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**Extending the clinical and genetic spectrum of ARID2 related intellectual disability. A case series of 7 patients.**

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## Abstract

In the last 3 years *de novo* sequence variants in the *ARID2* (AT-rich interaction domain 2) gene, a subunit of the SWI/SNF complex, have been linked to intellectual disabilities in 3 case reports including one which describes frameshift mutations in *ARID2* in 2 patients with features resembling Coffin-Siris syndrome.

*ARID2*-related intellectual disability is characterised by coarse facial features, intellectual disability, hypotonia, behavioural problems and hypoplasia of the fifth finger or toe nails. There is a significant overlap with clinical features apparent in Coffin-Siris syndrome. Coffin-Siris syndrome (CSS) is a rare congenital syndrome characterized by intellectual deficit, coarse facial features and hypoplastic or absent fifth fingernails and/ or toenails among other features. Mutations in a number of different genes encoding SWI/SNF chromatin remodelling complex proteins have been described but the underlying molecular cause remains unknown in approximately 40% of patients with CSS.

Here we describe 7 unrelated individuals, 2 with deletions of the *ARID2* region and 5 with *de novo* truncating mutations in the *ARID2* gene. Similarities to CSS are evident. Although hypertrichosis and hypoplasia of the fifth finger nail and distal phalanx do not appear to be common in these patients, toenail hypoplasia and the presence of Wormian bones might support the involvement of *ARID2*.



## Introduction

The AT-rich interaction domain 2 (ARID2) protein is a subunit of the SWI/SNF chromatin remodelling complex and is involved in transcriptional activation and repression of target genes [1] [2]. There are two major subfamilies of the complex: BAF (BRG1- or hBRM-associated factor) and PBAF (polybromo-associated factor) and ARID2 is a component of the latter. ARID2 is required for the stability of the SWI/SNF complex that catalyses nucleosome repositioning [PMID: 10466730] and enables DNA to be accessed by transcription factors and repair enzymes. [3] The ARID2 protein is highly expressed in the heart, stomach, oesophagus, fetal testis and fetal brain [4]. ARID2 interacts with several other subunits of the BAF complex such as SMARCE1, SMARCA4 and SMARCB1 [4, 5], all of which are implicated in Coffin Siris Syndrome (CSS). [6]

Coffin-Siris syndrome (CSS, OMIM: #135900) is a heterogeneous congenital anomaly syndrome characterized by intellectual disability, hypertrichosis, coarse facial appearance, sparse scalp hair, and hypoplastic or absent fifth fingernails or toenails. Other features may include feeding difficulties, poor growth, craniofacial abnormalities, spinal anomalies, and congenital heart defects. The *ARID1B* gene is most commonly associated with CSS and approximately 37% of patients harbour pathogenic mutations [7] within this gene. Loss of function mutations including microdeletions, nonsense variants, and frameshifts have all been described [6, 8-15] but no clearly pathogenic missense variants have been identified. As well as other genes encoding proteins of the SWI/SNF complex mentioned above, mutations in the *SOX11* gene have been described recently in patients with features resembling CSS. *SOX11* is also involved in regulation of transcription and acts downstream from the PAX6-BAF complex [16, 17]

Using whole exome sequencing (WES), Shang et al [18] identified four independent, novel, loss of function variants in *ARID2* gene in four patients, three of which were confirmed to be *de novo* while parental samples were not available for the fourth patient. All four of these

patients have intellectual disability and share other characteristics including attention deficit hyperactivity disorder, short stature, dysmorphic features and Wormian bones. More recently Bramswig et al [19] described 2 individuals with *ARID2* mutations and CSS-like phenotypes and Van Paemel et al [20] reported a case with partial deletion of the *ARID2* gene and significant phenotypic overlap with CSS.

Here we describe 7 additional unrelated individuals with mutations in or involving the *ARID2* gene and compare their findings to features considered to be typical of CSS whilst highlighting possible differentiating features.

## Materials and methods

### Patient selection & data acquisition

The UK-wide Deciphering Developmental Disorders (DDD) Study has collected DNA samples and phenotype information from 13632 children and adults over a 4 year period from April 2011. These individuals presented with an undiagnosed developmental disorder to 24 Regional Genetics Services in the UK and Republic of Ireland participating in patient recruitment. The primary aim of the DDD study has been to identify the underlying molecular diagnosis by performing SNP array comparative genomic hybridisation (array CGH, also known as chromosomal microarray) and exome sequencing on the samples obtained from family trios [21]. The DDD study has also compiled a carefully curated list of genes reported to be associated with developmental disorders: the DDG2P (Developmental Disorders Genotype-to-Phenotype) database [22]. As *ARID2* has only recently been identified as a potential developmental gene it was not included in the DDG2P database at the time of data collection for this paper.

A request was made via a Complementary Analysis Project ([www.ddduk.org](http://www.ddduk.org)) to access data from the first 4,293 trios sequenced and 5 DDD recruits were found to possess a protein-truncating variant within the *ARID2* gene. Patient 1 was identified by chromosomal microarray carried out as part of a screen for developmental delay while patient 7 was found and included in our cohort after performing a copy number variant overlap search on the DECIPHER website. DECIPHER is a secure online portal that contains a database of genetic and phenotypic data for patients with copy number and other genetic variants submitted by contributors worldwide. All genomic and phenotype data collected by the DDD Study are also held securely within this database. Additional clinical data were obtained by contacting Clinical Geneticists who recruited patients to the DDD study and the DECIPHER database. Consent for publication was obtained from all patients or legal guardians locally.



## Array CGH

Array CGH on patients 1 and 7 were carried out using OCG 8\*60K ISCA v2.0 and BlueGnome Cytochip 8x60 K v2.0 oligonucleotide arrays respectively. This was analysed using BlueFuse Multi v2.5 (9271), CytoChip v2 algorithm. Coordinates refer to human genome build GRCh37 (hg19). Fluorescence in situ hybridisation (FISH) using BAC probes (BlueGnome BlueFish) was used to confirm gains and losses detected by array-CGH and to test parents to confirm that the copy number variant had arisen *de novo* in the proband. Both copy number variants (CNVs) are recorded in the DECIPHER database [<https://decipher.sanger.ac.uk>; Decipher ID numbers: 284808 (patient 1) and 267546 (patient 7)].

## Exome sequencing

Five DDD Study participants underwent whole exome sequencing (WES) of family trios, consisting of an affected individual/proband and the unaffected parents. Trio analysis allows detection of *de novo* mutations predicted to be functionally significant, as described previously (Wright et al, 2015). Sequencing was performed using Agilent SureSelect 55MB Exome Plus with Illumina HiSeq technology. *De novo* variants were identified by the DeNovoGear program and single nucleotide variants (SNVs) annotated by variant effect predictor (VEP) software of the Ensembl database (<http://www.ensembl.org/Tools/VEP>). The variants are publicly available in the DECIPHER database (<https://decipher.sanger.ac.uk/gene/ARID2#variants>).

## Results

### Patient 1

#### *Phenotype*

Patient 1 was referred to the Clinical Genetics Service regarding his growth and development. He was born by an emergency caesarean section for placenta previa at term with a birth weight of 2.296kg (-4.0 SD). He had had laryngomalacia which required surgical correction and there was a history of feeding difficulty, vomiting in early infancy, constipation and sleep disturbance. He was moderately delayed on developmental assessment (walked at 20 months of age and said his first words at the age of 19 months). He was short (87.8 cm, -3.6 SD) with a normal head circumference (-0.05 SD) and dysmorphic features including thin upper lip, micrognathia and low set ears were described. His toenails were noted to be hypoplastic. (Figure 1 and Table 1).

#### *Genotype*

Array CGH revealed male pattern with a heterozygous loss of 246,499bp deletion at 12q12, location 46,013,532 to 46,260,030 bp (GRCh37/hg19) encompassing the *ARID2* gene only. (Figure 2 and Table 1)

### Patient 2

#### *Phenotype*

Fetal scans identified renal pelvis dilatation and polyhydramnios. He was born at term weighing 3300g (-0.53 SD). He presented in infancy with severe feeding difficulties and developmental delay: he smiled at 12 weeks, sat at 15 months and walked independently at 21 months. He was assessed at the age of 2 years when he was speaking with single words but mainly communicating non-verbally. His height was 82 cm (- 2.4 SD), weight 12.2 kg (-

0.3 SD) and OFC 50.7 cm (+0.3 SD). He has suffered from constipation and frequent infections during infancy. He exhibited generalised hypotonia and joint hypermobility with mildly elevated creatine kinase levels and myotonic dystrophy (DM1) was subsequently ruled out. On examination he had plagiocephaly, protruding ears, thin upper lip vermilion and downturned corners of the mouth. Array CGH was normal. (Figure 1 and Table 1)

### *Genotype*

A heterozygous *de novo* frameshift mutation in exon 10 of the *ARID2* gene was detected by exome sequencing [12:g.46231317\_46231318insT (GRCh37/hg19); NM\_152641.2 (ARID2\_v001):c.1158dup; NP\_689854.2: p. (Asn387\*)]. (Figure 3 and Table 1)

### Patient 3

#### *Phenotype*

Patient 3 was born at term with a birthweight of 3129g (-1.0 SD) and OFC 36 cm (+1.0 SD). Other than having a raised alpha-fetoprotein (AFP) level, the pregnancy was uncomplicated. He was referred to clinical genetics at 6 months for assessment of disproportionate growth parameters and developmental delay. Cranial ultrasound was normal. At 2 years of age he weighed 12 kg (-0.89 SD), his height was 83 cm (-2.06 SD) and OFC 52.8 cm (+2.0 SD). He was classed as moderately delayed in all areas of development: he smiled at 16 weeks, sat independently at the age of 10 months and walked between the ages 2 and 2.5 years. He started speaking in sentences at about the age of 5 years and he has a stammer. He is able to follow single stage commands, prefers routine and has a limited range of interests. He attends a special educational needs school with some time in a mainstream school with one to one assistance. He is sensitive to loud noises and crowds. On examination at 6 years 10 months his height was 114.5cm (-2.0 SD), 20.85kg (-1.0 SD) and OFC 55.5cm (+2.0 SD) demonstrating relative macrocephaly. He has a high broad forehead with down-slanting

palpebral fissures and slightly low-set posteriorly rotated ears. He has an alternating strabismus but satisfactory vision. His fingernails are normal but all toenails, especially digits 3, 4 and 5 are hypoplastic. MRI scan of his cranium at the age of 6 months demonstrated a mildly dysmorphic corpus callosum (curved with a thin elongated splenium) and delayed myelination (equivalent to 4 months). The local Geneticist performed Genetic investigations prior to recruitment into the DDD Study. These included testing for PTEN mutation and a RASopathy panel, which did not identify any variants. (Table 1 and Figure 1)

### *Genotype*

A heterozygous *de novo* stop gained variant was found in exon 4 of the *ARID2* gene [12: g.46205315 C>G (GRCh37/hg19); NM\_152641.2: c.399C>G; NP\_689854.2: p. (Tyr133\*)]. This patient was also found to have compound heterozygous missense variants in the *VDR* gene [12: g.48238711 C>T; NM\_001017536.1:c.1252C>T; NP\_001017536.1:p.(Arg418Cys) and 12:g.48272629 G>A; NM\_001017536.1:c.296+122C>T]. The patient does not exhibit any features of rickets, the phenotype associated with this gene and the variants were classed as likely benign and benign respectively. (Figure 3 and Table 1)

### Patient 4

#### *Phenotype*

Patient 4 was identified through the DDD Study. He was born at term to non-consanguineous parents weighing 3630 grams (+0.16 SD). Increased nuchal translucency was detected on the antenatal ultrasound scans during pregnancy. Significant speech and global developmental delays were noticed during early childhood and his behaviour was described as rigid with physical outbursts and high anxiety levels. He smiled at 14 weeks, sat independently at 8 months and walked unaided at the age of 2-2.5 years. He said his first words over the age of 5 years and is currently being assessed for autistic spectrum disorder.

Assessment at the age of 8 years revealed short stature with height 122.1cm (-1.37 SD), weight 30.85 kg (+0.94 SD) and head circumference 55cm (+0.65 SD) and on physical examination he was found to have micrognathia, webbed neck, hypoplastic fingernails and short toes. Differential diagnoses of Noonan syndrome, Pallister-Killian syndrome and Smith-Lemli-Opitz syndrome were considered but genetic investigations including karyotype, telomere screen, FISH of 12p on buccal swab and array CGH were reported as normal. Skeletal survey was suggestive of multiple epiphyseal dysplasia and skull X-ray showed Wormian bones. (Figure 1 and Table 1)

### *Genotype*

Whole exome sequencing revealed a heterozygous *de novo* stop gained mutation in *ARID2* gene in exon 15, at position 4624350 substituting a cytosine by thymine [12: g.46246350 C>T (GRCh37/hg19); NM\_152641.2: c.4444C>T; NP\_689854.2: p. (Gln1482\*)]. The base change at this position results in a premature stop codon and truncation of the protein. (Figure 3 and Table 1)

### Patient 5

#### *Phenotype*

Patient 5 was born at 38 weeks gestation weighing 2891g (-2.2 SD); his length was 53 cm (-2 SD) at 6 weeks. His mother reported decreased fetal movements and developed polyhydramnios. He was slow to feed in infancy owing to his poor swallow. He sat independently at age 10 months, walked at 2 years, required speech and language therapy (SALT) input from age 2 years and was able to speak in full sentences by the end of primary school. He attended mainstream school with support. He wears glasses for a combination of myopia and hypermetropia. When assessed by clinical genetics he was found to be short with height following the 0.4<sup>th</sup> centile. Dysmorphic features included thick eyebrows, ptosis,

prominent eyelashes, flat nasal bridge, short nose, broad nasal root and tip, anteverted nares, thick alae nasi, broad philtrum, high and broad forehead and slightly posteriorly rotated ears. In addition he was observed to have mild 5<sup>th</sup> finger clinodactyly and small nails on his 2<sup>nd</sup> toes which are flexed and curled under at the distal phalangeal joints. Mild joint laxity was also noted. (Figure 1 and Table 1)

### *Genotype*

A heterozygous *de novo* frameshift mutation was detected in exon 15 of the *ARID2* gene [12: g.46246590\_46246591insCAAG (GRCh37/hg19); NM\_152641.2: c.4687\_4690dup; NP\_689854.2: p. (Thr1564Lysfs\*5)]. (Figure 3 and Table 1) A maternally inherited missense variant (X: g.5447558 C>T, NM\_004463: c.2269G>A) in the *FGD1* gene was also identified by the DDD Study but this was classed as a variant of uncertain significance (VUS).

### Patient 6

#### *Phenotype*

Patient 6 was born at term to non-consanguineous parents. His birth weight was 3000 grams (-1.16 SD). He was able to walk independently at the age of 12 months but only started speaking over the age of 5 years. He attended special needs nursery and school. As an adult, he is able to speak in sentences but mainly uses single words and phrases and is functioning at the level of a 5 year old, having recently learned to write his name.

When assessed at age 18 years, his height was 182.2 cm (+0.74 SD), weight 83.2 kg (+1.5 SD) and OFC 57.3 cm (+0.04 SD). He was dysmorphic with coarse face, low anterior hairline, thick eyebrows, broad nasal tip, thick vermilion of the lower lip and micrognathia (Figure 1 Patient 6). He has pes cavus, joint hypermobility and myopia. Genetic testing for Fragile X syndrome was reported as negative. (Figure 1 and Table 1)

### *Genotype*

Whole exome sequencing identified a heterozygous *de novo* frameshift mutation at position 12: 46244551 in exon 15 of *ARID2*, GCT replacing a G at this position [12:g.46244551\_46244552, G>GCT (GRCh37/hg19); NM\_152641.2: c.2645\_2646insCT; NP\_689854.2: p. (Val883Leufs\*10)]. (Figure 3 and Table 1)

### Patient 7

#### *Phenotype*

Patient 7 was born at 42 weeks gestation weighing 3062 grams (-2.0 SD) and was noted to have stridor from birth. She was seen in the genetics clinic on account of her short stature and moderate learning difficulties and on assessment she was dysmorphic with downslanting palpebral fissures, low-set and posteriorly rotated ears, narrow and high palate, small chin and arachnodactyly. All toenails were hypoplastic. Generalised hypotonia was noted during infancy. Skeletal features comprising mild pectus carinatum, hyperkyphosis of the thoracic spine and hyperlordosis of the lumbar spine were also reported (Table 1). At the age of 6 months she required surgery for a laryngeal web. She went into puberty aged 16 years and although her stature was short through childhood her final adult height is 161.5 cm (-1 SD) and OFC 55cm (-0.5 SD). She suffered cerebral sinus thrombosis aged 22 and was investigated for homocystinuria although this was excluded. She is also reported to have anxiety. (Figure 1 and Table 1)

#### *Genotype*

Array CGH revealed a heterozygous 1.39 Mb deletion at 12q12-13.11 [12: 46189641 to 47575801, GRCh37/hg19] involving 7 protein coding genes including *ARID2*. The other genes (*AMIGO2*, *PCED1B*, *SCAF11*, *SLC38A1*, *SLC38A2*, *SLC38A4*) are not listed by DECIPHER as morbid and do not appear in the DDG2P database. (Figure 2 and Table 1)

In addition, this patient was also noted to have heterozygous 329.66 kb duplication at 7q11.23 (7: 75255680-75585337). The duplication was also detected in one of his unaffected parents and therefore was classed as benign. (Figure 2 and Table 1)



## Discussion

The *ARID2* gene encodes one of the DNA-binding proteins characterized by an AT-rich interactive domain. Members of the ARID family have been described to play a role in embryonic patterning, cell lineage gene regulation, the control of cell cycle and regulating the transcription and modification of chromatin structure [1-3]. The ARID2 protein functions as one of the unique subunits of the polybromo- and BRG1-associated factor (PBAF) protein subset within the SWI/SNF chromatin remodelling complex [23] and is expressed in all tissues [4].

Pathogenic variants in other members of the SWI/SNF chromatin remodelling complex, namely *ARID1B*, *SMARCA2*, *ARID1A*, *SMARCB1* and *SMARCE1*, have been reported in individuals with CSS [8, 11, 13, 24] while *ARID1B* variants have also been reported in non-syndromic intellectual disability with or without hypoplasia of the corpus callosum [6]. Shang et al [18] previously reported 4 unrelated individuals who possess likely pathogenic variants in *ARID2*, all of them sharing characteristics that included intellectual disability, hypotonia, short stature, failure to thrive and dysmorphic features: micro/retrognathia, downslanted palpebral fissures, frontal bossing and low set and posteriorly rotated ears. Patient 4 (S4 in Table 1) was reported to have an additional *de novo* variant in the *EP300* gene but she did not resemble features typical of Rubinstein-Taybi syndrome. More recently Bramswig et al. [19] reported two individuals with *de novo* frameshift *ARID2* variants and a phenotype resembling Coffin Siris syndrome. These patients have similar facial features to those described by Shang et al. Individual 1 in Bramswig's paper developed seizures from age 14 months. Although seizures are described in a proportion of cases with CSS [25], the finding of a *de novo* variant in *TRIM8* could also account for his seizures and be contributing to his severe intellectual disability.

Comparing the 7 patients presented in this paper with those previously reported (see table), all have a degree of developmental delay ranging from moderate to severe, which would be

in keeping with a diagnosis of CSS [25]. All 7 patients presented here were delayed in speech acquisition with 3 said to have had no words until after 5 years. The younger patient reported by Bramswig was using signs when assessed at 4.5 years while the second patient with a possible second pathogenic variant had no speech at 7 years. Three out of 7 cases were small for gestational age, which is similar to the proportion of CSS cases in other series with this finding [6]. Short stature (5<sup>th</sup> centile or less) is common in CSS and was present in 10 of 14 and none of the children assessed had heights above -1 standard deviation for their age. Head circumferences were all around the mean for age apart from patient 3 who has relative macrocephaly. We do not have information about growth during childhood for patient 6 who was assessed as a young adult but patient 7 had been small throughout childhood but achieved a normal adult height after late onset of puberty. Interestingly, the combination of short stature and dysmorphic features led to 2 patients being tested for Noonan syndrome and other RASopathies.

Before the identification of *ARID1B* and other genes associated with CSS, clinical diagnosis of this syndrome was always relatively difficult as the facial gestalt is not as well defined as in other syndromes and there was debate whether all patients diagnosed with CSS had the same diagnosis [8]. Hypoplasia of the distal phalanx and nail of the fifth finger is highly indicative but is not always present. Although Bramswig et al. described this sign in both of their cases, this feature was not apparent in the 7 cases described here, however toenail hypoplasia was marked in 4/7 and this has also been described in CSS. Other ectodermal features associated with CSS are less specific and include hypertrichosis and sparse scalp hair. None of our 7 cases were documented as having these findings. One feature not commented on previously but noticeable in this series and in published photographs of other *ARID2* cases is that almost all cases appear to have blonde hair and none have very dark coloured hair. This is possibly coincidental and simply a reflection of their ethnicity and it will be interesting to see if it is borne out when more cases are identified.

Individuals with CSS are said to have coarse facial features comprising bushy eyebrows, broad nasal tip and thickened alae nasi and lower lip vermilion. This tends to become more apparent with time. In our opinion, the younger patients in our cohort do not have this appearance but there is the impression of gradual coarsening in those patients over 5 years. This is also evident in pictures of patients 2 and 3 published by Shang et al, quite dramatically in the picture of individual 1 in the Bramswig paper and less markedly in individual 2 in the Bramswig paper and in photos of the patient presented by Van Paemel et al. All 7 patients presented here have similarities in facial appearance and these are also present in the photographs of 4 patients included in previous case series. Tall broad forehead, micro/retrognathia, downslanting palpebral fissures, low set and posteriorly rotated ears, and relatively short nose and long philtrum are consistent (Figure 1) and described in the previous case reports. On reviewing published pictures, other similarities are pronounced cupid's bow configuration of the upper lip vermilion in association with prominent philtral pillars in some cases while others have a wide mouth and broad shallow philtrum. In almost all cases the corners of the mouth appear down-turned. There is also relative malar hypoplasia with associated infraorbital fullness.

Behavioural phenotypes were described in 4 of the 7 patients ranging from anxiety, sleep disturbance, sensitivity to loud noises, 'routine driven' or rigid behaviour and physical outbursts, to being quiet and inappropriately affectionate. Aggressive behaviour, anxiety, tics and attention deficit disorder were also described by Shang et al. and hyperactivity and short attention span by Van Paemel et al. Therefore, behavioural issues appear to be a common problem. Other frequently reported but less specific findings are generalised hypotonia, joint hypermobility, feeding difficulties and a tendency to constipation. Of note, 2 patients required surgery for laryngeal anomalies. Skeletal anomalies including chest deformity, kyphosis and kyphoscoliosis are described in 4 of 14 patients while another was thought to have possible skeletal dysplasia. The presence of Wormian bones documented in 3 of 14 patients is intriguing and might be a useful diagnostic clue. Wormian bones are more often seen in

primary skeletal dysplasias and osteogenesis imperfecta and we speculate that *ARID2*, part of the SWI/SNF chromatin remodelling complex, might have an influence on the BMP pathway and bone formation. Mutations in the protease domain of Bone morphogenetic protein 1 (BMP1), a member of the BMP signalling pathway, have been reported in individuals with osteogenesis imperfecta which is characterised by the presence of Wormian bones [26]. In an analysis of stable *ARID2* knockdown cells, Xu et al. demonstrated that *ARID2*-containing complexes play a critical role in normal osteoblast differentiation and in the regulation of expression of at least *BMP4* and *FGFR2* genes [23]. While a previous study, using *BRG1* mutant cells, determined that the SWI/SNF chromatin remodelling complex is required for the BMP2-induced skeletal gene expression that controls osteoblast differentiation [27]. Bone morphogenetic protein (BMP) signalling has been shown to influence many processes in early development including cell growth, apoptosis and differentiation [28-30] and it is extensively regulated by various extracellular, intracellular and membrane modulators [31]

Patient 1 and 7 both have partial deletions of the *ARID2* gene whereas patients 2-6 were found to have truncating (frameshift and stop gained) mutations suggesting that haploinsufficiency of the *ARID2* gene through a loss of function mechanism is the likely molecular cause for these patients' phenotypes. This is also suggested by the types of mutations previously reported in the literature.[18, 19].

Although at the present time, approximately 40% of the individuals with CSS-like features do not receive a molecular diagnosis [10-12, 32], features in our patients overlap sufficiently to suggest that *ARID2* mutations could account for a proportion of these.

In summary, this case series brings to a total of 14 unrelated patients with disruption of the *ARID2* gene resulting in phenotypic features overlapping with those described in CSS. Intellectual disability, short stature, hypoplastic toe or fingernails with characteristic facial features which become more apparent with age are all in keeping with this diagnosis. The

presence of Wormian bones, skeletal and laryngeal anomalies appear to be additional findings while common non-specific findings such as feeding difficulties, constipation, joint laxity and refraction errors contribute to the morbidity associated with this disorder. Though less commonly associated with CSS, macrocephaly, seizures and non-verbal severe intellectual disability are all reported in single cases here and could be part of the spectrum or, alternatively, be due to a second disorder affecting these patients.

This case series provides further evidence that *ARID2* is a developmental gene associated with intellectual disability and further supports the contention raised by Braumswig et al, that disruption of the *ARID2* gene causes a Coffin-Siris like phenotype in affected individuals.

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## Figure legends

Figure 1. Patients 1-7, showing facial phenotypes in the upper panel, profiles in middle panel and limb phenotypes in lower panel.

Figure 2. Genomic location of deletions found in patients 1 and 2, indicated as red horizontal bars.

Figure 3. Schematic of *ARID2* mutations; stop gained variants are indicated by red arrows and frameshift variants by black arrows, respectively. Diagram extracted from the DECIPHER website.

<https://decipher.sanger.ac.uk/gene/ARID2#overview/protein-info>



Table 1. Clinical features and genotypes of patients carrying ARID2 mutations.

ID	P1 Decipher284808	P2 DDD 267395	P3 DDD 275526	P4 DDD 263830	P5 DDD 265539	P6 DDD 272339	P7 Decipher267546	S1	S2	S3	S4	B1	B2	VP
ARID2 variant	deletion exons 1-16	c.1158dup; p. (Asn387*)	c.399C>G; p. (Tyr133*)	c.4444C>T; p. (Gln1482*)	c.4687_4690dup; p.(Thr1564Lysfs*5)	c.2645_2646insCT; p.(Val883Leufs*10)	Deletion exons 4-21	c.2536delG; p.Val846Leufs*3	c.1028 T>A; p.Leu343*	c.4441delC; p.His1481Ilefs*4	c.4318 C>T; p.Q1440*	c.3411_3412delIAG; p.Gly1139Serfs*20	c.156delC; p.Arg53Glufs*5	Deletion exons 3-5
Age/Gender	4.5y/M	5y/M	7y/M	8.3y/M	15.5y/M	18y/M	23y/F	15y/F	8y/F	6y/M	8y/F	7y/M	4y/M	3.9y/F
Pregnancy	Placenta previa	PH Dec FM	Raised AFP	Increased NT	PH Dec FM							Two previous miscarriages, born at 36 weeks	PH, Short femur (3 <sup>rd</sup> centile) on antenatal scans	IUGR Placental calcifications
BW SD	-3sd	-0.53sd	-1sd	+0.16sd	-2.2sd	-1.16sd	-2sd	-2sd	-1sd	-1sd	-1sd	-1.6sd	+2.1sd	-1.4sd
Height/OFC SD	-3.6/-0.05	-2/+0.3	-2.06/+2	-1.37/+0.65	-2/nk	+0.74/+0.04	-1/-0.5	-2.5/nk	-1/nk	-2/nk	-2.5	-2.14/+0.68	-2.2/-0.4	-3.3/-2.0
Intellectual impairment	mod	mod	mod	+	+	+	mod	+	+	+	+	+	+	+
First words spoken	19m	2y	5y	>5y	2y	>5y	nk	1 y	nk	18m	nk	absent	absent	2y
Behaviour	Sleep disturbance	'Routine driven' Rigid, anxiety	'Routine driven'	Rigid, anxiety		Quiet	anxiety	ADHD, anxiety	ADHD, aggressive	Affinity for water, sensitive to loud noises	ADHD, tics	-	Hand flapping	Hyperactivity, short attention span Talks loudly
Dysmorphism	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Coarse features	-	-	+	+	+	+			+	+		+	+	+
Ectodermal features	TNHP (all) blonde	blonde	TNHP (3,4,5) blonde	FNHP blonde	TNHP (2) blonde	-	TNHP (all)	nk	blonde	blonde	nk	FNHP(5), TNHP (5) blonde Delayed dental eruption	FNHP (all) TNHP (especially 5th)	TNHP
Hand, foot & digital anomalies	-	-	-	Short toes	5 <sup>th</sup> finger & 2 <sup>nd</sup> toe HDP	-		nk	nk	nk	nk	Small hands, tapering fingers, small feet	HDP, tapering fingers, clinodactyly	Brachymesop halangia clinodactyly
Additional features	Laryngomalacia, feeding diff, constipation	Hypotonia, raised CPK, constipation	Alternating strabismus	Webbed neck, Wormian bones, ?MED on skel survey	Ptosis Mixed refractive errors JH	Pes cavus, JH, myopia	Hypotonia, PC, kyphosis, lordosis, DP, laryngeal web, CST, dislocatable hips at birth	Wormian bones, kyphoscoliosis	-	Plagiocephaly	Wormian bones, plagiocephaly, mild pectus excavatum	Seizures Bilateral inguinal hernia,	Bilateral hyperopia Constipation Umbilical hernia	
								Cleft palate, diaphragmatic eventration, atrial septal defect in 1/4						
Brain MRI	ND	ND	Delayed myelination, abnormal CC	ND	ND	ND	nk	Prominent lateral ventricles; small arachnoid cyst Mild periventricular leukomalacia				Dandy-Walker malformation	ND	nk
Other diagnoses considered		Myotonic dystrophy	PTEN RASopathy	Noonan, PKS, SLO								Mucopolysaccharidosis NPD CLS	FGFR3	SRS
Other variants identified			Compound het missense variant in VDR		Mat missense variant FGD1		Mat 7q11.23 gain		11q21.1 dup (421kb, inheritance undetermined)		EP300 (de novo, p.Y629N)	TRIM8 de novo c.1333c>t; p.Gln445*		

ADHD-attention deficit hyperactivity disorder; AFP-alphafetoprotein, CC-corpus callosum; CLS-Coffin-Lowry syndrome; CPK- creatine phosphokinase; CST-cerebral sinus thrombosis; Dec FM-decreased fetal movements; DP-delayed puberty; F-female; FNHP-fingernail hypoplasia; HDP-hypoplastic distal phalanges; JH-joint hypermobility; M- male; Mat- maternally inherited; MED- multiple epiphyseal dysplasia; ND- not done; nk-not known; NPD-Niemann-Pick disease; NT-nuchal translucency; PC-pectus carinatum; PH-polyhydramnios; PKS-Pallister Killian syndrome; SD-standard deviation; SLO- Smith Lemli Opitz syndrome; SRS- Silver-Russel syndrome; TNHP-toenail hypoplasia,

