



**HAL**  
open science

## Digestive involvement in a severe form of Snyder-Robinson syndrome: Possible expansion of the phenotype

Pauline Dontaine, Elisa Kottos, Martine Dassonville, Ovidiu Balasel, Véronique Catros, Julie Soblet, Pascale Perlot, Catheline Vilain

### ► To cite this version:

Pauline Dontaine, Elisa Kottos, Martine Dassonville, Ovidiu Balasel, Véronique Catros, et al.. Digestive involvement in a severe form of Snyder-Robinson syndrome: Possible expansion of the phenotype. *European Journal of Medical Genetics*, 2021, 64 (1), pp.104097. 10.1016/j.ejmg.2020.104097. hal-03038235

**HAL Id: hal-03038235**

**<https://hal.science/hal-03038235>**

Submitted on 15 Dec 2020

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Author contribution statement:

P.D., J.S. and V.C. wrote the manuscript. P.D., E.K., J.S. and C.V. contributed to variant interpretation and acquisition of phenotypic data. M.D., O.B. and P.P. contributed to the acquisition of phenotypic data. V.C. performed the spermine and spermidine dosage. All authors critically revised the manuscript.

Journal Pre-proof

## Digestive involvement in a severe form of Snyder-Robinson syndrome: possible expansion of the phenotype.

Pauline Dontaine<sup>1</sup>, Elisa Kottos<sup>1</sup>, Martine Dassonville<sup>2</sup>, Ovidiu Balasel<sup>3</sup>, Véronique Catros<sup>4</sup>, Julie Soblet<sup>1,5,6</sup>, Pascale Perlot<sup>7</sup>, Catheline Vilain<sup>1,5,6\*</sup>

### **Affiliations:**

<sup>1</sup> Department of Genetics, Hôpital Universitaire des Enfants Reine Fabiola, ULB Center of Human Genetics, Université Libre de Bruxelles, Brussels, Belgium.

<sup>2</sup> Department of Pediatric Surgery, Hôpital Universitaire des Enfants Reine Fabiola, Université Libre de Bruxelles, Brussels, Belgium.

<sup>3</sup> Department of Neonatology, Hôpital Universitaire des Enfants Reine Fabiola, Université Libre de Bruxelles, Brussels, Belgium.

<sup>4</sup> Univ Rennes, Inserm, CHU Rennes, Institut NUMECAN (Nutrition Metabolisms and Cancer), CRB Santé Rennes, F-35000 Rennes, France.

<sup>5</sup> Department of Genetics, Hôpital Erasme, ULB Center of Human Genetics, Université Libre de Bruxelles, Brussels, Belgium.

<sup>6</sup> Interuniversity Institute of Bioinformatics in Brussels, Université Libre de Bruxelles, Brussels, Belgium.

<sup>7</sup> Department of pediatrics, Hôpital Universitaire des Enfants Reine Fabiola, Université Libre de Bruxelles, Brussels, Belgium.

\* Corresponding author:

Catheline Vilain

cavilain@ulb.ac.be

Department of Genetics

Hôpital Universitaire des Enfants de la Reine Fabiola

15, avenue J. J. Crocq, 1020 Bruxelles, Belgium

00324772530

**Abstract:**

Snyder-Robinson syndrome (OMIM #309583) is a rare X-linked condition, caused by mutation in the *SMS* gene (MIM \*300105), characterized by a wide spectrum of clinical signs including developmental delay, epilepsy, asthenic habitus, dysmorphism, osteopenia, and renal or genital anomalies.

Here we describe two maternal half-brothers who both presented with severe neurodevelopmental delay, seizures, hearing loss, facial dysmorphism, renal and ophthalmologic anomalies, failure to thrive and premature death. A novel p.(Gly203Asp) variant was found at the hemizygous state in the two boys, and an elevated Spermidine/Spermine ratio confirmed the diagnosis of Snyder-Robinson syndrome. One of the brothers presented with gastrointestinal symptoms, with jejunal stenosis, enteral feeding intolerance, failure to thrive due to a dysfunctional gastrointestinal system, cholestasis and exocrine pancreatic insufficiency. Although more studies will be needed to understand its mechanisms, this observation lends further support to the possibility of severe digestive involvement in Snyder Robinson syndrome.

**Key words:**

Snyder-Robinson ; SMS ; failure to thrive ; new variant ; mendeliome

**Main text:****Introduction:**

Snyder-Robinson syndrome is a rare X-linked condition first described in 1969 by Snyder and Robinson (Snyder and Robinson, 1969). Its incidence is unknown, and less than thirty cases have been reported to date. The disorder is caused by a mutation in the *SMS* gene, located on the X chromosome, coding for an enzyme involved in polyamine metabolism called spermine synthase.

Spermine synthase works as a homodimer, the two sub-units being linked by their N-domain. The C-terminal and central domains of the enzyme contain the active sites (Wu et al., 2008). Spermine synthase catalyses the conversion of spermidine into spermine. Reported *SMS* missense mutations are spread throughout the gene (figure 1). Those damaging the N-domain are predicted to alter the stability of the homodimer, while those located in the C-domain are predicted to affect the active sites (Zhang et al, 2010).

Consequently, spermine levels in blood cells drastically decrease. Spermine is thought to have many important roles: protection from oxidative stress, regulation of ion channels including receptors mediating synaptic transmission in the mammalian brain, osteogenic and osteoclastic activity regulation, gene expression, protein synthesis, and maturation of the intestinal immune system (Pegg, 2014).

In parallel, spermidine levels increase in the blood cells. Spermidine is converted by spermine/spermidine N<sup>1</sup>-acetyltransferase (SSAT) into N<sup>1</sup>-acetylspermidine, then exported from the cell or oxidized by N<sup>1</sup>-acetylpolyamines oxidase into its precursor, producing two toxic by-products : aldehydes and H<sub>2</sub>O<sub>2</sub> (Murray-Stewart et al., 2018). Some studies

suggest that oxidative stress resulting from that catabolism may play an important physiopathological role in Snyder-Robinson syndrome (Li et al., 2017).

The diagnosis of Snyder-Robinson syndrome requires the identification of an *SMS* pathogenic variant, decreased or absent spermine synthase activity in white blood cells, or an increased spermidine/spermine ratio. Its highly variable phenotype includes developmental delay, intellectual disability, epilepsy, asthenic habitus, facial dysmorphism, osteopenia and genital and renal complications (See Table 1 and Supplementary Table 1). Recently, Larcher et al. (2019) described a patient with a complete loss of function of the spermine synthase who died at 4 months from abdominal sepsis. He presented severe prenatal and postnatal growth retardation, severe developmental delay, a thin habitus and facial dysmorphism. His multiple malformations included hypospadias, left multicystic kidney, congenital hypothyroidism and vertebral and cerebral anomalies. He also presented rectosigmoid hirschprung disease, gut malrotation and precirrhotic portal fibrosis.

Here we report two maternal half-brothers with a severe phenotype, one with intestinal, pancreatic and hepatic involvement. This observation provides further evidence that the phenotype of Snyder-Robinson syndrome could include a digestive involvement.

#### **Clinical report:**

The index patient (II.4 on pedigree, figure 2) was born after 34 weeks of gestation from healthy parents of Moroccan origin. The mother was 39-years-old and the father was 33-years-old. The mother suffered from gestational diabetes. The antenatal sonographies suggested jejunal stenosis. An amniocentesis was performed and the karyotype and CGH array showed normal results. He was delivered vaginally, and needed respiratory support by

non-invasive ventilation after a few minutes of mask ventilation. His Apgar score at 1, 5 and 10 minutes was 6/8/9. His birth weight was 2300 g (+0.11 SD), his head circumference was 32 cm (+0.58 SD) and his birth length was 50 cm (+2.10 SD).

Facial dysmorphism was noted from birth: he presented short and downslanted palpebral fissures, large low-set ears, a broad philtrum, micrognathia and a high palate. Prominent heels and long fingers were also observed. He had a right deviated trachea, a laryngomalacia and a bilateral uretero-hydronephrosis.

Patient presented axial and peripheral hypotonia, poor spontaneous movements, rare eye-opening and no sucking reflex. He presented clustered spasms from 9 months of age. A 24-hours EEG showed hypsarrhythmia with a right posterior epileptic focus. West syndrome was suspected and Vigabatrine was started, with good spasms control. A cerebral MRI was performed at the age of 2 months and 4 months, and showed widespread white matter high signal intensities in T2-weighted images and diffuse supratentorial cortical atrophy.

Auditory evoked potentials were concordant with a central hearing deficiency, and the ophthalmological evaluation showed bilateral polar cataract.

An extensive metabolic check-up was performed and showed no abnormality.

Jejunal stenosis was confirmed after birth, requiring multiple surgical interventions, during the first two months of life, for persistent jejunal diaphragms and malrotation. Despite several operations, gastric enteral feeding intolerance persisted, with recurrent vomiting, which was treated first with intravenous parenteral nutrition, then nasojejunal tube feeding, subsequently replaced by a jejunostomy with a discharge gastrostomy.

The patient presented neonatal jaundice during the first days of life that resolved after phototherapy. At one month, cholestasis began to appear, without resolution after temporary withdrawal of the parenteral nutrition. During the third surgical intervention at 3 months the liver was described as nodular and greenish. Liver biopsies showed persistent cholestasis, hepatic fibrosis and ductopenia, attributed to the prolonged parenteral nutrition. He was treated with ursodeoxycholic acid. He also presented an exocrine pancreatic deficiency, and needed pancreatic enzymes and liposoluble vitamins supplementation. Despite several attempts to nourish him properly with parenteral then enteral feeding, he failed to thrive and died at 10 months of age, in a state of severe undernutrition.

Patient 2 (II.2 on pedigree, figure 2) was born after a 39 weeks gestation, from the propositus's mother and another father of Moroccan origin. The mother was 33 years-old and the father was 60 years-old. The patient was delivered vaginally by vacuum extraction following fetal distress and needed ventilatory support using non-invasive ventilation for a few hours to counter neonatal respiratory distress syndrome. His Apgar score at 1, 5 and 10 minutes was 7/8/8. His birthweight was 2850 g (-1.15 SD), his birth length 49 cm (-0.54 SD), and his birth head circumference 32.5 cm (-1.39 SD).

Dysmorphic facial features were observed, such as micrognathia, low anterior hairline, short palpebral fissures, deeply set eyes, sloping forehead, macrotia and convex nasal ridge. Other findings included second and third left fingers camptodactyly, single transverse palmar crease on the right hand, fingers hypermobility, widely spaced nipples with a bell-curved thorax, sacral dimple, and a hoarse cry.

Patient developed a global developmental delay with axial hypotonia, peripheral hypertonia and absent speech. At his last neurological assessment at 2 years of age, he could not maintain a sitting position, had poor spontaneous movements, fluctuant interactions, and poor eye contact. A cerebral MRI, performed at 8 months of age, showed myelinisation delay and corpus callosum hypoplasia. At one year he developed myoclonic jerks involving all 4 limbs. Several EEGs were performed and showed generalised spike-and-wave discharges concomitant with myoclonia, suggesting generalised epilepsy. He was treated with Levetiracetam, later replaced by valproic acid, with good results.

An ophthalmological check-up was performed and showed a rod-cone dystrophy, a convergent strabismus and an intermittent nystagmus. Auditory evoked potentials conducted at one year showed bilateral transmissional hearing loss. Visual and somaesthetic potentials were within the normal range.

Surgery for hypospadias and cryptorchidism was performed. The patient presented right renal duplication with grade II bilateral ureterovesical reflux, with several urinary tract infection episodes.

Failure to thrive was observed and enteral feeding by nasogastric tube, later replaced by a gastrostomy, was required. The patient also presented severe constipation. From birth, he presented obstructive apnea syndrome, requiring constant cardiorespiratory monitoring. He was admitted several times for pneumonia and bronchiolitis with hypoxemia. A right deviated trachea was observed. At the age of one year he presented an infectious acute respiratory distress syndrome with severe pulmonary hypertension and circulatory shock that was complicated by colic infarction. A right hemicolectomy was required.

Patient died at the age of 3 years and 6 months from a septic shock during a bilateral pneumonia with severe respiratory insufficiency.

An extensive search for inborn errors of metabolism was performed, showing normal results. Previous genetic analysis by CGH array and karyotype showed no significant abnormality.

## **Methods:**

### *Mutation analysis:*

Clinical exome capture (3989 genes) was achieved using the SeqCap EZ Choice XL (NimbleGen, Madison, WI). Samples were sequenced in a paired-end 100 bp run on a Novaseq instrument (Illumina, San Diego, CA, USA). Bioinformatics pipeline was launched by the Brussels Interuniversity Genomics High Throughput core (BRIGHTcore, Brussels, Belgium) and filtering of the variants was accomplished using Highlander (<http://sites.uclouvain.be/highlander>). Details on pipeline and filtering are available on request.

### *Erythrocyte Polyamine Level Determination:*

Erythrocyte spermine and spermidine levels were determined from patient blood samples. Briefly 5 ml blood samples were collected in blood collection tubes containing a 0.129 M buffered sodium citrate solution and were centrifuged at 2,500 relative centrifugal force (g). After removal of plasma and buffy coat layer, the red blood cell pellet was washed with 0.14 M NaCl solution. One ml packed red blood cell pellet was removed and hemolyzed with 2 ml distilled water. Proteins were removed from the hemolysate by the addition of 2 ml ice cold 10% HClO<sub>4</sub>. After centrifugation at 3000 g free polyamines contained in red blood cells were

measured from 1 ml perchloric supernatant. For each sample 0.5 ml aqueous sodium carbonate saturated solution was added with 1 ml dansyl chloride dissolved in acetone. The samples were kept in the dark for 16 hours under a slight air depression to allow selective evaporation of acetone. Dansylated derivatives of polyamines were recovered by 2 successive dry extractions using cyclohexane. The residue was then resuspended in 1 ml acetonitrile. Ten microliters of this extract were used for high performance liquid chromatography analysis using the sensitive fluorimetric method previously described by Moulinoux (Moulinoux et al., 1984) and Quemener (QUEMENER et al., 1986). The supernatant fluid was stored frozen at  $-40^{\circ}\text{C}$ . High pressure performance liquid chromatography polyamine determination was performed on a 25 x 0,26 cm column packed with 10  $\mu\text{M}$  C-18 reversed-phase packing. Measurement of fluorescence was performed on a Thermo Scientific fluorimeter (365 nm for excitation; 520 nm for emission). The mobile phase for elution was a linear gradient between 20% acetonitrile in water (v/v) and acetonitrile (flow rate 2 ml/min, elution time 12 min). Circulating polyamine values were expressed in  $\text{nmol}/810^9$  erythrocytes.

## Results:

Massive parallel sequencing of a 3989 mendeliome gene panel allowed identification of a hemizygous c.608G>A (chromosome X: 21996180G>A (GCh37/hg19), NM\_004595.5) variant in the *SMS* gene, leading to the substitution of the Glycine 203 by an Aspartic acid p.(Gly203Asp) in both affected half-brothers and present in the mother at the heterozygous state. This variant has been submitted to the ClinVar database (<https://www.ncbi.nlm.nih.gov/clinvar/>) under the number SUB7094130.

A significantly elevated spermidine/spermine ratio above 19 (spermidine above 50 nmoles/8.109 erythrocytes (norm: 5-11) and spermine at 2.58 nmoles/8.109 erythrocytes (norm: 3.5-8.5)) confirmed pathogenicity of the variant and the diagnosis of Snyder-Robinson syndrome.

In duo analysis of the 3989 gene panel failed to identify any other disease-causing variant which could explain the severe gastrointestinal phenotype of patient 1. Analysis of the sequence data using CoNVaDING failed to identify any significant small CNVs (Johansson et al., 2016).

#### **Discussion:**

Here we describe two patients, both with a severe phenotype, one with a life-threatening digestive involvement, presenting a novel missense variant p.(Gly203Asp) in the *SMS* gene. This variant was absent from 183503 X chromosomes in the control population (<https://gnomad.broadinstitute.org/>) and replaces a highly conserved amino-acid. There is computational evidence in favour of pathogenicity of the variant, since 8 out of 9 tools (DANN, FATHMM-MKL, M-CAP, MVP, MutationAssessor, MutationTaster, REVEL and SIFT versus 1 benign prediction from DEOGEN2) predict a deleterious effect. The residue Gly<sup>203</sup> involved is located two amino-acids away from the residue Asp<sup>201</sup>, which is one of the important binding sites of the spermine synthase (Wu et al. 2008). Shift of Gly<sup>203</sup> to Aspartic acid could possibly change the conformation of the binding site or lead to an alteration of enzyme affinity for the substrate. Spermine synthase loss of function was confirmed by strongly elevated spermidine and decreased spermine in the blood cells of patient 1.

As for many genetic syndromes, Snyder Robinson Syndrome has a wide clinical variability. The reasons for this are unknown. So far, no clear genotype-phenotype correlation has been drawn and variable expressivity has been observed even within families (Arena et al., 1996). Some of the symptoms of our patients were similar to other cases described in the literature (See Table 1 and Supplementary Table 1), such as developmental delay, hypotonia, epileptic encephalopathy, similar facial dysmorphism, osteopenia, cryptorchidism and renal complications (Arena et al., 1996; de Alencastro et al., 2008; Peron et al., 2013).

Tracheomalacia, respiratory tract infections, corpus callosum anomalies and cerebral atrophy have already been described (Abela et al., 2016 ; Albert et al., 2015).

Ophthalmological involvement, such as pronounced myopia (de Alencastro et al., 2008; Kesler et al., 2009) and retinitis pigmentosa (Albert et al., 2015), has also been reported, although in a minority of Snyder-Robinson patients.

Compared to most cases reported, our patients were severely affected, with major developmental delay and premature death. In 2015 Albert and colleagues described two brothers with tracheomalacia, respiratory tract infections, hypoglycaemia, multiple fractures, delayed development with regression and absence of walk or speech, seizures, hearing loss, retinitis pigmentosa and nephrocalcinosis. Exome sequencing showed a maternally inherited *SMS* p.(Gln148Arg) variant. Gln<sup>148</sup> is one of the 8 residues involved in the binding of 5'-methylthioadenosine (MTA). MTA acts as an amine acceptor in the transfer of the aminopropyl group from decarboxylated S-adenosylmethionine to spermidine. In the absence of any other convincing variant, they hypothesized that the location of the variant could explain the severity of the symptoms (Albert et al., 2015). In the same way, the severe

phenotype of our patients could be linked to the proximity of the mutation p.(Gly203Asp) to the decarboxylated S-adenosylmethionine binding residue.

Besides constipation and spontaneously-resolving hypertransaminemia (Peron et al., 2013), gastrointestinal or digestive symptoms had not been reported in Snyder-Robinson syndrome until recently. In 2019, Larcher et al. described for the first time a severe case of Snyder-Robinson syndrome with rectosigmoid hirschprung disease, gut malrotation and precirrhotic portal fibrosis, suggesting that the phenotype of Snyder-Robinson syndrome could include a digestive involvement (Larcher et al., 2019). We report the second patient with Snyder-Robinson syndrome associated with severe digestive involvement, with jejuno-duodenal stenosis, major gastric intolerance, cholestasis, hepatic fibrosis and exocrine pancreatic deficiency. Although the possibility of a second genetic condition in patient 1 cannot be formally ruled out, there are some other elements in the literature suggesting that loss of function of SMS can impact the gastrointestinal tract and digestive system.

A few studies have tested the hypothesis that polyamines may play a role in the digestive metabolism. Fang et al. (2016) showed that administration of high doses of spermine supplements to weaning rats affected intestinal morphology and enhanced intestinal antioxidative capacity. Zahedi et al. (2012) studied the role of polyamines in liver injury after CCl<sub>4</sub>- administration to rats, showing that inhibition of SSAT and polyamine oxidase reduced the severity of hepatic lesions after CCl<sub>4</sub>- administration, thereby suggesting that toxic products of polyamine catabolism could contribute to hepatocytic death. Furthermore, Alhonen et al. (2000) reported that overexpression of SSAT could induce acute pancreatitis in mice, by polyamines depletion, in this case without involving reactive oxygen species.

In drosophilian models of Snyder-Robinson syndrome, Li and colleagues showed that the alteration of polyamine metabolism caused lysosomal dysfunction, as well as alteration in mitochondrial morphology and function, thus confirming that the physiopathology of Snyder-Robinson syndrome implicates oxidative stress (Li et al., 2017).

For patient 1, we may hypothesize that congenital jejunal stenosis (which could have occurred by chance), insufficient intestinal maturation due to spermine deficiency, and oxidative stress secondary to accumulation of spermidine and production of toxic metabolites contributed to the digestive intolerance and liver and pancreatic failure.

For patient 2, the need for enteral feeding and failure-to-thrive could be partially explained by the combination of severe hypotonia and frequent infections, while the constipation could be caused by multiple factors. Nevertheless, because shock-associated ischemic colitis is very unusual in children, we could also hypothesize that a vulnerability to oxidative stress contributed to the occurrence of a colic infarction during his circulatory shock.

### **Conclusion:**

We have described two severe cases of Snyder-Robinson syndrome, caused by a novel *SMS* mutation. One of our patients presented jejunal stenosis, hepatic fibrosis, pancreatic exocrine deficiency, enteral feeding intolerance and severe failure to thrive, offering further evidence that the phenotype of Snyder-Robinson syndrome could include digestive involvement. The presence of congenital jejunal stenosis, added to the oxidative stress characterizing this syndrome, could have contributed to the digestive intolerance and to the liver and pancreatic failure, but more studies are required to understand the mechanism of digestive injury in Snyder-Robinson syndrome.

**Acknowledgments:**

The ULB Center of Human Genetics, Hôpital Universitaire des Enfants Reine Fabiola and Hôpital Erasme are part of the European Reference Network on Rare Congenital Malformations and Rare Intellectual Disability (ERN-ITHACA) [EU Framework Partnership Agreement ID: 3HP-HP-FPA ERN-01-2016/739516].

**Accession number:**

The *SMS* variant c.608G>A has been submitted to the ClinVar database (<https://www.ncbi.nlm.nih.gov/clinvar/>) under the number SUB7094130.

**Conflict of interest:**

The authors have no conflict of interest to declare.

**Tables:****Table 1:**

| Clinical features             | Our patients | Total |
|-------------------------------|--------------|-------|
| Pre-natal growth retardation  | 0/2          | 5/20  |
| Post-natal growth retardation | 2/2          | 12/19 |
| Severe developmental delay    | 2/2          | 20/22 |
| Able to walk                  | 0/2          | 13/23 |
| Verbal communication          | 0/2          | 13/23 |
| Seizures                      | 2/2          | 14/18 |
| Regression                    | 0/2          | 4/21  |
| Microcephaly                  | 0/2          | 4/21  |
| Cerebral MRI anomalies        | 2/2          | 12/18 |
| Bone fractures                | 0/2          | 12/21 |
| Kyphoscoliosis                | 0/2          | 17/21 |
| Thin habitus                  | 2/2          | 21/21 |
| Chest deformity               | 2/2          | 12/15 |
| Long great toes               | 0/2          | 9/16  |
| Long hands/fingers            | 2/2          | 13/18 |
| Hyperextensible fingers       | 1/2          | 7/15  |
| Asymmetric face               | 0/2          | 11/21 |
| Abnormal palate               | 2/2          | 16/21 |
| Prominent lower lip           | 0/2          | 14/21 |
| Prognathism                   | 0/2          | 6/19  |

|                              |     |       |   |
|------------------------------|-----|-------|---|
| Abnormal ears                | 2/2 | 11/12 | Table 1:<br>Frequency of the clinical features of the Snyder-Robinson patients reported in the literature. A more complete description of each patient is available in Supplementary Table 1. |
| Digestive features           | 2/2 | 5/12  |   |
| Respiratory features         | 2/2 | 5/5   |   |
| Urogenital anomalies         | 2/2 | 9/18  |   |
| Hearing loss                 | 2/2 | 4/10  |   |
| Ophthalmologic abnormalities | 2/2 | 7/13  |   |
| Premature death              | 2/2 | 4/22  |   |

er-Robinson patients reported in the literature. A more complete description of each patient is available in Supplementary Table 1.

**Figures legends:**

## Figure 1:

Schematic representation of the *SMS* gene (transcript NM\_004595.5) and the pathogenic variants reported in the literature.

Green : missense variants. Orange : truncating variant. Blue : splicing variant. Red : variant of our patients.

The variants were collected from : Abela et al., 2016 ; Albert et al., 2015 ; Arena et al., 1996 ; Becerra-Solano et al., 2009 ; Cason et al., 2003 ; de Alencastro et al., 2008 ; Larcher et al., 2019 ; Lemke et al., 2012 ; Peron et al., 2013 ; Schwartz et al., 2011 ; Starks et al., 2018 ; Zhang et al., 2013.

## Figure 2:

Pedigree of the family. The black squares indicate affected males. The female carrier is indicated by a circle with a dot.

**References:**

- Abela, L., Simmons, L., Steindl, K., Schmitt, B., Mastrangelo, M., Joset, P., Papuc, M., Sticht, H., Baumer, A., Crowther, L.M., Mathis, D., Rauch, A., Plecko, B., 2016. N8-acetylspermidine as a potential plasma biomarker for Snyder-Robinson syndrome identified by clinical metabolomics. *J. Inherit. Metab. Dis.* 39, 131–137.  
<https://doi.org/10.1007/s10545-015-9876-y>
- Albert, J.S., Bhattacharyya, N., Wolfe, L.A., Bone, W.P., Maduro, V., Accardi, J., Adams, D.R., Schwartz, C.E., Norris, J., Wood, T., Gafni, R.I., Collins, M.T., Tosi, L.L., Markello, T.C., Gahl, W.A., Boerkoel, C.F., 2015. Impaired osteoblast and osteoclast function characterize the osteoporosis of Snyder - Robinson syndrome. *Orphanet J. Rare Dis.* 10.  
<https://doi.org/10.1186/s13023-015-0235-8>
- Alhonen, L., Parkkinen, J.J., Keinä, T., Sinervirta, R., Herzig, K.-H., Jä, J., Virtanen, A.I., 2000. Activation of polyamine catabolism in transgenic rats induces acute pancreatitis.
- Arena, J.F., Schwartz, C., Ouzts, L., Stevenson, R., Miller, M., Garza, J., Nance, M., Lubs, H., 1996. X-linked mental retardation with thin habitus, osteoporosis, and kyphoscoliosis: Linkage to Xp21.3-p22.12. *Am. J. Med. Genet.* 64, 50–58.  
[https://doi.org/10.1002/\(SICI\)1096-8628\(19960712\)64:1<50::AID-AJMG7>3.0.CO;2-V](https://doi.org/10.1002/(SICI)1096-8628(19960712)64:1<50::AID-AJMG7>3.0.CO;2-V)
- Becerra-Solano, L.E., Butler, J., Castañeda-Cisneros, G., McCloskey, D.E., Wang, X., Pegg, A.E., Schwartz, C.E., Sánchez-Corona, J., García-Ortiz, J.E., 2009. A missense mutation, p.V132G, in the X-linked spermine synthase gene (SMS) causes Snyder-Robinson syndrome. *Am. J. Med. Genet. Part A* 149, 328–335.  
<https://doi.org/10.1002/ajmg.a.32641>

- Cason, A.L., Ikeguchi, Y., Skinner, C., Wood, T.C., Holden, K.R., Lubs, H.A., Martinez, F., Simensen, R.J., Stevenson, R.R., Pegg, A.E., Schwartz, C.E., 2003. X-linked spermine synthase gene (SMS) defect: The first polyamine deficiency syndrome. *Eur. J. Hum. Genet.* 11, 937–944. <https://doi.org/10.1038/sj.ejhg.5201072>
- de Alencastro, G., McCloskey, D.E., Kliemann, S.E., Maranduba, C.M.C., Pegg, A.E., Wang, X., Bertola, D.R., Schwartz, C.E., Passos-Bueno, M.R., Sertié, A.L., 2008. New SMS mutation leads to a striking reduction in spermine synthase protein function and a severe form of Snyder-Robinson X-linked recessive mental retardation syndrome. *J. Med. Genet.* 45, 539–543. <https://doi.org/10.1136/jmg.2007.056713>
- Fang, T., Jia, G., Zhao, H., Chen, X., Tang, J., Wang, J., Liu, G., 2016. Effects of spermine supplementation on the morphology, digestive enzyme activities, and antioxidant capacity of intestine in weaning rats. *Anim. Nutr.* 2, 370–375. <https://doi.org/10.1016/j.aninu.2016.09.002>
- Johansson, L.F., van Dijk, F., de Boer, E.N., van Dijk-Bos, K.K., Jongbloed, J.D.H., van der Hout, A.H., Westers, H., Sinke, R.J., Swertz, M.A., Sijmons, R.H., Sikkema-Raddatz, B., 2016. CoNVaDING: Single Exon Variation Detection in Targeted NGS Data. *Hum. Mutat.* 37, 457–464. <https://doi.org/10.1002/humu.22969>
- Kesler, S.R., Schwartz, C., Stevenson, R.E., Reiss, A.L., 2009. The impact of spermine synthase (SMS) mutations on brain morphology. *Neurogenetics* 10, 299–305. <https://doi.org/10.1007/s10048-009-0184-2>
- Larcher, L., Norris, J.W., Lejeune, E., Buratti, J., Mignot, C., Garel, C., Keren, B., Schwartz, C.E., Whalen, S., 2019. The complete loss of function of the SMS gene results in a severe form of Snyder-Robinson syndrome. *Eur. J. Med. Genet.* 103777.

<https://doi.org/10.1016/j.ejmg.2019.103777>

Lemke, J.R., Riesch, E., Scheurenbrand, T., Schubach, M., Wilhelm, C., Steiner, I., Hansen, J., Courage, C., Gallati, S., Bürki, S., Strozzi, S., Simonetti, B.G., Grunt, S., Steinlin, M., Alber, M., Wolff, M., Klopstock, T., Prott, E.C., Lorenz, R., Spaich, C., Rona, S., Lakshminarasimhan, M., Kröll, J., Dorn, T., Krämer, G., Synofzik, M., Becker, F., Weber, Y.G., Lerche, H., Böhm, D., Biskup, S., 2012. Targeted next generation sequencing as a diagnostic tool in epileptic disorders. *Epilepsia* 53, 1387–1398.

<https://doi.org/10.1111/j.1528-1167.2012.03516.x>

Li, C., Brazill, J.M., Liu, S., Bello, C., Zhu, Y., Morimoto, M., Cascio, L., Pauly, R., Diaz-Perez, Z., Malicdan, M.C. V., Wang, H., Boccuto, L., Schwartz, C.E., Gahl, W.A., Boerkoel, C.F., Zhai, R.G., 2017. Spermine synthase deficiency causes lysosomal dysfunction and oxidative stress in models of Snyder-Robinson syndrome. *Nat. Commun.* 8.

<https://doi.org/10.1038/s41467-017-01289-7>

Moulinoux, J.P., Quemener, V., Le Calve, M., Chatel, M., Darcel, F., 1984. Polyamines in human brain tumors - A correlative study between tumor, cerebrospinal fluid and red blood cell free polyamine levels. *J. Neurooncol.* 2, 153–158.

<https://doi.org/10.1007/BF00177902>

Murray-Stewart, T., Dunworth, M., Foley, J., Schwartz, C., Casero, R., 2018. Polyamine Homeostasis in Snyder-Robinson Syndrome. *Med. Sci.* 6, 112.

<https://doi.org/10.3390/medsci6040112>

Pegg, A.E., 2014. The function of spermine. *IUBMB Life* 66, 8–18.

<https://doi.org/10.1002/iub.1237>

- Peron, A., Spaccini, L., Norris, J., Bova, S.M., Selicorni, A., Weber, G., Wood, T., Schwartz, C.E., Mastrangelo, M., 2013. Snyder-Robinson syndrome: A novel nonsense mutation in spermine synthase and expansion of the phenotype. *Am. J. Med. Genet. Part A* 161, 2316–2320. <https://doi.org/10.1002/ajmg.a.36116>
- QUEMENER, V., LE GALL, E., EDAN, C., MOULINOX, J.P., 1986. Red blood cell polyamine levels in children with acute leukemia: their clinical interest in disease staging and monitoring of therapy efficiency. *Cancer J.* 1.
- Schwartz, C.E., Wang, X., Stevenson, R.E., Pegg, A.E., 2011. Spermine Synthase Deficiency Resulting in X-Linked Intellectual Disability (Snyder–Robinson Syndrome), in: *Polyamines: Methods and Protocols*. Springer Science+Business Media, pp. 437–445. <https://doi.org/10.1007/978-1-61779-034-8>
- Snyder, R.D., Robinson, A., 1969. Recessive sex-linked mental retardation in the absence of other recognizable abnormalities. Report of a family. *Clin. Pediatr. (Phila)*. 8, 669–74. <https://doi.org/10.1177/000992286900801114>
- Starks, R., Kirby, P., Ciliberto, M., Hefti, M., 2018. Snyder-Robinson syndrome. *Autops. Case Reports* 8. <https://doi.org/10.4322/acr.2018.031>
- Wu, H., Min, J., Zeng, H., McCloskey, D.E., Ikeguchi, Y., Loppnau, P., Michael, A.J., Pegg, A.E., Plotnikov, A.N., 2008. Crystal structure of human spermine synthase: Implications of substrate binding and catalytic mechanism. *J. Biol. Chem.* 283, 16135–16146. <https://doi.org/10.1074/jbc.M710323200>
- Zahedi, K., Barone, S.L., Xu, J., Steinbergs, N., Schuster, R., Lentsch, A.B., Amlal, H., Wang, J., Casero Jr, R.A., Soleimani, M., Jr, C.R., Hepatocyte, S.M., 2012. Hepatocyte-specific

ablation of spermine/spermidine-N 1-acetyltransferase gene reduces the severity of CCl<sub>4</sub>-induced acute liver injury. *Am J Physiol Gastrointest Liver Physiol* 303, 546–560.

<https://doi.org/10.1152/ajpgi.00431.2011.-Activation>

Zhang, Z., Norris, J., Kalscheuer, V., Wood, T., Wang, L., Schwartz, C., Alexov, E., Van Esch, H., 2013. A Y328C missense mutation in spermine synthase causes a mild form of snyder-robinson syndrome. *Hum. Mol. Genet.* 22, 3789–3797.

<https://doi.org/10.1093/hmg/ddt229>

Zhang, Z., Teng, S., Wang, L., Schwartz, C.E., Alexov, E., 2010. Computational analysis of missense mutations causing Snyder-Robinson syndrome. *Hum. Mutat.* 31, 1043–1049.

<https://doi.org/10.1002/humu.21310>

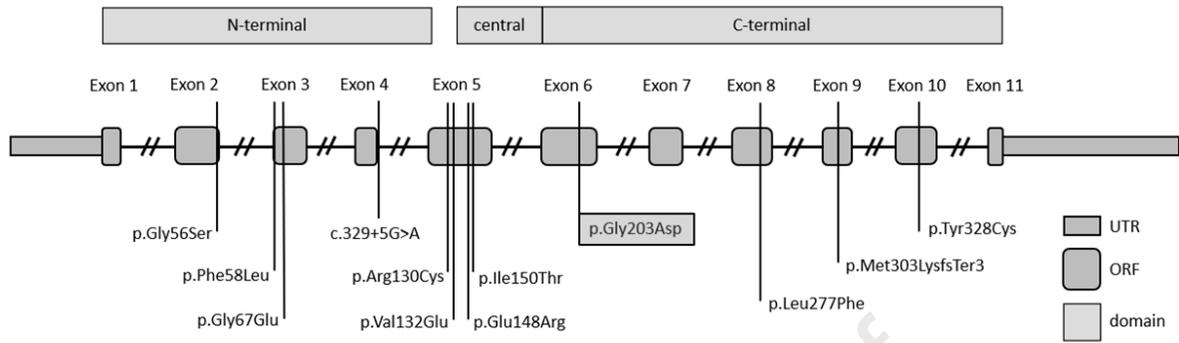
### **Supplementary material:**

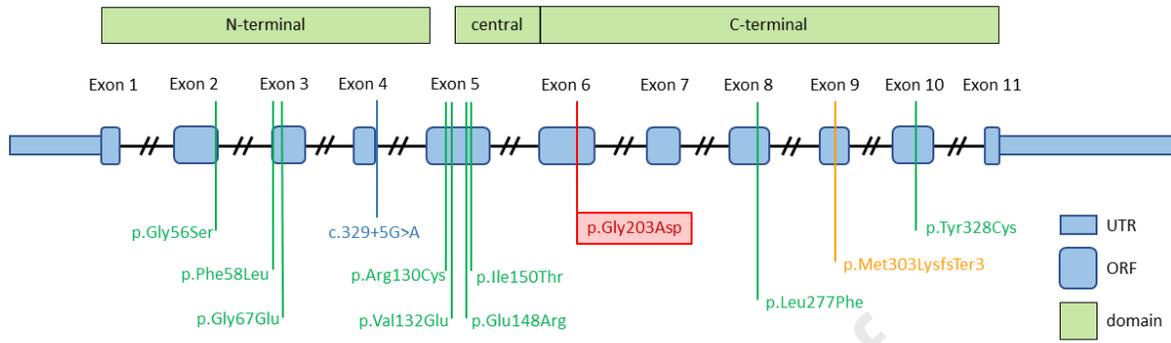
Supplementary Table 1:

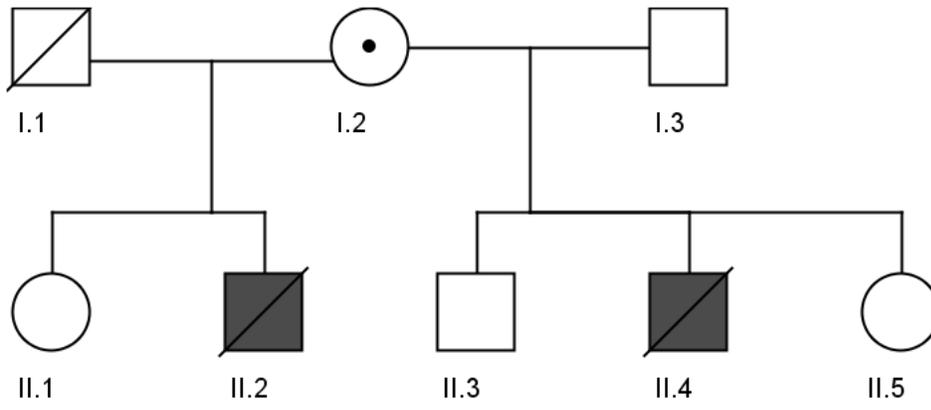
*Clinical description and SMS variants of the Snyder-Robinson patients reported in the literature.*

*+ : present ; - : absent ; blank : no data ; bold : coding variant in NM\_004595.5 ; italic : coding variant as described in the initial article ; NA : not applicable ; ND : not determined.*

*<sup>a</sup> variant found by Cason et al (2003), <sup>b</sup> reported by Arena et al (1996) and Kesler et al (2009)*







Journal Pre-proof