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Absence of *SBDS* mutations in sporadic paediatric acute myeloid leukaemia

Shwachman-Diamond syndrome (SDS, On-line Mendelian Inheritance in Man (OMIM) #260400) is an autosomal recessive condition, characterized by pancreatic exocrine insufficiency, skeletal abnormalities, bone marrow failure, and an increased risk of myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML), the latter occurring in 19–36% of patients (Shimamura, 2006). Compound heterozygous mutations in *SBDS* are identified in the majority of SDS patients. Of the two most frequently found mutations in *SBDS*, 183-184TA > CT and 258 + 2T > C, at least one is present in approximately 90% of affected individuals. These mutations are located in exon 2, and result from gene conversion with *SBDSP1*, the *SBDS* pseudogene (Boocock *et al*, 2003). Although its exact function remains unclear, the SBDS protein appears to have a role in ribosome maturation, and might have additional extraribosomal functions (Finch *et al*, 2011; Johnson & Ellis, 2011).

Because of the increased risk of AML, but lack of a clear genotype-phenotype relationship in SDS (Kuijpers *et al*, 2005), we hypothesized that compound heterozygous *SBDS* mutations might be present in seemingly sporadic paediatric AML. Furthermore, we hypothesized that heterozygous mutations in *SBDS* might be present at increased frequency in sporadic AML compared to healthy controls, and might thus be a risk factor for AML development. Given the significant toxicity of standard chemotherapy and transplantation conditioning regimens in SDS patients with MDS or AML (Shimamura, 2006), but the reduction in morbidity after reduced-intensity conditioning regimens (Bhatla *et al*, 2008), the identification of AML patients carrying *SBDS* mutations seems clinically relevant.

In leukaemic blast cells derived at diagnosis from 160 paediatric AML patients (median age: 9·6 years (range: 0–18·5 years); 90 (56·3%) male, 70 (43·7%) female), who were enrolled in consecutive AML-BFM (Berlin-Frankfurt-Münster), DCOG (Dutch Childhood Oncology Group)/MRC (UK Medical Research Council), and LAME (Leucémie Aiguë Myéloblastique Enfant) treatment protocols between 1987 and 2008 (Hollink *et al*, 2011), we specifically amplified *SBDS* and not *SBDSP1*, as previously described, and sequenced exon 2 of *SBDS* (Calado *et al*, 2007). Germline material of the AML patients was not available, and we assume that *SBDS* gene variants found in leukaemic blast cells were constitutional and not acquired variants.

Two AML patients carried a heterozygous 258 + 2T > C mutation (carrier frequency 0·013). This mutation disrupts the donor splice site of intron 2 and results in the use of a cryptic donor splice site in exon 2, leading to a frameshift and premature protein truncation at codon 84 (Boocock *et al*, 2003). Furthermore, 28 of 160 AML patients carried the silent variant 201A > G (carrier frequency 0·175) (Fig 1). No compound heterozygous mutations in exon 2 of *SBDS* were detected. Of 168 Dutch blood bank donors, one carried

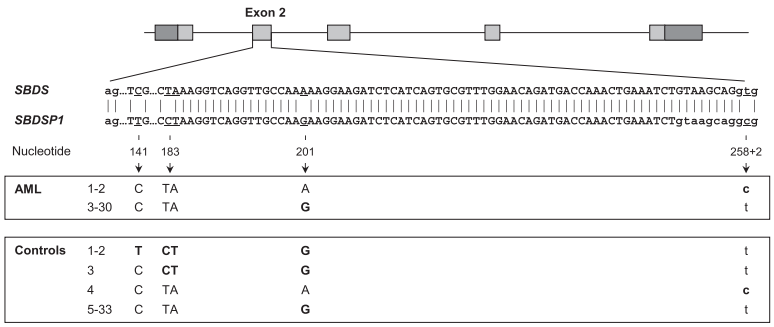

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Fig 1. Graphical representation of paediatric AML patients and controls carrying *SBDS* nucleotide changes, depicted in bold, resulting from gene conversion events with *SBDSP1* in and around exon 2. The absence of *SBDSP1*-like sequences at nucleotide 141, 183-184, and 201 in AML patients, and the absence of *SBDSP1*-like sequences at nucleotide 141, 183-184, 201, or 258 + 2 in controls, indicate the specificity of amplicons for *SBDS*. Figure adapted from Boocock *et al* (2003).

Table I. *SBDS* gene variants resulting from gene conversion in paediatric AML patients and controls. Values represent the number of individuals carrying a variant (carrier frequency).

Nucleotide change	Amino acid change	AML patients (n = 160)	Controls (n = 168)
Het. 141C > T	–	–	2 (0·012)
Het. 183-184TA > CT	K62X	–	3 (0·018)
Het. 201A > G	–	28 (0·175)	32 (0·190)
Het. 258 + 2T > C	C84fs3	2 (0·013)	1 (0·006)

AML, acute myeloid leukaemia; Het., heterozygous.

the heterozygous 258 + 2T > C (carrier frequency 0·006). Furthermore, 3 of 168 blood bank donors carried a heterozygous 183-184TA > CT (carrier frequency 0·018), introducing a premature stop codon at amino acid 62. The silent variants 141C > T and 201A > G were present in 2 (carrier frequency 0·012) and 32 (carrier frequency 0·190) controls, respectively (Table I). In previously published controls cohorts, 183-184TA > CT was present in 1 of 70 individuals (carrier frequency 0·014) (Nakashima *et al*, 2004) and 0 of 100 individuals (Boocock *et al*, 2003), whereas 258 + 2T > C was absent in three published controls cohorts of 70, 100 and 276 individuals each (Boocock *et al*, 2003; Nakashima *et al*, 2004; Calado *et al*, 2007).

We conclude that in a cohort of 160 paediatric AML patients, homozygous or compound heterozygous mutations in *SBDS* were absent, and heterozygous mutations in *SBDS* were present at frequencies comparable to healthy controls. Our findings confirm a previous report in which no mutations in exon 2 of *SBDS* were found in a smaller cohort of 48 children with *de novo* AML and 48 children with AML in remission (Majeed *et al*, 2005). Taken together, these results suggest that children with seemingly sporadic AML are unlikely to have underlying SDS.

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Authorship contributions

AMA, RTC, SK, NSY, RP, VHJV, MHE conceived and designed the experiments; AMA, SK performed the experiments; AMA, RTC, NSY, CMZ, SK, AB, KG, VH, GJLK, DR, JT, TWK, RP, VHJV, MHE contributed reagents, materials and analysis tools and wrote the paper.

Conflict of interest

The authors have no conflicts of interest to declare.

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Assessment of molecular remission rate after bortezomib plus dexamethasone induction treatment and autologous stem cell transplantation in newly diagnosed multiple myeloma patients

High-dose therapy supported by autologous stem cell transplantation (ASCT) is the standard approach in multiple myeloma (MM) in eligible patients under 65 years. The importance of complete response (CR) after ASCT for prolonged overall survival (OS) has been confirmed in a meta-analysis (Van de Velde *et al*, 2007). Previous data suggest that molecular remission (MolR) is associated with better long-term outcome and might serve as a surrogate for OS (Bakkus *et al*, 2004; Sarasquete *et al*, 2005; Ladetto *et al*, 2010, 2011; Putkonen *et al*, 2010). Not only polymerase chain reaction (PCR)-negativity but also attainment of low minimal residual disease (MRD) below the cut-off level of $\leq$0·01%–0·015% in allele-specific quantitative PCR (ASO-PCR) predicts a better outcome (Bakkus *et al*, 2004; Sarasquete *et al*, 2005; Putkonen *et al*, 2010). The International Myeloma Workshop Consensus Panel 1 has approved the molecular CR (sensitivity 10^{-5}) as an additional criterion in the International Myeloma Working Group criteria (Rajkumar *et al*, 2011).

The aim of this prospective phase II study was to explore response rates after bortezomib plus dexamethasone (VD) induction and further after ASCT, including MRD assessment by ASO-PCR in patients achieving at least near complete remission (nCR). Secondary objectives were overall response rate, duration of MolR and progression-free survival (PFS). Patients with previously untreated, symptomatic MM, aged between 18 and 65 years were eligible. Forty-seven patients were included between May 2009 and June 2011 in nine centres in Finland. Written informed consent was obtained. The study was approved by the Finnish Medicines Agency and the Ethics Committees of participating hospitals and it was registered with ClinicalTrials.gov, number NCT00861250. Induction treatment comprised four three-week cycles of bortezomib 1·3 mg/m^2 intravenously on days 1, 4, 8 and 11 plus dexamethasone 40 mg on days 1–4 (all