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New insights into *CC2D2A*-related Joubert syndrome

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ABSTRACT

Purpose In this study, we describe the phenotype and genotype of the largest cohort of patients with Joubert syndrome (JS) carrying pathogenic variants on one of the most frequent causative genes, *CC2D2A*.

Methods We selected 53 patients with pathogenic variants on *CC2D2A*, compiled and analysed their clinical, neuroimaging and genetic information and compared it to previous literature.

Results Developmental delay (motor and language) was nearly constant but patients had normal intellectual efficiency in 74% of cases (20/27 patients) and 68% followed mainstream schooling despite learning difficulties. Epilepsy was found in only 13% of cases. Only three patients had kidney cysts, only three had genuine retinal dystrophy and no subject had liver fibrosis or polydactyly. Brain MRIs showed typical signs of JS with rare additional features. Genotype–phenotype correlation findings demonstrate a homozygous truncating variant p.Arg950* linked to a more severe phenotype.

Conclusion This study contradicts previous literature stating an association between *CC2D2A*-related JS and ventriculomegaly. Our study implies that *CC2D2A*-related JS is linked to positive neurodevelopmental outcome and low rate of other organ defects except for homozygous pathogenic variant p.Arg950*. This information will help modulate patient follow-up and provide families with accurate genetic counselling.

INTRODUCTION

Joubert syndrome (JS) is a rare genetic neurological disorder first described in 1969¹ with a prevalence estimated between 1/80,000 and 1/100,000 by many authors.² It is characterised by a distinctive cerebellum and brain stem malformation: a cerebellar vermis hypoplasia/dysplasia combined with horizontally oriented, thickened superior cerebellar peduncles and a deepened interpeduncular fossa resulting in the pathognomonic ‘molar tooth sign’ (MTS) on axial cuts of brain MRIs.^{3–6} Patients present congenital cerebellar ataxia,

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Joubert syndrome (JS) is a complex neurological disorder that includes a specific cerebellum malformation and other organ defects. It is a genetically heterogeneous disease belonging to the group of ciliopathies. JS is known to be a severe disorder because it causes important neurodevelopmental disabilities and other organ complications (retinopathy, nephronophthisis, liver fibrosis). In our French cohort, we found multiple patients with encouraging neurodevelopmental features and rare other organ disorders and linked them to their genetic cause of JS: *CC2D2A* variants.

developmental delay and possible intellectual disability (ID), abnormal eye movements (mainly ocular motor apraxia and/or nystagmus) and occasionally breathing dysregulations such as alternate episodes of apnoea and tachypnoea.^{1 7–10} JS can be diagnosed based on these clinical features combined with the demonstration of MTS on neuroimaging. The neurological syndrome is frequently combined with other organ defects such as retinal dystrophy, chorioretinal colobomas, kidney disease (renal cysts and/or nephronophthisis) and liver fibrosis.^{2 11–13} Dysmorphic particularities can be observed as well as orofacial anomalies and polydactyly.

Nowadays, genetic advances have allowed to find more than 40 genes causing JS, and all these genes encode for proteins of the primary cilium thus categorising JS as a ciliopathy.^{14–16} The cilium is a hair-like cellular organelle operating like a cellular sensor for extracellular signals. Its nearly ubiquitous localisation explains the pleiotropic clinical features of JS.^{13 17 18} Moreover, studies have demonstrated that cilia play a crucial role in neurodevelopment.^{19–21}

JS is an autosomal recessive disorder—apart from *OFD1* hemizygous X-linked variants. Analysis of known causative genes in JS allows a molecular diagnosis in approximately 60% of cases.²² Genetic causes of JS can overlap with other ciliopathies such

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SV and LB contributed equally.

WHAT THIS STUDY ADDS

⇒ With this study, we showed that *CC2D2A* variants are associated with a better neurodevelopmental outcome with a small percentage of renal, retinal, or liver involvement. We also refuted the previous association between *CC2D2A* and ventriculomegaly and confirmed a slightly higher risk of epilepsy for *CC2D2A*-related JS. We built a genotype–phenotype correlation with homozygous p.Arg950* variant linked to a worse developmental outcome with renal and retinal complications.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ This study encompasses the largest cohort of patients with *CC2D2A*-related JS and details their phenotypes and clinical manifestations. This study will allow to establish specific monitoring recommendations for patients with *CC2D2A*-related JS and to give out specific prognosis information to patients and their families. This last point is particularly important in the emergence of prenatal genetic testing when morphological anomalies are detected on ultrasound examinations. This study could help the parents make their decision about the outcome of the pregnancy if *CC2D2A* variants were found.

as Meckel syndrome (MKS) (MIM PS249000). This striking clinical and genetic heterogeneity of JS raises the possibility of oligogenic inheritance and the existence of genetic modifiers modulating the different phenotypes. This has been demonstrated in other ciliopathies such as Bardet-Biedl syndrome.²³ However, some correlations between genotype and phenotype have been shown in various studies.^{14 18}

CC2D2A is one of the most common genes causing JS.²² The *CC2D2A* gene encodes a protein located in the transition zone of the cilium.²⁴ Its overall structure includes three predicted coiled-coil domains (allowing interaction with other proteins), a calcium-binding C2 domain (involved in vesicle fusion and trafficking) and a C-terminal region.^{25 26}

CC2D2A gene was first identified in 2008 in a consanguineous Pakistani family with autosomal recessive mental retardation and retinitis pigmentosa.²⁷ Later on, brain MRI images were reviewed and the MTS was detected in both siblings which allowed to link *CC2D2A* with JS.²⁸ Moreover, fundamental studies showed zebrafish *CC2D2A* mutants developed pronephric cysts.²⁵ Other studies have shown that *CC2D2A* protein controls transmembrane protein entrance into the cilium through a role in vesicle trafficking and fusion.^{29 30}

CC2D2A variants also cause around 10% of MKS, and there is a phenotype–genotype correlation in this overlap. Biallelic truncating variants lead to MKS, whereas genotypes containing at least one hypomorphic variant lead to JS.^{31 32}

In addition, *CC2D2A* has been involved in gene–phenotype correlation studies. It has been linked to JS with liver involvement and coloboma.³³ However, this was not validated by other JS cohorts where *CC2D2A* variants resulted in a lower rate of other organ defects.^{14 22} Moreover, another report demonstrated an association between *CC2D2A* variants and ventriculomegaly and seizures needing other JS cohorts' reports to be validated.³⁴

In this study, we describe the clinical and genotypic aspects of the largest cohort of *CC2D2A*-related JS cases consisting of 52 patients and 1 fetus.

Patients, materials and methods

We selected 53 JS subjects with pathogenic variants on *CC2D2A*, recruited from 2008 to 2020, from the French Reference Centre for Cerebellar Malformations and Congenital Diseases cohort consisting of 409 patients with confirmed JS. The criteria for diagnosis of JS were the following: (1) brain MRI demonstrating MTS with or without vermian hypoplasia/dysplasia and (2) clinical features of JS such as hypotonia, ataxia, developmental delay and oculomotor anomalies. The fetus was included in our study as his pathology examination observed the MTS and vermian hypoplasia. Before enrolment, all participants or their legal representatives signed a written consent form allowing genetic testing and anonymous recording of their genetic and clinical data for research purposes.

Molecular diagnosis of JS was first obtained by Sanger sequencing of strategically selected genes from 2008 to 2013, then using targeted next-generation sequencing of a JS gene's panel on a MiSeq Illumina sequencer from 2013 to 2019 and on a NextSeq sequencer since 2019. The actual panel consists of 38 known JS genes. The resulting sequencing data were analysed by Variant Studio software, and the likelihood of pathogenicity was predicted by SIFT, Polyphen2 and CADD softwares for missense variants and MaxEntScan software for splice variants. Candidate variants were confirmed by Sanger sequencing and segregation analysis with parental DNA. In parallel, partial deletions/duplications were detected using a home-made script (DeCoVa). Variants were then categorised into five different classes according to the American College of Medical Genetics and Genomics³⁵ guidelines. Only biallelic class 4 or 5 variants were considered responsible for the patient's phenotype. However, variants of unknown significance could be later classified as class 4 if recurrent in another patient with JS of our cohort or in the literature.

Clinical information of JS was either collected through a structured intake form filled by the medical practitioner prescribing the genetic tests or through medical records. Clinical information was also collected later to track the evolution of patients' phenotypes (this was possible for 33 patients out of 52). The last medical report available is used for patients' descriptions.

Eighteen brain MRIs were available and were reviewed by two neuropaediatricians and the reference centre's neuroradiologist, to confirm the MTS and look for additional features. If MRIs were unavailable, neuroimaging information was extracted from radiologist reports.

Data were then organised and statistically described using Excel software. We used Julia software to examine the association between *CC2D2A* and epilepsy using χ^2 exact tests.

RESULTS

Patients' demographic and general characteristics

There were 51 living patients, 1 deceased patient and 1 fetus, accounting for 45 families (8 pairs of siblings). Three out of 45 families had consanguineous parents (online supplemental table S1). The median age of living patients was 8 years old (4 months–63 years old) at the last medical report available. When a second medical evaluation was reported (33 patients), the median follow-up was 4 years and 11 months (7 months–12½ years). There were 29 men and 24 women. Patients had a various span of geographic origins, with a majority of people from European descent as well as from the Maghreb, Sub-Saharan Africa and Middle East origins.

Table 1 Clinical features of <i>CC2D2A</i> patients		
Neurological features		
Cerebellar ataxia	41/48	85%
Dysmetria	21/48	44%
Epilepsy	7/52	13%
Behavioural disorders	13/48	27%
Eye features		
Oculomotor apraxia	40/52	77%
Nystagmus	17/52	33%
Strabismus	11/52	21%
Coloboma	0/39	–
Microphthalmia	1/52	2%
Retinal involvement*	7/44	16%
Kidney features		
Renal cysts	3/45	7%
Impaired renal function	1/40†	3%
Osmolarity disorder/alteration	1/21	5%
Liver features		
Abnormal liver ultrasound‡	2/48	4%
Abnormal liver blood tests	1/48	2%
Additional features		
Orofacial region anomalies	0/53	–
Dysmorphic features§	8/53	15%
Polydactyly	0/53	–
Macrocephaly	2/30	7%
*Retinal involvement is defined by an abnormal electroretinogram and/or retinal anomalies with impaired visual acuity.		
†The only patient with renal failure was in his early 60s.		
‡One patient had homogeneous hepatomegaly and the other liver steatosis.		
§Dysmorphic features included mainly a protruding forehead.		

First clinical signs

The first relevant clinical signs observed were reported for 49 subjects out of 53. For 3/49, the first signs were antenatal (online supplemental tables S1,S4). Two siblings presented vermian hypoplasia on fetal ultrasounds. One fetus had vermian agenesis seen on ultrasound during the second trimester and molecular prenatal diagnosis (testing for sibling's variants) confirmed *CC2D2A*-related JS. This led to medical termination of pregnancy according to French law.

The first clinical signs of JS occurred in the first 3 months of life for 33/49 patients (65%). The most common signs were hypotonia and eye movement anomalies (online supplemental table S1). Overall, the first signs of JS were noticed before 2 years old in all the patients of our cohort.

Neurological features

Overall, 42/50 (86%) patients had an abnormal neurological examination, meaning that 8 patients did not have cerebellar ataxia and JS was solely suspected because of ocular motor anomalies and/or developmental delay.

Cerebellar ataxia was defined by either hypotonia observed in infants and/or gait disturbance in toddlers and beyond and was present in 41/48 patients (85%) (table 1). A follow-up evaluation was available for 33 patients and only 3 patients still had disabling cerebellar ataxia: two patients needed a wheelchair in crowded spaces and one patient had regular falls while walking on stable ground.

In our cohort, 7/52 (13%) patients had reported seizures. For 2/7, it had started in the neonatal period. No type of seizures dominated; 1/6 had epilepsy with febrile seizures, 4/6 had

generalised seizures, 1 patient's epilepsy ceased after the age of 5 years and 1 patient manifested their first seizures at 25 years old. None had drug-resistant epilepsy. When comparing *CC2D2A*-related JS (n=53) to the rest of the French JS cohort (n=409), we found a significant correlation using χ^2 test between *CC2D2A* and epilepsy with a p=0.03.

Behavioural disorders were noticed in 13/48 (27%) patients spanning from emotional dysregulation disorders, tantrums, aggressivity to attention deficit and hyperactivity as well as social communication disorder and restricted interests.

Other neurological features observed included spastic hyper-tonia, equinovarus feet and movement disorders such as hyperkinesia. These neurological signs were observed in patients bearing globally severe phenotypes. Additionally, joint hypermobility, impaired fine motor skills and exaggerated deep tendon reflexes were often described. Cranial circumference was reported in 30 patients and 2/30 had macrocephaly (≥ 2 SD).

Other organ features

An overall abnormal eye examination was registered in 52/52 of patients with *CC2D2A*-related JS. Neurologically based vision issues were mainly reported such as oculomotor apraxia, nystagmus and strabismus (table 1).

Vision acuity was impaired in 19/35 of cases. However, patients mostly had mild vision defects such as hypermetropia (8/19) and astigmatism (4/19). Three patients had amblyopia, which was a consequence of strabismus. Only one patient was clinically blind.

Funduscopy was found abnormal for 6/44 patients, with only 3 confirmed cases of retinitis pigmentosa; the other cases were either retina pallor (2/6) or mild changes in the retinal pigment epithelium (1/6). Seventeen patients underwent an electroretinogram which showed an absent response in only one case and decreased responses in three cases. Thirteen patients had a normal response on electroretinogram. Overall, retinal involvement was diagnosed in seven patients (16%).

In this study, 3/45 patients had kidney manifestations. Only 3/45 patients (7%) had renal cysts found on ultrasound including one patient with chronic renal failure with glomerular filtration rate at 35 mL/min and altered osmolarity, who was in his early 60s at diagnosis (table 1). The other two patients with cysts were an adolescent and a young adult in their early 20s.

Only 2/48 patients had liver manifestations. One patient had homogeneous hepatomegaly with normal liver function. Another patient had liver steatosis with persistently elevated aminotransferases.

None of the 53 subjects had polydactyly or orofacial defects. Eight out of 53 subjects had dysmorphic features. However, it does not appear that *CC2D2A* can be suspected based on a particular dysmorphic phenotype. Two out of 28 patients with reported hearing tests had impaired hearing supposedly due to recurring ear infections. Cardiac ultrasound was assessed in only 11 patients, and no cardiac malformations were detected.

Developmental features

Most of the patients (48/49) experienced developmental delay in either language or motor skills development. Only 1/49 achieved development milestones at a normal age. To assess independent walking, we excluded patients who were not walking under the age of 18 months old at the last medical report: out of 46 patients, 41 (89%) patients were able to walk independently. The median age for the onset of walking was 25 months (15

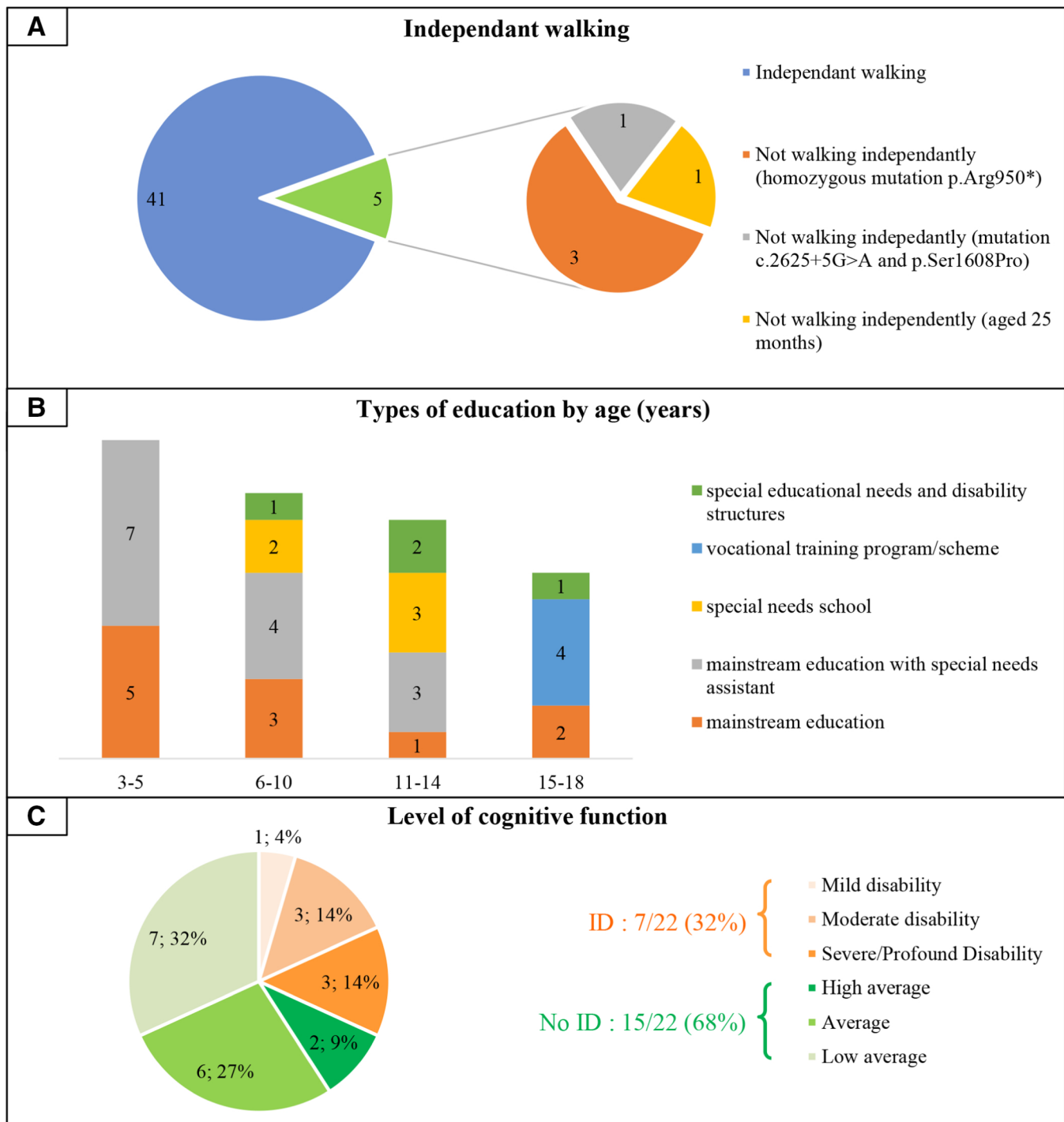


Figure 1 Developmental features. (A) Forty-one patients were walking independently over 18 months old. Five patients not walking independently are described in the pie chart on the right hand side. (B) Children aged 3 to 5 years were in preschool, 6 to 10 years were in primary school, 11 to 14 years were in middle school and 15 to 18 years were in high school. (C) High average=IQ scores between 110 and 119 and average=IQ scores between 90 and 109. Low average=IQ scores between 80 and 89. Mild disability=IQ scores between 55 and 70 and moderate disability=IQ scores between 40 and 54. Severe/profound disability=patients not ambulatory and unable to communicate. No patient had borderline intellectual functioning (IQ scores between 70 and 79), ID=intellectual disability (IQ score<70), no ID=IQ score≥70.

months–11 years). Among the five non-walking patients, one was over 2 years old at the last evaluation and was starting to walk with assistance. Three patients (3/5) who were not ambulatory carried the same homozygous variant p.Arg950* and their overall phenotype was more severe. The other non-ambulatory subject (1/5) was around 15 years old and carried the compound heterozygous variants c.2625+5G>A and p.Ser1608Pro (figure 1A).

Most patients started speaking at a later age than normal, but 78% (28/36) of patients could speak fluently with elaborate

sentences over the age of 5 years. The main issues described were articulation disorders such as dysarthria or phonemes confusion. Most patients developed a substantive vocabulary. When at a reading age (defined as over 7 years old), 77% (23/30) of patients were able to read but some patients read at a slower pace as they were inconvenienced by their ocular motor apraxia. Due to the kinetic cerebellar syndrome, children had a shaky and/or illegible handwriting. For the few who managed to do it legibly, the writing required a lot of concentration and was very tiring.

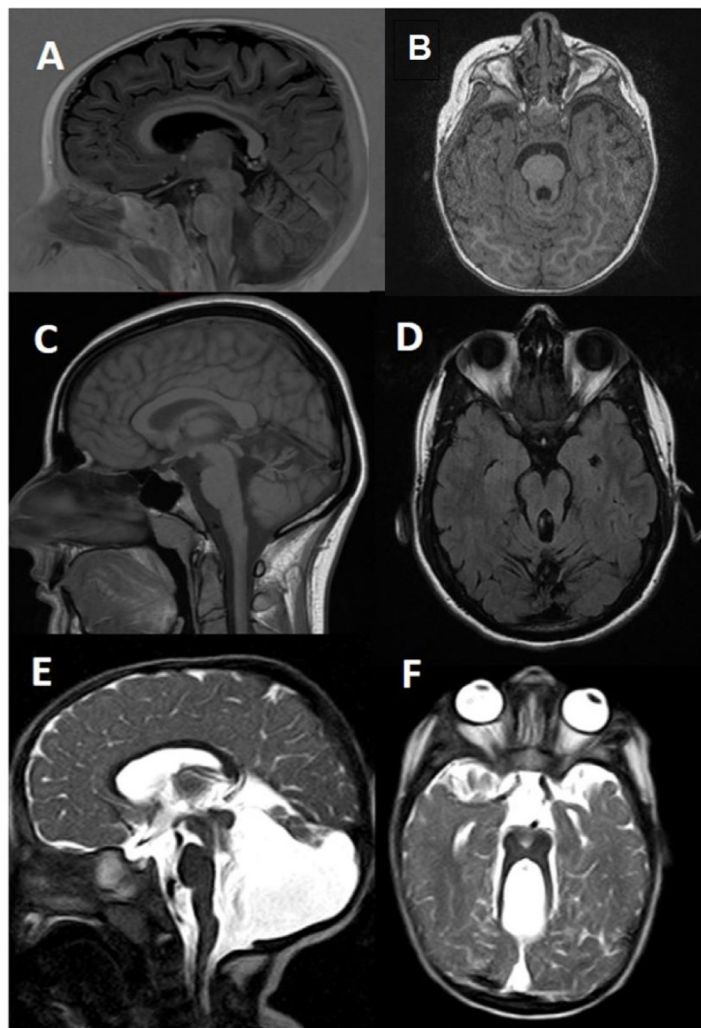


Figure 2 Neuroimaging spectrum of the *CC2D2A*-related Joubert syndrome (JS) subjects of our cohort. (A, B) Patient 1: no dysplasia of superior vermis, only underdevelopment of posterior vermis (A). molar tooth sign (MTS) (B). (C, D) Patient 2: severe hypoplasia/dysplasia of the vermis and atrophy (C). MTS present (D). Patient was 24 years old at MRI, 65 years old patient showed also atrophy of vermis and cerebellar hemisphere. Their clinical evolution was stable. (E, F) Patient 3: very dysplastic vermis with abnormal foliation, enlarged fourth ventricle and upward dislocation of the fastigium, retrocerebellar fluid collection and upward displacement of the cerebellar tentorium insertion (E). MTS present (F).

Intellectual functioning

Nineteen patients of the *CC2D2A* cohort had been prescribed standardised intelligence tests. Various tests were reported (Development Quotient, Wechsler Preschool and Primary Scale of Intelligence (WPPSI), and Wechsler Intelligence Scale for Children (WISC) tests).³⁵ ID was defined with an IQ score below or equal to 70 according to Diagnostic and Statistical Manual of Mental Disorders, Sixth Edition. The resulting scores were analysed and classified by our reference centre's neuropsychologist. A typical JS neurodevelopmental cognitive profile was defined by heterogeneous IQ results with lower grades in visuospatial and processing speed categories and higher scores in verbal comprehension and fluid reasoning (n=8/19).

Three patients were unable to carry out neuropsychological evaluations due to multiple disabilities and absence of communication skills. They were considered profoundly intellectually impaired. Interestingly, they all carried the homozygous p.Arg950* variants.

The total of subjects included in the classification was therefore 22, and 68% (15/22) had normal cognitive functions (figure 1C).

Patients attending mainstream schools with good grades and over the age of 7 were considered not intellectually deficient but were excluded from the classification if they did not have standardised tests (n=5). Overall, intellectual ability could be determined for 27 patients in total, out of which 74% (20/27) had normal cognitive functions.

Education

Overall, 25/38 (66%) were able to follow a mainstream education with or without a learning support assistant. However, the split of patients by age corresponding to the different levels of French educational system showed that, when ageing, patients had rising learning difficulties and many of them needed to be reoriented to special needs education (9/38 in special needs school or vocational training programmes) (figure 1B). In preschool, 7/12 needed a special needs assistant in class, suggesting the early onset of learning difficulties. The main learning difficulties assessed were geometry (due to visuospatial deficits) and multi-tasking (patients were qualified as slower than other children) but this could be improved by taking notes on a computer.

There were also very encouraging outcomes with patients exhibiting close to none learning difficulties. One patient over the age of 18 at the last medical assessment had just passed the French high school final examination (Baccalaureate). Another teenager, in his last year of high school had excellent grades and was planning to start medical school the following year.

By contrast, 4/38 patients (11%) attended special educational needs and disability institutions which welcome children with motor disability or ID. Among the four patients enrolled, one carried the p.Arg950* homozygous variant and was not ambulatory or communicative. The two others were ambulatory and communicative but had a moderate ID. Information available on the fourth patient did not explain his need for such a structure.

Neuroimaging features

In our study, 44/53, 83% of MRI images or neuropathology reports showed isolated MTS with or without vermis hypoplasia/dysplasia, without other malformations. Eight patients and one fetus presented additional features: 1/53 had periventricular nodular heterotopias, 3/53 patients had cerebellar hemispheres hypoplasia/dysplasia, 2/53 patients had thick corpus callosum and only 2/53 (4%) presented ventriculomegaly. The fetus had polymicrogyria. Only 18 MRIs were available for review; figure 2 shows the wide neuroimaging spectrum of *CC2D2A*-related JS.

Intrafamilial phenotypic variability

Within our cohort, we had seven pairs of living siblings. Contrary to previous literature, phenotypes were comparable among members of the same family, regarding extraneurological complications, cerebral malformations, neurodevelopmental outcome and IQ scores. For one pair of siblings, one had hyperactivity disorder and needed special education, whereas his older brother was in vocational training but had a lower IQ score (online supplemental table S2).

Genetic results

Genetic cause was defined by the presence of likely pathogenic or pathogenic variants in both copies of *CC2D2A*. In our JS

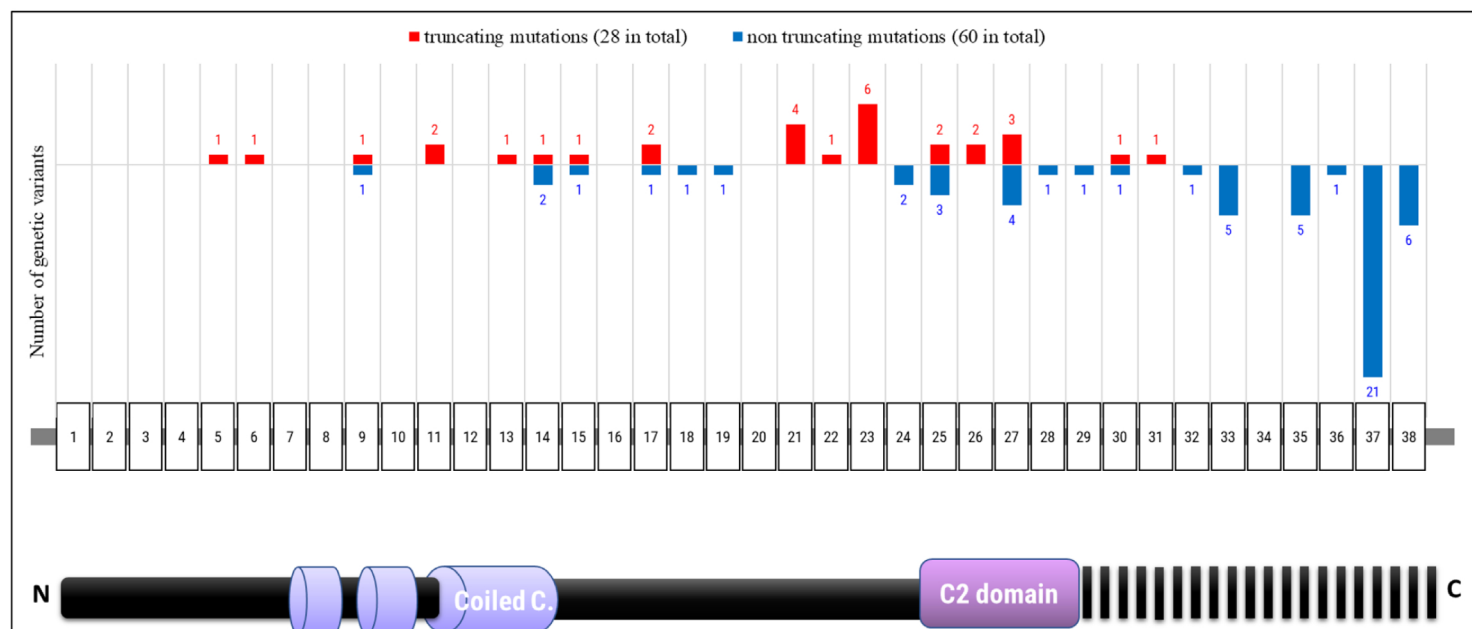


Figure 3 CC2D2A protein schematic and variant summary. CC2D2A protein is represented with the three coiled coil domains (coiled C.) and C2 domain. The gene 38 exons are represented above with a graphic representation of the affected exons in the discovered variants. Truncating variants are represented in red and non-truncating variants are represented in blue. Only 88 variants of the 45 families are represented in this graph, because the two deletions were not included.

cohort, *CC2D2A* was the most frequent gene, identified in 13% (53/409) of patients.

Fifty-seven different variants were identified in 45 families (29 missense, 11 splice site, 10 non-sense, 4 frameshift, 2 deletions and 1 synonymous) and 28 of them were novel variants (online supplemental table S3 and figure 1). Segregation analysis showed that 93% (42/45) bore compound heterozygous pathogenic variants.

Most of the probands had at least one hypomorphic *CC2D2A* variant (39/45, 87%) (online supplemental table S3). Nineteen probands (42%) harboured two non-truncating variants, and 18 (40%) were compound heterozygous for one truncating variant and one non-truncating variant. Two had a combination of a deletion and a missense variant. Finally, three families had the same homozygous non-sense p.Arg950* variant, and three different probands were compound heterozygous with one splicing variant and one non-sense variant.

Non-truncating variants were mostly located in the C2 domain or C-terminal region of the protein, whereas truncating variants were either located before or inside the C2 domain (figure 3 and online supplemental figure S1). This highlights the importance of the C2 domain on CC2D2A's function. The C-terminal region's function is unknown but highly conserved in species and studies suggest that it overlaps with a centrosomal protein implying a role in cell polarity. A recurrent variant (p.Asp1556Val) located on the 37th exon coding for the C-terminal region was found in 16 families. Interestingly, two patients harboured the same synonymous p.Ser1476=variant, unknown in gnomAD, which was shown to lead to an abnormal splicing and predicted in-frame deletion of exon 35 (available on request).

Genotype–phenotype correlations

As described earlier, a homozygous stop-gain variant was found in four patients from three families. Homozygous p.Arg950* variant was linked to a particularly severe phenotype (table 2), with a higher rate of retinal dystrophy (4/4), epilepsy (3/4), spastic paresis (3/4), and a worse neurodevelopmental outcome

overall. One subject had congenital hydrocephalus requiring ventriculoperitoneal shunting. The only patient with both retinal dystrophy and renal cysts bore the p.Arg950* variant.

Furthermore, another patient carried biallelic truncating variants and had a severe JS phenotype. He was a compound heterozygote carrying the c.2487–7_–3delTTTTC variant, suspected to lead to an out-of-frame exon deletion, and the previously reported variant p.Val1097Phefs*2. The subject was a few months old when information was collected, and he presented

Table 2 Clinical description of the patients harbouring the p.Arg950* homozygous variant

	Patient 1	Patient 2*	Patient 3	Patient 4
Age at medical report (in years)	Early adolescence	Middle childhood	Early childhood	Early adolescence
Sex	M	F	F	F
Country origin	Syria	Syria	nd	Algeria
Consanguineous parents	Yes	Yes	Yes	Yes
Affected sibling	Yes	Yes	No	No
Epilepsy	Yes	Yes	No	Yes (NN)
Stereotypies	Yes	Yes	No	No
Spastic paresis	Yes (P)	Yes (P)	No	Yes (T)
Developmental features	Not able to walk or speak	Not able to walk or speak	Not able to speak	Not able to walk or speak
Intellectual ability	ID severe/profound	ID severe/profound	nd	ID severe/profound
Retinal involvement†	Yes	Yes	Yes	Yes
Kidney/liver complications	No	No	No	Renal cysts
Brain MRI	MTS+(HC)	MTS+(HC)	MTS	MTS+(V)

*Patient 1 and patient 2 were siblings.

†Retinal involvement is defined by an abnormal electroretinogram and/or retinal anomalies on funduscopy.

HC, abnormal cerebellar hemispheres; ID, intellectual ability; MTS, molar tooth sign; nd, not determined; NN, neonatal (seizures); P, paraparesis with equinovarus feet; T, tetraparesis; V, ventriculomegaly.

a severe phenotype with neonatal seizures, axial hypotonia and peripheric hypertonia, clubfeet, nystagmus and microphthalmia. He deceased at 14 months, and his cause of death was unknown.

However, other severe phenotypes were pointed out, without genotype correlations. One subject bore a heterozygous compound variant including c.2625+5G>A (a splice-site variant suspected to lead to an out-of-frame deletion of exon 21 and premature stop codon, thus abbreviating the protein before the C2 domain) and a missense variant p.Ser1608Pro located on exon 38. This patient presented neonatal seizures which spontaneously resolved, and he could not walk or talk at 6 years old. He had persistent hypotonia, hyperkinetic movements, swallowing problems, pyramidal and extrapyramidal signs and self-mutilation behaviour. He had no other organ defects. His brain MRI demonstrated enlargement of the posterior fossa (figure 2; patient 3). He was related to the only fetus presented in our cohort study.

In addition, two patients with biallelic truncating variants did not bear a severe JS phenotype. One was a compound heterozygous for a non-sense variant p.Gln1274* located just after the C2 domain and c.247+1G>A, a splice-site variant predicted to lead to the deletion of the fifth exon and a premature stop codon. Another patient had two truncating variants: c.1765–1G>C suspected to lead to an out-of-frame deletion of exon 17 and a non-sense variant p.Arg1049*, truncating the protein inside the C2 domain. He was diagnosed with JS in his early 60s as he was hospitalised for a depressive state which led him to self-harm. The doctor, who examined him during his stay, noticed the gait disturbance and suspected a cerebellar stroke. The brain MRI revealed the MTS. He had presented moderate developmental delay and learning difficulties, but nowadays worked in the family-owned grocery store and was autonomous in his daily life. These two mild phenotypes suggest that their genotypes might be compatible with the presence of a functional protein through the absence of mRNA decay or unexpected splicing.

DISCUSSION

In this study, we show that most *CC2D2A* genotypes including at least one hypomorphic variant lead to a mild JS phenotype with rare extra neurological disorders and positive neurodevelopmental outcomes. Our study also brings to light a distinctively severe phenotype caused by homozygous p.Arg950* variants.

This study encompasses the largest number of subjects with JS caused by *CC2D2A* pathogenic variants. Bachmann-Gagescu et al. reported 20 subjects from 17 families,³⁴ Mougou-Zerelli et al reported 11 MKS cases and 2 JS cases³² and Gorden et al reported 10 patients.²⁵

In previous literature, JS is linked to ID: 72% of 54 patients with JS and 65% of 76 JS individuals were intellectually impaired in a 2016 and a 2017 study.^{36 37} In addition, in Bachmann-Gagescu et al.'s study,³⁴ 9/17 *CC2D2A*-related patients with JS could not walk independently (age range 2–9 years), 7/13 could not talk over the age of 3 years and all were enrolled in special education. This contrasts with our cohort, in which 89% of patients could walk, 78% could speak and 74% of subjects had normal cognitive function with some patients having better than average IQ scores.

Moreover, Bachmann-Gagescu et al.'s study indicated a significant correlation between epilepsy and *CC2D2A* (reported in 5/17 families (27%)) and between ventriculomegaly and *CC2D2A* (13/17 families (76%)).³⁴ In our study, only 13% (n=7/52) of patients manifested seizures,

but this percentage was still significantly higher than in other patients with JS without *CC2D2A* variants (n=17/307 (6%)). However, 3/7 patients had the same homozygous p.Arg950* variant so it is difficult to confirm the correlation between gene and phenotype. On the contrary, our work does not corroborate the previous association between *CC2D2A*-related JS and ventriculomegaly.

Our work thus seems to differ from previous associations as our patients had more encouraging neurodevelopmental outcomes even though they had a higher risk of epilepsy. One possible explanation for this could be that mild JS phenotypes are more difficult to detect and therefore were less represented in previous studies.

In a 2015 North American study describing the largest cohort of patients with JS, the retinal disease was present in 30% of patients, renal disease in 25% and liver disease in 14%.²² In our *CC2D2A*-related JS cohort, the prevalence of other organ defects is much lower (16%, 7% and 4%, respectively). This is demonstrated in other *CC2D2A*-related JS cohorts.^{15 22} Monitoring *CC2D2A* patients via systematic abdominal ultrasounds and blood and urinary tests stays the safest option but perhaps its frequency could be reduced.³⁸

We tried building genotype–phenotype correlations within *CC2D2A*. Most of our patients with JS had at least one non-truncating variant, supporting previous genotype–phenotype correlation of *CC2D2A* variants causing either JS or MKS.³²

In addition, p.Arg950* stop-gain homozygous variant led to a distinctive phenotype with a severe developmental outcome and higher risk of epilepsy, ventriculomegaly and retinal dystrophy. This same p.Arg950* homozygous variant was reported in Bachmann-Gagescu et al's study, and their subject had a similar phenotype. He could walk with aid at 4 years old but was not verbal. He had no seizures but manifested stereotyped movements. He also had retinal disease and ventriculomegaly on brain imaging. These findings confirm the deleterious state of this variant and could modulate prenatal counselling of families when p.Arg950* homozygous variant is found.

Phenotypical heterogeneity was also demonstrated in our cohort and in this way mirrors other JS studies. This raises the question of genetic modifiers and oligogenic inheritance in *CC2D2A*-related JS like it was shown in zebrafish.²⁵ However, as patients did not benefit from the same genetic tests it was not possible to compare phenotypes with additional heterozygous variants.

This study bears several limitations typical of retrospective research studies. It relied on other practitioners for accurate record keeping, and specific information was not always included in medical reports. Moreover, it can also exhibit information or misclassification bias as assessments were not all standardised. Taking into account the progressive aspect of JS, we tried to evaluate them later, but it was only possible in 33 cases as some patients were lost to follow-up.

In conclusion, this work offers new insights into phenotypic particularities of *CC2D2A*-related JS as it differs from the previous literature. It will allow practitioners to offer specific prognosis information to families and define precise recommendations for monitoring JS caused by *CC2D2A* variants.

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Figure S1: Schematic summary of genomic positions of the discovered variants on the *CC2D2A* gene

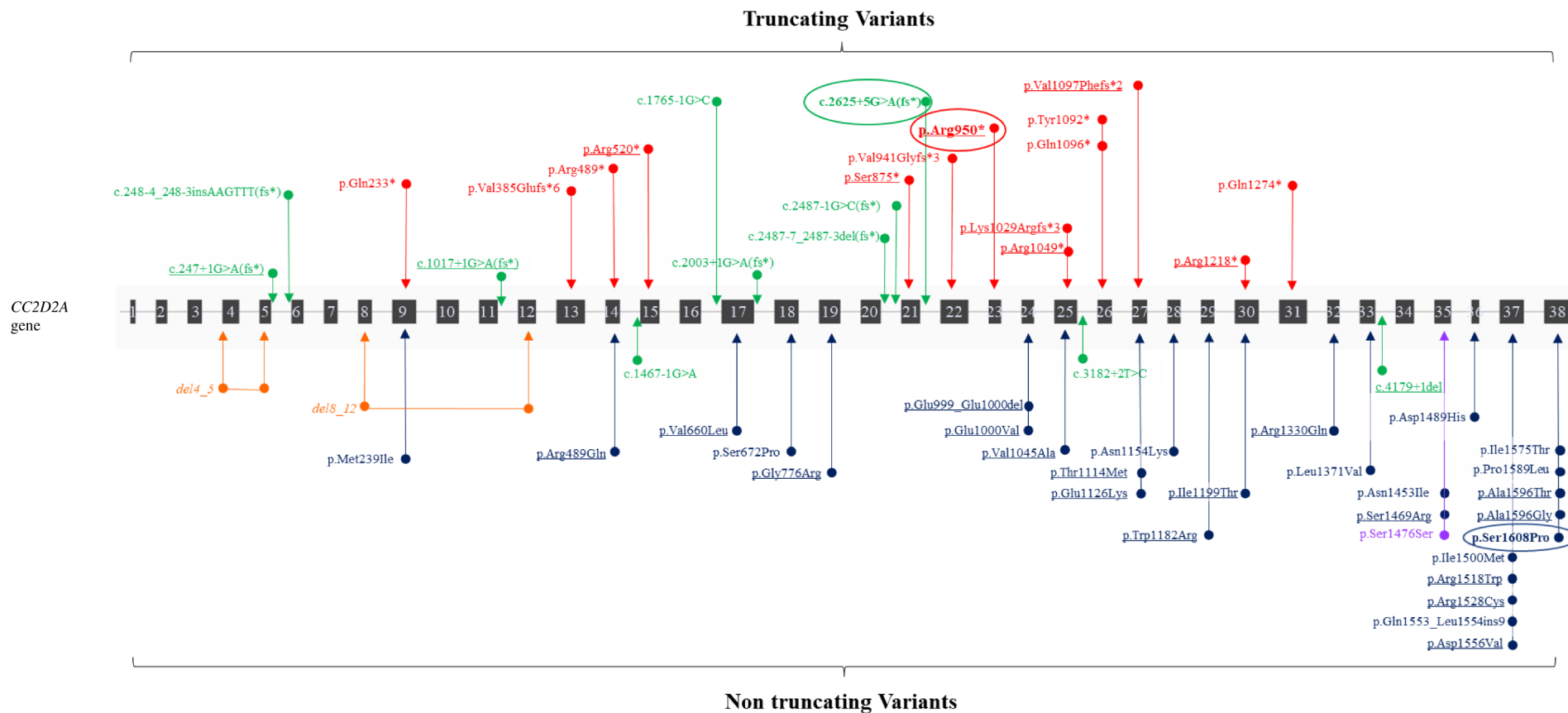


Table S1. Patients' characteristic and clinical first signs

Characteristics	Number of patients	Percentage
Age in years at last medical report		
< 5 years old	11/52	21%
5-9 years old	18/52	35%
10-14 years old	12/52	23%
15-19 years old	6/52	12%
≥20 years old	5/52	10%
Age at JS diagnosis ^c		
Antenatal	1/40 ^c	3%
< 2 years old	18/40	45%
2-4 years old	11/40	28%
5-9 years old	6/40	15%
10-14 years old	2/40	5%
≥ 15 years old	2/40	5%
Sex (Male/Female) ^b	29/24	55%/45%
Consanguineous parents	3/45	7%
Families with ≥ 1 affected sibling	8/45	18%
Clinical first signs		
<i>Antenatal</i>	3/49	6%
<i>Before 3 months old</i>	32/49	65%
Hypotonia (with seizures)	14/33 ^a (2/14)	-
Abnormal eye movements	30/33	-
Breathing dysregulations	6/33	-
More than one sign	15/33	-
<i>Between 3 and 24 months</i>	14/49	29%
Developmental delay and/or ataxia	12/14	-
Ocular motor anomalies	6/14	-
Both	4/14	-

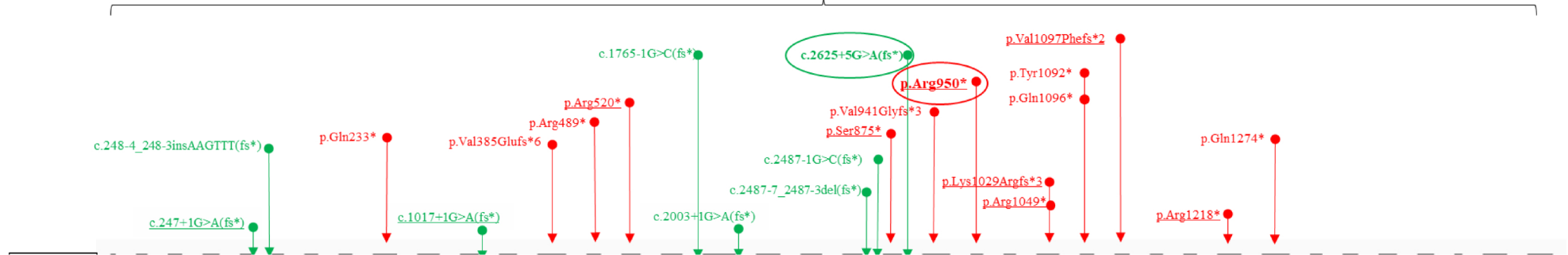
^a33 patients in total exhibited JS signs in the first three months of life. One of them already had presented antenatal anomalies

^b The only fetus was male

Figure S1: Schematic summary of genomic positions of the discovered variants on the *CC2D2A* gene.

Truncating variants (leading to an abbreviated or truncated protein) included nonsense variants and frameshift variants as well as canonical splice-site variants leading to an out of frame exon deletion modifying the reading frame (splice fs*). Non truncating variants or hypomorphic variants included missense variants, small in-frame indels and synonymous variants as well as splice-site variants leading to an in-frame exon deletion (splice in frame). Larger deletions were considered as a separate type of variants. Color code : Exon deletions: unknown protein effect, Splicing variants, Nonsense and frame shift variants, In frame and missense variants, Synonym variants. Circled variants were connected to severe phenotypes of the cohort. Previously reported pathogenic variants are underlined.

Truncating Variants



del4_5

del8_12

p.Val660Leu

p.Glu999_Glu1000del

p.Glu1000Val

p.Arg1330Gln

c.1179+1del
p.Asp1489His

p.Ile1575Thr

p.Pro1589Leu

p.Alal596Thr

p.Alal596Gly

p.Ser1608Pro

p.Ile1500Met

p.Arg1518Trp

p.Arg1528Cys

p.Gln1553_Leu1554ins9

p.Asp1556Val

p.Ser1476Ser

p.Trp1182Arg

Non truncating Variants

Families	GENE	Exon	HGVS coding DNA	HGVS Protein Predicted	Previously reported mutation
Family 1	CC2D2A	23	c.2848C>T	p.Arg950*	yes
Family 2	CC2D2A	37	c.4667A>T	p.Asp1556Val	yes
Family 3	CC2D2A	37	c.4667A>T	p.Asp1556Val	yes
Family 4	CC2D2A	37	c.4667A>T	p.Asp1556Val	yes
Family 5	CC2D2A	14	c.1466G>A	p.Arg489Gln	yes
Family 6	CC2D2A	11	c.1017+1G>A	p.?	yes
Family 7	CC2D2A	37	c.4552C>T	p.Arg1518Trp	yes
Family 8	CC2D2A	14	c.1465C>T	p.Arg489*	no
Family 9	CC2D2A	30	c.3596T>C	p.Ile1199Thr	yes
Family 10	CC2D2A	15	c.1558C>T	p.Arg520*	yes
Family 11	CC2D2A	24	c.2999A>T	p.Glu1000Val	yes
Family 12	CC2D2A	37	c.4667A>T	p.Asp1556Val	yes
Family 13	CC2D2A	18	c.2014T>C	p.Ser672Pro	no
Family 14	CC2D2A	23	c.2848C>T	p.Arg950*	yes
Family 15	CC2D2A	23	c.2848C>T	p.Arg950*	yes
Family 16	CC2D2A	27	c.3289del	p.Val1097Phefs*2	yes
Family 17	CC2D2A	17	c.1978G>C	p.Val660Leu	yes
Family 18	CC2D2A	33	c.4179+1del	p.?	yes
Family 19	CC2D2A	25	c.3145C>T	p.Arg1049*	yes
Family 20	CC2D2A	35	c.4407C>G	p.Ser1469Arg	yes
Family 21	CC2D2A	37	c.4667A>T	p.Asp1556Val	yes
Family 22	CC2D2A	39	c.4667A>T	p.Asp1556Val	yes
Family 23	CC2D2A	20	c.2487-7_2487-3del	p.?	no
Family 24	CC2D2A	25	c.3084del	p.Lys1029Argfs*3	yes
Family 25	CC2D2A	37	c.4667A>T	p.Asp1556Val	yes
Family 26	CC2D2A	21	c.2625+5G>A	p.?	no
Family 27	CC2D2A	27	c.3341C>T	p.Thr1114Met	yes
Family 28	CC2D2A	27	c.3376G>A	p.Glu1126Lys	yes
Family 29	CC2D2A	37	c.4667A>T	p.Asp1556Val	yes
Family 30	CC2D2A	31	c.3820C>T	p.Gln1274*	no
Family 31	CC2D2A	22	c.2822_2829+5del	p.Val941Glyfs*3	no
Family 32	CC2D2A	14	c.1467-1G>A	p.Gln490_Lys536del	no
Family 33	CC2D2A	37	c.4667A>T	p.Asp1556Val	yes
Family 34	CC2D2A	33	c.4179+1del	p.?	yes
Family 35	CC2D2A	14	c.1466G>A	p.Arg489Gln	yes
Family 36	CC2D2A	37	c.4667A>T	p.Asp1556Val	yes
Family 37	CC2D2A	29	c.3544 T>C	p.Trp1182Arg	yes
Family 38	CC2D2A	11	c.1017+1G>A,	p.Val294Glyfs*8	yes
Family 39	CC2D2A	6	c.248-4_248-3insAAGTTTT	p.?	no
Family 40	CC2D2A	29	c.3341C>T	p.Thr1114Met	yes
Family 41	CC2D2A	25	c.3134T>C	p.Val1045Ala	yes
Family 42	CC2D2A	9	c.717G>A	p.Met239Ile	no
Family 43	CC2D2A	26	c.3276C>A	p.Tyr1092*	no
Family 44	CC2D2A	27	c.3289del	p.Val1097Phefs*2	yes
Family 45	CC2D2A	17	c.1765-1G>C	fs*	no

Exon	HGVS coding DNA	HGVS Protein Predicted	Previously repr Bi allelic variant	
23	c.2848C>T	p.Arg950*	yes	homozygous
9	c.697C>T	p.Gln233*	no	compound heterozygous
37	c.4582C>T	p.Arg1528Cys	yes	compound heterozygous
33	c.4179+1del	p.?	yes	compound heterozygous
36	c.4465G>C	p.Asp1489His	no	compound heterozygous
35	c.4428C>T	p.Ser1476Ser	no	compound heterozygous
38	c.4786G>A	p.Ala1596Thr	yes	compound heterozygous
35	c.4407C>G	p.Ser1469Arg	yes	compound heterozygous
30	c.3652C>T	p.Arg1218*	yes	compound heterozygous
37	c.4667A>T	p.Asp1556Val	yes	compound heterozygous
33	c.4111C>G	p.Leu1371Val	no	compound heterozygous
26	c.3286C>T	p.Gln1096*	no	compound heterozygous
38	c.4766C>T	p.Pro1589Leu	no	compound heterozygous
23	c.2848C>T	p.Arg950*	yes	homozygous
23	c.2848C>T	p.Arg950*	yes	homozygous
37	c.4667A>T	p.Asp1556Val	yes	compound heterozygous
21	c.2487-1G>C	p.?	no	compound heterozygous
19	c.2326G>A	p.Gly776Arg	yes	compound heterozygous
32	c.3989G>A	p.Arg1330Gln	yes	compound heterozygous
38	c.4787C>G	p.Ala1596Gly	yes	compound heterozygous
27	c.3341C>T	p.Thr1114Met	yes	compound heterozygous
21	c.2624C>A	p.Ser875*	yes	compound heterozygous
27	c.3289del	p.Val1097Phefs*2	yes	compound heterozygous
37	c.4500T>G	p.Ile1500Met	no	compound heterozygous
33	c.4179+1del	deletion of exon 33	yes	compound heterozygous
38	c.4822T>C	p.Ser1608Pro	no	compound heterozygous
37	c.4634_4660dup	p.Gln1553_Leu1554ins9	no	compound heterozygous
38	c.4724T>C	p.Ile1575Thr	no	compound heterozygous
17	c.2003+1G>A	p.?	no	compound heterozygous
5	c.247+1G>A	p.?	yes	compound heterozygous
35	c.4358A>T	p.Asn1453Ile	no	compound heterozygous
28	c.3462C>A	p.Asn1154Lys	no	compound heterozygous
8_12	deletion	p.?	no	compound heterozygous
38	c.4766C>T	p.Pro1589Leu	no	compound heterozygous
37	c.4500T>G	p.Ile1500Met	no	compound heterozygous
25	c.3182+2T>C	p.Ile1006_Ser1061del	no	compound heterozygous
24	c.2996_3001del	p.Glu999_Glu1000del	yes	compound heterozygous
37	c.4667A>T	p.Asp1556Val	yes	compound heterozygous
37	c.4667A>T	p.Asp1556Val	yes	compound heterozygous
39	c.4667A>T	p.Asp1556Val	yes	compound heterozygous
13	c.1154_1155del	p.Val385Glu fs*6	no	compound heterozygous
4 et 5	deletion	p.?	no	compound heterozygous
35	c.4428C>T	p.Ser1476Ser	no	compound heterozygous
37	c.4667A>T	p.Asp1556Val	yes	compound heterozygous
25	c.3145C>T	p.Arg1049*	yes	compound heterozygous

Table S4: Age at clinical first signs/ JS diagnosis / last medical report of our cohort patients

Age at first clinical signs	Age at medical diagnosis of JS	Age at last medical report available
≥ 3 months old and < 2 years old	Toddler	Early childhood
≥ 3 months old and < 2 years old	Early childhood	Early childhood
< 3 months old	Infancy	Early childhood
≥ 3 months old and < 2 years old	Infancy	Toddler
≥ 3 months old and < 2 years old	Infancy	Infancy
≥ 3 months old and < 2 years old	Infancy	Infancy
≥ 3 months old and < 2 years old	Toddler	Toddler
< 3 months old	NA	Middle childhood
< 3 months old	NA	Toddler
< 3 months old	Early childhood	Early childhood
≥ 3 months old and < 2 years old	NA	Middle childhood
≥ 3 months old and < 2 years old	NA	Toddler
< 3 months old	Infancy	Infancy
< 3 months old	Infancy	Infancy
< 3 months old	Infancy	Infancy
< 3 months old	NA	Toddler
< 3 months old	NA	N/A
na	Early childhood	Early childhood
na	Early childhood	Early childhood
≥ 3 months old and < 2 years old	Infancy	Infancy
< 3 months old	Toddler	Early childhood
< 3 months old	Middle childhood	Middle childhood
antenatal	Toddler	Toddler
antenatal	NA	Infancy
< 3 months old	Toddler	Toddler
< 3 months old	Infancy	Early childhood
< 3 months old	Infancy	Infancy
≥ 3 months old and < 2 years old	Toddler	Infancy
< 3 months old	NA	Infancy
< 3 months old	NA	Early childhood
< 3 months old	Early childhood	Early childhood
antenatal	Term neonatal	NA
≥ 3 months old and < 2 years old	NA	Toddler
≥ 3 months old and < 2 years old	NA	Infancy
< 3 months old	Infancy	Toddler
na	N/A	N/A
< 3 months old	Infancy	Infancy
< 3 months old	Infancy	Infancy

< 3 months old	Infancy	Toddler
< 3 months old	Infancy	Infancy
< 3 months old	Early childhood	N/A
\geq 3 months old and < 2 years old	NA	Early childhood
\geq 3 months old and < 2 years old	Early childhood	Middle childhood
< 3 months old	Infancy	Toddler
< 3 months old	NA	Infancy
< 3 months old	Term neonatal	Early childhood
< 3 months old	Infancy	Infancy
< 3 months old	Infancy	Infancy
< 3 months old	Infancy	Infancy
< 3 months old	NA	Early childhood
< 3 months old	Middle childhood	Middle childhood
< 3 months old	Infancy	Infancy
na	NA	Middle childhood

Preterm neonatal - The period at birth when a newborn is born before the full gestation period

Term neonatal Birth - 27 days

Infancy 28 days – 12 months

Toddler 13 months – 2 years

Early childhood 2 – 5 years

Middle childhood 6 – 11 years

Early adolescence 12 – 18 years

Late adolescence 19 – 21 years