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ARTICLE

Expanding the phenotypic spectrum of variants in *PDE4D*/*PRKAR1A*: from acrodysostosis to acroscyphodysplasia

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Abstract

Acrodysostosis (MIM 101800) is a dominantly inherited condition associating (1) skeletal features (short stature, facial dysostosis, and brachydactyly with cone-shaped epiphyses), (2) resistance to hormones and (3) possible intellectual disability. Acroscyphodysplasia (MIM 250215) is characterized by growth retardation, brachydactyly, and knee epiphyses embedded in cup-shaped metaphyses. We and others have identified *PDE4D* or *PRKAR1A* variants in acrodysostosis; *PDE4D* variants have been reported in three cases of acroscyphodysplasia. Our study aimed at reviewing the clinical and molecular findings in a cohort of 27 acrodysostosis and 5 acroscyphodysplasia cases. Among the acrodysostosis cases, we identified 9 heterozygous de novo *PRKAR1A* variants and 11 heterozygous *PDE4D* variants. The 7 patients without variants presented with symptoms of acrodysostosis (brachydactyly and cone-shaped epiphyses), but none had the characteristic facial dysostosis. In the acroscyphodysplasia cases, we identified 2 *PDE4D* variants. For 2 of the 3 negative cases, medical records revealed early severe infection, which has been described in some reports of acroscyphodysplasia. Subdividing our series of acrodysostosis based on the disease-causing gene, we confirmed genotype–phenotype correlations. Hormone resistance was consistently observed in patients carrying *PRKAR1A* variants, whereas no hormone resistance was observed in 9 patients with *PDE4D* variants. All patients with *PDE4D* variants shared characteristic facial features (midface hypoplasia with nasal hypoplasia) and some degree of intellectual disability. Our findings of *PDE4D* variants in two cases of acroscyphodysplasia support that *PDE4D* may be responsible for this severe skeletal dysplasia. We eventually emphasize the importance of some specific assessments in the long-term follow up, including cardiovascular and thromboembolic risk factors.

Introduction

Acrodysostosis is a rare autosomal dominant condition first described by Pierre Maroteaux et al. in 1968 [1] and further reviewed by Robinow et al. in 1971 [2]. It consists in the association of (1) skeletal features characterized by short stature, facial dysostosis with nasal hypoplasia and peripheral dysostosis with severe brachymetatarsia, brachymetacarpia, brachydactyly, cone-shaped epiphyses, and advanced bone maturation, (2) inconstant resistance to multiple hormones including parathyroid hormone or thyrotropin, and (3) possible neurological involvement with moderate to mild intellectual disability [1, 2]. Differential diagnoses include Albright hereditary osteodystrophy (AHO) [MIM103580] and pseudopseudohypoparathyroidism [MIM 612463] due to loss of function variants in *GNAS* (α-stimulary subunit of the G-protein) and characterized by less severe skeletal involvement [3].

In 2011, the recurrent p.Arg368* variant in the *PRKAR1A* gene has been found in three individuals with acrodysostosis and resistance to multiple hormones [4]. *PRKAR1A* encodes the cyclic AMP (cAMP)-dependent regulatory subunit of protein kinase A. The mutated subunit impairs the protein kinase A response to cAMP [4],

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accounting for hormone resistance and skeletal abnormalities resembling those observed in AHO. We then identified *PDE4D* variants as another cause of acrodysostosis, most frequently without hormone resistance [5]. *PDE4D* is also involved in cAMP signaling pathway metabolism, encoding a class IV cAMP-specific phosphodiesterase, regulating cAMP concentration.

On the basis of these molecular basis, two distinct MIM references have been generated, namely ACRO1 (MIM 101800) for *PRKAR1A* variants and ACRO2 (MIM614613) for *PDE4D* variants. We previously reported some genotype–phenotype correlations. Indeed, patients with *PRKAR1A* variants tend to have less characteristic facial dysostosis, normal intelligence, and resistance to multiple hormones compared to patients with *PDE4D* variants who presented with characteristic facial dysostosis, intellectual disability, and subtle or absence of hormonal resistance [6, 7].

In recent studies, several acroscyphodysplasia cases [MIM 250215] [7, 8] were reported with a *PDE4D* variant. This entity consists of the association of severe growth retardation, micromelia predominating in the lower limbs, knee flexion, and severe brachydactyly altogether with a specific radiological appearance of the knees: cup-shaped metaphyses with embedded epiphyses [9]. This radiological aspect is reminiscent of the appearance of the cone-shaped epiphyses of small joints in acrodysostosis.

Studying 32 unrelated cases of acrodysostosis ($n = 27$) or acroscyphodysplasia ($n = 5$), we found *PDE4D* variants in 13 cases, including 2 cases of acroscyphodysplasia, and *PRKRA1A* variants in 9 acrodysostosis cases. We confirmed some genotype–phenotype correlations and involvement of *PDE4D* in acroscyphodysplasia. We also emphasize the importance of some specific assessments in the long-term follow up of these conditions.

Subjects and methods

Clinical ascertainment

Overall, 32 patients were recruited for this study, including 27 cases of acrodysostosis (comprising 10 patients previously described in Michot et al. [5]) and 5 cases of acroscyphodysplasia. Two patients had an affected parent. Inclusion criteria for acrodysostosis cases were a peripheral dysostosis with severe generalized brachydactyly, affecting metacarpals and phalanges, associated with cone-shaped epiphyses. Short stature, facial dysostosis, resistance to multiple hormones, and intellectual disability were not considered as mandatory. The acroscyphodysplasia cases were recruited on the sole criterion of a characteristic radiological aspect of the knees.

Among the 32 recruited patients, 18 were females and 14 were males. Thirty patients were sporadic cases, and in 2 cases, acrodysostosis was inherited from an affected mother.

Informed consent for participation, sample collection, and photograph publication were obtained using protocols approved by the Necker Hospital ethics board committee.

Clinical data collection

According to a literature review, a questionnaire with selected medical items was sent out to the referring physician, to collect details on the clinical and biological symptoms of the cases diagnosed with acrodysostosis and acroscyphodysplasia. Available data, photographs, and radiographs (when authorized by the patients or their legal representatives) were collected.

Hormonal screening

For complete screening of mineral metabolism, blood levels of creatinine, calcium, phosphorus, thyroxin (T4), thyrotropin, 25-hydroxyvitamin D, 1-25-dihydroxyvitamin D, parathyroid hormone (PTH), and fibroblast growth factor 23 (FGF23) levels were measured, along with urinary creatinine, calcium, and phosphorus excretion.

PRKAR1A and *PDE4D* molecular screening

Genomic DNA was obtained from peripheral blood leukocytes using standard procedures. The exons and exon–intron boundaries of *PRKAR1A* (GenBank NM_002734.4) and *PDE4D* (GenBank NM_001104631.1) were amplified using specific primers (available upon request). Amplification products were purified by ExoSapIT (Amersham, Buckinghamshire, UK) and directly sequenced using the Big Dye Terminator Cycle Sequencing Ready Reaction kit v.1.1 on an automatic sequencer (ABI3130xl; PE Applied Biosystems, Foster City, CA). Sequence analyses were performed using Seqscape software v2.5 (Applied Biosystems).

Results

Sequencing data

De novo heterozygous *PRKAR1A* variants were identified in 9 cases, including the p.Arg368* stop variant [4] in 5/9 and missense variants (c.786G>C (p.(Trp262Cys)), c.968A>C (p.(Tyr323Ser)), c.968A>G (p.(Tyr323Cys)), c.1117T>C (p.(Tyr373His))) in 4/9. All these variants were predicted as probably damaging using PolyPhen2 software and were not identified in 200 control chromosomes, nor indexed in gnomAD database. They altered a conserved amino acid,

located either in the catalytic domain or in the cAMP-binding domain B (Fig. 1).

PDE4D variants were identified in 11 acrodysostosis cases, including the two familial cases. They were all missense variants spread throughout the whole *PDE4D* coding sequence (Fig. 2), altering a conserved amino acid located in the catalytic domain and were predicted as probably damaging using PolyPhen2 software, except for the c.568T>G (p.(Ser190Ala)) and c.568T>C (p.(Ser190Pro)) predicted as benign. These last 2 variants occurred de novo and affected a serine residue predicted to be phosphorylated (UniProt database) and were absent from alleles in 200 ethnicity-matched controls and from gnomAD database.

De novo *PDE4D* variants (c.995T>C (p.(Phe332Ser)) and c.99T>G (p.(Ile333Met))) were identified in 2/5 patients with acroscyphodysplasia.

Clinical, biological, and radiological data of acrodysostosis cases

The clinical and available biological details are summarized in Table 1 for patients with identified variants.

Intrauterine growth retardation (IUGR) was observed in 8/9 with *PRKAR1A* variants, but only in a few patients with *PDE4D* variants ($n = 5/11$, but no available data in three cases). Postnatally, 8/9 patients with *PRKAR1A* variants developed growth deficiency, ranging from −2 SD to −6.7 SD, whereas only 4/11 individuals with *PDE4D* variants had postnatal short stature. However, patients with *PDE4D* variants reported herein were young at time of the study (6 were below 10 years of age). Data on pubertal development were not available for these young patients, but one boy had cryptorchidism treated by testosterone and one girl a female hypospadias. One boy with *PRKAR1A* variant (out of 3) also had cryptorchidism, which required surgery. In four adult women with *PRKAR1A* variants, one experienced a miscarriage and an intrauterine fetal death, whereas another one had an absence of the mammary glands development.

Brachydactyly involved all rays of fingers and toes, associated with cone-shaped epiphyses. Three patients also had single palmar crease, including two with *PRKAR1A* variants and one without any identified variant. A relative hyperplasia of the first ray of the feet was historically described and was indeed observed in 6/9 patients with *PRKAR1A* variants and 2/11 with *PDE4D* variants. One patient with *PDE4D* variant (patient 20) developed a macrodactyly of second and third rays of one foot that required amputation. Elder patients with *PRKAR1A* variants developed stiffness of the elbows and one underwent corrective surgery for carpal tunnel syndrome.

A flat face with malar hypoplasia was noted in all patients (Fig. 3). Nevertheless the distinctive nasal hypoplasia was described in only 3/9 patients with *PRKAR1A* variants,

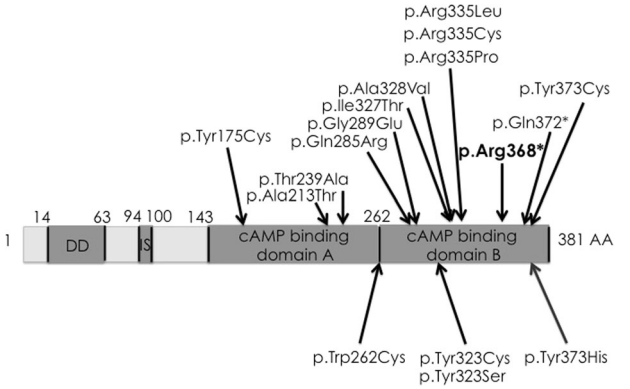

Fig. 1 Scheme of the *PRKAR1A* gene, modified from Linglart el al. [7]. Above are listed the previously published variants, including the recurrent p.Arg368* variant, which was found in four cases in this study. Below are the four new and unique variants described in this study

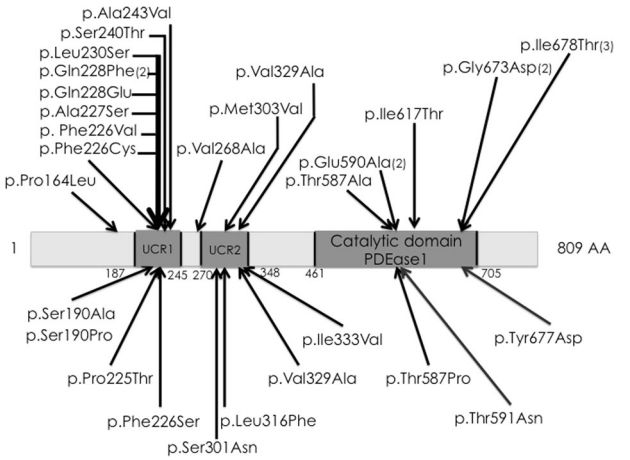

Fig. 2 Scheme of the *PDE4D* gene. Above are listed the previously published variants. Below are the eleven new and unique variants described in this study

whereas 11/11 patients with *PDE4D* variants had this typical facial feature. Noteworthy, the three patients with *PRKAR1A* variants and nasal hypoplasia harbored missense variants affecting the Tyrosine 323 (c.786G>C, c.968A>C and c.968A>G) and the tryptophane 262 residues, and not the recurrent stop variant affecting arginine 368 residue. Moreover, 3/9 patients with *PRKAR1A* variants and 5/11 with *PDE4D* had a prominent mandible. Two patients with *PRKAR1A* variants presented with delayed eruption of teeth and 1 with *PDE4D* variant had abnormal enamel.

Eight out of nine with *PRKAR1A* variant had normal intellectual development. Only patient 8 (who harbored a *PRKAR1A* variant affecting the tyrosine 323 residue (c.968A>C)) had a moderate intellectual disability with congenital axial hypotonia and triventricular cerebral dilatation. Noteworthy, the second patient with a variant affecting this residue, patient 9 (c.968A>G), was only 18 months old at the time of completion and besides he

Table 1 Clinical and biological data of the 20 acrodysostosis patients with identified variants described in this study

	PRKAR1A (n=9)	PDE4D (n=11)	Total
Paternal age at birth	34 to 40 y	19 to 50 y	mean : 35,2
Ethnicity	Caucasian, Asian, Maghreb	Caucasian, Turkish, South Arabia	–
Sex	6 F/3 M	4 F/7 M	10 F/10 M
Age	5,5 to 38 y	4,5 to 33 y	4,5 to 37 y
IUGR	8 (89%)	5 (45%)	13+ (65%)
Postnatal growth retardation	8 (89%)	4 (36%)	12+ (60%)
Actual height (SD)	−6,7 to −0,9 SD	−3 to +1,8 SD	−6,7 to +1,8 SD
Obesity (BMI)	3 (33%)	2 (18%)	5+ (25%)
Facial dysostosis			
Nasal hypoplasia	3 (33%)	10 (90%)	13+ (65%)
Depressed nasal bridge	8 (89%)	11 (100%)	19+ (95%)
Prominent mandibule	3 (33%)	5 (45%)	8+ (40%)
Peripheral dysostosis			
Severe brachydactyly	9 (100%)	11 (100%)	20+ (100%)
Short, broad metatarsals, metacarpals and phalanges	9 (100%)	10 (90%)	19+ (95%)
Cone-shaped epiphyses	8 (89%)	8 (72%)	16+ (80%)
Advanced bone age	4 (44%)	6 (54%)	10+ (50%)
Narrowing of caudal vertebral interpedicular distance	6 (66%)	2 (18%)	8+ (40%)
Hormonal screening			
Parathyroid	8 resistence to PTH (89%)	1 slight increased PTH (9%)	9 elevated (45%)
GH axis	2 abnormal (22%)	0	2 abnormal (10%)
External genitalia	1 cryptorchidism (33% M)	1 cryptorchidism (14% M); 1 feminine hypospadias	3 abnormal (15%)
Puberty	2 abnormal in female	0	2 abnormal (10%)
Thyroid	7 hypothyroidism (78%)	0	7 hypothyroidism (35%)
Mental retardation	1 (11%)	10 (90%)	11+ (55%)

See supplementary material for the full version of Table 1

had walked normally at 15 months of age, congenital hypotonia and poor sucking had been noticed before. Conversely almost all patients (10/11) with *PDE4D* variants had mild to moderate intellectual disability. Moreover, two patients with a *PDE4D* variant developed acute intracranial hypertension due to a thrombophlebitis (patients 11 and 12).

All patients with *PRKAR1A* variants had endocrine disorders: 8/9 had an increased level of parathyroid hormone (PTH), 7/9 presented with peripheral hypothyroidism, and 2/9 with growth hormone deficiency. Conversely, only 1 patient with *PDE4D* variant had slightly increased level of PTH and no patient experienced hypothyroidism.

Additional health issues were observed including (i) recurrent ENT infections ($n = 2$; both with *PRKAR1A* variants), (ii) chronic erysipelas with fixed edema of the lower limbs and hypogammaglobulinemia ($n = 1$; *PRKAR1A* variant), and (iii) deafness ($n = 3$; two *PRKAR1A* variants and one with *PDE4D*).

Moreover, both patients with *PRKAR1A* variant affecting the tyrosine 323 residue (c.968A>C and c.968A>G) had additional symptoms including cardiovascular malformations (one with external jugular vein stenosis and one with total anomalous pulmonary venous return with atrial septal defect). One (patient 8 (c.968A>C)) had severe laryngotracheomalacy with cricoid hemangioma, whereas the other (patient 9 (c.968A>G)) required the dilatation of a subglottic diaphragm in infancy.

Noteworthy, one of the elder patients (patient 5) with the p. Arg368* *PRKAR1A* variant died suddenly at 38 years of age, following severe bronchospasm during a surgical procedure. She had concomitant lactic acidosis, rhabdomyolysis, and anuric renal deficiency. No clear etiology was determined despite extensive metabolic and infectious work-up.

All patients had shortened tubular bones of the hands and feet, of all the digits, associated with cone-shaped epiphyses (Fig. 3). Besides these canonical signs, other radiological features were noticed: advanced bone age (in 4/9 with

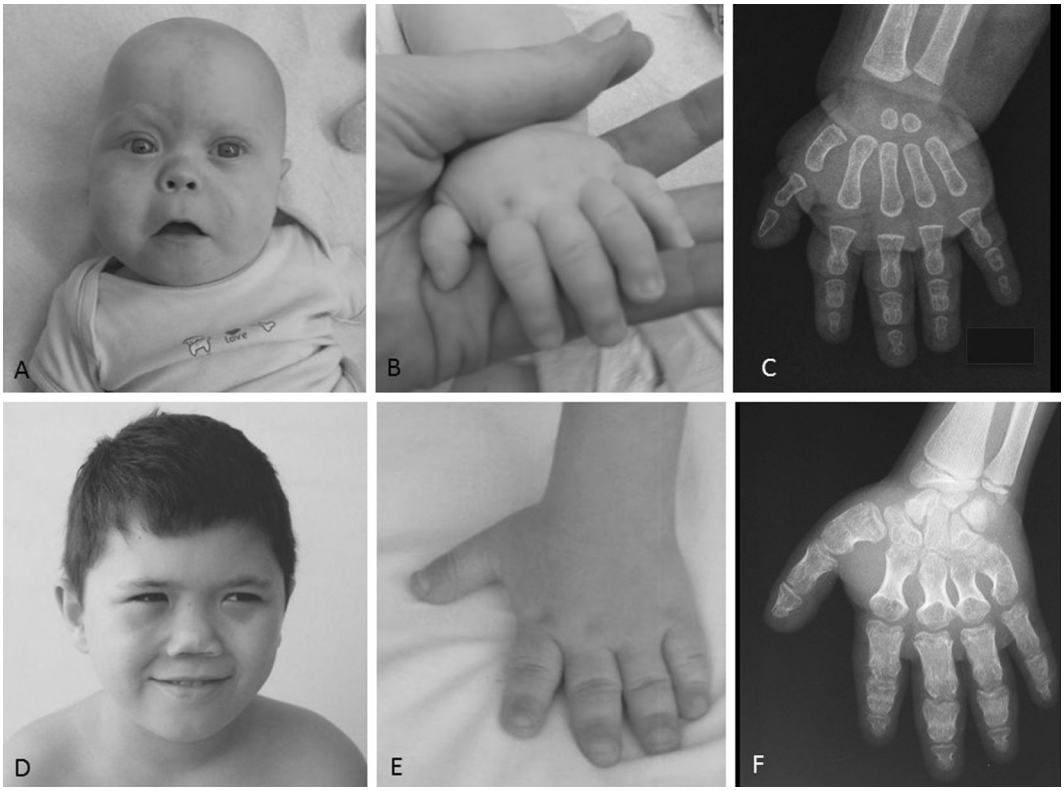

Fig. 3 Patients presenting with acrodysostosis. **a**, **b** Patient 5; general aspect and hand X-ray. **c**–**e** Patient 9; face, hand, and hand X-ray. **f**–**h** Patient 15; face, hand, and hand X-ray. Please note on **c** and **f** the typical facial gestalt with malar hypoplasia and nasal hypoplasia. Please note on **b**, **e**, and **h** the short broad metacarpals and phalanges, with cone-shaped epiphyses for patient 15

PRKAR1A variants and 6/11 with *PDE4D*), bilateral coxa valga (4/9 with *PRKAR1A* variants and 3/11 with *PDE4D*), irregular vertebral plates (2/9 with *PRKAR1A* variants and 2/11 with *PDE4D*), absence of widening of the interpedicular distance (3/9 with *PRKAR1A* variants and 2/11 with *PDE4D*). Moreover, 5/9 patients with *PRKAR1A* variants had skeletal abnormalities also affecting other epiphyses and one case had an important vertebral dysplasia in the first years of life, which progressively improved with age.

Clinical, biological, and radiological data of the five acroscyphodysplasia cases

All patients but one presented with postnatal growth retardation (−2 to −4 SD), after intrauterine growth retardation in only one case. Four patients have nasal hypoplasia, severe in two cases only; all five had a flat face with malar hypoplasia. They all had also micromelia and 3/5 had a severe brachydactyly with small phalanges and small metacarpals and metatarsals (two with moderate brachydactyly) (Fig. 4). Cone-shaped epiphyses were observed except in one young case. Two patients had knee stiffness. One patient presented with a peripheral hypothyroidism, but no other endocrine disorder was reported. All had an intellectual disability. Some non-specific MRI abnormalities were observed (one with thin corpus callosum, delayed myelinisation and cortical atrophy, another one with ventricular dilatation).

Among the 3 patients with no identified variants, 2 had a medical history of an early and severe infection and the last one of frequent infections.

Discussion

We describe here a large cohort of 27 patients with acrodysostosis. We identified variants in 74% of cases (20/ 27) including *PDE4D* variants in 11 and *PRKAR1A* in 9.

We further confirm genotype–phenotype correlations. Indeed, the 11 patients carrying *PDE4D* variants had the characteristic facial features with nasal hypoplasia, midface hypoplasia, and prominent mandible initially reported in acrodysostosis, and 10/11 had some degree of intellectual disability. This characteristic facial dysostosis and mild to moderate intellectual disability was observed, respectively, in only 3/9 and 1/9 cases carrying *PRKAR1A* variant. Noteworthy those patients had variants affecting the tryptophane 262 or the tyrosine 323 residues of PRKAR1A

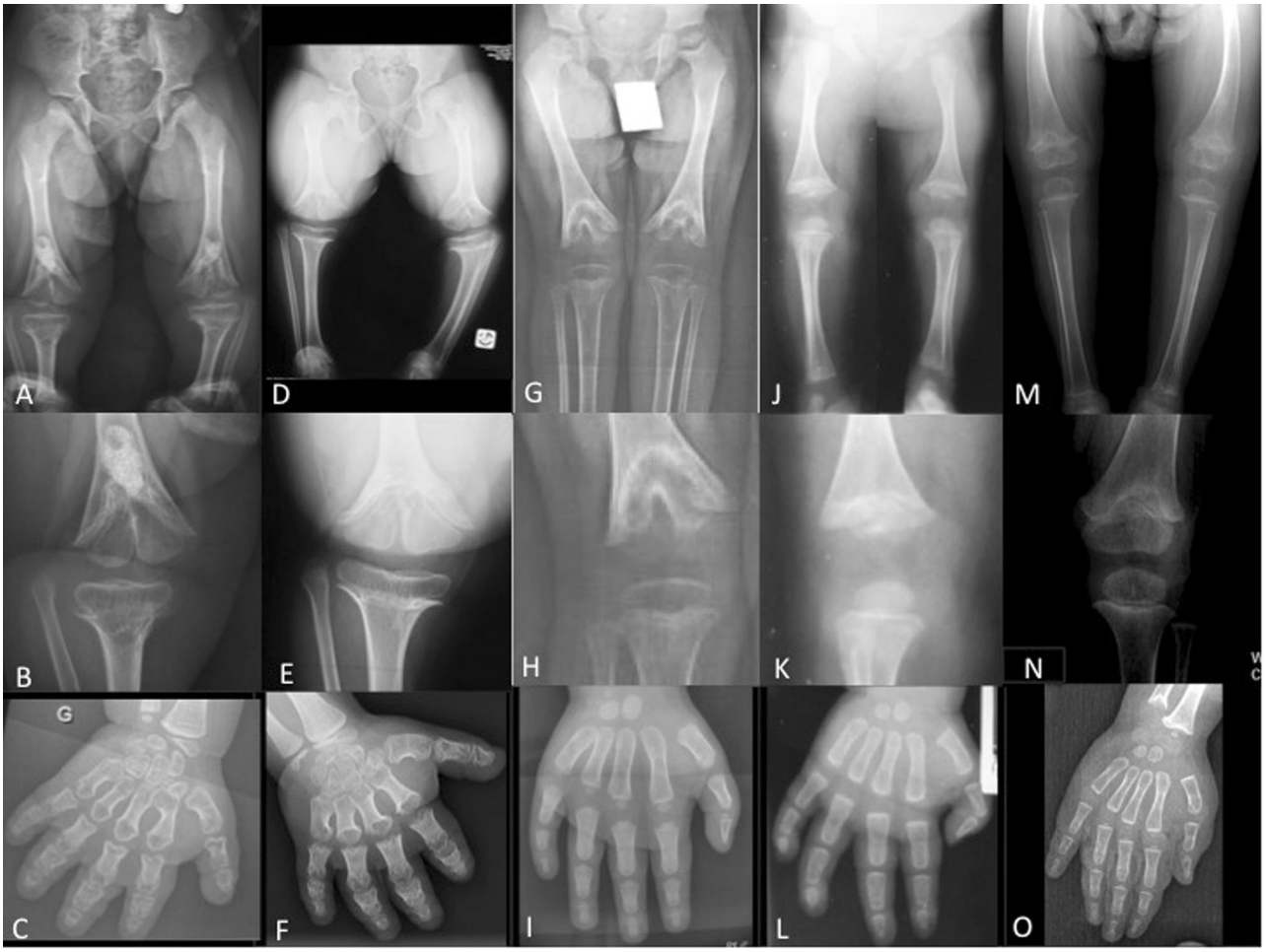

Fig. 4 X-rays of the acroscyphodysplasia cases showing the whole legs, the zoom on the knee and a hand. **a**–**c** 1st case. **d**–**f** 2nd case. **g**–**i** 3rd case. **j**–**l** 4th case. **m**–**o** 5th case. Please note the cup-shaped metaphyses with embedded epiphyses on knees and the aspect of the hands, similar to the acrodysostosis cases

(c.786G>C, c.968A>C, and c.968A>G), but not the recurrent p.Arg368* variant. Conversely, hormone resistance was consistently observed in patients carrying *PRKAR1A* variants (8/9 presenting with chronic resistance to parathyroid hormone and 7/9 had peripheral hypothyroidism), whereas only one case with *PDE4D* variant had a slightly increased level of PTH.

These results are in agreement with all cases reported so far ($n = 49$; 24 with *PRKAR1A* variants and 25 with *PDE4D* variants) [4, 6, 7, 10–16] (cf Table 2). In the literature, the peripheral bone dysostosis is consistently observed in all patients. The severe brachymetacarpia/brachymetatarsia and brachydactyly affect all the digits. The lumbar stenosis is also described in patients with *PRKAR1A* or *PDE4D* variants, but this data is scarcely specified in literature. Although short nose with flat face is consistently observed in all reported cases, severe nasomaxillary hypoplasia is more frequently reported in patients with *PDE4D* variants ($n = 24$ for whom data are specified), than in patients with *PRKAR1A* variants ($n = 6$ for whom data are specified). Intellectual disability is clearly reported in 24 cases of patients with *PDE4D* variants (of 25 reported in literature) but only in 5 with *PRKAR1A* variants (of 24 reported). Hormone resistance is conversely observed in only 6 patients carrying *PDE4D* variants (6/25 cases), whereas is present in 17 patients with *PRKAR1A* variants (of 24 cases (19 with the data available)) in literature.

We therefore confirm that molecular analysis shall be directed according to the symptoms: in case of facial dysostosis and moderate intellectual disability, first screen the *PDE4D* gene, and in case of less characteristic facial features and hormonal resistance first screen for *PRKAR1A* variants.

Our study also further highlights the risk of development of serious medical complications. Actually 3 patients with *PRKAR1A* and 2 with *PDE4D* variants developed progressive obesity which confirms previous publications [6]. cAMP signaling pathways, mediated by protein

Table 2 Comparison of the clinical and biological data of the patients of the present study to the literature

Patient	Linglart et al. [4] ($n=3$)	Lee et al. [11] ($n=2$)	Nagasaki et al. [15] ($n=1$)	Linglart et al. [7] ($n=11$ news)	Muhn et al. [14] ($n=4+7$)	Kaname et al. [10] ($n=1$)	Li et al. [12] ($n=1$)	Rhayem et al. [16] ($n=1$ new)	This study ($n=9$)	Lee et al. [11] ($n=3$)	Linglart et al. [7] ($n=2$ news)	Lynch et al. [6] ($n=8$)	Lindstrand et al. [13] ($n=5$)	Kaname et al. [10] ($n=7$)	This study ($n=11$)
Sex		2M		5M/6F	1M/3F	1M			6F/3M	2M/1F	2F			4M/3F	4F/7M
Age (death?)				3 to 26 y	11.5 to 31 y	3.5 to 39 y	12 y	9 y	5.5 to 37 y		3 to 26 y	2.5 to 41 y	3.5 to 14.5 y	3.5 to 39 y	4.5 to 33 y
Ethnicity	Europe and mixed Europe/ west Africa		Japanese		Vietnamese / Caucasian	Japanese and Korean	Chinese		Caucasian, Maghreb, Chinese					Japanese and Korean	Caucasian, Turkish, South Arabia
Gene sequencing															
PRKAR1A	p.Arg368* (×3)	p.Arg335Pro ; p. Ile327Thr	p. Thr239Ala	p.Arg368* (x6); p. Gln285Arg ; p.Gly289Glu ; p. Ala328Val; p. Arg335Leu; p.Gln372*	p.Arg368*, p. Ala213Thr, p. Tyr373Cys, p. Arg335Cys	p.Arg368*	p. Gly289-Glu	p. Tyr175-Cys	p.Arg368*, p. Tyr373His, p.323Ser, p. Trp262Cys, p. Tyr323Cys	/	/	/	/	/	/
PDE4D	/	/	/	/	/	/	/	/	/	p.Gln228Glu; p. Glu590Ala; p. Gly673Asp	p. Ala227Ser ; p. Glu590Ala	p.Ala243Val (×4) ; p. Ile617Thr; p. Val268Ala; p. Ser240Thr; p. Pro164Leu	p. Phe226Val; p.Met303Val; p.Val329Ala; p.Phe226Cys; p.Ile678Thr	p.Ile678Thr (×2); p. Gly673D; p. Gln228Phe (×2); p. Leu230Ser ; p.Thr587Ala	p. Phe226-Ser, p. PRo225T-hr, p. Ser190-Ala, p. Thr587Pr-o, p. Tyr677-Asp, p. Thr591As-n, p. Ser190-Pro, p. Ile333Val, p. Leu316-Phe, p. Val329Al-a, p. Ser301-Asn
IUGR	+ 3/3	NA	−	+ 8/11	NA		+		8	NA	+1/2				4
Postnatal growth retardation	+ 3/3	+ 2/2	+	+ 7/11	+ 3/4	− 1/1	+	−	8	+ 2/2	+1/2	− (7/8)	− (3/5)	+4/7	3
Actual height (SD)	−2.5 to −4	Mild	−3.1	−1 to −4.6	−1.1 to −3.3	−1.7	−2	+1	−6.7 to −0.9	Mild	0.4 to −3		−0.7 to −2.9	−0.2 to −5.9	−3 to +1.8
Obesity	− 3/3	NA		+ 2/11	NA	− 1/1	−		3	NA	+ 2/2	+ (4/8)	− (4/5)	− 7/7	1
Facial dysostosis															
Nasal hypoplasia	+ 3/3	+ 2/2	+	+ 11/11	+ 2/4	+ 1/1	+		3	+ 3/3	+ 2/2	+ (7/8)	+ (5/5)	+ 7/7	9
Depressed nasal bridge	NA	Midface hypoplasia		+ 5/11 severe hypoplasia	+ 1/4	+ 1/1 mild NM hypoplasia	+		8	Midface hypoplasia	− 2/ 2 severe hypoplasia			+ 7/7 severe NM hypoplasia	11
Prominent mandibule	NA	NA		NA	+ 1/4	NA			3	NA	NA			NA	5
Peripheral dysostosis															
	+ 3/3	+ 2/2	+	+ 11/11	+ 4/4	+ 1/1 mild			9	+ 3/3	+ 2/2	+ (7/8)	+ (5/5)	+ 7/7 severe	11

Table 2 (continued)

Patient	Linglart et al. [4] ($n = 3$)	Lee et al. [11] ($n = 2$)	Nagasaki et al. [15] ($n = 1$)	Linglart et al. [7] ($n = 11$ news)	Muhn et al. [14] ($n = 4+/7$)	Kaname et al. [10] ($n = 1$)	Li et al. [12] ($n = 1$)	Rhayem et al. [16] ($n = 1$ new)	This study ($n = 9$)	Lee et al. [11] ($n = 3$)	Linglart et al. [7] ($n = 2$ news)	Lynch et al. [6] ($n = 8$)	Lindstrand et al. [13] ($n = 5$)	Kaname et al. [10] ($n = 7$)	This study ($n = 11$)
Severe brachydactyly															
Short, broad metatarsals, metacarpals and phalanges	+ 3/3	+ 2/2	+	+ 11/11	+ 4/4	+ 1/1	+	+	9	+ 3/3	+ 2/2	+ (6/6)	+ (4/4)	+ 7/7	10
Cone-shaped epiphyses	+ 3/3	NA	+	+ 11/11	+ 4/4	NA		+	8	NA	+ 2/2	+ (2/6?)	NA	NA	7
Advanced bone age	+ 3/3	NA		+ 9/11	NA	+ 1/1	+		4	NA	+ 2/2	+ (2/2) and 1 N	NA	+ 7/7	6
X-rays															
Narrowing of caudal vertebral interpedicular distance	+ 3/3	+ 2/2			+ 1/4		NA	+	5	+ 3/3		−	+ (2/3)		1
Hormonal screening															
PTH (ng/L)	↑ 3/3		↑	↑ 11/11	↑ (2/2)	N 1/1	↑	↑	8↑		N 2/2	N (8/8)	↑ (4/5)	N 7/7	1 slightly↑
GH secretory response	Impaired 2/2		N		↑ HGH (1/3)				1 complete deficiency						N
Gonadotropic axis	Mild gonadotropin resistance		N		↑ FSH (1/3)				N						N
OGE	2M: bilateral cryptorchidism —1F: normal	cryptorchidism: 1/2M		M: normal (5/5)—F: 2/6 with irregular cycles	N				1 cryptorchidism	Unilateral undescended testis : 1/2	Normal	5M: 2 hypospadias; 3 cryptorchidism —3F: normal	4M:1 cryptorchidism		1 testicular ectopy; 1 feminine hypospadias
Free T4 (pmol/L)	N 3/3	Congenital hypothyroidism 1/2	N	N or ↓	NA		N	N	N or ↓	Congenital hypothyroidism 1/3	N or ↓	N (8/8)	N (4/4)		N
TSH (mUI/L)	↑ 3/3		↑	↑ 9/11	↑ (2/3)	↑ 1/1	↑	↑	8↑		N 2/2	N (7/8)	N (4/4)	N 7/7	N
Neurology															
Mental retardation		+ 2/2 (mild)	+ (mild)	− 11/11	+ 1/4 (mild)	+ 1/1 mild	−		1	+ 2/3 (1 mild; 1 significant)	+ 2/2	+ (8/8)	+ (5/5)	+ 7/7 (1 mild/ 6 severe)	9
Other	CNS calcifications 1/3	NA		+ 5/11 (behavioral troubles)	Coarse hair (1), sensineural deafness (1)				/	NA	− 2/2 (behavioral troubles)				Red hair, intracranial hypertension

kinase A (PKA), have an important role in the metabolic homeostasis including the regulation of adiposity [17]. In mice, targeted disruption of the RII beta gene, coding for a regulatory subunit of PKA, leads to a lean phenotype with resistance to diet-induced obesity [18]. Cushing syndrome with bilateral adrenocortical hyperplasia may be caused by inactivating variants of *PRKAR1A* leading to an increase of PKA activation. The patients with these inactivating *PRKAR1A* variants have a lower BMI than the ones without variants, confirming a link between germline defects of PKA and human obesity phenotypes [19].

Similarly, *PDE4D* which converts cAMP to AMP, countering activation of PKA, is an insulin-responsive gene important for metabolism regulation, including adipocyte lipolysis [20]. Rat adipocytes treated with the PDE4 inhibitor rolipram showed a significant increase in lipolysis and reversed in part prostaglandin E2 antilipolysis [20]. Furthermore, cAMP-dependent PKA pathway is also involved in insulin secretion and resistance. Overexpression of *PDE4D* in α-cells reduced insulin secretion, whereas inhibition of PDE4 activity by rolipram or knockdown of *PDE4D* restored it [21]. One patient with acrodysostosis has clearly been described with a metabolic syndrome and hypertension [22]. Although the physiopathological link between *PRKAR1A* and *PDE4D* variants and human obesity and insulin resistance will require further study, follow-up of patients should be careful on the BMI, blood pressure, and glucose status.

We also report two patients with *PDE4D* variants who developed intracranial hypertension and thrombophlebitis. One adult patient with acrodysostosis and one child with *PDE4D* variant have also been described with a deep vein thrombosis [13, 23]. Actually, several case–control studies have assigned an association between *PDE4D* variants and risk of ischemic stroke among different ethnicities [24]. Moreover, *PDE4D* is associated with inflammation and reduced *PDE4D* is assumed to predispose individuals to atrial fibrillation, which increases the risk of cardiogenic stroke [25]. More studies are necessary to assess the functional and physiological effects of the described *PDE4D* SNPs. We advise to pay special attention to the thromboembolic risk during follow-up. Actually we emphasize the importance of a multidisciplinary long-term follow up, including cardiovascular and thromboembolic risk factors and we propose a short checklist for medical management (Table 3).

In this study, we also investigated 5 rare cases of acroscyphodysplasia initially described by Verloes et al. in 1991 [9]. All 5 patients had the characteristic metaphyseal changes, but only 3 patients had very short hands and feet, considered as a main criterion for this diagnosis [9, 26]. Among these 3 patients, *PDE4D* variants were identified in the 2 with severe growth retardation (−3 and −4 SD), severe nasal hypoplasia and stiffness of the knees. This finding suggests that *PDE4D* screening should be considered in case of knee cup-shaped metaphyses with embedded epiphyses when associated with acrodysostosis-like brachydactyly and nasal hypoplasia. As few observations of typical knee cup-shaped metaphyses with embedded epiphyses have been described after repeated injuries or meningococcemia [26], the reported infections could be at least a part of the cause of the aspect of acroscyphodysplasia in 2/3 patients without identified *PDE4D* variants. A recent study has described *GNAS* variants in 2 cases of acroscyphodysplasia, supporting the hypothesis of a genetic heterogeneity for this condition [8].

The majority of described cases of acrodysostosis have been sporadic with no familial medical history. In our study, we described two cases of mother-to-child transmission of *PDE4D* variants (patients 17 and 18). Prior to *PRKAR1A* and *PDE4D* identification, several reports have described autosomal dominant inheritance, from mother [27–31] or father [32]. Only one report described concordant siblings with seemingly unaffected parents [33], but it has been supported by the observation of Lynch et al. [6]. They described three affected children with clinically unaffected parents, but with a *PDE4D* variant-carrying father. They raise the hypothesis of an imprinting of *PDE4D* gene to explain the variable expressivity, as in the mouse genome, *PDE4D* showed a paternal bias in transmission [34]. However, the familial cases we described with a maternal transmission of *PDE4D* variants and clear phenotype in the kindred rather opposes this hypothesis. More familial cases will be needed to further accord the resulting phenotypes regarding the parental inheritance.

Finally, neither *PDE4D* nor *PRKAR1A* variants were found in seven patients with characteristic skeletal features but no hormone resistance or facial dysostosis, supporting that other disease genes may account for these remaining acrodysostosis cases. Interestingly, both forms of acrodysostosis (ACRO1—MIM 101800—corresponding to variants of *PRKAR1A* and ACRO2—MIM614613—to variants of *PDE4D*) are due to variants of two genes of the same pathway of cAMP signaling. PRKAR1A is the cAMP-dependent regulatory subunit of protein kinase A and *PDE4D* is a class IV cAMP-specific phosphodiesterase, regulating cAMP concentration. The involvement of *PDE4D* in acrodysostosis further supports the key role of cAMP signaling pathway in skeletogenesis, as previously shown for Albright hereditary osteodystrophy due to *GNAS* variants. The conditions share several clinical features (metacarpal abnormalities, obesity, and resistance to hormones depending on generation of cAMP for acrodysostosis with *PRKAR1A* variants), but some manifestations are variable in severity (degree and extent of brachydactyly and

Table 3 Proposed management recommandations for the follow-up of patients presenting with acrodysostosis

Assessments	At diagnosis	Minimal frequency
Physical examination		
Vital parameters (arterial blood pressure)	X	Systematic per year
Physical examination (including facial gestalt, neurologic examination)	X	
Anthropometrics measurements (BMI)	X	
Joint mobility	X	
Vertebral static assessment	X	
Developmental milestones assessment	X	
Endocrine screening		
Calcemia, phosphoremia, PTH, 25-hydroxy-vitamine D, FGF23, calciuria/creatininuria	X	Systematic per year
T3, T4, TSH	X	Systematic per year
IGF1 (+/− GH stimulation test)	X	Once, if growth deficiency
Gonadotropic axis testing	X	Depending on puberty
Blood glucose level	X	Systematic per year
Others		
Genetics	X	Systematic per year
Ear, nose, and throat (deafness? infections?)	X	Systematic per year
Oral and maxillofacial (teeth? Nasal hypoplasia?)	X	Systematic per year
Polygraphy	X	If symptoms of sleep apnea syndrome
Thromboembolic risk factors	X	Systematic per year
Heart ultrasound	X	Once
X-rays	X	Depending on orthopedics
Cerebral MRI	X	Once, +/− depending on clinic
Psychological support	X	Systematic

degree of parathyroid hormone resistance) and some others are quite similar. This clinical variability may be possibly linked to the tissue-specificity of the imprinting of GNAS or to the tissue-specific expression of alternative isoforms of protein kinase A or of phosphodiesterase 4 [4]. Finally, the dysregulation of cAMP intracellular signaling in response to a number of membrane-impermeable hormones could be the underlying common mechanism of acrodysostosis. Further study on the genomes of patients without identified variants is required to elucidate the molecular basis in these cases, but it might be hypothesized that the putative other genes involved in acrodysostosis are linked to regulation of cAMP signaling.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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