Absence of acquired copy number neutral loss of heterozygosity (CN-LOH) of chromosome 7 in a series of 10 patients with Shwachman–Diamond syndrome

We report that acquired copy number neutral loss of heterozygosity (CN-LOH) of chromosome 7 was not identified in a series of 10 patients with Shwachman–Diamond syndrome (SDS).

Shwachman–Diamond syndrome (Online Mendelian Inheritance in Man reference 260400) is a rare autosomal recessive disease first reported in 1964 (Bodian *et al*,1964; Shwachman *et al*,1964) and characterized by exocrine pancreatic insufficiency, skeletal abnormalities and bone marrow (BM) dysfunction with an increased risk to develop myelodysplastic syndrome and/or acute myeloid leukaemia (MDS/AML). Almost 90% of SDS cases are caused by two common mutations, c. [183_184TA>CT] and c.[258 + 2T>C], in exon 2 of the *SBDS* gene, localized on chromosome 7. Clonal chromosome anomalies are often found in the BM of SDS patients; the most frequent are an isochromosome for long arms of chromosome 7, i(7)(q10) and an interstitial deletion of long arms of chromosome 20, del(20)(q11) (Maserati *et al*, 2009).

An earlier study analysed BM DNA of eight cases with the i(7)(q10) and demonstrated that all of them carried a double dose of the c.[258 + 2T>C] (Minelli *et al*, 2009). This result suggested that, as the c.[258 + 2T>C] mutation still allows the production of some amount of normal protein (Austin *et al*, 2005), this could contribute to the low incidence of MDS/AML observed in this subset of SDS patients.

Recently, Parikh *et al* (2012) described acquired CN-LOH for most of 7q in a patient with SDS, also showing that the clone of BM cells with CN-LOH contained two copies of the gene with the c.[258 + 2T>C] mutation. The presence of the CN-LOH in BM cells mimics the presence of i(7)(q10): both mechanisms produce a duplication of the c.[258 + 2T>C] mutation, together with the probable associated positive effects.

Parikh *et al* (2012) also outlined that the genetic variation of the CN-LOH could provide an explanation for clonal expansion of the affected haematopoietic progenitor cell.

Consequently, we investigated if, to provide the cell with an extra copy of the c.[258 + 2T>C] mutation, this could be a common mechanism in addition to the typical i(7)(q10) frequently observed in BM from SDS patients. We collected BM samples from 10 SDS patients in whom the cytogenetic analysis demonstrated a normal karyotype (eight cases) or the presence of the 20q deletion (two cases). The genotypes were c.[258 + 2T>C]/[183_184TA>CT] in seven cases and c.[258 + 2T>C]/[others] in three cases.

Morphological analysis of the BM aspirate, available for six patients, showed normal or hypoplastic cellularity in three, and normal or hyperplastic cellularity with minimal decrease of erythropoiesis, megakaryopoiesis and myelopoiesis in the remaining three. These data are in keeping with the karyotype-phenotype correlation discussed by Pressato *et al* (2012).

Microsatellite analysis, performed as reported in Minelli *et al* (2009), demonstrated a normal dosage between paternal and maternal alleles, excluding the presence of uniparental disomy related to chromosome 7, in all BM samples examined (Table I and Fig 1A). The method is able to identify the presence of the chromosomal anomaly if it is present in at least 20% of cells (Minelli *et al*, 2009).

To verify the reliability of the method, we sequenced exon 2 of the *SBDS* gene from the BM of two SDS patients with 50–70% cells with i(7)(q10) and, as expected, found that the mutant peak (C) was higher than the wild-type peak (T) (Fig 1B).

Exon 2 of the *SBDS* gene was then sequenced using BM and peripheral blood samples and, in all cases analysed, the mutant peak (C) and the wild-type peak (T) for the c.[258 + 2T>C] mutation were exactly the same height in both samples (Fig 1C) whereas Parikh *et al* (2012) observed an higher peak for the mutated allele when BM and fibroblasts were compared.

Thus, no evidence for CN-LOH was found in any of the patients studied. If the hypothesis that an increased level of the c.[258 + 2T>C] mutation on a SDS background (c.[183_184TA>CT]/c.[258 + 2T>C]) provides a selective advantage, we would expect to identify cases in whom a clone showing a (partial) loss of the c.[183_184TA>CT] or an homozygous c.[258 + 2T>C] is present.

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Table I. Result of molecular and morphological analyses on bone marrow samples in 10 Shwachman–Diamond syndrome patients.

Case	*SBDS* genotypes	Allelic ratio*		Bone marrow morphology
		Short arm	Long arm	
1	[c.258 + 2T>C]/[c.183_184TA>CT]	1·25 (2)	1·27 (2)	na
2	[c.258 + 2T>C]/[c.183_184TA>CT]	0·98 (2)	0·99 (2)	a
3	[c.258 + 2T>C]/[c.183_184TA>CT]	0·96 (3)	0·985 (3)	na
4	[c.258 + 2T>C]/[c.183_184TA>CT]	0·94 (2)	1·06 (3)	a
5	[c.258 + 2T>C]/[c.258 + 2T>C + 183_184TA>CT]	1·13 (3)	1·07 (3)	c
6	[c.258 + 2T>C]/[c.183_184TA>CT]	0·98 (2)	0·9 (4)	b
7	[c.258 + 2T>C]/[c.107delT]	1·01 (2)	0·95 (2)	d
8	[c.258 + 2T>C]/[c.101A>T]	1·15 (2)	1·16 (5)	c
9	[c.258 + 2T>C]/[c.183_184TA>CT]	0·86 (4)	0·92 (4)	na
10	[c.258 + 2T>C]/[c.183_184TA>CT]	0·99 (3)	0·95 (3)	na

na, not available.

*patient genotype is expressed as ratio between peak heights of chromosome 7 informative alleles of the short tandem repeat polymorphisms analysed (the number of these is given in brackets).

Bone marrow morphology is reported as follows: a, hypoplastic cellularity and absence of megakaryocytes; b, normal/hypoplastic cellularity, isolated megakaryocytes and limited dysmyelopoiesis (<10%); c, normal/hyperplastic cellularity, normal/few megakaryocytes and absence of dysmyelopoiesis; d, normal cellularity and megakaryocytes, erythro-myeloid component sufficiently present.

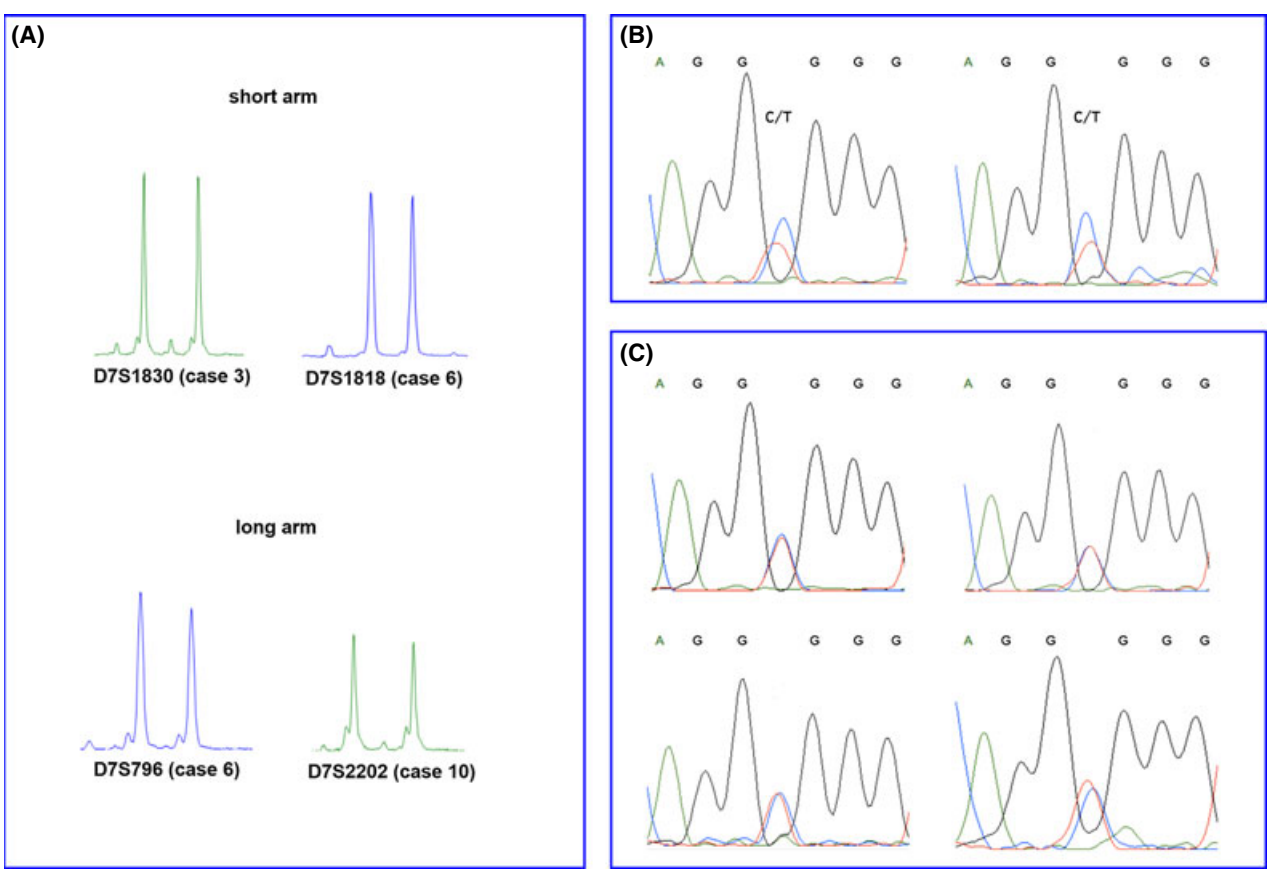

Fig 1. Examples of experimental investigations (A) Short tandem repeat polymorphisms analysis in three cases. (B) Chromatographs of the c.[258 + 2T>C] mutation from bone marrow samples of two Shwachman–Diamond syndrome (SDS) patients with 50% (left) and 70% (right) cells with i(7)(q10), the mutant peak (C) is higher than the wild-type peak (T) in both. (C) Chromatographs of the c.[258 + 2T>C] mutation from peripheral blood (left) and bone marrow (right) samples from two of ten SDS patients analysed in the present study. The mutant peak (C) and the wild-type peak (T) for the c.[258 + 2T>C] mutation were exactly the same height in both cases.

analysed the data. C. Danesino, A. Minelli and F. Pasquali designed the research study and wrote the paper. L. Sainati, D. Longoni, F. Poli, M. Cipolli, S. Perobelli, E. Nicolis, Z. Cannioto all contributed to collect the data.

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References

Austin, K.M., Leary, R.J. & Shimamura, A. (2005) The Shwachman-Diamond SBDS protein localizes to the nucleolus. *Blood*, **106**, 1253–1258.

Bodian, M., Sheldon, W. & Lightwood, R. (1964) Congenital hypoplasia of the exocrine pancreas. *Acta Paediatrica*, **53**, 282–293.

Maserati, E., Pressato, B., Valli, R., Minelli, A., Sainati, L., Patitucci, F., Marletta, C., Mastronuzzi, A., Poli, F., Lo Curto, F., Locatelli, F., Danesino, C. & Pasquali, F. (2009) The route to development of myelodysplastic syndrome/acute myeloid leukaemia in Shwachman-Diamond syndrome: the role of ageing, karyotype instability, and acquired chromosome anomalies. *British Journal of Haematology*, **145**, 190–197.

Minelli, A., Maserati, E., Nicolis, E., Zecca, M., Sainati, L., Longoni, D., Lo Curto, F., Menna, G., Poli, F., De Paoli, E., Cipolli, M., Locatelli, F., Pasquali, F. & Danesino, C. (2009) The isochromosome i(7)(q10) carrying c.258 + 2t>c mutation of the SBDS gene does not promote development of myeloid malignancies in patients with Shwachman syndrome. *Leukemia*, **23**, 708–711.

Parikh, S., Perdigones, N., Paessler, M., Greenbaum, B., Tooke, L.S., Biegel, J.A., Mason, P.J. & Bessler, M. (2012) Acquired copy number neutral loss of heterozygosity of chromosome 7 associated with clonal haematopoiesis in a patient with Shwachman-Diamond syndrome. *British Journal of Haematology*, **159**, 480–482.

Pressato, B., Valli, R., Marletta, C., Mare, L., Montalbano, G., Lo Curto, F., Pasquali, F. & Maserati, E. (2012) Deletion of chromosome 20 in bone marrow of patients with Shwachman-Diamond syndrome, loss of the EIF6 gene and benign prognosis. *British Journal of Haematology*, **157**, 503–505.

Shwachman, H., Diamond, L.K., Oski, F.A. & Khaw, K.T. (1964) The syndrome of pancreatic insufficiency and bone marrow dysfunction. *Journal of Pediatrics*, **65**, 645–663.

Ofatumumab monotherapy in relapsed/refractory mantle cell lymphoma – a phase II trial

Mantle cell lymphoma (MCL), an uncommon subtype of non-Hodgkin lymphoma (NHL), is considered one of the more aggressive lymphoid tumours. Despite the use of novel treatment regimens, MCL remains incurable and the focus of therapy is on the depth and duration of remission.

The chimeric CD20 monoclonal antibody rituximab is a commonly used therapy in lymphoma and rituximab monotherapy shows some efficacy in MCL (Foran *et al*, 2000). It has recently been shown to improve the overall survival in MCL when added to combination chemotherapy, without increasing treatment toxicity (Rule *et al*, 2011).

Ofatumumab is a fully human type 1 CD20 monoclonal antibody that targets a novel epitope on the CD20 molecule (Teeling *et al*, 2006). This allows closer binding to the cell surface, which is thought to contribute to both its increased ability to activate complement-dependent cytotoxicity (CDC) and the longer off-rate compared to rituximab.

Ofatumumab has shown single agent activity in the treatment of chronic lymphocytic leukaemia (CLL) (Coiffier *et al*, 2008) and relapsed/refractory follicular lymphoma (Hagenbeek *et al*, 2008) with overall response rates of 44% and 42%, respectively. No dose-limiting toxicities occurred in