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ORIGINAL ARTICLE

Insights into Mutation Effect in Three Poikiloderma with Neutropenia Patients by Transcript Analysis and Disease Evolution of Reported Patients with the Same Pathogenic Variants

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Abstract

Purpose Poikiloderma with neutropenia (PN) is a genodermatosis currently described in 77 patients, all presenting with early-onset poikiloderma, neutropenia, and several additional signs. Biallelic loss-of-function mutations in *USB1* gene are detected in all molecularly tested patients but genotype-phenotype correlation remains elusive. Cancer predisposition is recognized among PN features and pathogenic variants found in patients who developed early in life myelodysplasia ($n = 12$), acute myeloid leukemia ($n = 2$), and squamous cell carcinoma ($n = 2$) should be kept into account in management and follow-up of novel patients. This will hopefully allow achieving data clustered on specific mutations relevant to oncological surveillance of the carrier patients.

Methods We describe the clinical features of three unreported PN patients and characterize their *USB1* pathogenic variants by transcript analysis to get insights into the effect on the overall phenotype and disease evolution.

Results A Turkish boy is homozygous for the c.531delA deletion, a recurrent mutation in Turkey; an adult Italian male is compound heterozygous for two nonsense mutations, c.243G>A and c.541C>T, while an Italian boy is homozygous for the splicing c.683_693+1del variant. The identified mutations have already been reported in PN patients who developed hematologic or skin cancer. Aberrant mRNAs of all four mutated alleles could be identified confirming that transcripts of *USB1* main isoform either carrying stop codons or mis-spliced may at least partially escape nonsense-mediated decay.

Conclusions Our study addresses the need of gathering insights on genotype-phenotype correlations in newly described PN patients, by transcript analysis and information on disease evolution of reported patients with the same pathogenic variants.

Keywords Poikiloderma with neutropenia · *USB1* · transcript analysis · disease phenotype · cancer predisposition

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Introduction

Poikiloderma with neutropenia (PN, OMIM#604173) is a rare autosomal recessive disorder characterized by cutaneous alterations that appear during early infancy on the extremities as erythematous rash, blisters, and swelling, which may suddenly spread to the face, and then evolve into post-inflammatory poikiloderma, characterized by areas of hyper- and hypopigmentation, atrophy, and telangiectasias [1, 2]. Noncycling neutropenia is the second hallmark of the syndrome and usually develops in the early months of life manifesting with recurrent infections of the respiratory tract and the inner ear. Additionally, most patients have palmoplantar hyperkeratosis, onychodystrophy, fragile teeth, short stature, low bone density, and dysmorphisms, mainly saddle nose and midface hypoplasia [3–5]. Based on the occurrence of myelodysplasia (MDS) [4, 6–10], acute myeloid leukemia (AML) [3, 4], and squamous cell carcinoma (SCC) [4, 5] in respectively 12, 2, and 2 out of 77 PN patients so far described [2, 11], PN is thought to be a cancer-predisposing syndrome with tumors mostly developing since the second decade of life.

The U6 snRNA biogenesis 1 gene (*USB1*, OMIM*613276) on 16q21 encoding the U6 small nuclear RNA phosphodiesterase is the only causative gene for PN [12], and biallelic loss-of-function pathogenic variants have been identified in all patients with a consistent clinical diagnosis [2]. The USB1 protein contains two tetrapeptide motifs ($His_{120}LeuSer_{122}Leu$ and $His_{208}LeuSer_{210}Leu$) poised across an active site cleft between two symmetric juxtaposed lobes [8]. USB1 catalytic activity is directed on the 3′ end of U6 snRNA precursors; thus, USB1 protects U6 RNA from degradation by exosome and chaperones it into the spliceosome where it acts [13–16]. Nonetheless, the mechanistic insights into USB1 function remain to be unravelled given that splicing defects have been detected in mpn1Δ yeast cells, but not in PN lymphoblastoid cell lines [14]. To add further complexity, the ubiquitously expressed *USB1* gene presents different isoforms most of which have not yet been functionally characterized in physiologic and pathologic conditions. Due to the lack of functional studies on the variously defective USB1 proteins, only the mutational status of PN patients can be related to their clinical presentation, making it difficult to address genotype-phenotype correlations. Furthermore, owing to the relatively recent identification of the *USB1* gene [12, 17] and the provision thereafter of the genetic test [8, 18], long-term follow-up of PN patients is at the initial stage, precluding so far an extensive survey of the propensity of patients carrying different mutations to develop cancer.

A step forward to a better comprehension of genotype-phenotype correlations in PN patients is represented by transcript analysis of the mutant genes and the description of adult patients whose phenotype is fully expressed.

We report here the clinical and molecular findings, including transcript analysis, of three PN patients, including an adult one. We confirm that *USB1*-mutated transcripts carrying premature stop codons partially escape nonsense-mediated decay (NMD), predicting differently aberrant proteins of the main *USB1* isoform. We also provide the hematological characterization of the two described Italian patients focussing on mild and nonspecific bone marrow cell abnormalities, which should be regarded as premyelodysplastic changes to be carefully followed up.

Methods

Three male patients, referred to us by clinical geneticists and dermatologists, were enrolled in this study. The patients and their parents provided signed informed consent for genetic analysis and photos collection.

DNA Extraction and Mutation Analysis

Genomic DNA was isolated from the peripheral blood lymphocytes of the patients (no. #32, no. #48, and no. #49) and their parents using the Wizard Genomic DNA Purification Kit (Promega).

About 100 ng of DNA was used to separately amplify the seven exons and their flanking regions (± 20 bp) of *USB1* gene using GoTaq® Flexi DNA polymerase (Promega) and previously published primer sequences and PCR conditions [12]. Amplicons were sequenced using Big Dye Terminator v.1.1 Cycle Sequencing Kit according to the manufacture's protocol on the ABI PRISM 3130 sequencer (Applied Biosystems). Electropherograms were analyzed with ChromasPro software 1.42 (Technelysium Pty Ltd., technelysium.com.au/wp/chromaspro/) using the wild-type sequence of *USB1* gene (ENSG00000103005) as reference.

Sequence variants were described according to HGVS nomenclature guidelines (http://varnomen.hgvs.org/).

Pathogenic variants were included in LOVD database (https://databases.lovd.nl/shared/genes/USB1).

RNA Extraction, RT-PCR Analysis, and Semiquantitative RT-PCR

After collection of blood samples from PN patients no. #48 and no. #49 and controls, white blood cells were separated with Ficoll-Paque™ PLUS (GE Healthcare) density gradient, and used to extract total RNA with TRIreagent™ (Sigma), according to manufacturer's protocols. Following DNA-free DNase (Ambion) treatment, 500 ng of total RNA was used to synthesize cDNA using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems) with random

hexamers. All samples were reverse transcribed in two independent experiments.

PCR amplification of the *USB1* gene transcripts was performed for patients and positive controls using GoTaq® Flexi DNA polymerase (Promega); primers and conditions are listed in Table S1.

Nucleotide sequences were compared to the major *USB1* transcript reference sequence [GenBank: NM_024598.2].

Semiquantitative *USB1* and *GAPDH* RT-PCR analyses were performed on 2 μl of cDNA of patient no. #48, patient no. #49, four healthy controls, and a negative control using GoTaq® Flexi DNA polymerase (Promega); primers and conditions are listed in Table S2. The predicted sizes of *USB1* and *GAPDH* amplicons were 297 and 312 bp, respectively. Both reactions were simultaneously carried out in the GeneAmp PCR system 9700 (Applied Biosystems).

PCR products from 25, 28, 31, and 35 PCR cycles were loaded onto ethidium bromide-stained 2% agarose gel. Images were acquired with Gbox Chemi XT4 system (Syngene, Cambridge, UK). The densitometry of the bands was carried out using Gene Tools Gel Analysis software (Syngene).

Results

The clinical findings and the *USB1* genotypes of the three PN patients herein described are summarized in Table 1.

Clinical Data

Patient No. #32

Patient no. #32 is the only child of healthy nonconsanguineous parents of Turkish ethnicity (Fig. 1a, top). The pregnancy was uneventful and delivery was at the 36th week. Parameters of the newborn male were as follows: weight 2525 g, length 47 cm, and head circumference (occipitofrontal circumference (OFC)) 32.5 cm.

The parents informed that a cutaneous rash, which dropped away with sunlight, appeared on cheeks, ears, and extensor site of extremities when the infant was 4 months old. They also reported a history of several sinopulmonary infections during infancy.

When the child was referred for physical examination at 2.5 years, a diffuse poikiloderma with some atrophic areas and minimal desquamation and telangiectasia was evident on the face, ears, and limbs; in addition, the young boy showed photosensitivity, thin hair, nail dystrophy on feet, and minimal palmoplantar hyperkeratosis (Fig. 1a, bottom panels). No craniofacial dysmorphisms, gingivitis, or caries were observed. No osteopenia or fractures were revealed by radiographic images.

Time course laboratory examinations revealed moderate leukopenia (4.26×10^9/L), neutropenia (0.7×10^9/L), and slightly increased levels of LDH (10.43 ukat/L).

Patient No. #48

Patient no. #48 is a 36-year-old man born to nonconsanguineous Italian parents and he has a younger unaffected sister (Fig. 1b, top).

The pregnancy was uneventful, delivery was at the 36th week, and the infant weight at birth was 2900 g.

Personal history records hospitalization at 1 month for sepsis due to Gram+ infection, hemolytic anemia, and hypotonia. Neutropenia was present since the first years of life, but laboratory data cannot be traced back. Erythematous rash manifested early in infancy and then progressively evolved into poikiloderma during childhood. Photosensitivity with sun exposure was noted from first infancy and determined the onset of diffuse solar lentigines spread on photo-exposed skin areas. Growth delay was reported. He suffered from hypodontia and diffuse caries; no oral mucosal manifestations were present.

At age 15 years, radiographic images of the carpus revealed osteopenia, dysmorphic appearance of ulnar epiphysis, sclerosis of distal phalanges of the first and third digits, and soft tissue reduction of periungual phalanges. He also presented with delayed puberty and hormone therapy was started to treat hypogonadism.

Bone marrow aspiration at age 23 years documented hypocellularity with decreased number of neutrophils and increased myeloid precursors. Morphological analysis showed cells of myeloid lineage with dysplastic changes, but immunophenotypic analysis was normal without evidence of blasts. Bone marrow cytogenetic analysis on 20 cells showed a normal 46, XY karyotype.

At the last physical examination, the patient, 35 years old, presented with poikiloderma extending progressively at the trunk, face, and upper and lower limbs, and remarkable palmoplantar hyperkeratosis causing marked inability to walk and conditioning the use of specific plantar supports. Onychogryphosis and pachyonychia (Fig. 1b, bottom panels) were also present, often painful and exposed to local skin ulcerations and infections, promptly treated with local antiseptic solutions. He has thin and sparse hair, eyelashes, and eyebrows, and displays several craniofacial dysmorphisms, including macrocephaly, high forehead, hypertelorism, depressed nasal bridge, midface retrusion with mild prognathism, and low-set ears. Short stature and low muscle tone of the upper and lower extremities are noted. Laboratory investigations recorded leukopenia (1.55×10^9/L), severe (G4) neutropenia (0.45×10^9/L), anemia (HGB 123 g/L), and thrombocytopenia (124×10^9/L). Assay of clonogenic blood cells showed a normo-represented erythroid lineage and a slightly reduced myeloid lineage.

Table 1 Clinical details of *USB1*-mutated patients

Patient code	#32	#48	#49
Year of birth, sex	2010, M	1981, M	2011, M
Origin	Turkey	Italy	Italy
Birth parameters	Weight, 2525 g Length, 47 cm OFC, 32.5 cm	Weight, 2900 g Length, n.a. OFC, n.a.	Weight, 3144 g Length, 49 cm OFC, 36 cm
Poikiloderma (age at onset)	+ (4 m)	+ (n.a.)	+ (6 m)
Photosensitivity	+	+	–
Hair	Thin	Thin and sparse	Sparse
Dental defects	–	+	–
Hyperkeratosis	Palmoplantar	Palmoplantar	–
Nail dystrophy	+	+	+
Bone defects	–	Osteopenia Sclerosis of distal phalanges	–
Craniofacial dysmorphisms	–	Macrocephaly High forehead Hypertelorism Depressed nasal bridge Midface retrusion Mild prognathism Low-set ears	Frontal bossing Saddle nose Malar hypoplasia
Recurrent infections	+	+	+
Leukopenia	+	+	+
Neutropenia	+	+	+
Bone marrow evaluation	n.a.	Hypocellularity	Hypocellularity
Others	–	Hemolytic anaemia Hypotonia Growth delay Short stature Delayed puberty Hypogonadism	Hepatosplenomegaly
USB1 genotype	c.[531delA];[531delA]	c.[243G>A];[541C>T]	c.[683_693+1del];[683_693+1del]
USB1 protein	p.His179MetfsTer86	p.Trp81Ter p.Gln181Ter	p.Asp204_Gln231del

OFC, occipitofrontal circumference; +, sign present; −, sign absent; *n.a.*, data not available

Anti-nuclear antibody, anti-extractable nuclear antigene antibody, and antibodies to phospholipids were negative. CD3+,CD4+,CD8+,CD19+, and CD16/56+ cells were in the normal range.

Patient No. #49

Patient no. #49 is a 6-year-old boy, the second child of healthy, unrelated Italian parents (Fig. 1c, top).

The pregnancy was uneventful and delivery was at term by C-section for podalic presentation of the fetus.

Birth weight and length were 3144 g and 49 cm, respectively; OFC was 36 cm.

Cutaneous alterations, characterized by hypopigmented areas and diffuse xerodermia, developed at 6 months of life on the extremities and then spread to the face and ears. At 13 months, skin biopsy on the left leg confirmed poikilodermatous changes with hyperkeratosis, hypergranulosis, and slight acanthosis.

Since the age of 3 years, he suffered from frequent upper respiratory tract infections, but attended the kindergarten without major health issues.

At the time of clinical genetic assessment, he was 3 years and 8 months old and presented with poikiloderma, sparse hair, eyelashes and eyebrows, dystrophic nails, and thickened toenails (Fig. 1c, bottom panels). Photosensitivity was not observed.

He also had mild dysmorphic features including frontal bossing, saddle nose, and malar hypoplasia. No dental anomalies were noted. Normal psychomotor development was observed. His weight was 15 kg and height 95 cm (both at the 10th–25th percentile). Whole blood cell count evidenced leukopenia (3.31×10^9/L) and severe neutropenia (0.13×10^9/L),

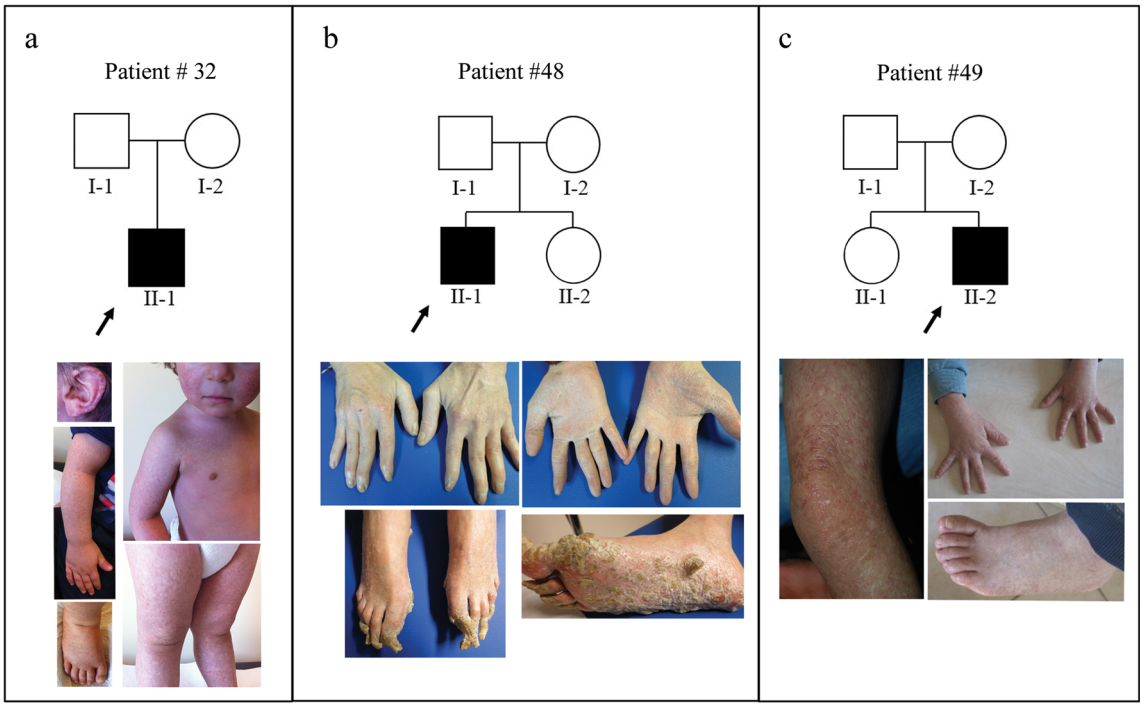

Fig. 1 Pedigrees and pictures showing ectodermal alterations of the three PN patients described here. **a** Skin alterations observed in patient #32 at age 2.5 years: clockwise poikiloderma on the helix, face and shoulder, legs, foot, and arms can be noted. **b** Close-up of cutaneous features observed on the extremities of patient #48. Compared to the hands, the feet are more involved as observed by onycogryphosis and severe hyperkeratosis of soles. Sclerodactyly-like changes of the hands and feet are probably due to phalange hypoplasia and not to a primitive skin disease. **c** Diffuse poikiloderma on the hands, foot, and arm observed in patient #49

and laboratory investigations showed increased levels of lactate dehydrogenase (LDH, 14.25 ukat/L), creatine phosphokinase (CPK, 4.45 ukat/L), aspartate amino transferase (AST, 1.02 ukat/L), and low level of creatinine (19.45 μmol/L).

Bone marrow aspiration evidenced decreased cellularity with reduced number of mature granulocytes and asynchronous maturation of erythroid lineage while bone marrow biopsy revealed a slight increment of myeloid precursors (Fig. 2a). A normal level of CD34+ naïve was apparent (Fig. 2b). No G-CSF therapy was started.

At his current age of 5 years, abdominal ultrasound revealed hepatosplenomegaly and laboratory analyses showed neutropenia (0.23×10^9/L), increased levels of LDH (11.95 ukat/L), creatine phosphokinase (4.9 ukat/L), and ferritin (0.90 nmol/L).

USB1 Molecular Analysis

USB1 sequence analysis enabled detecting different biallelic pathogenic variants in the three clinically diagnosed PN patients. As shown in Fig. 3, patient no. #32 was found to be homozygous for the exon 5 c.531delA alteration (Fig. 3a), for which both his parents are healthy carriers. Patient no. #48 was found to be compound heterozygote for exon 2 c.243G>A inherited from his father, and exon 5 c.541C>T inherited from his mother (Fig. 3b). Patient no. #49 was homozygous for exon 6 c. 683_693+1del variant, inherited by each of his healthy parents (Fig. 3c).

RT-PCR analysis allowed investigating the consequences of the *USB1* pathogenic variants at the transcript level. In all patients, the aberrant *USB1* transcripts were detected (Fig. 3d–f) and all predict to give rise to aberrant protein products.

In detail, in the homozygous patient no. #32, only the transcript carrying the r.531delA alteration was detected (Fig. 3d). The single nucleotide deletion causes a change in the reading frame starting from histidine 179, which is replaced by methionine, and leading to a stop codon 86 amino acids downstream (p.His179MetfsTer86). The predicted aberrant protein has the same amino acid length (264 aa) of the wild-type protein but a different composition in the last 85 residues.

In the compound heterozygous patient no. #48, transcripts carrying either the c.243G>A (r.243g>a) or the c.541C>T (r.541c>u) nonsense substitution have been detected (Fig. 3e) even if at a reduced level as compared with healthy controls as shown by semiquantitative analysis (Fig. S1). These aberrant transcripts that partially escape NMD, should lead to aberrant proteins of only 81 (p.Trp81Ter) and 181 amino acids (p.Gln181Ter), respectively.

Finally, only the aberrant transcript lacking the entire, 84-nucleotides long, exon 6 has been detected in the homozygous patient no. #49 (Fig. 3f, Fig. S1). The c.683_693+1del affects the guanine of the splicing GT

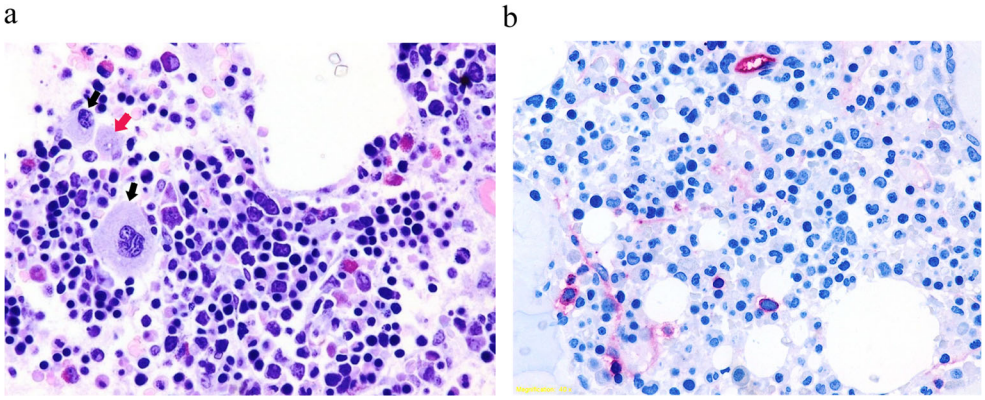

Fig. 2 Bone marrow images of patient #49. **a** Bone marrow biopsy showing areas of evaluable myelopoiesis with abundant granulopoietic precursors and few neutrophils, dispersed single pro-erythroblasts with erythroid cells in various stages of differentiation, normal (black arrows), and mildly dysplastic megakaryocytes (red arrow) (Giemsa staining, × 40 magnification). **b** CD34 immunohistochemistry showing few dispersed CD34+ positively stained cells (Gill's hematoxylin, × 40 magnification)

donor site at 5′ of intron VI causing the in-frame skipping of exon 6 in the RNA precursor (r.610_693del) (see the schematic in Fig. 3f). Translation of the exon 6-skipped messenger should result in an aberrant protein missing 28 amino acid residues (p.Asp204_Gln231del).

Discussion

All the pathogenic variants carried by the three herein characterized PN patients have been already reported. Most importantly, patients in the literature with the same mutations who

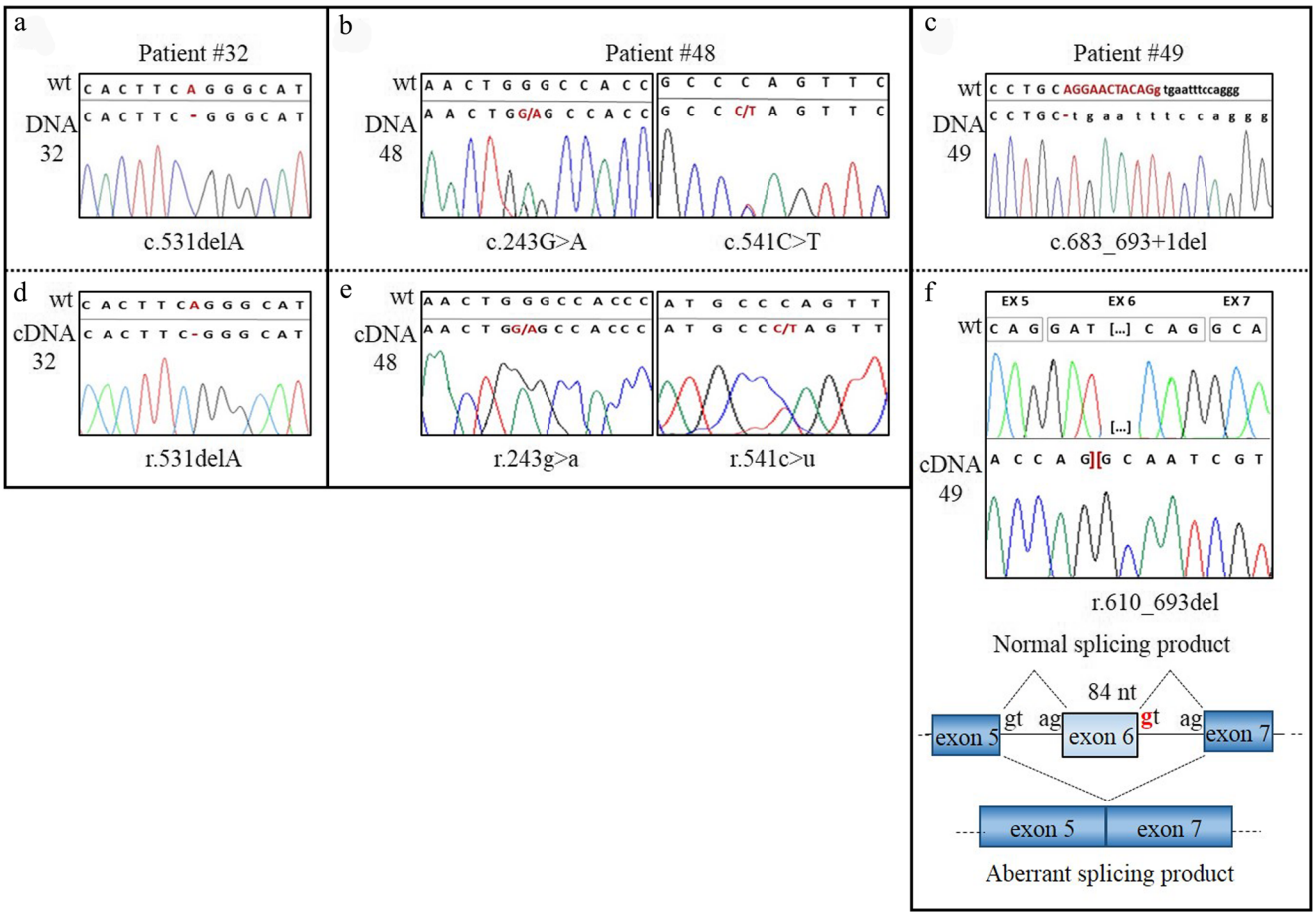

Fig. 3 *USB1* mutations and related aberrant transcripts identified in the PN patients. Electropherograms showing the *USB1* homozygous or heterozygous alterations detected in DNA (**a**, **b**, **c**) and RNA (**d**, **e**, **f**) of PN patients #32 (left), #48 (middle), and #49 (right). A schematic of the predicted in frame skipping of exon 6 caused by c.683_693+1del is provided (**f**, lower panel)

Table 2 Association between *USB1* mutations and cancer

This paper		Literature			
Pt	*USB1* mutations	Patient code (age)	*USB1* Mutations	Cancer (age of onset)	Ref.
#32	c.[531delA];[531delA]	DC107 II-1 DC107 II-2	c.[531delA];[531delA]	MDS MDS	[4]
		DC279 II-1 DC279 II-2	c.[531delA];[531delA]	– AML	[4]
		DC 215	c.[531delA];[531delA]	–	[4]
		No. 16 (12 years)	c.[531delA];[531delA]	–	[8]
		No. 17 (19 years)	c.[531delA];[531delA]	MDS (6 years)	[8]
		A (V-5) (6 years) B (V-2) (8 years)	c.[531delA];[531delA]	MDS (6 years) –	[9]
		C (V-4) (14 years)		–	
		Family 1 II-1 Family 1 II-5	c.[531delA];[531delA]	– –	[10]
		Family 4 II-2 Family 4 II-3	c.[531delA];[531delA]	– –	[10]
		Family 5	c.[531delA];[531delA]	–	[10]
		Patient 1 (28 years)	c.[531delA];[531delA]	–	[19]
		Patient 2 (35 years)	c.[531delA];[531delA]	–	[19]
		Patient (8 years)	c.[531delA];[531delA]	–	[20]
#48	c.[243G>A];[c.541C>T]	RT1 II-1	c.[243G>A];[243G>A]	AML	[3, 4]
		RT1 II-2		SCC (> 21 years)	[4]
		c16-01 (4.5 years)	c.[243G>A];[267T>A]	–	[18]
		Patient 1 (4 years) Patient 2	c.[243G>A];[243G>A]	– –	[21]
		Family 5 Pt 6	c.[541C>T];[541C>T]	SCC (13 years)	[5, 22]
		RT 3	c.[541C>T];[541C>T]	–	[4]
#49	c.[683_693+1del];[683_693+1del]	Patient 2 (> 26 years)	c.[502A>G];[683_693+1del]	MDS (14 years)	[6, 12]

MDS, myelodysplasia; *AML*, acute myeloid leukemia; *SCC*, squamous cell carcinoma

developed or not developed cancer are listed in Table 2 to discuss the relevance of this information to oncological follow-up of the newly described patients.

The c.531delA homozygous deletion detected in exon 5 of our Turkish patient no. #32 represents one of the most frequent *USB1* mutations, observed so far in 12 unrelated families of Turkish ethnicity, likely due to a founder effect [4, 8–10, 19, 20]. All the 18 reported patients share the major clinical signs of PN syndrome (poikiloderma, recurrent infections, neutropenia, palmoplantar hyperkeratosis, pachyonychia) and, in addition, four of them suffer from myelodysplasia [4, 8, 9] and one has developed acute myeloid leukemia [4] (Table 2). This recurrent mutation is predicted to lead to the loss of the second His_{208}-Leu-Ser_{210}-Leu tetrapeptide motif of the USB1 protein (p.His179MetfsTer86) determining the destruction of its 2H active site. Indeed, previously performed transcript analysis confirmed that the aberrant transcript of this variant does not fully undergo NMD being thus potentially translatable [8]. Bone marrow studies were not performed in patient no. #32, though a strict oncological surveillance has been recommended to the professionals in charge of child follow-up.

As regards the Italian patient no. #48 with compound heterozygosity, the c.243G>A transition has been described in three European families [4, 18, 21] in either homozygous (two families) or heterozygous (one family) state. To note, in one homozygous family with two affected sisters, the eldest died from AML [3] and the other developed a skin cancer [4] (Table 2). RNA analysis, first performed in the current work, could detect the aberrant transcript even of this early truncating mutation predicting a protein (p.Trp81Ter) comprising part of the N-terminal sequence repeat and lacking both His-Leu-Ser-Leu tetrapetide motifs which are essential for USB1 activity [8].

The second c.541C>T mutation of patient no. #48 has been described in homozygous state in two patients [4, 22] of which the one with the more severe phenotype developed a squamous cell carcinoma at 13 years of age [5] (Table 2). The aberrant transcript of this nonsense c.541C>T mutation has

been here assessed and its detection supports possible translation in a truncated protein lacking the second His-Leu-Ser-Leu motif (p.Gln181Ter).

Three out of seven patients carrying either mutation of patient no. #48 developed cancer, two developed skin cancer early in life [4, 5], and one AML (42%) (Table 2). Adult patient no. #48 is tumor free at age 36 years, though bone marrow studies had evidenced hypocellularity, dysplastic changes of myeloid cells, and decreased number of neutrophils. His overall phenotype is quite severe, with critical neutropenia, anemia, thrombocytopenia, remarkable ectodermal findings, craniofacial dysmorphisms, skeletal abnormalities, hypogonadism, and muscle hypotonia. Though the *USB1* genotype of our patient is first described, the history of patients carrying either of his mutations imposes careful dermatological screening for skin cancer.

The c.683_693+1del mutation detected in homozygous condition in the Italian boy no. #49 has been already described in heterozygous state in one Italian patient reported to suffer from myelodysplasia [6, 12] (Table 2). This splice site mutation like another described mutation, c.693+1G>T involving the first base of intron VI [8, 22], should lead to the loss of the second His-Leu-Ser-Leu domain of the USB1 protein encoded by the skipped exon 6, as predicted by the aberrant RNA here assessed. Bone marrow studies evidenced hypocellularity and decreased number of granulocytes, which at this early age may signal the propensity to myelodysplasia evolution. This is consistent with the marked hepatosplenomegalia displayed by patient no. #49.

Conclusions

Clinical characterization of three unrelated PN patients confirmed the clinical expressivity of PN, related both to the major poikiloderma and neutropenia signs and to other less frequently observed clinical findings. Our three patients display a phenotype that is graded as moderate in no. #32 (neutropenia), severe in no. #49 (neutropenia and dysplastic changes of myeloid and erythroid cells), and remarkably severe in the adult patient no. #48, who displays almost all clinical findings of the PN syndrome.

Molecular characterization of these three patients allowed confirming the presence of scarce amount of potentially translatable aberrant transcripts, predicting differently defective proteins. This information can be extended to published patients carrying the same mutation(s) not processed for transcript analysis, enhancing genotype-phenotype correlations which have so far remained elusive for PN, notwithstanding > 70 patients reported. Until studies on USB1 protein in PN patients are performed, a search of the transcripts of mutant alleles in patients who carry reported or recurrent mutations, if not previously performed, may be the first step to shed light on the effects of the mutation(s) and to provide a key to interpret the reported association of certain mutations with cancer development.

In the same way, the reported follow-up of PN patients with the same *USB1* genotype is crucial for management of novel-described patients and may direct targeted surveillance of hematological and skin cancer.

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Authorship Contributions EAC interpreted the molecular results, drafted, revised, and approved the final version; NHE contributed with clinical data and biological samples of family no. #32, drafted the clinical paragraph, and approved the final version; CG contributed with clinical data and biological samples of family no. #49, drafted the clinical paragraph, and approved the final version; PF contributed with clinical data and biological samples of family no. #48, drafted the clinical paragraph, and approved the final version; EDF performed molecular analyses; IN contributed with clinical data and biological samples of family no. #49, drafted the clinical paragraph, and approved the final version; EF contributed with clinical data and biological samples of family no. #49, drafted the clinical paragraph, and approved the final version; MG contributed with clinical data and biological samples of family no. #48, drafted the clinical paragraph, and approved the final version; CG supported the molecular work and software access; LL conceived and coordinated the study, and drafted, revised, and approved the final version of the manuscript.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests.

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