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ORIGINAL ARTICLE

Broadening the phenotypic spectrum of pathogenic *LARP7* variants: two cases with intellectual disability, variable growth retardation and distinct facial features

Iris HIM Hollink[1], Majid Alfadhel[2], Anwar S Al-Wakeel[2], Farough Ababneh[2], Rolph Pfundt[3], Stella A de Man[1,4], Rami Abou Jamra[5], Arndt Rolfs[5,6], Aida M Bertoli-Avella[5] and Ingrid MBH van de Laar[1]

In 2012 Alazami *et al.* described a novel syndromic cause of primordial dwarfism with distinct facial features and severe intellectual disability. A homozygous frameshift mutation in *LARP7*, a chaperone of the noncoding RNA 7SK, was discovered in patients from a single consanguineous Saudi family. To date, only one additional patient has recently been described. To further delineate the phenotype associated with *LARP7* mutations, we report two additional cases originating from the Netherlands and Saudi Arabia. The patients presented with intellectual disability, distinct facial features and variable short stature. We describe their clinical features and compare them with the previously reported patients. Both cases were identified by diagnostic whole-exome sequencing, which detected two homozygous pathogenic *LARP7* variants: c.1091_1094delCGGT in the Dutch case and c.1045_1051dupAAGGATA in the Saudi Arabian case. Both variants are leading to frameshifts with introduction of premature stop codons, suggesting that loss of function is likely the disease mechanism. This study is an independent confirmation of the syndrome due to LARP7 depletion. Our cases broaden the associated clinical features of the syndrome and contribute to the delineation of the phenotypic spectrum of *LARP7* mutations.
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INTRODUCTION

In the era of next-generation sequencing techniques in diagnostics, such as whole-exome sequencing and whole-genome sequencing, novel causes of intellectual disability and other genetic disorders are being discovered at a rapid pace.[1,2] Besides novel genetic causes, the clinical spectrum caused by pathogenic genetic variants will be expanded as patients with less specific phenotypes will be molecularly diagnosed. Knowledge on the clinical spectrum and phenotype related to pathogenic genetic variants is essential for appropriated interpretation of next-generation sequencing results in patients. Therefore, understanding of the associated phenotypes of novel and rare pathogenic variants and proper documentation in databases and scientific literature is of utmost importance.

Alazami *et al.*[3] described a novel cause of syndromic primordial dwarfism (OMIM 615071). They identified a Saudi family with a homozygous frameshift mutation in La ribonucleoprotein domain family, member 7 gene (*LARP7*). The LARP7 protein is a chaperone of the noncoding RNA 7SK. All nine family members affected by the same homozygous *LARP7* mutation had primordial dwarfism defined as anthropometric measurements of <3.5 s.d. below the mean in the absence of frank skeletal dysplasia with onset at birth. Besides primordial dwarfism, features of the patients included severe intellectual disability and distinct facial features as triangular face, deep-seated eyes, narrow palpebral fissures, broad nose, malar hypoplasia and a short philtrum. As all these patients were from the same family, additional cases are required to further elucidate the phenotype associated to *LARP7* mutations. A large study of Najmabadi *et al.* in 136 Iranian families with autosomal recessive intellectual disability also identified a frameshift mutation (p.Gln276fs) in *LARP7* in one family with a syndromic form of intellectual disability. Besides microcephaly, no further clinical characteristics of this case were reported.[4]

Here we describe two new cases with pathogenic variants in *LARP7* presenting with intellectual disability, variable growth retardation and distinct facial features. A comparison with the previously described Saudi family further delineates the phenotypic spectrum related to the loss of LARP7.

MATERIALS AND METHODS

The first patient was referred for consultation by a clinical geneticist of the Erasmus Medical Centre at the outpatient clinic of the Amphia Hospital in Breda for evaluation of his developmental delay. Genomic DNA was extracted from peripheral blood of the patient and parents using standard protocols.

[1]Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, The Netherlands; [2]Division of Genetics, Department of Pediatrics, King Saud bin Abdulaziz University for Health Sciences, King Abdullah Specialized Children's Hospital, King Abdulaziz Medical City, Riyadh, Saudi Arabia; [3]Department of Human Genetics, Division of Genome Diagnostics, Radboud University Medical Centre, Nijmegen, The Netherlands; [4]Department of Pediatrics, Amphia Hospital, Breda, The Netherlands; [5]Centogene AG, Rostock, Germany and [6]Albrecht-Kossel-Institute for Neuroregeneration, Medical University Rostock, Rostock, Germany
Correspondence: Dr IMBH van de Laar, Department of Clinical Genetics, Erasmus Medical Center, Wytemaweg 80, PO Box 2040, 3000 CA Rotterdam, The Netherlands.
E-mail: i.vandelaar@erasmusmc.nl
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Diagnostic whole-exome sequencing analysis was performed in trio (patient and both parents) at the Radboud University Medical Center in Nijmegen, the Netherlands, essentially as described before.[5] Exome capture was performed with the Agilent SureSelect Human All Exon v4 enrichment kit (Agilent Technologies, Santa Clara, CA, USA). Whole-exome sequencing was performed on the Illumina HiSeq platform (BGI, Copenhagen, Denmark). Data were analyzed with BWA (read alignment) and GATK (variant calling) software packages.[6,7] Variants were annotated using an in-house-developed pipeline. Prioritization of variants was done by an in-house-designed 'variant interface' and manual curation. Potentially causative variants were confirmed by Sanger sequencing.

DNA samples from the second patient and his parents were sent to Centogene (Rostock, Germany) for diagnostic whole-exome sequencing analysis. Exome sequencing was performed in the index and parents using genomic DNA, which was amplified with the Ion AmpliSeq Exome Kit (Life Technologies, Waltham, MA, USA). The DNA libraries were pooled, barcoded and sequenced using an Ion Torrent Proton sequencer (Life Technologies). The combination of different bioinformatics tools allowed variant calling and annotation. All candidate variants were required on both sequenced DNA strands and to account for $\geqslant 20\%$ of total reads at that site with a minimum depth of coverage of $20\times$. Common variants ($\geqslant 5\%$ in the general population) were discarded by comparison with the 1000G, the Exome Variant Server, the Exome Aggregation Consortium database an in-house exome variant database, to filter out both common benign variants and recurrent artifact calls. All detected variants were initially compared with our mutation database (CentoMD), The Human Gene Mutation Database and ClinVar to directly identify and annotate changes previously described in the literature. Then, variants were evaluated based on the suspected disease mode of inheritance and compatibility with the clinical phenotype provided for the index. Selected candidate variants were confirmed by conventional Sanger sequencing. Segregation of these variants with the disease was assessed for all available family members.

Written informed consent was obtained from the parents of both cases for publication of this case report. Consent for publication of photographs of the parents of case B was obtained and declined by the parents of case A.

RESULTS

Case A

The first patient is a 6-year-old boy. He is the first child of non-consanguineous Dutch parents with an unremarkable family history. The pregnancy was uneventful though mother reported little child movements. Mother smoked during pregnancy. He was born at a gestation of 40 weeks and 3 days with a weight of 2980 g (−1 s.d.), length of 45 cm (−3 s.d.) and head circumference of 34 cm (−1 s.d.).

His psychomotor development is delayed. He was able to walk by the age of 18 months, and spoke two-word sentences at 5 years. Neuropsychological evaluation at the age of 5.1 years showed a developmental age of 22 months (intelligence quotient equivalent of 37). He shows progression regarding motor skills, social behavior and self-independence; his language skills are relatively poor. He has a happy appearance but shows anxiety for loud unexpected noises. He drools and has premature changing of his teeth.

He had recurrent middle ear infections for which he had tympanostomy tubes and tonsillectomy. At the age of 3 years he had two episodes of convulsions during fever. An electroencephalogram showed an epileptic focus left occipital in the brain but there were no clinical signs of epilepsy. He was diagnosed with transient erythroblastopenia of childhood, requiring two blood transfusions at 3 and 5 years of age. He showed a slightly reduced growth from birth; at 6 years of age growth parameters were height at −2.5 s.d. (his target height based on parental height is 0 s.d.), weight at −1 s.d. and head circumference at −2 s.d.

Physical examination revealed dysmorphic facial features; prominent forehead, hypertelorism with downward eye slant, epicanthic folds, deep-set eyes, blue sclerae, upturned nose, long philtrum, thin upper lip and simple ears. He has hypermobility of the joints of the hands, clinodactyly of first toe.

An ultrasound of the heart and kidneys, total skeletal survey, magnetic resonance imaging of cerebrum, hearing screening and ophthalmologic examination revealed no abnormalities. Micro-array analysis showed a normal male profile and a mitomycin-C test revealed no aberrations. Extensive metabolic investigations in blood and urine were normal. No mutations were identified in the *ATRX* gene.

Whole-exome sequencing detected a homozygous frameshift variant (c.1091_1094del; p.Arg364fs) in *LARP7* (NM_015454.2; Figure 2). The presence of this variant was confirmed in the patient and in both parents using Sanger sequencing. This variant is novel and has not been reported previously. Additional compound heterozygous missense variants (c.7985T>C; p.Val2662Ala and c.4969G>A; p.Val1657Met, respectively, paternal and maternal inherited) were detected in *LAMA2* (NM_000426.3; cDNA). These variants are novel and their pathogenicity is unclear. *LAMA2* gene mutations are found in merosin-deficient congenital muscle dystrophy (OMIM 607855). This clinical picture is not present in our patient.

Case B

The second patient is a 2.5-year-old boy originating from Saudi Arabia. He is the first child of consanguineous (first cousins) parents of Saudi origin with no significant family history. During pregnancy there was unexplained intrauterine growth retardation. He was born at term with a weight of 2 kg, no other measurements are available. After birth, a cleft palate was noticed, which was partially repaired at 16 months of age. He had a laryngomalacy that resolved at 6 months of age. During his first year he had two episodes of aspiration pneumonia and recurrent otitis media, which disappeared after 1 year of age.

He has global developmental delay. His functioning is equivalent to the age of 1 year. His motor milestones were delayed with unsupported sitting at 9 months, standing at 2 years and walking independently at 27 months. He is not able to speak, and expresses his needs only by crying. There are no behavioral problems.

He showed reduced growth from birth; at 2.3 years of age growth parameters were height at −3 s.d. and weight at −3 s.d. In addition, he had microcephaly with a head circumference at −4 s.d.

Physical examination revealed dysmorphic features (Figure 1): a triangular face; puffy upper eyelids; sparse eyebrows; deep-set eyes; malar hypoplasia; broad nose; full lips; short and smooth philtrum; large mouth; widely spaced teeth; and a partially corrected cleft palate. He has right torticollis, and widely spaced and dysplastic nipples. He has no hypermobility of the joints. His second and fourth toes overlap the third toe bilaterally. He has a normal muscle tone. All of the following were unremarkable: hearing test; ophthalmologic examination; chromosomal analysis; skeletal survey including X-rays of the feet; and abdominal ultrasound. Brain magnetic resonance imaging is reported as unremarkable. Newborn screening was unremarkable. Ammonia and lactic acid, and total homocysteine were normal.

Whole-exome sequencing revealed a homozygous duplication (c.1045_1051dupAAGGATA; p.Thr351Lysfs*9) in *LARP7* as described by Alazami *et al.*[3] (Figure 2). The presence of this variant was confirmed in the patient (homozygote) and both parents (heterozygotes) using Sanger sequencing. Additional homozygous variants were detected, respectively, in *SMC2* (NM_001042550.1; c.2951A>G; p.N984S) and in *CDH7* (NM_017780.3; c.1405A>G; p.R469G).

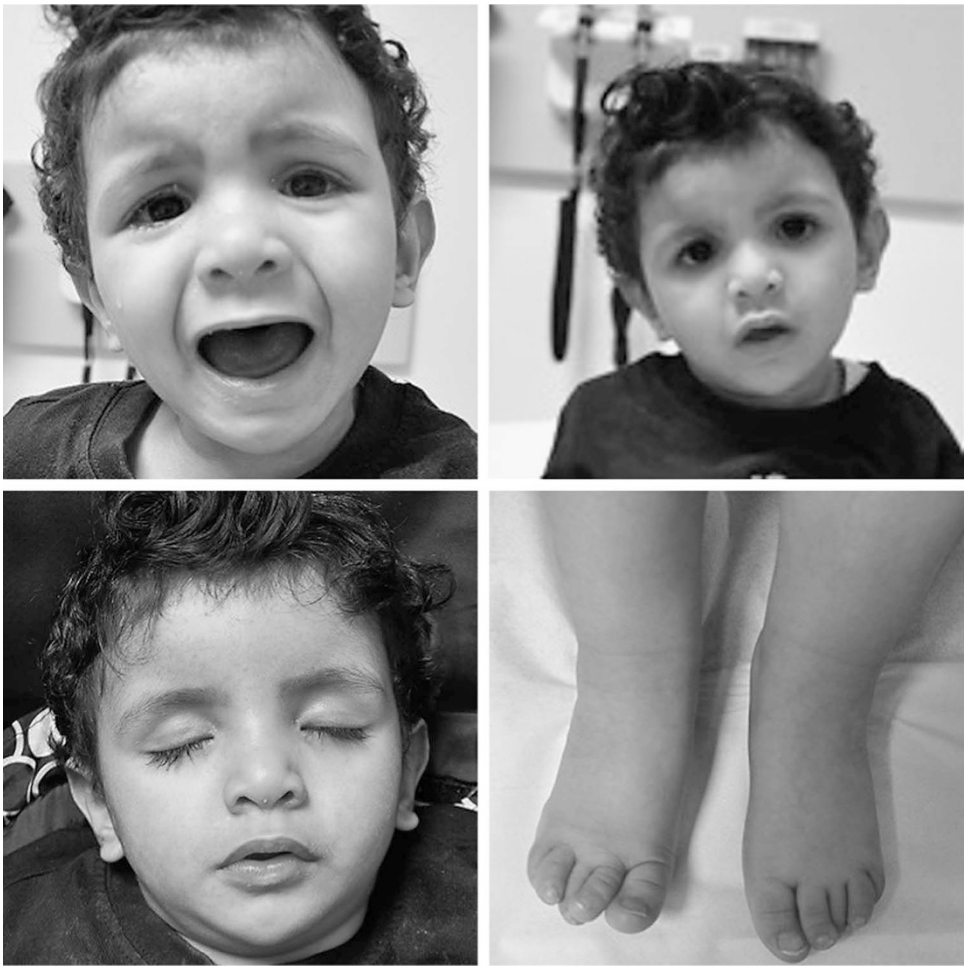

Figure 1 Photographs of case B. Case B at age 2.5 years showing triangular face with prominent forehead, deep-seated eyes with sparse eyebrows, a broad nose, full lips and overlapping toes. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

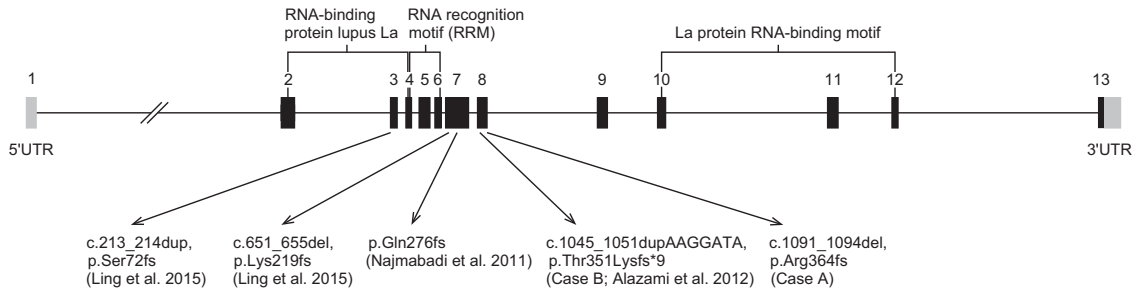

Figure 2 Schematic representation of the *LARP7* gene. Boxes represent exons 1–13 with the untranslated regions (UTRs) and the open reading frame. The functional domains are indicated above. The pathogenic variants found in our cases and mutations previously published are depicted.

The pathogenicity of both missense variants is unclear. The clinical picture of the disorders associated with *SMC2* and *CDH7* variants (respectively, Smith–McCort dysplasia and CHARGE syndrome) are not present in our patient. Moreover, the inheritance mode of CHARGE syndrome (autosomal dominant) is not compatible with the homozygous *CDH7* variant. Therefore, both variants do not seem causative for the disorder of case B.

DISCUSSION

We report on two novel cases with loss of function variants in *LARP7* presenting with intellectual disability, distinct facial features and variable growth retardation. Alazami *et al.*[3] showed in their original report loss of LARP7 protein expression caused by the homozygous frameshift mutation, which leads to the depletion of 7SK. In cells, 7SK is abundantly expressed and forms the 7SK small nuclear ribonucleoprotein (snRNP) complex. 7SK snRNP has a role in gene regulation through suppression of the positive transcription elongation factor b as well as competing with HMGA1 in transcriptional regulation. Okamura *et al.*[8] showed a critical role of 7SK snRNP in the maintenance of proliferation in primordial germ cells by cell cycle control of transcription. Furthermore, Dai *et al.*[9] recently discovered that knockdown of LARP7 resulted in premature differentiation of

Table 1 Clinical features of presented cases compared with the previously reported patients

	Case A	*Case B*	*Case reported by Ling* et al.	*Cases from Saudi family (n = 9); Alazami* et al.
Age (years)	6	2.5	2.5	Median 12 (range 5 to 22)
Gender	M	M	F	5 M, 4 F
Biometry at birth	At term	At term	At term	NA
Weight	2980 g	2.0 kg	2608 g	
Height	45 cm	NA	47.6 cm	
OFC	34 cm	NA	32.4 cm	
Biometry				
Height	107.4 cm (–2.5 s.d.)	81 cm (–3 s.d.)	72.7 cm (–4 s.d.)	< –3.5 s.d. (range –3.6 to –10.5)
Weight	16.5 kg (–1 s.d.)	9.1 kg (–3 s.d.)	7.6 kg (–5.5 s.d.)	< –2 s.d. (range –2 to –4.5)
OFC	48.4 cm (–2 s.d.)	44.5 cm (–4 s.d.)	46 cm (–1.0)	< –1.5 s.d. (range –1.5 to –7)
Developmental delay	Severe	Severe	Severe	Severe
Developmental age	22 months at 5.1 years (IQ 37)	1 year at 2.5 years	NA	Range 1 to 5 years
Motor skills	Walk at 18 months	Walk at 27 months	Walk at 2 years	
Language skills	Two-word sentences, echolalia	No single words	No single words	
Behavior	Happy, social		Anxious, agitated	
Self-mutilation	–	–	–	2/9
Hyperactivity	+	–	–	NA
Facial features				
Triangular face	–	+	+	9/9
Prominent forehead	+	+	+	+
Narrow and short palpebral fissures	–	+	–	7/9
Deep-seated eyes	+	+	+	9/9
Sparse eyebrows	–	+	–	9/9
Low-set ears	–	–	+	6/9
Malar hypoplasia	–	+	+	8/9
Broad nose	+	+	–	9/9
Short philtrum	–	+	–	9/9
Wide mouth	–	+	+	9/9
Full lips	+ (only full lower lip)	+	+	5/9
Widely spaced teeth	+	+	+	8/9
Skeletal features				
Scoliosis	+	–	NA	2/9
Other	Clinodactyly first toe	Second and fourth toes overlap over third toe	–	Mild epiphyseal changes in the proximal phalanges
Other features	Thickened skin over hands, TEC	Cleft palate, right torticollis	Poor balance with wide-based gait, hypersensitivity to touch and sound	Strabismus 4/9, thickened skin over hands and feet 5/9, short Achilles' tendon 2/9

Abbreviations: IQ, intelligence quotient; NA, not available; OFC, occipitofrontal head circumference; TEC, transient erythroblastic anemia of childhood.

embryonic stem cells via downregulation of Lin28. They proposed herewith a mechanism for global growth failure caused by premature differentiation of cells.

In our first case a novel frameshift variant in *LARP7* is located near the mutation originally reported by Alazami *et al.* (Figure 2). Complete loss of the LARP7 protein due to nonsense-mediated mRNA decay is also expected in our case. The second case harbors the identical frameshift variant as described by Alazami *et al.*, which might be a founder mutation in this area as the patient originated from the same geographic region in Saudi Arabia.

The currently presented cases have a severe developmental delay identical to the Saudi family (Table 1). Although language skills are relatively poor, motor skills seem less affected. Regarding behavior abnormalities, only hyperactive behavior was reported in the first case. In addition to severe developmental delay and intellectual disability reported until now in all cases with *LARP7* mutations, there seems to

be a recognizable facial phenotype. Cases have a prominent forehead with obvious deep-seated eyes, narrow palpebral fissures, sparse eyebrows and a broad nose despite Saudi Arabian or Caucasian origin.

In contrast, our cases do not have a height below −3.5 s.d. as present in all originally reported Saudi cases, though both have a short stature ranging from −2.5 to −3 s.d. (Table 1). This expands the phenotypic spectrum of *LARP7* mutations to a milder end of short stature not restricted to primordial dwarfism. In addition, microcephaly is not a requisite to make the diagnosis as the first case has no microcephaly, similar to some of the family members described by Alazami *et al.*

Additional previously unreported clinical features present in our cases were cleft palate, transient erythroblastic anemia of childhood and mild skeletal features as clinodactyly and overriding of toes. However, it remains to be determined whether these features are part of the spectrum of *LARP7* mutations. Furthermore, scoliosis was also seen in our first case and thick skin over his hands was also noticed. Taken together, the latter is seen now in more than half of the patients with *LARP7* mutations (Table 1). Interestingly, besides cleft palate no major congenital abnormalities are detected in patients with *LARP7* mutations until now.

During revision of our report Ling *et al.* published a single case of Alazami syndrome presenting with significant failure to thrive, short stature and developmental delay caused by compound heterozygous variants in *LARP7* (Figure 2 and Table 1).[10] The girl has comparable facial dysmorphic features and severe developmental delay. The report confirms our observation of expansion of the spectrum of growth retardation in Alazami syndrome not restricted to primordial dwarfism with absence of prenatal growth retardation and absence of microcephaly.

In conclusion, we present the clinical characteristics of two unrelated individuals with Alazami syndrome. Homozygous loss of function variants in the *LARP7* gene were identified in both patients, therefore this is an independent confirmation of this syndrome due to LARP7 depletion. Pathogenic *LARP7* variants should be considered in patients with severe intellectual disability characteristic facial features and short stature, even in the absence of primordial dwarfism. The identification of additional patients will shed light on the phenotypic and genetic diversity of loss of *LARP7* expression.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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