

## CORRESPONDENCE

# PIEZO1 mutations impact on early clinical manifestations of myelodysplastic syndromes

To the Editor:

Chronic anemia is the most frequent presentation of myelodysplastic syndromes (MDS), but precise diagnosis, still based mainly on marrow precursors' morphology, is frequently challenging. Indeed, MDS-like dysplasia may be observed in non-clonal cytopenias and hematologic disorders, including nutritional/copper deficiencies, toxin exposures, and inherited disorders like red blood cell (RBC) membrane defects. We herein investigated the genetic landscape of MDS patients defined "young" due to the atypical early onset of the disease ( $\leq 60$  years) and anemia as the main cytopenia. Genetic analysis by whole exome sequencing (WES) is recommended for young de novo MDS patients with no syndromic signs, to detect germline variants predisposing to myeloid malignancies, but could also help to make a differential diagnosis with RBC defects.

Twenty-one consecutive patients with a diagnosis of MDS were referred to the MDS Unit of the University of Florence, Italy. All patients underwent bone marrow (BM) aspiration for diagnostic evaluation. The 21 MDS patients were evaluated by WES on both BM DNA and saliva (S) DNA. Variants were considered germline when confirmed in S-DNA samples with variant allele frequency (VAF) at least  $\geq 0.30$ . To validate the presence of somatic variants detected by WES, all 21 patients underwent targeted NGS of BM-DNA, with a routine diagnostic panel including 27 genes more frequently mutated in myeloid neoplasms. Five cases and their relatives were examined in the Medical Genetics Laboratory of the University of Naples "Federico II" and CEINGE, Biotechnologie Avanzate, Italy, by a t-NGS panel composed of 86 genes that are causative of RBC defects. The pathogenicity of each variant was evaluated according to the guidelines of the American College of Medical Genetics and Genomics (ACMG). Osmotic gradient ektacytometry was performed using the Osmoscan LoRRca MaxSis (Mechatronics Instruments BV, Zwaag, The Netherlands) by using peripheral blood (PB) samples.<sup>1</sup> This test was used to measure RBC deformability and hydration status in the patients carrying *PIEZO1* and *KCNN4* mutations.

Median age of the entire group of 21 patients was 51 years (age range 21–60 years) with a male/female ratio of 2.5 (Supplementary Table 1). Patients were distributed according to the Revised International Prognostic Scoring System (IPSS-R) as follows: 28.6% Very Low, 52.4% Low, 0% Intermediate, 9.5% High, and 9.5% Very High risk categories. Moreover, 28.6% of cases presented neutropenia

and 52.4% thrombocytopenia. Morphological dysplasia compatible with MDS was confirmed by a centralized morphological revision of BM smears. Six of the 21 analyzed patients (28.6%) who presented anemia carried causative variants of RBC defects in both BM-DNA and S-DNA samples. Median age of this subset of "young" patients was 48.5 years (age range 36–58 years). Particularly, we found that five patients (P1, P2, P3, P5, and P6) carried pathogenic variants in *PIEZO1* causing dehydrated hereditary stomatocytosis type 1 (DHS1, Table 1). In publicly available databases, germline variants of *PIEZO1* are reported as extremely rare in the MDS population (<1%). Patient P4 showed a complex mode of inheritance with the co-segregation of three hereditary RBC defects: G6PD deficiency, DHS type 2 (DHS2), and familial pseudohyperkalemia (FP). Patients P3 and P6 presented cytogenetic alterations (both a 5q deletion) detected through conventional karyotype. MDS-associated somatic variants were present in 5/6 patients (Table 1), with the exception of P4, who did not present somatic mutations. Somatic variants were detected in BM-DNA by WES analysis and excluded in S-DNA, and they were validated by performing a t-NGS myeloid panel. The VAF of these somatic variants were all  $>0.20$  except for a second *TP53* variant in P6 (VAF of 0.05), revealed only by the t-NGS myeloid panel.

Five of the six patients were further investigated except for patient P6, whose DNA was retrospectively analyzed after his death. Iron overload was documented in all five patients through ferritin dosage (ng/mL) and/or transferrin saturation (%); furthermore, T2\* magnetic resonance imaging (MRI) revealed in the same patients mild hepatic and cardiac overload (Supplementary Table 2). Interestingly, 3/5 patients reported a personal history of gallstones, while all of them presented splenomegaly, a clinical feature that is not typical of MDS. Other germline myeloid predisposing alterations were present in P3 (*CHEK2* NM\_007194.3: c.1100delC, p.T367Mfs\*15). Samples of these five suspected MDS patients were further analyzed by applying a t-NGS hereditary anemia panel to PB-DNA, thus confirming the variants identified by WES. We further characterized the deformability and the hydration status of RBCs of these five patients by ektacytometry analysis (Supplementary Figure 1). Overall, all the patients showed a bell-shaped left shifted curve compared to healthy controls, indicating a substantial dehydration status of RBCs in accordance with the presence of causative variants in *PIEZO1*. RBC morphology on PB allowed detection of stomatocytes in 3/5 patients (Supplementary Figure 2). First and second degree relatives available for each

**TABLE 1** Assessment of pathogenicity of the germline variants detected in six patients with suspected MDS following ACMG guidelines and somatic variants.

Pt code	MDS diagnosis	Germline variant	HGVS nomenclature		Manual variant classification (ACMG guidelines)	HGMD ID	Somatic variant	HGVS nomenclature	
			cDNA level	Protein level				cDNA level	Protein level
P1	MDS-MLD	PIEZO1	c.6781A>G	p.S22261G	LP (PM1, PM2, PM7, PP1, BS2)	-	KRAS	c.437C>T	p.A146V
P2	MDS-SLD	PIEZO1	c.3667G>A	p.V1223I	LP (PM1, PM7, PP1, PP2)	-	TET2	c.795_796insT	p.H266Sfs*16
							CBL	c.1142G>A	p.C381Y
							SRSF2	c.284C>G	p.P95R
							U2AF1	c.626G>A	p.R209H
P3	MDS with isolated del 5q	PIEZO1	c.5863C>T	p.R1955C	LP (PM1, PM2, PM7, PP3, BS2)	CM163883	TET2	c.2599T>C	p.Y867H
		CHEK2	c.1100delC	p.T367Mfs*15	VUS (PV51, PM2)	CD993415			
P4	MDS not confirmed	G6PD	c.844G>C	p.D282H	LP (PM2, PP3, PP2, PP5)	CM890052	-	-	-
		KCNN4	c.44G>A	p.R15Q	LP (PM1, PM7, PP2, PP3)	-			
		ABCB6	c.575G>A	p.R192Q	VUS (PM1, PP3, PP5, BS2)	CM1411559			
P5	MDS-MLD	PIEZO1	c.6479C>T	p.P2160L	LP (PM1, PM2, PM7, PP3)	CM1911803	IDH2	c.419G>A	p.R140Q
P6	MDS with isolated del 5q	PIEZO1	c.6781A>G	p.S22261G	LP (PM1, PM2, PM7, PP1, BS2)	-	TP53	c.440T>A	p.V147D
							TP53	c.742C>T	p.R248W

Note: Genetic characteristics of the six patients in which at least one high confidence RBC variant was detected. For each patient germline and somatic variants are reported. Abbreviations: HGMD, Human Gene Mutation database (HGMD Professional 2020.3); HGVS, Human Genome Variation Society database; MDS-MLD, MDS with multilineage dysplasia; MDS-SLD, MDS with single lineage dysplasia; Pt, patient; VAF, variant allele frequency.

patient were subjected to t-NGS RBC analysis on PB-DNA. Variants concerning RBC defects were confirmed by familial segregation. The hemoglobin level was documented in first/second degree patients' relatives confirming the variable expressivity of DHS (Supplementary Table 3). Ektacytometry curves depicted in relatives with DHS variants highlighted the dehydrated conditions of the RBCs beyond the MDS diagnosis of the probands (Supplementary Figure 3).

Among these MDS suspected cases, only patient P4 did not present any recurrent MDS cytogenetic abnormalities, neither any acquired somatic mutations. Patient P4, after remarkable anemia for some years, underwent BM evaluation that suggested a diagnosis of MDS with a normal karyotype and was stratified as IPSS-R low risk. Anemia was accompanied by the novel appearances of mild thrombocytopenia and splenomegaly. He was hospitalized 1 year after MDS diagnosis for acute cholecystitis while anemia was treated with erythropoietin, with fluctuation of hemoglobin values, and periodical exacerbations of anemia. Despite marrow morphology being compatible with the diagnosis of MDS with single lineage (erythroid) dysplasia (although affecting <10% of cells), WES was performed and MDS was excluded. Erythroid dysplasia was due to DHS2/FP/G6PD deficiency; indeed, multi-locus RBC defects are a recurrent disease mechanism in patients affected by hereditary anemia.<sup>1</sup>

*PIEZO1* variants determine DHS1, whose phenotype ranges from asymptomatic to severe, with massive hemolysis. DHS1 patients show hemolytic anemia with a high reticulocyte count, a tendency to macrocytosis, and mild jaundice. Some patients with DHS1 present dysplasia and hyperplasia of erythroid marrow progenitors, which may mimic MDS.<sup>2</sup> In this study, five adult patients with a confirmed diagnosis of early onset of MDS presented isolated pathogenic germline variants in *PIEZO1* (Table 1). Their relatives (investigated for 4/5 *PIEZO1* mutated patients) presented a well-compensated hemolysis without anemia, while in one case a relative presented with erythrocytosis (P5 father). However, the MDS diagnosis was confirmed in all *PIEZO1* mutated patients.

We reckoned that the concomitant presence of inherited RBC defects and MDS could accelerate the appearance of anemia, determining an early clinical onset of MDS in our “young” patient cohort. Mutations of *PIEZO1* could assume a specific role in the pathogenesis of MDS. Indeed, *PIEZO1* activation was demonstrated to delay erythroid differentiation of normal and DHS1-derived human progenitor cells, also affecting reticulocyte maturation.<sup>3</sup> In addition, alterations of *PIEZO1* may affect not only erythropoiesis, but also innate and adaptive immunity. Recent evidence highlights a role for *PIEZO1* in regulation of iron metabolism in human and mice. DHS patients can exhibit hyperferritinemia (and even hemosiderosis), accompanied by very low values of plasma hepcidin. Overexpression and chemical activation in hepatoma cell lines of the R2456H and R2488Q *PIEZO1* gain-of-function mutants induced a stronger  $Ca^{2+}$  influx than in cells expressing WT *PIEZO1*. The increased  $Ca^{2+}$  signal was associated with ERK phosphorylation, inhibition of the BMP/SMADs pathway, and decreased expression of *HAMP*,

encoding hepcidin.<sup>4</sup> These events could trigger genomic instability and favor MDS. A significant increased risk of acute myeloid leukemia was observed in sickle cell disease, without correlation with the use of hydroxyurea.<sup>5</sup> Stress erythropoiesis in inherited RBC defects determines chronic inflammation, iron overload, enhanced marrow proliferation, and turnover of erythroid precursors. All these conditions may play a role in determining genomic instability and facilitating the development of hematological malignancies. While only recently the evaluation of the incidence and impact of clonal hematopoiesis in sickle cell disease has been attempted, the predisposition to develop myeloid malignancies in other inherited RBC defects is completely under investigation. Further investigations to better evaluate the specific pathogenetic role of *PIEZO1* mutations in MDS are ongoing in our center.

Our observations yield two main indications: The first one is that in a condition compatible with MDS, the presence of inherited RBC defects should be explored, especially in unusual early onset of MDS. Unilineage BM erythroid dysplasia with anemia can simulate MDS and can cause misdiagnosis of inherited RBC defects. It is recognized that patients with an unusual young onset of de novo MDS should be extensively studied at genetic level, because of the high incidence of germline predisposition.<sup>6</sup> Genetic studies by the use of WES or t-NGS for RBC defects should thus complete the diagnostic workflow of patients with early onset of MDS. The second suggestion is that further investigations are needed to clarify the role of *PIEZO1* as a predisposing factor for the development of clonal diseases like MDS.

#### AUTHOR CONTRIBUTIONS

Enrico Attardi and Valeria Santini identified and managed MDS cases. Enrico Attardi, Immacolata Andolfo, Achille Iolascon, and Valeria Santini designed the study, collected and interpreted the data, wrote the manuscript. Immacolata Andolfo, Roberta Russo, Barbara Eleni Rosato, Roberta Marra, Federica Del Giudice performed t-NGS and ektacytometry experiments, clinical data collection, and participated in the analysis interpretation. Marco Gabriele Raddi, Alice Brogi, Angela Consagra, Cristina Amato, and Alessandro Sanna helped in sample and data collection. Lucia Tiberi, Daniela Formicola, Rosangela Artuso performed WES experiments and participated in the analysis interpretation. Immacolata Andolfo, Roberta Russo, and Achille Iolascon edited the manuscript, helped in data interpretation, and gave helpful intellectual insights during the study. Valeria Santini and Achille Iolascon took responsibility for the integrity and the accuracy of the data presented; and all authors reviewed and approved the final version of this manuscript.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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