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SHORT REPORT

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Short stature with low insulin-like growth factor 1 availability due to pregnancy-associated plasma protein A2 deficiency in a Saudi family

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Abstract

In 2016 a new syndrome with postnatal short stature and low IGF1 bioavailability caused by biallelic loss-of-function mutations in the gene encoding the metalloproteinase pregnancy-associated plasma protein A2 (PAPP-A2) was described in two families. Here we report two siblings of a third family from Saudi Arabia with postnatal growth retardation and decreased IGF1 availability due to a new homozygous nonsense mutation (p.Glu886* in exon 7) in *PAPPA2*. The two affected males showed progressively severe short stature starting around 8 years of age, moderate microcephaly, decreased bone mineral density, and high circulating levels of total IGF1, IGFBP3, and the IGF acid-labile subunit (IGFALS), with decreased free IGF1 concentrations. Interestingly, circulating IGF2 and IGFBP5 were not increased. An increase in growth velocity and height was seen in the prepuberal patient in response to rhIGF1. These patients contribute to the confirmation of the clinical picture associated with PAPP-A2 deficiency and that the *PAPPA2* gene should be studied in all

patients with short stature with this characteristic phenotype. Hence, pediatric endocrinologists should measure circulating PAPP-A2 levels in the study of short stature as very low or undetectable levels of this protein can help to focus the diagnosis and treatment.

KEYWORDS
free, IGF1, bone mineral density, IGF1, IGFALS, *PAPPA2*, PAPP-A2, rhIGF1, short stature

1 | INTRODUCTION

Short stature can be due to simple or complex etiology affecting hormonal status and/or the growth plate.[1] Over the last decade, we have learned much regarding monogenic causes of short stature due to affectation of the growth hormone (GH)/insulin-like growth factor (IGF) axis,[2] which controls linear growth. Understanding the physiology, genetics, and etiology of growth defects is important for implementing correct treatment. Regulation of the growth plate is influenced by genes such as *GH1*, *GHR*, *IGF1*, *IGFR*, *IGF*2, and *IGFALS*.[2] In 2016, biallelic loss-of-function mutations in the *PAPP-A2* were reported to cause a new syndrome with postnatal short stature due to low IGF-1 bioavailability.[3]

GH stimulates IGF, IGFBP, and IGFALS production by the liver,[1] with IGF1 being the main factor stimulating linear growth. IGF1 is also produced in most other tissues and exerts a paracrine effect.[4] Approximately 95% of IGF1 circulates in ternary complexes (TC) that consist of IGF1, IGFBP3 or IGFBP5 and ALS. As free IGF1 (*f*IGF1) is the active molecule that promotes linear growth, it must be released from these complexes.[1] To liberate IGF1 from the ternary complex PAPP-A2 cleaves IGFBP3 and IGFBP5.

Mutations in the *PAPPA2* gene have been reported in five members of two unrelated families with short stature and high total IGF1, but low *f*IGF-1 levels.[3] Here we report two siblings of a family from Saudi Arabia carrying a new loss-of-function mutation in homozygosis in the *PAPPA2* gene associated with postnatal short stature. To our knowledge, these represent two of the seven cases reported to date worldwide.

2 | MATERIALS AND METHODS

ELISA was used to determine serum levels of ALS and insulin (BioVendor), IGF1, free IGF1, IGF2, total IGFBP4, and IGFBP5, intact IGFBP3 and IGFBP4, PAPP-A and PAPP-A2 (Ansh Labs, Webster). GH and total IGFBP-3 were measured by chemiluminiscence immunoassays (DiaSorin). All assays were performed according to the manufacturers' instructions and all intra- and inter-assay coefficients of variation were lower than 10%.

Whole-genome sequencing was performed in blood DNA from the index case by CENTOGENE. DNA sequencing and validations were performed by CAP/CLIA, commercially accredited laboratories. The average coverage depth was ~30X. A complete illustration of the pipeline, filtration processes, and prioritization steps have been previously published https://pubmed.ncbi.nlm.nih.gov/30202406/. The variant plus 150 bp on either side of each variant in the genomic region were amplified using specific primers PAPPA2 c.2656 FWD, ACTCTCCTCATCTCCCATCTC. PAPPA2 c.2656 REV CAGTGGTCACCAGGGTATAAAG, and bidirectionally sequenced using Sanger sequencing.

2.1 | Ethical statement

Written consent for these studies was obtained from all adults and parents of the index patient.

The laboratory procedures employed were reviewed and approved by the institutional review committee of the Hospital Infantil Universitario Niño Jesús (C.I: R-0017/19) and conform to the Helsinki Declaration.

3 | RESULTS

The index case, a Saudi boy presented to our endocrine clinic at the age of 14 years complaining of short stature. Poor growth was noticed around 8 years of age and it became progressively more apparent over time (Figure 1A). His delivery was at term (39 weeks) and uneventful. His parents are first cousins with a mid-parental height of 172 cm, which is above average in the Saudi society. Among his three surviving siblings, a 22-year-old brother had short stature and similar features (Figure 1B), the others are of normal height.

He and his elder brother, with the same genetic condition, had microcephaly, triangular face, small chin, and anterior pointed ears (Table 1), with mild learning difficulties. The index's height was 138.8 cm (−3.3 SDS). Growth velocity (GV) initially was 1.2 cm/year (Figure 1A). Bone age was delayed by 2 years and a skeletal survey showed osteopenia with no deformities. DXA scan showed osteoporosis with a lumbar spine bone mineral density (BMD) Z-score (adjusted for height) of −3.4. Brain MRI was normal.

Preliminary studies showed high serum IGF1 and IGFBP3 levels and no abnormalities in the sequence of chromosome 11.15. A genome sequence revealed a homozygous loss-of-function variant in the *PAPPA2* gene on chromosome 1 (GRCh37):g.176664905G > T; NM_020318.2:c.2656G > T; p.Glu886* (Figure 2A). This variant, presumably resulting in a null allele undergoing nonsense-mediated mRNA decay and coding for a truncated 886/1791 amino acid protein, has not been reported previously in gnomAD, ESP, or 1000 Genomes. This novel homozygous mutation in *PAPPA2* was highlighted as likely pathogenic using ACMG (American College of Medical Genetics) criteria, given its loss-of-function nature and the

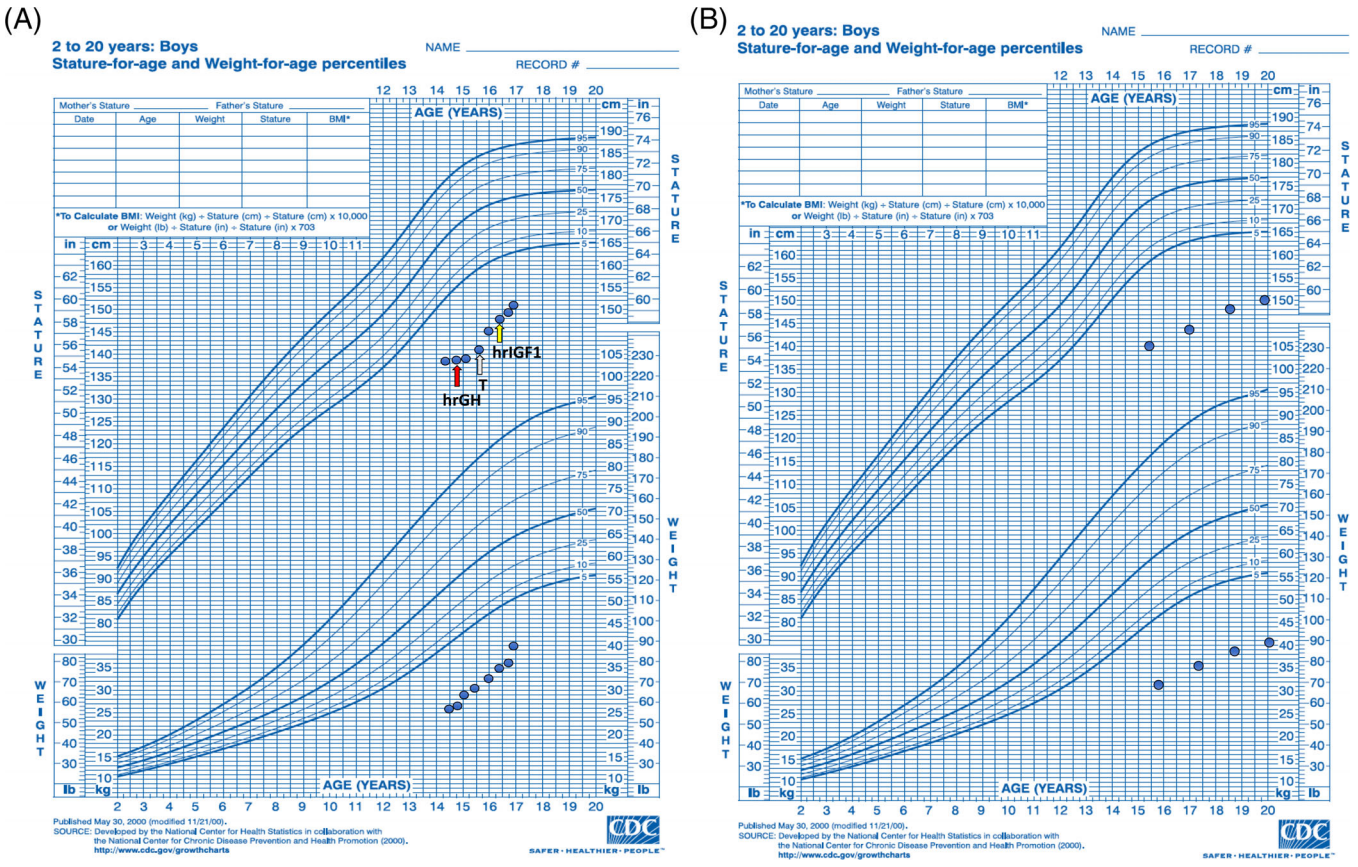

FIGURE 1 (A) Auxological characteristics of the index case showing the response to rhGH, testosterone (T), and rhIGF1; (B) Auxological characteristics of his affected adult brother depicting natural growth with no treatment [Colour figure can be viewed at wileyonlinelibrary.com]

clinical presentation in these patients similar to those previously reported with *PAPPA2* mutations.[3] Family segregation analysis revealed that the older brother with similar clinical features (Figure 2B, Table 1) is also homozygous for the mutation, while the parents and the two other living siblings are heterozygous carriers (Figure 2B).

Subsequent biochemical analysis showed significantly increased total IGF1, total IGFBP3, intact IGFBP3, and IGFALS levels in both affected siblings. PAPP-A2 levels were undetectable, while PAPP-A levels were in the normal range (Table 1). Normal values have been previously reported.[3] Before the genetic diagnosis, the patient received GH (0.035 mg/kg/day) and testosterone (50 mg IM monthly for 3 months due to pubertal delay). After testosterone treatment, he attained Tanner stage II. His GV was 6 cm after 1 year in response to rhGH and the advancement of puberty. He started treatment with rhIGF-1 (40 μg/kg twice daily gradually increasing to 80 μg/kg twice daily based on response) and after 7 months, he gained 4.9 cm of height (8.4 cm/year).

4 | DISCUSSION

To date short stature related to *PAPPA2* gene defects has been reported in three different families, including that reported here. The first report was of a 9-year-old girl and a 5-year-old boy from a Spanish family of non-consanguineous parents that presented with growth retardation compared with their target heights.[3] In the other family of consanguineous parents (cousins) from Palestine an adolescent girl with two younger brothers had growth retardation that warranted medical evaluation at different ages.[3] The cases reported here share clinical and laboratory data with those previously reported. Furthermore, the observed likely pathogenic variant in the *PAPPA2* gene (p.Glu886*), presumably a null allele undergoing nonsense-mediated mRNA decay, is consistent with the clinical observations and the diagnosis of PAPP-A2 deficiency, as well as with undetectable levels of PAPP-A2 in serum.[3]

Postnatal short stature is the main complaint for which these patients are clinically evaluated. To date, growth is described to be appropriate prenatally and during infancy, but with a progressive decline after ~6 years of age. Indeed, late detection of gene defects in this entity leads to more severe short stature as found here and in the previously reported Palestinian girl.[3] Three of the reported cases were born SGA, with one achieving good catch-up during the first 2 years of life. In all cases, a typical phenotype included small chin, long thin fingers, and modest microcephaly. Anterior rotated ears (33.3%) and delayed dental eruption (50%) have also been observed.[3]

Four of the seven reported patients are postpubertal.[3] Two girls had spontaneous onset of puberty at around 11 years of age with normal progression, while two males, including our index patient, had

TABLE 1 Clinical characteristics and management outcome in the patients

	Clinical characteristics	Index case	Affected brother
Growth data	Sex	M	M
	Age at examination (years)	14	22
	Gestational age (weeks)	39	36
	Birth weight (kg)	N/A (SGA)	N/A (SGA)
	Height (cm)	138.8 (−3.3)	153.7 (−3.6)
	BMI	14.68	17.84
	Head circumference (SDS)	−3.4	N/A
Clinical features	Small chin	Yes	Yes
	Thin long fingers	Yes	Yes
	Anterior rotated ears	Yes	No
	Delayed dental eruption	No	Yes
	Onset of puberty (years)	14.5	15
	Growth spurt	Still growing	N/A
Radiological data	BMD	Low	Not done
	Skeletal survey	Normal	Normal
Genetic results	*PAPP-A2* gene variant	(c.2656G > T, p.Glu886*)	(c.2656G > T, p.Glu886*)
Therapeutic data	Treatment	GH and rhIGF1	N/A
	Complications	None	N/A

Biochemical parameters

Subjects	IGF1 (μg/L)	fIGF1 (μg/L)	IGF2 (μg/L)	Total IGFBP3 (μg/L)	Intact IGFBP3 (μg/L)	Total IGFBP4 (μg/L)	Intact IGFBP4 (μg/L)	IGFBP5 (μg/L)	ALS (U/L)	Insulin (mU/L)	PAPP-A (μg/L)	PAPP-A2 (μg/L)	GH (CLIA) (μg/L)
Index	3193	2.37	95	8510	4347	37.52	7.19	466	4422	13.44	0.48	Undetectable	0.53
Affected brother	1751	0.40	124	5620	2535	51.94	6.91	409	3266	3.00	0.72	Undetectable	0.16
Father	342	0.21	135	3420	175	165.80	2.14	205	1925	1.30	0.95	0.04	0.13
Mother	778	3.20	189	4680	691	68.24	4.43	629	2673	2.55	1.27	0.02	0.01
Healthy brother	260	5.13	393	4010	216	96.67	6.94	446	2386	13.45	1.41	0.04	0.10
Healthy sister	301	0.86	346	5020	735	159.18	10.51	367	1257	4.97	0.67	0.02	0.38

Note: Biochemical parameters related to the family members: Index, affected brother, father, mother, and two healthy siblings.

Abbreviations: BMD, body mass index; GH, growth hormone; PAPP-A2, pregnancy-associated plasma protein A2-; rhIGF-1, recombinant human insulin-like growth factor-1; SGA, small for gestational age.

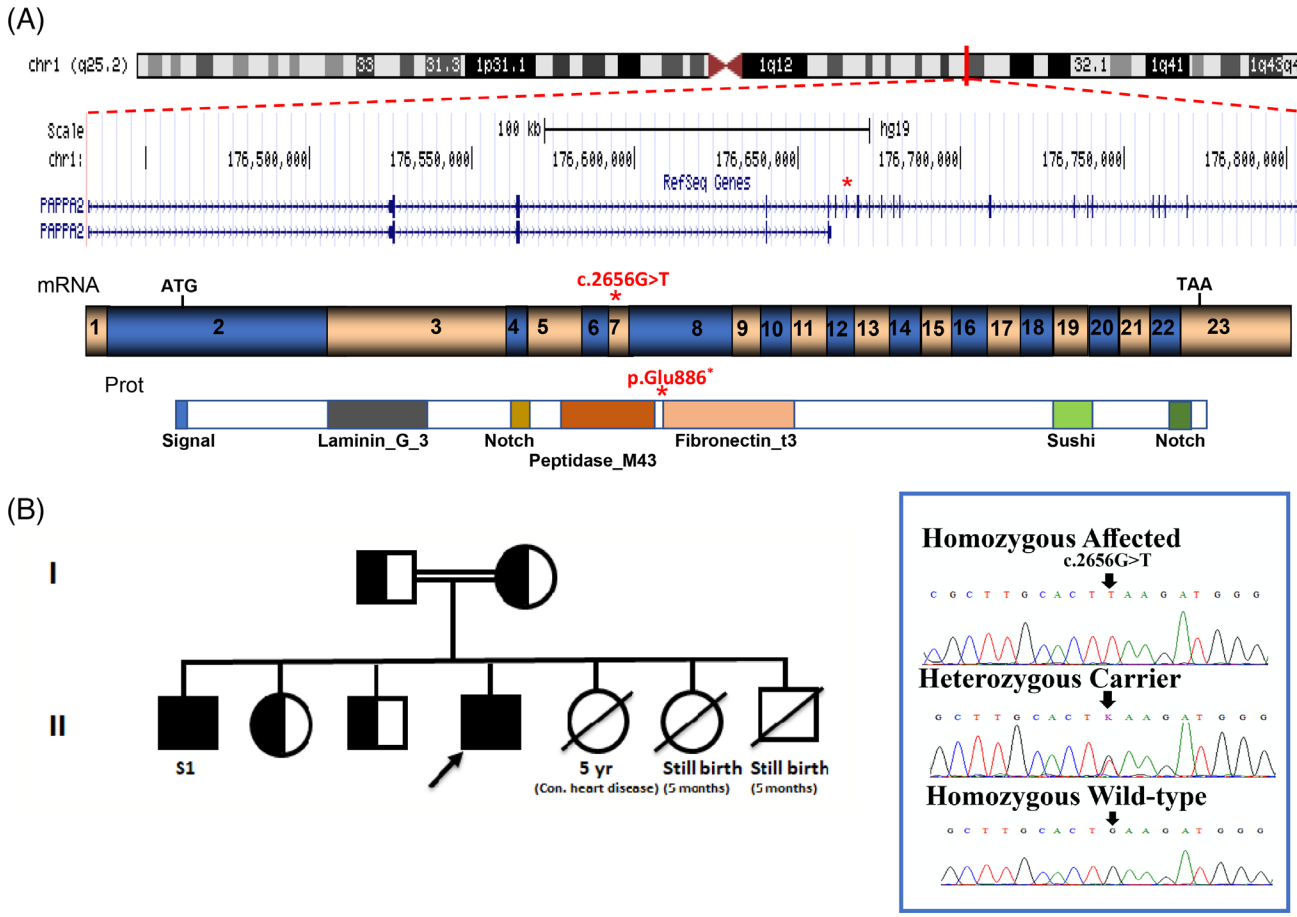

FIGURE 2 *PAPPA2* gene and family pedigree. (A) Schematic representation of the genomic structure of the *PAPPA2* gene on chromosome band 1q24, the encoded mRNA, and the protein with its relevant functional domains. The red asterisk shows the position of the variant identified in the present study. (B) Pedigree and representative Sanger sequencing trace of the mutation identified in an affected individual (top), a heterozygous carrier (middle), and a control (bottom) [Colour figure can be viewed at wileyonlinelibrary.com]

delayed puberty.[3] In two cases where pubertal onset and progression were normal, a pubertal growth spurt was not observed. The Spanish female patient began puberty while receiving rhIGF1 and was immediately treated with GnRH to stop pubertal development.[5] Thus, it is possible that pubertal delay is more common in males than in female PAPP-A2 deficient patients. The question regarding male fertility might be raised later as fertility is affected in PAPP-A2 deficient male mice but not females.[6]

All cases reported to date have high total IGF1, IGFBP3, IGFALS, and stimulated GH levels, very low free IGF1 and very low or undetectable levels of PAPP-A2[3]; however, the cases reported here did not have elevated IGF2 or IGFBP5. Fasting hyperinsulinism and impaired fasting glucose is reported in some pateints.[3] Our index case had a modest basal hyperinsulinemia, but his affected brother did not.

Skeletal surveys demonstrated thin long bones in all previous cases,[3] which was not observed in our patient. Densitometric scans in the two Spanish cases were in the osteoporotic range of decreased lumbar density (Z-score −2 to −2.3 *SD*).[3] One Palestinian subject had a significantly low BMD (−2.5). Our patient had a severely decreased lumbar density (Z-score −3.4).

The Spanish patients had a good response to rhIGF1 injections improving linear growth, BMD, and glucose metabolism, with no hypoglycaemia reported.[5,7] The two patients from Palestinian origin were subsequently treated with rhIGF1; however, to date the youngest patient has been reported to experience a modest growth response with rhIGF1. Treatment was discontinued in the second brother due to development of pseudotumor cerebri.[8] In the index case reported here, GH in combination with testosterone treatment was used before the genetic diagnosis. As expected testosterone improved growth; however, after 7 months of rhIGF1 treatment, growth velocity improved more dramatically (8.4 cm/year). Long-term outcomes will need further follow-up.

Advances in our understanding of short stature emphasize the importance of performing genetic studies, as well as taking into consideration the genetic potential of growth, or the patient's target height in the evaluation of short stature.[9] Identification of genetic causes of short stature provides guidance in treatment selection, helps in predicting prognosis and in indicating possible associated conditions that might need treatment, and supports genetic counseling.[10] Indications of genetic studies in cases of short stature should include

not only severe familial forms of isolated GH deficiency or specific syndromic forms of multiple pituitary hormone deficiencies, severe short stature (>−3SDS for the population or >3 *SD* lower than MPH), but also patients that are significantly far from their predicted target height.[2,9] Identification of the genetic bases in patients with idiopathic short stature should provide a more precise approach to treatment, as demonstrated with the discovery of PAPP-A2 deficiency.[11]

In conclusion, it is probable that PAPP-A2 deficiency is underdiagnosed as some patients may present normal height, or the physician does not correctly interpret the high IGF1 levels or the decline in growth velocity that these patients present during childhood. Thus, the *PAPP-A2* gene, as well as circulating levels of PAPP-A2, should be studied in all patients with short stature with these characteristics.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Data available on request due to privacy/ethical restrictions.

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