H syndrome mimicking Erdheim Chester disease: new entity and therapeutic perspectives

Biallelic pathogenic variants in the *SLC29A3* gene are responsible for autosomal recessive H syndrome, variably combining hyperpigmentation, hypertrichosis, hearing loss, hepatosplenomegaly, heart anomalies, hypogonadism, low height, diabetes mellitus and camptodactyly.[1] Tocilizumab (anti-IL-6 receptor) is considered to improve patient outcomes.[2]

However, other heterogenous *SLC29A3*-associated phenotypes have been reported with overlapping features, including hematologic disorders, auto-inflammatory manifestations and histiocytic infiltrates (HI) such as Rosai Dorfman disease (RDD) which have been classified in the R-group of the revised classification of histiocytoses.[3,4] Indeed, skin histiocytic infiltrate and lymphadenopathy were frequently described (68% and 24% respectively in a 79-patient cohort), whereas retroperitoneal fibrosis and mediastinal mass were unusual.[1]

SLC29A3 encodes for equilibrative nucleoside transporter 3 (ENT3) which allows membrane translocation of nucleosides in late endosomes, lysosomes and mitochondria.[5] A *SLC29A3*$^{-/-}$ mouse model displays systemic infiltrates in several organs notably associated with upregulation of the mechanistic target of rapamycin (mTOR) signaling.[6]

We herein describe four adult patients with pathogenic variants in *SLC29A3* and atypical presentation of HI mimicking Erdheim Chester disease (ECD) rather than R-group histiocytosis. Tocilizumab appeared effective in the regulation of their inflammatory manifestations, but ineffective regarding HI. One of these ECD-like patients was treated with cobimetinib (MEK inhibitor), allowing a fast reduction of HI, probably through the regulation of the mTOR pathway.

Immunohistochemistry was performed on an automated immunostainer (Ventana BenchMark Ultra, Roche, Meylan, France) using Ultraview Universal Kit. Sections were incubated with anti-pS6 (Ser235/236) (1:50, Cell Signaling, 4858), anti-p4E-BP1 (Thr37/46) (1:500, Cell Signaling, 2855) and anti-CD163 (10D6) (1:100, Novocastra-Leica, NCL-L-CD163). Staining was visualized with DAB solution.

Patients from French centers were identified and retrospectively included. Informed signed consent was obtained from each patient.

Next-generation sequencing (NGS) of a targeted panel of auto-inflammatory diseases-associated genes was performed in blood samples and revealed homozygous *SLC29A3* (NM_018344.6) disease-causing variants (Table 1).

Case 1

A 37-year-old female with a medical history of bilateral cochlear hearing loss presented anorexia, asthenia, and weight loss, as well as abdominal pain. Clinical examination found a cutaneous hyperpigmentation and hypertrichosis overlying the lower limbs (Figure 1A), hallux valgus, and camptodactyly. Blood test results showed a 100 mg/L C reactive protein (CRP), a characterized diabetes mellitus (hemoglobin HbA1c 7,7%) and low IGF-1 level 85 μg/L (normal range, 109-350 μg/L). Computed tomography (CT) scan and cardiac magnetic resonance imaging (MRI) found a right atrial, interatrial septum, periaortic, pleural, and perirenal tissue infiltrates (Figure 1B). Pleural and skin biopsy were performed and found CD163^{+}/CD68^{+}/S100^{-}/CD1a^{-}/OCT-2^{+} histiocyte infiltrates. Investigations on biopsy samples found no mutation in the BRAF-MEK-ERK pathway, or in *PIK3CA*, and negative p-ERK immunostaining.

The patient was treated with low-dose corticosteroids and tocilizumab (8 mg/kg/4 weeks) resulting in inflammation normalization, skin infiltration regression, but no other HI improvement. Although MAPK upregulation was not found, considering ECD-like HI, the patient was treated with cobimetinib (28-day cycle therapy: 40 mg/day for 21 days, and no treatment for 7 days), allowing a fast clinical response (within 3 months) with a significant reduction of interatrial septum infiltrate (26 mm to 13 mm), and resolution of periaortic and perirenal infiltrates (Figure 1C-F), as well as skin histiocytic infiltrate decrease in biopsy (Figure 1G, H) with no relapse at 1-year follow-up.

Case 2

A 42-year-old female presented a subacute 5-month evaluative spastic paraparesis. She had a history of bilateral cochlear hearing loss, micromelia, dysphonia and camptodactyly (Figure 2A). She also presented a history of child-onset inflammatory arthritis considered as juvenile idiopathic arthritis with corticosteroid dependence. Clinical examination revealed lower limbs pyramidal signs and sensory-motor deficit. Spinal cord MRI revealed an epidural mass with contrast enhancement between C5 and L5 complicated by dorsal medullar compression (Figure 2B), CT scan was unremarkable. Blood tests results found fluctuating inflammatory syndrome (CRP >10 mg/L). Infectious triggers were ruled out, and immunological assays were unremarkable.

A surgical biopsy was performed and revealed inflammatory infiltrates composed of plasma cells (CD138^{+}) and CD68^{+}/S100^{-}/CD1a^{-} macrophages. NGS analysis was per-

Table 1. Main characteristics of the enrolled patients in the study with mutation status.

Characteristic		Patient 1	Patient 2	Patient 3	Patient 4
Sex, M/F		F	F	M	F
Age in years at diagnosis		37	42	43	48
Genetic analysis	*SLC29A3* (NM_018344.6) disease-causing variants	Homozygous c.1088G>A p.(Arg363Gln).	Homozygous splice-site c300+1G>C	Homozygous c.1088G>A p.(Arg363Gln)	Heterozygous c.45delC (exon2); p.(Thr16Profs85) Heterozygous c1279G>A; p.(Gly427Ser)
	Mutations assessed in tissue biopsy (NGS)	Skin and pleural biopsy: no *BRAF*, MEK/ERK pathway or *PIK3CA* mutation	Epidural biopsy: no *BRAF* or *PIK3CA* mutation	*	Skin biopsy: no *BRAF* mutation
Age in years at symptom onset		16	4	5	5
Country of origin		Marocco	Algeria	Marocco	Martinique
Diabetes mellitus HbA1c (%)		yes 7.7	no *	no *	yes *
Anemia Hemoglobin (g/L) MCV (fL)		yes 109 70	yes 85 61	no * *	yes 80 67
Arthritis		no	yes	yes	no
Hyperpigmented skin		Lower limbs	0	Back	Lower limbs
Hearing loss		yes	yes	yes	yes
Micromelia		no	yes	no	yes
Hallux Valgus		yes	no	yes	Cubitus Valgus
Flat foot		no	no	yes	yes
Cardiac abnormalities		Pericardial effusion	Pericardial effusion	-	Pericardial effusion
Camptodactyly		yes	yes	yes	yes
Stature, height (cm)		163	147	170	160
Organomegaly		Splenomegaly	no	no	Hepatomegaly and splenomegaly
Sexual abnormalities		no	no	Gynecomastia	no
Ocular abnormalities		Exophtalmos	Red and painful eyes	Dilated scleral vessels	Glaucoma, exophthalmos, dilated scleral vessels
Facial telangiectasia		no	no	yes	no
Lymphadenopathy		no	no	no	yes
Tissue infiltrates Skin infiltrate** Peri-cardiac infiltrate** Peri-renal infiltrate** Bone involvement** Other infiltrate**		 yes yes yes no Pleural infiltrate	 no no no no Epidural infiltrate	 yes Peri-aortic infiltrate yes no Peritoneal infiltrate	 yes yes yes no Interstitial lung infiltrate
Inflammatory syndrome CRP⁺ (mg/L)		 100	 >10	 >20	 >100
Immunostaining Biopsy site CD68 CD163 PS100 Langherin CD1a BRAF pS6 / p4EBP OCT-2		 Skin and pleural + + - - - - + +	 Epidural + * - * - * * *	 Skin *** 	 Skin + + + * - * + +

*Data not available. **Imaging assessed (CT-scan, MRI or PET-scan). *** Skin biopsy was the only available tissue for this patient, and it revealed fibrotic tissue without histiocytes. x: Presence; 0: Absence; F: female; M: male; NGS: next-generation sequencing; Hb: hemoglobin; MCV: mean corpuscular volume; CRP⁺: C-reactive protein.

formed in biopsy and found no somatic mutation in *BRAF* or *PIK3CA*.

The patient was treated with corticosteroids (1 mg/kg/day) allowing rapid improvement of the neurological symptoms and epidural mass regression. Progression was marked by several flare-ups under corticosteroids at 15 mg/day. Tocilizumab (8 mg/kg/4 weeks) for 4 months allowed inflammatory syndrome normalization and corticosteroid sparing without arthritis flare. Epidural infiltrate persisted after treatment, but remained stable in spite of corticosteroid discontinuation.

Case 3

A 43-year-old man was refered to the Internal Medicine Department for suspected auto-inflammatory syndrome associated with arthritis. He was born in Morocco and had a history of bilateral cochlear hearing loss, camptodactyly complicating a child onset arthritis and papillary thyroid

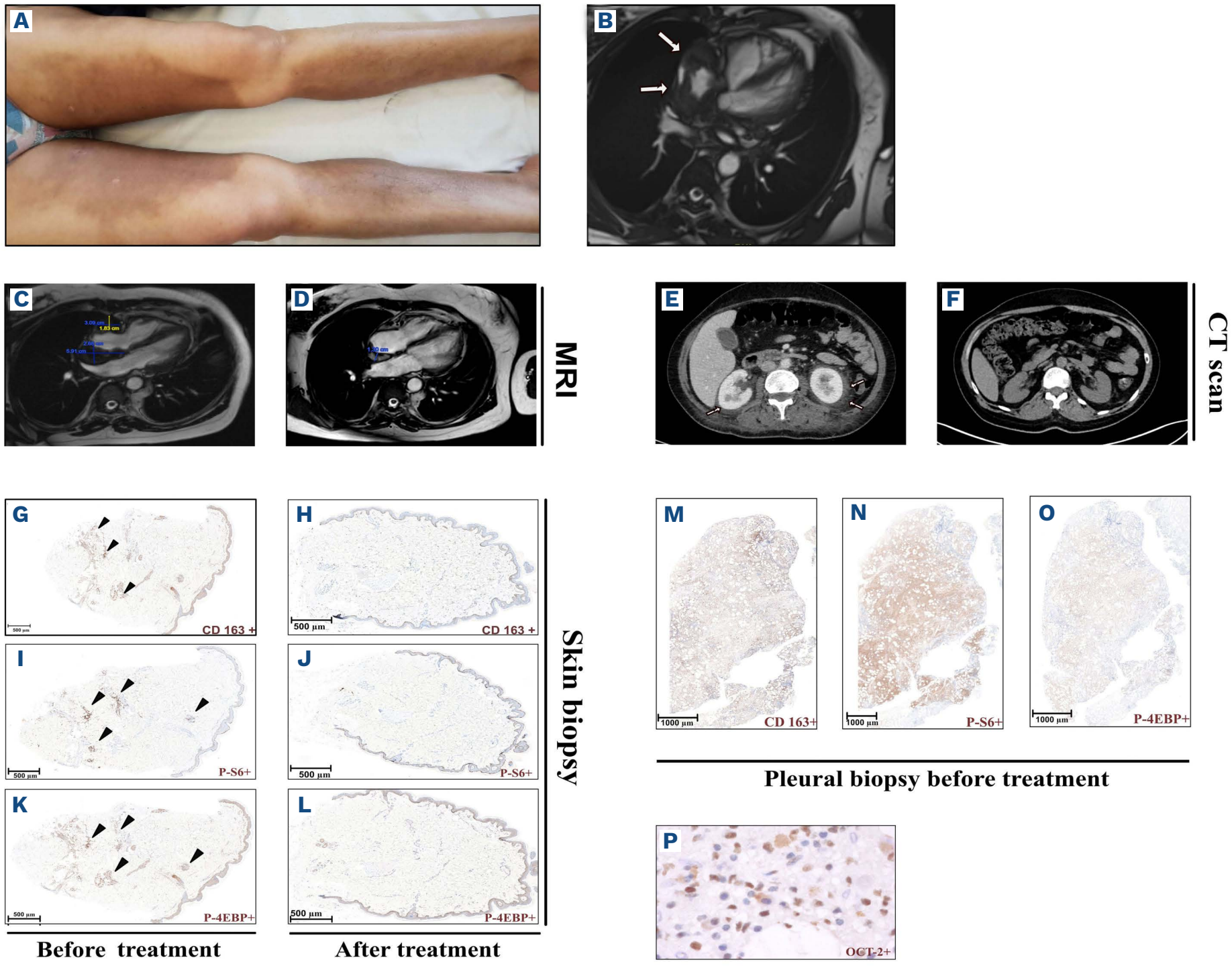

Figure 1. Patient #1 imaging and immunostaining before and during treatment. (A) Patient #1 lower limbs cutaneous infiltrates. (B) Heart cine 4 cavity magnetic resonance imaging (MRI) revealing right atrial histiocytic infiltrate of patient #1 before treatment (arrows). (C) Patient #1 heart cine 4 cavity MRI with measure of inter-atrial infiltrate before cobimetinib treatment and (D) under cobimentinib treatment (1-year duration). (E) Patient #1 injected abdominal computed tomography (CT) scan before cobimetinib treatment (arrows), and (F) non-injected abdominal CT scan under cobimentinib treatment (1-year duration). (G) Patient #1 skin biopsy with CD163^{+} immunostaining (magnification x2.4) showing histiocytic infiltrates (arrows) before cobimetinib treatment and (H) under cobimentinib treatment (1-year duration). (I) Patient #1 skin biopsy with p-S6 immunostaining (magnification x1.4), and (K) p-4EBP1 immunostaining (magnification x1.4) with mTOR pathway upregulation (arrows). (J) Patient #1 skin biopsy under cobimentinib treatment (1-year duration) with p-S6 immunostaining (magnification x1.4), and (L) p-4E-BP1 immunostaining with disappearance of mTOR pathway upregulation (magnification x1.4). (M) Patient #1 pleural biopsy before treatment with CD163^{+} immunostaining (magnification x1.2), (N) p-S6 immunostaining (magnification x1.2), and (O) p-4E-BP1 immunostaining (magnification x1.2). (P) Patient #1 skin biopsy before treatment with OCT-2 immunostaining (magnification x400).

carcinoma. Clinical examination revealed a gynecomastia, a hallux valgus, and depigmented back skin lesions.
CT scan found peri-aortic, perirenal, peritoneal and under-skin infiltrates. Blood test results found a fluctuating inflammatory syndrome (CRP >20 mg/L), an elevated luteinizing hormone and prolactinemia (14.7 UI/L; 417 UI/L respectively) with unremarkable immunological assay. A skin biopsy was performed in depigmented back lesions, but unfortunately uniquely contained fibrotic lesions without histiocytic infiltrate.
The patient was treated with tocilizumab (8 mg/kg/2 weeks) for 2 years with clinical improvement regarding auto-inflammatory manifestations (arthritis and inflammatory syndrome disappearance) but no improvement regarding the tissue infiltrate at CT scan.

Case 4

A 48-year-old women presented with multiple organ infiltrates and inflammatory syndrome. She had a history of childhood-onset bilateral hearing loss, bilateral micromelia with cubitus valgus deformity and camptodactyly. During adulthood, she developed systemic disorders such as a cutaneous infiltrate involving the back and left breast; mediastinum (Figure 2C, D), retroperitoneal (Figure 2E) and interstitial lung infiltrates on CT scan and recurrent pericarditis with fluctuating inflammatory syndrome (CRP ≥100 mg/L). She also developed endocrinopathy: diabetes mellitus and multi-nodular goiter. Immunological assay was unremarkable and infectious diseases were ruled out. A skin biopsy was performed and revealed $CD163^+/CD68^+/S100^+/CD1a^-/OCT\text{-}2^+$ histiocyte infiltrates. NGS analysis was performed in biopsy and found no somatic mutation in *BRAF*.
She was treated with tocilizumab (8 mg/kg/4 weeks) for 3 years allowing a regression of the cutaneous infiltrate and normalization of the inflammatory syndrome, but mediastinum and peri-renal infiltrate persisted.
Clinical improvement of patient #1 with cobimetinib could not be explained by a normalization of the MAPK pathway which was not upregulated.
Importantly, activation of mTOR pathway has been reported to play a pathological role in SLC29A3-deficiency and in ECD.[6,7] Moreover, cobimetinib has been shown to impact the mTOR pathway either by inhibiting MAPK which is interconnected with mTOR pathway or directly by inhibiting AKT.[8] Consequently, mTOR pathway downstream effectors (p-4E-BP and p-S6) were analyzed by immunohistochemistry on patient #4 before treatment on skin biopsy and on patient #1 before treatment on skin and pleural samples and during cobimetinib treatment (1-year therapy) on skin sample. We observed important p-4E-BP and p-S6 immunostainings before treatment, mainly co-localized with $CD163^+$ cells in patient #1 pleural and skin biopsy samples (Figura 1G-O) and in patient #4 skin biopsy sample (Figure 2F, G) Conversely, under therapy, histiocytic infiltrates, p4E-BP and p-S6 were dramatically decreased (Figure 1J, L). These findings strongly suggest that upregulation of the mTOR pathway contribute to HI pathogenesis, which can be normalized by cobimetinib treatment.
We describe herein a case series of adult patients showing ECD-like HI phenotypes in the context of *SLC29A3* disease-causing variants. Until now, heterogeneous H syndrome clinical presentations were described across *SLC29A3*-mutated cases, including HI which are R-group classified.[1] Patients in this study presented classical clinical features of H syndrome, with childhood or adolescent onset driven by previously described *SLC29A3* mutations.[3] In addition, three patients presented peri-renal and peri-cardiac/-aortic infiltrates, as commonly observed in ECD, whereas the absence of bone lesions and presence of skin

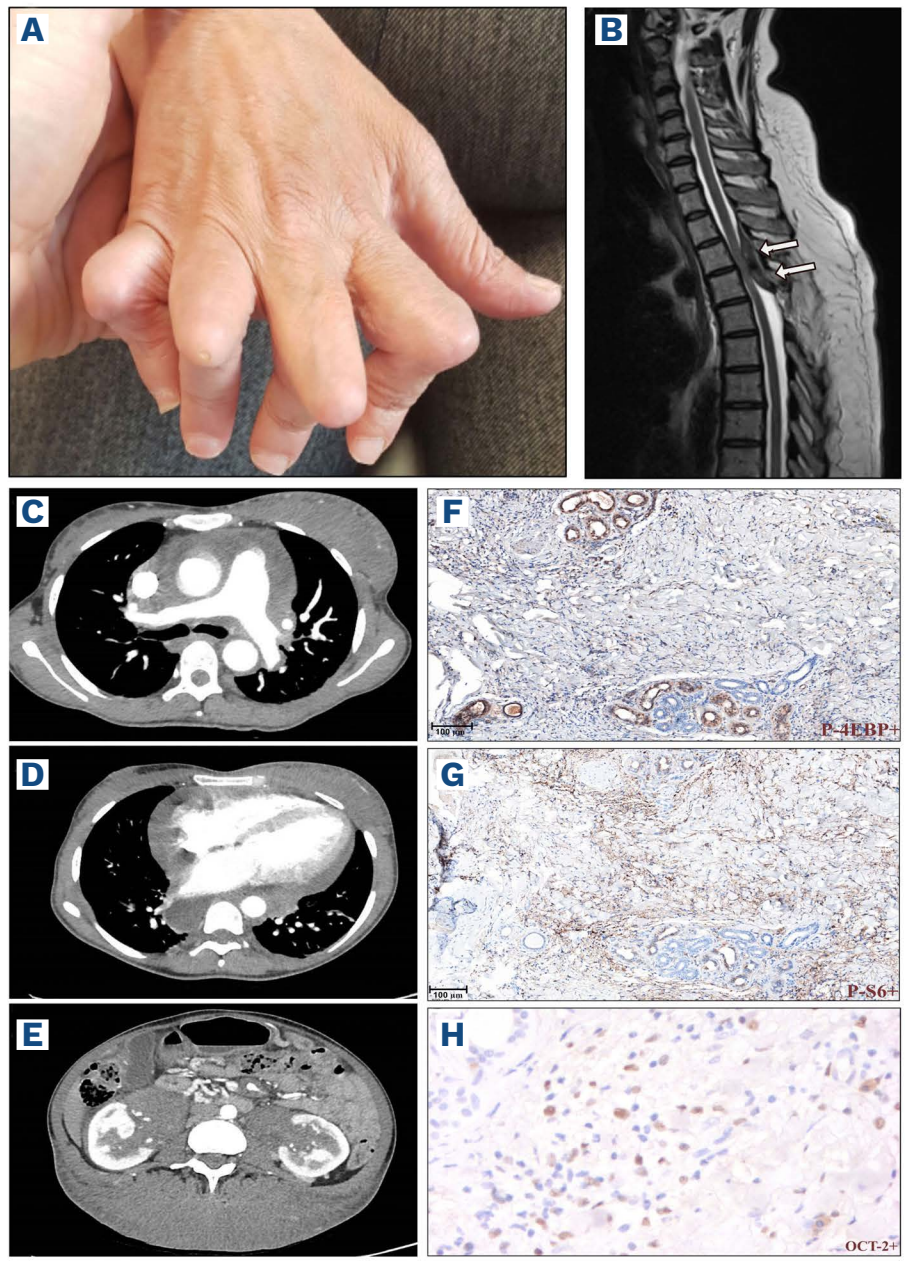

Figure 2. Clinical, imaging and pathological features of patients #2 and #4. (A) Patient #2 camptodactyly. (B) Patient #2 epidural infiltrate on T2 magnetic resonance imaging sequence (arrows). (C, D) Patient #4 thoracic computed tomography (CT) scan revealing mediastinum infiltrate. (E) Patient #4 injected abdominal CT-scan revealing retroperitoneal infiltrate. (F, G) Patient #4 skin biopsy before treatment with p-S6 and p-4EBP immunostaining (magnification x8.9). (H) Patient #4 skin biopsy before treatment with OCT-2 immunostaining (magnification x200).

infiltrates with hyperpigmentation are unusual in ECD. A recent study of 101 ECD focusing on clinical features and somatic mutations driving ECD in classical ECD (typical osseous involvement) and non-classical ECD (absence of typical osseous involvement) showed that 14% of patients had non-classical ECD.[9] Among these patients, 35% did not have alterations in MAPK-ERK pathway, more than 30% presented hairy kidney, and 14% presented cardiac or cutaneous involvement.[9] Considering these genotypic and phenotypic common points with our cases, we can hypothesize that a part of non-classical ECD could be driven by *SLC29A3* mutations or alterations in mTOR pathway. One patient presented an epidural mass inducing spinal cord compression, which is a rare complication of ECD.[10]

Histopathological characteristics were ECD-like features, for patients #1 and #2 with $CD68^+/CD163^+/S100^-/CD1a^-$ infiltrates, whereas for patient #4 S100 was positive. No emperipolesis was observed, contrasting with observations classically reported in R-group histiocytosis and some H syndrome histiocytic infiltrates. Interestingly, OCT-2 immunostaining was positive for patients #1 (Figure 1P) and #4 (Figure 2H). While OCT-2 staining should be negative on ECD samples, it is not specific of RDD, and this has been shown in some cases of Langerhans cell histiocytosis (5-10%) and of ALK^+ histiocytosis (60%).[11] Thus, OCT-2 immunostaining could be a pathological feature of interest in H syndrome histiocytosis and helpful to guide diagnosis towards H syndrome in case of histiocytic infiltrate with evocative clinical signs.

A SLC29A3$^{-/-}$ mouse model showed that this mutation affects macrophage and T-lymphocyte function via AMPK-mTOR-ULK signaling, leading to systemic abnormalities such as hypertrichosis, skeletal deformities, endocrinopathy and histiocytic infiltrates.[6] Indeed, because of lysosome trafficking disturbance, ENT3-deficient macrophages displayed impaired apoptotic cell clearance and proliferated via the MCSF/MCSFR (macrophage colony-stimulating factor) axis.[12]

Genetic analysis of ECD patients revealed that the *BRAF* V600E mutation is present in about half of the patients, while about 11% of patients displayed *PIK3CA* mutations.[7] Thus, the upregulation of RAS downstream pathway RAF/MEK/ERK as well as of the PI3K/AKT/mTOR pathway could induce ECD. Moreover, a 10-patient cohort clinical trial associating sirolimus and prednisone found clinical improvement (3 were *BRAF* V600E-mutated and 7 without documented mutation), as well as a 20-patient cohort long-term study (imaging or metabolic response in 65% of patients) underlying the role of PI3K/AKT/mTOR pathway in some ECD cases.[13,14] Considering these elements, we treated one patient with cobimentinib as a second-line therapy, which allowed rapid regression of HI despite the fact that the MAPK pathway was not hyperactivated. Immunostaining revealed that HI is associated with mTOR pathway upregulation which was normalized by cobimetinib. As the MAPK pathway is known to be involved in inflammatory signaling, MEK inhibitor could play here a double role with control of anti-inflammatory features via MAPK signaling and histiocytic infiltrate via mTOR signaling.[15]

Our report shows that an inherited monogenic condition can be associated to at least two types of histiocytoses and that a phenotype highly reminiscent of ECD can be associated with monogenic disease. Thus, it underlines the interest in searching for *SLC29A3* mutations in the context of atypical ECD-like histiocytosis. Morevoer, it expands the spectrum of overlapping histiocytoses, raising the question of reconsidering H syndrome histiocytosis as R-group members. Noteworthy, cobimetinib appears as a promising second-line therapy after tocilizumab. Finally, mTOR inhibitors could probably be tested to improve ECD-like histiocytosis in *SLC29A3*-mutated patients.

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Disclosures

No conflicts of interest to disclose.

Contributions

HL, MG-V, CD, GLG, YJ, LS, CB and PS followed the patients, analyzed the data and draft the manuscript. J-FE analyzed biopsies and performed the molecular analysis of gene mutation in patient #1 biopsy. GB analyzed *SLC29A3* mutations of patients and wrote the genetics paragraph. JT analyzed and collected biopsies. JV and Y-GG performed and analyzed pS6 and p-4E-BP immunostaining. All authors were involved in the critical analysis and final version of the manuscript.

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Data-sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request

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