Novel recurrent chromosome anomalies in Shwachman–Diamond syndrome

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Abstract
Background: Two chromosome anomalies are frequent in the bone marrow (BM) of patients with Shwachman–Diamond syndrome (SDS): an isochromosome of the long arm of chromosome 7, i(7)(q10), and an interstitial deletion of the long arm of chromosome 20, del(20)(q). These anomalies are associated with a lower risk of developing myelodysplasia (MDS) and/or acute myeloid leukemia. The chromosome anomalies may be due to an SDS-specific karyotype instability, reflected also by anomalies that are not clonal, but found in single cells in the BM or in peripheral blood (PB).

Procedure: Starting in 1999, we have monitored the cytogenetic picture of a cohort of 91 Italian patients with SDS by all suitable cytogenetic and molecular methods.

Results: Here, we report clonal chromosome anomalies that are different from the aforementioned, as well as changes found in single cells in BM/PB of the same patients.

Conclusions: Some of the newly recognized clonal anomalies in BM reported here are recurrent, especially unbalanced structural anomalies of chromosome 7, a further complex rearrangement of the del(20)(q) with duplicated and deleted portions, and an unbalanced translocation t(3;6), with partial trisomy of the long arm of chromosome 3 and partial monosomy of the long arm of chromosome 6. Firm conclusions on the possible prognostic relevance of these anomalies would require further study with larger patient cohorts, but our data are sufficient to suggest that these patients necessitate more frequent cytogenetic monitoring. The results on anomalies found in single cells confirm the presence of an SDS-specific karyotype instability.

Keywords
acute myeloid leukemia, chromosome anomalies, karyotype instability, myelodysplastic syndrome, Shwachman–Diamond syndrome

1 | BACKGROUND

Two chromosome anomalies are frequently acquired in the bone marrow (BM) of patients with Shwachman–Diamond syndrome (SDS): isochromosome of the long arm of chromosome 7, i(7)(q10), and interstitial deletion of the long arm of chromosome 20, del(20)(q).1 The role of these clonal changes in relation to the risk of these patients to develop myelodysplasia (MDS) and/or acute myeloid leukemia (AML) has been investigated, and both anomalies have been shown to be associated with a low risk.2,3 Starting in 1999, we monitored the cytogenetic picture (in BM and peripheral blood [PB]) in a cohort of Italian patients with SDS (in total 91 subjects). We report here clonal chromosome anomalies found in these patients different from the more frequent ones, associated or not with the i(7)(q10) or the del(20)(q). Some of these chromosome changes are recurrent, and we tried to infer their possible relevance in prognostic evaluations.

We have already postulated that clonal chromosome anomalies are due to an SDS-specific karyotype instability, which may be reflected by anomalies that are not clonal, but found in single cells both in the BM and PB.4,5 Therefore, we also report on the chromosome anomalies that we found in single cells in BM or PB of the same patients.
2 METHODS

2.1 Patients

For the analysis, we took into account the results of all cytogenetic analyses performed since 1999 in the follow-up of our cohort of 91 Italian patients with SDS, all with ascertained biallelic mutations of the SBDS gene. The cohort included 57 males and 34 females, with a median age of 20 years, excluding six patients who died during the study (three males and three females). More details on gender and age are presented in Supplementary File S1. A portion of the cytogenetic results has already been reported. The patients are identified by their unique patient number (UPN), both in the text and in the tables, as in our previous publications.

Informed consent to this study was obtained according to the principles of the Declaration of Helsinki from the patients or from patients’ parents.

2.2 Methods

Chromosome analyses were performed using routine methods and the Q-banding by fluorescence using quinacrine technique on BM direct preparations and 24–48 hr cultures, and on unstimulated and phytohaemagglutinin-stimulated PB cultures.

FISH on metaphases and interphase nuclei was carried out with standard procedures utilizing the probes and libraries described previously: these are able to detect the most frequent chromosome anomalies and monitor abnormal clones with less frequently identified anomalies.

The microarray-based comparative genomic hybridization (a-CGH) was performed with the 244 K genome-wide system (Agilent Technologies Inc., Santa Clara, CA, USA), according to the manufacturer’s instruction on DNA from BM and PB. The DNA was extracted using the Qiagen Flexigene kit (QIAGEN GmbH, Hilden, Germany), and competitor DNA was either purchased from Promega (Promega Corporation, Madison, WI, USA) or from Agilent as part of the labeling kit. Slides were scanned using Agilent’s microarray scanner G2565CA and microarray images were analyzed using Agilent’s Feature Extraction 10.7.3.1 or 12.0.2.2 software, and with Agilent’s Genomic Workbench software (5.0.14 and 7.0.4.0). All results refer to the genome assembly hg19.

3 RESULTS

We monitored all available patients at least once a year. Therefore, these longitudinal cytogenetic data provided a chromosome picture of the BM varying over time, specifically the proportion of cells with anomalies, the appearance of novel clonal anomalies, variation in their percentage, and even anomalies that disappeared. Clonal chromosome anomalies in BM were present in 41 out of 91 patients. In 19 of these, the anomaly was already present when they were enrolled into the cohort, whereas in 22 it was acquired during the follow-up. The i(7)(q10) was observed in 17 patients, and the del(20)(q) in 15, both of these changes were found in three, but in independent clones, as already mentioned. So, in total, the most frequently observed clonal anomalies were found in 35 patients. Other, different, clonal anomalies were found in the BM of 13 patients, in eight cases in the absence of i(7)(q10) or del(20)(q) (Table 1), and in five cases in association with one of these changes (Table 2). We defined these anomalies as clonal also when detected in very few cells, in some cases even only one or two cells, but observed repeatedly in analyses made during subsequent years. Tables 1 and 2 list the results obtained from the analyses in which informative results were found, and give the proportion of cells with an infrequent anomaly from each analysis. All structural anomalies were confirmed by FISH with libraries for painting of the involved chromosomes and with informative probes, also on interphase nuclei in some patients (UPN 53 and 58). Five patients with a clone bearing one of the more common changes also developed a clone with an infrequent anomaly (Table 2). We have already demonstrated that in all cases the additional anomaly is acquired in an independent clone. In Table 2, these patients are listed with their karyotype and the number of cells from each different clone.

Whenever available, the a-CGH results are also included in Tables 1 and 2, while details on the size of the material lost or gained and on the breakpoints are given in Supplementary Table S1. These data were instrumental in the precise definition of the unbalanced anomalies of patients UPN 17, 20, 23, 53, 78, and 86. The percentage of abnormal cells in BM given in the a-CGH results in Tables 1 and 2 was evaluated by the formula proposed by Valli et al. In UPN 17, 20, and 86, these percentages indicate the presence of more than one abnormal clone. The normal a-CGH results obtained in UPN 15, 28, 58, and 29 are due to the small number of BM cells bearing the chromosome anomaly, whereas the a-CGH result of UPN 1 provided evidence of i(7)(q10) and not of the clonal inversion of chromosome 7 present in a few cells.

Patients UPN 17 and 20, with a clone carrying the customary del(20)(q), developed another clone in which a different anomaly derived from a further rearrangement of the del(20)(q). This abnormal chromosome had the appearance of a chromosome 20 deleted both in the short and in the long arm. The a-CGH showed the derivative chromosome 20, der(20), to be a complex rearrangement with deleted and duplicated regions (Table 2). The rearrangement was almost identical in both patients, and has already been reported by our group. The a-CGH profiles have been reported as well in Valli et al. The different percentages observed in the deleted and duplicated regions in these two patients (Table 2) are due to the major deletion of the long arm present both in cells with the del(20)(q) and cells with the rearranged der(20), whereas other imbalances regard only the cells with the der(20). Of note, in both of these patients, another small clone with 47 chromosomes and a duplicated der(20) was also found (Table 2).

In patient UPN 86, who had an unbalanced complex translocation t(1;3) in the BM, the imbalances concerned chromosome 1, with two different deletions of the short arm present in one clone (31% of the cells), and a duplication of the short arm in 15% of the cells.

Table 3 lists the nonclonal chromosome anomalies detected in single cells in BM and PB in the absence of i(7)(q10) or del(20)(q) (UPN 8, 10,
### Table 1
Patients with clonal anomalies in the absence of i(7)(q10) or del(20)(q)

| UPN | Material (year)* | Age (years) | Karyotype/FISH | a-CGH results - % abnormal cells (year) | Clinical outcome |
|-----|------------------|-------------|----------------|----------------------------------------|-----------------|
| 12  | BM (2003)        | 24          | 45XX,del(5)(q13q31or33),t(11;17)(q12;p13), -17,+der(17)(11;17)(q12;p13), -18 (15)/45XX,del(5)(q13q31or33), -7,-11,-17. +der(17)(11;17)(q12;p13)x2,-18,+mar [5] | na                      | AML, dead (2003) |
| 15  | BM (2004)        | 11          | 46XX,del(7)(q22q32) [3]/46,XX [63] | Normal (2007) | No complications |
| 19  | BM (2004)        | 6           | 46XY,add(7)(p?) [2]/46XY [22] | na                      | MDS, HSCT (2004) |
| 28  | BM (2005)        | 10          | 46XXX,del(X)(q23) [2]/46XX [17] | Normal (2009) | No complications |
| 53  | BM (2010)        | 1           | 46XX,der(6)(t;3;6)(q24;q13) [11]/46,XX [3] | dup(3)(q24q29) del(6)(q13q27) – 76% (2010) | (2011) |
| 58  | BM (2008)        | 1           | 46XY,der(16)(t;16)(q21q23) [1]/46XY [6] | na                      | BM aplasia, HSCT (2011) |
| 78  | BM (2014)        | 13          | 46XY,der(6)(t;3;6;3)(q24;q13) [3]/46,XY [6] | na                      | MDS, AML, HSCT (2014) |
| 86  | BM (2015)        | 37          | 46XY,der(16)(t;16)(q21q23) [13]/46,XY [6] | del(1)(p36.31p36.21) – 31% (2014) | MDS |

BM, bone marrow. In some patients, multiple analyses were performed within the same year.

a-BM, unique patient number.

Details of the a-CGH results regarding the size of the duplication/deletion and the breakpoints are provided in Supplemental Table S1.

bResult obtained with FISH on mitoses.

cResult obtained with FISH on interphase nuclei.

dSmall deletion of chromosome 17 not detectable at chromosome analysis, but revealed by a-CGH.
| UPN | Material (year) | Age | Karyotype/FISH | a-CGH results on BM-% abnormal cells (year) | Clinical outcome (year) |
|-----|----------------|-----|----------------|--------------------------------------------|-------------------------|
| 1   | PB-PHA (2003)  | 8   | 46,XY,inv(7)(p13q21) [7]/46,XY [143] | del(7)(p22.3p11.2)dup(7)(q11.21q34.3)−32% (2001) | No complications |
|     |                |     |                |                                            |                         |
| 17  | BM (2005)      | 20  | 46,XY,del(20)(q11.21q13.31) [3]/46,XY +der(20) [9]/46,XY [11] | del(20)(p13q11.2) − 34%, dup(20)(q11.21q13.31) − 47%, del(20)(q11.21q13.31) − 55% (2008) | No haematological complications |
|     |                |     |                |                                            |                         |
| 20  | BM (2004)      | 31  | 46,XY,del(20)(q11.21q13.32) [18]/46,XY +der(20) [6]/47,XY +der(20) [1]/46,XY [2] | del(20)(p13q11.21) − 24%, dup(20)(q11.21q13.32) − 67%, dup(20)(q13.32q13.33) − 24% (2008) | No complications |
|     |                |     |                |                                            |                         |
|     |                |     |                |                                            |                         |
|     |                |     |                |                                            |                         |
| 23  | BM (2010)      | 9   | 46,XX,i(7)(q10) [5]/46,XX [16] | del(5)(q31.1q35.3)del(7)(p22.3p11.2)del(13)(q11.21q13.3)del(15)(q21.3)del(18)(q11.2q12.2)del(18)(q21.1q23.3)−81% (2011) | MDS, AML (2011), dead (2012) |
|     |                |     |                |                                            |                         |
| 29  | BM (2008)      | 4   | 46,XY,i(7)(q10) [4]/46,XY,del(7)(q34) [4]/46,XY [9] | Normal (2008) | MDS (2008), HSCT* (2008), dead (2008) |

BM, bone marrow; HSCT, hematopoietic stem cell transplantation; PB-PHA, peripheral blood PHA-stimulated cultures; UPN, unique patient number. Monitoring of patients UPN 17 and 20 was performed every year in 2003–2008 and 2004–2015, respectively, but only representative results are shown here. Details of the a-CGH results regarding the size of duplications/deletions and the breakpoints are provided in Supplemental Table 1.

*BM: bone marrow; PB-PHA: peripheral blood PHA-stimulated cultures.
*aResult obtained with FISH on mitoses.
*bImbalances showing the presence of the i(7)(q10).
*cDead due to dilative cardiomyopathy (2008).
*dHSCT: Haematopoietic stem cell transplantation.
| UPN  | Common anomaly present/age (years) | Material (year)/age (years) | Karyotype/FISH                                                                 | Clinical outcome (year)       |
|------|-----------------------------------|----------------------------|-------------------------------------------------------------------------------|------------------------------|
| 8    | –                                 | PB-PHA (2004)/16           | 46,XX,t(7;14)(p15;q11.1) [2]/46,XX [156]^a                                   | No complications             |
| 10   | –                                 | PB-PHA (2002)/6             | 46,XY,t(7;14)(p15;q13)[1]/46,XY [129]^a                                     | No complications             |
| 21   | –                                 | BM (2004)/1                 | 45,XX,-7[1]/46,XX [28]                                                       | No complications             |
| 30   | –                                 | BM (2005)/1                 | 46,XY,rea(2;?) [1]/46,XY [19]                                                | No complications             |
| 38   | –                                 | PB-PHA (2008)/1             | 46,XY,t(7;14)(p15;q13)[1]/46,XY [91]                                         | No complications             |
| 41   | –                                 | BM (2013)/8                 | 45,XX,-7[1]/46,XX,t(9;13)[q22;q22][1]/46,XX [10]                            | No complications             |
| 49   | –                                 | PB-PHA (2010)/21            | 45,XY,-2,der(20)(2;20)[p11;q13.3][1]/46,XY,t(3;7)[q12;p15][1]/46,XY [98]    | MDS (2010), HSCT (2010)     |
| 4    | del(20)(q) since 2005/16           | PB-PHA (2002)/13            | 46,XY,t(10;20)[q;p?][1]/46,XY [103]                                         | No complications             |
| 11   | i(7)(q10) since 2003/10            | PB-PHA (2004)/11            | 45,XY,-7[1]/46,XY [20]                                                      | MDS (2003)                   |
| 13   | del(20)(q) since 2009/11           | BM (2003)/5                 | 47,XY,+mar[1]/46,XY [21]                                                    | MDS (2013)                   |
| 25   | i(7)(q10) since 2004/23            | BM (2008)/27                | 45,XX,-7[1]/46,XX,inv(7)[p?q?][1]/46,XY [15]/46,XY[2]                      | BM aplasia (2012), HSCT (2012)|
| 35   | del(20)(q) since 2010/7            | BM (2010)/7                 | 46,XX,t(8;11)[q22;q21][1]/46,XX [22]                                        | No complications             |
| 46   | i(7)(q10) since 2009/2             | PB-PHA (2009)/2             | 46,XY,t(7;14)[p15;q11][1]/46,XY,inv(7)[p?q?][1]/46,XY [57]^a                | No complications             |
| 61   | del(20)(q) since 2011/’<1          | PB-PHA (2011)/’<1           | 46,XY,t(7;14)[p13;q12][1]/46,XY [99]                                        | BM aplasia (2012), HSCT (2013)|
| 84   | del(20)(q) since 2015/20           | BM (2015)/20                | 46,XY,der(6),der(7)rea(6;7) [6p23:25;7p12:7q32],del(20)[q11][1]/46,XY [4] | No complications             |

BM, bone marrow; HSCT, hematopoietic stem cell transplantation; PB-PHA, PHA stimulated peripheral blood cultures; UPN, unique patient number.

^aResult obtained with FISH on mitoses.
FIGURE 1  a-CGH profiles of BM of patients UPN 53 and 78, with partial trisomy of the long arm of chromosome 3 and partial monosomy of the long arm of chromosome 6

21, 30, 38, 41, and 49) or in association with these common changes (UPN 4, 11, 13, 25, 35, 46, 61, and 84). In Table 3, as in Tables 1 and 2, only the results of analyses that disclosed nonclonal anomalies are presented.

4 | DISCUSSION

In the clonal anomalies (different from i(7)(q10) and del(20)(q)) reported here the distribution of the chromosomes involved was markedly disparate. If we take into account both the numerical and structural changes listed in Tables 1 and 2, there were 28 different anomalies in which chromosome 7 was involved six times, chromosome 6 four times, and chromosomes 3, 5, 17, 18 and 20 twice each. This result was even more evident in nonclonal anomalies found in single cells (Table 3): chromosome 7 was involved 15 times, chromosomes 2 and 14 five times, and chromosome 20 twice.

According to the literature, the presence of i(7)(q10) and del(20)(q) is associated with a lower risk of developing MDS/AML. However, the
presence of one or more independent clones with other anomalies could be linked to a higher risk: in our cohort, MDS/AML developed in two out of five such cases (Table 2).

An analysis of all the patients with anomalies different from i(7)(q10) and del(20)(q) reported here showed that MDS/AML developed in six out of 13. A complex karyotype, with more than three different independent changes, was found in two patients when they had already developed MDS/AML (UPN 12 and 23), while structural anomalies (deletions, inversions, and unbalanced translocations) were present in the other cases.

Some changes are noteworthy, as they were recurrent, and they are as follows. First, structural rearrangements of chromosome 7, mainly unbalanced (deletions, inversions, or translocations), were present in five of our 13 patients (UPN 1, 15, 19, 23, 29), three of whom developed MDS/AML. Second, a further complex rearrangement of the more common del(20)(q), leading to duplicated and deleted portions, was identical in two patients (UPN 17 and 20). Their a-CGH profiles astonished us, as they were almost identical (Tables 2 and 3); neither developed MDS/AML. Third, an unbalanced translocation t(3;6), with partial trisomy of the long arm of chromosome 3 and partial monosomy of the long arm of chromosome 6 (Fig. 1), was not identical but very similar in two patients without i(7)(q10) or del(20)(q) (UPN 53 and 78) (Table 1), one of whom developed MDS/AML.

In a comparison of our results with those in the literature, we made the following observations. Anomalies of chromosome 7 have been recognized for some decades as the most frequent changes in the BM of SDS and they include the i(7)(q10). Besides this frequently observed change, in the literature there are at least 14 cases of monosomy 7, some of which were del(20)(q), four cases of deletion of the long arm of chromosome 7, and four cases of unbalanced translocation involving chromosome 7. Our results add one case of monosomy (UPN 12), one case of pericentric inversion (UPN 1), two with a deletion of the long arm (UPN 15 and 29), and two with an unbalanced translocation (UPN 19 and 23) (Tables 1 and 2).

No patient in the literature has been shown to bear the complex rearrangement seen in our subjects, UPN 17 and 20. Two patients reported by Donadieu et al. had an unbalanced translocation between chromosomes 3 and 6 that might be similar to our cases, UPN 53 and 78, although the definition of the translocation in Donadieu et al.’s study was incomplete.

Nonclonal chromosome changes, found in single cells (Table 3), were present in 15 of 91 patients in our cohort, with a frequency higher than control subjects. In particular, a translocation t(7;14) found in five cases, with a much higher frequency as compared to controls. These data support our former hypothesis of a peculiar SDS-associated karyotype instability, which may be linked to the acquisition of clonal anomalies in the BM and to variations in the risk of progression to MDS/AML (higher or lower according to the chromosome anomaly acquired).

Patients with SDS and rare, infrequent acquired chromosome anomalies in BM need more frequent cytogenetic monitoring due to a possible higher risk of developing MDS/AML; this monitoring should include standard cytogenetics, FISH, and more sophisticated methods to detect chromosome imbalances, such as array-CGH.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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