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

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To the Editor

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

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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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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 Berlin-Frankfürt-Münster, Dutch Childhood Oncology Group/UK Medical Research Council, and Leucemie Aique Myeloide Enfant AML 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, 1 carried 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; Calado *et al*, 2007; Nakashima *et al*, 2004).

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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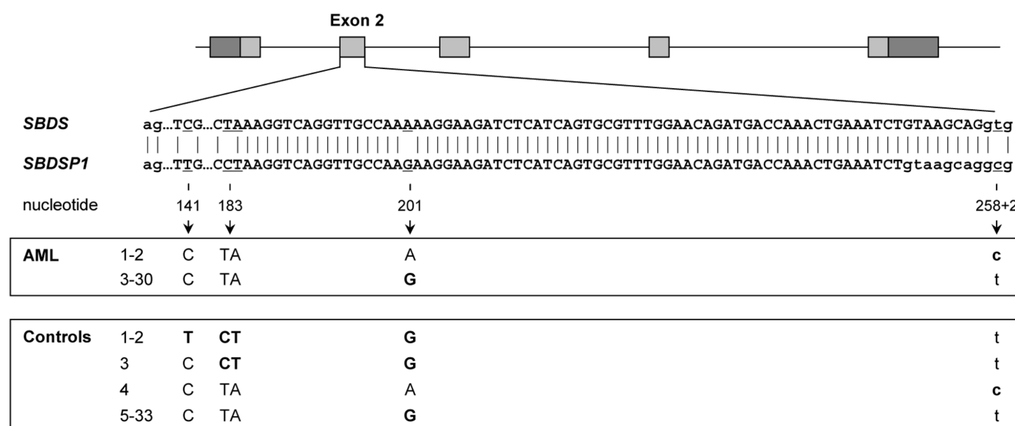

Figure 1.

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).

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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