CLINICAL REPORT

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Editor's Note

In this article Concolino and colleagues describe the phenotype in three sibs with the Clercuzio-type poikiloderma with neutropenia syndrome (PN). Most of the authors are also co-authors on the article by Volpi et al. published in the January issue of the *American Journal of Human Genetics* where the causative gene for PN is identified in this family. In the ensuing report herein Concolino et al. provide comprehensive phenotypic data on the sibs who were succinctly presented in the *AJHG* paper. Continuing on this theme, I would turn the reader's attention to a related article on this topic by Tanaka and colleagues, published in the June issue of the *Journal* [Tanaka et al., 2010. Identification of a homozygous deletion mutation in *C16orf57* in a family with Clericuzio-type poikiloderma with neutropenia, *Am J Med Genet* Part A 152A:1347–1348] where the authors identify a homozygous deletion in sibs previously reported in the *Journal* in 2008.

John C. Carey
Editor-in-Chief

Clericuzio-Type Poikiloderma With Neutropenia Syndrome in Three Sibs With Mutations in the *C16orf57* Gene: Delineation of the Phenotype

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We report on three sibs who have autosomal recessive Clericuzio-type poikiloderma neutropenia (PN) syndrome. Recently, this consanguineous family was reported and shown to be informative in identifying the *C16orf57* gene as the causative gene for this syndrome. Here we present the clinical data in detail. PN is a distinct and recognizable entity belonging to the group of poikiloderma syndromes among which Rothmund–Thomson is perhaps the best described and understood. PN is characterized by cutaneous poikiloderma, hyperkeratotic nails, generalized hyperkeratosis on palms and soles, neutropenia, short stature, and recurrent pulmonary infections. In order to delineate the phenotype of this rare genodermatosis, the clinical presentation together with the molecular investigations in our patients are reported and compared to those from the literature.

Key words: poikiloderma; genodermatosis; congenital neutropenia

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INTRODUCTION

Clericuzio-type poikiloderma with neutropenia (PN OMIM #604173) represents a well-defined phenotype characterized by

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poikiloderma, hyperkeratotic nails, generalized hyperkeratosis on palms and soles, neutropenia, short stature, and recurrent pulmonary infections [Clericuzio et al., 1991].

The dermatologic onset consists of the occurrence of a papular erythematous rash during the first months of life that spreads on to the limbs centrifugally. When the papulae vanish, hypo/hyper pigmentation and telangiectasia develop.

The differential diagnosis includes other poikiloderma conditions, that is, RAPADALINO and Rothmund–Thomson syndromes (RTS, OMIM #268400) [Van Hove et al., 2005]. Due to the overlap of some distinctive clinical features, PN patients may be misdiagnosed as RTS, even if cataract and digestive tract involvement (hallmark features of RTS) are not preset. In PN patients chronic neutropenia is a distinguishing manifestation. At the molecular level *RECQL4* helicase gene mutations are present in about 60–65% of RTS cases [Larizza et al., 2010] but are not present in all PN patients, strengthening the hypothesis of distinct genetic mechanism responsible for PN [Wang et al., 2003; Van Hove et al., 2005; Mostefai et al., 2008]. Recently, we demonstrated that mutations in the *C16orf57* gene are responsible for the PN phenotype in the reported three sibs [Volpi et al., 2010]. In this report we present the clinical data in more detail.

The sibship reported underlines the intrafamilial variable expressivity and the natural history of PN syndrome. Moreover, we compare the clinical features in our patients with the 11 so far published [Pianigiani et al., 2001; Wang et al., 2003; Van Hove et al., 2005; Mostefai et al., 2008] to better delineate the spectrum of this rare syndrome.

CLINICAL REPORTS

Family history and genetic testing: Three siblings, one male (Patient 1) and two females (Patients 2 and 3), from healthy consanguineous (Fig. 1) Italian parents were born at term after uneventful pregnancy, absence of perinatal problems; all growth parameters were in the normal range. At birth none showed skin manifestations and/or dysmorphic signs. As shown in the simplified pedigree (Fig. 1) [see full pedigree, Volpi et al., 2010] the siblings are born to double second cousins, and the father himself is son of a consanguineous marriage.

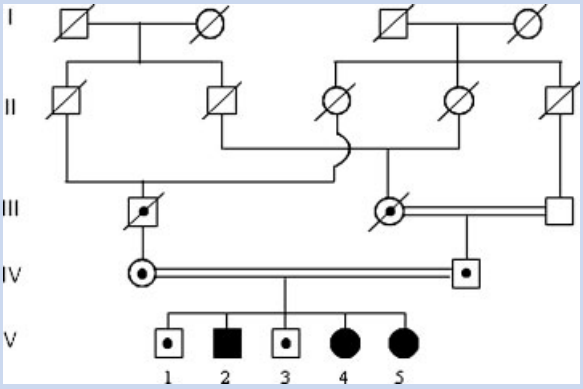

FIG. 1. Pedigree of the family.

Patient 1

The cutaneous manifestations began at 6 months of age as a rash involving primarily the cheeks, extensor surface of the arms, and then the knees (Fig. 1, V2). During his first months of life the child had recurrent pulmonary infections, otitis media, and sinusitis until the age of 3 years. He showed poor growth starting in infancy but no delay in psychomotor development. Skin findings including reticular pattern of hyper- and hypopigmentation with slight telangiectasia and atrophy of his cheeks, upper trunk, and extremities, allowed a clinical diagnosis of RTS at the age of 5 years. At the age of 6 years because of a pulmonary infection he was hospitalized and at that time he showed a weight of 17.7 kg (10th centile), a height of 106 cm (3rd centile), and normal mental development. During hospitalization the biological data as laboratory investigations showed leukopenia (2.5×10^3 μL) with neutropenia (700 mm^3), increased creatine phosphokinase (CPK, 250 U/L, n.v. < 180), and lactate dehydrogenase (LDH, 700 U/L, n.v. < 500) while viral serology, erythrocyte sedimentation rate, C reactive protein, immunoglobulin subclasses (IgA, IgM, IgG), complement factors (C3 and C4), AGA, EMA, tTG, and thyroid hormones were in the normal range for age. Bone marrow smears showed abnormal maturation of neutrophil lineage, with increased numbers of immature cells, but no abnormal clonality. The skeletal investigation, electromyography, electroencephalography, abdominal ultrasonography, echocardiography, and bone scintigraphy with Tc99m were normal. No ophthalmologic abnormality and specifically no cataracts were found.

The patient came to our attention for the first time at the age of 17 years and though a history of persistent neutropenia was reported, no recent infectious episodes were recorded. At that time his weight was 49 kg (<5th centile), his height 156 cm (<3rd centile), his head circumference 56 cm (50th centile), and Tanner stage was P5B5. Until age 3 years, growth velocity had always been under 5th centile; his genetic target for the height was 170 ± 8 cm. Puberty had begun at age 14 years without pubertal growth spurt.

The clinical exam at the age of 21 (Fig. 2) showed dysmorphic signs including eyebrows hypoplasia with normal hair, frontal bossing, widely spaced eyes without telecanthus, midface hypoplasia, small nose with depressed nasal bridge, and mild prognathism. Generalized fine hyperkeratosis with poikiloderma on the face and arms with several areas of skin atrophy was detected. Hyperkeratosis was more pronounced at extremities, with hyperkeratotic palms/soles and pachyonychia. A bilateral hyperlaxity of fingers (Fig. 2) and a joint stiffness in elbows were present.

The skin biopsy showed edema in the papillary derma, necrosis of keratinocytes with apoptosis, acute perivascular inflammation with granulomatous vasculitis of derma middle blood vessels. Standard karyotype on skin biopsy was normal. No screening for increased breakage was performed. No bone abnormalities were found. Skeletal X-rays were normal, and MRI showed normal intensity of medullar bone signal of explored segments. The bone age was appropriate for chronological age.

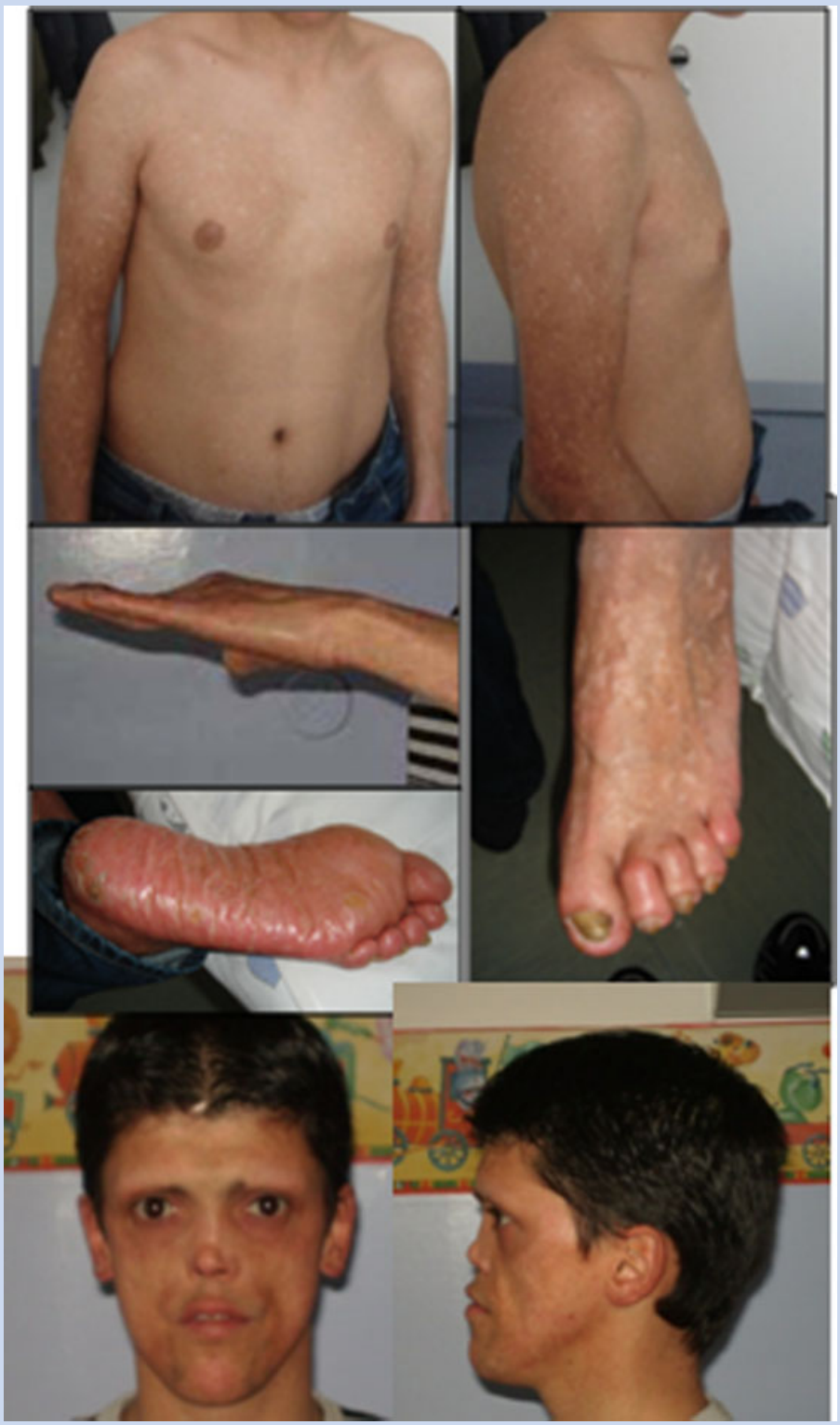

FIG. 2. Patient 1 at the age of 21 years. Note eyebrows hypoplasia, severe midface hypoplasia, the pattern of the poikiloderma, pachyonychia and plantar hyperkeratosis, hypermobile fingers.

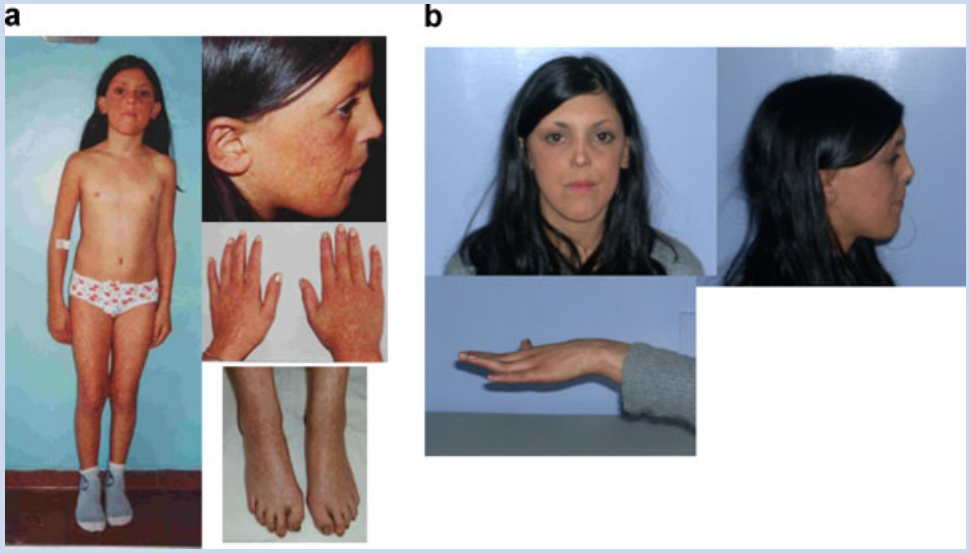

FIG. 3. Patient 2: (a) at the age of 10 years: note poikiloderma of face and arms and foot pachyonychia and (b) at the age of 16 years: note a mild midface hypoplasia with a small mandible, hypoplasia of lateral eyebrows, hypertelorism, long philtrum, hypermobile fingers with "beak of swan" appearance.

The mental development was normal. Mild splenomegaly was detected by abdominal ultrasonography. Laboratory investigations showed a leukopenia (GB 2.2×10^3 µL) with neutropenia (550 mm^3), elevated LDH (800 U/L, n.v. < 500), and CPK (280 U/L, n.v. < 180).

Patient 2

The skin manifestations appeared at 8 months of age with a rash involving primarily the face and the extensor surface of the arms evolving in classic poikiloderma (Fig. 1, V4). During the first years of life no history of recurrent infections was reported but neutropenia was found at the age of 2 years. At the age of 3 years she showed a reduction of height velocity with a height age of 2 years. At age 4 a high-resolution cytogenetic analysis confirmed a 46,XX karyotype.

When she first came to our attention at the age of 9 years and 11 months; the clinical examination showed a weight of 25 kg (10th centile), a height of 120 cm (<3rd centile), a head circumference of 52 cm (50th centile), prepubertal stage and mental development were normal. Exam showed poikiloderma on the face, arms, and thorax, nail dystrophy, hypoplasia of eyebrows, mild midface hypoplasia, and mild splenomegaly (Fig. 3). She showed leukopenia (3.5×10^9 µL) with neutropenia (600 mm^3). CPK and LDH were elevated. Viral serology, AGA, EMA, tTG, were normal. Normal levels of all immunoglobulin classes and of complement factors (C3 and C4) were found. Renal and cardiac functions were normal. Electromyography, electroencephalography, and bone scintigraphy with technetium were normal. Abdominal ultrasonography showed a spleen size of 16 cm.

Physical examination at the age of 16 years showed midface hypoplasia with a small mandible, hypoplasia of lateral eyebrows, hypertelorism (interpupillar distance >95th centile), long philtrum, hypermobile fingers with "beak of swan" appearance (Fig. 3b).

Patient 3

The child was evaluated at our hospital at the age of 8 years and 8 months (Fig. 1, V5). She showed the characteristic poikilodermatous rash that had first appeared at age 10 months as a facial rash. The rash was erythematous and macular with few atrophic areas. During the first months of life the girl had recurrent pulmonary infections, otitis media, and sinusitis until 3 years of age. Leukopenia (3.2×10^3 µL) was found with severe neutropenia (0.6×10^3 µL) at age 18 months. The short stature was discovered at the age of 3 years and her growth velocity was always under 5th centile in the following years. High-resolution karyotype was 46,XX.

Clinical examination showed a weight of 23 kg (10–25th centile), a height of 121.5 cm (<3rd centile), a head circumference of 52 cm (50–70th centile), prepubertal stage, and mental development were normal. The patient showed poikiloderma, microretrognathism, and a low posterior hairline (Fig. 4a). At the age of 12 years a dysmorphic examination revealed lateral hypoplasia of eyebrows, hypoplasia alae nasal, mild retromicrognatia, and hypermobile fingers with "beak of swan" appearance (Fig. 4b).

The routine laboratory investigations showed increased CPK and LDH, leukopenia (GB 3.2×10^3 µL) with marked neutropenia (500 mm^3). Levels of immunoglobulin classes and complement factors (C3 and C4) were normal. Abdominal and cardiac ultrasonography, electromyography, and muscular biopsy (histochemical and biochemical analyses) were normal.

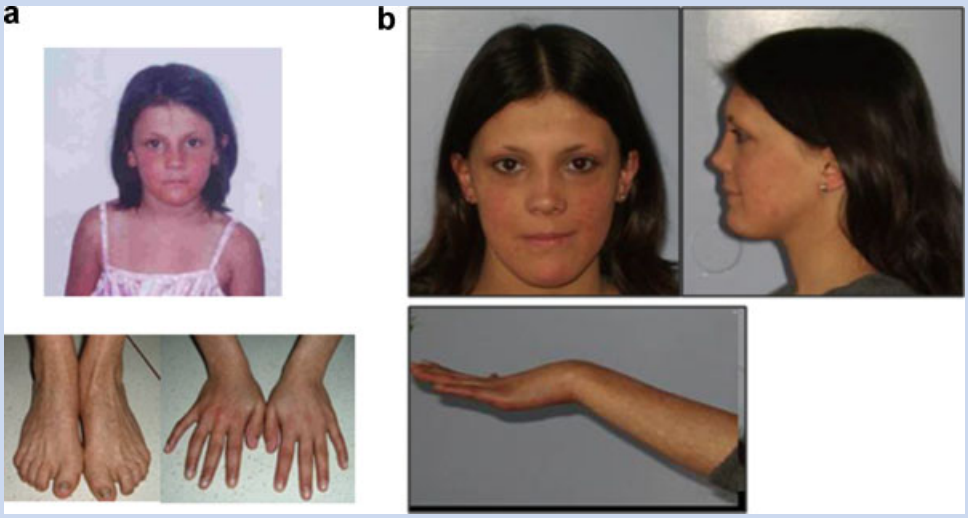

FIG. 4. Patient 3: (a) at the age of 8 years: note face poikiloderma. b: At the age of 12: note eyebrows hypoplasia, mild retromicrognatia, hypermobile fingers with "beak of swan" appearance.

In both sisters the bone age matched the chronological age and endocrine exams showed that IGF1 and IGFBP3 levels, arginine/L-dopa-stimulated growth hormone analysis, TSH, and T3 levels were in the normal range. The growth velocity was between 5 and 10th centile and the puberty started at the age of 11 and 12 years, respectively, without pubertal spurt. Their genetic target was 157 ± 8 cm.

At the 6-month follow-up, evaluation of the patients' neutropenia found a persistent neutropenia with a mean value of 700 mm^3 (range of 500–1,100 mm^3). Bone marrow smears showed myelodysplastic features, abnormal maturation of neutrophil lineage, and an increased number of immature cells. None of the three patients showed skin sensitivity to sun exposure and there were no evidence of cancer skin lesions. None of them developed cataracts.

To exclude a possible diagnosis of RTS, the *RECQL4* gene was capillary sequenced in the propositus, Patient 1, and no mutations were found in all exons and introns, in keeping with the finding that the three affected sibs do not share intragenic *RECQL4* SNPs (data not shown). Autozygosity mapping performed by a genome-wide SNP array from 18 family members identified a candidate region of 3.4 Mb mapping at 16q inherited identically by descent (IBD) in all affected family members. Next generation sequencing of the entire repeats-masked candidate region, including coding and noncoding sequence, revealed a homozygous change at IVS IV donor splice site of the *C16orf57* gene. Details of the multimethod procedure used to hunt and validate the causative gene have been reported [Volpi et al., 2010].

DISCUSSION

We report on the clinical features of three siblings affected with Clericuzio poikiloderma with neutropenia syndrome, all carrying the same homozygous c.504-2A > C mutation at the acceptor splice site of intron 4 of *C16orf57* gene [Volpi et al., 2010]. Our molecular data conclusively confirm the notion that this rare poikiloderma syndrome is a genetically distinct entity [Van Hove et al., 2005; Mostefai et al., 2008].

Since the first description by Clericuzio in 14 Navajo Indians [Clericuzio et al., 1991], 5 other kindreds have been described [Wang et al., 2003; Van Hove et al., 2005; Mostefai et al., 2008] with a total of 10 affected subjects, coming from different countries (2 Navajo, 1 Turkish/British, 2 Scottish, 2 Turkish, and 3 Moroccan). All reported patients, including ours, had a clinical diagnosis of PN and absence of *RECQL4* mutations. Moreover, an Italian patient, first reported as RTS and myelodysplasia [Pianigiani et al., 2001] but displayed a typical PN phenotype, was found to be a compound heterozygote for *C16orf57* mutations (c.502A > G and c.666-676 + 1del12) [Volpi et al., 2010]. This confirms that PN is a genetic disease likely occurring in all ethnicities and not restricted to the American Navajo population [Erickson, 1999].

We compared the phenotype of our affected sibs with that of previously described patients (Table I) including the first patients who have been molecularly assessed to delineate the spectrum of clinical findings. We suggest that this description may be helpful to recruit and select patient candidates to be tested for the *C16orf57* gene.

With regard to RTS, which enters in the differential diagnosis with PN, we underline the differential profile of the skin changes; they start in both syndromes in the first year of life with the same morphology and evolution of the lesions (from acute rash to chronic poikiloderma with prevalent acral distribution). Peripheral involvement of arms and legs at the onset, then spreading centrifugally has been described in most of the previously PN reported cases [Van Hove et al., 2005]. This observation has led to an emphasis on the initial localization of the rash as one of the main differential hallmarks between PN and RTS, where the rash starts on the face [Wang et al., 2003; Van Hove et al., 2005]. In our patients the skin

TABLE I. Clinical and Laboratory Findings in 14 Patients With Poikiloderma Neutropenia Syndrome

	Present report (one kindred)			Mostefai et al. [2008] (one kindred)			Van Hove et al. [2005] (one kindred)		Wang et al. [2003] (three kindreds)					Pianigiani et al. [2001] (one kindred)
	Ethnicity													
	Italian			Moroccan			Turkish		Navajo		Turkish/ British	Scottish		Italian
	1	2	3	1	2	3	1	2	1	2	1	1	2	1
Skin manifestations														
Age onset	6mo	8mo	10mo	14mo	15d	6mo	8mo	11mo	/	/	3mo	2mo	2mo	6–7mo
Initial localization	Cheeks	Face	Face	Face, extremities	Face, extremities	Face, extremities	Arms, legs	/	/	/	Lower extremities	Arms, legs	Arms, legs	/
Pachyonychia	+	+	+	+	+	−	+	+	+	+	+	+	+	+
Growth delay	++	+	+	+ (3y)	++ (3y)	/	−	/	/	/	/	/	/	+
Dysmorphic features	+	+	+	−	/	/	/	/	/	/	/	/	/	+
Recurrent infections														
Pneumonia	+	−	+	+	+	+	+	+	+	+	/	+	+	+
Otitis media	−	−	+	+	+	+	+	+	+	+	/	+	+	+
Other	−	−	S	FC; A	A	BL	GS	BL; CO	/	/	/	/	/	GS
Neutropenia														
Age onset	6mo	2y	18mo	14mo	12mo	6mo	7d	21d	/	/	20mo	20mo	20mo	/
Absolute number (mm^3)	500–1,100	600–800	350–700	220–884	345–1,541	183–5,200	150–500	/	/	/	300	100–900	100–900	355
Laboratory findings														
CPK	+	+/−	−	/	/	/	+	−	/	/	/	/	/	/
LDH	+	+	+	/	/	/	+	+	/	/	/	/	/	/
Splenomegaly	+	+	−	+	+	+	+	+	/	/	/	/	/	+

/, not reported; −, absent; +, present; S, sinusitis; FC, facial cellulitis; A, adenitis; BL, blepharitis; GS, gastroenteritis; CO, conjunctivitis; mo, months, y, years; d, days.

lesions manifested in the first year of the age but primarily involved the cheeks, then spreading to extensor surface of the arms and finally to the knees. The same onset was also reported in the Moroccan PN patients [Mostefai et al., 2008]. Thus, we posit out that the first localization of the rash may be variable and does not represent per se a criterion for establishing or excluding PN diagnosis. The histology on the skin was not diagnostic and was not a distinctive feature compared with RTS.

Isolated persistent severe neutropenia is the hallmark of this genodermatosis, appearing between the first weeks and 20 months of age. In Patients 2 and 3 the neutropenia was diagnosed at 2 years and 18 months, respectively, while in Patient 1 the first observation of neutropenia was at age 6, being unavailable previous measurements. We followed up the evolution of neutropenia in all three patients for 4 years, and we never found a significant variation in neutrophils absolute number (500–1,100 mm^3) suggesting that neutropenia is not cyclic as reported by Wang et al. [2003]. The leukopenia can explain the several pulmonary infections, otitis media, and sinusitis while it is not clear why the infective episodes became less frequent during the adolescence and adult age as observed in our patients though the neutropenia is persistent in the long-term follow-up. Besides neutropenia, myelodysplasia could be another distinctive feature of PN, as pointed out in previous reports [Pianigiani et al., 2001; Van Hove et al., 2005; Mostefai et al., 2008]. The detection of *C16orf57* mutations in the patient described by Pianigiani et al. [2001] is consistent with the opinion that a few patients previously reported as RTS [Rizzari and Conter, 1996] who also show myelodysplasia probably belong to this syndromic entity.

Increase of enzymes as LDH and CPK has been reported by Van Hove et al. [2005] and a possible muscle involvement has been suggested. Our three sibs all had a slightly and episodic increase of CPK (250–380 U/L), while LDH was significantly increased with values between 500 and 1,200 U/L. However, the electromyography and muscular biopsy did not show any abnormality either in our patients or in the patient described by Van Hove, preventing to ascertain the origin of these enzymatic increases. The increased CPK and LDH serum levels, in the absence of clinical evidence, appear to be distinctive biochemical markers of PN, as they have never been reported in RTS patients.

Growth delay is reported in some PN patients and it is probably due to recurrent infectious diseases common in this syndrome. Short stature, in our patients appeared in the first years of life, with a deceleration of growth velocity after the age of 3 years, but no sib had GH deficiency based on provocative GH test, or other apparent causes of short stature.

Moreover, dysmorphic features such as hypoplasia of eyebrows, frontal bossing, widely spaced eyes, midface hypoplasia, small nose, depressed nasal bridge, and prognatism are common signs in our siblings. Though an examination has not been made in all previously reported cases, the high similarity of craniofacial dysmorphisms between our male patient (Fig. 2) and the male patient described by Van Hove et al. [2005], in particular, midface hypoplasia is recognizable by photographs. Moreover, moderate facial dysmorphisms like "saddle nose" and hypertelorism are also reported in the other Italian compound heterozygote for *C16orf57* mutations [Pianigiani et al., 2001; Volpi et al., 2010]. Our clinical report points to the wide phenotypic heterogeneity of PN syndrome which deserves other observations to be further defined. Careful bone marrow smears examination and follow-up for the hematologic disease are needed to check the onset of myelodysplasia or leukemic transformation.

Genome-wide SNPs array-based homozygosity mapping and targeted next generation sequencing have proven to be the best strategy to appoint *C16orf57* as Clericuzio-type poikiloderma with neutropenia gene, allowing to provide a complete genetic counseling from carrier testing through at-risk relatives, and hence to plan out a targeted onco-hematologic surveillance for the affected patients.

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