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Acquired copy number neutral loss of heterozygosity of chromosome 7 associated with clonal haematopoiesis in a patient with Shwachman-Diamond syndrome

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Shwachman-Diamond syndrome (SDS) is an autosomal recessive disorder characterized by bone marrow (BM) failure, pancreatic insufficiency, and skeletal abnormalities. Mutations in the *SBDS* gene on chromosome arm 7q, explain 90% of SDS cases (Boocock *et al*, 2003). SBDS is essential for the assembly of mature ribosomes. Frequently SDS patients are compound heterozygotes for two common *SBDS* mutations (the 183_184 TA→CT and 258+2 T→C mutations). The null 183_184 TA→CT mutation results in a stop codon while the 258+2 T→C mutation causes a splicing error with only small amounts of full length protein produced (Austin *et al*, 2005). Homozygosity for the 183_184 TA→CT mutation has never been found.

Here we describe acquired copy number neutral loss of heterozygosity (CN-LOH) for most of 7q in an SDS patient. The clone of BM cells with CN-LOH contained 2 copies of the

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PM, MB, JAB, NP and SP designed the research study. SP, NP, LST, MP and BG performed the research. PM, MB, JAB, LST, NP and SP analysed the data. All authors contributed to writing the manuscript.

gene with the 258+2 T→C mutation, which increases the level of SBDS protein providing the likely explanation for clonal expansion of the affected haematopoietic progenitor cell.

Neutropenia was found in a neonatal female when a complete blood count was performed due to concerns of neonatal sepsis. In addition to haematological and infectious issues, this patient also developed exocrine pancreatic insufficiency. At four months of age she was diagnosed with SDS, which was confirmed by gene sequencing. A BM aspiration and biopsy were performed on the patient at 13 months of age and a follow-up examination was performed a year later. Karyotypes were prepared, and single nucleotide polymorphism (SNP) analysis of DNA isolated from the BM aspirates was accomplished with the HumanHap610 (first BM) or Omni1-Quad (second BM aspirate) genotyping beadchip (Illumina, San Diego, CA). The patient and her unaffected parents were recruited for a research study at the Children's Hospital of Philadelphia, Pennsylvania, USA. Samples from skin, BM and peripheral blood (PB) from the patient as well as PB from the parents were obtained after written informed consent. The Ethics Committee of the Hospital approved the study in accordance with the declaration of Helsinki.

SNP analysis of DNA from the parents PB was performed with the Illumina Human Omni1-Quad gene chip. Data analysis was performed with GenomeStudio Software (Illumina). Plots of two parameters, the log2R ratio and the B allele frequency, provided information regarding copy number and genotype. The comparison between the genotype data of the parents and the CN-LOH region in the patient was performed with GenomeStudio Software. Polymerase chain reaction and Sanger sequencing of BM DNA and DNA from cultured skin fibroblasts and PB was carried out by standard procedures (Boocock *et al*, 2003, Sanger and Coulson 1975).

The BM aspirate and biopsy performed on the patient at 13 months of age, showed normal cellularity, with normal erythropoiesis and megakaryopoiesis, with a decrease in myeloid progenitors. Standard cytogenetic analysis revealed a normal karyotype 46,XX. Genome wide (GW) SNP array analysis revealed CN-LOH in 20% of the cells from a region just below the centromere of chromosome 7 to the end of 7q (Figure 1). A similar percentage of cells with 7q CN-LOH and no other cytogenetic abnormalities were identified on a second BM one year later.

Genotype analysis of the parents showed that the mother was heterozygous for the 183_184 TA→CT mutation and the father was heterozygous for the 258+2 T→C mutation. The patient was heterozygous for both mutations. Analysis of 683 informative markers from 7q showed that the LOH was due to loss of the maternal alleles. The comparison of the 258+2 T→C sequencing peaks between fibroblast and BM samples from the patient showed that the 258+2 C peak was slightly higher than the 258+2 T peak in the BM sample but not in the skin sample (Figure 2). These results indicate that the cells with LOH of 7q detected in the BM of the patient have two copies of the *SBDS* 258+2 T→C mutation.

Our results are consistent with clonal expansion of cells, in the BM of an SDS patient, that have undergone a genetic event producing LOH for 7q, including the *SBDS* gene. Genetic analysis shows that the expanded clone contains 2 copies of the hypomorphic *SBDS* mutation while the constitutional genotype is compound heterozygous for 258+2 T→C and 183_184 TA→CT. This is the third example of somatic genomic changes leading to clonal expansion in the BM of SDS patients. Clonal cytogenetic abnormalities affecting 7q have been repeatedly observed in the BM of SDS patients (Dror *et al*, 2002, Shimamura 2006, Smith *et al*, 2002). The most frequent is an isochromosome 7q (i(7)(q10)), leading to 3 copies of 7q. Interestingly, all of the compound heterozygous SDS patients with i(7)(q10) clones analysed so far have an extra copy of the 258+2 T→C mutated gene (Minelli *et al*,

2009). In those cases, as in the case described here, there is an increase in the copy number of the gene producing some active protein. Another common cytogenetic event in SDS is an interstitial deletion in 20q removing a region that includes the *EIF6* gene (Pressato *et al*, 2012). It was recently shown that SBDS couples with the GTPase EFL1 to cause the release of EIF6 from the pre-60S ribosome subunit, an essential step in the formation of the mature ribosome (Finch *et al*, 2011). It was hypothesized that the decreased amount of EIF6 in these cells, in the context of SBDS deficiency, improved ribosome biogenesis. Again the deletion is associated with clonal expansion. Interestingly SDS has an increased likelihood of progression to myelodysplastic syndrome/acute myeloid leukaemia that is frequently associated with numerical and structural chromosome 7 anomalies. To the contrary, both i(7)(q10) and the 20q deletion in patients with SDS are associated with low risk of transformation of the affected cell clone (Minelli *et al*, 2009, Pressato, *et al*, 2012), though this relationship has been questioned (Dror *et al*, 2002). Interestingly this patient is another example of an emerging paradigm whereby, in the context of the aplastic BM, haematopoietic progenitor cells that partially overcome the disease causing genetic lesion due to acquired genomic changes have a survival or growth advantage, leading to clonal expansion. Somatic genomic changes and clonal expansion have been observed in other BM failure syndromes (May 2011). These changes often improve blood cell production and in some cases decrease the risk of leukaemic transformation. Such “natural gene therapy” may be instructive concerning possible drug targets.

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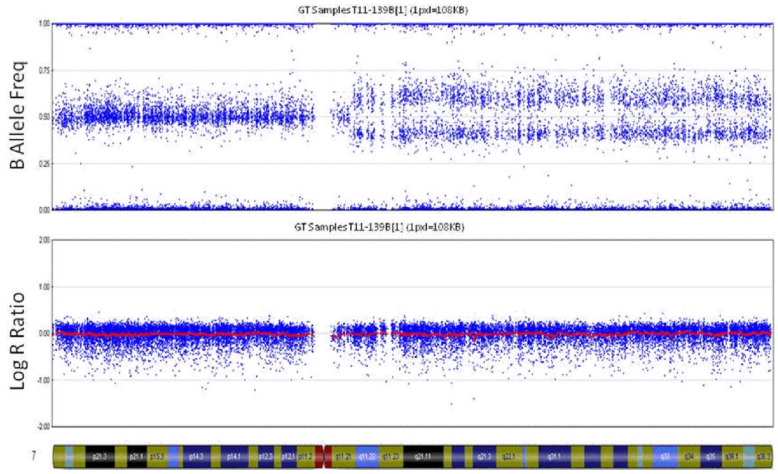

Figure 1. B allele frequency results and LOH analysis from BM of CHOP256.01 at chromosome 7

This figure shows BM mosaicism with approximately 20% of cells showing CN LOH of 7q.

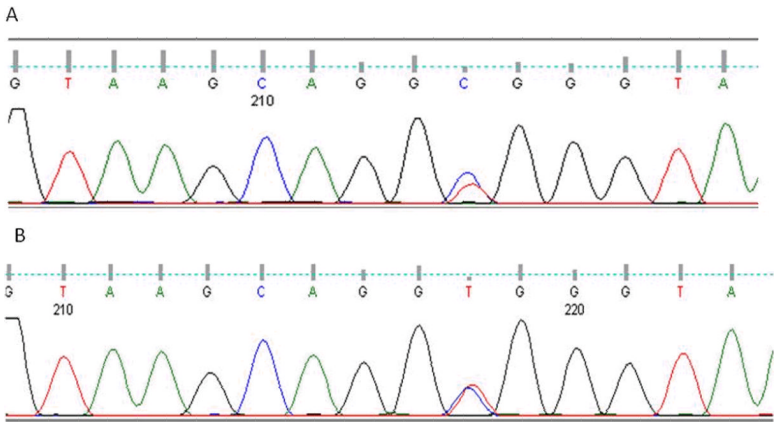

Figure 2. Chromatograph of the *SBSD* 258+2 T→C mutation from BM(A) and skin (B)
Note that in the BM the mutant peak (T red) is lower, whereas in the skin both the C (blue) and T (red) peaks are about similar in height.