

Project Title: Genetic and clinical heterogeneity in Shwachman-Diamond Syndrome

Supervisor: Antonella Minelli

Laboratory: Medical Genetics

Research Theme: Rare mendelian diseases.

Shwachman-Diamond Syndrome (SDS, OMIM #260400) is a rare inherited bone marrow failure syndrome (IBMFS) characterized by neutropenia, exocrine pancreatic insufficiency, skeletal alterations, developmental delay and, relevantly, by an increased risk for myelodysplasia (MDS) and acute myeloid leukaemia (AML).

The clinical picture of the syndrome shows wide variability (neutropenia and pancreatic insufficiency are seen in over 90% of cases while up to 15–20% of patients may develop MD and AML) and, similarly, the severity of the different clinical problems may vary greatly in different cases.

Patients carry mainly *SBDS* pathogenic variants, three of which are very common and observed in more than ninety percent of cases, and this fact prevents effective genotype-phenotype correlation.

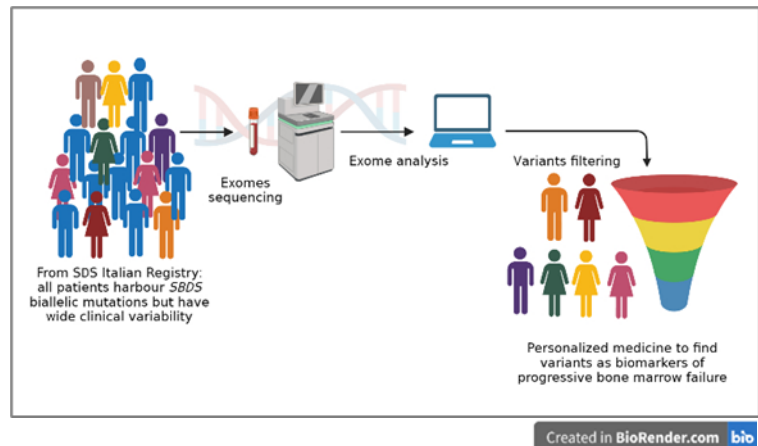
In my lab, within previous research projects, we performed exome sequencing in 16 *SBDS* mutated patients and addressed our research in identifying germinal pathogenic variants, additional to the biallelic *SBDS* mutations causing the disease, which might be relevant to explain i) clinical unexpected or uncommon symptoms, ii) the severity of common haematological signs as neutropenia or low platelet count, and to forecast the probability of progression of haematological abnormalities to myelodysplastic/leukemic forms.

This PhD project continues this research line, strongly supported by results already obtained.

In detail, we plan:

- To select 4-5 cases from the Italian Registry (in which more than 130 patients are entered, all with demonstrated biallelic *SBDS* pathogenic mutations) in whom the clinical picture includes some clinical signs not usually observed in SDS patients; this analysis will be carried on with the collaboration of Dr. M. Cipolli and Dr. E. Pintani who are in charge of the clinical follow up and register maintenance, respectively. Following the identification of such cases, appropriate counselling and signing informed consent, a blood sample will be obtained, and exome sequencing performed. The variants found will be analysed using tools described in ref. 1, with the aim to identify genetic variations (additional to *SBDS* mutations) which can explain the uncommon clinical sign observed.
- To continue and expand the search for genetic variants (additional to *SBDS* mutations) which can explain clinical severity of some haematological features as neutropenia: in a recent PhD Thesis, Dr. I. Taha stated: "Interestingly, Pearson correlation testing showed a negative correlation between the absolute neutrophil count and the number of variants identified in all patients ($R=-0.601$, $p=0.05$). According to our results, we presume that it is more likely to have a very low neutrophil count if the patient has a higher number of neutropenia-related variants. These novel findings, especially if confirmed in other studies with larger sample sizes in the future, can be utilized in genomic surveillance and personalized medicine for SDS patients". So, we plan to check for the presence of neutropenia related pathogenic variants in a larger sample of cases. Next step will be to extend the same approach to set of genes related to low platelet counts, and to the development of bone marrow insufficiency.

The relevance of the project relates to two main goals: first, obtaining genetic data immediately useful for clinical management of each single patient (personalized medicine), second, validating in SDS the model of genotype-phenotype correlation in which not only the mutations in the disease-causing genes are considered, but also other genomic potentially pathogenic variants. The study also impacts the scientific



community as any improvement in the knowledge of genetic data relating to clinical phenotype, may be immediately relevant for the ongoing new projects of “mutation related” therapies which starts in May 2023.

Techniques: WGS data analysis, Sanger Sequencing, DNA and RNA extraction, RT- qPCR.

Selected references:

1: Taha I, Foroni S, Valli R, Frattini A, Roccia P, Porta G, Zecca M, Bergami E, Cipolli M, Pasquali F, Danesino C, Scotti C, **Minelli A**. Case Report: Heterozygous Germline Variant in EIF6 Additional to Biallelic SBDS Pathogenic Variants in a Patient With Ribosomopathy Shwachman-Diamond Syndrome. *Front Genet.* 2022 Aug 12;13:896749. doi: 10.3389/fgene.2022.896749. PMID:36035165; PMCID: PMC9411639.

2: Taha I, De Paoli F, Foroni S, Zucca S, Limongelli I, Cipolli M, Danesino C, Ramenghi U, **Minelli A**. Phenotypic Variation in Two Siblings Affected with Shwachman-Diamond Syndrome: The Use of Expert Variant Interpreter (eVai) Suggests Clinical Relevance of a Variant in the KMT2A Gene. *Genes (Basel).* 2022 Jul 23;13(8):1314. doi: 10.3390/genes13081314. PMID: 35893049; PMCID: PMC9394309.

3: Morini J, Nacci L, Babini G, Cesaro S, Valli R, Ottolenghi A, Nicolis E, Pintani E, Maserati E, Cipolli M, Danesino C, Scotti C, **Minelli A**. Whole exome sequencing discloses heterozygous variants in the DNAJC21 and EFL1 genes but not in SRP54 in 6 out of 16 patients with Shwachman-Diamond Syndrome carrying biallelic SBDS mutations. *Br J Haematol.* 2019 May;185(3):627-630. doi: 10.1111/bjh.15594. Epub 2018 Sep 10. PMID: 30198570.

4: **Minelli A**, Nacci L, Valli R, Pietrocola G, Ramenghi U, Locatelli F, Brescia L, Nicolis E, Cipolli M, Danesino C. Structural variation in SBDS gene, with loss of exon 3, in two Shwachman-Diamond patients. *Blood Cells Mol Dis.* 2016 Sep;60:33-5. doi: 10.1016/j.bcmd.2016.06.007. Epub 2016 Jun 22. PMID: 27519942.