

Haploinsufficiency of *ARID1B*, a Member of the SWI/SNF-A Chromatin-Remodeling Complex, Is a Frequent Cause of Intellectual Disability

Juliane Hoyer,^{1,8} Arif B. Ekici,^{1,8} Sabine Endeke,^{1,8} Bernt Popp,¹ Christiane Zweier,¹ Antje Wiesener,¹ Eva Wohlleber,² Andreas Dufke,³ Eva Rossier,³ Corinna Petsch,¹ Markus Zweier,¹ Ina Göhring,¹ Alexander M. Zink,² Gudrun Rappold,⁴ Evelin Schröck,⁵ Dagmar Wiczorek,⁶ Olaf Riess,³ Hartmut Engels,² Anita Rauch,^{1,7} and André Reis^{1,*}

Intellectual disability (ID) is a clinically and genetically heterogeneous common condition that remains etiologically unresolved in the majority of cases. Although several hundred diseased genes have been identified in X-linked, autosomal-recessive, or syndromic types of ID, the establishment of an etiological basis remains a difficult task in unspecific, sporadic cases. Just recently, *de novo* mutations in *SYNGAP1*, *STXBPI*, *MEF2C*, and *GRIN2B* were reported as relatively common causes of ID in such individuals. On the basis of a patient with severe ID and a 2.5 Mb microdeletion including *ARID1B* in chromosomal region 6q25, we performed mutational analysis in 887 unselected patients with unexplained ID. In this cohort, we found eight (0.9%) additional *de novo* nonsense or frameshift mutations predicted to cause haploinsufficiency. Our findings indicate that haploinsufficiency of *ARID1B*, a member of the SWI/SNF-A chromatin-remodeling complex, is a common cause of ID, and they add to the growing evidence that chromatin-remodeling defects are an important contributor to neurodevelopmental disorders.

Intellectual disability (ID) is a severely incapacitating condition that imposes a significant burden on affected individuals and their families. The incidence is estimated at 2%–3%, and severe forms (intelligence quotient [IQ] < 50) account for about 0.5% of all newborns. It is now accepted, at least in developed countries, that the vast majority of cases are of genetic origin.¹ Some 10% of affected boys are estimated to have an X-linked condition, and in about 80% of X-linked families, the underlying genetic defect has now been uncovered through systematic sequencing of all X-chromosomal coding segments.^{1,2} Autosomal-recessive forms are amenable to positional cloning in consanguineous families, and this strategy has recently led to the identification of an important number of genes harboring recessive mutations.^{3,4} Investigating sporadic cases from nonconsanguineous couples is more difficult, but the discovery of several genes involved has been enabled by either synaptic candidate-gene approaches or the *de novo* occurrence of copy-number variants (CNVs) or chromosomal translocations.^{5–8} Whole-exome sequencing in 10 and 20 trios confirmed the power of this technique to identify ID-associated genes, and several studies proposed that many sporadic cases might arise from *de novo* mutations.^{9,10} Despite important advances in identifying the underlying genes, some 50% of all cases and the vast majority of unspecific patients remain undiagnosed.¹¹ This is probably a result of the enormous locus heterogeneity because most

genes with autosomal-dominant mutations only account for a few cases each.

The German Mental Retardation Network (MRNET) aims to systematically uncover the genetic basis of ID. Over several years, we recruited from eight different medical-genetics centers a large study group of affected individuals mostly of German origin. The study was approved by all institutional review boards of the participating institutions, and written informed consent was obtained from all participants or their legal guardians. We screened 1,986 of the individuals with array-based molecular karyotyping by using high-resolution platforms. In one male infant, we identified a 2.5 Mb deletion containing five genes in chromosomal region 6q25.3 (Figure 1) by using the high-resolution Genome-Wide Human SNP Array 6.0 (Affymetrix, Santa Clara, California) and the Affymetrix Genotyping Console Software (version 3.0.2). Segregation analysis of both parents revealed a *de novo* origin of the deletion. The familial relationship was confirmed. Because the phenotype of our patient resembled that of the patients with larger deletions,¹² we hypothesized that one of the five deleted genes would show haploinsufficiency and would represent a phenocritical gene responsible for the ID.

Using Sanger sequencing, we screened all five genes from this region, including *TFB1M* (MIM 607033), *NOX3* (MIM 607105), and three brain-expressed genes, *TIAM2* (MIM 604709), *CLDN20*, and *ARID1B*, for point mutations in

¹Institute of Human Genetics, University of Erlangen-Nuremberg, Erlangen 91054, Germany; ²Institute of Human Genetics, Rheinische Friedrich-Wilhelms-University, Bonn 53105, Germany; ³Institute of Human Genetics, University of Tuebingen, Tuebingen 72076, Germany; ⁴Department of Human Molecular Genetics, University of Heidelberg, Heidelberg 69120, Germany; ⁵Institut für Klinische Genetik, Technische Universität Dresden, Dresden 01307, Germany; ⁶Institut für Humangenetik, Universitätsklinikum Essen, Essen 45147, Germany; ⁷Institute of Medical Genetics, University of Zurich, Schwerzenbach 8603, Switzerland

⁸These authors contributed equally to this work.

*Correspondence: andre.reis@uk-erlangen.de

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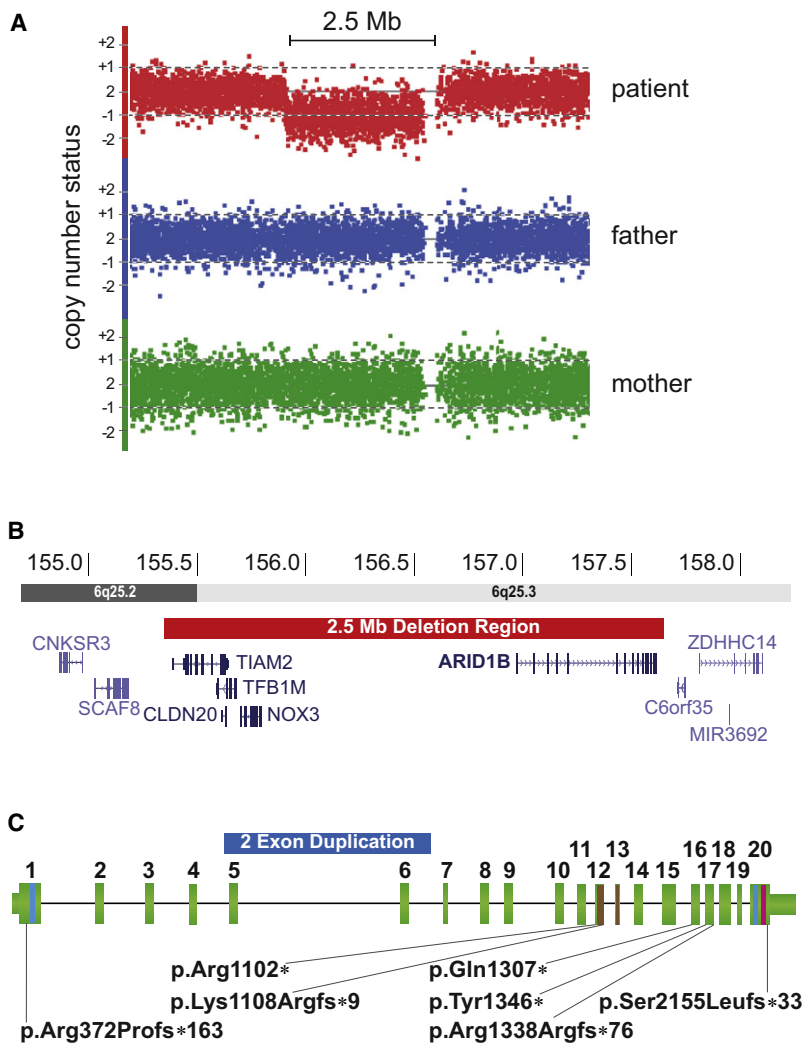


Figure 1. Summary of the Nine De Novo CNVs and Point Mutations Detected in *ARID1B*

(A) De novo deletion in patient 1 detected with molecular karyotyping by the Affymetrix SNP6.0 platform. The signal reduction of 1,568 markers indicating the deletion region is detected only in patient 1 (red dots) and is absent in both parents.

(B) 2.5 Mb deletion (red bar) in chromosomal region 6q25, which includes five RefSeq genes, among them *ARID1B*. Two gray scales are illustrating two different chromosomal bands as indicated in the horizontal bars. Genomic positions are in million bp.

(C) Genomic structure of *ARID1B*. Vertical green bars illustrate the exons with their respective number above. Narrow green bars illustrate the 5' and 3' UTRs. The three known *ARID1B* domains are indicated by colored lines (blue, LXXLL; brown, ARID; and magenta, BC-Box). The duplication in patient 2 is indicated by a blue bar encompassing exons 5 and 6. The localization of mutations in patients 3–9 is indicated by black lines leading to the mutation identifiers.

detected in another patient a 180 kb duplication encompassing exons 5 and 6 (hg18 chr6: 157,299,982–157,474,352). Using the SALSA multiple ligation probe amplification (MLPA) reagents EK5 (MRC-Holland, Amsterdam, The Netherlands) and copy-number calculation with Seqpilot software (JSI Medical Systems, Kippenheim, Germany), we confirmed this mutation by MLPA with customized probes for exons 4, 5, 6, 7, and 8 (Table S3, available online). We studied a common SNP in exon 6 by using cDNA generated from RNA isolated

from fresh blood leukocytes with the PAXgene Blood RNA System (PreAnalytics) in conjunction with the Superscript Reverse Transcriptase Kit (Invitrogen) and random hexamers. This analysis revealed that the duplication arose on the paternal allele and led to monoallelic expression of the wild-type allele (Figure 2A). Analysis of parental DNA confirmed the biological relationships and revealed that all mutations arose de novo. Given that all mutations occurred de novo and were predicted to cause loss of function, we hypothesized that haploinsufficiency of *ARID1B* would underlie the ID in patients with 6q25.3 microdeletion syndrome.

121 individuals with moderate to severe ID without a known genetic cause; these individuals were from the Erlangen subgroup of the German Intellectual Disability Network (MRNET). Microarray analyses for CNVs >200 kb had been previously performed in 82 (68%) of the individuals, and no obvious pathogenic CNV was found. For sequencing, we used Applied Biosystems (ABI) BigDye Terminator chemistry and purification with Agencourt AMPure and CleanSEQ kits (Beckman-Coulter) on an ABI 3730 sequencer with Sequencing Analysis v.3.6.1 (Applied Biosystems) and Sequencher 4.9 (Gene Codes Corporation) software packages. Although no mutation was detected in *TFB1M*, *NOX3*, *TIAM2*, or *CLDN20*, we identified a c.3919C>T (p.Gln1307*) nonsense mutation in exon 16 of *ARID1B* in patient 3 and an 11 bp deletion in exon 20 of *ARID1B* (NM_020732.3) in patient 4; the latter mutation (c.6463_6473del [p.Ser2155Leufs*33]) lead to a frameshift and a premature termination codon after 33 residues. We used original, nonamplified DNA samples for independent PCR and bidirectional sequencing to confirm the mutations. In addition, we reanalyzed the genomic region of *ARID1B* for CNVs <200 kb in this subgroup, and we de-

Given the relatively high frequency of mutations identified in the first subgroup, we extended the mutation screen (consisting of exons 2–20, including flanking intronic regions) to another 766 ID-affected individuals from the MRNET consortium (for a detailed description of the study group, see supplemental data). As a result of its high GC content, we amplified exon 1 with the Fast Amplification Kit (QIAGEN) by using a set of nested PCR fragments with different sequencing primers (Table S4). In this group, we further identified four nonsense mutations and one

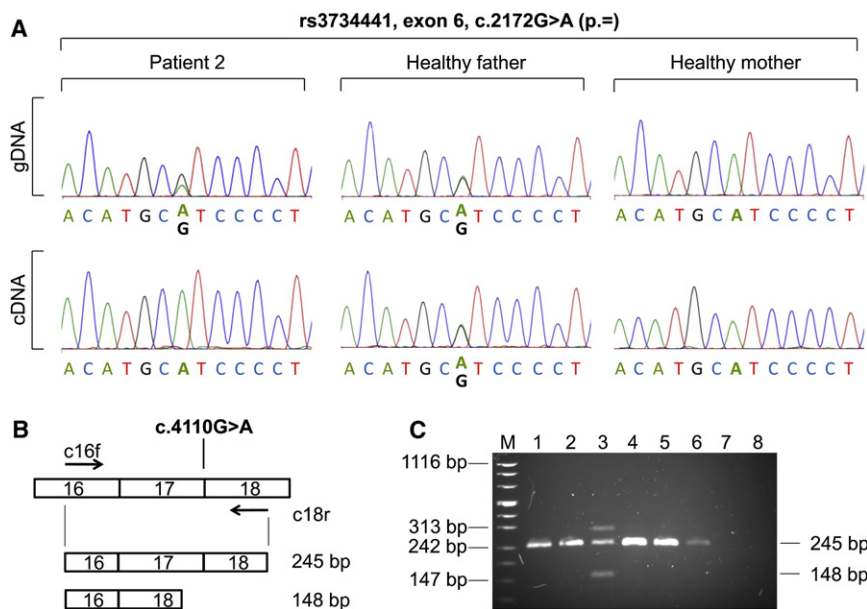


Figure 2. Transcript Analysis for the *ARID1B* Mutations in Patients 2 and 7

(A) Partial sequence electropherograms of *ARID1B* exon 6 obtained from gDNA and cDNA from patient 2 with a de novo duplication of exons 5 and 6 and his healthy parents. Patient 2 and his father are heterozygous for rs3734441 (c.2172G>A on exon 6) at the gDNA level. Note that the amount of guanine is doubled in the patient. At the cDNA level, the father shows biallelic expression, whereas monoallelic expression of adenine in patient 2 indicates that the duplication leads to a null allele. (B and C) Synonymous variant in the last codon of exon 17 induces exon skipping of exon 17 in patient 7. Using primers located in exons 16 and 18 (c16f and c18r, respectively), indicated by arrows for RT-PCR on RNA from peripheral blood leukocytes, resulted in an additional aberrant product of 148 bp in the patient (lane 3), whereas both parents (lanes 1 and 2) and controls (lanes 4–6) showed only the expected 245 bp fragment (lane 7, genomic DNA control; lane 8, no

template control; M, size standard). Exon skipping in the patient was verified by the sequencing of amplified products and is predicted to result in a frameshift and premature stop codon after 76 amino acids (p.Arg1338Argfs*76). In the patient's lane, an additional third band with a molecular size of ~310 bp represents a heteroduplex.

synonymous variant in the last base pair of exon 17; these mutations are predicted to affect the consensus splice donor site (Table 1 and Table S1). The skipping of exon 17 was confirmed by RT-PCR from RNA extracted from the patients' blood (Figures 2B and 2C) and was predicted to cause a frameshift resulting in a premature translational termination (p.Arg1338Argfs*76). All mutations were shown to have occurred de novo, and the parental relationships were confirmed by a forensic set of microsatellites (Promega) in all instances. These mutations confirmed the initial hypothesis of the causative role of *ARID1B* in ID and increased the total number of bona fide mutations to eight (0.9%) in the entire cohort of 887 individuals. In addition, we observed 101 unique variants not annotated in dbSNP (build 132) (Table S1). We investigated segregation in 18 out of 23 cases with missense variants—when parental material was available—and could show segregation from a healthy parent (10 maternal and 8 paternal) in all instances. The remaining five variants are not located in any known domain and were not suspicious when investigated with various prediction programs (Table S2), suggesting that these 23 missense variants are benign. However, we cannot exclude some milder effect on protein function. Our findings thus indicate that *ARID1B*-haploinsufficiency-causing mutations, but not missense variants, are a common cause of ID. This association is further supported by recent reports on microaberrations affecting *ARID1B*. One case with a frameshift intragenic 281 kb deletion affecting the ARID domain was reported in a patient with autism,¹³ a complex chromosomal translocation leading to a fusion gene of *ARID1B* and *MRPP3* was found in an individual with ID,¹⁴ and one translocation, four

larger deletions (including *ARID1B*), and 3 intragenic deletions were reported by Halgren et al.¹⁵ in patients with ID.

The phenotype associated with nonsense and frameshift mutations in *ARID1B* is shown in Figure 3 and summarized in Table 1. All individuals presented with moderate to severe psychomotor retardation, and most showed evidence of muscular hypotonia. In many of the patients, expressive speech was reported to be more severely affected than receptive function. Although no distinct recognizable facial gestalt could be discerned, consistent findings in most of the patients were an abnormal head shape and low-set, posteriorly rotated, and abnormally shaped ears. Even though many other minor anomalies such as down-slanting palpebral fissures, a bulbous nasal tip, a thin upper lip, minor teeth anomalies, and brachydactyly or single palmar creases were observed frequently, gross malformations such as congenital heart defects, structural brain anomalies, or cleft palate were only rarely observed. The majority of patients had short stature of postnatal onset or body height within the lower normal range. With regard to these aspects, the phenotype of patients with point mutations overlaps with that observed in patients with large genomic deletions on chromosome 6q or intragenic deletions within *ARID1B*^{12,15} (such as in patient 1), further confirming that haploinsufficiency of *ARID1B* is indeed responsible for most of the symptoms. However, either autism spectrum disorder or autistic traits were reported in five patients with larger genomic or intragenic deletions^{13,15} but were observed in only one of our patients. Three of our five patients who underwent a cerebral magnetic resonance imaging (MRI) scan had minor

Table 1. Clinical Data from Patients with ARID1B Deletions or Mutations

	Nagamani et al.¹² (N = 4)	Halgren et al.¹⁵ (N = 8)	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
<i>ARID1B</i> defect	deletion of several genes, including <i>ARID1B</i>	one translocation, four larger deletions (including <i>ARID1B</i>), and three intragenic deletions	deletion of five genes (chr6: 155,364,154–157,681,073*)	duplication of exons 5 and 6 (chr6: 157,299,982–157,474,352*)	c.3919C>T (p.Gln1307*)	c.6463_6473del (p.Ser2155 Leufs*33)	c.3304C>T (p.Arg1102*)	c.3323_3324delAA (p.Lys1108 Argfs*9)	c.4110G>A (p.Arg1338 Argfs*76)	c.4038T>A (p.Tyr1346*)	c.1114 dupC (p.Arg372 Profs*163)
Inheritance	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo	de novo
Sex	2 F, 2 M	6 F, 2 M	F	F	M	M	F	F	F	M	M
Age at last follow-up examination	10–33 months	3–46 years	3 years, 3 months	4 years, 11 months	3 years, 5 months	7 years, 3 months	12 years, 8 months	4 years	6 years, 3 months	17 years	20 years
Birth parameters (weight, length, OFC)	3 ≤ 3 rd ct 2 ≤ 5 th ct 3 < 3 rd ct	3/7 ≤ 10 th ct 5/6 ≤ 10 th ct 5 ≤ 10 th ct	50 th ct 3 rd –10 th ct 50 th –75 th ct	25 th ct 50 th ct 25 th ct	50 th ct 25 th ct 25 th ct	50 th ct 50 th ct 50 th ct	25 th ct 25 th ct 50 th ct	3 rd –10 th ct 50 th ct 3 rd –10 th ct	50 th –75 th ct >97 th ct 75 th ct	10 th –50 th ct 50 th ct 10 th –50 th ct	10 th ct 50 th ct 50 th ct
Length and/or height and OFC	2/4 < 3 rd ct 4/4 < 3 rd ct	7/7 ≤ 5 th ct 1/5 < 3 rd ct	10 th ct 10 th –25 th ct	3 rd –10 th ct <3 rd ct	3 rd –10 th ct 50 th ct	<3 rd ct 25 th –50 th ct	<3 rd ct 25 th –50 th ct	10 th –25 th ct 75 th ct	25 th –50 th ct 75 th ct	50 th ct >97 th ct	<3 rd ct <3 rd ct
Developmental delay	4/4	8/8	severe	moderate	severe	moderate IQ = 50 (tested)	severe	mild to moderate	moderate to severe	moderate	moderate
Speech	1/4 spoke two words at 33 months	8/8 were severely impaired or absent	–	first words at 3–4 years and sentences at 4 years, 11 months	single words	–	first words at >5 years and two-word sentences at 12 years	at age of 24 months, corresponding to age of 17 months	single words	short sentences and sufficient working vocabulary	delayed
Age of walking	1/4 at 23 months	1/1 at 30 months	28 months	24 months	20 months	+	27 months	24 months	24 months	18 months	20 months
Muscular hypotonia	2/4	7/7	+	+	+	+	+	+	+	–	–
MRI scan anomaly	2/3 (2 with ACC)	4/5 with ACC or HCC	retrocerebellar cyst	delayed myelination	NA	–	–	NA	asymmetric calvaria	NA	–
Seizures	1/4	3/7	–	–	–	–	+**	–	+**	+	–
Hearing loss	4/4	?	–	–	–	–	+(unilateral)	–	–	–	–
Heart malformation	1/4 (ASD)	?	–	+	–	+(ASD)	–	–	–	–	–

Table 1. Continued

	Nagamani et al.¹² (N = 4)	Halgren et al.¹⁵ (N = 8)	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9
Cleft palate	0/4 with cleft palate and 2/4 with palatal anomalies	?	–	– and high palate	–	+	+	–	– and high palate	–	–
Abnormal shape of head	3/4 with plagiocephaly	5/5 with low hairline	plagiocephaly and frontal bossing	plagiocephaly and frontal bossing	prominent forehead	–	brachycephaly and low forehead	frontal bossing	low forehead	–	brachycephaly
Low-set and/or posteriorly rotated ears	4/4	2/2	+	+	+	–	+	+	+	+	+
Abnormally shaped ears	2/4	?	+	+	+	+	+	–	+	–	+
Downslanting palpebral fissures	2/4	?	+	–	+	+	+	–	–	–	–
Strabism	+	2	+	+	–	+	–	–	–	–	–
Bulbous nasal tip	3/3	3/3	+	+	–	+	+/-	+/-	+	–	–
Thin upper lip	2/4	3/4	–	+	+	+	+	+	–	–	+
Teeth anomalies	?	?	–	small and pointed	pointed	small	first teeth small and widely spaced	–	small	malocclusion and delayed second dentition	NA
Retro/micrognathia	2/3	?	+	+	–	–	–	+	–	+	–
Hand and feet anomalies	1/4 with clinodactyly and 0/4 with single palmar crease	?	–	single palmar creases and brachydactyly V	brachydactyly	single palmar creases, sandal gaps, and hypoplastic nails	sandal gaps, clinodactyly V, and hypoplastic toe nails V	long toes	–	–	single palmar creases, clinodactyly V, deep set thumbs, and Hallux valgus
Other abnormalities	2/4 with retinal anomalies; 1/4 with genitourinary anomaly	5/7 with AuSD or autistic traits, 4 with myopia/hypermotropia, 1 with cataracts, 4 with hypertrichosis, and 5 with feeding problems	clitoris hypertrophy and long philtrum	ataxic gait, sparse hair, sacral dimple, and three hemangiomas	allergy, recurrent infections, autistic features, and aggression	cryptorchism and myopia	allergy, myopia, megaureter, wide mouth, dry hair, and hypothyreosis	hypertrichosis and myopia	hypertrichosis	myopia, skin hypopigmentation, and hypertelorism	unilateral myopia, blocked nasolacrimal duct, dermoid cyst, atlanto/occipital abnormalities, discreet rhizomelic shortening of arms and legs, scoliosis, and cryptorchism

The following abbreviations are used: N, number of patients; F, female; M, male; ct, percentile; OFC, occipital-frontal circumference; IQ, intelligence quotient; MRI, magnetic resonance imaging; +, present; –, absent; NA, not analyzed; ACC, agenesis of corpus callosum; HCC, hypoplastic corpus callosum; ASD, atrial septum defect; AuSD, autism spectrum disorder; post., posteriorly; *, hg18; and **, occurrence of singular seizure.



Figure 3. Facial Appearance of Patients with Deletions or Mutations in *ARID1B*

Note consistently low-set, posteriorly rotated, and abnormally shaped ears and other frequent dysmorphisms such as frontal bossing, downslanting palpebral fissures, a bulbous nasal tip, and a thin upper lip.

- (A and B) Patient 1 at age 3 years, 3 months.
 (C and D) Patient 2 at age 4 years, 11 months.
 (E and F) Patient 3 at age 3 years, 5 months.
 (G) Patient 4 at age 5 months.
 (H and I) Patient 4 at age 7 years, 3 months.
 (J) Patient 5 at age 4 years, 11 months.
 (K and L) Patient 5 at age 12 years, 8 months.
 (M and N) Patient 6 at age 6 years, 3 months.
 (O and P) Patient 7 at age 3 years, 10 months.
 (Q and R) Patient 8 at age 17 years.

unspecific anomalies such as retrocerebellar cysts, delayed myelination, and asymmetric calvaria, but none showed a hypoplastic or aplastic corpus callosum, which was considered a hallmark of the *ARID1B* deficiency by Halgren et al.¹⁵ (it was only reported in patients with larger deletions there). In addition, hearing loss was also more consistently observed in patients with larger deletions¹² than in our patients with point mutations in *ARID1B*. These two aspects might therefore be more related to the contribution of additional genes affected by chromosomal aberrations than to haploinsufficiency of *ARID1B* itself.

ARID1B is highly expressed in the brain and in embryonic stem cells and encodes AT-rich interactive domain-containing protein 1B, also known as BAF250b, the largest subunit of the mammalian SWI/SNF-A chromatin-remodeling complex. This complex facilitates DNA access with

the use of transcription factors and the transcription machinery.¹⁶ BAF250b has a DNA-binding domain known as ARID (AT-rich interaction domain) and is thought to target the complex to specific genes.¹⁷ BAF250b and its ortholog BAF250a (encoded by *ARID1A* [MIM 603024]) associate with E2F transcription factors and play important roles in cell-cycle control.¹⁸ Recently, it has been shown that BAF250b is also part of an E3-ubiquitin-ligase complex targeting histone H2B at lysine 120 for monoubiquitination in vitro.¹⁶ Histone H2B ubiquitination has been shown to be required for transcriptional activation in vitro¹⁹ and associates with transcriptionally active genes in vivo.^{20,21} BAF250b interacts with Elongin B/C through its B/C box and with Cullin 2 (CUL2 [MIM 603135]) through both the ARID1B and B/C boxes and assembles the complex in a manner similar to that of the

well-characterized Von Hippel-Lindau (VHL) complex, which targets the hypoxia inducible factor HIF1 α (MIM 603348).¹⁶

We conclude that *ARID1B* haploinsufficient mutations are a relatively frequent cause of moderate to severe ID, and our findings add to the growing evidence of a role of altered chromatin remodeling in the pathogenesis of ID.^{4,22–24}

Supplemental Data

Supplemental Data include a description of the study group, four tables, and supplementary references and can be found with this article online at <http://www.cell.com/AJHG>.

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Web Resources

The URLs for data presented herein are as follows:

Berkeley *Drosophila* Genome Project (BDGP), <http://www.fruitfly.org/>

Human Splicing Finder (HSF), <http://www.umd.be/HSF/>

NetGene2 server, <http://www.cbs.dtu.dk/services/NetGene2/>

Online Mendelian Inheritance in Man (OMIM), <http://www.omim.org>

PANTHER 7.0, <http://www.pantherdb.org/>

PolyPhen-2, <http://genetics.bwh.harvard.edu/pph2/>

SIFT, <http://sift.jcvi.org/>

SNAP, <http://www.roslab.org/services/SNAP/>

SpliceView, http://zeus2.itb.cnr.it/~webgene/wwwspliceview_ex.html

UCSC Genome Browser, <http://genome.ucsc.edu/>

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