Loss-of-function variants in SRRM2 cause a neurodevelopmental disorder

Cuinat, Silvestre; Nizon, Mathilde; Isidor, Bertrand; Stegmann, Alexander; van Jaarsveld, Richard H.; van Gassen, Koen L.; van der Smagt, Jasper J.; Volker-Touw, Catharina M.L.; Holwerda, Sjoerd J.B.; Terhal, Paulien A.

Published in:
Genetics in Medicine

DOI:
10.1016/j.gim.2022.04.011

Publication date:
2022

Genetics in Medicine (2022) 24, 1774–1780

ELSEVIER

Genetics in Medicine

www.journals.elsevier.com/genetics-in-medicine

BRIEF REPORT

Loss-of-function variants in *SRRM2* cause a neurodevelopmental disorder

ARTICLE INFO

Article history:
Received 24 January 2022
Received in revised form
6 April 2022
Accepted 6 April 2022
Available online 14 May 2022

Keywords:
Molecular genetics
Neurodevelopment
Spliceosome
SRRM2
Intellectual disability

ABSTRACT

Purpose: *SRRM2* encodes the SRm300 protein, a splicing factor of the SR-related protein family characterized by its serine- and arginine-enriched domains. It promotes interactions between messenger RNA and the spliceosome catalytic machinery. This gene, predicted to be highly intolerant to loss of function (LoF) and very conserved through evolution, has not been previously reported in constitutive human disease.

Methods: Among the 1000 probands studied with developmental delay and intellectual disability in our database, we found 2 patients with de novo LoF variants in *SRRM2*. Additional families were identified through GeneMatcher.

Results: Here, we report on 22 patients with LoF variants in *SRRM2* and provide a description of the phenotype. Molecular analysis identified 12 frameshift variants, 8 nonsense variants, and 2 microdeletions of 66 kb and 270 kb. The patients presented with a mild developmental delay, predominant speech delay, autistic or attention-deficit/hyperactivity disorder features, overfriendliness, generalized hypotonia, overweight, and dysmorphic facial features. Intellectual disability was variable and mild when present.

Conclusion: We established *SRRM2* as a gene responsible for a rare neurodevelopmental disease.

Introduction

RNA splicing is a highly conserved process that transforms pre-messenger RNAs (mRNAs) into mature mRNAs. Constitutive splicing involves removing intronic sequences, whereas alternative splicing modifies the exonic composition of transcripts in a finely regulated manner, thus increasing the diversity of proteins produced. This process is accomplished by the spliceosome that includes 5 small nuclear ribonucleoprotein particles (snRNPs: U1, U2, U4, U5, and U6) and hundreds of other protein factors.[1] Among these, SR proteins are an evolutionarily conserved family characterized by 1 or 2 N-terminal RNA recognition motif (RRM) followed by a downstream arginine- and serine-rich region (RS domain, with at least 50 amino acids and 40% serine and arginine content).[2] This classification excludes many splicing factors containing RS domains but lacking RRM motif. Thus, these splicing factors are designated as SR-related proteins. SRm300, encoded by the *SRRM2* gene (OMIM 606032), is one of them.

SRRM2 encodes SRm300, which is a nuclear ubiquitous protein of 2752 amino acids that forms a complex with SRm160 encoded by *SRRM1*. This complex is involved in pre-mRNA maturation as one of the main catalytic components of the spliceosome and promotes interaction between pre-mRNA and splicing factors such as snRNPs.[3,4] *SRRM2* is highly conserved through evolution and has

*Correspondence and requests for materials should be addressed to Silvestre Cuinat, CHU de Nantes Hôtel-Dieu, Service de Génétique Médicale, 9 quai Moncousu, 44093 Nantes cedex 1, France. *E-mail address:* silvestre.cuinat@chu-nantes.fr or Benjamin Cogné, CHU de Nantes Hôtel-Dieu, Laboratoire de génétique moléculaire, 9 quai Moncousu, 44093 Nantes cedex 1, France. *E-mail address:* benjamin.cogne@chu-nantes.fr

A full list of authors and affiliations appears at the end of the paper.

doi: https://doi.org/10.1016/j.gim.2022.04.011

many orthologs, for example in yeast (Cwc21p) and *Caenorhabditis elegans* (Rsr-2).[5-7]

SRRM2 shows a high probability of intolerance to loss-of-function (LoF) variants (probability of loss of function intolerance = 1, observed/expected ratio = 0.06) in Genome Aggregation Database (gnomAD) (v2.1.1) and belongs to the 0.1% most intolerant human protein-coding genes with a residual variation intolerance score of −4.5 (15th position among 17,000 genes).[8] *SRRM2* variants have not been reported in patients. However, by a statistical approach, Kaplanis et al[9] studied the enrichment in de novo variants among 31,000 trio-exome sequencing (ES) performed for neurodevelopmental disorder and identified *SRRM2* as one of the 28 genes significantly enriched in LoF variants.

To date, no formal phenotype association has been reported with *SRRM2*. Here, we report 22 patients with LoF heterozygous variants in *SRRM2* and describe the phenotype associated with this spliceosomopathy.

Materials and Methods

The phenotype of each patient was evaluated by a clinical geneticist in each collaborating center. Patients 1 to 3, 17, and 20 to 22 were diagnosed by singleton ES with familial segregation analysis by Sanger sequencing, except for patient 17 for whom parental samples were unavailable. Patients 4 to 14, 16, 18, and 19 were diagnosed using trio ES. Next-generation sequencing was performed at each local center, using the following platforms: HiSeq for patients 1 to 3, 5 to 9, 12, 17, 20, and 21; NextSeq for patients 13 and 14; and NovaSeq for patients 11, 15, 17 to 19, and 22 (Illumina). SOLiD system sequencing (Life Technologies) was used for patient 10. The deletions of patients 15 and 22 were discovered by single-nucleotide variant (formerly single-nucleotide polymorphisms)–array analysis using a CytoSNP-850K BeadChip (Illumina). Details of the sequencing method, kits, and tools used by each center are described in the Supplemental material and method. Variant interpretation and classification was done adhering to American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines. All single-nucleotide variants and indel nomenclature were verified in VariantValidator and submitted in ClinVar (see Web Resources) with the following accession numbers: SCV002016268.1, SCV002016269.1, then SCV002055998 to SCV002056014.

Results

Genetic results

Among the 1000 probands with developmental delay and intellectual disability (ID) in the clinical exome database from Nantes hospital, we found that 2 patients had protein truncating variants in *SRRM2*. Given the high predicted LoF intolerance of *SRRM2* (NM_016333.4) (probability of loss of function intolerance = 1; observed/expected ratio = 0.06 in gnomAD v2.1.1), we performed segregation analysis by Sanger and showed that the 2 variants were de novo (maternity, paternity, and the identity of the sample were confirmed by microsatellite analysis).

With the web-based tool GeneMatcher (see Web Resources), 20 new patients with likely gene-disrupting variants in *SRRM2* were identified. Of a total of 22 patients, 12 had frameshift variants, 8 had nonsense variants, and 2 had a 66 kb and 270 kb microdeletion involving 3 genes (arr [GRCh37]16p13.3 [2747761_2813511]x1) and 13 genes, respectively, (arr[GRCh37]16p13.3 [2,763,528-3,032,566] x1) of which *SRRM2* is the only one predicted to be haploinsufficient. Of note, other patients in Decipher (see Web Resource) with a neurocognitive phenotype have slightly larger deletions involving *SRRM2*, but the hypothesis of a recurrent breakpoint remains to be explored.

All these variants are absent from gnomAD database (v2.1.1). Of 22, 19 were confirmed to be de novo and 1 was suspected to be in mosaic state in the asymptomatic mother (2 of 108 reads), but an orthogonal validation of the mosaicism will be necessary to confirm it. Parental segregation analysis was lacking for 1 patient, and 1 variant was inherited from a father with developmental delay. One nonsense variant is located in exon 2 of 15, the other 19 frameshift and nonsense variants are located in the large exon 11 of 15 with a predicted degradation by nonsense-mediated decay (Figure 1A). No variant better explained the phenotype of these patients. Twenty *SRRM2* LoF variants were found among 74,000 ES/genome sequencing in 11 local databases, ie, 0.027%. In these databases, no *SRRM2* LoF variant was found in patients without a neurodevelopmental disorder.

Patient phenotype

Clinical phenotypes are summarized in Table 1; the detailed individual table is provided in Supplemental material and method. All patients had developmental delay predominantly on language acquisition (16/19). The age at which the first words were spoken ranged from 1 year to 4 years (mean of 22 months, interquartile range of 12 months), and the age at which the first sentences were spoken ranged from 2 years to 7 years (mean of 3 years 6 months, interquartile range of 15 months). Motor delay was less common: 8 of 22 patients started to walk after 18 months, but all of them started to walk before 24 months. When present and evaluable, ID was mild (16/20) and IQ ranged from 50 to 70. Of 20 patients, 4 patients did not have ID and neurocognitive evaluation was not available for 2 patients. No moderate or severe ID was observed. Although autistic features seemed frequent (9/22), some patients were reported to have a friendly sociable personality (8/22), sometimes excessively. Attention-deficit/hyperactivity disorder was also

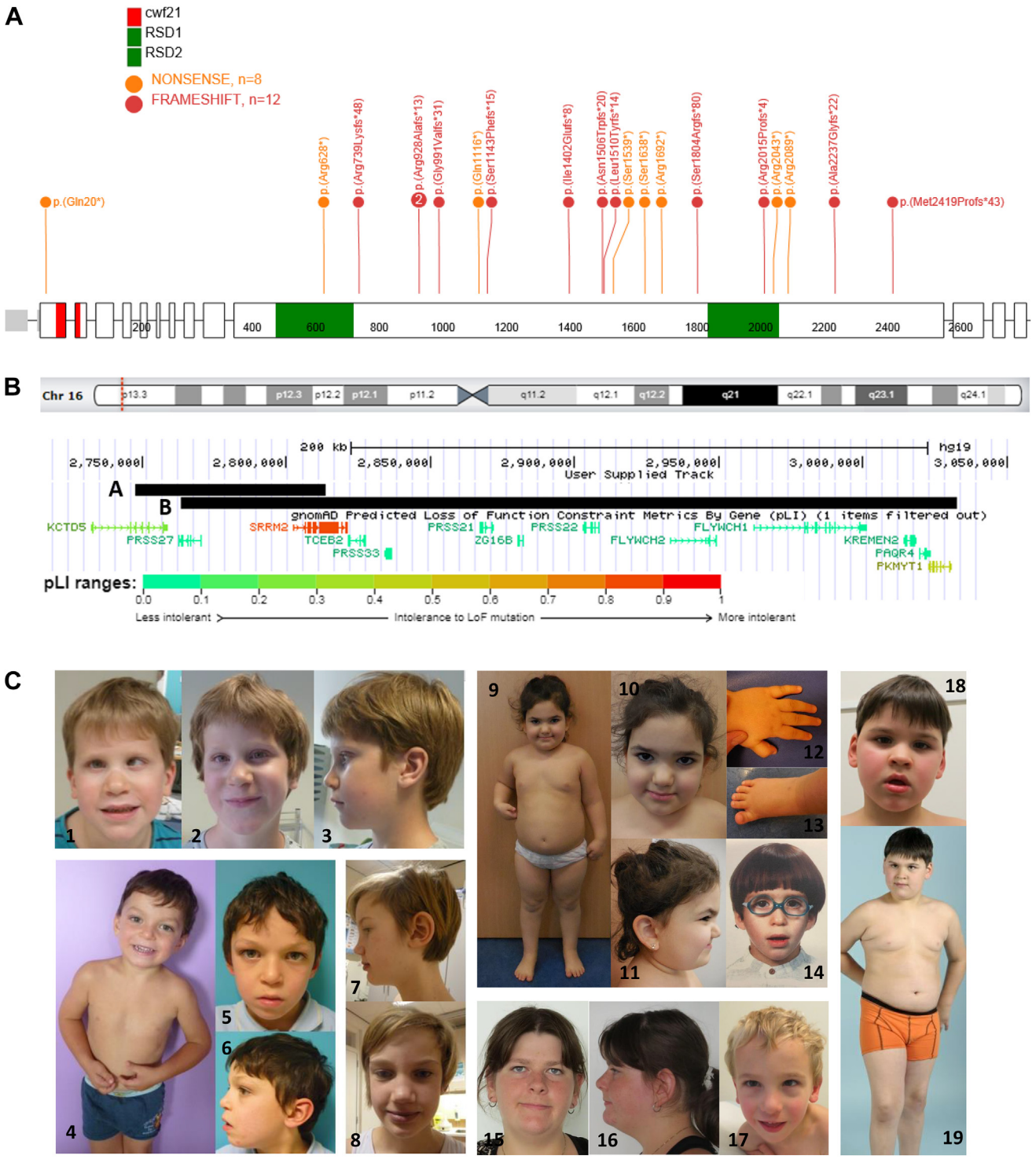

Figure 1 **Molecular data and clinical pictures.** A. Genetic findings of individuals with *SRRM2* variants. A schematic view of *SRRM2* exon sequence (NM_016333.4) and the effects of variants identified in this study. Domains representation: cwf21 domain in red, Rich Serine and Arginine Domain 1 and 2 in green. The amino acid numbering is based on PeCan Data of Proteinpaint (see Web Resources). B. A schematic view of large deletions involving *SRRM2* detected by SNP array analysis and based on UCSC browser (see Web Resources). The 66 kb deletion of patient 22 involves 3 genes (arr[GRCh37] 16p13.3 [2747761_2813511] x 1) and the 270 kb deletion of patient 15 involves 13 genes (arr[GRCh37] 16p13.3 [2,763,528-3,032,566] x 1). In both cases, *SRRM2* is the only gene predicted to be haploinsufficient. C. Images of individuals with *SRRM2* LoF. Patient 1 at 3.5 years (1), 8.5 years (2, 3), patient 2 at 4 years (4) and 6.5 years (5, 6), patient 10 at 13 years (7, 8), patient 7 at 4 years (9-13), patient 3 at 4 years (14), patient 14 at 19 years (15, 16), patient 15 at 4 years (17), patient 11 at 5 years (18) and 8 years (19). Note the deep-set eyes, bulbous nasal tip, and smooth philtrum, which are among the most recurrent characteristics in our cohort. gnomAD, Genome Aggregation Database; LoF, loss of function; pLI, probability of loss of function intolerance.

Table 1 Clinical findings of individuals with *SRRM2* LoF variants ($N = 22$)

Patient Characteristics	Values
Gender	
Male	14
Female	8
Age at the time of study (y)	4-28 y (mean = 11 y)
Neurodevelopment and behavior	
Developmental delay	22
Language delay	16/19
Walking delay	8
Intellectual disability	16/20
Autistic features	9
ADHD features	6
Hypersociability/friendliness	8
Anxiety	2
Hyperphagia	4
Feeding difficulties	5
Other neurological findings	
Neonatal hypotonia	4
Hypotonia at the time of the study	9
Distal hyperlaxity	4
Coordination trouble/dyspraxia	5
Growth	
Overweight	12
Obesity	7
Tall stature	4
Morphological features	
Facial dysmorphism	20
Small/short hands and feet	6
Ophthalmologic abnormalities	
Strabismus	4
Hypermetropia	3
Other visceral and skeletal abnormalities	
Unilateral hypoplastic kidney	1
Complex congenital heart defect	1
Micropenis, small testes	1/14 males
Spina bifida	1
Scoliosis with hemivertebra	1

$N = 22$ unless indicated otherwise.
ADHD, attention-deficit/hyperactivity disorder; *LoF*, loss of function.

reported (6/22). Global hypotonia seems to be recurrent (9/22), sometimes present at birth (4/22) and then accompanied by neonatal feeding difficulties (5/22). Postnatal overweight was observed in 12 of 22 patients, with obesity in 7 of 22, sometimes before age 4 years. An eating disorder with hyperphagia, food obsession, and lack of satiety was reported for 4 of them.

Mild dysmorphic features were observed (Figure 1B), such as hypotonic face (5/22), epicanthus (3/22), deep set eyes (10/22), large everted ears (7/22) or low-set posteriorly rotated ears (4/22), uplifted horizontal ear lobule (4/22), broad bulbous nasal tip (9/22), smooth philtrum (6/22), thin upper lip (7/22), broad chin (6/22), short neck (3/22), microcephaly (1/22), and macrocephaly (2/22). Other features included broad short hands (6/22) and feet (6/22), mild visual impairment with strabismus (4/22) or hypermetropia (3/22), urogenital abnormalities with small testes and micropenis (1/14 males), shawl scrotum (1/14 males), and unilateral kidney hypoplasia (1/22). One patient had complex congenital heart defect and thoracic hemivertebra, but in this case, valproate exposure during pregnancy could be involved in this phenotype.

Discussion

We identified a neurodevelopmental disorder caused by LoF variants in *SRRM2*. The individuals showed a mild to moderate developmental delay predominantly on language, autistic or attention-deficit/hyperactivity disorder features, overfriendliness, global hypotonia, overweight, and characteristic facial features. ID was variable. The main differential diagnoses evoked and investigated were fragile X syndrome (10/22), Prader-Willi syndrome (4/22), and myotonic dystrophy type 1 (3/22).

We did not identify pathogenic missense variants in *SRRM2* in this study. *SRRM2* has a low genetic constraint regarding missense variants in gnomAD (v2.1.1) with a z score of −6.28 (o/e = 1.43). Indeed, SRm300 contains serine-arginine–enriched RS domains, involved in splicing regulation by promoting protein-protein and protein-RNA interactions, with a poorly conserved structure.[10] However, SRm300 also contains a conserved cwf21 domain that seems to promote interaction between mRNA and the spliceosome catalytic machinery. Cwc21p ortholog in *Saccharomyces cerevisiae* is made of only 135 amino acids, and Rsr-2 in *Caenorhabditis elegans* is made of 425 amino acids. Both contain the cwf21 domain.[5-7] Although we only identified LoF variants in *SRRM2*, it is not excluded that missense variants in this conserved cwf21 domain could alter protein function.

We identified 4 C to T transition at arginine codon CGA leading to premature stop codon. Arginine is encoded by 6 different codons of which 4 are hypermutable because of the presence of CpG dinucleotide, including the CGA codon. Because *SRRM2* is enriched in arginine codons, it is not surprising to identify such events in this gene. Indeed, Schulze et al[11] observed that arginine substitutions underlie 20.0% of all pathogenic single-nucleotide variants, and arginine was the most commonly substituted amino acid in genes linked to syndromic autism spectrum disorder. Interestingly, in their list of 18,295 genes, *SRRM2* appears to be the third most enriched in the CGA codon, reinforcing the hypothesis that *SRRM2* may be, as well as other proteins with RS domains, a high-risk gene for nonsense variants.

Based on gnomAD constraint data, *SRRM2* is predicted to be haploinsufficient (pLI = 1, o/e = 0.06). Given that in our study we identified 22 patients with LoF variants and an overlapping, although nonspecific, neurodevelopmental disorder, haploinsufficiency is the most probable pathomechanism. *SRRM2* haploinsufficiency is not the first model of spliceosomopathy, but unlike *SF3B4* [OMIM 605593]- or *EFTUD2* [OMIM 603892]-related disorders, no patient has mandibulofacial dysostosis or major skeletal abnormalities.

ID in spliceosomopathies can be the main symptom, with the example of heterogeneous nuclear ribonucleoprotein–related disorders (OMIM 300986, OMIM 617391, and OMIM 616580). Several heterogeneous nuclear ribonucleoproteins are involved in neuronal proliferation, differentiation, and plasticity.[12] RNA sequencing data from patients with *HNRNPR* variants showed aberrant intronic retention in multiple *HOX* genes, considered as fundamental regulators of embryonic development.[13] Paradoxically, other spliceosomopathies are nonsyndromic and organ-specific, such as isolated retinitis pigmentosa 18 (*PRPF3* [OMIM 607301]), nonsyndromic deafness DFNB109 (*ESRP1* [OMIM 618013]), or spinal muscular atrophy (*SMN1* [OMIM 253300]).

SRm300 is known as a splicing factor, but unlike SRm160, it does not appear to be essential for constitutive splicing.[4] However, the early embryonic lethality shown in knockout mice and *Caenorhabditis elegans*[7] indicates *SRRM2* as an important gene during development. SRm300 appears to be involved in the final phase of the splicing process (initiation of the second transesterification step)[14] and was the only SR-related protein found at the heart of the spliceosome catalytic complex after purification.[15] Its interaction with Prp8p and U5-snRNP could allow precise selection of 3′ splice sites and provide fine control of alternative splicing by promoting the use of weak 3′ splice sites.[14] RNA sequencing studies, searching for abnormally spliced transcripts, might help to understand why *SRRM2* LoF appears to specifically affect neurodevelopment, without other major visceral manifestations.

Another interesting pathway to consider is SRm300 interaction with SON (*SON DNA* binding protein). *SON* haploinsufficiency is responsible for a syndromic neurodevelopmental disorder, and splicing aberrations have been found in these patients.[16] Similar to SRm300, SON seems to act as an alternative splicing facilitator, preferentially for weak splice sites, interacting with SR proteins and RNA polymerase. However, it should be considered that splicing alteration may not be the only mechanism involved. Together, SON and SRm300 structure the nuclear speckles,[17] a subtype of nuclear bodies in interchromatin domain, enriched in pre-mRNA splicing factors, and whose functions remain unclear. In a recent study, disruption of nuclear speckles by knocking down *SRRM2* ortholog in mouse hepatocytes reduced chromatin interactions, mostly in the highly active compartments, leading to a wide dysregulation of gene expression.[18] Moreover, SRm300 interacting with Cactin and Dhx8 could also be involved in sister chromatid cohesion and cell division cycle, by promoting Sororin (Cdca5) splicing.[19] Therefore, it would be interesting to investigate speckles integrity and chromatid cohesion by microscopy in patients with *SRRM2* LoF.

This study presents the molecular and clinical description of a neurodevelopmental disorder caused by LoF variants in *SRRM2* and aims to provide a molecular diagnosis for these patients on the basis of exome or genome data. Further molecular and functional studies will be necessary to characterize the pathophysiology of the disease, estimate its prevalence, and identify potential pathogenic missense variants. To improve the identification and management of this syndrome, the clinical data need to be enriched, in particular data concerning the neurocognitive profile and the long-term outcome of these patients.

Data Availability

The data that support the findings of this study are available upon request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Author Information

Conceptualization: B.C.; Methodology: B.C.; Data Curation: B.C., S.C.; Data Collection: all coauthors; Photo Collection: B.I., M.N., I.V., B.B.A.D.V., E.H.G., J.J.v.d.S., L.B.O., C.B.A., M.S.; Writing-original draft: B.C., S.C.; Writing-review and editing: all coauthors.

Ethics Declaration

In each participating center, clinical assessment was performed by at least 1 expert clinical geneticist. Written consent for genetic testing was obtained from all patients and parents included in this study. Additional written consent for the publication of photographs was obtained for individuals 1, 2, 3, 7, 10, 11, 14, and 15.

This study was approved by the CHU de Nantes ethics committee (Research Programme "Génétique Médicale DC-2011-1399). Our local consent form has been approved by the ethics committee of Nantes UMC, i.e., Consultative Committee on Data Processing for Research in the Health Field (CCTIRS) and French National Commission for Data processing and individual liberties (CNIL). All these activities are subject to the competent Ethics Committee (Comité de Protection des Personnes) and are the subject of a declaration/authorization with the French Ministry in charge of research. All other collaborating institutions have received approval from their local Institutional Review Board.

Web Resources

gnomAD: https://gnomad.broadinstitute.org/. Accessed May 1, 2022.
OMIM: https://www.omim.org/. Accessed May 1, 2022.
Proteinpaint: https://proteinpaint.stjude.org. Accessed May 1, 2022.
GeneMatcher: https://genematcher.org/. Accessed May 1, 2022.

Variant Validator: https://variantvalidator.org/service/validate/. Accessed May 1, 2022.
ClinVar: https://www.ncbi.nlm.nih.gov/clinvar/. Accessed May 1, 2022.
UCSC: https://genome.ucsc.edu/. Accessed May 1, 2022.
Decipher: https://www.deciphergenomics.org/. Accessed May 1, 2022.

Conflict of Interest

This work was carried out within the framework of Nantes University Medical Center activity without additional funding. One patient was diagnosed in the context of work in a private company (AiLife Diagnostics, Pearland, Texas). The other authors declare no conflicts of interest.

Additional Information

The online version of this article (https://doi.org/10.1016/j.gim.2022.04.011) contains supplementary material, which is available to authorized users.

Authors

Silvestre Cuinat[1,*], Mathilde Nizon[1,2], Bertrand Isidor[1,2], Alexander Stegmann[3,4], Richard H. van Jaarsveld[5], Koen L. van Gassen[5], Jasper J. van der Smagt[5], Catharina M.L. Volker-Touw[5], Sjoerd J.B. Holwerda[5], Paulien A. Terhal[5], Sarah Schuhmann[6], Georgia Vasileiou[6], Mohamed Khalifa[7], Alaa A. Nugud[7], Hemad Yasaei[8], Lilian Bomme Ousager[9,10], Charlotte Brasch-Andersen[9,10], Wallid Deb[1,2], Thomas Besnard[1,2], Marleen E.H. Simon[5], Karin Huijsdens-van Amsterdam[5], Nienke E. Verbeek[5], Dena Matalon[11], Natalie Dykzeul[11], Shana White[11], Elizabeth Spiteri[11], Koen Devriendt[12], Anneleen Boogaerts[12], Marjolein Willemsen[3,4], Han G. Brunner[3,4], Margje Sinnema[4], Bert B.A. De Vries[3], Erica H. Gerkes[13], Rolph Pfundt[3], Kosuke Izumi[14], Ian D. Krantz[14], Zhou L. Xu[14], Jill R. Murrell[15], Irene Valenzuela[16], Ivon Cusco[16,17], Eulàlia Rovira-Moreno[16,17], Yaping Yang[18], Varoona Bizaoui[19], Olivier Patat[20], Laurence Faivre[21,23], Frederic Tran-Mau-Them[22,23], Antonio Vitobello[22,23], Anne-Sophie Denommé-Pichon[22,23], Christophe Philippe[22,23], Stéphane Bezieau[1,2], Benjamin Cogné[1,2,*]

Affiliations

[1]Service de Génétique Médicale, Centre Hospitalier Universitaire de Nantes, Nantes, France; [2]Université de Nantes, Inserm UMR 1087 / CNRS UMR 6291, Institut du thorax, Nantes, France; [3]Department of Human Genetics, Radboud University Medical Center, Nijmegen, The Netherlands; [4]Department of Clinical Genetics, Maastricht University Medical Center, Maastricht, The Netherlands; [5]Department of Genetics, University Medical Center Utrecht, Utrecht, The Netherlands; [6]Institute of Human Genetics, Universitätsklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany; [7]Genetic Department, Dubai Health Authority, Latifa Women and Children Hospital, Dubai, United Arab Emirates; [8]Dubai Genetics Center, Pathology and Genetics Department, Dubai Health Authority, Dubai, United Arab Emirates; [9]Department of Clinical Genetics & Human Genetics, Odense University Hospital, University of Southern Denmark, Odense, Denmark; [10]Department of Clinical Research, Odense University Hospital, University of Southern Denmark, Odense, Denmark; [11]Department of Pediatric, Division of Medical Genetics, Stanford University and Health Care, Palo Alto, CA; [12]Center for Human Genetics, University Hospital Leuven, KU Leuven, O&N I Herestraat 49, Leuven, Belgium; [13]University Medical Center Groningen, Department of Genetics, University of Groningen, Groningen, The Netherlands; [14]Division of Human Genetics, Department of Pediatrics, Children's Hospital of Philadelphia, Philadelphia, PA; [15]Division of Genomic Diagnostics, Department of Pathology and Laboratory Medicine, Children's Hospital of Philadelphia, Philadelphia, PA; [16]Department of Clinical and Molecular Genetics, Hospital Vall d'Hebron, Barcelona, Spain; [17]Centro de Investigación Biomédica en Red de Enfermedades Raras (CIBERER), Barcelona, Spain; [18]AiLife Diagnostics, Pearland, TX; [19]Clinical Genetics and Neurodevelopmental Disorders, Centre Hospitalier de l'Estran, Pontorson, France; [20]Department of Medical Genetics, Toulouse University Hospital, Toulouse, France; [21]Centre de référence Anomalies du Développement et Syndromes malformatifs, FHU-TRANSLAD, GAD, CHU Dijon et Université de Bourgogne, Dijon, France; [22]Unité Fonctionnelle Innovation en Diagnostic Génomique des Maladies Rares, FHU-TRANSLAD, CHU Dijon Bourgogne, Dijon, France; [23]Inserm UMR1231, GAD, Université de Bourgogne, Dijon, France

References

1. Wahl MC, Will CL, Lührmann R. The spliceosome: design principles of a dynamic RNP machine. *Cell*. 2009;136(4):701–718. http://doi.org/10.1016/j.cell.2009.02.009.
2. Manley JL, Krainer AR. A rational nomenclature for serine/arginine-rich protein splicing factors (SR proteins). *Genes Dev*. 2010;24(11):1073–1074. http://doi.org/10.1101/gad.1934910.
3. Sawada Y, Miura Y, Umeki K, Tamaoki T, Fujinaga K, Ohtaki S. Cloning and characterization of a novel RNA-binding protein SRL300 with RS domains. *Biochim Biophys Acta*. 2000;1492(1):191–195. http://doi.org/10.1016/s0167-4781(00)00065-8.

4. Blencowe BJ, Baurén G, Eldridge AG, et al. The SRm160/300 splicing coactivator subunits. *RNA*. 2000;6(1):111–120. http://doi.org/10.1017/s1355838200991982.
5. Grainger RJ, Barrass JD, Jacquier A, Rain JC, Beggs JD. Physical and genetic interactions of yeast Cwc21p, an ortholog of human SRm300/SRRM2, suggest a role at the catalytic center of the spliceosome. *RNA*. 2009;15(12):2161–2173. http://doi.org/10.1261/rna.1908309.
6. Khanna M, Van Bakel H, Tang X, et al. A systematic characterization of Cwc21, the yeast ortholog of the human spliceosomal protein SRm300. *RNA*. 2009;15(12):2174–2185. http://doi.org/10.1261/rna.1790509.
7. Fontrodona L, Porta-de-la-Riva M, Morán T, et al. RSR-2, the *Caenorhabditis elegans* ortholog of human spliceosomal component SRm300/SRRM2, regulates development by influencing the transcriptional machinery. *PLoS Genet*. 2013;9(6):e1003543. http://doi.org/10.1371/journal.pgen.1003543.
8. Petrovski S, Wang Q, Heinzen EL, Allen AS, Goldstein DB. Genic intolerance to functional variation and the interpretation of personal genomes. *PloS Genet*. 2013;9(8):e1003709. http://doi.org/10.1371/journal.pgen.1003709.
9. Kaplanis J, Samocha KE, Wiel L, et al. Evidence for 28 genetic disorders discovered by combining healthcare and research data. *Nature*. 2020;586(7831):757–762. http://doi.org/10.1038/s41586-020-2832-5.
10. Jeong S. SR proteins: binders, regulators, and connectors of RNA. *Mol Cells*. 2017;40(1):1–9. http://doi.org/10.14348/molcells.2017.2319.
11. Schulze KV, Hanchard NA, Wangler MF. Biases in arginine codon usage correlate with genetic disease risk. *Genet Med*. 2020;22(8):1407–1412. http://doi.org/10.1038/s41436-020-0813-6.
12. Bain JM, Cho MT, Telegrafi A, et al. Variants in HNRNPH2 on the X chromosome are associated with a neurodevelopmental disorder in females. *Am J Hum Genet*. 2016;99(3):728–734. http://doi.org/10.1038/s41436-020-0813-6.
13. Duijkers FA, McDonald A, Janssens GE, et al. HNRNPR variants that impair homeobox gene expression drive developmental disorders in humans. *Am J Hum Genet*. 2019;104(6):1040–1059. http://doi.org/10.1016/j.ajhg.2019.03.024.
14. Gautam A, Grainger RJ, Vilardell J, Barrass JD, Beggs JD. Cwc21p promotes the second step conformation of the spliceosome and modulates 3′ splice site selection. *Nucleic Acids Res*. 2015;43(6):3309–3317. http://doi.org/10.1093/nar/gkv159.
15. Konarska MM. A purified catalytically competent spliceosome. *Nat Struct Mol Biol*. 2008;15(3):222–224. http://doi.org/10.1038/nsmb0308-222.
16. Kim JH, Shinde DN, Reijnders MRF, et al. De novo mutations in SON disrupt RNA splicing of genes essential for brain development and metabolism, causing an intellectual-disability syndrome. *Am J Hum Genet*. 2016;99(3):711–719. http://doi.org/10.1016/j.ajhg.2016.06.029.
17. Ilik İA, Malszycki M, Lübke AK, Schade C, Meierhofer D, Aktaş T. SON and SRRM2 are essential for nuclear speckle formation. *Elife*. 2020;9:e60579. http://doi.org/10.7554/eLife.60579.
18. Hu S, Lv P, Yan Z, Wen B. Disruption of nuclear speckles reduces chromatin interactions in active compartments. *Epigenetics Chromatin*. 2019;12(1):43. http://doi.org/10.1186/s13072-019-0289-2.
19. Zanini IMY, Soneson C, Lorenzi LE, Azzalin CM. Human cactin interacts with DHX8 and SRRM2 to assure efficient pre-mRNA splicing and sister chromatid cohesion. *J Cell Sci*. 2017;130(4):767–778. http://doi.org/10.1242/jcs.194068.