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A 79-kb paternally inherited 7q32.2 microdeletion involving *MEST* in a patient with a Silver-Russell syndrome-like phenotype

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ABSTRACT

Maternal uniparental disomy of human chromosome 7 [upd(7)mat] is well-characterized as a cause of the growth disorder Silver-Russell syndrome (SRS). However, the causative gene is not currently known. There is growing evidence that molecular changes at the imprinted *MEST* region in 7q32.2 are associated with a phenotype evocative of SRS. This report details a patient with a SRS-like phenotype and a paternally inherited microdeletion of 79 kilobases (35-fold smaller than the previously reported smallest deletion) in the 7q32.2 region. This microdeletion encompasses only five genes, including *MEST,* which corroborates the hypothesis that *MEST* plays a central role in the 7q32.2 microdeletion growth disorder, as well as further implicating *MEST* in upd(7)mat SRS itself.

INTRODUCTION

Silver-Russell syndrome (SRS; OMIM 180860) is a well-established genetic condition primarily characterized by pre- and post-natal growth restriction with relative macrocephaly. Hypomethylation of the imprinting control region 1 (ICR1) on 11p15 (~30-60% of cases; 11p15 LOM) and maternal uniparental disomy of chromosome 7 [~5-10% of cases: upd(7)mat] are the most common identifiable causes of Silver-Russell syndrome; though consideration of other molecular etiologies is ongoing (including chromosome 14q32 abnormalities; Gicquel et al., 2005; Netchine et al., 2007; Schönherr et al., 2006; Wakeling et al., 2017). Though overall the features of these two molecular subgroups are very similar, subtle differences have been elucidated. For example, body asymmetry is more often a feature of 11p15 LOM patients when compared to upd(7)mat patients (77% vs 29%; Wakeling et al., 2017), whereas developmental delay is more often a features of upd(7)mat patients when compared to 11p15 LOM patients

(65% vs 20%; Wakeling et al, 2010). The specific gene(s) responsible for the upd(7)mat phenotype remain unknown.

Among the upd(7)mat group, there have been patients characterized with segmental uniparental disomy within the 7q region, including 7q31-qter (Eggermann et al., 2008; Hannula, Lipsanen-Nyman et al., 2001). This suggests that this region contains at least one imprinted gene important for growth in the SRS phenotype. Four imprinted genes (*MEST*, *CPA4*, *COPG2,* and *KLF14*) and one imprinted non-coding RNA (*MEST1T1*) are located within the MEST-differentially methylated region (DMR) (Parker-Katiraee et al., 2007). Given that paternally inherited knockout MEST mice demonstrate pre- and post-natal growth restriction, *MEST* has been long regarded as a compelling candidate gene for SRS (Kobayashi et al., 1997; Lefebvre et al., 1998). However, *MEST1T1* and *CPA4* have also been discussed as candidate genes for SRS, and there have been no patients with SRS phenotypes with point mutations or aberrant methylation patterns in any of these genes to date (Bentley et al., 2003; Blagitko et al., 1999; Kayashima et al., 2003; Meyer et al., 2003a; 2003b; Riesewijk et al., 1998).

The key role of the 7q32.2 locus was further corroborated by the discovery of two patients with *de novo* 7q32.2 microdeletions on the paternal allele and SRS features (Carrera et al., 2016; Eggermann et al., 2012). However, these two deletions were 3.7 and 2.8 Mb in size and encompass all the proposed candidate genes (including *MEST*, *MEST1T1*, *CPA4, and COPG2*) in this region, as well as many others. Herein, we report the case of a 79 kb paternally inherited 7q32.2 microdeletion leading to an SRS-like phenotype; a deletion that is 35 fold smaller than the smallest previously reported microdeletion. This case further

elucidates candidate genes in this SRS-like microdeletion phenotype, as well as provides support that these genes contribute to the upd(7)mat SRS phenotype itself.

CLINICAL REPORT

The proband is the first child of healthy non-consanguineous Northern European Caucasian parents (Fig. 1a). The pregnancy was complicated by an undiagnosed maternal bleeding disorder. Fetal growth was in the 45th percentile for weight in the third trimester. Spontaneous vaginal delivery occurred at 39 weeks and 4 days gestational age. Birth weight was 2950 g (-1.0 standard deviations [SD]), birth length was 45 cm (-3.1 SD), and head circumference at 4 days of life was 34.9 cm (-0.2 SD). Her discharge from hospital was delayed due to difficulty establishing feeding. There was no neonatal hypoglycemia. The neonatal period was uncomplicated apart from a prolonged time to regain birth weight (3 weeks).

She was admitted to hospital at 6 months of age for failure to thrive attributed to poor intake and cow's milk protein allergy. She was started on a hypoallergenic extensively hydrolyzed infant formula with supplemental breast feeding after maternal initiation of a dairy-free diet.

Thereafter she was followed by Gastroenterology, a Registered Dietitian, as well as an Occupational Therapist. She had chronic difficulties with selective eating, abdominal pain with vomiting, diarrhea, food aversion, and failure to thrive throughout childhood. Workup was extensive and negative. It included: fructose/lactose breath testing, sweat chloride, fecal elastase, celiac screening (tissue transglutaminase-IgA and total IgA), and skin allergy testing. Biopsy of the duodenum, stomach and esophagus did reveal mild histological findings consistent with gastroesophageal reflux disease. She was started on a course of lansoprazole with improvements in her vomiting and abdominal pain. When weaned off, her symptoms returned but gradually faded as she aged.

Overall, her growth remained suboptimal throughout her childhood: weight was consistently below the 1st percentile (-3.90 to -2.26 SDs), height ranged from the 3rd to the 8th percentile (-1.90 to -1.20 SDs), and BMI was consistently below the 1st percentile (-3.40 to -2.04 SDs). Linear growth velocity was normal. Head circumference, while initially close to the 50th percentile at 4 days of age (-0.2 SD), was measured at the 2.5th percentile at 8 years of age. All of these growth parameters were in the context of mid-parental height of 94th percentile (+1.57 SD) and a sibling who plotted above the 97th percentile for height and weight at 3 years and 8 months of age. Her bone age was 7 years 4 month at a chronological age of 8 years 9 months giving her predicted adult height of 156.5 cm (-1.02 SD, 15.4%), which was below her genetic potential. At 7 years of age, she had a calculated consistent intake of 150% of her estimated daily requirements and therefore her growth issues were not attributable to caloric deficits.

Other ongoing medical issues included frequent upper and lower respiratory tract infections, and a chronic wheeze for which she was prescribed salbutamol (only used during intercurrent illness). Notably, at 8 years of age, she was diagnosed by Endocrinology with benign ketotic hypoglycemia, which is due to lower glycogen stores causing episodes of mild hypoglycemia secondary to prolonged fasting. A prolonged fast excluded adrenal insufficiency and other causes of ketotic hypoglycemia. Two illnesses in her childhood required hospital admission: 1) influenza A and constipation with transaminitis and hyponatremia (4 years 9 months); 2) adenoviral gastroenteritis and dehydration (7 years).

At seven years of age, the proband met 5 of the 6 criteria for diagnosis of SRS (negative for limb asymmetry) as per the Netchine-Harbison SRS scoring system (Azzi et al., 2015) though she did attain this score with a pattern that was unique. She met the criteria for being born

small for gestational age in length, but not weight; she met the criteria for relative macrocephaly at birth by comparison of birth length, but not weight; and she met the criteria for postnatal growth failure at 24 months by comparison to mean parental height (which was between the 90-97th percentile) rather than in absolute measurements. Her findings were felt to be more in keeping with upd(7)mat SRS phenotype given her score and lack of limb asymmetry (as expected given there is no mosaicism). She was never treated with growth hormone therapy.

The proband was assessed by a clinical geneticist at eight years of age. She was of normal intellect with no developmental or behavioural concerns apart from poor fine motor dexterity; but she was reportedly reading at the level of a 13-year-old. She was babbling at 6 months of age and was walking at 12 months of age. On examination, she had borderline microcephaly and a very thin body habitus (BMI 12.9 kg/m^2, -2.04 SD). There was no craniofacial disproportion. She was distinctive in appearance with normal eye spacing but short palpebral fissures, dental irregularities (large teeth for mouth with sequelae of crowding), and micrognathia (Fig. 1a). One café-au-lait spot on her hip. She did not have limb asymmetry, or true clinodactyly, though there was some lateral deviation at the 5th PIP joints. Prominent heels were not specifically noted at this assessment but had been noted previously.

SRS testing demonstrated normal 11p15.5 gene dosage and normal H19 DMR methylation (via methylation-specific multiplex-ligation-dependent probe amplification [MS-MLPA] studies; ME-030-C3 probe mix BWS/RSS kit, MRC Holland [Amsterdam, The Netherlands]). UPD testing via short tandem repeat [STR] analysis confirmed bi-parental inheritance of chromosome 7. However, SNP-oligonucleotide microarray analysis using the Affymetrix CytoscanHD platform demonstrated a 79 kb deletion at 7q32.2 (arr[GRCh37]

7q32.2(130071996_130151083)x1) that involves five genes: the 5' end of *CEP41*, *MEST*, *MEST1T1*, *MIR335*, and the 3' end of *COPG2* (Fig. 1c, Supplemental Fig 1a). Review of this region within the Database of Genomic Variants and gnomAD structural variation database did not show deletions affecting these genes, indicating they are very rare in control populations (Collins et al., 2020; MacDonald et al., 2014). Microarray follow-up studies demonstrated that her deletion was paternally inherited (healthy father with a measured height of 179.4 cm), and her unaffected sibling was confirmed to have a normal copy number for this region (growth above 97th percentile). Methylation studies via MS-MLPA at 7q32 (ME-034-B1probe mix, MRC Holland [Amsterdam, The Netherlands]) demonstrated one copy number loss at *MEST* in both the father and proband, with complete methylation at *MEST* in the proband and absence of methylation in the father (Fig 1b, Supplemental Figure 1).

DISCUSSION

Herein we present the smallest deletion ever published at 7q32.2, further describing this SRS-like microdeletion phenotype for which only two other patients have been reported so far. At only 79 kb, it is 2% the length of the next smallest reported deletion. As such, the critical role of this imprinted region in the etiology of this SRS-associated growth deficiency can be further narrowed to a 79 kb region containing only five genes (5' end of *CEP41*, *MEST*, *MEST1T1*, *MIR335*, and the 3' end of *COPG2)*, three of which are imprinted and expressed on the paternal allele (*MEST*, *MEST1T1*, and *COPG2*).

Functionally, the deletion found in our proband should result in loss of the paternally expressed *MEST*, *MEST1T1*, *COPG2* genes; this would be comparable to the functional absence of expression of these genes in upd(7)mat. Therefore, if any of these genes were partially responsible for the upd(7)mat phenotype in SRS, we would anticipate that the two phenotypes

would be more similar, when compared to other genetic causes of SRS. The proband met 5 of the 6 criteria for diagnosis of SRS as per the Netchine-Harbison SRS scoring system (Azzi et al., 2015), though in a pattern that was unique. For example, her birth weight did not meet criteria for small for gestational age (though her length did). This pattern of higher birth weight is more commonly seen in upd(7)mat SRS than in 11p15 LOM SRS (Bruce et al., 2009). Overall, the phenotype of our patient more closely resembles the upd(7)mat phenotype of SRS (summarized in Table 1) as expected given the location of this deletion.

While the two previously reported patients with 7q32.2 microdeletions also grossly recapitulated the upd(7)mat phenotype, they had additional features, including congenital heart malformations and hearing deficits (Carrera et al., 2016; Eggermann et al., 2012). These features are not typical of SRS and were attributed to haploinsufficiency of other genes found in these large deletions. Our patient, who has a much smaller deletion encompassing only five genes, has a more "pure" SRS-like phenotype without a congenital heart malformation, hearing deficit, or global neurodevelopmental delay, thereby corroborating the hypothesis that those additional features are due to haploinsufficiency of other genes found in the larger deletions.

Four imprinted genes (*MEST*, *CPA4*, *COPG2,* and *KLF14*) and one imprinted non-coding RNA (*MEST1T1*) are regulated by the *MEST*:alt-TSS-DMR, and reasonably, *CPA4* has been considered as a candidate genetic cause of upd(7)mat (Bentley et al., 2003). The small deletion identified in our proband suggests that the gene responsible for the growth deficiency found in the 7q32.2 phenotype — and by extension, upd(7)mat SRS itself — can be narrowed down to the three imprinted genes found in her deletion: *MEST*, *MEST1T1*, and *COPG2*. Combined with the mouse studies that demonstrate pre- and post-natal growth restriction in paternally inherited *Mest* knockout mice, our case report provides further evidence that loss of

paternally expressed *MEST* may be the cause of this growth phenotype (Lefebvre et al., 1998). However, no point mutations in *MEST* or these other genes have been identified as the etiology of SRS, and characterization of additional patients with deletions or point mutations are necessary to corroborate this hypothesis.

In summary, the identification of a patient with the smallest deletion ever published narrows down the genetic etiology of the 7q32.2 microdeletion growth deficiency phenotype to a small region containing five genes: *CEP41*, *MEST*, *MEST1T1*, *MIR335*, and *COPG2*. Given that the deletion includes *MEST*, this case remains consistent with the hypothesized function of this gene in the etiology of upd(7)mat SRS. The future identification of individuals who have pathogenic variants in the paternally inherited copy of *MEST* would ultimately provide proof that this gene is the fundamental cause of the upd(7)mat SRS growth phenotype.

DATA AVAILABILITY STATEMENT:

The data that support the findings of this study are available from the corresponding author upon reasonable request.

CONFLICT OF INTEREST

The authors state no conflict of interests.

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AUTHOR CONTRIBUTIONS

GG planned and supervised this study. GG, MJ, and CZ performed clinical work. DJS, MBB, CY, and DD performed and interpreted laboratory data. KMV drafted the manuscript and all authors worked on the final version of the manuscript. All the co-authors read and approved the final version of the manuscript.

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FIGURE LEGENDS

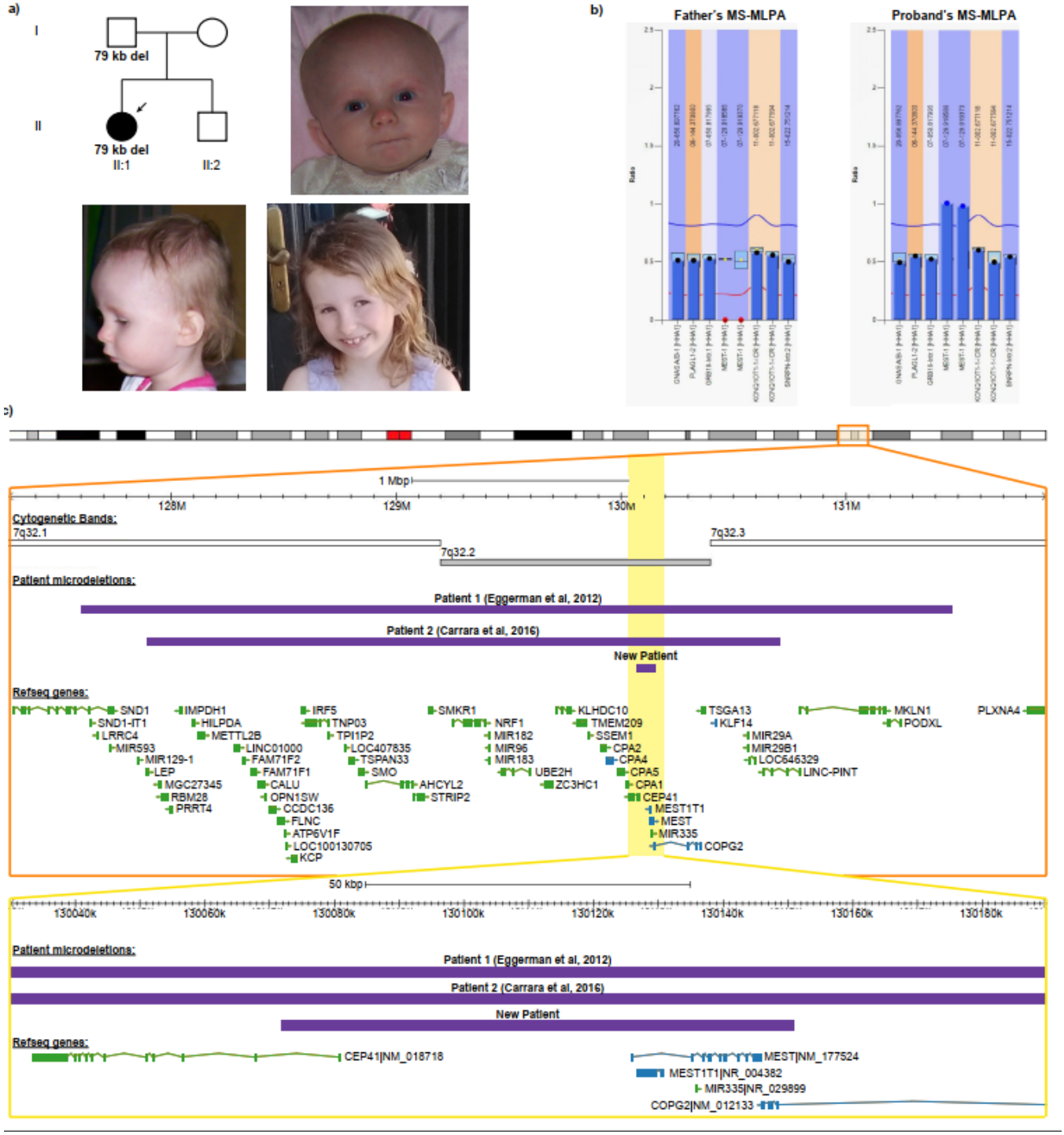
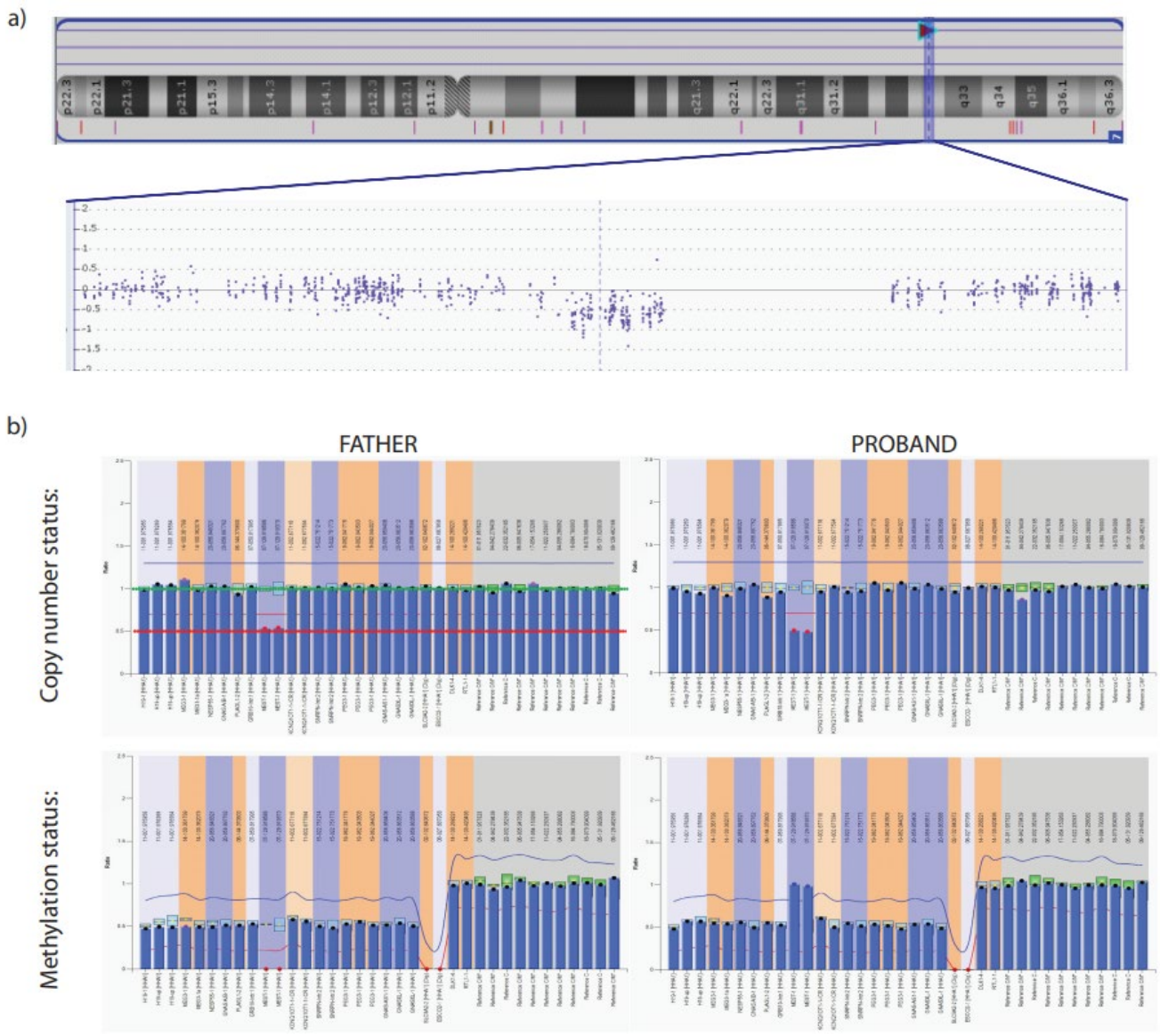


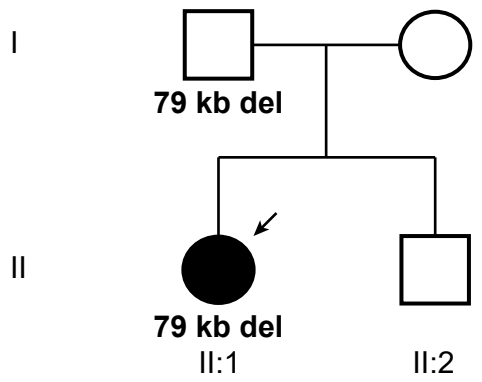
Figure 1. Extent of 7q32.1-3 microdeletions in three patients. a) Pedigree and photographs. Arrow marks our proband with growth retardation phenotype as depicted in text.

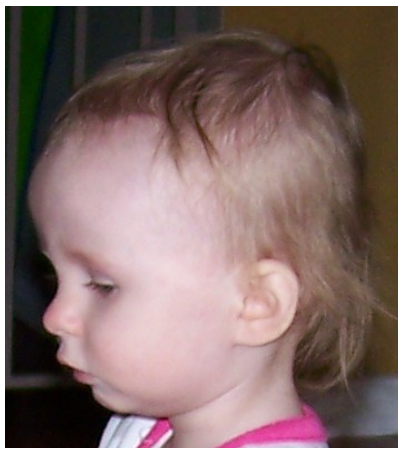
79 kb del indicates the presence of 7q32.2(130071996_130151083) deletion in family member. Photographs of index patient at 6 months, 1.5 years, and 9 years of age include relative macrocephaly, prominent forehead, micrognathia, downturned mouth, triangular face. b) Methylation status at 7q32.2 as determined by HhaI digested MS-MLPA demonstrating absence of methylation at *MEST* in the father (left panel) and complete methylation in the proband (right panel). c) Illustration of the 7q32.2 locus depicting the positions of the three documented microdeletions (purple bars). Top panel represents 7q32.1-3 locus with the 58 indicated genes (genes in blue depict imprinted genes within the *MEST*-DMR). Yellow shading indicates the enlarged region in the bottom panel demonstrating the smallest deletion with the five involved genes, three of which are imprinted: *MEST*, *MEST1T1*, and *COPG2*. Image modified from the DGV (https://dgv.tcag.ca), NCBI Build 37 (hg19). Top panel: chr7:127,290,000-131,899,999. Bottom panel: chr7:130,030,000-130,189,999.



Supplemental Figure 1. a) CytoScan HD data of the 7q32.2 microdeletion. b) Multilocus methylation-specific multiplex ligation-dependent probe amplification with copy number status (upper panel) determined by undigested MS-MLPA, and methylation status (lower panel) determined by HhaI digested MS-MLPA. The father (left panel) and proband (right panel) have a copy number loss at *MEST* with absence of methylation in the father, and complete methylation in the proband.

a)





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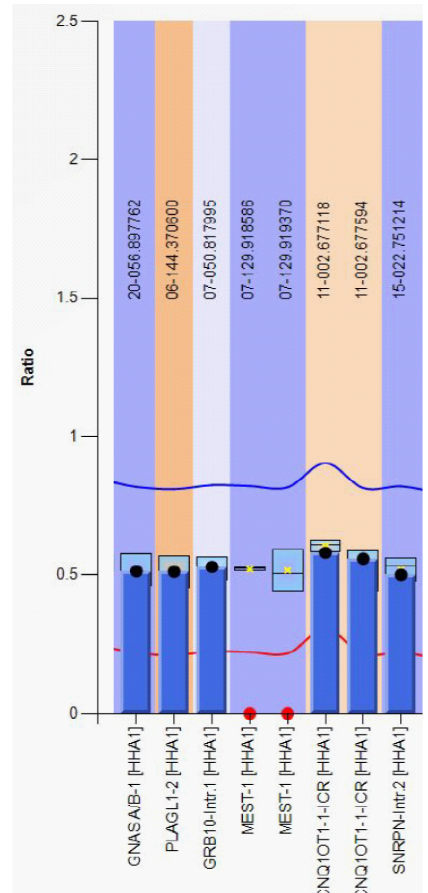

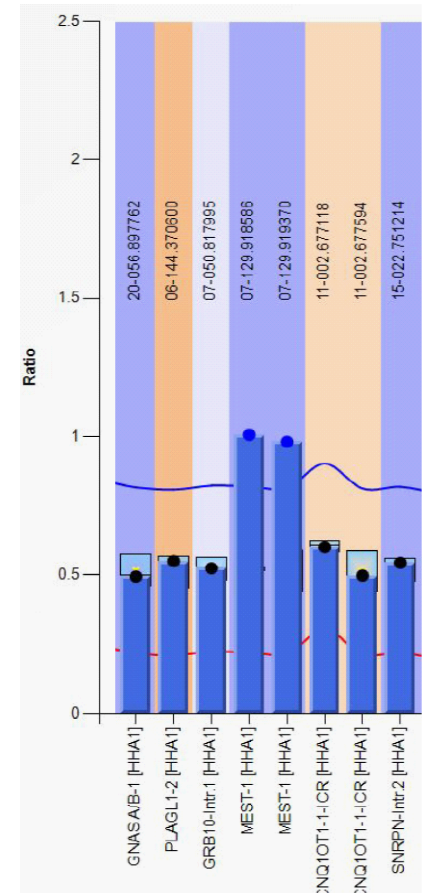

c)

Table 1. Overview of the major clinical features in three patients with 7q32.2 microdeletions compared with other SRS genotypes

	Total SRS (Wakeling *et al*, 2017)	11p15 loss of methylation (Wakeling *et al*, 2017)	Maternal UPD(7) (Wakeling *et al*, 2017)	Deletion Patient 1 (Eggermann *et al*, 2012)	Deletion Patient 2 (Carrera *et al*, 2016)	Present Patient
Molecular Features						
Description of the Molecular Change	Various	Loss of methylation on chromosome 11p15	Maternal uniparental disomy of chromosome 7			
Origin of change				De novo paternal chromosome affected	De novo paternal chromosome affected	Paternally inherited
Size and position of the deletion				3.7 Mb chr7:127599298-131471494	2.8 Mb chr7:127889335-130708391	79 kb chr 7:130071998-130151083 (CEP41, MEST, MESTIT1, MIR335, COPG2)
***MEST* included**				Yes	Yes	Yes
Netchine-Harbison clinical scoring system						
Born small for gestational age	91.7% (60)	100% (35)	72.7% (11)	- (-1.58 SD)	+ (-3.05 SD)	+ (-3.1 SD in length)
Relative macrocephaly at birth	85.7% (209)	99.1% (112)	85.2% (27)	- (-1.98 SD)	- (14 9/12 years: -2.75 SD; 17 years: -3.13 SD)	+ (when compared to length, not weight)
Postnatal height ≤ -2SD	84.2% (317)	83.8% (173)	80.9% (47)	- (-0.74 SD)	- (14 9/12 years: -1.67 SD; 17 years: -1.23 SD)	+ (given <-2SD from MPTH)
Protruding forehead	88.1% (201)	93.7% (126)	100.0% (27)	+	+	+ (infancy and early childhood)
Feeding difficulties and/or low body mass index	70.4% (307)	71.7% (173)	87.2% (47)	+	+ (first months only)	+
Body asymmetry	57.3% (473)	77.4% (226)	29% (62)	-	-	-
Other features						
Triangular face	93.9% (164)	98.7% (74)	50.0% (16)	Slightly	Slightly	+ (infancy and early childhood)
Delayed closure of fontanelle	42.6% (47)	44.4% (36)	36.0% (11)	NR	NR	-
Low set and/or posteriorly rotated ears	49.3 (266)	50.0% (140)	68.8% (48)	+	Large ears with unfolded helix	-
Downturned mouth	47.7% (176)	57.0% (114)	25.7% (39)	-	-	+
Irregular/crowded teeth	36.9% (195)	28.6% (105)	38.9% (36)	-	-	+
Micrognathia	61.7% (115)	74.7% (79)	25.9% (27)	NR	NR	+
Low muscle tone	56.3% (103)	67.2% (61)	47.4% (19)	+ (severe truncal hypotonia)	-	-
5th finger clinodactyly	74.6% (319)	80.7% (176)	56.3% (48)	-	-	-
Syndactyly of toes	29.9% (264)	41.8% (141)	16.7% (48)	-	-	-
Prominent heels	44.3% (61)	25.7% (35)	100% (12)	NR	NR	+
Shoulder dimples	65.6% (61)	77.1% (35)	66.7% (12)	NR	NR	-
Scoliosis and/or kyphosis	17.6% (227)	10.0% (97)	16.3% (43)	NR	+	-
Male genital abnormalities	40.0% (85)	44.4% (63)	21.4% (14)	NR	NR	NA
Motor delay	36.6% (254)	30.5% (141)	58.3% (36)	+	+	-
Speech delay	39.7% (189)	31.7% (101)	63.9% (36)	+ (severe)	+	-
Autism spectrum disorder/PDD	18.0% (61)	5.7% (35)	58.3% (12)	+ (severe global developmental delay)	+ (33% global disability)	-
High pitched/squeaky voice	45.2% (42)	39% (26)	71.0% (7)	-	-	-
Excessive sweating	53.8% (106)	51.4% (70)	70.4% (27)	NR	NR	-
Hypoglycemia	22.3% (103)	21.7% (69)	29% (0)	NR	NR	+ (benign ketotic hypoglycemia)
Other				Pulmonary stenosis, mild hearing impairment	Hypermetropia, thin upper lip, intraventricular septal defect, moderate hearing impairment requiring hearing aids	Short palpebral fissures

+ denotes present, - denotes absent, NR denotes not reported, MPTH denotes mid-parental target height, NA denotes not applicable.

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Journal title	American Journal of Medical Genetics: Part A
Submission ID or DOI	21-0564.R1
Provide a brief explanation of and reason for the changes requested	To address review comments, we required an additional investigation. Drs. Dyment and Chen were instrumental in dete

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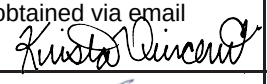
Dimitri	Stavropoulos	PhD	N	Genome Diagnostics, Depa	james.stavropoulos		obtained via email 	19-Jan-2022
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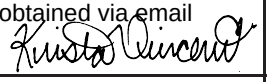
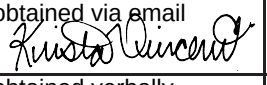
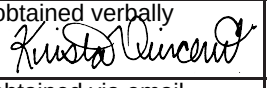
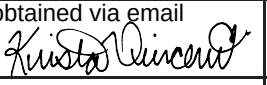
Mary	Jiang	MD	N	University of Ottawa, Ottaw	MJiang@cheo.on.c		obtained via email 	04-Jan-2022
Caroline	Zuijdwijk	MD	N	University of Ottawa, Ottaw	czuijdwijk@cheo.on	0000-0003-03	obtained via email 	05-Jan-2022
David	Dyment	MD, PhD	N	Department of Medical Ger	ddyment@cheo.on.		obtained verbally 	06-Jan-2022
Gail	Graham	MD	N	Department of Medical Ger	gailegraham@roger		obtained via email 	04-Jan-2022

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Author Name**		Conflict of Interest statement	Acknowledgements	Contribution statement††
Given Name	Family Name			
Chen	Yang	None to declare	N/A	Data curation (supporting), formal analysis (supporting)
David	Dyment	None to declare.	N/A	Conceptualization (supporting); methodology (supporting)

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