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PEG10 loss of function causes Silver-Russell syndrome: a familial case with paternal deletion

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Silver-Russell syndrome (SRS, MIM#180860) is an imprinting disorder characterized by prenatal and postnatal growth retardation, relative macrocephaly at birth, prominent forehead, feeding difficulties, and body asymmetry. Clinical diagnosis is based on at least 4 out of 6 clinical signs (Netchine-Harbison clinical scoring system). The main molecular mechanisms are loss of methylation at the paternal *H19/IGF2:IG-DMR* (30-60%) at the 11p15 chromosomal region and maternal uniparental disomy of chromosome 7 (5-10%). While it is well known that deregulation of 11p15 imprinted genes (*IGF2*, *H19*, and *CDKN1C*) contributes to the SRS phenotype, those on chromosome 7 (*GRB10*, *PEG10*, and *MEST*) are still in discussion. We report two brothers clinically diagnosed with SRS (postnatal growth delay, relative macrocephaly at birth, feeding difficulties, and SRS facies). One patient also has distal tremors and a treated growth hormone deficiency. CGH-array revealed a deletion of 109 Kb including *PEG10* and *SGCE* genes, inherited from their unaffected father. Whole Exome Sequencing did not disclose other causative variants. PEG10 functions as a transcriptional repressor of cyclin-dependent kinase inhibitors, including *CDKN1C*, for which maternal gain-of-function variants are linked to SRS. Real-time PCR studies showed a downregulation of *PEG10* and an upregulation of *CDKN1C* expression only in the affected brothers. Interestingly, *IGF2* was upregulated in the patient 1 under GH administration. Our findings provide the first evidence supporting the role of *PEG10* in the pathogenesis of Silver-Russell Syndrome, mediated by a gain-of-function effect on the *CDKN1C* expression, offering new insights into the molecular mechanisms underlying this condition.

Keywords Silver-Russell Syndrome, PEG10, SGCE, Maternal uniparental disomy of chromosome 7, CDKN1C, IGF2, 7q21 deletion

Abbreviations

SRS	Silver-russell syndrome
NH-CSS	Netchine-harbinson clinical soring system
DMR	Differentially methylated region
LoM	Loss of methylation
IC1	Imprinting center 1
UPD	Uniparental disomy
WES	Whole exome sequencing
ACMG	American college of medical genetics
MS-MLPA	Methylation-specific multiplex ligation probe-dependent amplification
RT-qPCR	reverse transcription quantitative real-time PCR
Pt	Patient
BW	Birth weight
BL	Birth lenght

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OFC	Occipital-frontal circumference
IUGR	Intrauterine growth restriction
GH	Growth hormone
BMI	Body mass index
OI	Osteogenesis imperfecta
DYT11	Myoclonic dystonia 11
NDNC8	Nail-disorder nonsyndromic congenital 8

Silver–Russell syndrome (SRS) is a rare (1:30.000-100.000) imprinting disorder characterized by pre- and postnatal growth retardation, relative macrocephaly at birth associated with a triangular face and a prominent forehead, body asymmetry, and feeding difficulties. Clinical diagnosis is based on the occurrence of at least 4 out of 6 clinical signs, in accordance with the Netchine–Harbison Clinical Scoring System (NH-CSS)[1]. The Silver Russell syndrome is a paradigmatic imprinting disorder, where specific genes undergo to a monoallelic expression of a single parental allele, while the other one is silenced (imprinted). Loss of methylation of the paternal allele at *H19/ IGF2:IG-DMR* in the 11p15.5 region (IC1_LoM, 30%–60% of cases), leading to *IGF2* gene downregulation, and maternal uniparental disomy of chromosome 7 (UPD(7)mat, 5%–10% of cases) involving *GRB10:alt-TSS-DMR*, *PEG10:TSS-DMR*, and *MEST:alt-TSS-DMR*[1,2] are the frequent etiopathogenic mechanisms. Further rare abnormalities, such as epimutations in *MEG3:TSS-DMR* or UPD(14)mat, UPD(16)mat, UPD(20)mat, and pathogenic variants within the *IGF2*, *PLAG1*, *HMGA2,* and *CDKN1C* genes have been described in approximately 4-5% of SRS patients[3,4]. However, about 40% of clinical SRS cases remain molecularly undiagnosed[1]. Cohorts of individuals with IC1_LoM and UPD(7)mat have a very similar phenotype, even if among the UPD(7)mat group, a minor frequency of body asymmetry and a higher incidence of neurodevelopmental delay were observed[3,5,6]. Most of the reported studies on the UPD(7)mat have associated the onset of the SRS phenotype with the combined altered expression of the three imprinted genes *GRB10, MEST, and PEG10*. However, SRS patients with segmental UPD(7)mat not involving the three DMRs and SRS, SRS-like patients with a genetic defect impairing singularly *GRB10*[7–10] or *MEST*[11–13] have been described. Here, we refer to the first familial case involving the *PEG10* gene. *PEG10* has aroused interest due to its maternal imprinting and critical role in early development, particularly in placental and fetal growth[14]. *PEG10* is known to be expressed only from the paternal allele, predominantly in the placenta, though its paternal expression is also observed in various other tissues postnatally[15]. Despite its essential role in growth regulation, the precise contribution of *PEG10* to the development of SRS has remained unclear.

In this study, we report a novel paternal deletion involving both the *PEG10* and *SGCE* genes in two siblings with a strong clinical suspicion of SRS. By investigating the molecular effects of the *PEG10* deletion, we aim to elucidate the mechanisms underlying the clinical manifestations of SRS and deepen our understanding of its genetic basis.

Materials and methods

Methylation-Specific Multiplex Ligation Probe-dependent Amplification (MS-MLPA)

The MRC-Holland kit (MRC Holland, Amsterdam, Netherlands) ME-030 BWS/RSS, ME032-UPD7-UPD14, and ME031-GNAS were used to investigate the methylation status at SRS imprinted loci. The analyses were performed according to the manufacturer's protocols. Four control samples were included in each experiment. Raw data were analyzed using Coffalyser.Net software (version 140,701, MRC Holland).

CGH array

Whole-genome array-CGH analysis was performed using the GenetiSure Postnatal Research CGH+SNP Microarray 2x400K platform (Agilent) to detect copy number variants (CNVs) and loss of heterozygosity. Labeling and hybridization were performed according to the manufacturer's protocol, and CNVs were detected by the Agilent Cytogenomics v5.0.2.5 analysis software. The map positions refer to the Human Genome Building 37 (hg19) assembly. Detected CNVs were compared with the Database of Genomic Variants (http://projects.tcag.ca/variation/, release March 2016) to exclude common copy number polymorphisms (minor allele frequency >1%).

Whole Exome Sequencing (WES)

WES was performed at the BIODIVERSA srl service (Milan, Italy). WES libraries were prepared with the SureSelect XT PreCap Human All Exon V8 kit (Agilent Technologies Inc., Santa Clara, CA, USA) and sequenced on a NovaSeq 6000 system (Illumina Inc, San Diego, CA) to generate 150 bp paired-end reads with a mean target coverage of ≥100×. FASTQ files were quality-checked with FastQC v0.11.9 and processed using the DRAGEN Bio-IT Platform v3.8.4 in WES mode, which performed read alignment to GRCh38, duplicate marking, realignment, base recalibration, and germline variant calling. Variant annotation was performed using WANNOVAR[16], incorporating functional predictions and population frequency data. A virtual panel of 2508 growth-related genes was designed to disclose causative variants by reviewing the literature and using PanelApp[17]. All variants identified were filtered by minor allele frequency (< 1%) in the 1000 Genomes, Genome Aggregation Databases, and Exome Aggregation Consortium databases. The interpretation of the variants was based on the classification by the InterVar, VarSome, and Franklin by Genoox databases[18,19] in accordance with the American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines[20,21]. All the variants herein reported were confirmed by Sanger sequencing.

Reverse Transcription Quantitative Real-Time PCR (RT-qPCR) and statistical analysis

Total RNA of patients, their relatives, and healthy controls was collected using Tempus Blood RNA tubes (Thermo Fisher Scientific) and isolated using the Tempus Spin RNA Isolation kit (Thermo Fisher Scientific). RNA was reverse-transcribed into cDNA using the SuperScript VILO cDNA Synthesis Kit (Thermo Fisher, Waltham, MA, USA). RT-qPCR reactions for the *PEG10* (F_GACCCCATCCTTCCTGTCTTC, R_CCCCTCTTCCACTCCTTCTTT), *IGF2* (F_CCGGCTTCCAGACACCAAT, R_GGTAAGCAGCAATGCAGCAC), and *CDKN1C* (F_CGGCGATCAAGAAGCTGTC, R_GCTGATCTCTTGCGCTTGG) genes were carried out using the SYBR Green Universal Master Mix (Thermo Fisher Scientific). They were performed using the QuantStudio 12K Flex Real-Time PCR System (Thermo Fisher Scientific). Data were analyzed using the QuantStudio 12K Flex Software v1.2.3 (Thermo Fisher Scientific). The amounts of mRNA were calculated using the 2^-ΔΔCt method, normalized to the housekeeping genes *HMBS* and *ACTB*, and replicated three times. We established the proper range of gene expression in 12 healthy adult controls (6 males and 6 females, 20-40 years). Statistical analysis was performed using GraphPad Prism 9.0 software. Data obtained from expression studies were analyzed using the Kruskal-Wallis test and Dunn's multiple comparisons test.

Results

Clinical presentation

The two affected brothers were born to healthy unrelated parents with a normal height (mother 160 cm; father 175 cm; target height 170.6 cm, -0.6 SDS). Both pregnancies occurred spontaneously and were complicated by maternal cholestasis. Data on gestational age as well as birth weight, birth length, and head circumference were obtained from birth charts and converted to SD according to Bertino et al[22]. Auxological data were evaluated according to Tanner growth charts[23]. Growth charts of the two boys are displayed in Supplementary Figure 1. Parents did not allow the publication of the photos. The main clinical features of the sibs are summarized in Figure 1.

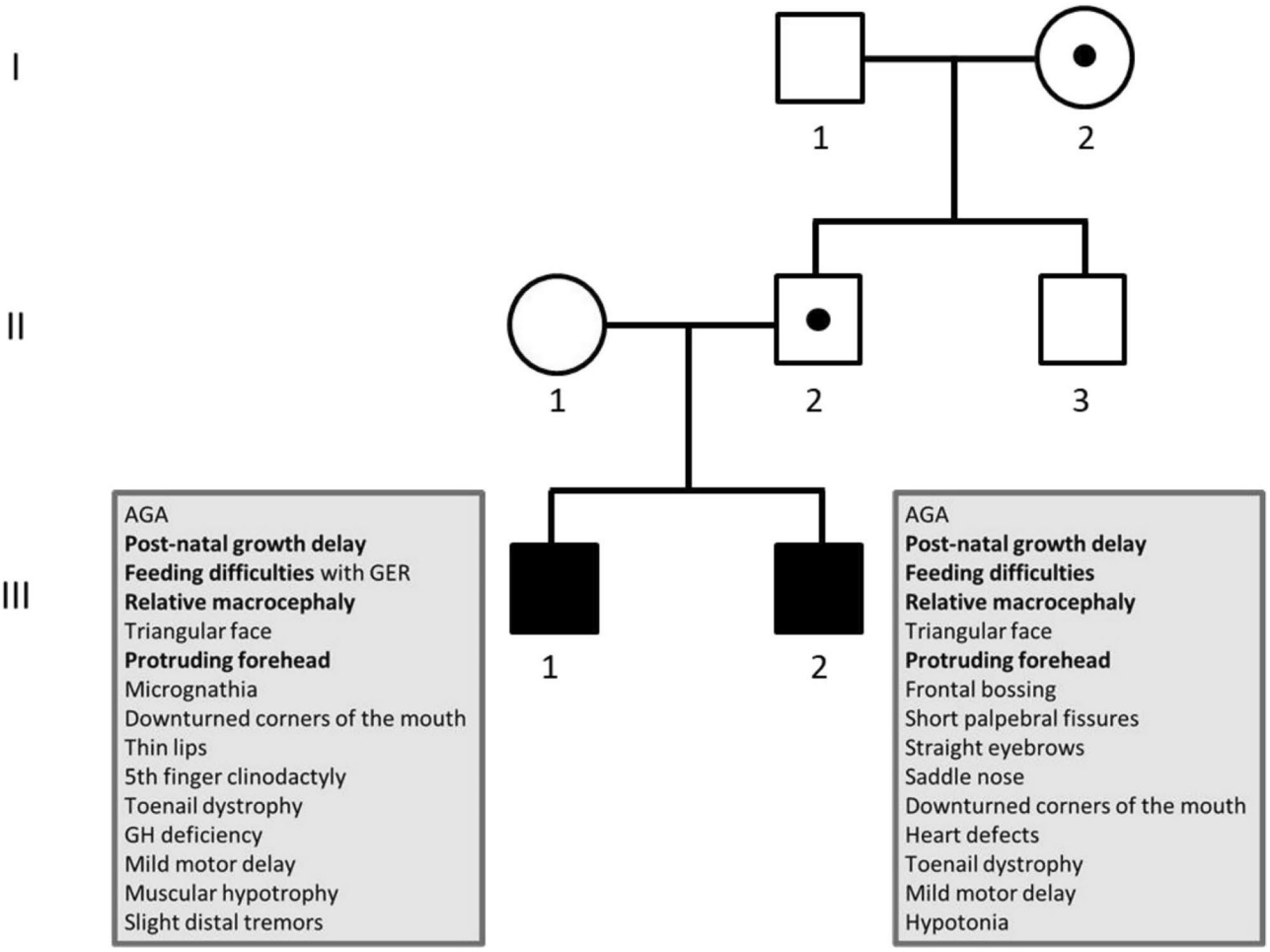

Fig. 1. Main clinical features of our patients and segregation of the 7q21.3 deletion. The family pedigree shows that the deletion is present in both affected siblings, as well as in the unaffected father and paternal grandmother, while it is absent in all other family members. The clinical features of the sibs are summarized in the boxes. AGA = appropriate for gestational age; GER = gastroesophageal reflux.

Patient 1 (Pt1) was born at 36 + 6 weeks of gestation with a birth weight (BW) of 2830 g (−0.07 SDS), a birth length (BL) of 47 cm (−0.56 SDS), and an occipital-frontal circumference (OFC) of 35.5 cm (1.66 SDS). Facial dysmorphisms included a triangular face with a prominent forehead and frontal bossing, micrognathia, downturned corners of the mouth, and thin lips. He experienced feeding difficulties with gastroesophageal reflux and episodes of hypoglycemia. Muscular hypotrophy, fifth finger clinodactyly, left valgus foot and knee, toenail dystrophy, extra hair whorls, and fetal pads were also observed. At 24 months, he weighed 8.6 kg (−3.79 SDS), measured 79 cm in length (−2.45 SDS), and had an OFC of 49 cm (0.2 SDS). He fulfilled 4 out of 6 NH-CSS criteria. Due to a severe growth delay, two growth hormone (GH) tests were performed at the age of four years, revealing an insufficient GH peak, which is suggestive of GH deficiency. GH treatment was therefore started, resulting in an improvement in growth velocity.

A mild motor delay was reported with autonomous walking at 18 months and difficulties in fine motor skills. Slight distal tremors were first noticed at the age of seven, along with easy fatigue. At the last clinical assessment at the age of 8 years and 9 months, his weight was 22 kg (−1.87 SDS), his height was 128.2 cm (-0.4 SDS), and his OFC was 54 cm (1.3 SDS). Body Mass Index (BMI) was 13.8 Kg/m2 (-1.65 SDS according to World Health Organization, WHO).

Patient 2 (Pt2) was born at 35 + 2 weeks of gestation with a BW of 2580 g (0.12 SDS), a BL of 47 cm (0.12 SDS), and an OFC of 35 cm (1.76 SDS). At birth, he required resuscitation with CPAP. Feeding difficulties, requiring a nasogastric tube for 20 days after birth, muscular hypotonia, right-sided aortic arch, atrial septal defect, phimosis, broad halluces with toenail dystrophy, and varus feet were reported. At the last evaluation, when the child was 4 years and 8 months old, his weight was 13.7 kg (−2.33 SDS), his height was 94.4 cm (−2.5 SDS), and his OFC was 51.4 cm (0.3 SDS). BMI was 15.6 Kg/m^2 (0.13 SDS).

He displayed a triangular face, protruding forehead, frontal bossing, saddle nose, downturned corners of the mouth, straight eyebrows, and short palpebral fissures. He reached an NH-CSS of 4/6. Furthermore, a mild motor delay was observed, with independent walking achieved at 16 months and a wide-based gait.

Brain Magnetic Resonance Imaging (MRI) was normal. Because of short stature, 2 GH stimulation tests were performed at the age of 2 and at the age of 4 years, which revealed a normal GH peak (9.64 ng/ml).

These patients undergo auxo-endocrinological assessments every six months and neurological evaluations every year as part of their follow-up.

Molecular analysis

Following the Pt2's SRS clinical diagnosis, methylation was analysed by MS-MLPA in the imprinted regions on chromosomes 11, 7, 14, and 20, without disclosing (epi)genetic or genetic alterations. In line with our diagnostic workflow[3], sequencing of the SRS-related genes, *HGMA2*, *IGF2*, *PLAG1*, *CDKN1C*, and *IGF1R* was carried out. After the birth of the second child, displaying light cardiac anomalies, a high-resolution CGH Array revealed a paternal deletion of approximately 109 Kb at 7q21.3, arr[GRCh37]7q21.3(94141411x2,94191291_94300309x1,94306364x2) encompassing the entire *SGCE* gene, associated to the autosomal dominant Myoclonic Dystonia 11 (DYT11, MIM#159900), as well as the maternally imprinted *PEG10* gene (Figure 2). As depicted in Figure 1, the deletion, also present in the eldest brother, was segregated from the healthy father and paternal grandmother and was absent in their paternal uncle. The familial segregation was consistent with the hypothesis of an imprinting disorder. To rule out the occurrence of an alternative etiology, WES was analysed, but no pathogenic or likely pathogenic variant correlated to the growth disorder, GH deficit, and myoclonic dystrophy was identified. Table 1 summarizes the clinical features of patients previously reported in the literature who carry deletions with a maximum size of 2.5 Mb at 7q21.3; Figure 3 depicts the exact deletion sizes and gene content, demonstrating that our case is the smallest reported to date and the only one including only *PEG10* and *SGCE* genes.

Interestingly, a paternal missense variant in the *COL7A1* gene (NM_000094):c.7048C>T, resulting in the substitution of proline 2350 with a serine, was disclosed in both probands. Alterations in this gene are associated with different phenotypes, including the autosomal dominant Nail-Disorder Nonsyndromic Congenital 8 (NDNC8, MIM#607523), characterized by toenail dystrophy. Since the nail phenotype of the father was not assessed, the variant was classified as of Unknown Significance (PM2, PM1, PP3).

According to the paternal expression of the gene in this tissue, the paternally inherited deletion of *PEG10* was expected to affect its expression. RNA expression level was then studied in blood samples of the patients, their parents, and paternal uncle, while the paternal grandmother was not available for the collection of a new sample for the RNA study. The RT-qPCR analyses showed a downregulation of the *PEG10* expression in both probands compared to healthy controls, but not in their relatives (Figure 4). The next step was aimed at correlating the loss of function of *PEG10* with the SRS phenotype. Given that PEG10 regulates the expression of cyclin-dependent kinase inhibitor genes, including *CDKN1C*, and *IGF2* is the main SRS causative gene, we investigated the expression of these genes by RT-qPCR analyses. Both *CDKN1C* and *IGF2* turned out to be upregulated in the Pt1, taking GH, while only *CDKN1C* was overexpressed in Pt2 (Figure 4). A normal expression of both genes was observed in their relatives.

The deletion of *SGCE* leads to SGCE myoclonus-dystonia (*SGCE-M-D*), a maternal imprinting disorder, characterized by a brief muscle contractions, repetitive movements, occurring mainly in the upper part of the body. The disease has a variable penentrance and an onset before age of 18 years. The deletion of *SGCE* may explain the motor impairment observed in patient 1.

Discussion

The investigation of the etiology of Silver-Russell syndrome uncovered many (epi)genetic and genetic defects. Although UPD(7)mat was the first molecular alteration identified in clinically suspected SRS[24], the main deregulated genes on chromosome 7 and their role in pre- and postnatal development remain incompletely understood. To date, three maternally imprinted domains with a putative clinical relevance have been identified

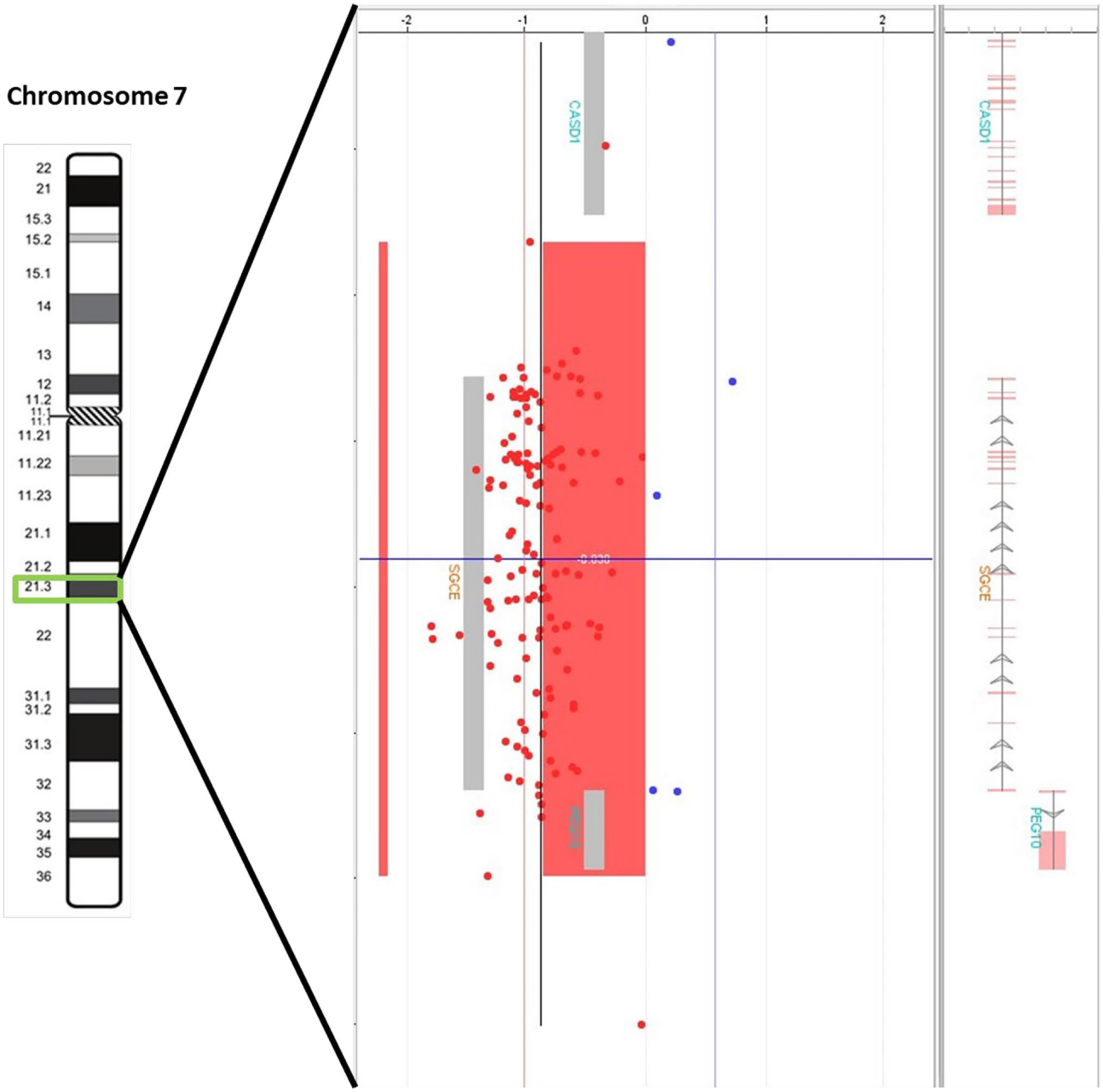

Fig. 2. Genomic profile of the 7q21.3 deletion. Array-CGH profile of chromosome 7 (hg19 genome assembly) showed an interstitial deletion of 109 Kb in the long arm of the chromosome (7q21.3), involving the maternally imprinted PEG10 and SGCE genes.

on chromosome 7: *GRB10:alt-TSS DMR* at 7p12.1; *PEG10:TSS DMR* at 7q21.3; and *MEST:alt-TSS DMR* at 7q32[25]. *GRB10* is a growth suppressor gene biallelically expressed in most fetal and postnatal tissues but only maternally expressed in the placenta[26], while *MEST* is a paternally expressed gene whose involvement in growth deficit is not well documented[10]. Maternal duplications of *GRB10* and paternal deletions of *MEST* are linked to SRS-like clinical features[7–9,11–13]. Recently, an intragenic paternal deletion of *GRB10* has been reported in a couple of monozygotic twins with postnatal SRS clinical features[10]. Furthermore, segmental UPD(7q)mat involving the *MEST* gene was found associated with classic or partial SRS phenotype[27,28]. While molecular and clinical evidence support the involvement of *GRB10* and *MEST* genes in the etiology of SRS, the role and function of the maternally imprinted *PEG10* gene have remained unclear[10].

For the first time, we describe a paternally inherited deletion encompassing *PEG10* and *SGCE* genes in two siblings with a strong clinical suspicion of SRS, providing new evidence for *PEG10* involvement in SRS pathogenesis. As shown in Figure 3, larger deletions in the region, typically spanning the *COL1A2* gene, have been documented previously and are associated with Osteogenesis Imperfecta (OI)[29–32]as well as SGCE-related myoclonus dystonia. Patients with these larger deletions commonly exhibited bone fractures, dentinogenesis

	This study	Asmus et al. 2007	Peall et al. 2014	Dale et al. 2011	Saugier-Veber et al. 2010	Grünewald et al. 2008
CNV details Size (Mb) n. of affected genes	0.109 2	1.63 10	2.3/2.0/1.9/0.7 16/16/15/4	0.17 2	1.88 15	1.09/1.35 5/3
Included *PEG10* gene	Yes	yes	4 yes /1 no	no	no	yes/probably
Inheritance	Pat #	NA	NA	Pat #	Pat	Pat #/DN*
n. of patients	2	1	5	2	2	3
Placental defects	-	-	-	-	+ / 1 NA	NA
IUGR/SGA	-	-	+	-	+ / 1 NA	NA
Relative macrocephaly at birth	++	NA	NA	-	- / 1 NA	NA
Protruding forehead	++	NR	NR	-	-	NR
Short stature	++	+	+++++	-	++	NR
OI features Blue sclerae Joint laxity Dental anomalies Bone fracture Hearing loss	[a] - - - - -	- + + + +	- + - - -	[a] - - - - -	- + - - -	- +++ - + -
Thin body/feeding difficulties	++	-	NR	-	+	NR
Facial dysmorphism	++	-	NR	-	-	NR
Microcephaly	-	-	++	-	++	NR
Motor delay	++	-	-	++	+	NR
Speech delay	-	-	+	++	+	NR
Myoclonus-Dystonia	-[§]	+	+++++	++	++	3
Cognitive impairment	-	-	+	++	++	NR

Table 1. Clinical and molecular features of the reported patients with a contiguous 7q21.3 gene deletion smaller than 2.5 Mb. Legends: NA = not available; NR = not reported; Pat = paternal; DN = de novo; IUGR = intrauterine growth restriction; SGA = small for gestational age; OI = osteogenesis imperfecta; + present; - absent; # unaffected; * onset on the paternal allele; [a] not include *COL1A2* gene; [§] only tremors in one patient.

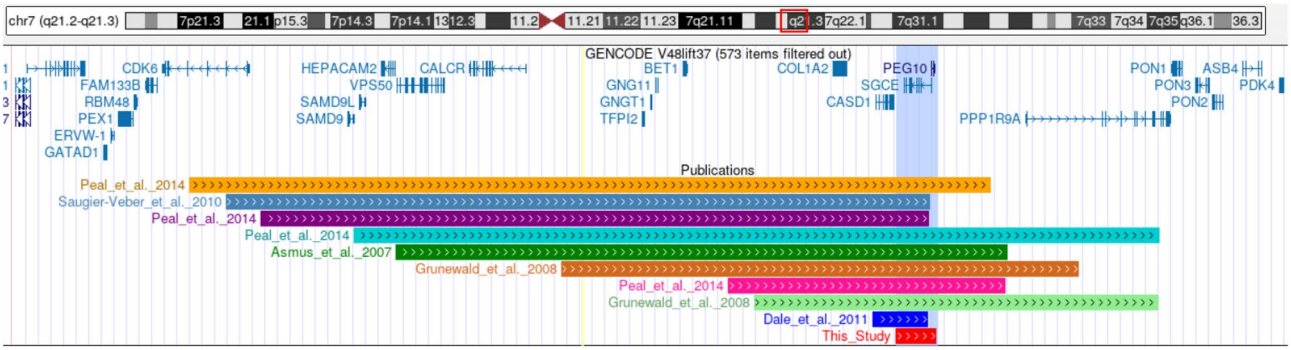

Fig. 3. Representation of contiguous gene deletions in the 7q21.3 chromosomal region involving PEG10 and SGCE genes. In the figure are displayed only the deletions smaller than 2.5 Mb. The deletion reported in this study is red highlighted.

imperfecta, hearing loss, short stature, and joint laxity[29], except for the patients described by Dale et al., in which a 170 Kb deletion affecting only *SGCE* and *CASD1* genes was reported[33]. In contrast, our family presents the smallest deletion reported so far at 7q21.3, affecting only *PEG10* and *SGCE* genes. Unlike those with larger deletions, our patients exhibit no OI-related features, aside from short stature, most likely because *COL1A2* and other genes are not impacted.

The *PEG10* gene is expressed only from the paternal allele, predominantly in the placenta of both humans and mice, but also shows high postnatal expression in various tissues such as adipose, kidney, brain, muscle, and lung[14,15]. In mouse models, embryos lacking *PEG10* appear morphologically normal at embryonic day 9.5 (E9.5), but by E10.5 they exhibit severe growth retardation of both the embryo and placenta[34]. In humans, *PEG10* expression is low during early gestation but increases significantly around weeks 11–12, suggesting that PEG10 plays an essential role in later stages of placental development[35]. Clinically, hypermethylation and aberrant *PEG10* expression have been associated with several pregnancy complications, including spontaneous miscarriage, intrauterine growth restriction (IUGR), and preeclampsia[36,37]. Doria et al. found a reduced expression of *PEG10* in fetal tissues at the third trimester, comparing fetuses from miscarriages versus control

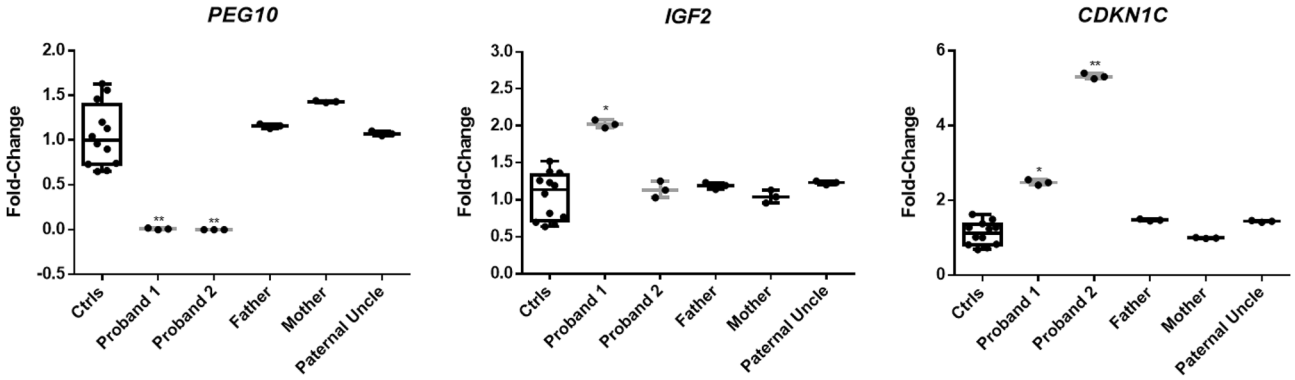

Fig. 4. Expression studies of PEG10, IGF2, and CDKN1C genes. Results from qPCR studies in blood from the probands and their relatives for different genes. The expression of the target genes was normalized against the housekeeping gene HMBS. Each experiment was replicated three times. The control group consisted of blood expression derived from 12 healthy individuals (* < 0.05, ** < 0.01, *** < 0.001). Error bars represent standard deviation.

fetuses. Despite the critical role of *PEG10* in both mouse and human placental development, our patients did not present any structural placental abnormalities or IUGR, and were born appropriate for gestational age. However, both patients were born preterm, and patient 2 required resuscitation. These observations may reflect distinct features of early human placental development[30,38]. Indeed, most previously reported cases involving paternal deletions at 7q21.3, which affect *PEG10*, *SGCE*, and additional genes, have not been associated with placental abnormalities or IUGR, supporting the idea that our patients' isolated deletion of only *PEG10* and *SGCE* may underlie the observed lack of placental phenotype aside from short stature.

Besides the placental function, *PEG10* has been reported as an oncogene implicated in tumor cell proliferation, metastasis, and apoptosis[15]. Several studies showed that *PEG10* is upregulated in different cancer types, with its overexpression enhancing cancer cell proliferation[15]. Conversely, reduced expression of *PEG10* is associated with marked growth inhibition of tumor cells[15,39]. The oncogenic properties of *PEG10* are linked to its ability to bind DNA via a CCHC-type zinc finger domain[40]. Other studies have shown that *PEG10* functions as a transcriptional repressor by binding to the promoters of key cell cycle-related genes active during the G0/G1 phase. These include cyclin-dependent kinase inhibitors (such as p16, p18, p21, p27, and p57) as well as specific cyclins (CCNE1, CCNE2, CCND1)[15,39–41]. Downregulation of *PEG10* in tumor cells leads to G0/G1 phase arrest, mediated by the upregulation and accumulation of the above inhibitory factors. The cited studies highlight the role of *PEG10* in promoting cell cycle progression from G0/G1 to S phase[41]. In an attempt to characterize the molecular impact of the paternal *PEG10* deletion in our patients, we evaluated the expression of *PEG10* and SRS genes *IGF2* and *CDKN1C* by RT-qPCR studies. Consistent with the maternal imprinting of *PEG10* in blood[42], its downregulated expression in UPD(7)mat SRS[10], and the segregation pattern of the deletion within the family, we observed a complete absence of *PEG10* expression in the two affected brothers, whereas expression levels were normal in both parents and paternal uncle. Although the literature indicates that *PEG10* does not bind the promoters of cell-growth genes such as *IGF2*, *PLAG1*, and *HMGA2*[40], we assessed *IGF2* expression due to its central role in SRS. Interestingly, *IGF2* expression was normal in Pt2 but also increased compared to the healthy relatives in Pt1. We can hypothesize that *IGF2* upregulation in Pt1 may be attributed to the ongoing GH therapy, according with the positive physical response observed. However further experiments will be required to sustain this observation. Human *IGF2* expression is regulated by GH through promoter binding, and GH-treated patients often exhibit increased serum IGF2 levels after treatment initiation[43–45]. The normal *IGF2* expression in Pt2 contrasts with the reduced expression typically reported in SRS patients with UPD(7)mat and UPD(7q)mat[10]. These discrepancies may be explained by hypothesizing that a complementary effect of the three chromosome 7 imprinted genes is necessary to obtain a complete phenotype and classic gene downregulation. These findings support the idea that the deregulation of *PEG10* in SRS does not involve the *IGF2* gene, aligning with previous reports on the known targets of PEG10 binding[40]. To enforce this consideration, there is also the occurrence of GH deficit only in one of the two affected children. Regarding the *CDKN1C* gene, and in line with the role of *PEG10* in regulating cell cycle progression[40], RT-qPCR analysis revealed upregulation of *CDKN1C* expression in both patients, with higher levels in Pt2, likely because he has not yet initiated growth hormone therapy. The finding is consistent with the established role of *CDKN1C* as a growth inhibitor and the documented pathogenic gain-of-function variants in *CDKN1C* in other SRS cases[46–49]. In contrast, unexpected downregulation of *CDKN1C* expression was recently reported in SRS patients with UPD(7)mat and UPD(7q)mat [10]. Additionally, rare paternal microdeletions affecting the *KCNQ1OT1:TSS-DMR* in the IC2 region of 11p15.5 not involving the *CDKN1C* gene, and maternal microduplications of IC2 have been linked to upregulation of gene expression and have been reported in cases of SRS or growth retardation phenotype[50–53]. Therefore, an increased functionality of *CDKN1C*, as observed in our patients, may contribute to cell cycle arrest and ultimately may result in growth restriction.

In conclusion, our findings suggest considering the *PEG10* gene as a gene involved in the pathogenesis of Silver-Russell Syndrome and provide new insights into the molecular mechanisms underlying the disorder. The

paternal deletion of *PEG10* and *SGCE* identified in our patients, along with the observed upregulation of the key growth-related gene *CDKN1C*, suggests that this genetic alteration contributes to the characteristic SRS clinical phenotype, and that locus-specific structural alterations involving *PEG10* and *SGCE* may represent a previously underappreciated mechanism contributing to SRS in a subset of patients. This also allows us to hypothesize the existence of an intricate interplay between imprinted gene dosage and growth regulatory pathways mediated by *CDKN1C* in the syndrome's molecular etiology. However, a limitation of this study is the use of blood samples for gene expression analysis, as PEG10 shows higher expression in placental and brain tissues. Altogether, these observations invite further investigation of paternal deletions of *PEG10* and *SGCE*, utilizing relevant tissue samples to provide more definitive evidence.

Data availability

Original data supporting the current study are available upon request in Zenodo at https://doi.org/10.5281/zenodo.16737114.

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Author contributions

MB, GP, MM made clinical suspicion in pediatric age; GP, AEMA, FN and MM carried on the clinical follow-up for endocrinological and neurological phenotype; SG, SR and AV excluded the molecular diagnosis of SRS; LB detected the microdeletion in 7q21 by array-CGH; AV and SR conceived and designed the study; AV and PT performed RT-qPCR and WES molecular analyses, LC carried on bionformatic data analysis; AV, GP and SR drafted the paper; LL and MM supervised the manuscript; all the authors read and approved the manuscript **.**

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Declarations

Competing interests

The authors declare no competing interests.

Ethics

The study was approved by the Ethical Committee of the Istituto Auxologico Italiano, IRCCS (2017_05_16_05).

Consent for publication

The family agreed for publication by signing an informed consent template.

Additional information

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