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Shwachman-Diamond syndrome with clonal interstitial deletion of the long arm of chromosome 20 in bone marrow: haematological features, prognosis and genomic instability

Roberto Valli,[1] Antonella Minelli,[2] Marta Galbiati,[3] Giovanna D'Amico,[3] Annalisa Frattini,[1,4] Giuseppe Montalbano,[1] Abdul W. Khan,[1] Giovanni Porta,[1] Giorgia Millefanti,[1] Carla Olivieri,[2] Marco Cipolli,[5] Simone Cesaro,[6] Francesco Pasquali,[1] Cesare Danesino,[2] Gianni Cazzaniga[3] and Emanuela Maserati[1]

[1]Genetica Umana e Medica, Dipartimento di Medicina e Chirurgia, Università dell'Insubria, Varese, [2]Genetica Medica, Fondazione IRCCS Policlinico S. Matteo and Università di Pavia, Pavia, [3]Immunology and Cell Therapy, Centro Ricerca Tettamanti, Paediatric Clinic, University of Milan Bicocca/MBBM, Monza, [4]Istituto di Ricerca Genetica e Biomedica, CNR, Milano, [5]Cystic Fibrosis Center, AOU Ospedali Riuniti, Ancona, and [6]Oncoematologia Pediatrica, Azienda Ospedaliera Universitaria Integrata, Verona, Italy

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Correspondence: Emanuela Maserati, Dipartimento di Medicina e Chirurgia, Università dell'Insubria, Via J. H. Dunant, 5, I 21100 Varese, Italy.
E-mail: emanuela.maserati@uninsubria.it

Summary

In Shwachman-Diamond syndrome (SDS), deletion of the long arm of chromosome 20, del(20)(q), often acquired in bone marrow (BM), may imply a lower risk of developing myelodysplastic syndrome/acute myeloid leukaemia (MDS/AML), due to the loss of the *EIF6* gene. The genes *L3MBTL1* and *SGK2*, also on chromosome 20, are in a cluster of imprinted genes, and their loss implies dysregulation of BM function. We report here the results of array comparative genomic hybridization (a-CGH) performed on BM DNA of six patients which confirmed the consistent loss of *EIF6* gene. Interestingly, array single nucleotide polymorphisms (SNPs) showed copy neutral loss of heterozygosity for *EIF6* region in cases without del(20)(q). No preferential parental origin of the deleted chromosome 20 was detected by microsatellite analysis in six SDS patients. Our patients showed a very mild haematological condition, and none evolved into BM aplasia or MDS/AML. We extend the benign prognostic significance of del(20)(q) and loss of *EIF6* to the haematological features of these patients, consistently characterized by mild hypoplastic BM, no or mild neutropenia, anaemia and thrombocytopenia. Some odd results obtained in microsatellite and SNP-array analysis demonstrate a peculiar genomic instability, in an attempt to improve BM function through the acquisition of the del(20)(q).

Keywords: Shwachman Diamond syndrome, del(20)(q), genomic instability, *EIF6* gene, risk of MDS/AML/BM aplasia.

Shwachman-Diamond syndrome (SDS) is an autosomal recessive disorder (Online Mendelian Inheritance in Man identification 260400) that is caused by mutations of the *SBDS* gene in at least 90% of cases (Maserati *et al*, 2009). It implies a wide spectrum of clinical signs and is characterized by exocrine pancreatic insufficiency, skeletal and neurodevelopmental abnormalities, bone marrow (BM) failure with peripheral cytopenias and an increased risk to develop myelodysplastic syndromes (MDS) and/or acute myeloid leukaemia (AML) (Dror, 2005). SDS is a ribosomopathy, as SBDS protein cooperates with the GTPase EFL1 to catalyse the removal of factor EIF6 from nascent 60S ribosomal subunit during ribosome biogenesis (Valli *et al*, 2017a; Warren, 2018). In a small proportion of cases, biallelic mutations of two other genes involved in ribosome biogenesis may cause SDS, or an SDS-like condition: *DNAJC21* (Dhanraj *et al*, 2017; D'Amours *et al*, 2018) and *EFL1* (Stepensky *et al*, 2017). Further, an SDS-like phenotype may be caused by monoallelic mutations of the gene *SRP54*, which produces a protein that is a key member of the cotranslational protein-targeting pathway (Carapito *et al*, 2017).

The most frequent clonal chromosome anomalies in BM of patients with SDS are 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) (Pressato *et al*,

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2012). Since 1999, we have followed a cohort of 96 Italian patients with SDS, including 18 patients with the exclusive del(20)(q) and two other patients who retain this abnormality in combination with i(7)(q10). We previously demonstrated by array-based comparative genomic hybridization (a-CGH) on DNA from BM, the loss of the Eukaryotic Initiation Factor 6 (*EIF6*) gene in all 6 of the patients tested patients from this group of 20 patients (Valli *et al*, 2013). EIF6 protein is necessary for ribosome biogenesis, and in mammals it is required for insulin and growth factor-stimulated translation; its physiological significance impacts on cancer and SDS (Brina *et al*, 2015). We postulated that *EIF6* hemizygosity in SDS patients permits more efficient ribosome biogenesis, leading to a lower risk of developing MDS and/or AML (Valli *et al*, 2013). The indirect evidence that *EIF6* hemizygosity affects ribosome biogenesis, has been demonstrated by polysomal profiles in knock-out heterozygous mice for the orthologue eIF6 gene (Gandin *et al*, 2008).

The genes *L3MBTL1* and *SGK2* are located in a cluster of imprinted genes on chromosome 20, and their loss might be related to dysregulation of erythropoiesis and megakaryopoiesis (Aziz *et al*, 2013). In six patients with del(20)(q) (five of which were included in our aforementioned a-CGH analysis), a possible preferential parental origin of the deleted chromosome 20 was excluded (Nacci *et al*, 2017). However, the authors noted that the haemoglobin concentration (Hb) and red blood cell count were higher in their SDS patients carrying del(20)(q) in comparison with 20 SDS patients without clonal del(20)(q) (Nacci *et al*, 2017).

We here report the results of a-CGH of six more patients, all with biallelic mutations of *SBDS* and del(20)(q). In addition, the analysis of parental origin of the deleted chromosome 20 in six patients was also examined. The overall results were compared with essential haematological data.

Furthermore, investigations on single nucleotide polymorphisms (SNPs) in arrays were performed on 14 patients with the del(20)(q) and/or other chromosome changes, to obtain further evidence of chromosome anomalies and instability.

Materials and methods

All the patients of this study presented the main typical phenotypic signs of SDS, and the diagnosis was confirmed by mutation analysis of *SBDS*. The BM of six patients of our cohort was used to perform chromosome and a-CGH analyses (Unique Patient Number (UPN) 1, 6, 35, 82, 84 and 85). Microsatellite study was performed to identify the parental origin of the deleted chromosome 20 in patients UPN 1, 14, 35, 82, 84 and 85. SNP-arrays were performed on 14 cases (UPN 1, 2, 13, 14, 20, 24, 29, 35, 36, 40, 54, 58, 65 and 84), seven of whom carry del(20)(q), two carry del(20)(q) in combination with i(7)(q10), six with i(7)(q10) alone and one exhibited an unbalanced translocation t(1;16)(q21;q23) (Table I). Results of chromosome analysis and/or a-CGH of patients UPN 1, 2, 6, 13, 14, 17, 20, 24, 29, 35, 36, 40, 65 and 68 have already been partially reported (Maserati *et al*, 2006, 2009; Pressato *et al*, 2010; Valli *et al*, 2013) (Table I).

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

Chromosome analyses were performed on BM with routine methods. Fluorescence *in situ* hybridization (FISH) on BM nuclei was carried out according to standard techniques with the following BAC probes, informative for the deletion detected in each patient: RP11-17F3 (UPN 6, 13, 17, 20, 35, 65, 82), CTD-2559C9 (UPN 13, 17), CTD-3092L7 (UPN 14) and XL Del(20q) probe (Metasystems, Altlussheim, Germany) (UPN 68, 84).

The a-CGH was performed on DNA from BM samples with the 244 K genome-wide system (Agilent Technologies Inc., Santa Clara, CA, USA), according to the manufacturer's instructions. All map positions in the results refer to the genome assembly map hg19.

The parental origin of the deleted chromosome 20 was determined by microsatellite analysis, as described by Nacci *et al* (2017), on the same DNA used for a-CGH. The short tandem repeat (STRs) polymorphisms used were chosen based on their heterozygosity (always above 80%): D20S484, D20S195, D20S890, D20S601, D20S847, D20S884, D20S891.

Table I. Bone marrow clonal anomalies of the patients studied in this report and the a-CGH/SNP arrays performed.

UPN	Clonal anomalies	a-CGH*	SNP array*
1	i(7)(q10)/del(20)(q)†	+¶/present paper	+
2	i(7)(q10)†	+¶	+
6	del(20)(q)†	+ present paper	−
13	del(20)(q)†	+**	+
14	i(7)(q10)/del(20)(q)†	+**	+
17	del(20)(q)‡	+**	−
20	del(20)(q)‡	+**	+
24	i(7)(q10)‡	+¶	+
29	i(7)(q10)‡	+ not informative ‡	+
35	del(20)(q)‡	+ present paper	+
36	i(7)(q10)‡	+¶	+
40	i(7)(q10)§	+§	+
54	i(7)(q10)	+¶	+
58	der(1)t(1;16)(q21;q23)	+ not informative	+
65	del(20)(q)	+**	+
68	del(20)(q)	+**	−
82	del(20)(q)	+ present paper	−
84	del(20)(q)	+ present paper	+
85	del(20)(q)	+ present paper	−

a-CGH, array comparative genomic hybridization; SNP, single nucleotide polymorphism; UPN, unique patient number.

*+ = performed; − = not performed.

†Cytogenetic analysis results reported in Maserati *et al* (2006).

‡Cytogenetic analysis results reported in Maserati *et al* (2009).

§Chromosome analysis and a-CGH results reported in Pressato *et al* (2010).

¶a-CGH confirms the i(7)(q10) (Maserati *et al*, 2009 and unpublished data).

**Valli *et al* (2013).

SNP-array analysis was performed on the DNA samples of 14 patients listed in Table I, genotyped by the Affymetrix® Cytogenetics Whole-Genome 2.7M Array (6 cases) or Affymetrix CytoScan HD Array (8 cases) (Affymetrix, Santa Clara, CA, USA) according to the manufacturer's protocol. Analysis of copy number variations and copy number neutral loss of heterozygosity (cnLOH) regions was performed with the Chromosome Analysis Suite (Affymetrix®, Santa Clara, CA, USA) software v.3.1.0.15 and based on hg19 assembly. Amplifications ≥20 kb and deletions ≥15 kb, containing a minimum of 20 markers in the region, were considered as significant. Detection of cnLOH was limited to aberrations longer than 2000 kb. Unfortunately, UPN 20 was analysed only for gains and losses, and not for cnLOH.

Results

Chromosome analysis on BM of the 12 patients used here for a-CGH (monitored at least once per year) showed the presence of the clonal interstitial deletion of chromosome 20.

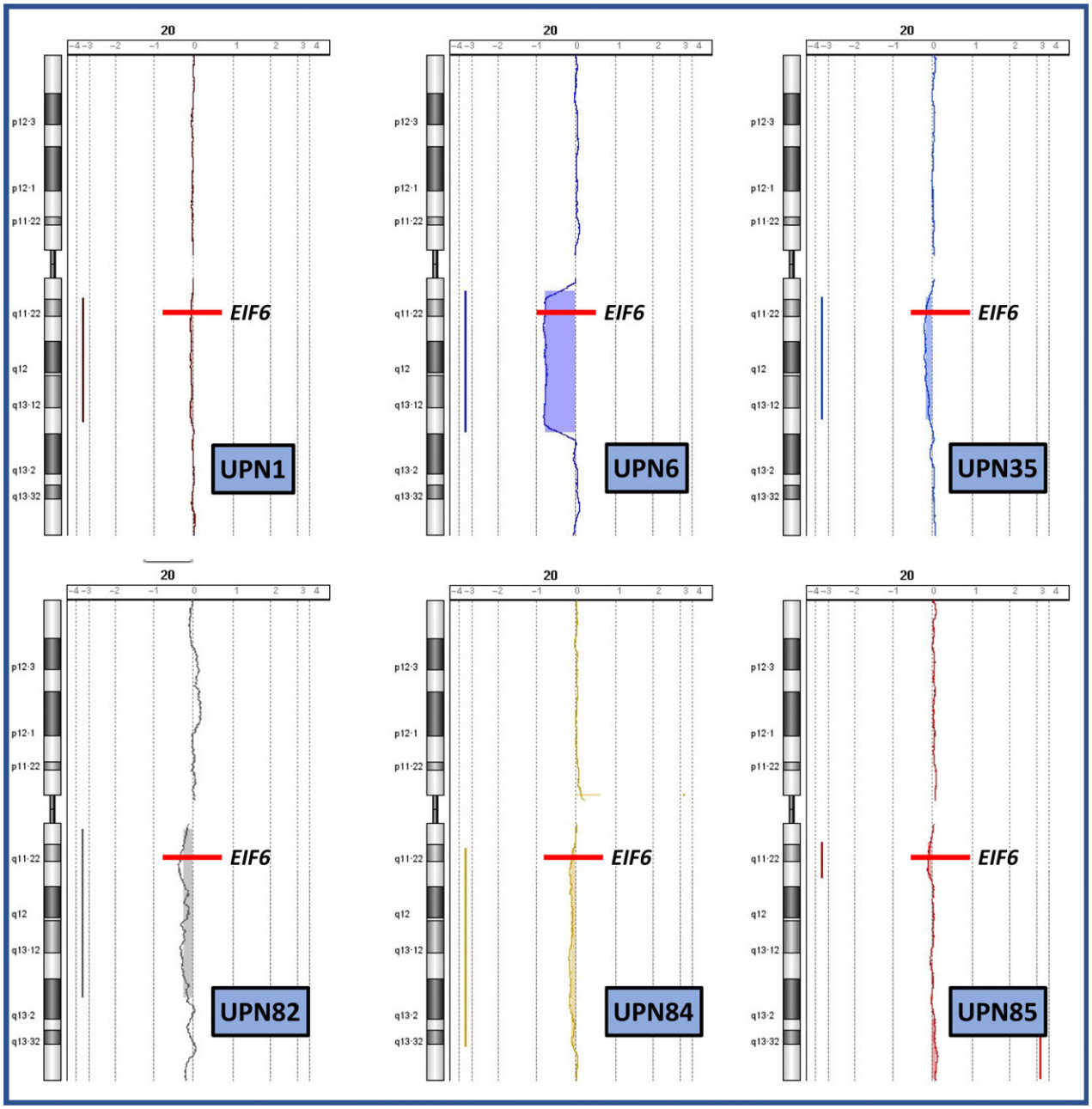


Fig 1. Array comparative genomic hybridization profiles of chromosome 20 in the newly investigated six patients with del(20)(q). Patients are identified by a Unique Patient Number (UPN). The profiles show extension of the interstitial deletion and the loss of the *EIF6* gene. [Colour figure can be viewed at wileyonlinelibrary.com]

The a-CGH results of the newly analysed six patients UPN 1, 6, 35, 82, 84 and 85 are shown in Fig 1. The positions of the breakpoints leading to the deletions are listed in Table II, which also gives the proportion of BM abnormal cells, as calculated with the formula suggested by Valli *et al* (2011). The proportion of cells containing the deletion was consistent with the evaluations obtained by FISH on nuclei with informative long arm probes (data not shown). The value of these proportions, variable at the time of our analyses, ranged from 9·5% to 82% (Table II).

SNP-array genotyping was performed on 14 SDS patients (Tables I and SI with raw data). Copy number variation analysis confirmed the presence of i(7)(q10) and del(20)(q), already identified by chromosome analyses and a-CGH (Maserati *et al*, 2009; Pressato *et al*, 2010; Nacci *et al*, 2017) (Table III). Interestingly, cnLOH regions involving the long arm of chromosome 20 were detected in three patients, namely UPN 1, 40 and 54. Two of them (UPN 40 and 54) were without del(20)(q) (Table I) and one case (UPN 1) showed del(20)(q) in a small proportion of cells (Table IV).

Microsatellite analysis on BM of the newly analysed patients with del(20)(q) (UPN 1, 14, 35, 82, 84 and 85) showed a different dosage of the paternal and maternal alleles in nine patients, providing evidence of the parental chromosome 20 deletion. The origin was paternal in two of these new cases, and maternal in one. In three patients (UPN 35, 82 and 85), unexpected discordant results were obtained, with some STRs indicating a paternal origin and others a maternal origin (Table IV).

There were some discordances between SNP array and cytogenetic/a-CGH results with regard to the start/stop points of the del(20)(q) and the abnormal cell proportion (Tables II, III, and SI). These differences are probably due to a different sensitivity of the technologies. Moreover, in some patients (UPN 1, 14, 20, 24 and 84) the material used for SNP array and a-CGH analyses was sampled at different dates, when the size of the abnormal clone might have changed.

Discussion

The results obtained from a-CGH of the 12 patients listed in Table II confirm that all deletions of the long arm of chromosome 20 in SDS are interstitial, that their proximal breakpoints are clustered in a rather small region of about 2600 kb, while the distal breakpoints are more variable. Excluding patients UPN 14 and 85, who presented very small deletions with the loss of 4150 and 4700 kb respectively, the distal breakpoints in the other 10 patients cluster in a segment of 11 227 kb, and the material lost is in the range 14 008 (UPN 68)–26 863 kb (UPN 13). One of these 10 patients (UPN 68) had two interstitial deletions present, with a segment of 2103 kb conserved between them.

The *EIF6* gene was lost in all 12 patients with the del(20)(q); moreover, three additional patients out of 14 analysed by SNP-array showed cnLOH encompassing the region containing *EIF6* (UPN 1, 40 and 54). These data on cnLOH further extend the involvement of this region in instability events; indeed, the minimal cnLOH region length was about 2200 kb and included the *EIF6* gene in all cases (Fig 2). Other recurrent cnLOH were identified, although their involvement in the disease could not be ascertained (Table III).

The *L3MBTL1* and *SGK2* genes were lost in all cases except UPN 14 and 85, i.e., the patients with the smallest deletions. The imprinted genes *L3MBTL1* and *SGK2* are expressed normally only if paternal in origin, thus suggesting possible differences in the BM status among SDS patients with either maternal (UPN 14, 20, 65, and 68) or paternal (UPN 1, 6, 13, 17, and 84) deleted chromosome. Considering the 12 available informative patients altogether, including those already reported (Nacci *et al*, 2017), the deleted chromosome 20 was of paternal origin in five cases and maternal

Table II. Results of array comparative genomic hybridization on bone marrow samples from the 12 patients with Shwachman-Diamond syndrome and acquired del(20)(q) listed in Table I. The bands involved and the start/stop points of the deletion provided by the bp positions give the details of the chromosome anomaly (genome assembly hg19).

UPN	Band	Deletion start (bp)	Deletion stop (bp)	% abnormal cells
1	q11.21–q13.13	31891819	48287277	9·50%
6	q11.21–q13.13	30922628	49497969	82%
13	q11.21–q13.32	30876455	57739620	55%
14	q11.21–q11.23	31163090	35309412	18·20%
17	q11.21–q13.31	31205853	55894884	47%
20	q11.21–q13.32	31294381	57252363	66·57%
35	q11.21–q13.13	31798183	47884947	20%
65	q11.21–q13.13	30157286	49497910	43%
68	q11.21–q13.12	31262228	43141623	16%
	q13.12–q13.13	45244728	47373188	
82	q11.21–q13.2	30020250	52206444	31·13%
84	q11.22–q13.33	32620650	58600338	15·50%
85	q11.21–q11.23	31814242	36538658	13·90%

UPN, unique patient number.

Table III. Recurring gains, losses (A) and copy number neutral loss of heterozygosity (B), found in ≥2 patients, derived from single nucleotide polymorphism-array analysis of 14 patients (Table I). Minimal common regions have been listed.

Patients	CN	Type of genomic variation	Chr	Bands	Size (kb)	Genomic location
(A) Recurring gains and losses						
1, 24, 36, 40, 54	1	Loss (mosaic)	7	whole p arm		
1, 24, 36, 40, 54	3	Gain (mosaic)	7	whole q arm		
13, 14, 20, 65, 84	1	Loss (mosaic)	20	q11.22–q11.23	3055	chr20:32758000-35813438
(B) Recurring cnLOH regions						
1, 13	2	LOH	2	p13.2–p12	3·061	chr2:72502269-75563148
13, 54	2	LOH	6	p22.1–p22.1	1·912	chr6:27363586-29275298
58, 84	2	LOH	3	p21.31–p21.2	2·189	chr3:49685591-51874275
1, 84	2	LOH	7	q11.21–q11.21	2·444	chr7:64140053-66583570
4, 13	2	LOH	8	p12–p12	2·022	chr8:32803572-34825223
2, 14, 24	2	LOH	8	q11.1–q11.21	2·091	chr8:46944404-49035329
14, 24, 36	2	LOH	10	q22.1–q22.2	2·369	chr10:74480275-76849397
29, 36	2	LOH	11	p11.2–p11.12	3·545	chr11:48018354-51563636
14, 29	2	LOH	14	q23.3–q24.1	2·055	chr14:65865670-67920573
1, 40, 54*	2	LOH	20	q11.22–q11.23	2·230	chr20:32738611-34968575
54, 40	2	LOH	X	q11.1–q12	5·140	chrX:62018109-67158519
36, 24, 54	2	LOH	X	q13.1–q21.1	6·336	chrX:71523649-77859592

Chr, chromosome; CN, copy number state; cnLOH, copy number neutral loss of heterozygosity; LOH, loss of heterozygosity.
*cnLOH 20q: see also Fig 2.

Table IV. Basic haematological data of the Shwachman-Diamond syndrome cohort included in Table I, and parental origin of the del(20)(q).

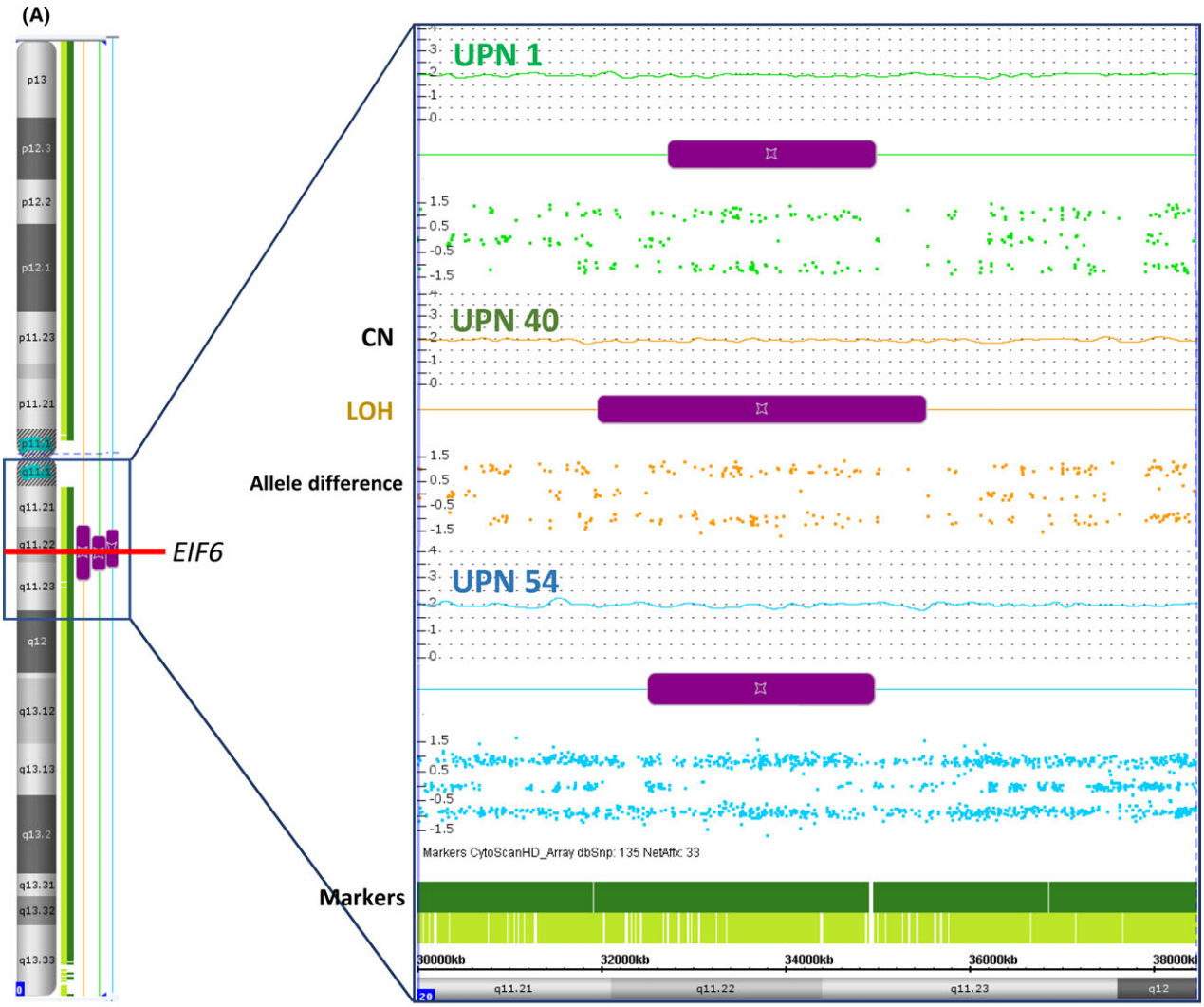
UPN	Age (years)	% abnormal cells	BM morphology	Neutropenia‡	Anaemia§	Thrombocytopenia¶	Parental origin of del(20)(q)
1*	9	9·5%	Almost normal	Mild	No	Mild	Maternal
6	18	82·7%	Almost normal	No	No	Mild	Paternal
13	11	55%	Almost normal	Mild	Mild	Mild	Paternal
14*	11	18.2%	Mild hypoplasia	Mild	No	No	Paternal
17†	23	48·5%	Mild hypoplasia	No	No	Mild	Paternal
20†	31	66%	Mild hypoplasia	Mild	No	Mild	Maternal
35	13	20%	Mild hypoplasia	Mild	Mild	No	Discordant
65	12	43%	Normal	No	No	Mild	Maternal
68	20	13·9%	Severe hypoplasia	No	No	Mild/No	Maternal
82	15	31·1%	Mild hypoplasia	Mild	Mild	Mild	Discordant
84	14	15·5%	Mild hypoplasia	Severe	No	Mild	Paternal
85	20	13·9%	Almost normal	Mild	No	Mild	Discordant

BM, bone marrow; UPN, unique patient number.
*Patient also had an independent clone with i(7)(q10).
†Patient also had a subclone with a rearrangement of the del(20)(q), with deleted and duplicated portions of chromosome 20 (Valli *et al*, 2017b).
‡Mild: neutrophil count 0·5–1·5 × 10^9/l, severe <0·5 × 10^9/l.
§Mild: Haemoglobin concentration 80–120 g/l, severe <70–80 g/l.
¶Mild: platelet count 50–150 × 10^9/l, severe <50 × 10^9/l.

in four (Table IV). So, no preferential parental origin of the deleted chromosome 20 was detected, and, therefore, the expression of these genes may be hardly relevant to the haematological phenotype of the patients with the del(20)(q).

In three patients (UPN 35, 82, 85) it was not possible to establish the parental origin of the deleted chromosome 20, because microsatellite analysis gave unexpectedly discordant results (Table IV). The simplest explanation may be a mitotic recombination between the two chromosomes 20 in the region of, or near to *EIF6*.

Both the discordant results obtained by microsatellite analysis and the cnLOH found by SNP-array may represent attempts to rearrange the chromosome, aiming to delete *EIF6* on chromosome 20. We hypothesize that the BM cells of SDS patients tend to attempt an improvement of their function, impaired by the defective SBDS protein, through the acquisition of the del(20)(q). Their attempt is successful when the deletion arises, while cnLOH might represent an unsuccessful attempt. The discordant microsatellite results might also represent attempts to rearrange chromosome 20,



(B)

Minimal Common LOH Region	Genes
Chr20:32738611-34968575 (2230 Kb)	*ASIP, AHCY, ITCH, MIR644A, DYNLRB1, MAP1LC3A,PIGU, TP53INP2, NCOA6, HMGB3P1,GGT7, ACSS2, GSS, MYH7B, MIR499A, MIR499B, TRPC4AP, EDEM2, PROCR, MMP24, MMP24OS, EIF6, FAM83C, UQCC1, GDF5, CEP250, C20orf173, ERGIC3, SPAG4, CPNE1, RBM12, NFS1, ROMO1, RBM39, PHF20, SCAND1, CNBD2, NORAD, EPB41L1, AAR2*

Fig 2. Copy number neutral loss of heterozygosity (cnLOH) of chromosome 20. (A) Detail of 20q cnLOH in UPN 1, 40 and 54. (B) List of genes included in minimal common LOH region. CN, copy number state; LOH, loss of heterozygosity; UPN, unique patient number. [Colour figure can be viewed at wileyonlinelibrary.com]

even in cases in which the *EIF6* gene was then successfully deleted. We previously postulated a peculiar kind of karyotype instability in the BM of SDS patients many years ago, when molecular data were not available (Maserati *et al*, 2000). We also reported evidence that several different cell lines, with different chromosome changes, can be found in BM of SDS patients (Pressato *et al*, 2015). This is case when the i(7)(q10) and the del(20)(q) are acquired subsequently in different clones, or clones with further rearrangements of del (20)(q), or together with i(7)(q10) or del(20)(q), other different anomalies in independent clones (Pressato *et al*, 2015; Valli *et al*, 2017b).

Table IV provides some basic information about the haematological condition of our patients with del(20)(q) at the time of a-CGH analysis, the criteria used to consider the level of severity of the condition, together with their age and proportion of BM cells bearing the del(20)(q). The definitions used to describe BM morphology come from the pathologists who analysed the preparations. In particular, "normal" and "almost normal" BM are based on standard generally accepted criteria (Greer *et al*, 2013). The benign prognostic significance of del(20)(q) and loss of *EIF6* may be sustained by the haematological features of these patients: BM picture and peripheral blood counts give evidence of a very mild condition in all our patients, both with regard to the BM morphology and the absent or mild peripheral blood cytopenias. Nevertheless, exceptions are represented by two borderline patients, with some more severe symptoms: patient UPN 68, with severely hypoplastic BM, but with no neutropenia, no anaemia and almost no thrombocytopenia (platelet count 143×10^9/l), and patient UPN 84, who had an almost normal BM morphologically, with neutropenia which may be considered severe (neutrophil count $0{\cdot}46 \times 10^9$/l), but no anaemia and mild thrombocytopenia (platelet count 127×10^9/l). Interestingly, these two patients are among those showing lower percentages of BM cells with the del(20)(q) (Table IV). Although some other patients with similar low percentages are present in our cohort (e.g. UPN 1 and 85), we do not know in which BM cell lineages the clonal anomaly is present, and this could vary from case to case.

We postulate that the loss of the *EIF6* gene due to the del(20)(q), as confirmed by the results reported here, is a good prognostic sign in general, in addition to the already suggested lower risk of transformation into MDS/AML (Pressato *et al*, 2012). To date, none of our 12 patients have encountered either this complication or evolution to severe BM aplasia. More extensive study on larger samples of patients, possibly with analysis of their follow-up, may further confirm this conclusion.

The 12 patients with del(20)(q) reported here are quite old for a disorder that is usually diagnosed in infants or children (Dror, 2005), as their age range is (in 2018), 14–44 years, with an average of 23 years. Also, this fact should be taken into account with regard to the risk of MDS/AML evolution, as it is well known that this risk increases with age (Maserati *et al*, 2006; Shimamura, 2006; Maserati *et al*, 2009; Pressato *et al*, 2010). Similarly, it is noteworthy that none of our patients showed haematogical features indicating progression towards BM severe aplasia. The lower risk of SDS patients with del(20)(q) to develop MDS/AML and to become frankly aplastic is further supported by these considerations concerning their age.

A comparison between the haematological data of the cohort of patients with del(20)(q) reported here and patients without this anomaly is not really feasible. Several facts would make it not convincing. First, the BM morphology and the cytopenias in SDS are quite variable. Second, the haematological condition varies considerably over time. Third, all patients with important cytopenias are transfused and treated, even for long periods. Nevertheless, in order to attempt a rough comparison, we randomly chose 25 patients from our entire cohort of 96 Italian patients, without any criteria besides the absence of del(20)(q). This cohort comprised 11 females and 14 males, with an age range of <1–38 years. The BM karyotype was normal in 20/25 patients, whereas a clonal i(7)(q10) was present in five of them at the time of blood sampling. Neutropenia was present in all but four patients and, among the other 21 patients, it was severe in 10 (neutrophil count $0{\cdot}058–0{\cdot}4 \times 10^9$/l and mild in 11 cases (neutrophil count $0{\cdot}5–1{\cdot}24 \times 10^9$/l). Hb was normal in 3/25 patients: anaemia was severe in two (53–71 g/l) and mild in 20 (79–129 g/l). Thrombocytopenia was present in 15/25 patients: severe in four (platelet count $9{\cdot}0–42 \times 10^9$/l) and mild in 11 ($81–143 \times 10^9$/l). Although these data are not statistically evaluable, they certainly show some relevant differences from the cohort of patients that have del(20)(q).

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Conflict of interest

The authors declare that they have no conflict of interest.

Author contributions

RV, AM, MG, GD, AF, GM, AWK, GP, CO performed the research and analyzed the data. MC, SC, FP, CD, GC, EM designed the research, analyzed the data, and wrote the paper.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table SI. Raw SNP array data: data from SNParray analysis of SDS patients by Affymetrix® Cytogenetics Whole-Genome 2.7M Array and Affymetrix CytoScan HD Array.

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