end of treatment (*data not shown*). However, we found a potential negative prognostic role of loss of 1p (EFS: median of 2 months *vs.* 14.5, P<0.05 and OS: median of 3 *vs.* 28 months, P<0.05) which was maintained for OS when we grouped these patients with our high-risk aberrations (median of 3 *vs.* 28 months, P<0.01; Figure 2B). In addition there was a borderline significant adverse effect for deletion 14q in patients with gain 1q regarding EFS (median of 13 *vs.* 15 months, P=0.06, Figure 2C).

Concordance of aberrations detected by iFISH and CN array

Of all 118 AL amyloidosis patients, iFISH analysis results were available for genomic regions 1q21, 5p15/5q35, 8p21, 9q34, 11q22.3 or 11q23, 13q14, 15q22, 17p13, and 19q13 in 99.8% of cases (1178 of 1180 single iFISH results). Balanced translocations detected by iFISH are not detectable by CN analysis. Overall concordance between iFISH and CN array results was 98% (range: 92-100%, Table 2). In 28 patients, a discordance was observed in 32 single iFISH results attributable almost exclusively to subclone aberrations (<35%) detectable by iFISH, but hardly perceptible by CN array.

Discussion

To our knowledge, this is the first report on a genome-wide CN survey of a large cohort of AL amyloidosis patients. The frequencies of aberrations detected by iFISH in the study herein were comparable to those in previous patient cohorts of studies from our group and others.[2-6,23] The most frequent chromosomal aberration detected in AL amyloidosis was translocation t(11;14), followed by gain of 11q22.3 or q23, deletion 13q14, gain of 1q21, and gain 19q13. The seemingly higher frequency of 36% of gain 1q in our patient cohort was comparable to iFISH results of probe 1q21 in MM,[9-15] yet slightly higher than previously published results regarding AL amyloidosis patients ranging between 23% and 28%.[6,23,24] This as well as the slightly higher frequencies of translocation t(11;14) and deletion 13q14 are partly attributable to our inclusion criteria, which only considered patients with at least one cytogenetic aberration detectable by iFISH and patients with a high enough number of purified clonal (i.e., aberrant) plasma cells, namely of 60% and above, for performing CN array analysis.

Hyperdiploidy and translocation t(11;14) were shown to be mutually exclusive in AL[5,6] and MM.[9,25-30] CN analysis grouped more patients into the HD group as compared to iFISH. So obviously, the rate of non-hyperdiploidy karyotypes of 75% was lower than that detected in previous

iFISH trials.[6] Nevertheless, the significant association between NHD status and translocation t(11;14) and the absence of association to monosomy 13 and IgH translocation with an unknown partner fully confirmed previous iFISH results. Furthermore, our results support the concept of hyperdiploidy or non-hyperdiploidy as the two major pathogenetic pathways in these plasma cell dyscrasias.[28,30] The previously described association of gain 1q21 with hyperdiploidy was found in our cohort only when the HD group was defined by iFISH (comparable to the classification in Bochtler *et al*.[6]). When CN results defined HD, this association was no longer significant. As far as associations of clinical parameters are concerned, the higher frequency of intact immunoglobulin as well as a higher degree of plasmacytosis and greater age in HD as compared to NHD patients, as reported earlier,[5,6] could also be confirmed by our study.

Translocation t(11;14) group

Our CN analysis showed that gain of 11q detected by iFISH in patients not belonging to the HD group with a translocation t(11;14) are almost always due to an unbalanced translocation der(11)t(11;14)(q13;q32), and are not caused by trisomy of chromosome 11. In the majority of patients, the translocation breakpoint was localized within the region of *CCDN1* and *MYEOV*. Patients in this group showed less genomic complexity indicated by a lower number of aberrations, i.e., genetic instability, which is comparable to findings in MM[6,9] and reinforces the concept that the occurrence of t(11;14) as an initiating event in plasma cell ontogenesis leads to a more stable phenotype.

Hyperdiploid karyotype

Detecting hyperdiploidy by CN array identified six additional patients, five of whom had not less than trisomies of chromosome 11 and 18, whereas the sixth of these patients showed a trisomy of chromosomes 9 and 17. Thus, the percentage of HD patients in our AL amyloidosis cohort is slightly underestimated by the score of Wuilleme *et al*.,[20] as already considered by Avet-Loiseau *et al*. for MM.[31]

When CN aberrations were used to define hyperdiploidy, no significant difference could be detected between the appearance of gain 1q21 in HD and NHD patients. Future studies that include trisomies of chromosomes 11 and 18 to define HD patients could investigate if the association of gain 1q to HD is more a matter of bias due to the probes for chromosomes used for classification or actually exists.

Gain of 1q21

Gain of the region investigated by the iFISH probe for chromosome 1, i.e., 1q21, almost exclusively resulted from a gain of the whole long arm of chromosome 1 but not due to trisomy 1. Gain 1q21 has been demonstrated to be of adverse prognostic impact in melphalan/dexamethasone treated AL amyloidosis patients,[25] and is considered by some authors to be a progression marker in AL[23,24] as well as in MM.[32-35] The association of partial deletions of chromosomes 14q and 16q - not attributable to t(4;14) or t(14;16), respectively - with gain 1q21 has not been noted before in clonal plasma cells. Deletions of material of chromosome 14q and 16q have been described in MM with significant adverse prognostic effect, but others found no prognostic significance of partial deletion of chromosome 16q.[11,13,36-38] Two tumor suppressor genes are located in the minimal common region of the deletions on chromosome 14q: *JDP2* has been described as a *MYC* collaborating gene that has been implicated in suppressing p53 function.[39] Furthermore, it is downregulated in many human cancers[40] and plays a pivotal role during myeloid and lymphoid commitment from hematopoietic progenitors.[41] *MLH3* is involved in DNA mismatch repair, however, data on the role of the mismatch repair pathway in MM is scarce and discordant.[42,43] Interestingly, Walker *et al*.[12] also described a deletion of region 14q24.1-q31.1 in 15% of the MM patients in their study in which gene expression differences were also analyzed. Within the deleted region 14q24.1-q31.1 a total of 33 genes were underexpressed, although it was not documented whether these included *JDP2* and *MLH3*. As *MLH3* is also involved in the pathogenesis of hereditary tumor syndromes we wanted to rule out germline deletions of *MLH3* in our patients. Therefore, we checked for homozygous deletions in this region as most of the AL patients in our study were included in a recent genome-wide association study.[44] Using genotype data on blood DNA from 15 patients with partial loss of chromosome 14q, we checked the *MLH3* locus on 14q24 for stretches of homozygous single nucleotide polymorphisms between rs1548807 (at nucleotide 75,479,582, genome build 37) and rs7303 (75,520,065) spanning the gene. None of the 15 patients were homozygous over the *MLH3* locus ruling monoallelic deletion.

Comparison of the iFISH probe set with genome-wide CN array analysis results

We were able to show the reliability of the iFISH panel that is used in the diagnostics of AL amyloidosis patients by the high concordance with the CN results (median: 98%). This high concordance is in line with similar studies investigating MM and chronic lymphocytic leukemia patients by array-based Comparative Genomic Hybridization that report a concordance of 91.8% and 95.5% for overall results, respectively.[15,45] As demonstrated by the CN array analysis data of this study, our iFISH panel covers most relevant aberrations. CN analysis, in contrast to iFISH, is able to clearly distinguish whole chromosome gains from partial gains and thus accurately identify trisomies, which is particularly relevant in the context of hyperdiploid karyotypes. Additionally, the use of magnetic-activated cell sorting of the CD138-positive plasma cells to conduct iFISH and CN array analysis contributed to the high concordance of CN and iFISH results in our study.

Potential prognostic role of the new CN array findings

Our survival analyses imply a possible prognostically adverse effect of loss of 1p and deletion 14q. However, these results should be interpreted with caution as our patients were not treated homogenously (see Table 1), and the cohort size as well as the number of patients with the respective CN aberration are rather small for prognostic assessments.[24,46,47]

Deletions on chromosome 1p were rarely detectable in our AL cohort (3%), which is contrary to the findings in MM, where it was found in up to 25% and 30% in a cohort of newly diagnosed and relapsed MM patients,[13,15] respectively, and has been shown to be associated with adverse prognosis.[13,48] Deductive reasoning suggests that

the next step will be to evaluate our findings in a larger cohort of homogenously treated AL amyloidosis patients, e.g., by suitable iFISH probes, and evaluate a potential additional prognostic effect of the deletions on chromosome 1p and 14q.

In conclusion, we report for the first time on a genome-wide CN array analysis of a large cohort of 118 AL amyloidosis patients. We were able to detect hitherto unrecognized associations between prognostically relevant chromosomal aberrations in addition to the confirmation of known associations between CN aberrations. With respect to a potentially adverse prognostic effect, the concomitant partial deletion of 14q and gain 1q as well as the observed effect of a partial deletion of chromosome 1p should be further investigated to validate the results of this study.

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