

## ARTICLE OPEN



# ARID2-related disorder: further delineation of the clinical phenotype of 27 novel individuals and description of an epigenetic signature

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Rare genetic variants in *ARID2* are responsible for a recently described neurodevelopmental condition called ARID2-related disorder (ARID2-RD). *ARID2* belongs to PBAF, a unit of the SWI/SNF complex, which is a chromatin remodeling complex. This work aims to further delineate the phenotypic spectrum of ARID2-RD, providing clinicians with additional data for better care and aid in the future diagnosis of this condition. We obtained the genotypes and phenotypes of 27 previously unreported individuals with ARID2-RD and compared this series with findings in the literature. We also assessed peripheral blood DNA methylation profiles in individuals with ARID2-RD compared to epigenotypes of controls, unresolved cases, and other neurodevelopmental disorders. The main clinical features of ARID2-RD are developmental delay, speech disorders, intellectual disability (ID), behavior problems, short stature, and various dysmorphic and ectodermal features. Genome-wide differential methylation analysis revealed a global hypermethylated profile in ARID2-RD that could aid in reclassifying variants of uncertain significance. Our study doubles the number of reported individuals with *ARID2* pathogenic variants to 53. It confirms loss-of-function as a pathomechanism and shows the absence of a clear genotype-phenotype correlation. We provide evidence for a unique DNA methylation epigenotype for ARID2-RD and further delineate the ARID2-associated phenotype.

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## INTRODUCTION

Gene expression is critically regulated by the accessibility of the DNA by the transcription machinery. It depends on the level of chromatin compaction, which can be either euchromatin or heterochromatin, and on nucleosome positioning, composition, and modification. Chromatin remodeling is a dynamic process involving two major ATP-dependent chromatin remodeling complexes (CRCs). Among CRCs, the evolutionarily conserved BAF (BRG1/BRM-associated factor, also known as mammalian SWI/SNF) complexes promote gene expression by increasing access to DNA and thus facilitating transcription, replication, and repair [1]. The BAF complex binds to DNA and histones and effectuates nucleosome displacement to enhance DNA accessibility, thereby initiating the transcription machinery. BAF complexes also act as

tumor suppressors as somatic loss-of-function variants in one or more of their components are involved in almost 25% of all cancers [2]. They are organized into three broad subfamilies: canonical BAF (cBAF), polybromo-associated BAF (PBAF), and the GLTSCR1 or GLTSCR1L-containing and BRD9-containing (GBAF) complexes, also known as non-canonical BAF (ncBAF) [2]. The cBAF complex includes the AT-Rich Interaction Domain (ARID) proteins ARID1A and ARID1B, while ARID2 is a component of the PBAF complex [3]. *ARID2* (OMIM\*609539), also known as *BAF200*, contains an AT-rich DNA interaction domain consensus located at the N-terminus, followed by RFX, GLN (glutamine-rich region), and two classic C2H2 zinc fingers at the C-terminus, which directly bind to DNA or interact with proteins. *ARID2* is ubiquitously expressed and participates in many biological processes, such as

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transcriptional regulation, tumorigenesis, development, and differentiation through tissue-specific gene expression and control of cell proliferation [4].

Germline pathogenic variants in genes encoding proteins from the BAF complexes cause a spectra of disorders of which the majority consists of Coffin-Siris (CSS, OMIM#135900) and Nicolaides-Baraitser (NCBRS, OMIM#601358). NCBRS is caused by pathogenic variants in the helicase domain of *SMARCA2* (OMIM#600014) [3]. More recently, de novo *SMARCA2* missense variants clustering outside the helicase domain have been associated with a distinct syndrome with ID and blepharophimosis (OMIM#619293) [5]. In contrast, the phenotypic spectrum of CSS is broader, including developmental delay affecting intellectual and motor functions, hypotonia, growth impairment, coarse features, feeding difficulties in infancy, and hypoplastic to absent fifth distal phalanges of fingernails and toenails [6, 7]. Pathogenic variants in several genes, including *ARID1A* (OMIM#603024), *ARID1B* (OMIM#614556), *SMARCA4* (OMIM#603254), *SMARCB1* (OMIM#601607), *SMARCE1* (OMIM#603111), *SOX11* (OMIM#600898), *DPF2* (OMIM#601671), *SMARCC2* (OMIM#601734), *SOX4* (OMIM#184430), *SMARCD1* (OMIM#60), *BICRA* (OMIM#605690), and *ARID2* (OMIM#609539) are responsible for CSS or CSS-like [8].

In 2015, *ARID2* was first associated to a BAF-related ID syndrome called CSS-6 (OMIM#617808). To date, 26 individuals harboring causative heterozygous *ARID2* loss-of-function variants have been reported [8–18]. The core phenotype described consists of developmental delay, speech disorders, ID, behavior problems, short stature, and various dysmorphic and ectodermal features referring to CSS.

In recent years, high-throughput DNA sequencing has led to the identification of many variants of unknown significance, necessitating further tools for better variant classification. Functional tests can be used to assess the pathogenicity of these variants, including specific methylation signatures called episignature [19]. Because of the interplay between histone positioning, composition, and modification, and DNA methylation at CpG dinucleotides, these biomarkers can sometimes be used to stratify variants in diseases directly or indirectly affecting DNA methylation [20]. *ARID2* plays a role in chromatin remodeling. We thus hypothesized that individuals with an *ARID2*-related disorder (*ARID2*-RD) may carry a recognizable DNA methylation pattern.

Here, we present evidence for an epigenetic signature associated with *ARID2*-RD, and we further delineate its phenotypic spectrum, with the report of 27 previously unreported, unrelated individuals harboring *ARID2* loss-of-function variants and a review of 26 previously published individuals.

## MATERIALS AND METHODS

### Individuals

Written informed consent for publication of clinical and genetic data was obtained from all individuals harboring variants of interest in *ARID2* or their legal guardians. Written informed consent for publication of medical photographs was obtained for individuals for whom these are provided. In 22 of 27 individuals, *ARID2* variants were identified using exome or genome sequencing (ES, GS). An intellectual disability gene panel (IDGP) identified one variant. In the remaining 4/27 individuals, *ARID2* deletions were identified using chromosomal microarray analysis (Table S1). The identified *ARID2* variants were deposited in ClinVar (Table S1) [21]. The supplementary data provide details about the methods used for IDGP, ES, GS, and chromosomal microarray analysis.

## RESULTS

### Identification of novel *ARID2*-RD individuals through genotype-first approach

Twenty-four affected individuals harboring *ARID2* pathogenic or likely pathogenic variants and interstitial deletions encompassing *ARID2* were recruited through the French network for rare

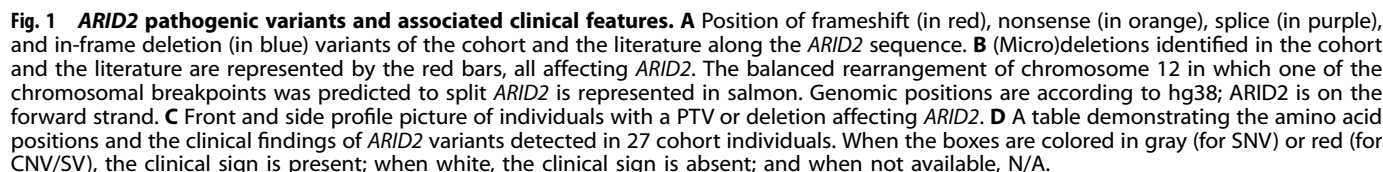
developmental disorders (AnDDI-Rares) and GeneMatcher [22]. Eighteen different single nucleotide variants or indels were identified in the coding sequence of *ARID2* (i.e., 12 frameshift, four nonsense, two splice variants), in addition to six interstitial deletions encompassing *ARID2* (Fig. 1A, B and D). Twenty-one of the twenty-seven (78%) variants were confirmed to have occurred de novo. Inheritance from a healthy parent carrying the pathogenic variant in a mosaic state was detected in two cases (individuals I-21 and I-23), and in I-27 the variant was inherited from the affected mother. In the remaining three cases, one or both parents were unavailable for testing (individuals I-2, I-9, and I-22).

### Pathogenic variants in *ARID2* are associated with a hypermethylated episignature

Next, we sought to investigate whether *ARID2*-RD individuals with pathogenic variants in *ARID2* are associated with a detectable change in DNA methylation compared to unaffected controls, and if affected individuals showed any stratification correlating with the severity of the disease. The analysis led to the identification of 215 probes (Table S3) whose differentially methylated CpG successfully permitted to separate *ARID2* cases (I-2, I-6, I-8, I-10, I-12, I-13, I-14, I-15, I-17, I-18, I-21, I-23, I-25, I-26) from unaffected controls. With unsupervised clustering methods, specifically hierarchical clustering (heatmap) and multidimensional scaling (MDS) methods, we were able to confirm that based on differential methylation from the selected probes, *ARID2* cases could be distinguished from controls (Fig. S1). To test the robustness and sensitivity of our *ARID2* episignature, we performed 14 rounds of leave-one-out cross-validation using unsupervised hierarchical and MDS clustering methods. All the cases used for testing clustered together with the remaining training cases showing the robustness and sensitivity of the episignature (Fig. S2).

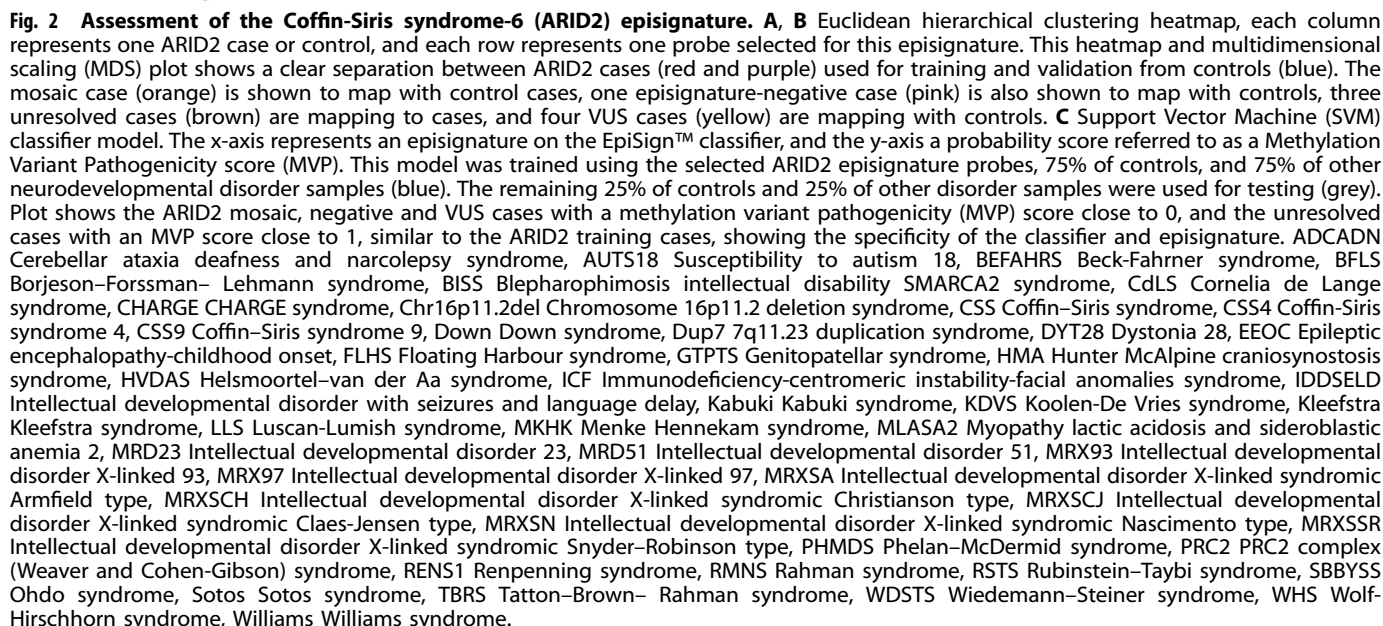
Next, we validated the *ARID2* episignature with three additional cases with pathogenic variants in *ARID2* (I-1, I-7, I-11). We performed hierarchical clustering and MDS and confirmed that all the validation cases clustered together with the training cases. With the support vector machine (SVM) classifier model, all the validation samples scored close to one, indicating the presence of the *ARID2* episignature (Fig. S3). Using the SVM classifier, we screened all unresolved cases in the EpiSign™ Knowledge Database (EKD), a database including cases and DNA methylation data used by the EpiSign™ classifier. When a clinical case is sent in, and no episignature is found, it gets labeled as unresolved. Every time a new episignature is discovered, we screen all unresolved cases to find matches. With the *ARID2* episignature, we found three cases (I-3, I-4, I-24) with an MVP score close to 1, and they all clustered with *ARID2* cases in hierarchical clustering and MDS plots. After contacting the clinical centers that submitted the cases, we confirmed that all three individuals carried pathogenic variants in *ARID2* (i.e., one frameshift, one in-frame deletion, and one intragenic deletion encompassing exons 7 through 10).

One of the most interesting utilities of episignatures is that they assist in reclassifying variants of uncertain significance (VUS). Therefore, we evaluated the episignature associated with two phenotypically unaffected individuals bearing mosaic variants (I-21\* and I-23\*) which are parents of two affected cases included in our cohort, three VUS cases (I-28, I-29, I-30) identified through GeneMatcher and the AnDDI-Rares network, and three unresolved cases (I-3, I-4, I-24), and compared them to the training cohort (discovery + validation cases) for assessment. The heatmap shows a clear separation between the *ARID2* discovery and validation cases used for training and the controls (Fig. 2A). The two healthy mosaic individuals and the three VUS cases clustered with controls. The three unresolved affected individuals clustered with the *ARID2* cases. The MDS plot shows the same results as the heatmap (Fig. 2B). The mosaic case (I-23\*) is shown to map between cases and controls in MDS, although closer to controls. The SVM classifier model was trained using the selected *ARID2* episignature probes,



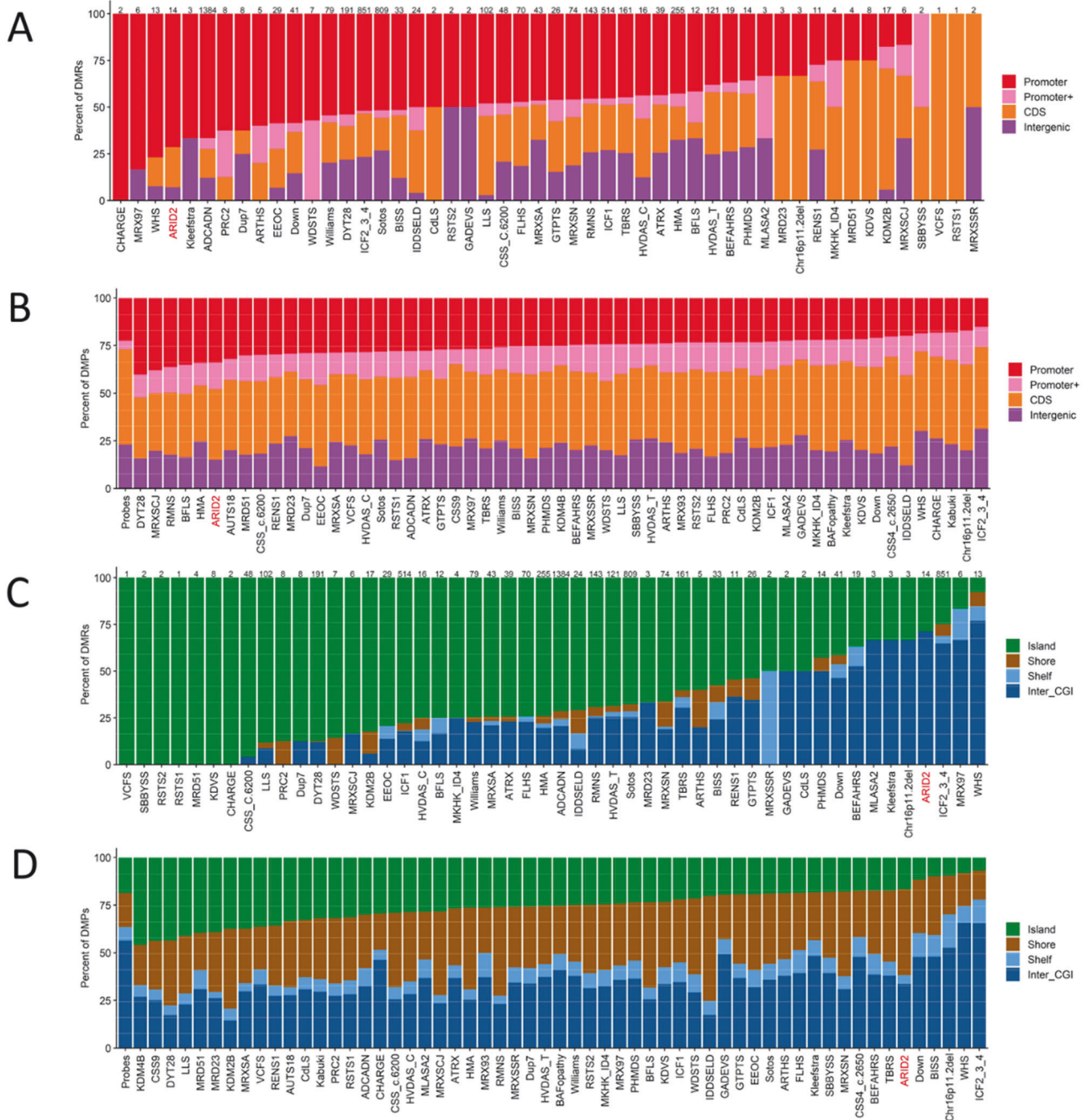
However, the MVP classifier is also showing that cases with an autosomal dominant cerebellar ataxia, deafness, and narcolepsy (ADCADN) (OMIM #604121) epismature are responding on the ARID2 epismature based on the MVP score being higher than 0, some as high as 0.75. This could be explained by the effect size on DNA methylation across a significant proportion of the CpG probes due to DNMT1 involvement in the ADCADN disorder. Therefore, this epismature showed the largest changes in methylation between cases and controls and overlapped with several other cohorts [23]. However, this does not affect our ARID2 epismature as there is still a separation between controls, ARID2 cases, and ADCADN cases when the cases are plotted together using unsupervised clustering methods (Figure S4).

Finally, we investigated the overlap of the *ARID2* genome-wide DNA methylation changes in cases with pathogenic *ARID2* variants and the other 56 episignatures described by Levy et al. [24]. Clustering analyses were performed using the top 500 DMPs for each cohort, for cohorts with less than 500 DMPs, the total number of DMPs was used to assess the similarity in genome-wide methylation profiles. We detected a predominant hypermethylation profile (Fig. 4A). Levy et al. [24] showed that 66% ( $n = 37$ ) of the episignatures showed hypomethylation and 34% ( $n = 19$ ) hypermethylation in the other 56 EpiSign™ disorders. *ARID2* had



To investigate the relation between the 57 cohorts, we showed the DMP overlap and the hypomethylation or hypermethylation levels with a binary tree where each node corresponds to a cohort.

Our clinical cohort comprised 27 individuals harboring pathogenic variants in *ARID2* (i.e., 24 individuals identified through AnDDI-Rares and GeneMatcher, and three individuals from the EKD). All individuals in this series presented with psychomotor delay, and 67% had

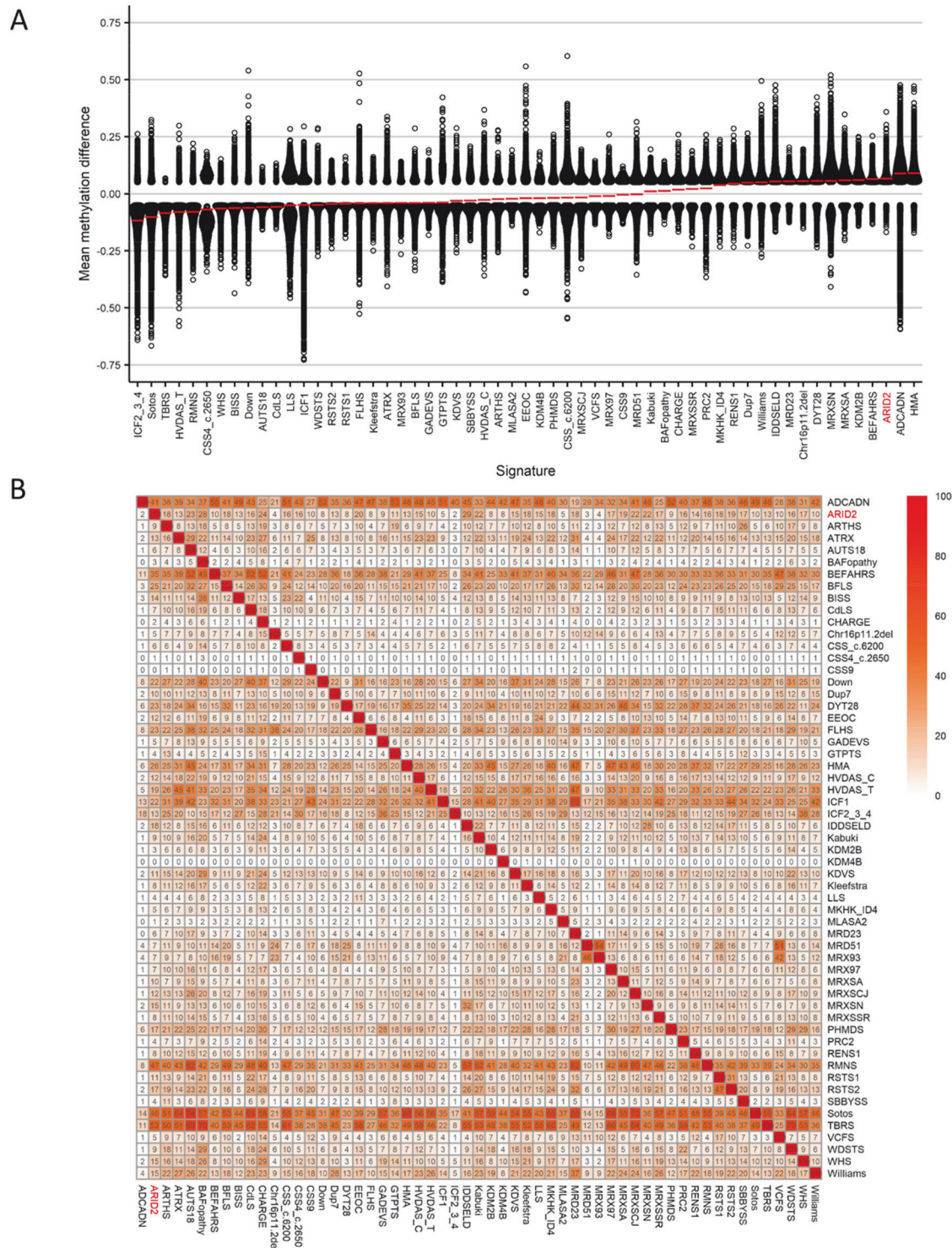


**Fig. 3 Differentially methylated probes (DMPs) annotated in the context of CpG islands and genes.** **A** DMPs in relation to genes. **B** DMPs in relation to genes. **C** DMPs in CpG islands and **(D)** DMPs in CpG island. Promoter, 0–1 kb upstream of the transcription start site (TSS); Promoter +, 1–5 kb upstream of the TSS; CDS, coding sequence; Intergenic, all other regions of the genome. Island, CpG islands; Shore, within 0–2 kb of a CpG island boundary; shelf, within 2–4 kb of a CpG island boundary; Inter\_CGI, all other regions in the genome. The Probes column in both (b,d) represents the background distribution determined in the Levy et al. [9] study of all array probes after initial filtering and used as input for DMP analysis.

hypotonia. Most had speech disorders (88%), behavioral abnormalities (64%), ID (70% in total, of which 79% mild and 21% moderate), and ectodermal features with dental, hair, and nail anomalies (73%, 50%, and 61.5%). Few have short stature (15%). The clinical characteristics of this series are summarized in Table 1, Fig. 1C, and given in detail in Table S1. Shared facial features included a coarse face, wide mouth, down-slanted palpebral fissures, and low-set, posteriorly rotated ears (Fig. 1D). Detailed reports, including variant classification scores, are available in Table S1. We did not identify a clear correlation between symptom severity and variant location (Fig. 1A; D, Table S4).

In the literature [8–13, 15–18] (Table 1, Table S4), the *ARID2* associated phenotype mainly consists of psychomotor delay (96%), hypotonia (70%), speech disorders (87%), behavior problems (65%), variable range of ID from mild to severe (96%), ectodermal features (hair 50%, and nail anomalies 38%), and few dental anomalies (24%). Short stature is frequent (60%). facial features (96%) and coarse face (71%) are also often reported.

Overall, the clinical assessment of the newly identified individuals allowed us to define the phenotypic spectrum of

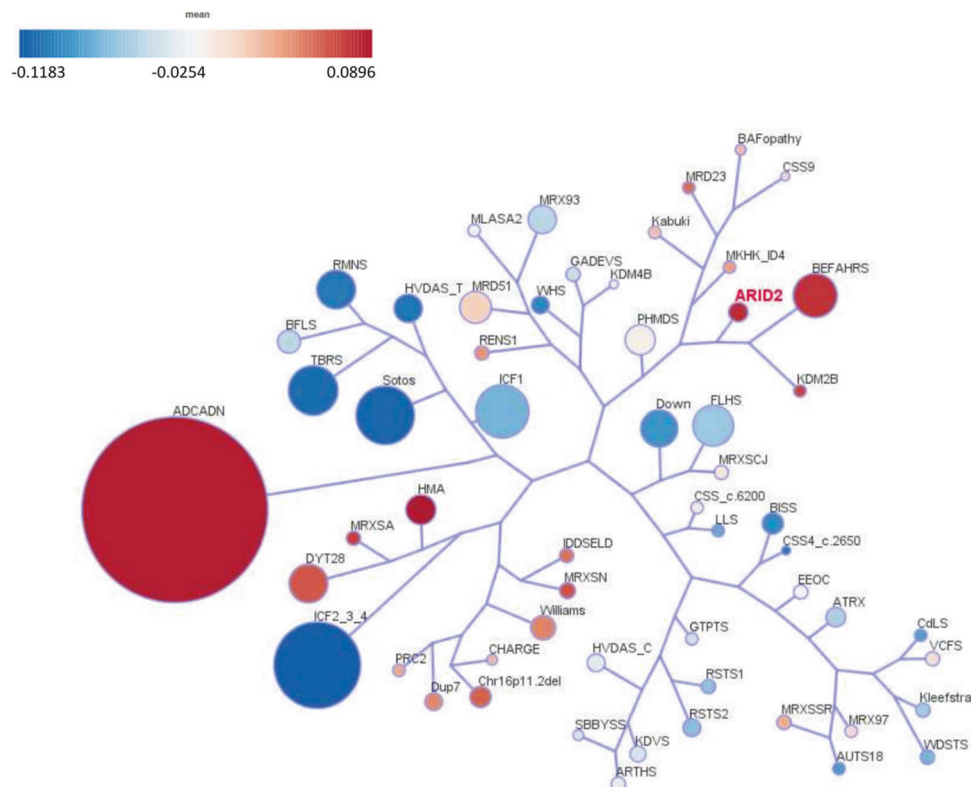


**Fig. 4 Relationships between the ARID2 cohort and 56 other EpiSign™ disorders. A** Global methylation profiles of all differentially methylated probes (DMPs, FDR < 0.05) for each cohort, sorted by mean methylation. Each circle represents one probe, and red lines show the mean methylation. **B** Heatmap showing the percentage of probes shared between each paired cohort. Colors indicate the percentage of the y-axis cohort's probes that are also found in the x-axis cohort's probes.

ARID2-RD, suggesting that ID is often mild, and when absent, individuals without an ID seem to have learning disabilities or a heterogeneous cognitive profile. Short stature is less frequently observed than previously reported (range -2.9 to 2.38 SD). Also, we did not note any apparent correlation between the severity of the disease and the type/localization of the variants (Fig. 1A; D).

## DISCUSSION

ARID2-RD is a rare monogenic disorder. Our study increases the total number of individuals reported with ARID2 pathogenic variants from 26 to 53 and further delineates the phenotypic spectrum of the ARID2 associated CSS 6 to be rather mild in many cases. There was no apparent genotype-phenotype correlation



**Fig. 5** Tree and leaf visualization of Euclidean clustering of all 57 cohorts using the top  $n$  DMPs for each group, where  $n = \min(\# \text{ of DMPs}, 500)$ . Cohort samples were aggregated using the median value of each probe within a group. A leaf node represents a cohort, with node sizes illustrating relative scales of the number of selected DMPs for the corresponding cohort, and node colors are indicative of the global mean methylation difference.

deducible despite the larger patient number. Consistent with previous reports, we observed loss-of-function as pathomechanism and we provide evidence of a unique DNA methylation epistatotype for *ARID2*-RD.

### Molecular description

All variants reported here and in the literature (i.e., 30 frameshifts, 13 nonsense, one in-frame deletion, and two splice variants) are distributed throughout the gene. The majority of the variants result in a premature termination codon (PTC). They are predicted to result in nonsense-mediated decay and hence loss-of-function, as is the assumed pathomechanism for all *ARID2* pathogenic variants reported. This is consistent with the “probability of being loss-of-function intolerant” (pLI) score of 1 [25] and the loss-of-function observed/expected upper bound fraction (LOEUF) score of 0.225, reflecting the high intolerance of this gene to loss-of-function variants. The lack of a clear genotype-phenotype correlation observed in our series and the literature (Fig. 1D, Table S1, Table S4) is an additional indirect argument indicating that the deleteriousness of *ARID2* variants results from haploinsufficiency after nonsense-mediated mRNA decay. Likewise, *ARID2* haploinsufficiency is the consequence of 17 chromosomal rearrangements encompassing *ARID2* reported so far, including the seven *ARID2* deletions in our study (Fig. 1B–D) [9–11, 15, 17, 18, 26].

Similarly, we do not observe a contiguous gene deletion phenotype but a *ARID2* haploinsufficiency phenotype. Genetic background may partially account for phenotypic variability, including ID differences, where polygenic contributions can impact global IQ scores [27]. Moreover, multiple genetic diseases could also contribute to phenotypic variability, as exemplified by the genetic findings in individual 11 of this study and individual 1 published by Bramswig et al. (listed as individual B1 in Fig. 1A and Table S4) [28]. They harbored the same pathogenic variant (Fig. 1B,

Table S1); however, individual B1 had also a de novo nonsense variant in the *TRIM8* gene explaining a more severe ID. On average, multiple diseases are expected in 4.3% of diagnosed patients with already identified genetic disorders [29, 30].

### Clinical description

The phenotype of *ARID2*-related disorders primarily involves neurodevelopmental issues that have been consistently observed in affected individuals (Table S1) [8–12, 14–18, 26, 28]. However, individuals in our group had less severe intellectual disability (ID) compared to previously reported cases. Importantly, three individuals in our group (I-5, I-17, I-25) did not exhibit ID, but they did show signs of developmental delay, learning disability, a heterogeneous cognitive profile, or speech disorder. This emphasizes the significance of early intervention. This variability did not seem related to the variant’s position in the gene. Neither our cohort nor the literature reported any specific MRI abnormalities. Cerebral atrophy, ventriculomegaly, or white matter hyperintensities are described.

It is important to pay special attention to behavioral problems as they are common in this disorder, with 64.5% of cases suffering from them according to Gazdag et al. [10]. In our study, and generally, the most common disorder was ADHD (40% and 35%, respectively). However, low frustration tolerance (20% and 17%) and anxiety disorders (12% and 15%) were also reported.

Individuals with *ARID2* loss-of-function variants share dysmorphic facial features, including coarse facies (67.5%), down-slanting palpebral fissures, low-set, and posteriorly rotated ears, as well as relatively short nose and long philtrum [28]. (Fig. 1C, Table S1). Several articles mentioned a resemblance between the dysmorphic facial features linked to *ARID2* and those in RASopathies [11, 15], such as Noonan syndrome, as in I-7 of this series (Fig. 1C).

**Table 1.** Clinical findings and their distribution for 49 new and already published individuals with ARID2-related disorder.

| Clinical Finding <sup>a</sup>                                       | This Series     | Literature     | Total           |
|---------------------------------------------------------------------|-----------------|----------------|-----------------|
| Sex (males/females, <i>n</i> (%))( <i>N</i> = 27 + 26)              | 13 (48)/14 (52) | 17 (65)/9 (35) | 30 (57)/23 (43) |
| <b>Neurodevelopmental disorders</b>                                 |                 |                |                 |
| Hypotonia <i>n</i> (%) ( <i>N</i> = 27 + 20)                        | 18 (67)         | 14 (70)        | 32 (68)         |
| Psychomotor delay <i>n</i> (%) ( <i>N</i> = 24 + 25)                | 24 (100)        | 24 (96)        | 48 (98)         |
| Speech disorder <i>n</i> (%) ( <i>N</i> = 25 + 15)                  | 22 (88)         | 13 (87)        | 35 (87.5)       |
| Intellectual disability <i>n</i> (%) ( <i>N</i> = 20 + 23)          | 14 (70)         | 22 (96)        | 36 (83)         |
| Epilepsy <i>n</i> (%) ( <i>N</i> = 26 + 24)                         | 2 (8)           | 2 (8)          | 4 (8)           |
| Brain MRI anomalies <sup>b</sup> <i>n</i> (%) ( <i>N</i> = 18 + 13) | 11 (61)         | 8 (62)         | 19 (61)         |
| <b>Behavioral problems</b> <i>n</i> (%) ( <i>N</i> = 25 + 23)       | 16 (64)         | 15 (65)        | 31 (64.5)       |
| Autism spectrum disorder <i>n</i> (%) ( <i>N</i> = 25 + 23)         | 0 (0)           | 2 (9)          | 2 (4)           |
| Attention deficit/hyperactivity disorder(%) ( <i>N</i> = 25 + 23)   | 10 (40)         | 7 (30)         | 17 (35)         |
| Low frustration tolerance <i>n</i> (%) ( <i>N</i> = 25 + 23)        | 5 (20)          | 3 (13)         | 8 (17)          |
| Aggressive behavior <i>n</i> (%) ( <i>N</i> = 25 + 23)              | 3 (12)          | 3 (13)         | 6 (12.5)        |
| Anxiety disorder <i>n</i> (%) ( <i>N</i> = 25 + 23)                 | 3 (12)          | 4 (17)         | 7 (15)          |
| <b>Feeding difficulties</b> <i>n</i> (%) ( <i>N</i> = 19 + 26)      | 12 (63)         | 8 (31)         | 20 (44)         |
| <b>Morphologic features</b>                                         |                 |                |                 |
| Facial features <sup>n</sup> (%) ( <i>N</i> = 27 + 26)              | 27 (100)        | 25 (96)        | 52 (98)         |
| Coarse face <i>n</i> (%) ( <i>N</i> = 19 + 21)                      | 12 (63)         | 15 (71)        | 27 (67.5)       |
| <b>Ophthalmologic Features</b>                                      |                 |                |                 |
| Ptosis <i>n</i> (%) ( <i>N</i> = 23 + 23)                           | 2 (9)           | 3 (13)         | 5 (11)          |
| Myopia <i>n</i> (%) ( <i>N</i> = 23 + 18)                           | 4 (17)          | 6 (33)         | 10 (24)         |
| Hyperopia <i>n</i> (%) ( <i>N</i> = 23 + 17)                        | 6 (26)          | 3 (18)         | 9 (22.5)        |
| Strabismus <i>n</i> (%) ( <i>N</i> = 23 + 18)                       | 5 (22)          | 7 (39)         | 12 (29)         |
| Astigmatism <i>n</i> (%) ( <i>N</i> = 23 + 17)                      | 2 (9)           | 1 (6)          | 3 (7.5)         |
| <b>Deafness</b> <i>n</i> (%) ( <i>N</i> = 20 + 12)                  | 2 (10)          | 3 (25)         | 5 (16)          |
| <b>Ectodermal features</b>                                          |                 |                |                 |
| Dental, gingival, jaw anomalies <i>n</i> (%) ( <i>N</i> = 26 + 25)  | 19 (73)         | 6 (24)         | 25 (49)         |
| Hair anomalies/Fair-hair <i>n</i> (%) ( <i>N</i> = 24 + 20)         | 12 (50)         | 10 (50)        | 22 (50)         |
| Nail anomalies <i>n</i> (%) ( <i>N</i> = 26 + 21)                   | 16 (61.5)       | 8 (38)         | 24 (51)         |
| <b>Skeletal features</b>                                            |                 |                |                 |
| Bone anomalies (X-rays) <i>n</i> (%) ( <i>N</i> = 16 + 19)          | 3 (19)          | 6 (32)         | 9 (26)          |
| Hand or feet anomalies <i>n</i> (%) ( <i>N</i> = 25 + 26)           | 11 (44)         | 15 (58)        | 26 (51)         |
| Short stature <-2 sd <i>n</i> (%) ( <i>N</i> = 27 + 25)             | 4 (15)          | 15 (60)        | 19 (36.5)       |
| <b>Cardiologic features</b> <i>n</i> (%) ( <i>N</i> = 15 + 15)      | 3 (20)          | 4 (27)         | 7 (23)          |

<sup>a</sup>The number of responders is detailed for every feature, and their frequencies/distributions were calculated according to that number, e.g., for hypotonia, (*N* = 27 + 20) means data were available for 27 individuals from our series and 20 individuals from the literature and 18(67) means 18 individuals in our series presented with hypotonia, which represents 67% of individuals in our series.

<sup>b</sup>MRI brain findings include cerebral atrophy, ventriculomegaly, or white matter hyperintensities.

Ectodermal abnormalities in CSS, such as specific nail abnormalities (i.e., small nails on the 5th finger or toe), are found in half of individuals with *ARID2* variants. Note also that 49% of all reported individuals have dental, gingival, and jaw abnormalities such as gingival overgrowth, persistence of primary teeth, tooth malposition, macrodontia, and retrognathia (Table 1, Fig. 1C, D).

Feeding problems, which affect 63% of our cohort and 44% of individuals, are also a common feature of this disorder. On the other hand, short stature is less frequent in our cohort (11%) than reported in the literature (60%) [8–12, 14–18] (Table 1, Table S1). These relatively low percentages indicate that growth abnormalities are not consistent in ARID2-related disorders.

### Epigenetic signature

We identified a robust and sensitive episignature for ARID2-RD, consisting of the methylation profile in 215 differentially methylated

CpG probes, which allows to discriminate individuals with ARID2-RD vs. unaffected controls. The ADCADN episignature that already overlaps with several other cohorts [23] is also responding on the ARID2 episignature, but does not affect the specificity of our ARID2 episignature. An episignature is helpful to 1) reclassify VUS, which are increasing in number due to high throughput sequencing techniques [31]; 2) screen unresolved cases to find matches as demonstrated by the cases of I-3 (c.836\_839dup, p.Asp280Glufs\*4), I-4 (c.988\_1008del, p.(Leu330\_Gly336del)), and I-24 (g.(?\_45891780)\_(45905075\_?)del). 3) Detect variant in a mosaic state as shown in I-23\* (12 :46123620-46298861)x1).

The gnomAD constraint score for missense variants is rather high (*Z* = 4.49) with three regions particularly depleted of predicted deleterious missense variants at the N-terminal (amino acid 1–455, regional missense constraint (RMC) = 0.46, *p* value = 2.57 × 10<sup>−17</sup>) and the C-terminal (aa 1634–1710, RMC = 0.29,

$p$ -Value =  $1.32 \times 10^{-5}$ ; and aa 1758–1835, RMC = 0.21,  $p$ -Value =  $1.03 \times 10^{-6}$ ) part of the protein, according to Decipher. These metrics suggest that deleterious missense variants with loss-of-function effect may also be causative for ARID2-RD. However, no such variants have been identified to date. In our cohort four missense VUS in I-28 (c.1150 G > A; p.(Ala384Thr) and c.4585 G > A; p.(Gly1529Arg)), I-29 (c.484 G > T; p.(Val162Leu)), and I-30 (c.335 A > C; p.(Glu112Ala)) were negative for being associated with a loss-of-function effect. Indeed, a negative epismutation result does not rule out the implication variants in a different (e.g. gain-of-function) ARID2-related disease. However, in our study other criteria contributed to the reclassification of the VUS in I-28 (c.1150 G > A; p.(Ala384Thr) and c.4585 G > A; p.(Gly1529Arg)), I-29 (c.484 G > T; p.(Val162Leu)), and I-30 (c.335 A > C; p.(Glu112Ala)) variants as being nonpathogenic such as the inheritance from an unaffected parent (c.1150 G > A and c.4585 G > A identified in individual I-28 were inherited from the healthy father and healthy mother respectively, c.484 G > T identified in individual I-29 was inherited from the healthy father; c.335 A > C identified in individual I-30 was inherited from the healthy father), or the predicted impact on the protein was likely\_benign and ambiguous for I-28, likely\_pathogenic for I-29 and ambiguous for I-30 according to Alphasense score [32].

We found 12 DMRs that were hypermethylated. One of the DMRs overlapped with *MC4R* associated with autosomal dominant and recessive obesity, a phenotype not described in young individual with ARID2-RD. However, it may be a phenotype that only develops with time, and it would be interesting to monitor weight trends in individuals with ARID2-RD. Another DMR overlapped *TRAK1* associated with autosomal recessive developmental and epileptic encephalopathy. Further investigation is needed to study the possible involvement of these regions and genes in the pathological mechanisms of ARID2-RD.

Finally, when we investigated the overlap of the ARID2 genome-wide DNA methylation changes in individuals with pathogenic ARID2 variants and other 56 established epismutations, we found an overlap with Intellectual developmental disorder, seizures, and language delay (IDDSLED), BAFopathy, CHARGE, Intellectual developmental disorder with autism and macrocephaly (AUTS18), Kabuki syndrome, Intellectual developmental disorder, X-linked 93 (MRX93) and Intellectual developmental disorder, X-linked 97 (MRX97). These neurodevelopmental syndromes share features with ARID2-RD, such as hypotonia, psychomotor delay, speech disorders, ID, and behavioral disorders. And some of them share morphologic features such as BAFopathy. These findings indicate possible underlying pathophysiological processes associated with ARID2-RD, such as post-translational binding of a ubiquitin-like protein shared by some of the proteins (*ARID2*, *CHD8*, *KMT2D*, *TET3*, and *ZNF711*) carrying a specific motif. These results warrant further investigations.

In conclusion, our study reviewed features of 53 individuals with ARID2-RD, including 27 unreported cases. The pathomechanism is ARID2 haploinsufficiency and there is no clear relationship between genotype and phenotype. We have also identified a unique DNA methylation pattern, which may aid in the diagnosis of ARID2-RD in the future. Additionally, our findings broaden the clinical spectrum of this disorder by describing milder phenotypes than previously reported, specifically regarding cognitive abilities and growth.

## DATA AVAILABILITY

The raw DNA methylation data for the samples are unavailable due to institutional and ethical restrictions. References sites web: <http://anddi-ares.org/> <https://genematcher.org/> <https://pecan.stjude.cloud/variants/proteinpaint>.

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Conceptualization: E.C., A.V., B.S.; Data Curation: C.H., E.C., C.P., KR, L.VDL., R.R., M.A.L.; Investigation: C.H., C.B., A.BOU., A.G., A.BON., K. S., B. O., A. BA., A. R., M.F., C.D., M.V., M.N., B.C., S.W., C.M., B.K., A.S., A.L.B., A.S.D.P., F.T.M.T., C.P., D.G., R.P., K.W.G., B.A.M., J.S., O.P., A.BOU., D.T., G.W.E.S., M.A., M.T., L.P., C.P., L.L., E.V.D.S., A.BE., F.C., T.R., M.T., A.G., V.P., D.B., C.T.R., B.I., E.C.; Formal Analysis: KR, L.VDL., R.R., M.A.L.; Methodology: KR, L.VDL., B.S.; Validation, and Visualization: KR, L.VDL.; Supervision: E.C., A.V., B.S.; Project administration: B.S.; Writing original draft: C.H., KR, L.VDL., E.C., A.V.; Writing review and editing: all the authors.

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## COMPETING INTERESTS

The authors declare no competing interests.

## ETHICS APPROVALS

The study was conducted in accordance with the regulations of the Western University Research Ethics Board (REB116108 and REB106302).

## ADDITIONAL INFORMATION

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