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TITLE:

LARP7 Variants and Further Delineation of the Alazami Syndrome Phenotypic Spectrum among Primordial Dwarfisms: 2 Sisters

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ABSTRACT

Alazami syndrome (AS) (MIM# 615071) is an autosomal recessive microcephalic primordial dwarfism (PD) with recognizable facial features and severe intellectual disability due to depletion or loss of function variants in *LARP7*. To date, 15 patients with AS have been reported. Here we describe two consanguineous Algerian sisters with Alazami PD due to *LARP7* homozygous pathogenic variants detected by whole exome sequencing. By comparing these two additional cases with those previously reported, we strengthen the key features of AS: severe growth restriction, severe intellectual disability and some distinguishing facial features such as broad nose, malar hypoplasia, wide mouth, full lips and abnormally set teeth. We also report significant new findings enabling further delineation of this syndrome: disproportionately mild microcephaly, stereotypic hand wringing and severe anxiety, thickened skin over the hands and feet, and skeletal, eye and heart malformations. From previous reviews, we summarize the main etiologies of PD according to the involved mechanisms and cellular pathways, highlighting their clinical core features.

Keywords: Alazami syndrome; primordial dwarfism; LARP7; intellectual disability.

INTRODUCTION

Primordial dwarfism (PD) encompasses a highly clinically and genetically heterogeneous group of disorders mainly characterized by severe pre- and post-natal growth retardation. More specifically, PD is defined by both height and weight being several standards of deviation (SD) below the age-adjusted mean and without major skeletal dysplasia(1).

Various molecular mechanisms underlie this group of disorders(2,3), including impaired mitotic mechanics(4–6), abnormal insulin-like growth factor 1 (IGF1) or 2 expression(7), abnormal response to DNA damage(8,9), defective spliceosomal machinery(10), abnormal replication licensing(11,12), and transcriptional regulation abnormalities(13). The latter mechanism is involved with depletion or loss of function mutations of La RibonucleoProtein domain family 7 (*LARP7,* MIM# 612026)(14). The LARP7 is a chaperone protein required for both stability and function of the RNA(15).

Pathogenic variants in *LARP7* are responsible for Alazami syndrome (AS) (MIM# 615071)(16), a recently described autosomal recessive PD associated with recognizable facial features and severe intellectual disability (ID). To date, 15 patients have been reported (13,17–19).

Here, we describe two additional consanguineous Algerian sisters with Alazami PD due to *LARP7* pathogenic variants. We strengthen some key features of AS and report significant new findings in comparison with previously reported cases to further delineate this syndrome. We focus on differences between AS and other PD disorders and suggest a simple diagram to map six main PD disorders, including AS, sorted by two core clinical features.

MATERIAL AND METHODS

The patients were referred for clinical suspicion of Rett syndrome without *MeCP2* variant to the reference center for developmental anomalies at the University Hospital of Montpellier, France.

Written informed consent was obtained from the parents of both sisters for Whole Exome Sequencing (WES), as was consent for publication of this case report and photographs.

Genomic DNA was extracted from whole blood of the two affected children and their unaffected parents. Samples from one affected child (patient 1) and her mother underwent WES performed by the Centre National de Génotypage, Institut de Génomique, Commissariat à l'Energie Atomique. After complete DNA quality control (quantification in duplicate, DNA integrity evaluation, absence of PCR inhibitors verification), genomic DNA (3 µg) was captured by an in-solution enrichment method (Human All Exon v5 – 50 Mb, Agilent Technologies, Santa Clara, CA, USA). Library preparation and exome enrichment (~20.000 targeted genes) was performed automatically by using NGSx (Perkin Elmer, MA, USA) and Bravo (Agilent Technologies, CA, USA), respectively, according to the manufacturer's instructions (SureSelect, Agilent Technologies CA, USA). After normalisation and quality control, exome-enriched libraries were sequenced by using the Illumina HiSeq2000 system (Illumina, CA, USA) as paired-end 100-bp reads. Samples were sequenced as pools of 4 samples per lane, to obtain an average coverage of 70 to 80 X, with at least 80% of the target nucleotides covered at 30 X. Image analysis and base-calling involved use of the Illumina Real Time Analysis (RTA) Pipeline. Sequence-quality parameters were assessed daily during the 12 days of sequencing. Standard bioinformatics analysis of sequencing data was based on the Illumina pipeline (CASAVA1.8.2) to generate a FASTQ file for each sample. Reads were mapped against the genomic sequences (human genome version hg19) of the targeted genes with the alignment algorithm ELANDv2 (multiseed and gapped alignments). Genetic variation annotation and filtration involved use of the Dijon in-house pipeline, as recently

described in Nambot et *al.*(20) To further filter out the variants, we applied one set of criteria: "gene known to be related to a human disease in OMIM database (table S1: column I)" and "allele frequence < 0.01 in all public databases ESP, ExAC, GnomAD" (table S1: columns O to R)". We analyzed each survived variant after filtration through its biological consistency and phenotypic relevance. Candidate variant and familial segregation were confirmed by Sanger sequencing for both affected sisters (patients 1 and 2) and each parent.

PATIENT REPORTS

Patients 1 and 2

The sisters were the only children of consanguineous Algerian parents. The family history was negative. Both pregnancies were uneventful but without medical follow-up. Both sisters were born at term, at home. Birth measurements were not reported. The sisters did not present feeding difficulties in infancy. Clinical examination was performed when the sisters were age 26 and 22. Clinical features are summarized and compared with previously reported patient features in Table 1.

Both sisters presented severe growth retardation and disproportionate mild microcephaly: occipitofrontal circumference (OFC) decrease was less severe than height decrease (at least 2 SD discrepancy between OFC and height) (Table 1). Both presented severe ID, including absence of speech. They did not recognize known people from unknown people. They both presented a severe anxiety. They had autistic behaviors such as stereotypic hand wringing, but gripping and handling were possible (Figure 1a). Both had lower-limb spasticity and increased tendon reflexes. Upper-limb joint mobility (wrist and elbow) was abnormally decreased.

The sisters had poor appetite, very selective eating behavior (almost exclusively dairy products) and chronic constipation. Physical examination revealed facial features in both (Figure 1b). They featured proximally placed fourth toes and thumbs (Figure 2a), thickened

skin over the hands (Figure 1c) and feet and cutis marmorata (Figure 1d). Pubertal development milestones were normal for both sisters (first periods at age 12).

Patient 1

In addition to the facial features shared with her sister, patient 1 presented sparse eyebrows, narrow and short palpebral fissures with deep-set eyes, a bifid tip on the nose and a short philtrum. She had neurosensorial disability, with low vision on left side and a keratoconus on the right eye, which caused blindness on this side. She needed a Botox injection to treat the spasticity. She had experienced tonicoclonic seizures at age 6 and received valproate for 1 year. She could smile. Daytime toilet training was achieved at age 4.

Patient 2

Patient 2 began to walk at age 3 and a half. Toilet training was never achieved. She could not smile.

Paraclinical investigations

Skeletal X-rays showed slender bones with increased cortical thickness (Figure 2b), a thin calvarium with low-density skullcap, fingerprint marks (Figure 2c) and high vertebrae with small intervertebral disks.

Metabolic screening and micro-array comparative genomic hybridization findings were unremarkable. Molecular genetic study was negative for genes involved in Rett Syndrome (MIM#312750)(21), congenital variant of Rett Syndrome (MIM#613454)(22) and Early Infantile Epileptic Encephalopathy type 2 (MIM#300672)(23)(*MeCP2*, *FOXG1* and *CDKL5*, respectively) as was chromosome breakage study. Brain MRI was normal but CT-scan revealed small brains without calcification. Abdominal and renal ultrasonography findings were normal. Otoacoustic emissions were normal.

RESULTS

On WES, patient 1 showed a homozygous frameshift variant in exon 7 of *LARP7*: c.524_525insTT (p.(Ala176Leufs*37)) (NM_001267039.1 ; SCV000743091). This homozygous variant was confirmed in both sisters. Each parent was heterozygous for this *LARP7* variant.

Because the *LARP7* variant is a frameshift variant in a gene whose loss of function is a known mechanism of disease, totally absent from controls in population databases (Exome Sequencing Project, 1000 Genomes Project and Genome Aggregation Database), detected as homozygous for this recessive disorder, we classified this variant as pathogenic with a strong level of evidence.(24)

After filtration strategy, 124 variants were left (listed in table S1), including 50 variants in genes known to be related to an autosomal dominant disease and 74 variants in genes known to be related to an autosomal recessive disease.

DISCUSSION

AS was defined by Alazami *et al.* in 2012(13) as a novel autosomal-recessive PD syndrome characterized by severe growth restriction with onset at birth and severe ID, related to pathogenic variants in *LARP7*. The original phenotypic spectrum has been expanded and includes recognizable facial features, such as triangular face, prominent forehead, deep-set eyes, sparse eyebrows, broad nose, widely spaced teeth and wide mouth(19) and behavioral concerns such as anxiety and hypersensitivity to hearing stimuli(18).

We report two new patients with AS. They strengthen previous descriptions because our patients share several key features with those in the literature: severe growth restriction and some distinguishing facial features. They highlight the facial pattern (including broad nose, malar hypoplasia, wide mouth and abnormally set teeth) as a core feature of AS. However, we highlight some additional features that are summarized in Table 1.

The discrepancy between height and OFC decrease severity is noteworthy and observed in all patients previously reported. This suggests that mild, even absent, microcephaly may fully match AS. This feature clearly differentiates AS from other microcephalic PDs such as Seckel syndrome and Microcephalic Osteodysplastic Primordial Dwarfisms (MOPDs)(5,28).

Our patients present potentially misleading behavioral diagnostic features: Rett-like stereotypical hand wringing and severe anxiety. In addition to other behavioral concerns previously described, these features seem to be entirely part of the phenotypic spectrum of AS. Interestingly for the categorization among other PD, these behavioral features clearly differentiated from Meier-Gorlin and Silver-Russel syndromes(11,29,30).

Cutis marmorata, seen in other PDs such as MOPD type 2, has never been reported and seems rare in AS, whereas thickened skin over the hands and feet appears more frequent and could be a recognizable physical sign (Figure 1c).

The skeletal (slender bones with increased cortical thickness and abnormally set toes and fingers), eye (keratoconus) and heart (atrial septal defect) malformations are remarkable and could be searched in other AS patients.

We suggest that these additional clinical findings belong to the AS phenotypic spectrum considering that they are in the overlapping part of the two sisters phenotypes. It could be interesting to further investigate all previously reported patients with AS in order to confirm the presence or absence of these apparently novel findings.

Nevertheless, multiple diagnosis is reported in about 1.4% to 7.2% of patients with complex phenotypes who undergo WES(31). Furthermore, prospective annual reanalysis of WES has been showed to improve diagnostic yield, highlighting that truly pathogenic variants can be missed by WES(20). Thus, we could not completely exclude the possibility of a multiple molecular diagnosis in these siblings that would have not been identified by WES at time of analyzing. It could also be argued that one or several other potentially modifier variants

detected in the WES might contribute to these additional finding. In this regard, we carefully analyzed all survived variants and attempted to identify those which could be relevant candidate as potentially modifying the causing variant. Among all 124 variants listed in table S1, none had both sufficient biological consistency and phenotypic relevance to be pointed out.

Figure 3, based on previous reviews(1,2,32) summarizes the main etiologies of PD: Seckel syndrome, MOPDs, Meier-Gorlin syndrome, IGF1 and IGF1-receptor deficiency syndromes, Silver-Russel syndrome and AS. In an attempt to map AS and these different syndromes in a practical simple manner, we present a streamlined diagram sorted by two core features: ID and microcephaly severity. We mentioned as well the involved mechanisms and cellular pathways. In this regard, AS is a PD with severe ID and mild microcephaly or normal OFC and an LARP7-related syndrome involving transcriptional regulation abnormalities. MOPD and Seckel syndrome both are PDs with severe ID and severe microcephaly. Both MOPD and Seckel syndrome may be linked to an abnormal mitosis and are part of the Centromeric Protein J- and Pericentrin-related syndromes**.** MOPD may also be linked to a defective spliceosomal machinery and is an RNU4ATAC-related syndrome. Seckel syndrome may also be linked to a perturbed DNA damage response and is part of the ATR-, ATRIP- and RBBP8-related Seckel syndromes. Meier-Gorlin syndrome and IGF1 and IGF1-receptor deficiency syndromes are both PDs with severe microcephaly and normal cognitive development (CD) or mild ID, related to abnormal replication licensing and abnormal GH-IGF1 signaling, respectively. Silver-Russel syndrome is a PD with normal or subnormal CD and normal OFC due to abnormal IGF2 expression.

In conclusion, we suggest further delineation of the AS phenotypic spectrum, which includes PD with possibly disproportionate mild even absent microcephaly, commonly severe ID, some recognizable facial and skeletal features and frequent behavioral autistic or Rett-like

features. This delineation might be helpful for the clinical diagnosis process, including for "WES as a first-line test and back to phenotype" approach.

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FIGURE LEGENDS

Figure 1: Patients 1 and 2: clinical features.

a. Behavioral pictures at age 26 and 22, respectively (upper left picture), and age 24 (upper right picture). Both presented autistic behaviors such as stereotypic hand wringing.

b. Front, lateral and global views at age 21 (patient 1) and 17 years (patient 2). Both presented facial features: broad nose, malar hypoplasia, wide mouth, full lips and abnormally set teeth. This pattern of facial features is shared with most patients with Alazami syndrome previously reported. It highlights this facial pattern as a core feature of Alazami syndrome. In addition, patient 1 presented sparse eyebrows, narrow and short palpebral fissures with deep-set eyes, a bifid tip of nose and a short philtrum. Note disproportionate mild microcephaly compared with severe short stature.

c. Palmar side of hands: both sisters presented thickened skin over the hands (and feet, not shown).

d. Skin pictures: both sisters presented extended cutis marmorata.

Figure 2: Skeletal features and X-rays views.

a. Both sisters presented abnormally set fourth toes and proximally set thumbs (left upper pictures).

b. Skeletal X-rays showing slender bones (black arrows) with increased cortical thickness (pairs of white arrows). Only patient 2 X-rays are shown (lower pictures), from left to right: front views of the hips, left knee, left forearm and right arm.

c. Head X-rays showing a thin calvarium with low-density skullcap and fingerprint marks (white arrows). Only patient 2 X-rays are shown.

Figure 3: Main etiologies of primordial dwarfism (PDs) according to two core features and linked to the involved mechanisms and cellular pathways.

As an example, Microcephalic Osteodysplastic Primordial Dwarfisms (MOPDs) and Seckel syndrome both are PDs with severe intellectual disability and severe MC. Both MOPDs and Seckel syndrome may be linked to abnormal mitosis and are part of the Centomeric Protein J- and Pericentrin-related syndromes**.** MOPD may also be linked to a defective spliceosomal machinery and is an RNU4ATAC-related syndrome. Seckel syndrome may also be linked to a perturbed DNA damage response and is part of the ATR-, ATRIP- and RBBP8-related Seckel syndromes. For further description of this figure, see the Discussion.

PD: primordial dwarfism, ID: intellectual disability, CD: cognitive development, MC: microcephaly, OFC: occipito-frontal circumference, MOPD: Microcephalic Osteodyplastic Primordial Dwarfism.

271 **Table 1.** Comparison of clinical features of presented patients and previously reported

272 patients with Alazami syndrome.

	Alazami *et al.* (13)	**Najmabadi *et al.*** (17)	**Ling *et al.*** (18)	**Hollink *et al.*** (19)	**Patient 1**	**Patient 2**	**Total**
ID (severe)	10/10	2/2	1/1	2/2	Yes (No speech)	Yes (No speech)	17/17
Biometry							
Weight (SD)	-2 to -4.5	NR	-5,5	-1 to -3	-2.8	-3.5	NC
Height (SD)	-4 to -10.5	NR	-4	-2.5 to -3	-6.5	-6.8	NC
OFC (SD)	-1.5 to -7	<-2	-1	-2 to -4	-3.5	-3.5	NC
DMM	8/9	NR	1/1	0/2	+	+	11/14
Facial features							
Triangular face	9/9	NR	1/1	1/2	-	-	11/14
Prominent forehead	+ (NC)	NR	1/1	2/2	-	-	NC
Narrow and short palpebral fissures	7/9	NR	0/1	1/2	+	-	8/14
Deep-set eyes	9/9	NR	1/1	2/2	+	-	12/14
Sparse eyebrows	9/9	NR	0/1	1/2	+	-	11/14
Low set ears	6/9	NR	1/1	0/2	-	-	7/14
Malar hypoplasia	8/9	NR	1/1	1/2	-	+	10/14
Broad nose	9/9	NR	0/1	2/2	+	+	12/14
Short philtrum	9/9	NR	0/1	1/2	+	-	11/14
Wide mouth	9/9	NR	1/1	1/2	+	+	13/14
Full lips	5/9	NR	1/1	2/2	+	+	10/14
Widely spaced teeth/tooth misalignment	8/9	NR	1/1	2/2	+	+	13/14
Skeletal, muscle and skin features							
Scoliosis	2/9	NR	NR	1/2	+	-	4/13
Hypermobility of distal tips	NR	NR	0/1	1/2	+	-	2/5
Abnormally set toe(s)/fingers	NR	NR	0/1	1/2	+	+	3/5
Skeletal anomalies	NR	NR	0/1	1/2	+	+	3/5
Thickened skin over the hands and feet	5/9	NR	0/1	1/2	+	+	8/14
Cutis marmorata	NR	NR	0/1	0/2	+	+	2/5
Behavioral features							
Anxiety	NR	NR	1/1	1/2	-	-	2/5
Self-mutilation of hands	2/9	NR	1/1		-	-	3/12
Stereotypic	NR	NR	0/1	0/2	+	+	2/5

behavior including hand wringing							
Selective eating behavior	NR	NR	0/1	0/2	+	+	2/5
Other features							
Seizures	NR	NR	0/1	0/2	+	-	1/5
Ocular anomalies	NR	NR	0/1	0/2	-	+*	1/5
Strabismus	4/9	NR	0/1	0/2	-	-	4/14
Atrial septal defect	NR	NR	0/1	0/2	-	+	1/5
Disturbed sleep/sleep apnea episodes	NR	NR	0/1	0/2	+	+	2/5

NR: not reported, NC: non-countable, DMM: disproportionate mild microcephaly,

**Keratoconus*

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<u>Supplemental data:</u>

Table S1: List of all survived variants after filtration strategy (lines 3 to 52: variants in genes related to an autosomal dominant disease; lines 53 to 126: variants in genes related to an autosomal recessive disease).

See Excel table S1 attached

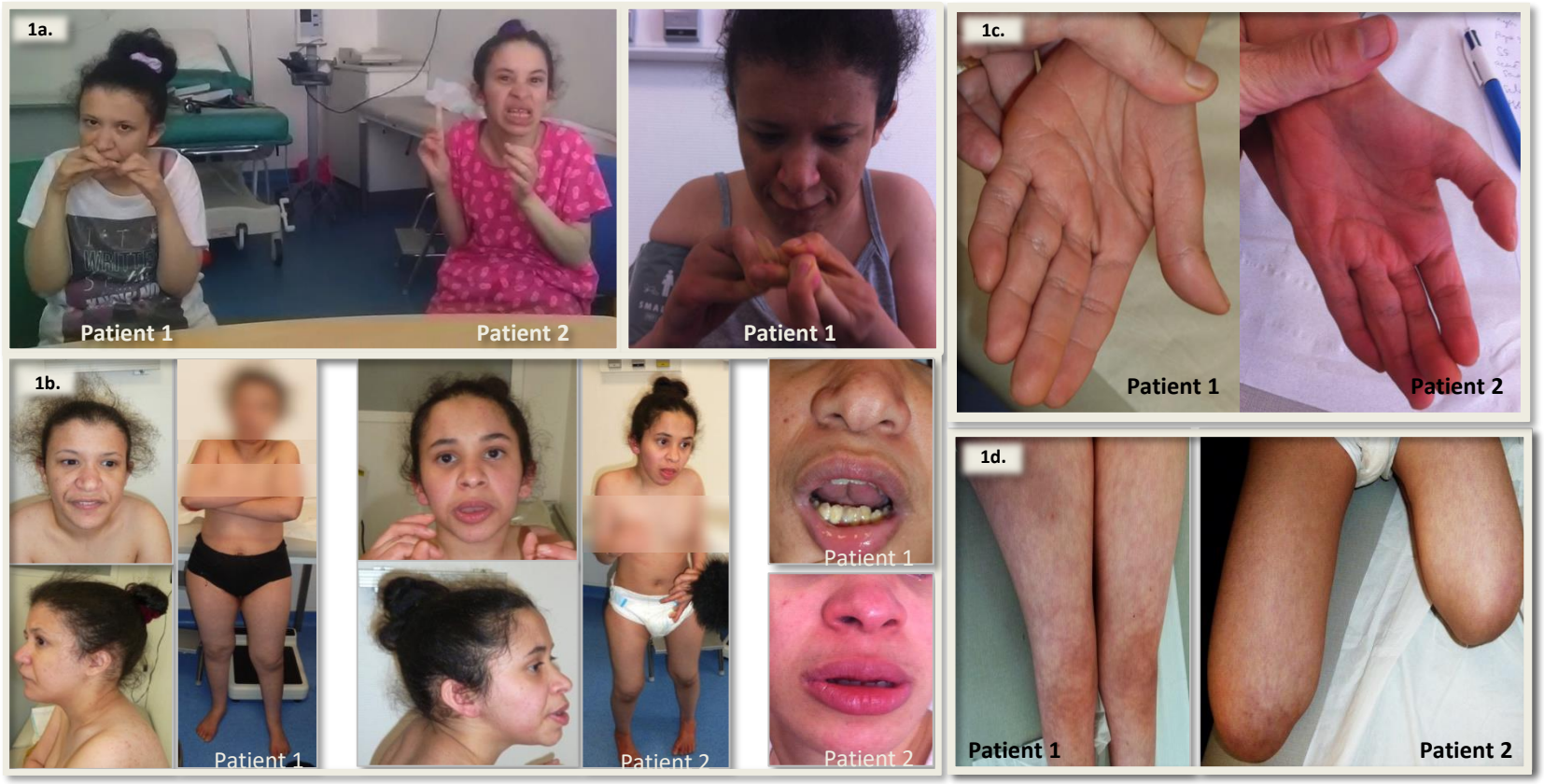

Figure 1: Patients 1 and 2: clinical features.

a. Behavorial pictures at age 26 and 22, respectively (upper left picture), and age 24 (upper right picture). Both presented autistic behaviors such as stereotypic hand wringing.

b. Front, lateral and global views at age 21 (patient 1) and 17 years (patient 2). Both presented facial features: broad nose, malar hypoplasia, wide mouth, full lips and abnormally set teeth. This pattern of facial features is shared with most patients with Alazami syndrome previously reported. It highlights this facial pattern as a core feature of Alazami syndrome. In addition, patient 1 presented sparse eyebrows, narrow and short palpebral fissures with deep-set eyes, a bifid tip of nose and a short philtrum. Note disproportionate mild microcephaly compared with severe short stature.

c. Palmar side of hands: both sisters presented thickened skin over the hands (and feet, not shown).

d. Skin pictures: both sisters presented extended cutis marmorata.

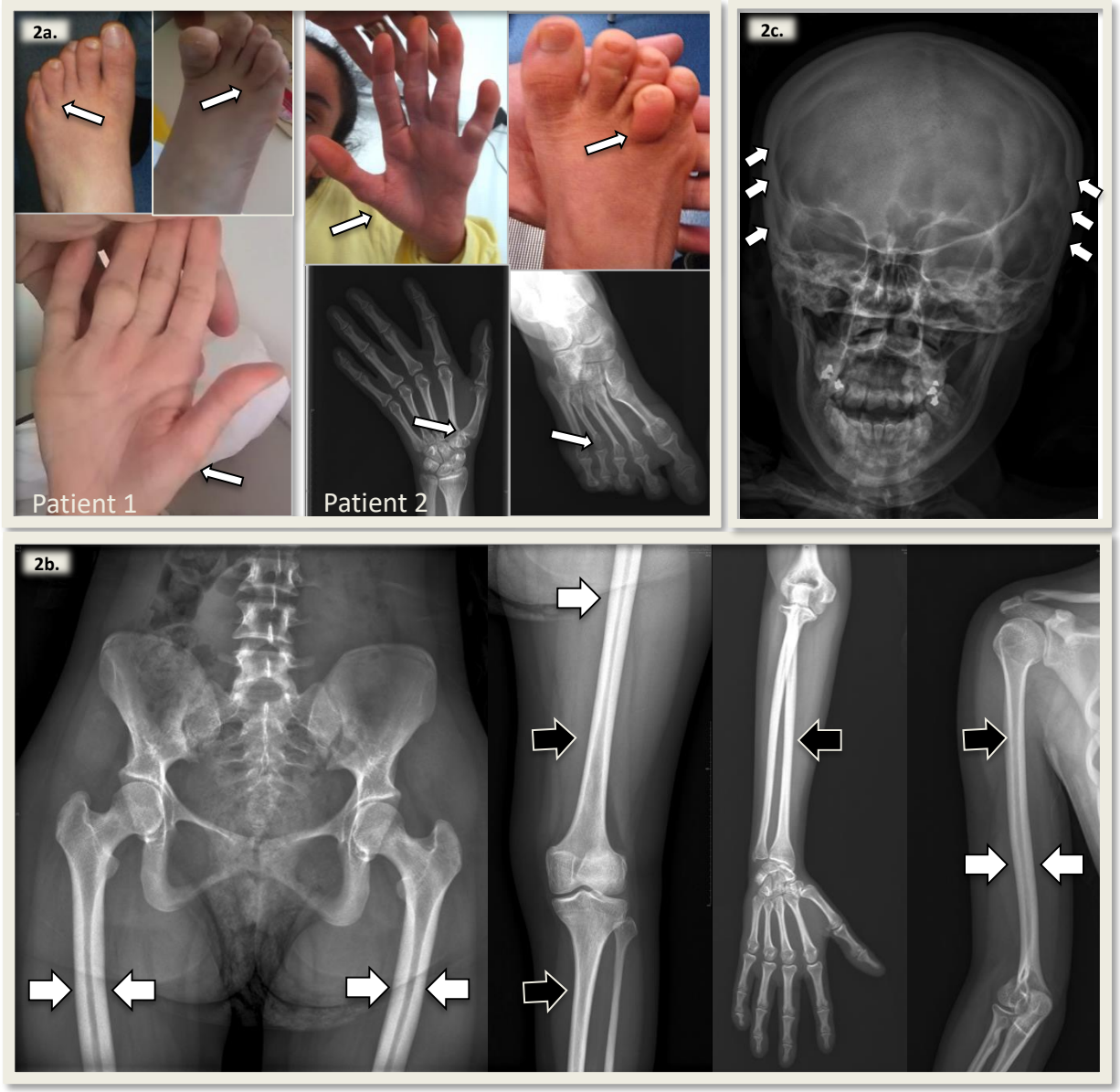

Figure 2: Skeletal features and X-rays views.
a. Both sisters presented abnormally set fourth toes and proximally set thumbs (left upper pictures).
b. Skeletal X-rays showing slender bones (black arrows) with increased cortical thickness (pairs of white arrows). Only patient 2 X-rays are shown (lower pictures), from left to right: front views of the hips, left knee, left forearm and right arm.
c. Head X-rays showing a thin calvarium with low-density skullcap and fingerprint marks (white arrows). Only patient 2 X-rays are shown.

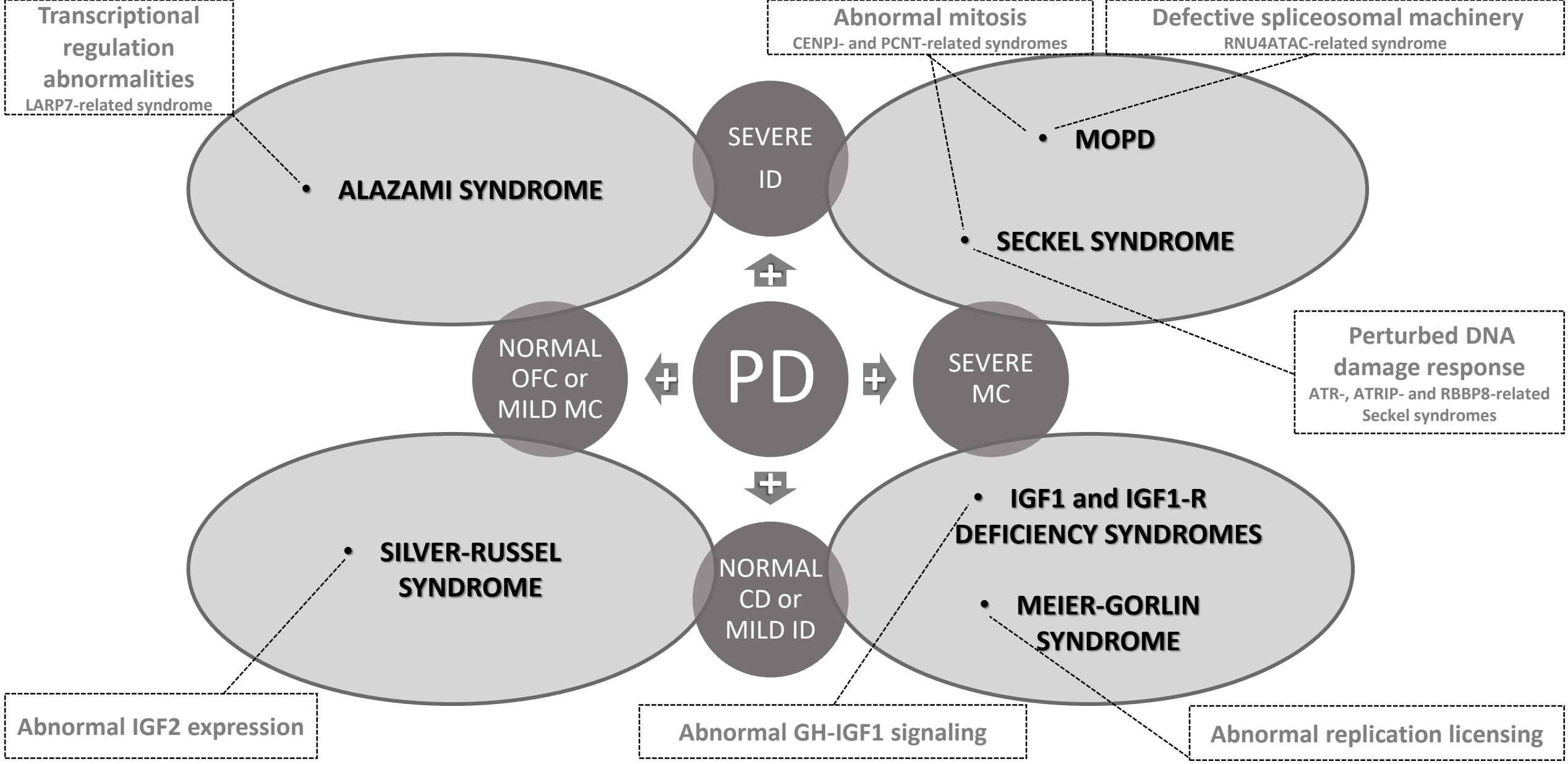

Figure 3: Main etiologies of primordial dwarfism sorted with two core-features and linked to the involved mechanisms and cellular pathways.
For instance, MOPD and Seckel syndrome both are PD with severe ID and severe MC. Both MOPD and Seckel syndrome may be linked to a abnormal mitosis and are part of the CENPJ- and PCNT-related syndromes. MOPD may also be linked to a defective spliceosomal machinery and is a RNU4ATAC-related syndrome. Seckel syndrome may also be linked to a perturbed DNA damage response and is part of the ATR-, ATRIP- and RBBP8-related Seckel syndromes. For further description of this figure, see in discussion.
PD: primordial dwarfism, ID: intellectual disability, CD: cognitive development, MC: microcephaly, OFC: occipito-frontal circumference, MOPD: Microcephalic Osteodyplastic Primordial Dwarfism.