Short stature in *PRMT7* Mutations: first evidence of response to growth hormone treatment

Running title: Short-term rGH effectiveness in SBIDDS

Rodari Giulia[1,2], Villa Roberta[3], Porro Matteo[4], Gangi Silvana[5], Iascone Maria[6] , Elli Francesca[1], Giacchetti Federico[1], Profka Eriselda[1], Collini Valentina[7], Dall'Antonia Alberta[7], Arosio Maura[1,2], Mantovani Giovanna[1,2], Bedeschi Maria Francesca[3], Giavoli Claudia[1,2]

[1]*Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Endocrinology Unit, Milan, Italy*
[2]*University of Milan, Department of Clinical Sciences and Community Health, Milan, Italy*
3Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Medical Genetic Unit, Milan, Italy
4Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Pediatric Physical Medicine & Rehabilitation Unit, Milan, Italy.
5Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Neonatal Intensive Care Unit (NICU), Milan, Italy6[7]University of Milan, Milan, Italy
6Laboratorio di Genetica Medica, ASST Papa Giovanni XXIII, Bergamo, Italy
7University of Milan, Milan, Italy

Address all correspondence to:
Giulia Rodari, ORCID: 0000-0002-2200-9538
tel. +39 (2) 55033381
Email: rodarigiulia@gmail.com, giulia.rodari@unimi.it.
University of Milan, Department of Clinical Sciences and Community Health, Fondazione IRCCS Ca' Granda Ospedale Maggiore Policlinico, Endocrinology Unit, Milan, Italy.

Key words: PRMT7, SBIDDS, short stature, GH, growth hormone therapy

Word Count: 2538

Grant Support
No Grant Support was used for this project.

Competing Interest
The authors have no relevant conflicts to declare.

Abstract

Protein arginine methyltransferase 7 (*PRMT7*) pathogenetic variants have been associated with the human disorder of Short Stature, Brachydactyly, Intellectual Developmental Disability and Seizures syndrome (SBIDDS). Only 15 cases have been described in the literature.

Here we report two female dizygotic twins with novel compound heterozygous deleterious variants of *PRMT7* and describe the associated endocrine manifestations and short-term response to recombinant growth hormone (rGH) treatment. They were born at 36+3 weeks from a dichorionic diamniotic twin pregnancy. Twin A was appropriate for gestational age while Twin B was small for gestational age. Whole exome sequencing analyses showed the same novel compound heterozygous genetic defects in the *PRMT7* gene (c.1220G>A of maternal origin; c.1323+2T>G of paternal origin, Fig.1). Due to severe short stature and growth impairment, at six years of age, endocrine investigations were performed to rule out growth hormone (GH) deficiency, and revealed GH deficiency (GHD) in Twin A and an appropriate GH response in Twin B. Therefore, both started rGH, albeit at different dosages according to the underlying diagnosis. Both showed a satisfactory short-term response to treatment with height gain (ΔHT) of +0.52 SDS (Twin A) and +0.88 SDS (Twin B) during the first year. In conclusion, our findings expand the knowledge about the endocrine manifestations associated with *PRMT7* pathogenetic variants, including GH deficiency and rGH response. Further studies are needed to investigate long-term outcomes and establish whether *PRMT7* genetic defects can be included among syndromic short stature treatable with rGH.

Introduction

Homozygous/compound heterozygous mutations in Protein Arginine Methyltransferase 7 (*PRMT7,* OMIM*610087) gene have been linked to a human disorder known as Short Stature, Brachydactyly, Intellectual Developmental Disability and Seizures syndrome (SBIDDS) (OMIM #617157) (1; 2; 3; 4; 5; 6). *PRMT7* encodes for an arginine methyltransferase that methylates arginine residues on various protein substrates and is involved in several biological processes, such as DNA transcription and repairing, RNA splicing, cell differentiation, and metastasis.

So far, only 15 patients with pathogenetic variants in *PRMT7* have been described. Akawi et al. initially identified six individuals belonging to three families with compound heterozygous pathogenic variants in *PRMT7*. These patients showed a syndromic condition characterized by mild intellectual disability (ID), facial dysmorphisms, microcephaly, short stature, and brachydactyly. Their clinical phenotype was considered a phenocopy of Albright hereditary osteodystrophy/Pseudohypoparathyroidism AHO/PHP (OMIM#103580; Orphanet 457059) (1). In 2017, Kernohan et al. described a 6-year-old male with similar clinical findings along with cryptorchidism and seizures who was found to have a homozygous deletion in the transcription start site of the *PRMT7* gene (2). In 2017, Agolini et al. reported three additional patients with severe/moderate ID, short stature, brachydactyly, mild dysmorphic features and possible genotype/ phenotype correlation (3). More recently, in 2019, Valenzuela et al. described a 2-year-old girl with short stature, psychomotor delay, hearing loss and brachydactyly due to two alterations in *PRMT7* for whom parental segregation studies detected biallelic mutation inheritance (4). Birnbaum et al. reported two male siblings with a novel *PRMT7* homozygous deleterious variants and brain calcifications, delayed myelination and congenital orbital tumor (5). Finally, a very recent report by Poquérusse et al. described a novel homozygous substitution in *PRMT7* in two brothers from a consanguineous Iraqi family with associated compound heterozygous defects in the dysplasia-associated perlecan-encoding *HSPG2* gene (OMIM*****142461) (6). No endocrine evaluation was performed in all these patients.

Over the past few decades, *PRMT7* has been the subject of extensive research. In murine models, *PRMT7*-null mice showed reduced body size and shortened metatarsal bones. These mice had high mortality rates shortly after birth, and the few mice that survived developed increased fat mass as adults. The phenotypes of *PRMT7* alterations in humans are mainly represented as SBIDDS syndrome. The similarities in phenotype between

PRMT7-null mice and humans with *PRMT7* pathogenetic variants demonstrate the crucial role of *PRMT7* in the development of skeletal muscle, neurons, bone and adipose tissues. Even though short stature can be considered as a distinctive feature of the syndrome, its underlying pathogenetic aspects are yet to be established. Indeed, postnatal growth restriction of prenatal onset, mild intellectual disability (ID), facial dysmorphisms and brachydactyly resemble phenotypically conditions associated with methylation disturbances.

Here we report the case of two dizygotic twins diagnosed by whole exome sequencing (WES) analysis of DNA from whole peripheral blood. Of note, the parents also have one unaffected son (born before the two sisters).

Patients and methods

In accordance with hospital ethics committee standards, parents signed written informed consent for DNA analysis and for publication of the present article with accompanying images. No ethical approval was required for this clinical case series as patients were treated in accordance with latest guidelines and local regulations for recombinant growth hormone (rGH) therapy.

Clinical characteristics

The study included two prepubertal twins born after in vitro fertilization at 36+3 weeks from a dichorionic diamniotic pregnancy complicated by threatened abortion at 13 weeks of gestational age. Amniocentesis performed at 25 weeks of gestation showed in both a normal female karyotype, confirmed after birth at chromosomal microarray analysis. Twin A was born appropriate for gestational age (AGA, birth weight 1990 gr, -1.73 SDS according to Bertino charts, (7)), whereas Twin B had postnatal growth restriction of prenatal onset, was born small for gestational age (SGA, birth weight 1425 gr, -2.8 SDS according to Bertino charts (7)), and needed non-invasive ventilation support for respiratory distress syndrome. Postnatally, they both showed global developmental delay and progressive decline in growth. No episode of seizure has been reported. Twin A also had bilateral epicanthus, convergent strabismus and bilateral hypermetropic astigmatism, while Twin B broad forehead, sparse eyebrows, hypertelorism, depressed nasal bridge and thin lips.

Genetic evaluation and testing

At 1.7 years of age, both children underwent genetic evaluation. Poor growth, global developmental delay and shared mild dysmorphic features suggested a possible genetic condition. The karyotype and array-based comparative genomic hybridization (aCGH) were unremarkable. At 4 years, both twins and their parents were examined by whole exome sequencing (WES) through next generation sequencing (NGS). WES identified the same compound heterozygous genetic defects in the *PRMT7* gene in both twins: the missense variant NM_019023.5:c.1220G>A p.(Cys407Tyr) of maternal origin and the splicing variant NM_019023.5:c.1323+2T>G of paternal origin.

Given the still poorly defined role of the methyltransferase *PRTMT7* in humans and the presence of phenotypic features resembling those found in some imprinting disorders, Methylation-Sensitive Multiplex Ligand-dependent Probe Amplification (MS-MLPA, using the assay SALSA MS-MLPA Probemix ME034) analysis was performed to investigate the methylation and copy number status of known imprinted loci associated with imprinting disorders and/or Multilocus imprinting disorders (MLID). The probe panel included genes involved in Transient Neonatal Diabetes Mellitus 1 (TNDM1), Silver Russel syndrome (SRS), Beckwith-Wiedemann syndrome (BWS), Prader-Willi syndrome (PWS), Angelman syndrome (AS), Kagami-Ogata syndrome, Temple syndrome and Pseudohypoparathyroidism type B (PHP1B, recently named as inactivation PTH/PTHrp signaling disorder 3, iPPSD3, according to the novel proposed classification of conditions related to the impairment of the PTH/PTHrp signaling pathway). No epigenetic alterations at imprinted loci were found in either patient.

Auxological evaluation and hormonal testing

The twins came to our attention at 3.5 years of age. At 6 years of age, they both showed severe short stature and progressive growth impairment (Fig.2a (8)). For this reason, endocrine assessment was planned to rule out growth hormone (GH) deficiency. GH deficiency and reduced IGF-I levels were found in Twin A (peak GH at arginine test: 4.61 µg/L; peak GH at glucagon test: 5.14 µg/L, IGF-I 53 ng/mL, -2.02 SDS) whereas an appropriate GH response, even in the presence of reduced IGF-I concentrations, was observed in Twin B (peak GH at arginine test: 11.8 µg/L, IGF-I 52 ng/mL, -2.05 SDS). Other blood tests were normal. In both, skeletal

maturation, evaluated according to the standards of Tanner-Whitehouse (9), was consistent with chronological age.

An MRI of whole brain and pituitary region was performed showing normal hypothalamic-pituitary region with corpus callosum thickening in Twin A (Fig.2b) and dysmorphic features of corpus callosum and cerebellar vermis with a pars intermedia cyst in Twin B (Fig.2c).

Laboratory Methods/Hormone assays

Growth hormone values were assessed with a chemiluminescence method (Immulite 2000, Siemens Medical Solutions Diagnostics, Los Angeles, CA) with a detection limit of 0.01 μg/L and calibrated to the WHO International Standard IS 98/574.

Insulin-like growth factor 1 (IGF-I) levels were measured by a chemiluminescent immunometric assay (Immulite 2000 IGF-I; Siemens Medical Solutions Diagnostics, Los Angeles, CA), with an intra- and interassay coefficient of variation of 2.9 and 7.4%, respectively and calibrated according to IS 02/254 standard. IGF-I concentrations were compared with those from an appropriate age- and sex-adjusted range and expressed as SDS. All the other biochemical parameters were measured by standard procedures.

Results

Growth Hormone Therapy and Response

Twin A was treated with rGH at the replacement dosage of 0.025 mg/kg/day, whereas Twin B with 0.035 mg/kg/day, as indicated in SGA (9). After two-year therapy, Twin A and B showed a good response to treatment with a total ΔHT of +0.82 SDS and +1.42 SDS, respectively (Table 1) (8). Growth hormone therapy was increased at each visit according to body weight changes. No sign of pubertal development was recorded and bone age showed a regular progression during the first two years of treatment.

Growth Curves are displayed in Fig.2a. In Twin B hand and wrist X-Ray assessment ruled out the presence of brachydactyly with cone-shaped epiphysis of the first metacarpal and intermediate phalanx of the second finger (Fig.2c).

Safety and adverse events

Neither twin experienced adverse effects over 2 years of therapy. Parameters of glucose metabolism remained unchanged. IGF-I levels were always within the reference range for age and sex.

Hormonal and metabolic parameters are shown in Table 2.

Discussion

To the best of our knowledge, this represents the first report of short-term good response to rGH treatment in SBIDDS Syndrome, either with or without underlying GH deficiency. We also investigated for the first time the methylation effects of *PRMT7* in the context of SBIDDS by MS-MLPA analysis for MLID and didn't find any epigenetic alteration at known imprinted loci associated with imprinting syndromes in either twin.

In SBIDDS Syndrome, growth delay, though not always present at birth (3), seems to be constantly reported later in life, usually occurring after 6 years of age (2; 4; 6) with an adult height usually falling under the 3rd centile (3). Despite being a distinctive feature of the syndrome usually found in all the patients described to date (Table 3), the underlying pathogenetic aspects of short stature are far to be clear. Given the role of *PRMT7* as methyltransferase, to investigate its contribution to growth impairment and phenotypic overlap with AHO/PHP, genetic analysis through MS-MLPA involving genes related to imprinting disorders was performed. AHO/PHP, resulting from genetic or epigenetic alterations in the *GNAS* locus (10), can sometimes be attributed to imprinting-associated altered methylation patterns (11; 12). In our case series, molecular investigations through MS-MLPA targeting imprinted genes related to imprinting disorders was normal.

Indeed, the striking height gain observed in our patients over the two-year rGH treatment is highly suggestive of a possible role of GH/IGF-I axis alterations as contributors of growth impairment though, to date, only one study performed an unspecified endocrinological evaluation for short stature, which was negative (4). In Twin A we found an insufficient response to GH stimulation tests, confirming the presence of GHD. This endocrine manifestation has never been described before in the spectrum of SBIDDS Syndrome, even though *PRMT7*-null murine models showed growth impairment and increased fat mass, as frequently encountered in human GHD. On the other hand, Twin B was found to be GH sufficient at stimulation test but, being born SGA from IUGR, was treated with rGH as indicated in Europe since 2003 in SGA children over 4 years of age in the absence of an appropriate catch-up growth (13).

Studies on rGH effects have clearly shown that responsiveness to therapy is highly related to the entity of the GH/IGF-I axis defect. However, there are few standardised definitions of "good" and "poor" short-term auxological response in the literature. Nonetheless, it is of paramount importance to optimize first year growth during rGH treatment, as it represents the strongest indicator of future adult height outcomes (14; 15). In 2010, Ranke et al. defined a first-year "poor response" as a height gain less than 0.4 SDS in severe GHD (GH peak after stimulation test<5 ng/dL) and less than 0.3 SDS in mild GHD or SGA (GH peak>5 ng/dL) (16). Second-year "poor response" was defined as a height gain less than 0.15 SDS and less than 0.12 SDS in severe GHD and mild GHD or SGA, respectively (16). Instead, Bang et al. in 2011 defined a first-year "poor response" as a height gain below 0.5 SDS (17). In this context, our patients showed a good response after 1- and 2-year therapy according to both the above reported criteria. Moreover, bone age showed regular progression during treatment.

These findings suggest the potential role of rGH treatment in the management of growth disorders associated with *PRMT7* pathogenetic variants, as observed in other syndromic short stature namely Noonan Syndrome (18) or aggrecan (*ACAN*) deficiency (19). The diagnostic process as well as the subsequent clinical response may indicate the presence of an underlying growth and developmental disorder of multifactorial origin, with endocrine involvement (with variable GH/IGF-I axis associated defect) being only one aspect of a more complex impairment in which several signalling pathways can be implied (1). Indeed, GH/IGF-I axis defects represent a continuum ranging between severe GHD to complete GH resistance (20), with the entity of idiopathic short stature (ISS) situated midway, probably including some children with mild GHD and some with partial GH resistance. In this context, both twins had low concentrations of IGF-I, thus supporting a possible underlying role of GH/IGF-I axis impairment of variable entity.

Moreover, Twin B, who was treated with higher doses in the absence of GHD, showed the best clinical response to treatment. This seems to reflect a dose-related response with greater efficacy from higher (pharmacological) rGH doses than physiological replacement, as frequently encountered in other syndromic short stature, namely Noonan Syndrome (18; 21), Turner Syndrome (22) or children born SGA (23). Furthermore, studies conducted in patients with ISS treated with rGH showed good response in the first year of treatment, though followed by a relatively reduced growth in the ensuing years (24).

In the literature, studies on SGA children treated with rGH have shown that most patients reached a normal height after the first years of rGH treatment and remained in the normal range until adult height. Nonetheless, at the end of GH treatment, a slight decrease in height SDS was found because of early onset of puberty and/or acceleration of bone maturation. This would lead to attainment of adult height at a relatively young age compared with peers and/or a reduced pubertal height gain (15). Only long-term follow-up will probably show whether this good response reflects a faster bone age progression or a sustained height gain with an appropriate target height retrieval.

Another aspect to take into consideration is the safety profile, especially in the presence of underlying genetic abnormalities potentially associated with risk of malignancies. The long-term safety of rGH therapy in disease categories at higher risk of developing cancer per se should be considered in treatment decision-making. Long-term follow-up is ongoing in these patients to provide data on adult height and safety profile. As far as short-term-safety is concerned, in both children parameters of glucose metabolism remained normal and IGF-I levels were in the normal range for sex and age over the whole treatment period.

In conclusion, this report expands the knowledge in the field of *PRMT7*-associated manifestations, with particular focus on short stature. The diagnostic evaluation as well as the subsequent response to treatment may indicate the presence of an underlying growth impairment of multifactorial origin, with endocrine involvement (with variable GH/IGF-I axis associated defect) being only an aspect of a more complex impairment in which several signalling pathways can be involved. This study demonstrates for the first time the effectiveness of rGH in two children with *PRMT7* deleterious variants: the striking and sustained good response leads to wonder if SBIDDS Syndrome should be probably counted among syndromic short stature treatable with growth hormone. Given the rarity of the disease as well as the shortage of data in the literature, it is difficult to determine which proportion of affected children could benefit from rGH treatment. However, every patient with SBIDDS Syndrome presenting with growth failure should undergo through endocrine assessment and be treated with rGH according to the current guidelines (25). Further studies are needed to identify the causal mechanisms and potential benefits of rGH in an attempt to have a more individualized treatment approach for these patients and to confirm rGH long-term efficacy and safety.

Data Availability Statement

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request

References

1. Akawi N, McRae J, Ansari M, Balasubramanian M, Blyth M, Brady AF et al. *Discovery of four recessive developmental disorders using probabilistic genotype and phenotype matching among 4,125 families.* Nature Genetics. 2015; 47(11):1363-1369.

2. Kernohan KD, McBride A, Xi Y, Martin N, Schwartzentruber J, Dyment DA et al. *Loss of the arginine methyltranserase PRMT7 causes syndromic intellectual disability with microcephaly and brachydactyly.* Clin Genet. 2017; 91(5):708-716.

3. Agolini E, Dentici ML, Bellacchio E, Alesi V, Radio FC, Torella A et al. *Expanding the clinical and molecular spectrum of PRMT7 mutations: Three additional patients and review*. Clinical Genetics, 2018; 93:675–681.

4. Valenzuela I, Segura-Puimedon M, Rodríguez-Santiago B, Fernández-Alvarez P, Vendrell T, Armengol L et al. *Further delineation of the phenotype caused by loss of function mutations in PRMT7.* Eur J Med Genet. 2019; 62 (3):182-185.

5. Birnbaum R, Yosha-Orpaz N, Yanoov-Sharav M, Kidron D, Gur H, Yosovich K et al. *Prenatal and postnatal presentation of PRMT7 related syndrome: Expanding the phenotypic manifestations.* Am J Med Genet A. 2019; 179 (1):78-84.

6. Poquérusse J, Whitford W, Taylor J, Alburaiky S, Snell RG, Lehnert K et al. *Novel PRMT7 mutation in a rare case of dysmorphism and intellectual disability.* J Hum Genet. 2022; 67 (1):19-26.

7. Bertino E, Spada E, Occhi L, Coscia A, Giuliani F, Gagliardi L et al. *Neonatal Anthropometric Charts: The Italian neonatal study compared with other European studies.* J Pediatr Gastroenterol Nutr. 2010; 51:353-361.

8. Growth Calculator 4. . [Online] http://www.weboriented.it/gh4/..

9. Tanner JM. *Assessment of Skeletal Maturity and Prediction of Adult Height (TW2 Method).* Academic Press, 1983, Vol. 2.

10. Linglart A, Levine MA, Jüppner H. *Pseudohypoparathyroidism.* Endocrinol Metab Clin North Am. 2018; 47 84):865-888.

11. Mantovani G, de Sanctis L, Barbieri AM, Elli FM, Bollati V, Vaira V et al. *Pseudohypoparathyroidism and GNAS epigenetic defects: clinical evaluation of albright hereditary osteodystrophy and molecular analysis in 40 patients.* J Clin Endocrinol Metab. 2010; 95 (2):651-658.

12. Linglart A, Maupetit-Méhouas S, Silve C. *GNAS -Related Loss-of-Function Disorders and the Role of Imprinting.* Horm Res Paediatr. 2013; 79 (3):119-129.

13. Clayton PE, Cianfarani S, Czernichow P, Johannsson G, Rapaport R, Rogol A. *Management of the child born small for gestational age through to adulthood: a consensus statement of the International Societies of Pediatric Endocrinology and the Growth Hormone Research Society.* J Clin Endocrinol Metab, 2007; 92 (3):804-810.

14. De Ridder MA, Stijnen T, Hokken-Koelega AC. *Prediction of adult height in growth-hormone-treated children with growth hormone deficiency.* J Clin Endocrinol Metab. 2007; 92 (3): 925-31.

15. Van Pareren Y, Mulder P, Houdijk M, Jansen M, Reeser M, Hokken-Koelega A. *Adult height after long-term, continuous growth hormone (GH) treatment in short children born small for gestational age: results of a randomized, double-blind, dose-response GH trial.* J Clin Endocrinol Metab. 2003. 88 (8):3584-3590.

16. Ranke MB, Lindberg A, KIGS International Board. *Observed and predicted growth responses in prepubertal children with growth disorders: guidance of growth hormone treatment by empirical variables.* J Clin Endocrinol Metab. 2010. 95(3):1229-1237.

17. Bang P, Ahmed SF, Argente J, Backeljauw P, Bettendorf M, Bona G et al. *Identification and management of poor response to growth-promoting therapy in children with short stature.* Clin Endocrinol (Oxf). 2012. 77(2):169-181.

18. Kelnar CJ. *Growth hormone therapy in noonan syndrome.* Horm Res. 2000;53:77-81.

19. Muthuvel G, Dauber A, Alexandrou E, Tyzinski L, Andrew M, Hwa V et al. *Treatment of Short Stature in Aggrecan Deficient Patients with Recombinant Human Growth Hormone: One-Year Response.* J Clin Endocrinol Metab. 2021.

20. Savage MO, Burren CP, Rosenfeld RG. *The continuum of growth hormone–IGF-I axis defects causing shortstature: diagnostic and therapeutic challenges.* Clin Endocrinol (Oxf). 2010; 72(6): 721-728.

21. Ozono K, Ogata T, Horikawa R, Matsubara Y, Ogawa Y, Nishijima K. *Efficacy and safety of two doses of Norditropin((R)) (somatropin) in short stature due to Noonan syndrome: a 2-year randomized, double-blind, multicenter trial in Japanese patients.* Endocr J, 2018;65 (2):159-174.

22. Dahlgren J, Albertsson-Wikland K. *GH Responsiveness in Children With Noonan Syndrome Compared to Turner Syndrome.* Front Endocrinol (Lausanne). 2021; 12:737893.

23. Chatelain P, Colle M, Nako JP, Le Luyer B, Wagner K, Berlier P et al. *Optimization of growth hormone dosing in children born small for gestational age: an open-label, randomized study of children during the fourth year of therapy.* Horm Res Paediatr, 2012; 77(3):156-63.

24. Deodati A, Cianfarani S. *Impact of growth hormone therapy on adult height of children with idiopathic short stature: systematic review.* BMJ. 2011; 11;342:c7157.

25. Collett-Solberg PF, Ambler G, Backeljauw PF, Bidlingmaier M, Biller BMK, Boguszewski MCS et al. *Diagnosis, Genetics, and Therapy of Short Stature in Children: A Growth Hormone Research Society International Perspective.* Horm Res Paediatr. 2019;92(1):1-14.

Acknowledgments

The Authors would like to thank the patients and their parents for their kind cooperation. This work has been generated within the European Reference Network on Rare Congenital Malformations and Rare Intellectual Disability (ERN-ITHACA) (EU Framework Partnership Agreement ID: 3HP-HP-FPA ERN-01-2016/739516).

Author Contributions

Rodari G wrote the first draft of the manuscript; Mantovani G, Arosio M and Giavoli C have revised the text; Elli F, Bedeschi MF and Giavoli C have critically reviewed the text and provided substantial scientific contributions. All authors approved the final version of the manuscript.

Funding

The research did not receive any specific grant from any funding agency in the public, commercial or not-for-profit sector.

Ethical approval

All procedures performed in this study were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Conflicts of interest

The authors have no conflicts of interest to disclose.

Figures Legend

Fig. 1: Whole exome sequencing analysis showing the compound heterozygous genetic defects in the PRMT7 gene

Fig. 2: Growth Curves according to Cacciari Growth Charts. Red arrow: rGH start (a), Brain MRI. White arrow: dysmorphic features of corpus callosum (b) and X-ray of Hand and Wrist for Bone Age Assessment (c) of Twin A (left panel) and B (right panel)

Table 1. Auxological Data at baseline and after 1 and 2 years of rGH therapy

	CA[1], years	BA[2], years	HT[3], cm (SDS)	GV[4], cm/year (SDS)	ΔHT[1]-HT[1]baseline, cm (SDS)	ΔTH[5]-HT[1] (SDS)	rGH[6] dose (mcg/kg/day)
Twin A (GHD[7])							
baseline	6.6	6.3	105 (-2.75)	3.8 (-2.5)	-	3.42	0.025
1 year	7.6	6.7	112.3 (-2.23)	7.5 (2.1)	7.3 (0.52)	2.90	0.022
2 years	8.7	7.2	119.6 (-1.95)	6.2 (0.9)	14.6 (0.82)	2.62	0.022
Twin B (SGA[8])							
Baseline	6.6	6.7	104 (-2.95)	4.3 (-2.0)	-	3.62	0.035
1 year	7.6	7.9	113.8 (-2.07)	9.5 (4.1)	9.4 (0.88)	2.74	0.032
2 years	8.7	8.7	122.1 (-1.53)	7.9 (2.9)	18.1 (1.42)	2.20	0.033

[1]Chronological Age; [2]Bone Age; [3]Height; [4]Growth Velocity; [5]Target Height; [6]Recombinant Growth Hormone; [7]Growth Hormone Deficiency; [8]Small for Gestational Age

Table 2. Hormonal and biochemical at baseline and after 1 and 2 years of rGH therapy

	IGF-I[1], ng/mL (nv 65-225)	IGF-I[1], SDS	Glycemia, mg/dL (nv 80-100)	Insulin, IU/L (nv 1.8-24)	TSH[2], mIU/L (nv 0.26-4.2)	Free T4[3], pg/mL (nv 8-17)
Twin A (GHD[4])						
Baseline	53	-2.02	83	8.0	1.76	10.6
1 year	172	0.66	90	10.4	-	-
2 years	164	0.49	91	11.2	1.5	10.3
Twin B (SGA[5])						
Baseline	52	-2.05	84	7.3	1.83	12.7
1 year	149	0.1	89	11.8	-	-
2 years	170	0.61	92	8.4	1.37	11.8

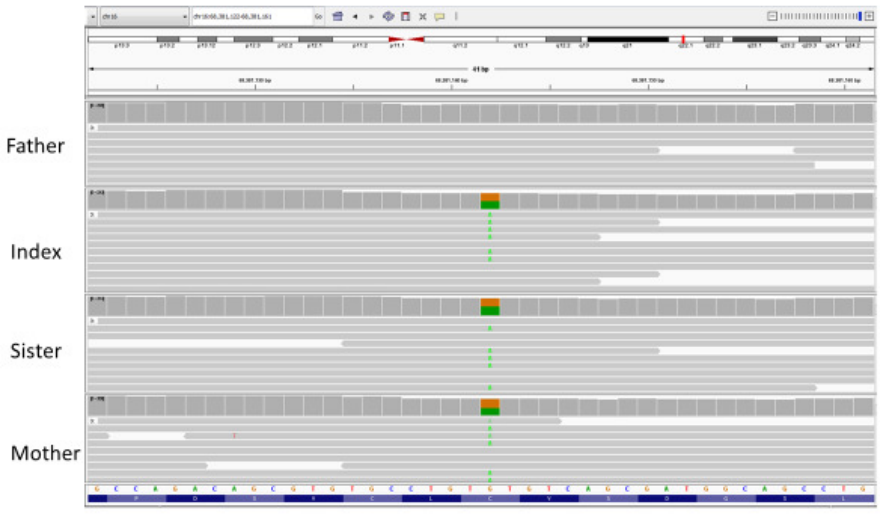
[1]Insulin-like Growth Factor-I; [2]Thyroid Stimulating Hormone; [3]Thyroxine; [4]Growth Hormone Deficiency; [5]Small for Gestational Age

Table3. Summary of previously reported auxological data

	Akawi et al 1-6	Kernohan et al 7	Agolini et al 8-10	Valenzuelaa et al 11	Birnbaum et al 12-13	Poquérusse et al 14-15
Short stature	X	X	X	X	X	X
Length at birth	NA	46 cm, -2.06 SD	Case 1: 47 cm, -2.05 SD Case 2: 49 cm, -0.98 SD Case 3: 48 cm, -1.31 SD	45 cm, -2.3 SD	NA	NA
Height at last follow-up	Case 1: 151.5 cm, -1.98 SD (adult height) Case 2: NA Case 3: 146.6 cm, -2.8 SD (adult height) Case 4: 150 cm, -1.4 SD (14 years) Case 5: 132 cm, -2.0 SD (9 years 7 months) Case 6: 102 cm, -2.9 SD (6 years 2 months)	110 cm, -1.93 SD (8 years)	Case 1: 90.3 cm, -2.37 SD (3 years and 7 months) Case 2: 160 cm, -1.92 SD (adult height) Case 3: 145.5 cm, -2.77 SD (adult height)	66 cm, -2 SD (10 years)	Case 1: NA Case 2: length<4 SD (21 months)	Case 1: 3rd centile (6 years) Case 2: 0.4th centile (5 years)

Fig. 1

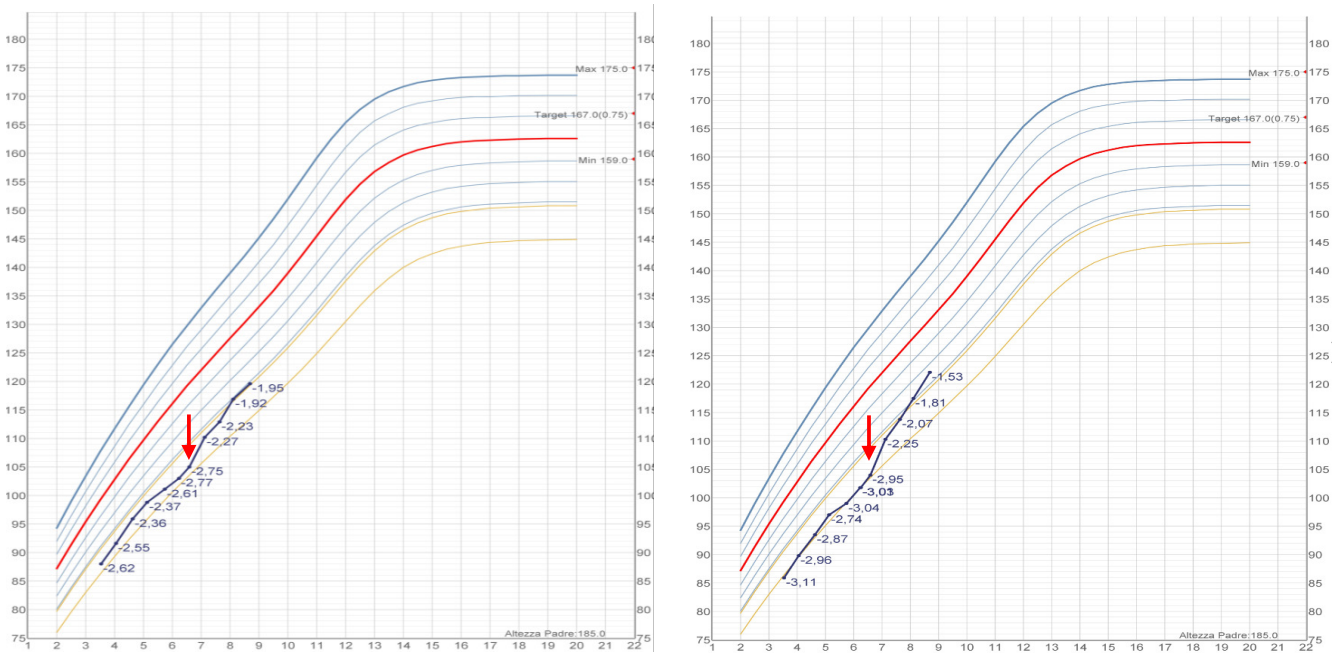
Chr16(GRCh37):g.68381142 (NM_019023.3:c.1220G>A), p.Cys407Tyr, maternal



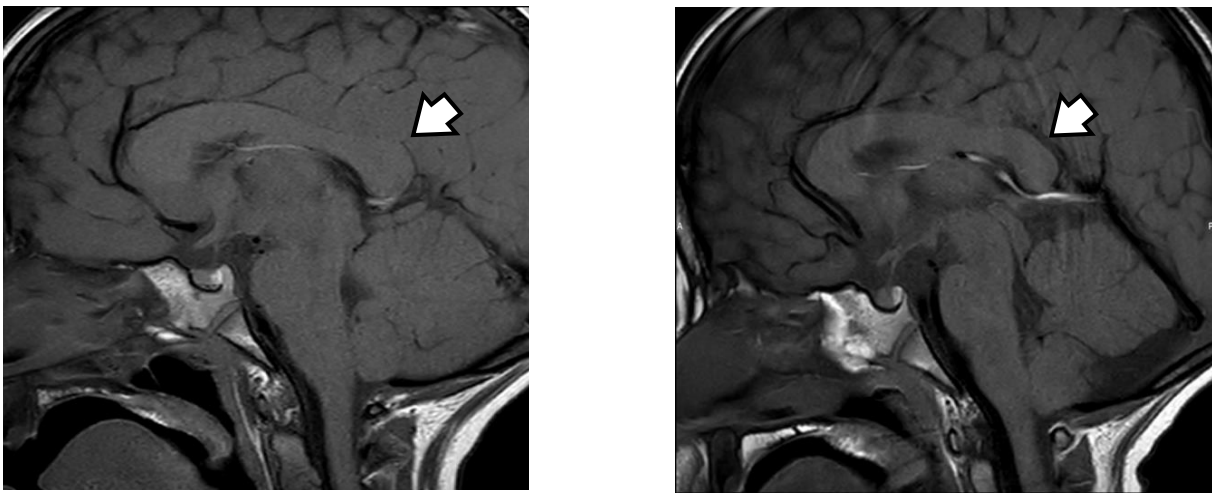
Chr16(GRCh37):g.68381583 (NM_019023.3:c.1323+2T>G), paternal

Fig. 2

a.


Max 175.0
Target 167.0(0.75)
Min 159.0
-1,95
-1,92
-2,23
-2,27
-2,75
-2,77
-2,61
-2,37
-2,36
-2,55
-2,62
Altezza Padre:185.0
Max 175.0
Target 167.0(0.75)
Min 159.0
-1,53
-1,81
-2,07
-2,25
-2,95
-3,03
-3,04
-2,74
-2,87
-2,96
-3,11
Altezza Padre:185.0

b.



c.