



OPEN ACCESS

Original research

# Advancing in Schaaf-Yang syndrome pathophysiology: from bedside to subcellular analyses of truncated MAGEL2

Laura Castilla-Vallmanya <sup>1,2,3</sup> Mónica Centeno-Pla <sup>1,2,4</sup>  
Mercedes Serrano <sup>2,3,5</sup> Héctor Franco-Valls,<sup>1</sup> Raúl Martínez-Cabrera,<sup>1</sup>  
Aina Prat-Planas <sup>1,2,3</sup> Elena Rojano,<sup>6</sup> Juan A G Ranea,<sup>3,6</sup> Pedro Seoane,<sup>3,6</sup>  
Clara Oliva,<sup>2,4</sup> Abraham J Paredes-Fuentes <sup>2,4</sup> Gemma Marfany <sup>1,2,3</sup>  
Rafael Artuch <sup>2,3,4</sup> Daniel Grinberg <sup>1,2,3</sup> Raquel Rabionet <sup>1,2,3</sup>  
Susanna Balcells <sup>1,2,3</sup> Roser Urreizti <sup>2,3,4</sup>

► Additional supplemental material is published online only. To view, please visit the journal online (<http://dx.doi.org/10.1136/jmg-2022-108690>).

For numbered affiliations see end of article.

## Correspondence to

Dr Roser Urreizti, Centro de Investigación Biomédica en Red de Enfermedades Raras, Madrid 28029, Spain; [roserrreizti@gmail.com](mailto:roserrreizti@gmail.com)

LC-V, MC-P and MS are joint first authors.

RR, SB and RU are joint senior authors.

Received 9 May 2022  
Accepted 27 July 2022



© Author(s) (or their employer(s)) 2022. Re-use permitted under CC BY-NC. No commercial re-use. See rights and permissions. Published by BMJ.

**To cite:** Castilla-Vallmanya L, Centeno-Pla M, Serrano M, et al. *J Med Genet* Epub ahead of print: [please include Day Month Year]. doi:10.1136/jmedgenet-2022-108690

## ABSTRACT

**Background** Schaaf-Yang syndrome (SYS) is caused by truncating mutations in *MAGEL2*, mapping to the Prader-Willi region (15q11-q13), with an observed phenotype partially overlapping that of Prader-Willi syndrome. *MAGEL2* plays a role in retrograde transport and protein recycling regulation. Our aim is to contribute to the characterisation of SYS pathophysiology at clinical, genetic and molecular levels.

**Methods** We performed an extensive phenotypic and mutational revision of previously reported patients with SYS. We analysed the secretion levels of amyloid- $\beta$  1–40 peptide ( $A\beta_{1-40}$ ) and performed targeted metabolomic and transcriptomic profiles in fibroblasts of patients with SYS (n=7) compared with controls (n=11). We also transfected cell lines with vectors encoding wild-type (WT) or mutated *MAGEL2* to assess stability and subcellular localisation of the truncated protein.

**Results** Functional studies show significantly decreased levels of secreted  $A\beta_{1-40}$  and intracellular glutamine in SYS fibroblasts compared with WT. We also identified 132 differentially expressed genes, including non-coding RNAs (ncRNAs) such as *HOTAIR*, and many of them related to developmental processes and mitotic mechanisms. The truncated form of *MAGEL2* displayed a stability similar to the WT but it was significantly switched to the nucleus, compared with a mainly cytoplasmic distribution of the WT *MAGEL2*. Based on the updated knowledge, we offer guidelines for the clinical management of patients with SYS.

**Conclusion** A truncated *MAGEL2* protein is stable and localises mainly in the nucleus, where it might exert a pathogenic neomorphic effect.  $A\beta_{1-40}$  secretion levels and *HOTAIR* mRNA levels might be promising biomarkers for SYS. Our findings may improve SYS understanding and clinical management.

## INTRODUCTION

In 2013, truncating mutations in *MAGEL2* (OMIM 605283) were associated with a new clinical entity,<sup>1</sup> first described as a Prader-Willi-like syndrome and currently named as Schaaf-Yang syndrome (SYS, OMIM 615547). *MAGEL2* is one of the

## WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ *MAGEL2*-truncating mutations cause Schaaf-Yang syndrome (SYS), but the functional effects of the truncated *MAGEL2* protein have been poorly defined.

## WHAT THIS STUDY ADDS

⇒ By expressing truncated *MAGEL2* in cell lines, we now know that a truncated version of the protein is retained in the nucleus, thus exerting a novel behaviour in addition to the loss of some of its main functions. Patients' fibroblasts show reduced levels of excreted amyloid- $\beta$  1–40 and intracellular glutamine as well as an altered transcriptomic profile, including overexpression of the major regulator *HOTAIR*.

## HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Based on a comprehensive review of genetic and clinical aspects of all reported cases, families and physicians will benefit from the Clinical Management Recommendations that we provide here.

five maternally imprinted protein-coding genes contained in the Prader-Willi region (15q11-q13). Lack of expression of the paternal alleles in this region causes Prader-Willi syndrome (PWS; OMIM 176270). In contrast, non-sense or frameshift mutations in the paternal allele of *MAGEL2* are predicted to encode a truncated protein lacking the MAGE Homology Domain (MHD) and have been associated with SYS.<sup>1</sup> Since then, over a hundred patients with SYS have been reported and phenotypically described.<sup>1–28</sup> Patients with SYS and PWS show overlapping clinical phenotypes, including neonatal hypotonia, intellectual disability (ID), developmental delay (DD), early feeding difficulties, endocrinological disturbances (hypogonadism and other hormonal imbalances) and sleep disorders. However, some of the clinical criteria for PWS diagnosis, such as hypopigmentation, characteristic

facial dysmorphism, small hand and feet, hyperphagia, obesity and obsessive-compulsive behaviours, are frequently absent in patients with SYS, who, on the other hand, present more frequently with severe ID, autism spectrum disorder (ASD) behaviours and joint contractures.<sup>29–31</sup> Some truncating variants in *MAGEL2* have also been associated with Chitayat-Hall syndrome (CHS).<sup>32</sup> However, a systematic review of all patients with SYS and CHS showed that there is no discernible genetic or clinical difference between both syndromes, and the latter has been renamed as SYS in OMIM.<sup>17</sup> In contrast, two particular *MAGEL2* truncating mutations have been recurrently identified in patients affected by lethal arthrogryposis multiplex congenita (AMC), a much more severe phenotype, distinct from SYS.<sup>4 10 27 33 34</sup> All in all, there is no specific constellation of symptoms pathognomonic or specific for any of these clinical syndromes; furthermore, they probably conform to a clinical continuum, therefore denoting the need to address clinical denomination according to molecular findings.

*MAGEL2* shows a wider expression in human fetal tissues than in adult tissues, where it is predominantly present in brain (according to GTEx<sup>35</sup>). In adult mice, it also becomes mostly restricted to the central nervous system, specifically to the amygdala and the hypothalamus, and predominantly in the suprachiasmatic, the paraventricular and the supraoptic nuclei.<sup>36–38</sup> It is a single-exon gene that encodes one of the largest proteins of the type II MAGE protein family consisting of 1249 amino acids. At a structural level, the N-terminal region of *MAGEL2* contains a proline-rich domain, whose function remains unclear.<sup>39</sup> At the C-terminus, from amino acids 1027 to 1195, there is the MHD, a highly conserved 170-amino acid sequence present in both type I and type II MAGEs, crucial for protein–protein interaction.<sup>40</sup> Through it, *MAGEL2* recognises and binds the coiled-coil domain of the E3 ubiquitin ligase TRIM27. The MHD is also crucial for binding VPS35, a subunit of the retromer cargo-selective complex. *MAGEL2*, TRIM27 and USP7 form the MUST complex, which is recruited to endosomes through direct binding of *MAGEL2* to VPS35 and plays a role in retrograde endosomal transport.<sup>41 42</sup> These specialised endosomes participate in endosomal export pathways that deliver membrane protein cargoes either to the trans-Golgi network through retrograde pathways or to the plasma membrane through recycling pathways.<sup>43</sup>

A dysfunction of the retrograde transport could be disturbing for many cellular processes. Loss of *MAGEL2* expression causes a reduction in secretory granules protein levels due to impaired endosomal protein trafficking and subsequent lysosomal degradation, resulting in a reduction of circulating bioactive hypothalamic hormones.<sup>36</sup> A well-coordinated trafficking network is also key for the correct regulation of amyloid precursor protein (APP) cleavage.<sup>44</sup> APP family members are relevant for neuronal differentiation and migration during cortical development<sup>45 46</sup> and proper neuromuscular junction formation and neurotransmission.<sup>47</sup> Many studies support a model where retromer deficiency leads to increased APP cleavage, A $\beta$  peptide production and exocytosis.<sup>48–50</sup> In addition, protein levels of the glucose transporter GLUT1 in the cell membrane are reduced after VPS35 and SNX27 inhibition, showing that they are also tightly regulated by the retromer.<sup>51</sup>

Here, we have performed an extensive literature revision and based on it, we have expanded the clinical and genetic delineation of SYS and developed a standardised set of guidelines for its clinical management. We also contribute to the knowledge of the cellular phenotype by assessing the effect of a recurrent truncating variant on *MAGEL2* protein stability and subcellular

localisation using heterologous expression vectors. Finally, we have performed a transcriptomic and metabolomic characterisation of fibroblasts derived from patients with SYS and interpreted the results in the context of molecular and clinical findings.

## RESULTS

### Clinical management of patients with SYS requires a coordinated multidisciplinary approach

We performed a systematic revision of all the published patients with SYS to date, who were all carriers of *MAGEL2* mutations (table 1 and online supplemental table 1), carefully inspecting both the molecular and phenotypic data, with the aim to propose a set of guidelines for SYS clinical management.

The literature-based recommendations have been divided into two life periods: the perinatal period (first 28 days of life) and infancy/adolescence. They include the most relevant medical problems associated with each period and the specific concerns or interventions advisable for each area. A schematic version of the different clinical areas and tests included in the guidelines are represented in figure 1, and a detailed, printable version is supplied as online supplemental table 2 (both in Spanish as online supplemental figure 1 and online supplemental table 3).

In most instances, pregnancy was uneventful (only polyhydramnios has been reported in some cases), although there is a high rate of caesarean sections (over 50% of the patients where delivery information is available). Clinical symptoms appear early in life showing a complex phenotype that includes neuromuscular symptoms, respiratory and endocrinological problems, feeding difficulties and dysmorphic traits. Other clinical issues can appear later and may affect almost any organ or system, requiring a coordinated multidisciplinary approach.

### SYS variants are mostly truncating and located in the C-terminal domain

To date, 61 different variants have been associated with SYS or AMC (figure 2). Mutation p.(Gln666Profs\*47), present in 80 individuals, is considered a recurrent mutational hotspot. These variants are mostly truncating and predominantly located in the C-terminal region of the protein, leading to a partial or a total lack of the MHD domain and compromising the functions that *MAGEL2* carries out through this domain. Three different missense variants and a small in-frame duplication have also been associated with SYS phenotypes (in grey in figure 2). These atypical variants are not predicted to encode a truncated form of the protein, and there are no functional studies to support their pathogenicity. Thus, their clinical implication remains to be proven.

### Fibroblasts from patients with SYS show altered gene expression patterns

To better understand the effect of *MAGEL2* truncating mutations on gene expression patterns, we performed an mRNA whole transcriptome analysis (mRNASeq) on fibroblasts from six healthy donors and three SYS subjects: a girl carrying p.(Gln638\*) and a boy and an unrelated girl, both carrying p.(Gln666Profs\*47). Using the ExpHunter Suite, we identified 132 differentially expressed genes, 76 upregulated and 56 downregulated (online supplemental table 4 and online supplemental figure 2A,B). The top 10 upregulated and downregulated genes, showing the most significant changes in the expression fold, are listed in table 2. Four genes were tested by quantitative PCR (qPCR) in fibroblasts from five patients with SYS and four control individuals,

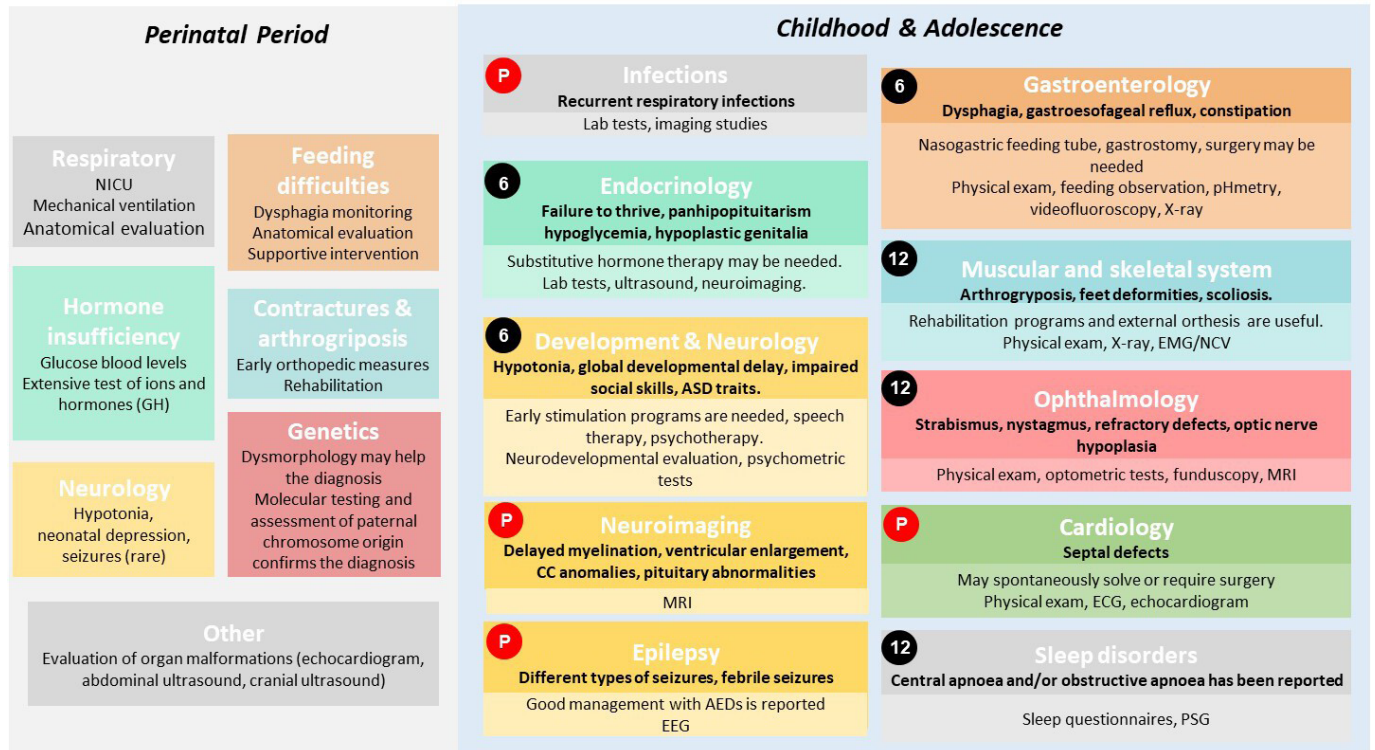
**Table 1** Clinical overview of patients with MAGEL2-related disorders

		Total	
	Diagnostic	SYS	AMC
	Individuals	122	13
	Gender	43 F; 42 M; 38 UnK	7 F; 5 M; 1 UnK
	Protein change	59 different mutations	p.Gln666Serfs*36; p.Leu708Trpfs*7
Phenotype (HPO term)			
Pregnancy complications			
Decreased fetal movement	HP:0001558	12/37 (32.4%)	4/4
Polyhydramnios	HP:0001561	7/16 (43.75%)	5/5
Neurology and neurodevelopment			
Neurodevelopmental delay	HP:0012758	100/100 (100%)	
Intellectual disability	HP:0001249	92/97 (94.8%)	
Autistic behaviour	HP:0000729	55/76 (72.4%)	
Abnormality of brain morphology	HP:0012443	19/32 (59.4%)	1/1
Infantile lethargy/weak cry	HP:0001254/HP:0001612	15/24 (62.5%)	
Epilepsy	HP:0001250	24/79 (30.4%)	
Respiratory			
Respiratory difficulties	HP:0002098	60/74 (81.8%)	1/1
Apnoea	HP:0002104	11/18 (61.6%)	
Sleep apnoea	HP:0010535	53/75 (70.7%)	
Respiratory failure requiring mechanical ventilation	HP:0004887	44/73 (60.3%)	
Respiratory distress requiring endotracheal intubation	HP:0004887	16/50 (32.0%)	
Recurrent respiratory infections	HP:0002205	5/6 (83.3%)	
Feeding and growth			
Feeding difficulties	HP:0011968	92/106 (86.8%)	1/1
Poor suck	HP:0002033	72/81 (88.9%)	
Dysphagia	HP:0002015	35/50 (70.0%)	
Nasogastric tube feeding in infancy	HP:0011470	41/63 (65.1%)	
Gastrostomy tube feeding in infancy	HP:0011471	26/54 (48.5%)	
Hyperphagia	HP:0002591	17/63 (27.0%)	
Increased body weight/obesity	HP:0004324/HP:0001513	24/79 (30.4%)	
Gastro-oesophageal reflux	HP:0002020	38/76 (50.0%)	
Chronic constipation	HP:0012450	46/76 (60.5%)	
Physical characteristics			
Neonatal hypotonia	HP:0001319	54/79 (68.4%)	2/2
Congenital contractures	HP:0002803	93/106 (87.7%)	
AMC	HP:0002804	15/31 (48.4%)	11/11
Abnormality of the eye	HP:0000478	41/52 (78.8%)	
Scoliosis	HP:0002650	38/66 (57.6%)	
Short stature	HP:0004322	34/49 (69.4%)	
Small hands	HP:0200055	30/49 (61.2%)	
Camptodactyly of finger	HP:0100490	27/54 (50.0%)	4/4
Tapered fingers	HP:0001182	15/44 (34.1%)	
Small feet	HP:0001773	14/38 (36.8%)	
Bilateral clubfoot	HP:0001776	8/27 (29.6%)	4/4
Facial dysmorphism	HP:0001999	64/70 (91.4%)	5/5
Endocrinology			
Hypopituitarism	HP:0040075	7/13 (53.8%)	1/1
Growth hormone deficiency	HP:0000824	16/22 (72.7%)	
Hypothyroidism	HP:0000821	8/27 (29.6%)	
Hypoglycaemia	HP:0001943	14/22 (63.6%)	
Temperature instability	HP:0005968	39/62 (62.9%)	
Diabetes insipidus	HP:0000873	5/17 (29.4%)	
Hypogonadism	HP:0000135	40/80 (50%)	2/2
Cryptorchidism	HP:0000028	8/12 (66.7%)	
Other alterations			
Congenital heart defect	HP:0001627	7/20 (35.0%)	
Bradycardia	HP:0001662	3/4 (75.0%)	
Sleep disturbance	HP:0002360	13/13 (100%)	
Death in infancy/childhood	HP:0001522/HP:0003819	10 cases	13 cases

AMC, arthrogyposis multiplex congenita; F, female; HPO, Human Phenotype Ontology; M, male; SYS, Schaaf-Yang syndrome; UnK, unknown.



## Schaaf-Yang Syndrome Clinical Management



Numbers in black dots state the recommended periodicity for clinical evaluation in months for every specialty for all the individuals. A P in a red dot means that those complementary exams and management tools need to be personalized, and applied only if patient's personal characteristics require them, always following the medical team criteria.

**Figure 1** Schematic guidelines for Schaaf-Yang syndrome clinical management. AEDs, anti epileptic drugs; ASD, autism spectrum disorder; CC, corpus callosum; EEG, electroencephalogram; EMG/NCV, electromyogram/nerve conduction velocity; GH, growth hormone; NICU, neonatal intensive care unit; PSG, polysomnography. Perinatal period: Boxes include the medical area of disease. Medical problems and management requirements are detailed below. Childhood and adolescence: Boxes include medical area of disease. Medical problems and diagnosis are detailed below in bold. Management recommendations and complementary examinations are below, not in bold letters with lighter colours at the bottom. Numbers in black dots state the recommended periodicity for clinical evaluation in months for every specialty for all the individuals. In contrast, a P in a red dot means that those complementary examinations and management tools need to be personalised, and applied only if patient's personal characteristics require them, always following the medical team criteria. For instance, MRI and cardiology are recommended at diagnosis/birth for all patients, but follow-up depends on comorbidities. Please see online supplemental table 2 for more details. A Spanish version of this figure is included as online supplemental figure 1.

which confirmed significant upregulation of *HOTAIR* and *PITX1* and downregulation of *TBX5* (online supplemental figure 2C).

Enrichment analysis on the 132 identified DEGs highlighted a group of five genes related to 'collagen formation' and various mitosis-related REACTOME categories, such as 'Resolution of Sister Chromatid Cohesion' and 'Mitotic Spindle Checkpoint' (online supplemental figure 2D). Consistently, most of those genes were also present in the 'Kinetochore' and 'Chromosome, centromeric region' categories according to Gene Ontology cellular component enrichment (online supplemental table 5).

#### SYS fibroblasts show decreased $A\beta_{1-40}$ peptide secretion levels

Given *MAGEL2* function in the retromer, the truncation of *MAGEL2* could affect APP cleavage and  $A\beta_{1-40}$  peptide production rates. Thus, levels of  $A\beta_{1-40}$  and  $A\beta_{1-42}$  peptides were measured by ELISA in the extracellular medium from SYS, PWS and control fibroblasts in the search for a potential biomarker of truncated *MAGEL2* function. SYS fibroblasts showed significantly decreased extracellular levels of the  $A\beta_{1-40}$  processed peptide, both compared with PWS and control fibroblasts. No differences were observed in the PWS group compared with the control (figure 3A). Levels of  $A\beta_{1-42}$  peptide were extremely low

and no differences were observed between conditions (data not shown).

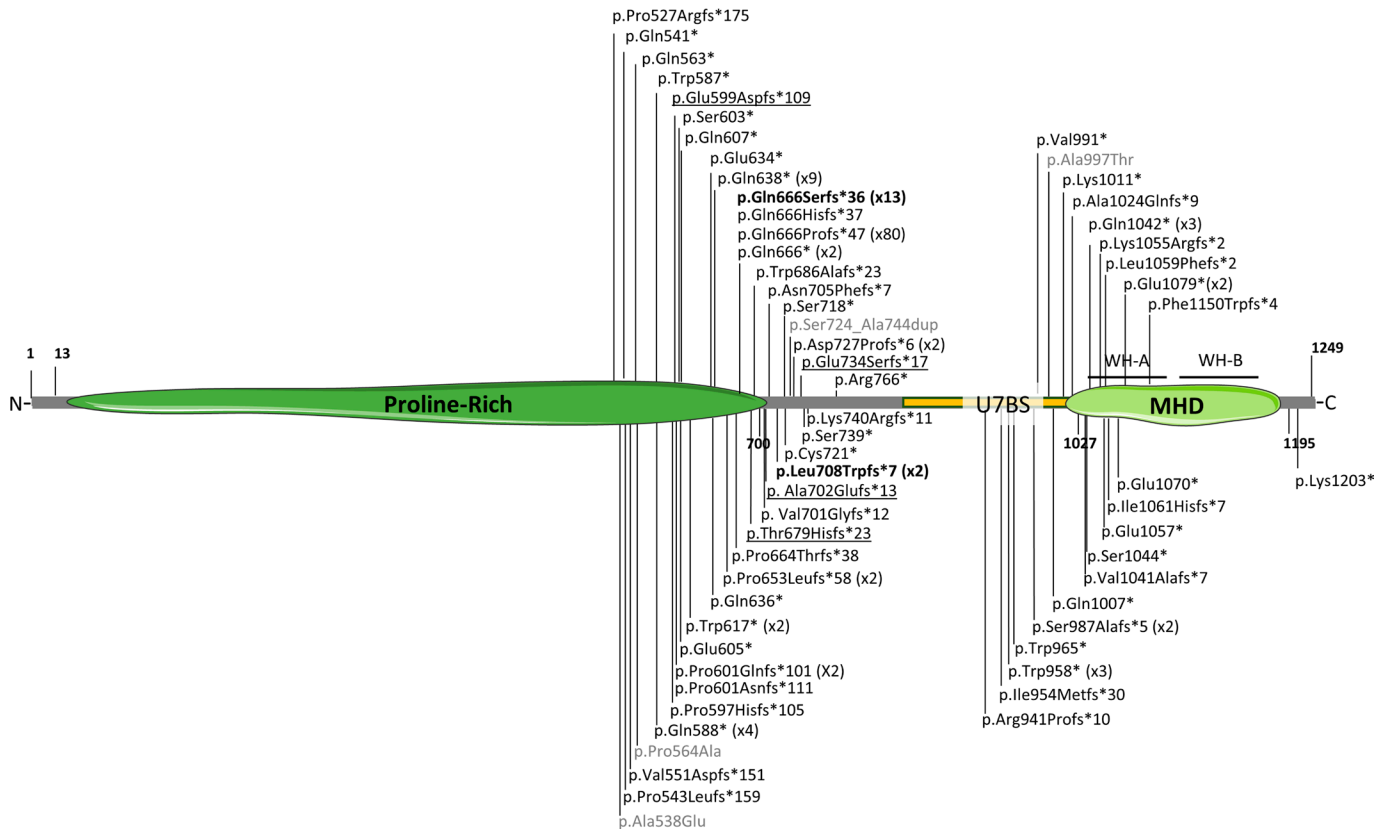
#### SYS fibroblasts show altered levels of organic acids and amino acids (metabolic profiling in fibroblasts)

Mass spectrometry analysis of intracellular metabolites from extracts of SYS (n=4), PWS (n=9) and control (n=5) fibroblasts showed a robust and significant decrease in glutamine levels for SYS fibroblasts (figure 3B), as well as a significant increase of suberic, sebacic, adipic and malic organic acids levels (online supplemental figure 3).

We investigated if any of the genes involved in these metabolites' pathways were differentially expressed in the transcriptomic analysis (online supplemental table 4). The only differentially expressed gene involved in glutamine (GO:0006541) or organic acid (GO:0006082) metabolic processes was *ME1* (ENSG00000065833), encoding the NADP-dependent malic enzyme protein, which was upregulated in SYS fibroblasts.

#### Truncated form of *MAGEL2* remains stable in the cell

To evaluate the stability and recycling of a truncated form of *MAGEL2*, HEK293T cells were transfected with haemagglutinin



**Figure 2** Schematic representation of *MAGEL2* disease-associated variants. MHD, MAGE Homology Domain. The number in brackets indicates the number of individuals carrying recurrent mutations. In bold, mutations p.Gln666Serfs\*36 (x13) and p.Leu708Trpfs\*7 (x2) associated with arthrogyriposis multiplex congenita. In grey, atypical, non-truncating variants. Previously misreported variants c.1797\_1820del (p.Glu599Aspfs\*109 (c.1797\_1810del)), c.224delC (p.Thyr679Hisfs\*23 (c.2034delC)), c.2015delC (p.Ala702Glufs\*13 (c.2105delC)) and c.390delA (p.Glu734Serfs\*17 (c.2199delA))<sup>10</sup> are underlined. All variants are referenced to the *MAGEL2* hg38 transcript NM\_019066.5. The complete list of variants, their cDNA annotation and reference are collected in online supplemental table 4. Image created with Servier Medical Art (smart.servier.com).

(HA)-tagged expression vectors containing either the wild-type (WT) cDNA sequence of *MAGEL2* (*MAGEL2*-WT) or the c.1912C>T; p.Gln638\* (*MAGEL2*-Gln638\*) mutation, which encodes a protein 611 amino acids shorter than the WT form and has been reported in nine patients (online supplemental table 1). Blocking of the proteasomal degradation pathway with MG132 (online supplemental figure 4A) or the lysosomal pathway with bafilomycin (online supplemental figure 4B) showed that *MAGEL2* is mostly degraded via proteasome with no differences between WT and truncated *MAGEL2* (online supplemental figure 4A,B). A time-course of cycloheximide treatment (2, 4, 6, 12 hours) showed also a similar stability and half-life for both *MAGEL2* forms (online supplemental figure 4C).

### Increased nuclear localisation of the truncated form of *MAGEL2*

To determine the subcellular localisation of the p.Gln638\* truncated form of *MAGEL2*, HEK293T, SAOS-2 and HeLa cells were transfected with the expression vectors *MAGEL2*-WT or *MAGEL2*-Gln638\*. Immunocytochemistry assays detecting HA showed that in SAOS-2 cells transfected with the *MAGEL2*-Gln638\* construct and quantified by Mander's coefficient there was a shift of the protein towards the nucleus, while the *MAGEL2*-WT form was mainly located in the cytoplasm (figure 3C,D). A similar localisation pattern was observed in HEK293T and HeLa cells (data not shown), supporting the idea that presence of variant p.Gln638\*, which leads to the lack of

part of the C-terminal sequence, affects protein subcellular localisation independently of the cell type. This result is consistent with predictions obtained following the Scandinavian Protocol, which combines different online tools to predict protein subcellular localisation.<sup>52 53</sup>

### DISCUSSION

Since its first description in 2013,<sup>1</sup> more than 150 SYS individuals carrying *MAGEL2*-truncating mutations have been published. Most publications include one or a reduced group of patients, often with scarce clinical descriptions, hampering the definition of the syndrome's characteristics. Families, already burdened by the solitude of having an ultrarare condition, lack clear and established clinical guidelines for their treatment and follow-up. Once available, such evidence-based recommendations will help reduce inequity in healthcare and empower both families and clinicians facing such a rare disease. To develop the recommendations, we performed an extensive revision of all the SYS subjects published so far at both the phenotypic and genetic level and elaborated a comprehensive and detailed follow-up programme. Despite the comprehensive revision, to date, no clear underlying phenotype-genotype correlation was observed, with the exception of two particular variants [p.(Gln666Serfs\*36) and p.(Leu708Trpfs\*7)] which are associated with the much more severe phenotype of AMC leading to perinatal death.

Genetically, SYS is caused by non-sense or frameshift mutations. Only one *MAGEL2* missense variant (c.1613C>A;

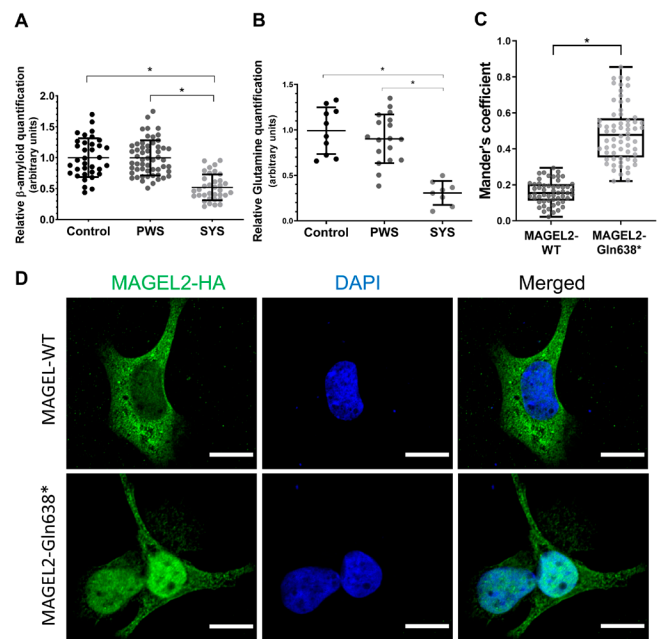
**Table 2** Top 10 upregulated and downregulated DEGs identified after mRNASeq analysis of skin fibroblasts in patients with SYS and controls

	Gene	ID	Log <sub>2</sub> FC	FDR
Upregulated	<b>HOTAIR</b>	ENSG00000228630	7.470	8.02E-30
	<b>MTRNR2L1</b>	ENSG00000256618	5.523	1.14E-05
	<b>PTPRD</b>	ENSG00000153707	4.625	1.23E-06
	<b>SLITRK4</b>	ENSG00000179542	4.524	8.81E-03
	<b>GATA2-AS1</b>	ENSG00000244300	4.304	1.41E-18
	<b>PITX1</b>	ENSG00000069011	4.234	8.16E-03
	<b>CLDN1</b>	ENSG00000163347	4.145	1.12E-06
	<b>ISL2</b>	ENSG00000159556	4.088	1.93E-03
	<b>RARB</b>	ENSG00000077092	4.000	1.49E-02
	<b>VANGL2</b>	ENSG00000162738	3.975	8.09E-07
Downregulated	<b>ANGPTL1</b>	ENSG00000116194	-7.084	3.66E-06
	<b>TBX5</b>	ENSG00000089225	-6.982	9.18E-39
	<b>PI16</b>	ENSG00000164530	-6.385	1.29E-03
	<b>TBX5-AS1</b>	ENSG00000255399	-5.457	5.44E-12
	<b>INMT</b>	ENSG00000241644	-5.041	2.56E-03
	<b>CRLF1</b>	ENSG00000006016	-5.029	6.80E-04
	<b>ASPA</b>	ENSG00000108381	-4.813	1.02E-03
	<b>WSP2</b>	ENSG00000064205	-4.420	3.87E-08
	<b>KY</b>	ENSG00000174611	-4.248	1.30E-11
	<b>APOD</b>	ENSG00000189058	-4.213	6.00E-05

Genes analysed by quantitative PCR are given in bold. DEGs, differentially expressed genes; FC, fold change; FDR, false discovery rate ; mRNASeq, mRNA sequencing; SYS, Schaaf-Yang syndrome .

p.Ala538Glu) has been reliably described as potentially disease-associated.<sup>17</sup> While at the moment of its publication, this change was classified as ‘disease causing’ based on available in silico and frequency data, currently, updated information, including a gnomAD V3.1 Amish MAF of 0.03, supports its reclassification as ‘likely benign’ according to the American College of Medical Genetics (ACMG) guidelines.<sup>54</sup> Clinically, the patient carrying this variant presented with DD, ASD and dysmorphic traits. While her presentation shares some traits with SYS, the high frequency of this variant in the Amish population, together with the non-specific character of these traits, would suggest that it is not the main cause of the disease. Two other missense mutations have been identified in two different patients: p.Pro564Ala and p.Ala997Thr (with two and four carriers in gnomAD v3.1.1, respectively). However, their potential causality was not further discussed and there are no clinical descriptions of the patients.<sup>10</sup> An in-frame duplication in the paternal chromosome (p.Ser724\_Ala744dup, two carriers in gnomAD v3.1) has also been identified in a patient with a clinical presentation sharing some resemblances with SYS, but the scarcity of available information makes it difficult to fully understand the clinical role of this variant.<sup>21</sup> We have also noticed some missannnotations, probably due to the fact that, originally, *MAGEL2* was predicted to encode a 529-amino acid protein (instead of the current 1249 amino acids) lacking the N-terminal domain (hg38). This is the case for reported variants p.(Thr76Hisfs\*23) and p.(Glu131Serfs\*17),<sup>10</sup> which we have relabelled as p.(Thr679Hisfs\*23) and p.(Glu734Serfs\*17). To sum up, while the pathogenicity of *MAGEL2*-truncating mutations has been clearly established and documented, it remains to be clarified whether any *MAGEL2* missense or in-frame mutation is really a causal variant for SYS.

The phenotypic overlap between SYS and PWS suggests that the alteration of *MAGEL2* may contribute to some aspects of



**Figure 3** Molecular and cellular biomarkers for Schaaf-Yang syndrome (SYS). (A) Amyloid- $\beta$  1–40 ( $A\beta_{1-40}$ ) peptide levels in control, Prader-Willi syndrome (PWS) and SYS fibroblasts’ extracellular medium. Data obtained from at least three independent experiments (SYS: n=5; PWS: n=9; control: n=5). (B) Glutamine levels in control, PWS and SYS fibroblasts (SYS: n=6; PWS: n=9; control: n=6). Values from two independent experiments have been normalised to the mean of the control group. Horizontal lines represent mean values and error bars represent the SD. Statistical analyses were performed using one-way analysis of variance and Tukey’s multiple comparisons test in GraphPad Prism. \* $p < 0.001$ . (C) Colocalisation quantification using Mander’s coefficient between the haemagglutinin (HA) fluorescence signal and DAPI (4’,6-diamidino-2-phenylindole) in *MAGEL2*-wild-type (WT) and *MAGEL2*-Gln638\* transfected cells normalised by the total HA intensity. n=118 from six independent experiments. \* $p < 0.001$ . (D) Representative immunofluorescence images of SAOS-2 cells transfected with *MAGEL2*-WT and *MAGEL2*-Gln638\* plasmids, stained with anti-HA (green, tagging *MAGEL2*) or DAPI (blue, cell nuclei). Scale bar represents 15  $\mu$ m.

the PWS phenotype, but the extent of this contribution is still an open question. Two patients carrying atypical deletions involving only *MAGEL2*, *NDN* and *MKRN3* did not show a full PWS phenotype: one patient showed only mild delayed motor skills and the other displayed obesity, DD and high pain threshold.<sup>55,56</sup> At the same time, neither does the deletion lead to SYS, further supporting that it is the presence of the truncated form of the *MAGEL2* protein that leads to some of the particular aspects of SYS. As our results show, the truncated protein is synthesised, it is stable and it is not degraded any faster than the WT form in the first 12 hours. We propose that this truncated form could be exerting toxic effects in addition to the negative effects caused by the lack of WT *MAGEL2* protein.

The role of *MAGEL2* in the regulation of endosomal protein trafficking and recycling has been widely studied.<sup>41,42,57</sup> Consistent with this, loss of paternal *MAGEL2* expression in *Magel2*<sup>PA/m+</sup> mice and neuronal cell models derived from patient with PWS leads to decreased levels of secretory granule proteins, which lead to reduced levels of circulating bioactive hormones and of mature secretory granules in neurons.<sup>36</sup> Also, dental pulp stem cell-derived neurons from several patients with PWS and



one patient with SYS showed impaired trafficking of M6PR (cation-dependent mannose-6-phosphate receptor), indicating impaired endosome-mediated retrograde transport. We hypothesised that this impaired trafficking could also be reflected in an aberrant A $\beta$  peptide production and exocytosis.<sup>48–50</sup> Indeed, A $\beta$ <sub>1–40</sub> peptide extracellular levels of SYS fibroblasts were significantly decreased compared with those of PWS and control groups, which showed no difference between them. This result supports the idea that the truncated form of the protein may have a different effect on protein trafficking than the complete loss of the protein itself and establishes A $\beta$ <sub>1–40</sub> levels as a potential biomarker for the disease in patient-derived cells.

Despite being its most deeply studied function, dysregulation of protein trafficking and recycling is not the only consequence of MAGEL2 malfunction. The subcellular localisation of the heterologously expressed full MAGEL2 protein<sup>41 42</sup> and its C-terminal part alone<sup>39</sup> has been previously studied, both presenting a mainly cytoplasmic localisation. Our immunocytochemistry assays in different transfected cell lines showed that, in contrast, the heterologously expressed MAGEL2-Gln638\* (N-terminal part of the protein) was predominantly located inside the nucleus. While the lack of a properly functioning MAGEL2 antibody hampers the validations of these experiments in the endogenous MAGEL2, they point to new functions of the truncated protein. The increased nuclear localisation of the protein suggests that, in addition to the loss of the MAGEL2 normal functions, including the translocation of YTHDF2 to the nucleus,<sup>39</sup> SYS-associated mutations could involve new functions of unknown consequences.

To explore if the truncated form of MAGEL2 could be affecting the expression of other genes, we applied an unbiased transcriptomic approach in fibroblasts. We found an enrichment in genes related to mitosis, nuclear function and localisation, as well as in developmental and neurological processes.

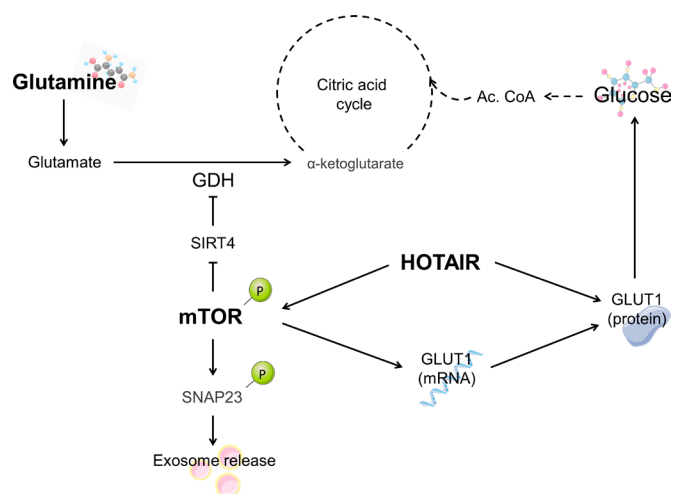
Previous studies on the full-length and C-terminal parts of the MAGEL2 protein have shown that this protein is involved in RNA metabolic processes.<sup>39</sup> In our transcriptomic analysis, *HOTAIR* expression was clearly increased in SYS fibroblasts. *HOTAIR* is a well-known long non-coding RNA that mediates transcriptional silencing in trans. This gene's promoter contains binding sites for numerous transcription factors, including AP1 (activator protein 1), Sp1 (specificity protein 1) and NF- $\kappa$ B (Nuclear Factor Kappa B Subunit 1).<sup>58</sup> It is able to exert epigenetic functions through H3K27 trimethylation and H3K4 demethylation, among other regulatory functions, and has a widely studied role in cancer (reviewed in Ref. 59). *HOTAIR* promotes transcriptional silencing of the *HOXD* locus, which plays an essential role in determining body axes and orchestrating organ formation during vertebrate development.<sup>60</sup> *HOTAIR* is also a regulator of mTOR, increasing its phosphorylation and mTOR-mediated exosome release,<sup>61</sup> and its regulation of GLUT1 levels (figure 4).<sup>62</sup> While our mRNASeq data do not show an increase in mTOR levels, SYS-derived fibroblasts have been reported to show an increase in mTOR expression and activity.<sup>63</sup>

Endocrine and metabolic alterations in PWS have been widely studied.<sup>64</sup> However, little is known regarding metabolic impairment in SYS. A study including five female and four male patients with SYS showed that they may present some but not all the endocrine alterations also observed in PWS: patients benefit from growth hormone (GH) therapy, show increased ghrelin levels and present a high risk of developing diabetes mellitus.<sup>10</sup> A review on the literature on endocrine abnormalities in MAGEL2-related syndromes<sup>26</sup> showed that the most common hormonal alterations involve GH, thyroid-stimulating hormone,

adrenocorticotrophic hormone, antidiuretic hormone and gonadotropins, probably caused by hypothalamic impairment.

The targeted metabolomic analysis of SYS-derived and PWS-derived fibroblasts was aimed at finding biomarkers that assess the lysosomal, peroxisomal, mitochondrial and cytosolic metabolic pathways. The most relevant finding was a significant reduction in glutamine levels in SYS fibroblasts compared with the other two groups, whereas glutamate levels remained unchanged. Glutamine supplies nitrogen and carbon for biosynthetic reactions in rapidly proliferating cells,<sup>65</sup> but in many contexts its key role relies on providing glutamate (figure 4) which has a wider range of metabolic functions than glutamine itself. An example of the relevant functions of glutamine and glutamate is the glutamate/GABA-glutamine cycle in neurons.<sup>66</sup> In fact, it has been stated that alterations of the GABAergic system may play an important role in aspects of the pathophysiology of PWS.<sup>67</sup> A hypothesis to explain the decrease in glutamine levels in SYS fibroblasts could be a dysregulation in the retrieval and recycling of a glutamine transporter, such as SLC1A5, whose retrieval and recycling is promoted by the retromer and whose degradation is enhanced on retromer knockout.<sup>68</sup> Another explanation could be related to the hyperactivation of the mTOR pathway, as mTOR plays a role in the glutamine metabolism by increasing glutamate dehydrogenase activity through the inhibition of SIRT4 (figure 4).<sup>69</sup> The hyperactivation of mTOR could drive a glutamate depletion which would be compensated by an increase in glutamine deamidation.

In conclusion, our results support the hypothesis that the SYS-specific phenotype might be explained by a neomorphic effect of the truncated protein, rather than by a dose-reduction situation. This is supported by the subtle changes in gene expression patterns and metabolite levels observed in fibroblasts, which suggest novel effects for the truncated protein, which warrant further exploration. In addition, we provide a comprehensive phenotypical delineation of the syndrome and a standardised set of guidelines aimed at the improvement of clinical management of patients with SYS. Finally, we propose to improve SYS



**Figure 4** Schematisation of the relationship between the different biomarkers identified in the Schaaf-Yang syndrome (SYS) fibroblasts. The increased levels of *HOTAIR* could lead to the upregulation of mTOR (mammalian target of rapamycin) previously seen in the literature as well as the deregulation of glucose uptake through GLUT1 (glucose transporter 1). Ac.CoA, Acetyl-coenzyme A; GDH, glutamate dehydrogenase; SIRT4, sirtuin 4; SNAP23, Synaptosome Associated Protein 23.

diagnosis by testing the pathogenicity of doubtful missense mutations through the in vitro overexpression system.

An extended version of the methodology is included as online supplemental material. Briefly, we performed a systematic review of the literature indexed in PubMed from the date of the first clinical description of pathology associated with variants in *MAGEL2* until February 2022 using the terms: ‘*MAGEL2*’, ‘*SYS*’ and ‘Schaaf-Yang syndrome’. All mutations have been referenced to the *MAGEL2* hg38 main transcript NM\_019066.5. Fibroblasts were obtained from skin biopsies of 7 *SYS* individuals, 9 patients with PWS and 11 controls (online supplemental table 6). All cell cultures (fibroblasts, HEK293T, HeLa and SAOS-2) were cultured in standard cell culture conditions. The cDNA sequences of interest were cloned in the mammalian expression vector pcDNA<sup>TM</sup>3.1<sup>(+)</sup> (Invitrogen, ThermoFisher Scientific) including a HA tag and transfected into the different cell lines. Cells were treated with MG132, bafilomycin or cycloheximide. Whole RNA was extracted from fibroblasts and RNA-Seq was performed by LEXOGEN and analysed with the ExpHunter Suite.<sup>70</sup> Expression levels of four selected genes were analysed by qPCR. Protein was extracted using RIPA buffer, resolved by SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and immunoblotted following standard biochemical techniques. For immunocytochemistry, cells were fixed in 4% paraformaldehyde (PFA), permeabilised and blocked. Coverslips were incubated with anti-HA primary antibody and DAPI. For ELISA quantification, the supernatant of the collected media (without FBS (fetal bovine serum)) was obtained and analysed by the  $\text{A}\beta_{1-40}$  high-sensitive ELISA or the  $\text{A}\beta_{1-42}$  high-sensitive ELISA (IBL International). For metabolomics, amino acids were analysed using ultrahigh-performance liquid chromatography–tandem mass spectrometry and organic acids with gas chromatography–mass spectrometry. Statistical analysis was performed using R-Studio. Differences were considered significant if  $p < 0.05$ .

All skin donors or their legal representative gave their written informed consent. Their samples and data were obtained in accordance with the Declaration of Helsinki 1964, as revised in October 2013 (Fortaleza, Brazil).

#### Author affiliations

<sup>1</sup>Department of Genetics, Microbiology and Statistics, IBUB, Facultat de Biologia, Universitat de Barcelona, Barcelona, Spain

<sup>2</sup>Institut de Recerca Sant Joan de Déu, Esplugues de Llobregat, Barcelona, Spain

<sup>3</sup>Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Instituto de Salud Carlos III, Madrid, Spain

<sup>4</sup>Clinical Biochemistry Department, Hospital Sant Joan de Déu, Esplugues de Llobregat, Barcelona, Spain

<sup>5</sup>Neurology Department, Hospital Sant Joan de Déu, Esplugues de Llobregat, Barcelona, Spain

<sup>6</sup>Department of Molecular Biology and Biochemistry; Institute of Biomedical Research in Málaga (IBIMA), University of Málaga, Málaga, Spain

**Twitter** Raquel Rabionet @krabionet and Roser Urreiziti @Roser\_uf

**Acknowledgements** We would like to thank the patients’ associations Associació Síndrome Opitz C, AESYS (Asociación Española del Síndrome Schaaf-Yang) for their generous financial support and Associació PWS-Catalunya and all the families who kindly donated samples. We thank Monica Cozar for her technical assistance.

**Contributors** MC-P, AP-P, RR, DG and RU gathered and reviewed all the clinical and mutation data previously published and elaborated the corresponding figures and tables. HF-V, LC-V, MC-P, RMC, SB and GM performed and analysed the heterologous expression experiments, WB and ICC. HF-V, RM-C, LC-V, MC-P and RU performed amilod Beta experiments. RA, CO, AJP-F, MC-P and RU performed and analyzed the metabolomics studies. LC-V, ER, JAGR, DG, SB and PS performed and analysed the mRNASeq experiments and further analysis. LCV, MS, RR, SB and RU drafted the manuscript. RU acts as a guarantor for this manuscript. All authors have critically reviewed and approved the manuscript.

**Funding** This research was funded through the Spanish Ministerio de Ciencia e Innovación (SAF2016-75946R and PID2019-107188RB-C21), the Instituto de Salud Carlos III-CIBERER (ACCI19P2AC720-1) and donations from Associació Síndrome Opitz C and Asociación Española del Síndrome Schaaf-Yang (AESYS). MC-P is supported by a Carmen de Torres fellowship from IRSJD. Funding sources were not involved in the study design, collection, analysis and interpretation of data, writing of the report or in the publication of the article.

**Competing interests** None declared.

**Patient consent for publication** Not applicable.

**Ethics approval** The study was approved by the Institutional Review Board (IRB00003099) of the Bioethical Commission of the University of Barcelona (5 October 2020) and Hospital Sant Joan de Déu (PIC-111-19).

**Provenance and peer review** Not commissioned; externally peer reviewed.

**Data availability statement** Data are available on reasonable request.

**Supplemental material** This content has been supplied by the author(s). It has not been vetted by BMJ Publishing Group Limited (BMJ) and may not have been peer-reviewed. Any opinions or recommendations discussed are solely those of the author(s) and are not endorsed by BMJ. BMJ disclaims all liability and responsibility arising from any reliance placed on the content. Where the content includes any translated material, BMJ does not warrant the accuracy and reliability of the translations (including but not limited to local regulations, clinical guidelines, terminology, drug names and drug dosages), and is not responsible for any error and/or omissions arising from translation and adaptation or otherwise.

**Open access** This is an open access article distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited, appropriate credit is given, any changes made indicated, and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>.

#### ORCID iDs

Laura Castilla-Vallmanya <http://orcid.org/0000-0002-2260-9664>

Mónica Centeno-Pla <http://orcid.org/0000-0002-8881-0264>

Mercedes Serrano <http://orcid.org/0000-0002-2056-2428>

Aina Prat-Planas <http://orcid.org/0000-0003-3040-6549>

Abraham J Paredes-Fuentes <http://orcid.org/0000-0002-7163-2385>

Gemma Marfany <http://orcid.org/0000-0001-7941-983x>

Rafael Artuch <http://orcid.org/0000-0002-3422-9685>

Daniel Grinberg <http://orcid.org/0000-0001-9859-2590>

Raquel Rabionet <http://orcid.org/0000-0001-5006-8140>

Susanna Balcells <http://orcid.org/0000-0003-1211-1907>

Roser Urreiziti <http://orcid.org/0000-0003-3617-7134>

#### REFERENCES

- Schaaf CP, Gonzalez-Garay ML, Xia F, Potocki L, Gripp KW, Zhang B, Peters BA, McElwain MA, Drmanac R, Beaudet AL, Caskey CT, Yang Y. Truncating mutations of *MAGEL2* cause Prader-Willi phenotypes and autism. *Nat Genet* 2013;45:1405–8.
- Soden SE, Saunders CJ, Willig LK, Farrow EG, Smith LD, Petrikon JE, LePichon J-B, Miller NA, Thiffault I, Dinwiddie DL, Twist G, Noll A, Heese BA, Zellmer L, Atherton AM, Abdelmoity AT, Safina N, Nyp SS, Zuccarelli B, Larson IA, Modrcin A, Herd S, Creed M, Ye Z, Yuan X, Brodsky RA, Kingsmore SF. Effectiveness of exome and genome sequencing guided by acuity of illness for diagnosis of neurodevelopmental disorders. *Sci Transl Med* 2014;6:265ra168.
- Urreiziti R, Cueto-Gonzalez AM, Franco-Valls H, Mort-Farre S, Roca-Ayats N, Ponomarenko J, Cozzuto L, Company C, Bosio M, Ossowski S, Montfort M, Hecht J, Tizzano EF, Cormand B, Vilageliu L, Opitz JM, Neri G, Grinberg D, Balcells S. A de novo nonsense mutation in *MAGEL2* in a patient initially diagnosed as Opitz-C: similarities between Schaaf-Yang and Opitz-C syndromes. *Sci Rep* 2017;7:44138.
- Fountain MD, Aten E, Cho MT, Juusola J, Walkiewicz MA, Ray JW, Xia F, Yang Y, Graham BH, Bacino CA, Potocki L, van Haeringen A, Ruivenkamp CAL, Mancias P, Northrup H, Kukulich MK, Weiss MM, van Ravenswaaij-Arts CMA, Mathijssen IB, Levesque S, Meeks N, Rosenfeld JA, Lemke D, Hamosh A, Lewis SK, Race S, Stewart LL, Hay B, Lewis AM, Guerreiro RL, Bras JT, Martins MP, Derksen-Lubsen G, Peeters E, Stumpel C, Stegmann S, Bok LA, Santen GWE, Schaaf CP. The phenotypic spectrum of Schaaf-Yang syndrome: 18 new affected individuals from 14 families. *Genet Med* 2017;19:45–52.
- Palomares-Bralo M, Vallespin E, Del Pozo Ángela, Ibañez K, Silla JC, Galán E, Gordo G, Martínez-Glez V, Alba-Valdivia LI, Heath KE, García-Miñaur S, Lapunzina P, Santos-Simarro F. Pitfalls of trio-based exome sequencing: imprinted genes and parental mosaicism-MAGEL2 as an example. *Genet Med* 2017;19:1285–6.
- Enya T, Okamoto N, Iba Y, Miyazawa T, Okada M, Ida S, Naruto T, Imoto I, Fujita A, Miyake N, Matsumoto N, Sugimoto K, Takemura T. Three patients with Schaaf-Yang syndrome exhibiting arthrognosis and endocrinological abnormalities. *Am J Med Genet A* 2018;176:707–11.



- 7 Hidalgo-Santos A, Del Carmen DeMingo-Delemany M, Moreno-Macián F, Roselló M, Orellana C, Martínez F, Caro-Llopis A, León-Cariñena S, Tomás-Vila M. A Novel mutation of *MAGEL2* in a patient with Schaaf-Yang syndrome and hypopituitarism. *Int J Endocrinol Metab* 2018;16:e67329.
- 8 Kleindendorst L, Pi Castán G, Caro-Llopis A, Boon EMJ, van Haelst MM. The role of obesity in the fatal outcome of Schaaf-Yang syndrome: early onset morbid obesity in a patient with a *MAGEL2* mutation. *Am J Med Genet A* 2018;176:2456–9.
- 9 Matuszewska KE, Badura-Stronka M, Śmigiel R, Cabała M, Biernacka A, Kosinska J, Rydzanicz M, Winczewska-Wiktor A, Sasiadek M, Latos-Bielerska A, Żemojtel T, Płoski R. Phenotype of two Polish patients with Schaaf-Yang syndrome confirmed by identifying mutation in *MAGEL2* gene. *Clin Dysmorphol* 2018;27:49–52.
- 10 McCarthy J, Lupo PJ, Kovar E, Rech M, Bostwick B, Scott D, Kraft K, Roscioli T, Charrow J, Schrier Vergano SA, Lose E, Smiegel R, Lacassie Y, Schaaf CP. Schaaf-Yang syndrome overview: report of 78 individuals. *Am J Med Genet A* 2018;176:2564–74.
- 11 McCarthy JM, McCann-Crosby BM, Rech ME, Yin J, Chen C-A, Ali MA, Nguyen HN, Miller JL, Schaaf CP, Hormonal SCP. Hormonal, metabolic and skeletal phenotype of Schaaf-Yang syndrome: a comparison to Prader-Willi syndrome. *J Med Genet* 2018;55:307–15.
- 12 Bayat A, Bayat M, Lozoya R, Schaaf CP. Chronic intestinal pseudo-obstruction syndrome and gastrointestinal malrotation in an infant with schaff-yang syndrome - Expanding the phenotypic spectrum. *Eur J Med Genet* 2018;61:627–30.
- 13 Poliak N, Rajan P. *MAGEL2* gene mutation and its associated phenotypic features in a five-month-old female. *J Pediatr Neonatal Care* 2018;8.
- 14 Tong W, Wang Y, Lu Y, Ye T, Song C, Xu Y, Li M, Ding J, Duan Y, Zhang L, Gu W, Zhao X, Yang X-A, Jin D. Whole-Exome sequencing helps the diagnosis and treatment in children with neurodevelopmental delay accompanied unexplained dyspnea. *Sci Rep* 2018;8:5214.
- 15 Gregory LC, Shah P, Sanner JRF, Arancibia M, Hurst J, Jones WD, Spoudeas H, Le Quesne Stabej P, Williams HJ, Ocaka LA, Loureiro C, Martinez-Aguayo A, Dattani MT. Mutations in *MAGEL2* and *L1CAM* are associated with congenital hypopituitarism and arthrogryposis. *J Clin Endocrinol Metab* 2019;104:5737–50.
- 16 Negishi Y, Ieda D, Hori I, Nozaki Y, Yamagata T, Komaki H, Tohyama J, Nagasaki K, Tada H, Saitoh S. Schaaf-Yang syndrome shows a Prader-Willi syndrome-like phenotype during infancy. *Orphanet J Rare Dis* 2019;14:277.
- 17 Patak J, Giffert J, Byler M, Neerukonda V, Thiffault I, Cross L, Amudhavalli S, Pacio-Miguez M, Palomares-Bralo M, Garcia-Minaur S, Santos-Simarro F, Powis Z, Alcaraz W, Tang S, Jurgens J, Barry B, England E, Engle E, Hess J, Lebel RR. *MAGEL2*-related disorders: a study and case series. *Clin Genet* 2019;96:493–505.
- 18 de Andrade G, de Oliveira Silva T, Nascimento do I, Boath A, Cunha K, Chermont A. Schaaf-Yang syndrome: a novel variant in *MAGEL2* gene in the first Brazilian preterm neonate. *Int J Case Rep Images* 2020;11.
- 19 Ahn H, Seo GH, Oh A, Lee Y, Keum C, Heo SH, Kim T, Choi J, Kim G-H, Ko T-S, Yum M-S, Lee BH, Choi IH. Diagnosis of Schaaf-Yang syndrome in Korean children with developmental delay and hypotonia. *Medicine* 2020;99:e23864.
- 20 Xiao B, Ji X, Wei W, Hui Y, Sun Y. A recurrent variant in *MAGEL2* in five siblings with severe respiratory disturbance after birth. *Mol Syndromol* 2020;10:286–90.
- 21 Marbach F, Elgizouli M, Rech M, Beygo J, Erger F, Velmans C, Stumpel CTRM, Stegmann APA, Beck-Wödl S, Gillessen-Kaesbach G, Horsthemke B, Schaaf CP, Kuechler A. The adult phenotype of Schaaf-Yang syndrome. *Orphanet J Rare Dis* 2020;15:294.
- 22 Llamas-Paneque A, Gómez-García A, Rivas-Iglesias C, Garzón- Castro M, Hernández-Iñiguez M, Recalde-Báez M. Schaaf-Yang syndrome: an example of genomic imprinting and expanding phenotype. *J Mol Genet Med* 2020;14.
- 23 Bertoli-Avella AM, Beetz C, Ameziane N, Rocha ME, Guatibonza P, Pereira C, Calvo M, Herrera-Ordóñez N, Segura-Castel M, Diego-Alvarez D, Zawada M, Kandaswamy KK, Werber M, Paknia O, Zielske S, Ugrinowski D, Warnack G, Kampe K, Iuraşcu M-I, Cozma C, Vogel F, Alhashem A, Hertecant J, Al-Shamsi AM, Alswaid AF, Eyaïd W, Al Mutairi F, Alfares A, Albalwi MA, Alfadhel M, Al-Sanna NA, Reardon W, Alanay Y, Rolfes A, Bauer P. Successful application of genome sequencing in a diagnostic setting: 1007 index cases from a clinically heterogeneous cohort. *Eur J Hum Genet* 2021;29:141–53.
- 24 Nunes S, Xavier M, Lourenço C, Melo M, Godinho C. Schaaf-Yang syndrome: a real challenge for prenatal diagnosis. *Cureus* 2021;13:e20414.
- 25 Duan Y, Liu L, Zhang X, Jiang X, Xu J, Guan Q. Phenotypic spectrum and mechanism analysis of Schaaf Yang syndrome: a case report on new mutation of *MAGEL2* gene. *Medicine* 2021;100:e26309.
- 26 Halloun R, Habib C, Ekhlevitch N, Weiss R, Tiosano D, Cohen M. Expanding the spectrum of endocrinopathies identified in Schaaf-Yang syndrome - A case report and review of the literature. *Eur J Med Genet* 2021;64:104252.
- 27 Laquerrière A, Jaber D, Abiusi E, Maluenda J, Mejlachowicz D, Vivanti A, Dieterich K, Stoeva R, Quevarec L, Nolent F, Biancalana V, Latour P, Sternberg D, Capri Y, Verloes A, Bessieres B, Loeuillet L, Attie-Bitach T, Martinovic J, Blesson S, Petit F, Beneteau C, Whalen S, Marguet F, Bouligand J, Héron D, Viot G, Amiel J, Amram D, Bellesme C, Bucourt M, Fèvre L, Jouk P-S, Khung S, Sigaudy S, Delezoide A-L, Goldenberg A, Jacquemont M-L, Lambert L, Layet V, Lyonnet S, Munnich A, Van Maldergem L, Piard J, Guimiot F, Landrieu P, Letard P, Pelluard F, Perrin L, Saint-Frison M-H, Topaloglu H, Trestard L, Vincent-Delorme C, Anthor H, Barnerias C, Benachi A, Bieth E, Boucher E, Cormier-Daire V, Delahaye-Duriez A, Desguerre I, Eymard B, Francannet C, Grotto S, Lacombe D, Laffargue F, Legendre M, Martin-Coignard D, Mégarbané A, Mercier S, Nizon M, Rigonnet L, Prieur F, Quélin C, Ranjatoelina-Randrianaivo H, Resta N, Toutain A, Verhelst H, Vincent M, Colin E, Fallet-Bianco C, Granier M, Grigorescu R, Saada J, Gonzales M, Guiochon-Mantel A, Bessereau J-L, Tawk M, Gut I, Gitiaux C, Melki J. Phenotypic spectrum and genomics of undiagnosed arthrogryposis multiplex congenita. *J Med Genet* 2022;59:559–567.
- 28 Stranneheim H, Lagerstedt-Robinson K, Magnusson M, Kvarnang M, Nilsson D, Lesko N, Engvall M, Anderlid B-M, Arnell H, Johansson CB, Barbaro M, Björck E, Bruhn H, Eisfeldt J, Freyer C, Grigelioniene G, Gustavsson P, Hammarsjö A, Hellström-Pigg M, Iwarsson E, Jemt A, Laaksonen M, Enoksson SL, Malmgren H, Naess K, Nordenskjöld M, Oscarson E, Pettersson M, Rasi C, Rosenbaum A, Sahlin E, Særdh E, Stöberg T, Tesi B, Tham E, Thonberg H, Töhönen V, von Döbeln U, Vassiliou D, Vonlanthen S, Wikström A-C, Wincent J, Winqvist O, Wredenberg A, Ygbeg S, Zetterström RH, Marits P, Soller MJ, Nordgren A, Wirta V, Lindstrand A, Wedell A. Integration of whole genome sequencing into a healthcare setting: high diagnostic rates across multiple clinical entities in 3219 rare disease patients. *Genome Med* 2021;13:40.
- 29 Costa RA, Ferreira IR, Cintra HA, Gomes LHF, Guida LdaC. Genotype-phenotype relationships and endocrine findings in Prader-Willi syndrome. *Front Endocrinol* 2019;10:864.
- 30 Fountain MD, Schaaf CP. Prader-Willi Syndrome and Schaaf-Yang Syndrome: Neurodevelopmental Diseases Intersecting at the *MAGEL2* Gene. *Diseases* 2016;4. doi:10.3390/diseases4010002. [Epub ahead of print: 13 01 2016].
- 31 Thomason MM, McCarthy J, Goin-Kochel RP, Dowell LR, Schaaf CP, Berry LN. Neurocognitive and neurobehavioral phenotype of youth with Schaaf-Yang syndrome. *J Autism Dev Disord* 2020;50:2491–500.
- 32 Jobling R, Stavropoulos DJ, Marshall CR, Cyttrynbaum C, Axford MM, Londero V, Moalem S, Orr J, Rossignol F, Lopes FD, Gauthier J, Alos N, Rupps R, McKinnon M, Adam S, Nowaczyk MJM, Walker S, Scherer SW, Nassif C, Hamdan FF, Deal CL, Soucy J-F, Weksberg R, Macleod P, Michaud JL, Chitayat D, Chitayat-Hall CD. Chitayat-Hall and Schaaf-Yang syndromes: a common aetiology: expanding the phenotype of *MAGEL2*-related disorders. *J Med Genet* 2018;55:316–21.
- 33 Mejlachowicz D, Nolent F, Maluenda J, Ranjatoelina-Randrianaivo H, Giuliano F, Gut I, Sternberg D, Laquerrière A, Melki J. Truncating mutations of *MAGEL2*, a gene within the Prader-Willi locus, are responsible for severe arthrogryposis. *Am J Hum Genet* 2015;97:616–20.
- 34 Guo W, Lai Y, Yan Z, Wang Y, Nie Y, Guan S, Kuo Y, Zhang W, Zhu X, Peng M, Zhi X, Wei Y, Yan L, Qiao J. Trio-whole-exome sequencing and preimplantation genetic diagnosis for unexplained recurrent fetal malformations. *Hum Mutat* 2020;41:432–48.
- 35 GTEx Consortium. The Genotype-Tissue expression (GTEx) project. *Nat Genet* 2013;45:580–5.
- 36 Chen H, Victor AK, Klein J, Tacer KF, Tai DJ, de Esch C, Nuttle A, Temirov J, Burnett LC, Rosenbaum M, Zhang Y, Ding L, Moresco JJ, Diedrich JK, Yates JR, Tillman HS, Leibel RL, Talkowski ME, Billadeau DD, Reiter LT, Potts PR. Loss of *MAGEL2* in Prader-Willi syndrome leads to decreased secretory granule and neuropeptide production. *JCI Insight* 2020;5. doi:10.1172/jci.insight.138576. [Epub ahead of print: 03 09 2020].
- 37 Kozlov SV, Bogenpohl JW, Howell MP, Wevrick R, Panda S, Hogenesch JB, Muglia LJ, Van Gelder RN, Herzog ED, Stewart CL. The imprinted gene *MAGEL2* regulates normal circadian output. *Nat Genet* 2007;39:1266–72.
- 38 Lee S, Kozlov S, Hernandez L, Chamberlain SJ, Brannan CI, Stewart CL, Wevrick R. Expression and imprinting of *MAGEL2* suggest a role in Prader-Willi syndrome and the homologous murine imprinting phenotype. *Hum Mol Genet* 2000;9:1813–9.
- 39 Sanderson MR, Fahlman RP, Wevrick R. The N-terminal domain of the Schaaf-Yang syndrome protein *MAGEL2* likely has a role in RNA metabolism. *J Biol Chem* 2021;297:100959.
- 40 Tacer KF, Potts PR. Cellular and disease functions of the Prader-Willi Syndrome gene *MAGEL2*. *Biochem J* 2017;474:2177–90.
- 41 Hao Y-H, Doyle JM, Ramanathan S, Gomez TS, Jia D, Xu M, Chen ZJ, Billadeau DD, Rosen MK, Potts PR. Regulation of WASH-dependent actin polymerization and protein trafficking by ubiquitination. *Cell* 2013;152:1051–64.
- 42 Hao Y-H, Fountain MD, Fon Tacer K, Xia F, Bi W, Kang S-HL, Patel A, Rosenfeld JA, Le Caignec C, Isidor B, Krantz ID, Noon SE, Pfothner JP, Morgan TM, Moran R, Pedersen RC, Saenz MS, Schaaf CP, Potts PR. USP7 acts as a molecular rheostat to promote WASH-Dependent endosomal protein recycling and is mutated in a human neurodevelopmental disorder. *Mol Cell* 2015;59:956–69.
- 43 Burd C, Cullen PJ. Retromer: a master conductor of endosome sorting. *Cold Spring Harb Perspect Biol* 2014;6. doi:10.1101/cshperspect.a016774. [Epub ahead of print: 01 Feb 2014].
- 44 JZA T, Gleeson PA. The role of membrane trafficking in the processing of amyloid precursor protein and production of amyloid peptides in Alzheimer's disease. *Biochim Biophys Acta Biomembr* 1861;2019:697–712.
- 45 Herms J, Anliker B, Heber S, Ring S, Fuhrmann M, Kretschmar H, Sisodia S, Müller U. Cortical dysplasia resembling human type 2 lissencephaly in mice lacking all three APP family members. *Embo J* 2004;23:4106–15.
- 46 López-Sánchez N, Müller U, Frade JM. Lengthening of G2/mitosis in cortical precursors from mice lacking beta-amyloid precursor protein. *Neuroscience* 2005;130:51–60.
- 47 Wang P, Yang G, Mosier DR, Chang P, Zaidi T, Gong Y-D, Zhao N-M, Dominguez B, Lee K-F, Gan W-B, Zheng H. Defective neuromuscular synapses in mice lacking amyloid precursor protein (APP) and APP-like protein 2. *J Neurosci* 2005;25:1219–25.

- 48 Small SA, Kent K, Pierce A, Leung C, Kang MS, Okada H, Honig L, Vonsattel J-P, Kim T-W. Model-guided microarray implicates the retromer complex in Alzheimer's disease. *Ann Neurol* 2005;58:909–19.
- 49 Mecozzi VJ, Berman DE, Simoes S, Vetanovetz C, Awal MR, Patel VM, Schneider RT, Petsko GA, Ringe D, Small SA. Pharmacological chaperones stabilize retromer to limit APP processing. *Nat Chem Biol* 2014;10:443–9.
- 50 Muhammad A, Flores I, Zhang H, Yu R, Staniszewski A, Planel E, Herman M, Ho L, Kreber R, Honig LS, Ganetzky B, Duff K, Arancio O, Small SA. Retromer deficiency observed in Alzheimer's disease causes hippocampal dysfunction, neurodegeneration, and Abeta accumulation. *Proc Natl Acad Sci U S A* 2008;105:7327–32.
- 51 Kvainickas A, Jimenez-Orgaz A, Nägele H, Hu Z, Dengjel J, Steinberg F. Cargo-selective SNX-BAR proteins mediate retromer trimer independent retrograde transport. *J Cell Biol* 2017;216:3677–93.
- 52 Emanuelsson O, Brunak S, von Heijne G, Nielsen H. Locating proteins in the cell using TargetP, SignalP and related tools. *Nat Protoc* 2007;2:953–71.
- 53 Laurila K, Vihinen M. Prediction of disease-related mutations affecting protein localization. *BMC Genomics* 2009;10:122.
- 54 Richards S, Aziz N, Bale S, Bick D, Das S, Gastier-Foster J, Grody WW, Hegde M, Lyon E, Spector E, Voelkerding K, Rehml HL, ACMG Laboratory Quality Assurance Committee. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of medical genetics and genomics and the association for molecular pathology. *Genet Med* 2015;17:405–24.
- 55 Kanber D, Giltay J, Wiecek D, Zogel C, Hochstenbach R, Caliebe A, Kuechler A, Horsthemke B, Buiting K. A paternal deletion of MKRN3, MAGEL2 and Ndn does not result in Prader-Willi syndrome. *Eur J Hum Genet* 2009;17:582–90.
- 56 Buiting K, Di Donato N, Beygo J, Bens S, von der Hagen M, Hackmann K, Horsthemke B. Clinical phenotypes of Magel2 mutations and deletions. *Orphanet J Rare Dis* 2014;9:40.
- 57 Doyle JM, Gao J, Wang J, Yang M, Potts PR. MAGE-RING protein complexes comprise a family of E3 ubiquitin ligases. *Mol Cell* 2010;39:963–74.
- 58 Cantile M, Di Bonito M, Cerrone M, Collina F, De Laurentis M, Botti G. Long non-coding RNA HOTAIR in breast cancer therapy. *Cancers* 2020;12. doi:10.3390/cancers12051197. [Epub ahead of print: 09 05 2020].
- 59 Liguori G, Cerrone M, De Chiara A, Tafuto S, Tracey de Bellis M, Botti G, Di Bonito M, Cantile M. The role of lncRNAs in rare tumors with a focus on Hox transcript antisense RNA (HOTAIR). *Int J Mol Sci* 2021;22:10160.
- 60 Tsumagari K, Baribault C, Terragni J, Chandra S, Renshaw C, Sun Z, Song L, Crawford GE, Pradhan S, Lacey M, Ehrlich M. DNA methylation and differentiation: Hox genes in muscle cells. *Epigenetics Chromatin* 2013;6:25.
- 61 Yang L, Peng X, Li Y, Zhang X, Ma Y, Wu C, Fan Q, Wei S, Li H, Liu J. Long non-coding RNA HOTAIR promotes exosome secretion by regulating Rab35 and SNAP23 in hepatocellular carcinoma. *Mol Cancer* 2019;18:78.
- 62 Wei S, Fan Q, Yang L, Zhang X, Ma Y, Zong Z, Hua X, Su D, Sun H, Li H, Liu Z. Promotion of glycolysis by HOTAIR through GLUT1 upregulation via mTOR signaling. *Oncol Rep* 2017;38:1902–8.
- 63 Crutcher E, Pal R, Naini F, Zhang P, Lausch M, Kim J, Bajic A, Schaaf CP. mTOR and autophagy pathways are dysregulated in murine and human models of Schaaf-Yang syndrome. *Sci Rep* 2019;9:15935.
- 64 Cassidy SB, Schwartz S, Miller JL, Driscoll DJ. Prader-Willi syndrome. *Genet Med* 2012;14:10–26.
- 65 Morris CR, Hamilton-Reeves J, Martindale RG, Sarav M, Ochoa Gautier JB. Acquired amino acid deficiencies: a focus on arginine and glutamine. *Nutr Clin Pract* 2017;32:305–47.
- 66 Cheung G, Bataveljic D, Visser J, Kumar N, Moulard J, Dallérac G, Mozheiko D, Rollenhagen A, Ezan P, Mongin C, Chever O, Bemelmans A-P, Lübke J, Leray I, Rouach N. Physiological synaptic activity and recognition memory require astroglial glutamine. *Nat Commun* 2022;13:753.
- 67 Rice LJ, Lagopoulos J, Brammer M, Einfeld SL. Reduced gamma-aminobutyric acid is associated with emotional and behavioral problems in Prader-Willi syndrome. *Am J Med Genet B Neuropsychiatr Genet* 2016;171:1041–8.
- 68 Curnock R, Calcagni A, Ballabio A, Cullen PJ. TFEB controls retromer expression in response to nutrient availability. *J Cell Biol* 2019;218:3954–66.
- 69 Csibi A, Fendt S-M, Li C, Poulogiannis G, Choo AY, Chapski DJ, Jeong SM, Dempsey JM, Parkhitko A, Morrison T, Henske EP, Haigis MC, Cantley LC, Stephanopoulos G, Yu J, Blenis J. The mTORC1 pathway stimulates glutamine metabolism and cell proliferation by repressing SIRT4. *Cell* 2013;153:840–54.
- 70 Jabato FM, Córdoba-Caballero J, Rojano E, Romá-Mateo C, Sanz P, Pérez B, Gallego D, Seoane P, Ranea JAG, Perkins JR. Gene expression analysis method integration and co-expression module detection applied to rare glaucide metabolism disorders using ExpHunterSuite. *Sci Rep* 2021;11:15062.

# **Supplementary Table 2: Management and surveillance proposed protocol for Schaaf-Yang Syndrome**

**(English version)**



Perinatal period		
	Description	Special problems and their management
<b>Respiratory difficulties</b>	They frequently require Neonatal ICU, mainly due to the need for respiratory assistance during the perinatal period that varies from oxygen therapy to invasive mechanical ventilation, ranging from a few hours of support to a few months. Laryngeal stridor, glossoptosis, tracheomalacia, and pulmonary hypoplasia have also been reported.	NICU. Mechanic ventilation. Evaluation by <b>otorhinolaryngologists</b> and <b>pulmonologists</b> to rule out anatomical abnormalities is essential. Respiratory support from <b>oxygen therapy</b> to <b>invasive mechanical ventilation</b> and need of tracheostomy.
<b>Feeding difficulties</b>	Feeding difficulties with frequent episodes of choking or ineffective sucking are very common. The combination of hypotonia, lethargy, dysphagia, and a high palate can make oral feeding very difficult to achieve.	<b>Nutritionists</b> and <b>dysphagia specialists</b> are needed. An anatomical evaluation to rule out <b>malformations</b> is necessary. Supportive nutritional intervention may be necessary, ranging from a nasogastric tube to a permanent gastrostomy or parenteral nutrition.

Supplementary Table 2: Management and surveillance proposed protocol for Schaaf-Yang Syndrome

Perinatal period		
	Description	Special problems and their management
<b>Contractures</b>	Clubfoot, arthrogyriposis, contractures, and other joint movement restrictions are frequently present at birth. Fetal hypokinesia can lead to severe arthrogyriposis.	Early intervention through orthopedics may be essential to ameliorate the prognosis. It is important to refer to specialists on <b>rehabilitation</b> and <b>children's orthopedics</b> .
	Several endocrine abnormalities have been detected during the perinatal period, including diabetes insipidus, hyponatremia, growth hormone (GH) deficiency, hypoglycemia, and hypocalcemia (see Endocrinology section).	Blood <b>glucose</b> levels need to be controlled during the perinatal period. Laboratory tests at this age should include a comprehensive analysis of <b>ions, glucose, and hormones</b> to rule out frequent abnormalities. Refer to the pediatric <b>endocrinologist</b> .
<b>Hormonal problems</b>	In the perinatal period, an evaluation by a child neurologist and a geneticist/dysmorphologist is recommended. A detailed physical examination and a standardized protocol are recommended to rule out congenital malformations that include echocardiogram, abdominal ultrasound, cranial ultrasound...	

Supplementary Table 2: Management and surveillance proposed protocol for Schaaf-Yang Syndrome

Childhood and Adolescence		
SPECIALTY	STUDIES AND FOLLOW-UP	SPECIFIC CARE AND RECOMMENDED STUDIES
<b>Endocrinology (E)</b>	<p>Every 6 months/ Physical exam Assessments: Laboratory Ultrasound Neuroimaging (MRI)</p>	<p>E1 <b>Failure to thrive</b> during infancy and childhood, but also excessive increase in weight, trend to obesity, and hyperphagia beginning after infancy. Dietary intervention may be necessary.</p> <p>E2 <b>Short stature</b> (-1.5 SD to -5 SD), sometimes GH may be needed (consider the increased risk of obstructive apnea when prescribing GH).</p> <p>E3 <b>Diabetes insipidus</b> has been described presenting with polyuria, low urine density, hyposthenuria, and hypernatremia. Hormone replacement therapy may be required.</p> <p>E4 <b>Panhypopituitarism</b> caused by pituitary gland hypoplasia has been described, but also with normal MRI. It is necessary to monitor thyroid function, somatomedin C, GH, adrenal hormones, testosterone, LH and FSH. Hyperprolactinemia has been detected. Hormone treatment, including GH, levothyroxine, and hydrocortisone, may be necessary. Hormone replacement therapy. Adolescence → estrogens.</p> <p>E5 <b>Hypoglycemia</b>. Guarantee the intake and maintenance of glucose. Rule out hyperinsulinemia.</p> <p>E6 <b>Temperature</b> instability. Support measures.</p> <p>E7 Hypoplastic genitalia, micropenis and cryptorchidism. Assess testosterone, surgery.</p>

Supplementary Table 2: Management and surveillance proposed protocol for Schaaf-Yang Syndrome



Childhood and Adolescence		
SPECIALTY	STUDIES AND FOLLOW-UP	SPECIFIC CARE AND RECOMMENDED STUDIES
<b>Gastroenterology (G)</b>	Every 6 months/  Physical exam Feeding Observation Assessment: pHmetry Video fluoroscopy Image (Rx) Laboratory	<p>G1 <b>Feeding</b> problems are almost constant. Initial feeding difficulties are due to dysphagia, recurrent respiratory aspiration, and sometimes a nasogastric tube and gastrostomy are required.</p> <p>G2 Early-onset chronic <b>constipation</b> and <b>gastroesophageal reflux</b> are also common.</p> <p>G3 Infrequently reported complications: Intestinal pseudo-obstruction, velopharyngeal insufficiency, eosinophilic esophagitis, and food allergies.</p>
<b>Muscular and skeletal (MS)</b>	Every 12 months/  Physical exam Assessment: Image (Rx) Electromyogram and nerve conduction	<p>MS1 Abnormal muscle tone is very frequent, especially hypotonia.</p> <p>MS2 <b>Arthrogryposis</b> is very common: contractures, shortening of limbs, elbows, knees, hips. Camptodactyly, clinodactyly, brachydactyly of the fingers, adducted thumbs.</p> <p>MS3 <b>Club feet</b> and <b>equinovarus</b> feet have been repeatedly described.</p> <p>MS4 <b>Scoliosis</b>, kyphosis, lordosis and asymmetric chest. Early rehabilitation programs and external bracing may be needed. Periodic X-ray monitoring of the hip in children who cannot walk and images of the spine X-ray as a whole is recommended. Consider surgery if necessary.</p> <p>MS5 Less frequently: mesomelic and rhizomelic shortening of the limbs, hip dysplasia, and distal muscular atrophy of the limbs.</p>

Supplementary Table 2: Management and surveillance proposed protocol for Schaaf-Yang Syndrome

Childhood and Adolescence		
SPECIALTY	STUDIES AND FOLLOW-UP	SPECIFIC CARE AND RECOMMENDED STUDIES
<b>Neurodevelopmental problems and intellectual development (ND)</b>	<p>Every 6-12 months</p> <p>Physical exam Assessment: Scales and developmental assessments either on the patient or completed by the parents</p>	<p>ND1 Abnormal muscle tone and <b>arthrogryposis</b> are very common. Hypotonia is the most prevalent.</p> <p>ND2 <b>Gross motor development</b> may be severely impaired by delayed acquisition of head control, sitting position, and gait (not achieved by many patients).</p> <p>ND3 <b>Fine motor development</b> is also atypical due to motor abnormalities, arthrogryposis, and camptodactyly.</p> <p>The purposeful use of the hands, which is based on cognition and social skills, may be impaired. Rehabilitation programs, early stimulation and, later, occupational therapy programs are essential.</p> <p>ND4 <b>Social skills</b> and communication are often severely affected. Only some of the patients can develop, speech and language (generally poor).</p> <p>ND5 <b>ASD traits and behavioral abnormalities</b> are very frequent and can interfere with communication skills. Evaluation by speech therapists is recommended. Alternatives to oral communication, such as pictographs or electronic devices, can be of help.</p> <p>Psychotherapy may be necessary for patients with ASD, to treat not only the communicative difficulties but also rigidity, repetitive behaviors, hypersensoriality,...</p>

Supplementary Table 2: Management and surveillance proposed protocol for Schaaf-Yang Syndrome

<b>Childhood and Adolescence</b>		
<b>SPECIALTY</b>	<b>STUDIES AND FOLLOW-UP</b>	<b>SPECIFIC CARE AND RECOMMENDED STUDIES</b>
<b>Epilepsy (E)</b>	<p>Every 12 months</p> <p>Clinical interview Assessment: EEG</p>	<p>E1 Febrile seizures have been reported.</p> <p>E2 Epilepsy was found in less than 50% of patients, including different types of seizures (partial, generalized). Different AEDs were used successfully.</p>
<b>Neuroimaging (NI)</b>	<p>At the time of diagnosis.</p> <p>Afterwards, neuroimaging studies if new symptoms are present: MRI</p>	<p>NI1 Delayed myelination, ventricular enlargement, abnormalities of the corpus callosum (thinning, dysplasia or agenesis).</p> <p>NI2 Normal or hypoplastic pituitary gland is frequently reported.</p> <p>NI3 Rarely described: global cerebral atrophy, increased T2 signal in the caudate nucleus or putamen, globus pallidus, hypoplastic vermis, localized cerebellar hemorrhages.</p>
<b>Sleep disorders (SD)</b>	<p>Every 12 months</p> <p>Assessment: Sleep questionnaires Polysomnography (PSG)</p>	<p>SD1 Central and/or obstructive apnea have been reported and can worsen the cognitive and behavioral phenotype, as well as can be the cause of premature death. Periodic PSG is recommended.</p>

Supplementary Table 2: Management and surveillance proposed protocol for Schaaf-Yang Syndrome



Childhood and Adolescence		
SPECIALTY	STUDIES AND FOLLOW-UP	SPECIFIC CARE AND RECOMMENDED STUDIES
<b>Infections (I)</b>	<p>Every 12 months</p> <p>Assessment: Laboratory Lung imaging studies</p>	<p>I1 <b>Recurrent respiratory infections</b> have been reported in patients with respiratory assistance but also without respiratory support. Chronic lung disease has also been reported due to repeated bronchopneumonia or recurrent aspirations. Imaging studies may be required to evaluate the lung parenchyma and rule out malformations. Invasive studies are rarely necessary.</p> <p>I2 No predisposition to infections in other organs or systems is reported.</p>
<b>Cardiology (C)</b>	<p>At the time of diagnosis and if new symptoms develop</p> <p>Physical exam Assessment: ECG Echocardiography</p>	<p>C1 <b>Septal defects (CSA)</b> have been reported as the most common structural cardiac defects, sometimes solving spontaneously.</p> <p>C2 Bradycardia has been described in a 1-month-old baby.</p>
<b>Ophthalmology (O)</b>	<p>Every 12 months</p> <p>Physical exploration Assess: Acuity assessment Fundus Imaging techniques</p>	<p>O1 <b>Strabismus</b> and <b>nystagmus</b> have been described as oculomotor disorders.</p> <p>O2 Refractive errors have been reported.</p> <p>O3 Hypoplasia or atrophy of the optic nerve can be present.</p> <p>O4 Vision is usually difficult to assess in patients with SYS. Pediatric ophthalmologists are required. Lack of eye tracking has been described, however probably features of ASD underlie the abnormal ocular pursuit.</p> <p>O5 Rare: Xerophthalmia, caused by sleep with opened eyes.</p>

Supplementary Table 2: Management and surveillance proposed protocol for Schaaf-Yang Syndrome

# **Supplementary Table 3: Propuesta de protocolo de estudio y seguimiento para el Síndrome de Schaaf-Yang**

**(Spanish version)**

Periodo perinatal		
	Descripción	Problemas especiales y su manejo
<b>Dificultades respiratorias</b>	<p>Con frecuencia precisan UCI Neonatal, principalmente debido a la falta de reactividad neonatal. La necesidad de asistencia ventilatoria durante el período perinatal varía desde la oxigenoterapia hasta la ventilación mecánica invasiva, desde unas horas de apoyo hasta algunos meses. También se han notificado estridor laríngeo, glosoptosis, traqueomalacia e hipoplasia pulmonar.</p>	<p>UCIN. Ventilación mecánica. Evaluación por <b>otorrinolaringólogos y neumólogos</b> para descartar anomalías anatómicas. Soporte respiratorio desde <b>oxigenoterapia</b> hasta <b>ventilación mecánica invasiva</b> y necesidad de traqueostomía.</p>
<b>Dificultades de alimentación</b>	<p>Son muy frecuentes las dificultades para alimentarse con frecuentes episodios de atragantamiento o succión ineficaz. La hipotonía, el letargo, la disfagia y un paladar alto, todos juntos pueden dificultar la nutrición por boca.</p>	<p>Se necesitan <b>nutricionistas y especialistas en disfagia</b>. Es importante realizar una evaluación anatómica para descartar <b>malformaciones</b>. Puede ser necesaria una intervención nutricional de apoyo, desde una sonda nasogástrica hasta una gastrostomía permanente o nutrición parenteral.</p>

Supplementary Table 3: Propuesta de protocolo de estudio y seguimiento para el Síndrome de Schaaf-Yang



Periodo perinatal		
	Descripción	Problemas especiales y su manejo
Contracturas	El pie zambo, la artrogriposis, las contracturas y otras restricciones de movimiento articular están presentes con frecuencia al nacer. La hipocinesia fetal puede provocar una artrogriposis grave.	La intervención temprana con medidas ortopédicas puede resultar esencial para obtener un mejor pronóstico. Es importante remitir a especialistas en <b>rehabilitación y ortopedia infantil</b> .
	Se han detectado varias alteraciones endocrinológicas durante el período perinatal, como la diabetes insípida, hiponatremia, deficiencia de hormona del crecimiento (GH), hipoglucemia e hipocalcemia (ver sección de Endocrinología).	Controlar los niveles de <b>glucosa</b> en sangre durante el período perinatal. Las pruebas de laboratorio a esta edad deben incluir un análisis exhaustivo de <b>iones, glucosa y hormonas</b> para descartar alteraciones prevalentes. Consultar con el <b>endocrinólogo</b> infantil.
Problemas hormonales	En la época perinatal se recomienda la evaluación por un neurólogo infantil y un genetista/ dismorfólogo. Se recomienda un examen físico detallado y un protocolo estandarizado para descartar malformaciones orgánicas que incluyen ecocardiograma, ecografía abdominal, ecografía craneal....	

Supplementary Table 3: Propuesta de protocolo de estudio y seguimiento para el Síndrome de Schaaf-Yang

Infancia y Adolescencia		
ESPECIALIDAD	SEGUIMIENTO Y ESTUDIOS	CUIDADOS ESPECÍFICOS Y ESTUDIOS RECOMENDADOS
<b>Endocrinología (E)</b>	Cada 6 meses/ Exploración física Valorar: Laboratorio Ecografía Neuroimagen (RMc)	<p>E1 <b>Fallo de medro</b> durante la infancia y la niñez, pero también <b>sobrepeso</b> temprano, obesidad e hiperfagia que comienzan después de la infancia. Puede ser necesaria una intervención dietética.</p> <p>E2 <b>Estatura baja</b> (de -1,5 DE a -5 DE), a veces necesita GH (considerar la apnea obstructiva cuando se prescribe GH)</p> <p>E3 Se ha descrito <b>diabetes insípida</b> que cursa con poliuria, densidad urinaria baja, hipostenuria e hipernatremia. Terapia hormonal sustitutiva.</p> <p>E4 Se ha descrito <b>panhipopituitarismo</b> causado por hipoplasia de la glándula pituitaria, pero también con resonancia magnética normal. Es necesario controlar la función tiroidea, somatomedina C, GH, hormonas suprarrenales, testosterona, LH y FSH. Se ha detectado hiperprolactinemia. Puede ser necesario un tratamiento con hormonas, que incluyen GH, levotiroxina e hidrocortisona. Terapia hormonal sustitutiva. Adolescencia → estrógenos.</p> <p>E5 <b>Hipoglicemia</b>. Garantizar la ingesta y mantenimiento de glucosa. Descartar hiperinsulinemia.</p> <p>E6 Inestabilidad de <b>temperatura</b>. Medidas de apoyo.</p> <p>E7 Genitales hipoplásicos, micropene y criptorquidia. Valorar testosterona, cirugía.</p>

Supplementary Table 3: Propuesta de protocolo de estudio y seguimiento para el Síndrome de Schaaf-Yang

Infancia y Adolescencia		
ESPECIALIDAD	SEGUIMIENTO Y ESTUDIOS	CUIDADOS ESPECÍFICOS Y ESTUDIOS RECOMENDADOS
<b>Gastroenterología (G)</b>	<p>Cada 6 meses/ Exploración física Observación de la alimentación Valorar: pHmetría Videofluoroscopia Imagen (Rx) Laboratorio</p>	<p>G1 Los problemas de <b>alimentación</b> son casi constantes. Las dificultades iniciales de alimentación se deben a disfagia, aspiración respiratoria recurrente y, en ocasiones, se requiere sonda nasogástrica y gastrostomía.</p> <p>G2 También son frecuentes el <b>estreñimiento</b> crónico de aparición temprana y el <b>reflujo gastroesofágico</b>.</p> <p>G3 Complicaciones informadas con poca frecuencia: pseudoobstrucción intestinal, insuficiencia velofaríngea, esofagitis eosinofílica y alergias alimentarias.</p>
<b>Muscular y esquelético (MS)</b>	<p>Cada 12 meses/ Exploración física Valorar: Imagen (Rx) Electromiograma y Conducción nerviosa</p>	<p>MS1 El tono muscular anormal es muy frecuente, sobre todo la hipotonía.</p> <p>MS2 La <b>artrogriposis</b> es muy frecuente: contracturas, acortamiento de extremidades, codos, rodillas, caderas. Camptodactilia, clinodactilia, braquidactilia de los dedos, pulgares en aducción.</p> <p>MS3 Se han descrito repetidamente los <b>pies zambos y equinovaros</b>.</p> <p>MS4 <b>Escoliosis</b>, cifosis, lordosis y tórax asimétrico. Es posible que se necesiten programas de rehabilitación temprana y ortesis externa. Se recomienda el control periódico de rayos X de la cadera en los niños que no pueden caminar y de la columna en total. Cirugía cuando sea necesario.</p> <p>MS5 Con menor frecuencia: acortamiento mesomélico y rizomélico de las extremidades, displasia de cadera y atrofia muscular distal de las extremidades.</p>

Supplementary Table 3: Propuesta de protocolo de estudio y seguimiento para el Síndrome de Schaaf-Yang

Infancia y Adolescencia		
ESPECIALIDAD	SEGUIMIENTO Y ESTUDIOS	CUIDADOS ESPECÍFICOS Y ESTUDIOS RECOMENDADOS
<b>Problemas de neurodesarrollo y desarrollo intelectual (ND)</b>	<p>Cada 6-12 meses</p> <p>Exploración física</p> <p>Valorar: Escalas y evaluaciones del desarrollo sobre el paciente o completadas por los padres</p>	<p>ND1 El tono muscular anormal y la <b>artrogriposis</b> son muy frecuentes. La hipotonía es la más prevalente.</p> <p>ND2 El desarrollo de la <b>motricidad gruesa</b> puede verse gravemente afectado por el retraso en la adquisición del control de la cabeza, la sedestación y la marcha (no logrado por muchos pacientes).</p> <p>ND3 El desarrollo de la <b>motricidad fina</b> también es anormal debido a anomalías motoras, artrogriposis y camptodactilia.</p> <p>El uso propositivo de las manos, que se basa en la cognición y las habilidades sociales, puede verse alterado. Los programas de rehabilitación, estimulación temprana y, posteriormente, los programas de terapia ocupacional son fundamentales.</p> <p>ND4 Las <b>habilidades sociales</b> y la comunicación suelen verse gravemente afectadas. Solo algunos pacientes pueden desarrollar un lenguaje escaso.</p> <p>ND5 Los <b>rasgos de TEA</b> y las <b>alteraciones del comportamiento</b> son muy frecuentes. Esto puede interferir con las habilidades de comunicación. Se recomienda la evaluación de los terapeutas del habla. Las alternativas a la comunicación oral, como pictogramas o dispositivos electrónicos, pueden ayudar. La psicoterapia puede ser necesaria para los pacientes con TEA, para tratar no solo la esfera comunicativa sino también la rigidez, las conductas reiterativas, la hipersensorialidad,...</p>

Supplementary Table 3: Propuesta de protocolo de estudio y seguimiento para el Síndrome de Schaaf-Yang

<b>Infancia y Adolescencia</b>		
<b>ESPECIALIDAD</b>	<b>SEGUIMIENTO Y ESTUDIOS</b>	<b>CUIDADOS ESPECÍFICOS Y ESTUDIOS RECOMENDADOS</b>
<b>Epilepsia (E)</b>	<p>Cada 12 meses</p> <p>Entrevista clínica</p> <p>Valorar: EEG</p>	<p>E1 Se han notificado convulsiones febriles.</p> <p>E2 Epilepsia en menos del 50% de los pacientes, diferente tipología (parcial, generalizada), diferentes FAEs utilizados con éxito.</p>
<b>Neuroimagen (NI)</b>	<p>En el momento diagnóstico.</p> <p>Posteriormente, valorar si aparecen nuevos síntomas: MRI</p>	<p>NI1 Mielinización retrasada, agrandamiento ventricular, anomalías del cuerpo caloso (adelgazamiento, displasia o agenesia).</p> <p>NI2 Con frecuencia se informa de glándula pituitaria normal o hipoplásica.</p> <p>NI3 Raramente descrito: atrofia cerebral global, aumento de la señal T2 en el núcleo caudado o en el putamen, globo pálido, vermis hipoplásico, hemorragias cerebelosas puntiformes.</p>
<b>Problemas de sueño (SD)</b>	<p>Cada 12 meses</p> <p>Valorar: Cuestionarios de sueño Polisomnografía (PSG)</p>	<p>SD1 Se ha informado de apnea central y / o apnea obstructiva que pueden empeorar los problemas cognitivos y de comportamiento, así como ser la causa de muerte prematura.</p> <p>Se recomienda PSG periódica.</p>

Supplementary Table 3: Propuesta de protocolo de estudio y seguimiento para el Síndrome de Schaaf-Yang



Infancia y Adolescencia		
ESPECIALIDAD	SEGUIMIENTO Y ESTUDIOS	CUIDADOS ESPECÍFICOS Y ESTUDIOS RECOMENDADOS
<b>Infecciones (I)</b>	Cada 12 meses  Valorar: Laboratorio Estudios de imagen pulmonars	I1 Se han notificado <b>infecciones respiratorias recurrentes</b> en los pacientes que necesitan asistencia respiratoria y en los que no la tienen, así como enfermedad pulmonar crónica debida a bronconeumonía repetida o aspiraciones recurrentes. Es posible que se requieran estudios de imágenes para verificar el parénquima pulmonar y descartar malformaciones. Rara vez son necesarios estudios invasivos.  I2 No se informa de una predisposición a infecciones en otros órganos o sistemas.
<b>Cardiología (C)</b>	Al diagnóstico y si se desarrollan nuevos síntomas  Exploración física Valorar: ECG Ecocardiografía	C1 Los <b>defectos septales (CSA)</b> se han informado como las anomalías cardíacas estructurales más frecuentes, que en ocasiones se resuelven espontáneamente.  C2 Se ha descrito bradicardia en un bebé de 1 mes.
<b>Oftalmología (O)</b>	Cada 12 meses  Exploración física Valorar: Valoración de la agudeza Fondo de ojo Técnicas de imagen	O1 Se han descrito <b>estrabismo y nistagmo</b> , como trastornos oculomotores.  O2 Se han descrito defectos de refracción.  O3 Hipoplasia o atrofia del nervio óptico.  O4 La vista es normalmente difícil de evaluar en pacientes con SYS. Se requieren oftalmólogos pediátricos. Se ha descrito falta de seguimiento con los ojos, probablemente los rasgos de TEA subyacen a este síntoma.  O5 Raras: Xeroftalmia, causada por dormir con los ojos abiertos.

Supplementary Table 3: Propuesta de protocolo de estudio y seguimiento para el Síndrome de Schaaf-Yang

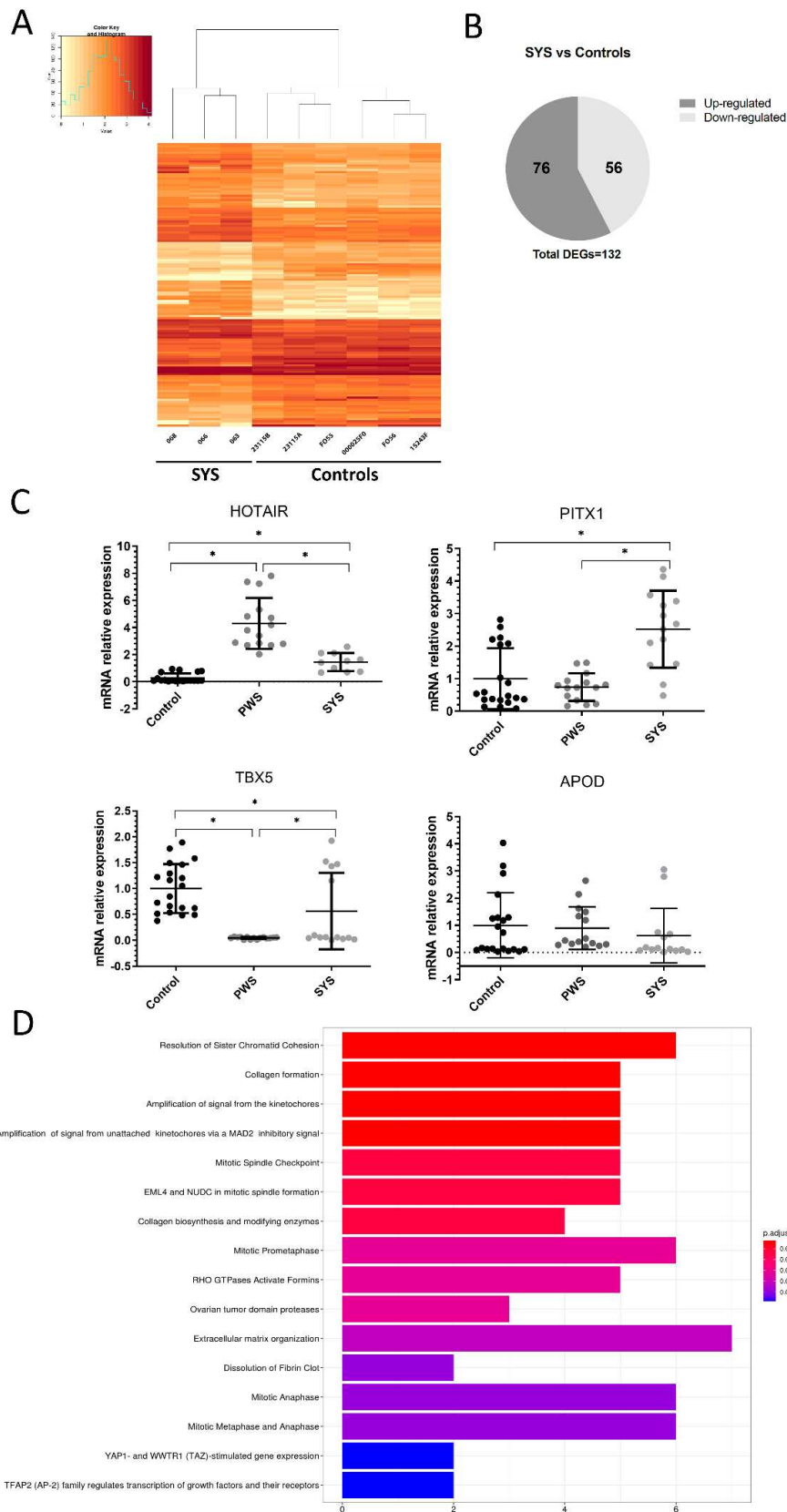
## Advancing in Schaaf-Yang syndrome pathophysiology: from bedside to subcellular analyses of truncated MAGEL2

Laura Castilla-Vallmanya<sup>1,2,3#</sup>, Mónica Centeno-Pla<sup>1,3,4#</sup>, Mercedes Serrano<sup>2,3,5#</sup>, Héctor Franco-Valls<sup>1</sup>, Raúl Martínez-Cabrera<sup>1</sup>, Aina Prat-Planas<sup>1,2,3</sup>, Elena Rojano<sup>6</sup>, Juan A. G. Ranea<sup>2,6</sup>, Pedro Seoane<sup>2,6</sup>, Clara Oliva<sup>3,4</sup>, Abraham J. Paredes-Fuentes<sup>3,4</sup>, Gemma Marfany<sup>1,2,3</sup>, Rafael Artuch<sup>2,3,4</sup>, Daniel Grinberg<sup>1,2,3</sup>, Raquel Rabionet<sup>1,2,3#</sup>, Susanna Balcells<sup>1,2,3#</sup>, Roser Urreizti<sup>2,3,4#</sup>

### SUPPLEMENTARY FIGURES:

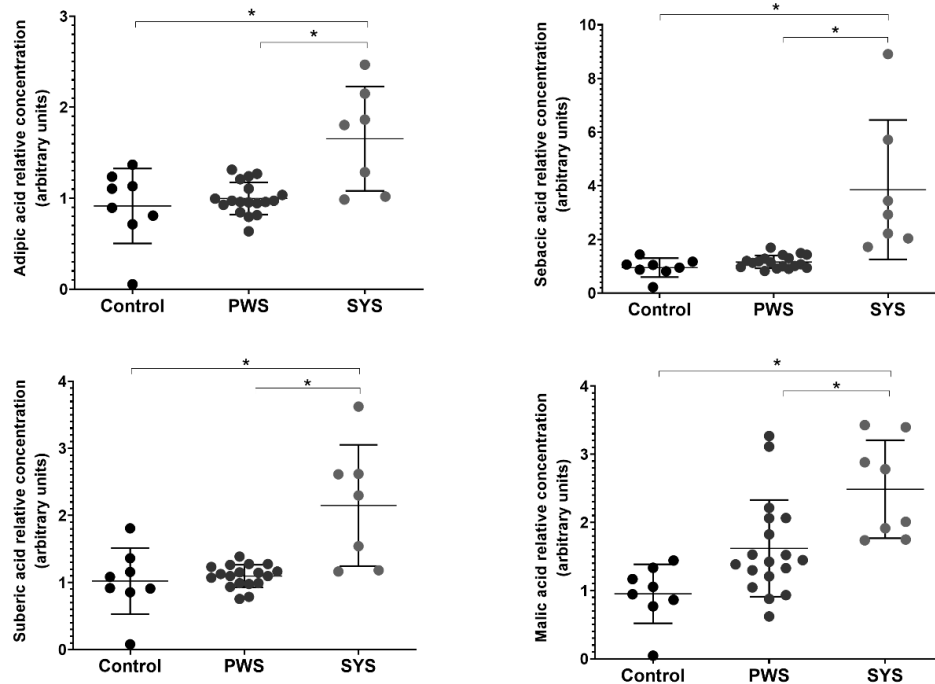


Supplementary Figure 1: Spanish version of Figure 1.

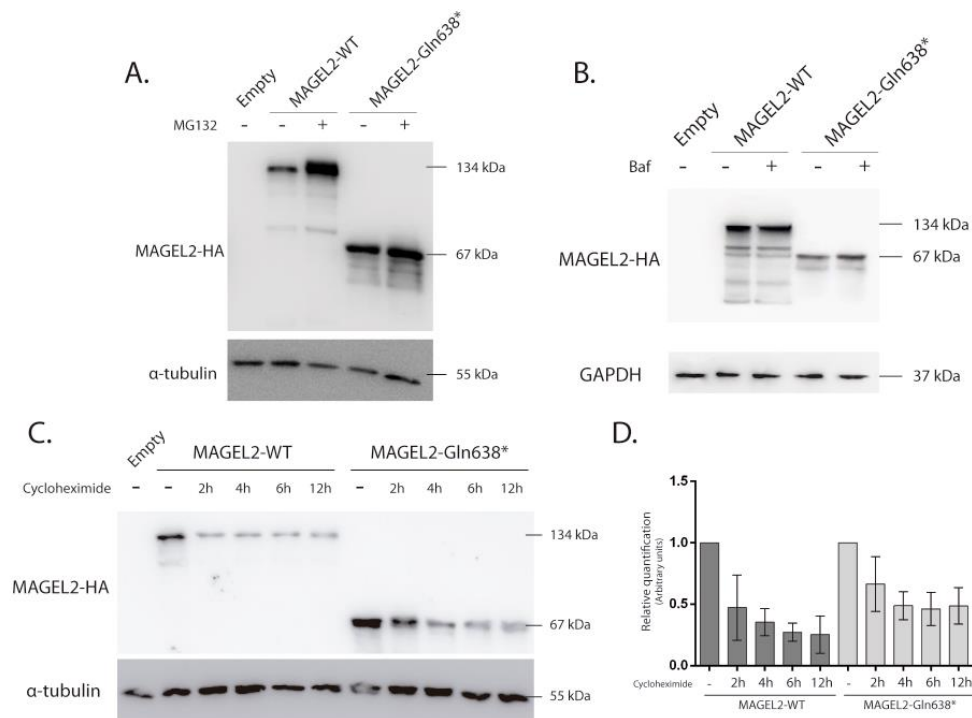


Suppl. 2

**Supplementary Figure 2:** A) Heatmap of the DEGs identified in SYS and control fibroblasts. B) Proportion of up- and down-regulated transcripts in SYS and control fibroblasts. C) qPCR analysis of *HOTAIR*, *PITX1*, *TBX5* and *APOD* in 5 SYS patients, 5 PWS patients and 7 control individuals. Values from 3 replicates have been normalised to the mean of the control group. Horizontal lines represent mean values and error bars represent the standard deviation. Statistical analyses were performed using One-Way ANOVA and Tukey's multiple comparisons test in GraphPad Prism.\* $p < 0.001$ . D) REACTOME enrichment analysis of DEGs based on over-representation analysis (ORA) obtained with Cluster Profiler. The length of the bar represents the number of genes observed in each category. Colour indicates adjusted p-value of each category.



**Supplementary Figure 3:** Targeted metabolomics in fibroblasts. Adipic, Suberic, Sebamic and Malic acid are significantly increased in SYS derived fibroblasts compared to controls and PWS derived fibroblasts. (SYS: n=6; PWS: n=9; control: n=6). Values from 2 replicates have been normalised to the mean of the control group. Horizontal lines represent mean values and error bars represent the standard deviation. Statistical analyses were performed using One-Way ANOVA and Tukey's multiple comparisons test in GraphPad Prism.\* $p < 0.001$ .



**Supplementary Figure 4: Degradation rate and stability of MAGEL2-WT and MAGEL2-Gln638\* proteins.** A) Representative Western Blot of heterologously expressed MAGEL2 proteins with or without MG132 treatment (10 $\mu$ M for 16 hours) (n=3). B) Representative Western Blot of heterologously expressed MAGEL2 proteins with or without Baf treatment (1 $\mu$ M for 16 hours) (n=3). C) Representative Western Blot of heterologously expressed MAGEL2 proteins with or without Cx treatment (150 $\mu$ M) during 2, 4, 6 or 12 hours (n=3). D) Quantification of three independent experiments normalised to the expression levels of MAGEL2-WT or MAGEL2-Gln638\* without Cx treatment.



## SUPPLEMENTARY MATERIAL AND METHODS:

**Literature review:** A Cochrane Library search and a PubMed database search was performed from the date of the first clinical description of pathology associated with variants in *MAGEL2* till May 31, 2022, using the following terms: 'MAGEL2', 'SYS', 'Schaaf-Yang syndrome', and different combinations of these terms. Concerning the data extraction, it included first author, publication year, molecular data (identified mutation and residue changes, *de novo* or inherited condition), pregnancy and perinatal information, multiorganic clinical data, complementary exams information, neuroimaging, and age and cause of death. Unfortunately, in some clinical series, much clinical information is not detailed.

**RNA-sequencing and data analysis:** Whole RNA was extracted from fibroblasts using the High Pure RNA Isolation Kit (Roche). RNA-Seq was performed by LEXOGEN, Inc. using the QuantSeq 3' messenger RNA (mRNA)-Seq FWD kit for library preparation. ExpHunter Suite [1] was used to analyse the expression data and perform functional enrichment analyses. The differential expressed genes (DEGs) are obtained using an adjusted p-value of 0.05, a minimum log fold change of 1 and Deseq2 and EdgeR packages give as DEG the analyzed gene. These DEGs are used as input for a functional enrichment analyses using REACTOME database and an adjusted p-value of 0.05.

**qPCR analysis of selected genes:** Whole RNA was extracted from fibroblasts using the High Pure RNA Isolation Kit (Roche). The extracted RNA was reverse transcribed using the High Capacity cDNA reverse transcription kit (Applied Biosystems) following the manufacturer's instructions. qPCR was performed using LightCycler® 480 SYBR Green I Master (04887352001, Roche). The human *GAPDH* gene served as a housekeeping gene and the efficiency of each reaction was calculated according to the standard curve. Melting-curves were conducted at the end of amplification to ensure data quality. Each sample was performed in triplicate. Primer sequences are available on demand.

**Cell culture:** Fibroblasts were obtained from skin biopsies of seven SYS patients, nine PWS patients and eleven controls (**Supplementary Table 7**). Corresponding informed consent and institutional ethics approval were obtained (Ethics Committee of the Universitat de Barcelona, IRB00003099). Fibroblasts were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Sigma-Aldrich, Merck) supplemented with 10% Foetal Bovine Serum (FBS) (Gibco, LifeTechnologies), 1% Penicillin–Streptomycin (Gibco, LifeTechnologies) and 1% GlutaMAX (Gibco, LifeTechnologies). Conditioned medium

Suppl. 5

for ELISA was only supplemented with 1% Penicillin–Streptomycin (Gibco, LifeTechnologies) and was collected after 72 h. HEK293T, HeLa and SAOS-2 cells were cultured in DMEM (Sigma-Aldrich, Merck) supplemented with 10% FBS (Gibco, LifeTechnologies) and 1% Penicillin–Streptomycin (Gibco, LifeTechnologies). The cDNA sequences of interest (ENST00000650528.1) were cloned in the mammalian expression vector pcDNA<sup>TM</sup>3.1<sup>(+)</sup> (Invitrogen, ThermoFisher Scientific) including a hemagglutinin (HA) tag at the C-terminal end (courtesy of the CRG). The vectors were transfected into 60% confluent HEK293T, HeLa and SAOS-2 cells using Lipofectamine<sup>TM</sup> 3000 (Invitrogen, ThermoFisher Scientific) and Opti-MEM<sup>TM</sup> (Gibco, LifeTechnologies). Cells were treated with 10 µmol/L MG132 (Calbiochem) or 1 µmol/L bafilomycin (Sigma-Aldrich, Merck) for 16 h or 150 µmol/L cycloheximide (Sigma-Aldrich, Merck) for 2, 4, 8 or 12 h prior to total protein extraction.

**Protein extraction and western blotting:** Total protein extraction was performed using RIPA buffer supplemented with protease inhibitors (04693159001, Roche, Merck) and N-ethylmaleimide (Sigma-Aldrich, Merck) and quantified using the Pierce<sup>TM</sup> BCA Protein Assay kit (ThermoFisher Scientific). Twenty µg of total protein per lane were run in 10% acrylamide/bis-acrylamide gels, transferred to a PVDF membrane (Millipore, Merck) and blocked with 5% skimmed milk in TBS-Tween 1X. Different antibodies were used: Anti-HA tag (ab18181, Abcam), anti- $\alpha$ -Tubulin (T5168, Sigma-Aldrich), anti-GAPDH (sc-47724, Santa Cruz Biotechnology) and anti-Mouse IgG (Fc specific)–Peroxidase (A0168, Sigma-Aldrich). Results were visualised using the LAS-4000 Luminescent Image Analyzer (Fujifilm) and the Luminata<sup>TM</sup> Forte Western HRP Substrate (WBLUF0100, Millipore). The detected bands were quantified using ImageJ.[2]

**Immunocytochemistry:** Cells were fixed in 4% Paraformaldehyde (PFA), permeabilized with 0,1 mol/L glycine and 0,1% Triton X-100 in PBS and blocked with 0,3 mol/L glycine, 0,05% Triton X-100 and 10% Normal Donkey Serum (#S30-100M, Merck Millipore) in PBS. Coverslips were incubated with anti-HA primary antibody (ab18181, Abcam), Donkey anti-Mouse Cy2 antibody (#715-225-150, Jackson ImmunoResearch) and DAPI (#D1306, Invitrogen) and mounted with MOWIOL (#475904, Millipore). Images were acquired using a Zeiss confocal microscope LSM 880 and analysed with ImageJ.[2]

**ELISA analysis:** The supernatant of the conditioned medium was obtained after short centrifugation. Total protein concentration was quantified using the Pierce<sup>TM</sup> BCA Protein Assay kit (ThermoFisher Scientific) and A $\beta$ <sub>1-40</sub> and A $\beta$ <sub>1-42</sub> amyloid peptide using the Amyloid-beta (1-40) High Sensitive ELISA and Amyloid-beta (1-42) High Sensitive

Suppl. 6

ELISA (IBL International GmbH). Statistical analyses were performed using One-Way ANOVA and Tukey's multiple comparisons test in GraphPad Prism.

**Metabolomics analyses:** Amino acid analyses were performed using a ultra-high-performance liquid chromatography–tandem mass spectrometry (UPLC-MS/MS) method, as previously reported.<sup>[3]</sup> Gas chromatography-mass spectrometry (GC-MS) analyses were performed to identify different organic acids, as described in [4, 5]. Statistical analyses were performed using One-Way ANOVA and Tukey's multiple comparisons test in RStudio (<https://www.rstudio.com/>).

**Ethical issues:** For the patients whose fibroblasts have been studied, parents gave their written informed consent. Their samples and data were obtained in accordance with the Helsinki Declaration of 1964, as revised in October 2013 (Fortaleza, Brazil). The study was approved by the Institutional Review Board (IRB00003099) of the Bioethical Commission of the University of Barcelona (October 5, 2020) and Hospital Sant Joan de Déu (PIC-111-19).

## REFERENCES

1. Jabato FM, Cordoba-Caballero J, Rojano E, et al. Gene expression analysis method integration and co-expression module detection applied to rare glucide metabolism disorders using ExpHunterSuite. *Sci Rep* 2021;11(1):15062.
2. Rueden CT, Schindelin J, Hiner MC, et al. ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics* 2017;18(1):529.
3. Casado M, Sierra C, Batllori M, et al. A targeted metabolomic procedure for amino acid analysis in different biological specimens by ultra-high-performance liquid chromatography-tandem mass spectrometry. *Metabolomics* 2018;14(6):76.
4. Divry P, Vianey-Liaud C, Cotte J. Routine gas chromatographic/mass spectrometric analysis of urinary organic acids. Results over a three-year period. *Biomed Environ Mass Spectrom* 1987;14(11):663-8.
5. Wilkins J, Sakrikar D, Petterson XM, et al. A comprehensive protocol for multiplatform metabolomics analysis in patient-derived skin fibroblasts. *Metabolomics* 2019;15(6):83.

**Antecedentes:** el síndrome de Schaaf-Yang (SYS) es causado por mutaciones truncantes en *MAGEL2*, localizado en la región de Prader-Willi (15q11-q13), con un fenotipo observado que se solapa parcialmente al del síndrome de Prader-Willi. *MAGEL2* desempeña un papel en el transporte retrógrado y la regulación del reciclaje de proteínas. Nuestro objetivo es contribuir a la caracterización de la fisiopatología del SYS a nivel clínico, genético y molecular.

**Métodos:** Hemos realizado una extensa revisión fenotípica y molecular de pacientes con SYS previamente publicados. Hemos analizado los niveles de secreción del péptido  $\beta$ -amiloide 1-40 ( $A\beta_{1-40}$ ) y hemos realizado perfiles metabolómicos y transcriptómicos específicos en fibroblastos de pacientes con SYS ( $n = 7$ ) en comparación con los controles ( $n = 11$ ). También transfectamos líneas celulares con vectores que codifican *MAGEL2* de tipo salvaje (WT) o mutado para evaluar la estabilidad y la localización subcelular de la proteína truncada.

**Resultados:** Los estudios funcionales muestran niveles significativamente reducidos de  $A\beta_{1-40}$  secretado y glutamina intracelular en fibroblastos SYS en comparación con los de controles sanos. También identificamos 132 genes expresados diferencialmente, incluidos ncRNA como HOTAIR, muchos de ellos relacionados con procesos de desarrollo y mecanismos mitóticos. La forma truncada de *MAGEL2* mostró una estabilidad similar a la normal, pero se trasladó significativamente al núcleo, en comparación con una distribución principalmente citoplásmica de la proteína *MAGEL2* completa. Basados en conocimientos actualizados, ofrecemos pautas para el manejo clínico de los pacientes con SYS.

**Conclusión:** La proteína *MAGEL2* truncada estudiada es estable y se localiza principalmente en el núcleo, donde podría ejercer un efecto patogénico neomórfico. Los niveles de secreción de  $A\beta_{1-40}$  y los niveles de ARNm de HOTAIR podrían ser biomarcadores prometedores para SYS. Nuestros hallazgos pueden mejorar la comprensión de SYS y su manejo clínico.