Lack of splice factor and cohesin complex mutations in pediatric myelodysplastic syndrome

Myelodysplastic syndromes (MDS) represent a heterogeneous group of hematologic disorders, with distinct subtypes defined by cytogenetics, the number of affected lineages, severity of cytopenia, cellular dysplastic morphology, and blast counts.[1] Extensive next-generation sequencing has recently been performed in adult MDS; these studies revealed that mutations most frequently occurred in genes involved in RNA splicing, the cohesin complex, chromatin modification, DNA methylation, transcriptional regulation, and signal transduction.[2-10] In contrast, relatively little is known about recurrently affected genes and pathways in childhood MDS and their contribution to disease pathogenesis.[11,12]

We performed for the first time within this disease group a systematic investigation of the importance of mutations and recurrently affected pathways found in a variety of hematologic diseases using deep sequencing, and compared our findings with published data from adult MDS patients. DNA samples from 24 primary and 14 secondary pediatric MDS cases were analyzed. Information on patients' characteristics is provided in *Online Supplementary Table S1*. Institutional review board approval for these studies was obtained in the participating centers. Thirty-nine samples were sequenced using the TruSight Myeloid Sequencing Panel (Illumina, San

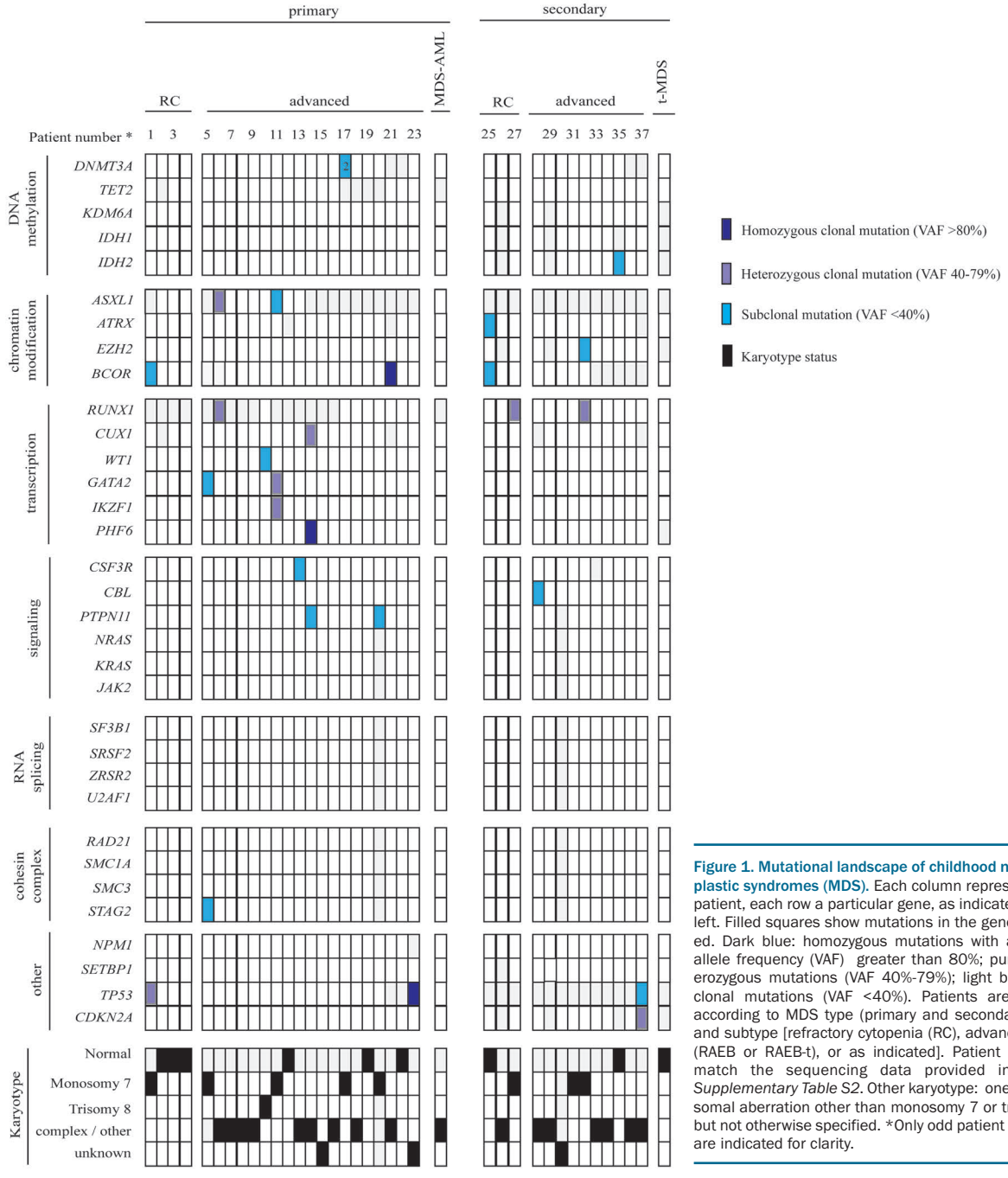

Figure 1. Mutational landscape of childhood myelodysplastic syndromes (MDS). Each column represents one patient, each row a particular gene, as indicated on the left. Filled squares show mutations in the gene indicated. Dark blue: homozygous mutations with a variant allele frequency (VAF) greater than 80%; purple: heterozygous mutations (VAF 40%-79%); light blue: subclonal mutations (VAF <40%). Patients are divided according to MDS type (primary and secondary MDS) and subtype [refractory cytopenia (RC), advanced MDS (RAEB or RAEB-t), or as indicated]. Patient numbers match the sequencing data provided in *Online Supplementary Table S2*. Other karyotype: one chromosomal aberration other than monosomy 7 or trisomy 8, but not otherwise specified. *Only odd patient numbers are indicated for clarity.

Diego, CA, USA) on the MiSeq platform and prepared according to the TruSight DNA Amplicon Sequencing Panel guide (Illumina). Average gene coverage was 2805. The MDS sample from patient 5 was analyzed using whole exome sequencing (WES), as previously described,[13] with an average gene coverage of 78. Further details on the patient cohort, on the bioinformatic analyses, and candidate mutation selection are provided in *Online Supplementary Table S1* and Figure S1.

In total, we found 28 mutations in 18 genes (Figure 1). Details on the mutations, frequency, the variant coverage, and variant allele frequencies (VAFs) in healthy individuals as assessed by population-based sequencing efforts, as well as predicted effects of the alterations for each patient are shown in *Online Supplementary Table S2*. *TP53, BCOR* and *RUNX1* mutations were present in 3 patients. *ASXL1, GATA2, PTPN11* were mutated twice and mutations in *WT1, DNMT3A, CUX1, STAG2, IKZF1, CSF3R, PHF6, ATRX, CBL, EZH2, IDH2, CDKN2A* were found in single patients only. Twenty-one patients (55%) had none of these mutations, but 13 of them had a cytogenetic abnormality (Figure 1 and *Online Supplementary Table S1*), most commonly monosomy 7. Overall, at least one genetic or cytogenetic aberration was present in 30 of the 38 (79%) patients. This percentage is identical to that previously reported for adult MDS.[7] There was no difference in frequencies or type of mutations between primary and secondary MDS samples. Unfortunately, no material of the primary diseases was available in the secondary MDS cases, thus, the possibility that these MDS cases are minimal residual diseases (MRDs) of the primary malignancies [e.g. of the acute myeloid leukemia (AML) patients] cannot be excluded. This represents an interesting question to be investigated in independent studies.

A previous report by Hirabayashi *et al.*[11] suggests a lack of mutations in the splice factor-encoding genes in pediatric MDS, as evidenced by Sanger sequencing of mutational hotspots in *SF3B1, U2AF35* and *SRSF2*. This finding contrasts strongly with results in adult MDS patients, in which mutations in genes involved in RNA splicing are the most common abnormality (Table 1), occuring as clonal mutations and early in disease evolution.[4,7,9] Because the resolution of conventional Sanger sequencing is low, and given the previously reported impact of subclonal mutations in adult MDS on patient survival,[7, 14] we assessed whether subclonal aberrations in the splice factor-encoding genes *SF3B1, SRSF2, ZRSR2* and *U2AF1* could be detected in our cohort. None of the pediatric MDS cases in our study had clonal or subclonal mutations in these genes, corroborating the findings by Hirabayashi *et al*. Another mechanism that is recurrently affected in adult MDS is the formation of the cohesin complex (Table 1).[10] We found only one mutation in *STAG2* in our pediatric MDS cohort (Figure 1), while other genes of this complex, such as *SMC1A, SMC3* and *RAD21*, were not mutated. On the other hand, we identified both clonal and subclonal mutations in genes involved in chromatin modification, DNA methylation, signaling and transcription (Figure 1 and *Online Supplementary Table S2*), in frequencies comparable to those reported for adult MDS (Table 1).[7]

In summary, our study shows that approximately 45% of pediatric MDS patients carry at least one mutation, primarily occurring in genes associated with chromatin modification, DNA methylation and transcription, but rarely in genes involved in RNA splicing and function of the cohesin complex. Because the latter mechanisms are most commonly affected in adult MDS, these data point towards a clear distinction between the pathogenesis of pediatric and adult patients, which may have implications for future therapy approaches in the different age groups.

Table 1. Comparison of frequencies of affected pathways in pediatric and adult myelodysplastic syndromes.

Pathway	Frequency (%) in pediatric MDS[1]	Frequency (%) in adult MDS[2]
DNA methylation	8.0	37.0
Chromatin modification	18.0	22.0
Transcription	20.0	14.0
Signaling	10.0	15.7
RNA splicing	0.0	47.4-55.2
Cohesin complex	2.5	4.1-8.0
Other pathways	7.5	7.0

MDS: myelodysplastic syndromes. [1]Data of this study. [2]Data based on Papaemmanuil et al.[7], Kon et al.[10] and Yoshida et al.[9]

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