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JARID2 haploinsufficiency is associated with a clinically distinct neurodevelopmental syndrome

Eline A. Verberne, MD[1], Shuxiang Goh, MBBS, BSc[2], Jade England, MD, MSc[3], Manon van Ginkel, BSc[1,4], Louise Rafael-Croes, MD[4], Saskia Maas, MD[1], Abeltje Polstra, PhD[1], Yuri A. Zarate, MD, MBA[5], Katherine A. Bosanko, MS, CGC[5], Kieran B. Pechter, PhD[6], Emma Bedoukian, MS, CGC[7], Kosuke Izumi, MD, PhD[7], Ayeshah Chaudhry, MBBS, FRCPC[8,9], Nathaniel H. Robin, MD[10], Megan Boothe, MD[10], Natalie C. Lippa, MS, CGC[11], Vimla Aggarwal, MBBS[11], Darryl C. De Vivo, MD[12], Anna Lehman, MD, FRCPC[13], Causes Study[13], Sylvia Stockler, MD[14], Ange-Line Bruel, PhD[15], Bertrand Isidor, MD, PhD[16], Jennifer Lemons, MS, CGC[17], David F. Rodriguez-Buritica, MD[17], Christopher M. Richmond, BBiomedSc, MBBS[18,19], Zornitza Stark, BM BCh, DM[18,20], Pankaj B. Agrawal, MD, MMSc[21], R. Frank Kooy, PhD[22], Marije E. C. Meuwissen, MD, PhD[22,23], David A. Koolen, MD, PhD[24], Rolf Pfundt, PhD[24], Agne Lieden, PhD[25,26], Britt-Marie Anderlid, MD, PhD[25,26], Dagmar Glatz[27], Marcel M. A. M. Mannens, PhD[1], Madhura Bakshi, MBBS, FRACP[2], Frédérick A. Mallette, PhD[27], Mieke M. van Haelst, MD[1] and Philippe M. Campeau, MD[3]

Purpose: *JARID2*, located on chromosome 6p22.3, is a regulator of histone methyltransferase complexes that is expressed in human neurons. So far, 13 individuals sharing clinical features including intellectual disability (ID) were reported with *de novo* heterozygous deletions in 6p22–p24 encompassing the full length *JARID2* gene (OMIM 601594). However, all published individuals to date have a deletion of at least one other adjoining gene, making it difficult to determine if *JARID2* is the critical gene responsible for the shared features. We aim to confirm *JARID2* as a human disease gene and further elucidate the associated clinical phenotype.

Methods: Chromosome microarray analysis, exome sequencing, and an online matching platform (GeneMatcher) were used to identify individuals with single-nucleotide variants or deletions involving *JARID2*.

Results: We report 16 individuals in 15 families with a deletion or single-nucleotide variant in *JARID2*. Several of these variants are likely to result in haploinsufficiency due to nonsense-mediated messenger RNA (mRNA) decay. All individuals have developmental delay and/or ID and share some overlapping clinical characteristics such as facial features with those who have larger deletions involving *JARID2*.

Conclusion: We report that *JARID2* haploinsufficiency leads to a clinically distinct neurodevelopmental syndrome, thus establishing gene–disease validity for the purpose of diagnostic reporting.

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INTRODUCTION

The *JARID2* (jumonji, AT rich interactive domain 2; OMIM 601594) gene is located on chromosome 6p22.3 and encodes a protein that regulates the activity of various histone methyltransferase complexes.[1–3] JARID2 forms a complex together with polycomb repressive complex 2 (PRC2) that is essential to recruit polycomb group proteins to its target genes. PRC2 can lower gene transcription by catalyzing the di- and trimethylation of lysine 27 on histone H3 (H3K27me2/3). By the regulation of epigenetic changes, the JARID2-PCR2 complex is necessary to control development, differentiation, and survival of embryonic cells.[4,5] *JARID2* also regulates pluripotency and embryonic stem cell differentiation through Nanog expression and β-catenin.[6] In addition, *JARID2* has an important function in the Notch-1 pathway, which is essential for development of the central nervous system and other tissues.[7] By the methylation of H3-K9 and repression of cyclin D1, *JARID2* also regulates

Correspondence: Mieke M. van Haelst (m.vanhaelst@amsterdamumc.nl) or Philippe M. Campeau (p.campeau@umontreal.ca). Affiliations are listed at the end of the paper.
These authors contributed equally: Eline A. Verberne, Shuxiang Goh, Jade England
These authors contributed equally: Mieke M. van Haelst, Philippe M. Campeau

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cardiomyocytes proliferation and migration of neural progenitor cells.[8]

JARID2 is crucial in embryogenesis and morphogenesis, and multiple malformations can arise from its dysregulation in mice. In the mouse, *Jarid2* is involved in the development of the cardiovascular system, the liver, in hematopoiesis and in neural tube fusion.[9] In human embryogenesis, *JARID2* is expressed in neurons, especially in the dorsal root ganglion, and in adults it is expressed in the neurons of the cerebral cortex.[10]

De novo coding single-nucleotide polymorphisms in *JARID2* have been found once per study in two autism studies[11,12] (p.Arg827Gln and p.Met1181LeufsTer3) and once in a schizophrenia study[13] (p.Gly769Ser). However, these single findings did not reach significance in those large studies.

In another study, *JARID2* was found to be in linkage disequilibrium with nonsyndromic cleft lip and/or palate. Mouse models showed that *Jarid2* is expressed in the merging palatal shelves at the time of fusion, supporting its involvement in palatal development.[14] A more recent case–control study found that a deep-intronic *JARID2* single-nucleotide variant was protective for nonsyndromic cleft lip and/or palate in a Brazilian cohort.[15]

Chromosomal deletions in 6p22–p24 involving *JARID2* have been identified by karyotype[16–19] and chromosome microarray analysis[20–22] in 15 individuals, of whom 13 have a complete deletion of *JARID2*. These individuals have a common phenotype of borderline intellectual functioning to severe intellectual disability (ID) and share characteristic facial features. These features include prominent supraorbital ridges, deep set eyes, infraorbital dark circles, and midface hypoplasia. Apart from *JARID2*, all of the reported deletions involve other neighboring genes as well (henceforth referred to as *JARID2*-plus deletions), which has complicated the identification of the critical gene(s). Based on the smallest region of overlap (involving the genes *JARID2* and *DTNBP1*) in four individuals with de novo 6p22.3-24.1 deletions, it has been proposed that *JARID2* is a likely candidate gene contributing to the phenotype. This was supported by the finding that *JARID2* expression in leukocytes is significantly reduced in these individuals compared with controls.[20] Because of the characteristic facial appearance in these individuals, Baroy et al. propose that *JARID2* haploinsufficiency may represent a clinically recognizable neurodevelopmental syndrome.[20]

We describe 16 individuals with developmental delay and/or ID and overlapping clinical features with a deletion or single-nucleotide variant of *JARID2*. Seven individuals have partial deletions of *JARID2* that are predicted to lead to nonsense-mediated messenger RNA (mRNA) decay and one individual has a complete deletion of *JARID2*. Five individuals have a single-nucleotide variant in *JARID2* that leads to a frameshift, stop codon, or splice site alteration, and three individuals have a missense variant. We thus confirm that *JARID2* haploinsufficiency leads to a clinically distinct neurodevelopmental syndrome.

MATERIALS AND METHODS

Subjects

Sixteen individuals from 15 unrelated families with a *JARID2* deletion or single-nucleotide variant were identified in a diagnostic setting. A collaboration to further analyze and report these cases was established through GeneMatcher, an online platform that facilitates connections between clinicians and researchers who share an interest in the same gene.[23] Clinical information was collected by reviewing the medical records. The characteristics of these individuals were compared to evaluate if there was a common phenotype.

Ethics statement

Approval to share clinical and genetic information was received from local institutional review boards (including the IRB of CHU Sainte-Justine and Medical Research Ethics Committee of Amsterdam UMC). Informed consent to publish clinical data was obtained from all families. For individuals where pictures are shown, a signed consent for the publication of photographs was obtained.

Microarray analysis

Comparative genomic hybridization (CGH) array and single-nucleotide polymorphism (SNP) array were performed independently at different centers. CGH array was performed on an Agilent 180K oligoarray in individual 1 and her parents and on an Agilent 105K oligoarray in individual 2 and her parents. CGH array was performed in individual 3 and his parents on an Oxford Gene Technology (OGT) 180K oligonucleotide platform. For individual 4 and his parents, SNP array was performed using an Illumina HumanCytoSNP-12 (v2.1) BeadChip. Illumina CytoSNP-850k SNP array was performed in individual 5 and her father (individual 6), mother, and brother. For individual 7, an oligo-SNP array was performed with Affymetrix CytoScan HD. SNP array with Illumina CytoSNP-12 (v2.1) was performed for individual 8, with parental microarrays performed on an Illumina Infinium Global Screening Array-24 (v2.0) kit.

Exome sequencing

Individual 9 had a commercial Autism/ID Xpanded Panel based on exome capture done at GeneDx lab. This panel uses a trio approach and includes more than 2300 genes associated with autism spectrum disorder and/or ID. Individual 10 had proband-only exome sequencing performed through GeneDx. Individual 11 was enrolled through an IRB-approved research exome sequencing protocol. The process for variant filtering and variant prioritization has been previously described.[24,25] Trio-based exome sequencing was completed with clinical confirmation by Sanger sequencing of the *JARID2* variant. Individual 12 underwent trio-based exome sequencing as part of a research study (CAUSES Study, approved by University

of British Columbia [REB#H15-00092]). Sequencing was performed at Ambry Genetics on an Illumina platform and analysis was performed by the research team at University of British Columbia. Individual 13 had solo exome sequencing performed with an in-house pipeline.[26] Parental inheritance was assessed through Sanger sequencing. Individual 14 had trio exome sequencing performed clinically at the Children's Hospital of Philadelphia. Exons were captured with the Agilent SureSelect XT Clinical Research Exome Version 1 kit (per manufacturer's protocol) and sequenced on the Illumina HiSeq 2500 platform. Sequencing data were processed using an in-house custom-built bioinformatics pipeline.[27–29] Individual 15 had a clinical diagnostic exome done with an in-house protocol[30] and her parents were assessed only for the variants identified. Individual 16 also had a clinical exome performed with the same protocol as individual 15.[30]

RESULTS

Clinical characteristics

We identified 4 females and 12 males with a median age of 9.5 years old (range 3.2 to 39 years) with a deletion or single-nucleotide variant in *JARID2*.

Development and behavior

All individuals have various degrees of developmental delay. Mild to moderate ID was diagnosed in 11/15 (73%) of them. Three individuals had borderline intellectual functioning and one had learning difficulties. Features of autism are noted in more than half of the cohort (9/16 [56%]) and a formal diagnosis of autism spectrum disorder was established in three of these individuals. Behavior abnormalities are present in 7/16 individuals (44%) and include an aggressive demeanor, tendency to obsessive/compulsive and perseverative behavior, attention deficit–hyperactivity disorder (ADHD), and trouble with socialization. Rare manifestations that are only observed in one individual include phonic processing disorder, speech sound disorder, motor dyspraxia, severe stutter, and developmental coordination disorder. One individual also presents two psychotic episodes at the age of 16 years (Table 1, Supplementary table 3).

Neurologic manifestations

Gait disturbance in individuals with *JARID2*-plus deletions was reported in the past by Baroy et al.[20] and Di Benedetto et al.[22] but we only identified one individual with a clumsy gait and frequent tripping in our cohort. Hypotonia is found in 5/16 individuals (31%) and only one individual has bradykinesia and bradyphrenia. We identified epilepsy in 3/16 individuals (19%) of the cohort. One individual developed acute epileptic encephalopathy at around age 2 years. Another individual has refractory focal epilepsy and absences. The third individual had epilepsy that resolved at 3 years of age. Nine individuals have been evaluated by brain magnetic resonance image (MRI) or computed tomography (CT) scan. Four individuals have various constitutional anomalies, including benign external hydrocephalus, posterior fossa cyst/mega cisterna magna, periventricular hyperintensities, and arachnoid cyst, but no consistent finding is observed (Table 1, Supplementary table 3).

Dysmorphism

Dysmorphic facial features are observed in 15/16 individuals (94%) (Fig. 1, Supplementary table 3). Dysmorphisms that are observed in more than two individuals are presented in Table 1. The most common features are a high anterior hairline and deep set eyes (6/16 individuals [38%]). Full lips are found in 5/16 (31%) individuals and a broad forehead, infraorbital dark circles, bulbous nasal tip, or depressed nasal bridge in 4/16 individuals (25%). Other less frequently identified dysmorphisms include prominent supraorbital ridges, midface hypoplasia, and a short philtrum (3/6 individuals [19%]). Abnormalities involving hands or feet are found in 5/16 individuals (31%) and include pes planus, clinodactyly of the 4th and 5th toes, persistent fetal pads, single palmar crease, camptodactyly of the 5th digit, syndactyly of the 2nd and 3rd toes, and tapering of the fingers.

Other

Several individuals have had perinatal complications, such as neonatal hyperbilirubinemia (three individuals) and neonatal feeding problems (two individuals). There are five individuals that have a tall stature and four individuals are overweight. One individual has microcephaly, while two have macrocephaly. Only one individual has a cardiac anomaly (tricuspid regurgitation). Musculoskeletal anomalies are observed in five individuals: three individuals have joint hyperlaxity, one has scoliosis, and one has congenital torticollis. Dental anomalies are seen in two individuals: one had hypodontia and the other prominent upper central incisors and irregularly spaced teeth. One individual has a bifid uvula and a submucous cleft palate. Cutaneous findings are inconsistent throughout the cohort. One individual has café au lait macules, one has acanthosis nigricans in the neck and axillae (secondary to obesity) with hirsutism, and another has a patch of prominent capillaries on the upper back. Refractory errors and strabismus are noted in four individuals. There are no individuals with hearing impairment or inner ear anomalies (Table 1, Supplementary table 3).

Genetic variants

Deletions

Microarray analysis revealed whole or partial deletions of *JARID2* in eight individuals (Figs. 2 and 3, Table 2). All deletions occurred de novo or were inherited from an affected parent, although for two individuals inheritance was not determined. Two de novo deletions were identified that involve only exon 2 of *JARID2* (individuals 1 and 3) and two that involve exon 2 and 3 (individuals 2 and 4). Individual 5 was found to have a 140-kb deletion comprising exons 2–5 of *JARID2*. Her father (individual 6) has a similar deletion, with differences in breakpoints due to inherent measurement uncertainty of the array platform. The error margins of their

Table 1 Clinical summary.

Individual	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	
																	nb male/total (%)
Gender	F	F	M	M	F	M	M	M	M	M	M	M	M	M	F	M	12/16 (75%)
																	Median age (range)
Age (years)	17	19	9	3.5	7	38	4	10	12.5	7.3	23	4	3.2	8	39	10.8	9.5 (3.2–39)
Type variant	Del	Del	Del	Del	Del	Del	Del	Del	FS	NS	FS	NS	SS	Mis	Mis	Mis	
Inheritance	dn	dn	dn	dn	P[a]	NA	NA	dn	dn	NA	dn	dn	dn	dn	dn	dn	
Clinical information																	nb affected/nb assessed (%)
Growth																	
Age at assessment (years)	16	7	9	3.5	7	38	5	9.6	12.6	7.3	23	4	1.4	8	39	10.8	
Height	N	↑	N	N	↑	NA	↑	N	↑	N	N	N	N	↑	N	N	
Weight	N	N	↑	N	N	NA	↑	N	N	N	N	N	N	↑	N	↑	
Head circumference	N	NA	NA	N	N	NA	↑	N	N	↓	NA	N	N	↑	N	N	
Microcephaly	–	NA	NA	–	–	NA	–	–	–	+	NA	–	–	–	–	–	1/12 (8%)
Macrocephaly	–	NA	NA	–	–	NA	+	–	–	–	NA	–	–	+	–	–	2/12 (17%)
Development/behavior																	
Intellectual disability	+	–	+	–	+	+	+	–	+	+	+	+	NA	+	–	+	11/15 (73%)
Developmental delay	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	16/16 (100%)
Behavior abnormalities	–	+	–	–	–	–	+	–	+	+	+	–	–	+	+	–	7/16 (44%)
Autistic features	+	–	+	+	–	–	+	–	+	+	–	+	–	+	+	–	9/16 (56%)
ASD diagnosis	–	–	–	–	–	–	–	–	+	–	–	–	–	+	+	–	3/16 (19%)
Neurologic																	
Hypotonia	–	–	–	+	–	–	+	–	–	+	–	+	+	–	–	–	5/16 (31%)
Gait disturbance	–	–	–	–	–	–	–	–	–	–	–	+	–	–	–	–	1/16 (6%)
Epilepsy	–	–	–	–	–	+	+	–	–	–	+	–	–	–	–	–	3/16 (19%)
MRI abnormalities	NA	NA	NA	–	–	NA	–	–	+	NA	–	+	+	+	NA	NA	4/9 (44%)
Dysmorphisms																	
Broad forehead	–	–	+	–	+	+	–	–	–	–	–	–	+	–	–	–	4/16 (25%)
High anterior hairline	+	+	–	+	+	+	–	–	+	–	–	–	–	–	–	–	6/16 (38%)
Prominent supraorbital ridges	–	–	–	–	+	+	–	–	–	–	+	–	–	–	–	–	3/16 (19%)
Deep set eyes	+	+	–	–	+	+	–	–	–	–	–	+	+	–	–	–	6/16 (38%)
Infraorbital dark circles	+	–	+	–	+	+	–	–	–	–	–	–	–	–	–	–	4/16 (25%)
Midface hypoplasia	–	+	–	–	+	+	–	–	–	–	–	–	–	–	–	–	3/16 (19%)
Depressed nasal bridge	+	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–	4/16 (25%)
Bulbous nasal tip	+	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	4/16 (25%)
Short philtrum	+	+	–	–	–	–	–	–	+	–	–	–	–	–	–	–	3/16 (19%)
Full lips	+	–	–	+	–	–	–	–	+	+	+	–	–	–	–	–	5/16 (31%)
Hand/foot abnormalities	+	–	+	–	+	–	+	–	–	–	–	–	–	–	+	–	5/16 (31%)
Other																	
Cardiac anomalies	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	1/16 (6%)
Musculoskeletal anomalies	–	–	–	+	–	–	–	+	–	–	+	+	+	–	–	–	5/16 (31%)
Dental anomalies	–	–	–	–	+	–	–	+	–	–	–	–	–	–	–	–	2/16 (13%)
Cleft lip/palate	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1/16 (6%)
Eye/vision anomalies	–	–	+	–	–	–	–	–	–	+	–	–	–	+	+	–	4/16 (25%)
Cutaneous anomalies	+	–	–	–	+	–	–	–	+	–	–	–	–	–	–	–	3/16 (19%)
Perinatal complications	–	–	+	+	–	+	–	–	+	NA	–	+	–	+	+	–	7/15 (47%)

nb number, *ASD* autism spectrum disorder, *Del* deletion, *dn* de novo, *F* female, *FS* frameshift, *M* male, *Mis* missense, *MRI* magnetic resonance image, *NA* not available, *NS* nonsense, *p* paternal, *SS* splice site, + yes, – no, ↑ over 2 SD, ↓ under 2 SD, *N* between −2 SD and +2 SD.
[a]Individual 6 is father.

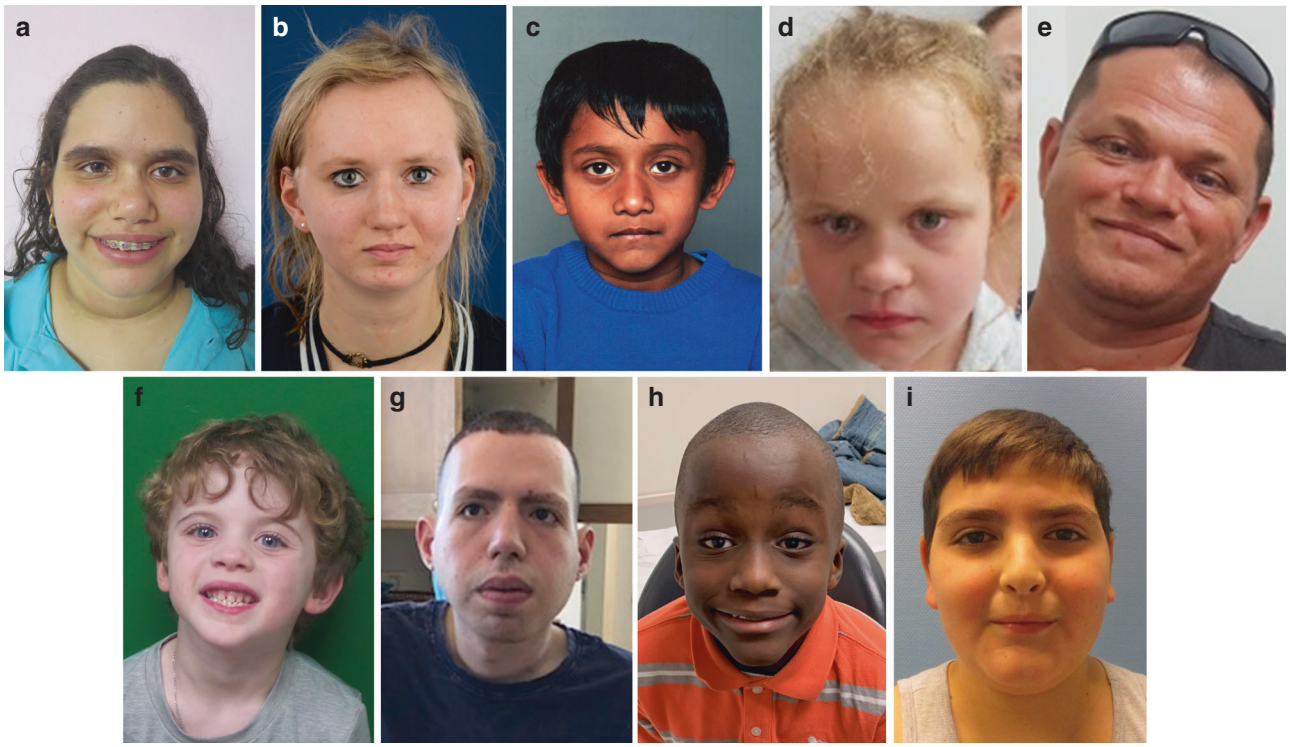

Fig. 1 Facial appearance of patients with JARID2 deletions and single-nucleotide variants. Individual 1 (**a**), individual 2 (**b**), individual 3 (**c**), individual 5 (**d**), individual 6 (who is the father of individual 5) (**e**), individual 7 (**f**), individual 11 (**g**), individual 14 (**h**), and individual 16 (**i**). Some individuals share physical features similar to others in the literature with *JARID2*-plus deletions, including high anterior hairline, broad forehead, deep set eyes, infraorbital dark circles, depressed nasal bridge, bulbous nasal tip and full lips.

breakpoints lie fully within the intronic region. The mother of individual 5 has a normal female microarray profile and the healthy brother of individual 5 has a normal targeted array for the familial deletion. Individual 7 has a deletion that includes exons 1 and 2 of *JARID2*. Individual 8 has a de novo deletion encompassing all of *JARID2* and the distal end of *DTNBP1* (involving the last three exons).

The intragenic *JARID2* deletions are likely to result in a frameshift that will lead to a premature stop codon. The predicted effect would be a loss of normal protein function through nonsense-mediated mRNA decay. Complete deletion of *JARID2*, as identified in one individual, is predicted to be pathogenic.

Single-nucleotide variants

We identified single-nucleotide variants of *JARID2* in eight individuals (Fig. 3 and Table 2). Two de novo frameshift variants were identified (individuals 9 and 11). Two individuals have a nonsense variant, of which one is de novo (individual 12). For the other one inheritance could not be determined because of adoption (individual 10). One individual (individual 13) has a de novo variant c.2731 +1G>C that is predicted to affect splicing since it affects a canonical splice site nucleotide. However, functional testing was not performed. These five variants are predicted to be pathogenic and lead to protein loss of function due to a splicing aberration or nonsense-mediated mRNA decay.

There are three individuals with a de novo missense variant (individuals 14, 15, and 16). The missense variants affect highly conserved residues as shown in Fig. 3. Pathogenicity predictions for missense and splice site variants are shown in Supplementary table 1. Multiple pathogenicity prediction tools classified missense variants as pathogenic; all were considered pathogenic by DANN, FATHMM-MKL, MutationTaster, and SIFT although other tools predicted they were benign. They all had CADD scores above 20 (26.5, 31, and 24.6, respectively), which means they are classified among the top 1% of variants in the genome with respect to pathogenicity probability.

Individuals with a different phenotype or other explanatory variants

We identified two other individuals with deletion or single-nucleotide variation in *JARID2* but they presented with a different phenotype or had other variations that could explain their phenotype. One individual with rhabdomyolysis had a de novo missense variant (c.3362A>G, p.[Asp1121Gly]) in *JARID2*. Another individual with a de novo missense variant in *JARID2* (c.2480G>A, p.[Arg827Gln]) was reported with a phenotype similar to our patients. The individual had ID, global developmental delay, autistic features, hypotonia, pes planus, and delayed myelination on MRI. He also had short stature, dysplastic semicircular canals, cardiac anomalies, feeding and breathing difficulties at birth, and some

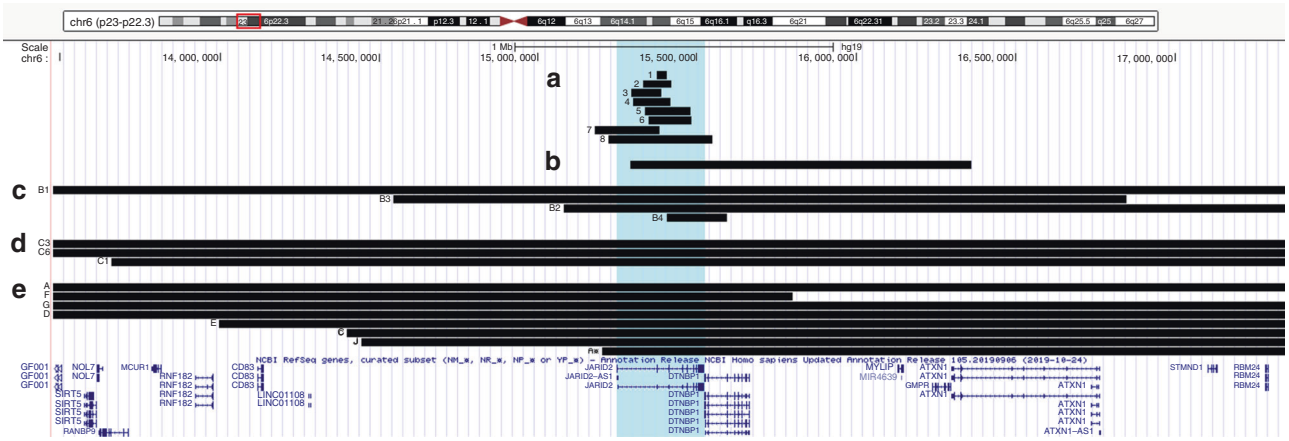

Fig. 2 Localization of deletions reported in this study and previously reported JARID2-plus deletions. Deletions reported in this study (**a**) and *JARID2*-plus deletions previously reported by Di Benedetto et al.[22] (**b**), Baroy et al.[20] (**c**), Celestino-Soper et al.[21] (**d**) and deletions from Swaay et al.[19], Davies et al.,[16] Davies et al.,[17] and Zirn et al.[18] as labeled by Celestino-Soper et al.[21] (**e**). Blue shade covers the *JARID2* gene region. For some of the reported individuals exact breakpoints were not given: the minimal size of the deleted region is presented in this figure. Data were uploaded into UCSC Genome Browser (genome.ucsc.edu).

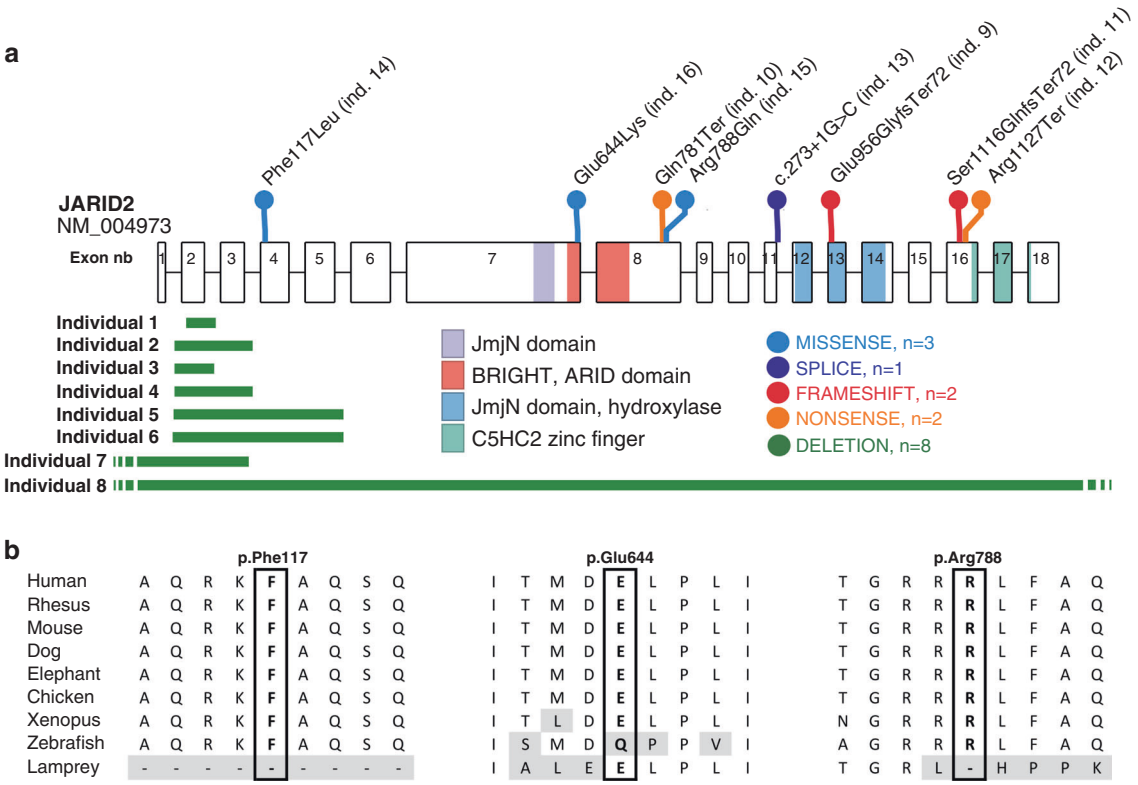

Fig. 3 Graphical overview of the JARID2 single-nucleotide variants and deletions. Location of the single nucleotide variants and deletions (introns not drawn to scale) (**a**) and conservation of the amino acids affected by missense variants (**b**). All variants based on NM_004973.4.

dysmorphisms that were not overlapping those of our patients (telecanthus, epicanthal folds, narrow palpebral fissures, broad nose, and long philtrum). Trio exome sequencing showed another de novo variant in *TLK2* (c.887T>C, p.[Leu296Pro]). This variant was further reclassified as likely pathogenic by the diagnostic laboratory and is currently the main candidate to explain the clinical phenotype. We are uncertain if the *JARID2* variant contributes to or exacerbates the phenotype, so we did not include this individual in our previous analyses. Bioinformatic predictions of these variants, as well as variants that were previously reported in the literature, are presented in Supplementary table 2.

Table 2 Variant summary.

Individual	Variant	Inheritance	Type	Position (Hg19)	Size (Mb)	Exon/intron
1	arr[GRCh37] 6p22.3(15374392_15405436)x1	De novo	Deletion	15374392–15405436	0.03	Exon 2
2	arr[GRCh37] 6p22.3(15330889_15419256)x1	De novo	Deletion	15330889–15419256	0.09	Exons 2–3
3	arr[GRCh37] 6p22.3(15291644_15388348)x1	De novo	Deletion	15291644–15388348	0.1	Exon 2
4	arr[GRCh37] 6p22.3(15298601_15417235)x1	De novo	Deletion	15298601–15417235	0.12	Exons 2–3
5	arr[GRCh37] 6p22.3(15334789_15479224)x1	Paternal (individual 6 is father)	Deletion	15334789–15479224	0.14	Exons 2–5
6	arr[GRCh37] 6p22.3(15346717_15481262)x1	Unknown	Deletion	15346717–15481262	0.14	Exons 2–5
7[a]	arr[GRCh37] 6p23p22.3(15177338_15382780)x1	Unknown	Deletion	15177338–15382780	0.205	Exons 1–2
8	arr[GRCh37] 6p22.3(15222515_15547476)x1	De novo	Deletion	15222515–15547476	0.32	All (exons 1–18)
9[b]	c.2866dupG, p.(Glu956GlyfsTer72)	De novo	Frameshift	15511547		Exon 13
10[c]	c.2341C>T, p.(Gln781Ter)	Unknown	Nonsense	15501533		Exon 8
11	c.3344dupG, p.(Ser1116GlnfsTer71)	De novo	Frameshift	15513546		Exon 16
12[d]	c.3379C>T, p.(Arg1127Ter)	De novo	Nonsense	15513582		Exon 16
13	c.2731+1G>C	De novo	Splice site	15507648		Intron 11
14	c.351T>G, p.(Phe117Leu)	De novo	Missense	15452264		Exon 4
15[e]	c.2363G>A, p.(Arg788Gln)	De novo	Missense	15501555		Exon 8
16	c.1930G>A, p.(Glu644Lys)	De novo	Missense	15497386		Exon 7

All variants based on NM_004973.4.
[a]Individual also has a maternally inherited pathogenic *PKP2* variant (associated with arrhythmogenic right ventricular cardiomyopathy [ARVC]).
[b]Individual also has a likely benign duplication of chromosome 15q13.3 (0.432 Mb).
[c]Individual also has a c.5046 G>C, p.(Leu1682Phe) variant in *ZNF292*.
[d]Individual also has compound heterozygous likely pathogenic variants in *MRFP* and a maternally inherited variant of unknown significance in *ZNF711* (c.1199G>A, p.[Arg400Lys]).
[e]Individual also has two de novo *TNRC18* variants: c.2291A>T, p.(His764Leu) and del(7)(p22.1p22.1).

DISCUSSION

We describe 16 individuals from 15 families with a deletion or single-nucleotide variant of *JARID2*. All individuals described in this paper have developmental delay and the majority have ID. The four individuals without ID however have borderline intellectual functioning and/or learning difficulties. Other common characteristics include hypotonia, autistic features, and behavior abnormalities, especially aggressive behavior. In some patients, we report similar physical features to previously reported cases in the literature with *JARID2*-plus deletions, including high anterior hairline, broad forehead, deep set eyes, infraorbital dark circles, depressed nasal bridge, bulbous nasal tip, and full lips. Patients with deletions tend to have more overlapping facial features than individuals with missense variants. This may be because missense variants cause a more moderate loss-of-function effect on JARID2. Our cohort is not large enough to determine if this trend is significant.

The identified *JARID2* deletions are predicted to lead to a loss of normal protein function, as well as the frameshift, nonsense, and splice site variants that were detected. Hence, these cases confirm the hypothesis by Barøy et al. that it is *JARID2* haploinsufficiency that leads to a clinically distinct neurodevelopmental syndrome.

It is noteworthy that in one case (individual 5) the *JARID2* deletion was inherited from an affected parent (individual 6). As some individuals only have a mild developmental delay or borderline intellectual functioning, we expect further patients to be identified with a pathogenic *JARID2* variant inherited from a mildly affected parent. There are no segmental duplications within *JARID2* that could explain the potentially recurrent breakpoint within intron 1, but there are Alu sequences that could potentially mediate Alu/Alu recombination.

Thus far, there has only been one other report of de novo intragenic *JARID2* deletions. That study described five individuals with ID and de novo intragenic *JARID2* deletions (as well as two duplications), all of them involving only exon 6 (exon 5 in NM_004973.4, 177 nucleotides).[31] Further in silico investigation showed that heterozygous loss or gain of *JARID2* exon 6 does not predict a frameshift and is likely to be tolerated. Additionally, they found a high frequency (>14%) of *JARID2* exon 6 copy-number variants (CNVs) in control populations.[32] The authors therefore concluded that these CNVs are unlikely to be causative for ID, although they might have a contributory effect. The *JARID2* deletions in our patients, however, were predicted to lead to a frameshift and no comparable losses were found in control populations reported in the Database of Genomic Variants (DGV, http://dgv.tcag.ca, accessed 26 May 2020).

The DGV contains only one individual with a *JARID2*-plus deletion (deletion of exons 7–18 of *JARID2* and a partial deletion of the adjacent gene *DTNBP1*).[33] This partial deletion of *JARID2* and *DTNBP1* is similar to the deletion identified in one of the patients reported by Baroy et al. (Fig. **2**, B4)[20] who had an IQ of 74. Possibly, healthy population databases might contain data on people with borderline intellectual functioning. One other exonic deletion is reported in the DGV that encompasses only exon 1 of *JARID2*.[34] There are no deletions that involve all of *JARID2* in the DGV. Finally, a small deletion (133 kb) involving the first three exons of *JARID2* was previously reported in an individual with isolated talipes equinovarus and his unaffected father who were reported to be cognitively normal and without a history of developmental delay (Gurnett, personal communication).[35]

Interestingly, there is one previous report of an individual with a de novo probably pathogenic missense variant in *JARID2* (c.2255C>T, p.[Pro752Leu]) from a cohort of 92 patients with syndromic ID.[36] Although no pathogenic *JARID2* single-nucleotide variants were described previously, pathogenic variants in other members of the JmjC domain–containing family of proteins have been associated with human diseases, including neurodevelopmental disorders.[37–40] Because JARID2 bears most resemblance to JARID1 proteins, pathogenic variants in *KDM5C* (*JARID1C*, OMIM 314690), associated with X-linked ID (OMIM 300534), and pathogenic variants in *KDM5B* (*JARID1B*, OMIM 605393), causing a form of autosomal recessive ID (OMIM 618109), are of most interest. In addition to the JmjC domain, these JARID1 proteins contain a Jumonji N (JmjN) domain, AT rich interaction domain (ARID), and a zinc finger (ZF) as well.[6]

Furthermore, expected and observed counts of single-nucleotide changes in gnomAD show that *JARID2* is extremely intolerant to loss-of-function variants (probability of loss of function intolerance [pLI] score 1; observed/expected [o/e] ratio 0.09, 90% confidence interval [CI]: 0.05–0.19). Also, fewer missense variants are observed than expected (o/e ratio 0.73 [90% CI: 0.68–0.78] with a *Z*-score of 2.69) (https://gnomad.broadinstitute.org/, accessed 20 May 2020). Regarding further bioinformatic analysis of *JARID2* as a dominant disease gene, the %HI score (from DECIPHER) is 12.14%. High %HI ranks (e.g., 0–10%) indicate a gene is more likely to exhibit haploinsufficiency. The *JARID2* P(AD) score is 0.996 (from DOMINO, wwwfbm.unil.ch/domino, accessed 20 May 2020). A P(AD) score of ≥0.95 is highly associated with autosomal dominant inheritance through haploinsufficiency, gain-of-function, or dominant-negative effects.[41]

Conclusion

We propose that *JARID2* should be considered as a critical gene in the 6p22–p24 region with haploinsufficiency resulting in developmental delay and/or borderline intellectual functioning to severe intellectual disability. In addition to *JARID2* deletions, loss-of-function single-nucleotide variants in this gene result in a similar neurodevelopmental syndrome. Currently, there are only three tests available in the Genetic Testing Registry that offer *JARID2* sequencing (https://www.ncbi.nlm.nih.gov/gtr/all/tests/?term=jarid2, accessed 14 May 2020). Our data provide further evidence for establishing gene–disease validity for the purpose of diagnostic reporting and we suggest adding *JARID2* to ID gene panels.

In summary, we propose that haploinsufficiency of *JARID2* be considered as a new, clinically distinct neurodevelopmental syndrome.

SUPPLEMENTARY INFORMATION

The online version of this article (https://doi.org/10.1038/s41436-020-00992-z) contains supplementary material, which is available to authorized users.

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DISCLOSURE

The authors declare no conflicts of interest.

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[1]Department of Clin Genet, Amsterdam UMC, Amsterdam Reproduction and Development Research Institute, University of Amsterdam, Amsterdam, The Netherlands; [2]Department of Clin Genet, Liverpool Hospital, Sydney, Australia; [3]Department of Pediatrics, University of Montreal, Montreal, QC, Canada; [4]Department of Pediatrics, Dr. Horacio E. Oduber Hospital, Oranjestad, Aruba; [5]Section of Genetics and Metabolism, Department of Pediatrics, University of Arkansas for Medical Sciences, Little Rock, AR, USA; [6]Division of Genomic Diagnostics, Children's Hospital of Philadelphia, Philadelphia, PA, USA; [7]Roberts Individualized Medical Genetics Center and the Division of Hum Genet, Children's Hospital of Philadelphia, Philadelphia, PA, USA; [8]Department of Laboratory Medicine and Genetics, Trillium Health Partners, Mississauga, ON, Canada; [9]Department of Laboratory Medicine and Pathobiology, University of Toronto, Toronto, ON, Canada; [10]Department of Genetics, University of Alabama at Birmingham (UAB), Birmingham, AL, USA; [11]Institute for Genomic Medicine, Columbia University Irving Medical Center, New York, NY, USA; [12]Departments of Neurology and Pediatrics, Columbia University Irving Medical Center, New York, NY, USA; [13]Department of Medical Genetics, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada; [14]Department of Pediatrics, Faculty of Medicine, University of British Columbia, Vancouver, BC, Canada; [15]Équipe Génétique des Anomalies du Développement (GAD), CHU Dijon-Bourgogne, Dijon, France; [16]Service de génétique médicale, CHU de Nantes, Nantes, France; [17]Department of Pediatrics, UTHealth McGovern Medical School, Houston, TX, USA; [18]Victorian Clin Genet Services, Murdoch Children's Research Institute, Melbourne, VIC, Australia; [19]School of Medicine, Griffith University, Gold Coast, QLD, Australia; [20]Department of Paediatrics, University of Melbourne, Melbourne, VIC, Australia; [21]Divisions of Newborn Medicine and Genetics & Genomics, Department of Pediatrics, Manton Center for Orphan Disease Research, Boston Children's Hospital, Boston, MA, USA; [22]Department of Medical Genetics, University of Antwerp, Antwerp, Belgium; [23]Department of Medical Genetics, University Hospital Antwerp, Antwerp, Belgium; [24]Department of Hum Genet, Radboud Institute for Molecular Life Sciences and Donders Institute for Brain, Cognition and Behaviour, Radboud university medical center, Nijmegen, The Netherlands; [25]Department of Clin Genet, Karolinska University Hospital, Stockholm, Sweden; [26]Department of molecular medicine and surgery, Karolinska Institutet, Stockholm, Sweden; [27]Département de Médecine, Faculté de médecine, Université de Montréal, and Centre de Recherche de l'Hôpital Maisonneuve-Rosemont, Montreal, QC, Canada.