BICRA, a SWI/SNF Complex Member, Is Associated with BAF-Disorder Related Phenotypes in Humans and Model Organisms

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Summary

SWI/SNF-related intellectual disability disorders (SSRIDDs) are rare neurodevelopmental disorders characterized by developmental disability, coarse facial features, and fifth digit/nail hypoplasia that are caused by pathogenic variants in genes that encode for members of the SWI/SNF (or BAF) family of chromatin remodeling complexes. We have identified 12 individuals with rare variants (10 loss-of-function, 2 missense) in the *BICRA* (*BRD4 interacting chromatin remodeling complex-associated protein*) gene, also known as *GLTSCR1*, which encodes a subunit of the non-canonical BAF (ncBAF) complex. These individuals exhibited neurodevelopmental phenotypes that include developmental delay, intellectual disability, autism spectrum disorder, and behavioral abnormalities as well as dysmorphic features. Notably, the majority of individuals lack the fifth digit/nail hypoplasia phenotype, a hallmark of most SSRIDDs. To confirm the role of BICRA in the development of these phenotypes, we performed functional characterization of the zebrafish and *Drosophila* orthologs of *BICRA*. In zebrafish, a mutation of *bicra* that mimics one of the loss-of-function variants leads to craniofacial defects possibly akin to the dysmorphic facial features seen in individuals harboring putatively pathogenic *BICRA* variants. We further show that Bicra physically binds to other non-canonical ncBAF complex members, including the BRD9/7 ortholog, CG7154, and is the defining member of the ncBAF complex in flies. Like other SWI/SNF complex members, loss of *Bicra* function in flies acts as a dominant enhancer of position effect variegation but in a more context-specific manner. We conclude that haploinsufficiency of *BICRA* leads to a unique SSRIDD in humans whose phenotypes overlap with those previously reported.

Introduction

Chromatin structure plays a key role in the regulation of gene expression throughout development by controlling the accessibility of regulatory sequences.[1–5] Genetic variants in genes that encode chromatin modifying proteins have been shown to cause neurological disorders such as Coffin-Siris syndrome (CSS [MIM: 135900]),

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Nicolaides-Baraitser syndrome (MIM: 601358), Weaver syndrome (MIM: 277590), and autism (MIM: 209850) as well as to play a role in various types of cancer.[6–11] Many chromatin-modifying proteins operate in large complexes such as the Switch/Sucrose Non-Fermentable (SWI/SNF) complex first identified in yeast[12] and the Polycomb Repressive Complex (PRC), which was originally identified in flies.[13] Although a number of chromatin-modifying genes have been shown to cause human diseases,[14–18] many genes that encode components of these chromatin-modifying complexes have yet to be associated with a genetic disorder.

SWI/SNF-related intellectual disability disorders (SSRIDDs) are rare neurodevelopmental disorders characterized by developmental delay (DD), intellectual disability (ID), coarse facial features, and hypoplasia of the fifth digit.[19,20] Pathogenic variants in genes that encode several members of the SWI/SNF complex have been shown to cause SSRIDDs. These genes include *SMARCA4* (MIM: 603254), *SMARCB1* (MIM: 601607), *SMARCC2* (MIM: 601734), *SMARCD1* (MIM: 601735), *SMARCE1* (MIM: 603111), *DPF2* (MIM: 601671), *ARID1A* (MIM: 603024), *ARID1B* (MIM: 614556), and *ARID2* (MIM: 609539).[6,16,17,19,21–23] Variants in *SMARCA2* (MIM: 600014), another member of the SWI/SNF complex, cause a related disorder called Nicolaides-Baraitser syndrome.[14]

The SWI/SNF complex alters the position of nucleosomes along DNA strands to regulate the accessibility of regulatory elements to transcription factors that control gene expression.[24,25] The SWI/SNF complex has long been divided into two sub-complexes—the BRG1/BRM-Associated Factor (BAF) complex and Polybromo BRG1/BRM Associated Factors (PBAF) complex—based upon their composition.[26–30] Recently, a new version of the complex called the non-canonical BAF (ncBAF) complex has been identified.[31,32] Currently, no phenotypic studies have been conducted in humans, mice, or zebrafish on ncBAF-specific genes.

One of the critical proteins that defines the ncBAF complex is BICRA (MIM: 605690) (previously known as *GLTSCR1* [*Glioma tumor suppressor candidate region gene 1*]).[31,32] In humans, sequence and copy number variants (CNVs) affecting *BICRA* have been identified in a variety of cancers, particularly oligodendrogliomas.[33–37] However, nothing is known about *BICRA*'s role in development or in the function of the nervous system.[35] Hence, understanding the function of *BICRA* may help us unravel the function of the ncBAF complex *in vivo*.

Here we present molecular and clinical data from 12 individuals with putatively pathogenic variants (10 loss-of-function, 2 missense) in *BICRA* whose symptoms are similar to those seen in individuals with other SSRIDDs. These individuals exhibit moderate developmental delay (particularly of speech and motor skills), mild to moderate intellectual disability, and dysmorphic facial features, but lack fifth digit/nail hypoplasia. We also present data from *in vivo* studies of *BICRA* and the ncBAF complex in zebrafish and *Drosophila*. In zebrafish, a mutation of *bicra* that mimics one of the individuals' loss-of-function variants causes craniofacial defects, providing a possible link with the dysmorphic facial features seen in our *BICRA* cohort. We show that the *BICRA* fly ortholog, *Bicra*, is expressed in neurons and glia in the larval and adult brain. In S2 cells, Bicra binds to other SWI/SNF complex members. Haploinsufficiency of fly *Bicra* causes an enhancement of position effect variegation at telomeres like members of the BAF and PBAF complexes, but not at the *white (w)* locus. We conclude that fly Bicra is a SWI/SNF complex member, like its human ortholog, and that haploinsufficiency of *BICRA* leads to a unique neurodevelopmental BAFopathy in humans whose phenotype overlaps with those seen in other SSRIDDs.

Subjects and Methods

Contact for Reagent and Resource Sharing

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Hugo J. Bellen (hbellen@bcm.edu).

Subject Recruitment

Genetic investigations occurred in a clinical setting. Consent for publication was obtained from parents of all subjects, and procedures were followed in accordance with guidelines specified by Institutional Review Boards and Ethnic Committees of the respective institutions. Detailed case reports are provided in the Supplemental Note.

Next Generation Sequencing of Affected Individuals

Trio exome sequencing (ES) and variant filtering was done as follows. After informed consent, we collected blood samples from the probands and their parents and extracted DNA using standard procedures. To investigate the genetic cause of the disease, ES was performed in the affected proband. Exome data are summarized in Table 1 and in the Supplemental Note. Across the performing labs, the minimum average depth of coverage was 100× across assays, and minimum proportion of the target at >10× coverage was 95%. Sequence alignment to the human reference genome (UCSC hg19), variants calling, and annotation were performed as described elsewhere.[38] After removing all synonymous changes, we filtered single-nucleotide variants (SNVs) and indels, only considering exonic and donor/acceptor splicing variants. In accordance with the pedigree and phenotype, priority was given to rare variants (<1% in public databases, including 1000 Genomes project, NHLBI Exome Variant Server, Complete Genomics 69, and Genome Aggregation Database [gnomAD v.2.1.1]) that fit a *de novo* model.

Drosophila melanogaster

The following fly lines were used: *Mi{ET1}Bicra*^{MB00611}*/TM3* (BDSC: 22851), *Mi{MIC}Bicra*^{MI14493}*/TM3* (BDSC: 59523), *Mi{MIC}Bicra*^{MI03400} (BDSC: 37342), *Mi{PT-GFSTF.1}Bicra*$^{MI03400\text{-}GFSTF.1}$ (BDSC: 61768), *In(1)w*$^{m4\ h}$*; hup*^{1}*/CyO* (BDSC: 9576), *CH321-05L16 (Bicra Genomic Rescue [GR]*, Genetivision), *Df(3R)BSC806* (BDSC: 27378), *y w; P{hsp26-pt-T}39C-5* (BDSC: 44259) *y w; P{hsp26-pt-T}39C-27* (BDSC: 44260). All flies were cultured at

Table 1. Genetic Variants in *BICRA* Subjects

Subject #	BICRA Variant (NM_015711.3)	Amino Acid Change	Inheritance	CADD Score
S1	c.936delC	p.Ala313Profs*30	*de novo*	N/A
S2	c.1574delG[a]	p.Ser525Thrfs*199	non-maternal	N/A
S3	c.1993C>T	p.Gln665*	*de novo*	38
S4	c.2075_2078delCTCA	p.Thr692Argfs*31	*de novo*	N/A
S5	c.2479_2480delinsA	p.Ala827Thrfs*15	*de novo*	N/A
S6	c.3247dupT	p.Cys1083fs*26	*de novo*	N/A
S7	c.4369C>T	p.Gln1457*	*de novo*	35
S8	~126 kb loss Chr19(GRCh37):g.48,030,624–481,56,194	deletion	*de novo*	N/A
S9	~167.8 kb loss Chr19(GRCh37):g.48,117,952–48,285,752	deletion	*de novo*	N/A
S10	~202 kb loss Chr19(GRCh37):g.48,003,600–48,206,000	deletion	*de novo*	N/A
S11	c.192G>C	p.Glu64Asp	*de novo*	24.9
S12	c.4267G>A	p.Glu1423Lys	*de novo*	22

[a]Also has a maternal BICRA missense variant

22°C, unless otherwise noted, on standard cornmeal and molasses medium in plastic vials. Both male and female flies were used in complementation tests and only male flies were used for position effect variegation assays.

Fly Stocks

All fly strains used in this study were generated in house or obtained from the Bloomington *Drosophila* Stock Center (BDSC).

Figures 4B and 4C: *Mi{PT-GFSTF.1}Bicra*$^{MI03400\text{-}GFSTF.1}$

Figure 4D: *In(1)w*^{m4h}*; +*

Figure 4D′: *In(1)w*^{m4h} *Mi{MIC}Bicra*^{MI14493}*/+*

Figure 4D″: *In(1)w*^{m4h}*; Mi{ET1}Bicra*^{MB00611}*/+*

Figure 4E: *P{hsp26-pt-T}39C-27; +*

Figure 4E′: *P{hsp26-pt-T}39C-27; Mi{MIC}Bicra*^{MI14493}*/+*

Figure 4E″: *P{hsp26-pt-T}39C-27; Mi{ET1}Bicra*^{MB00611}*/+*

Figure 4F: *P{hsp26-pt-T}39C-5; +*

Figure 4F′: *P{hsp26-pt-T}39C-5; Mi{MIC}Bicra*^{MI14493}*/+*

Figure 4F″: *P{hsp26-pt-T}39C-5; Mi{ET1}Bicra*^{MB00611}*/+*

Figure S1A: *Mi{MIC}Bicra*^{MI14493}*/Df(3R)BSC806, Mi{ET1}Bicra*^{MB00611}*/Df(3R)BSC806, Mi{PT-GFSTF.1}Bicra*$^{MI03400\text{-}GFSTF.1}$*/Df(3R)BSC806, Mi{MIC}Bicra*^{MI14493}*/Mi{ET1}Bicra*^{MB00611}

Figure S1B: *Mi{MIC}Bicra*^{MI14493}*/Mi{ET1}Bicra*^{MB00611}*, CH321-05L16 (Bicra GR); Mi{MIC}Bicra*^{MI14493}*/Mi{ET1}Bicra*^{MB00611}

Confocal Microscopy for Larval and Adult Brains

Larval and adult brains were dissected in 1× PBT (PBS+ 0.2% Triton X-100) and fixed in 3.7% formaldehyde in PBS for 30 min at room temperature. The samples were then washed in 0.2% Triton X-100 and were then stained with rabbit anti GFP:AlexaFlour488 (1:500, Invitrogen), rat:anti Elav (1:50, Developmental Studies Hybridoma Bank), mouse anti Repo (1:20, Developmental Studies Hybridoma Bank) overnight at 4°C, donkey: anti rat Cy3 (1:200, Jackson ImmunoResearch), donkey: anti mouse Alexa Fluor 647 (1:200, Jackson ImmunoResearch), and mounted in Vectashield (Vector Labs) and imaged with a Leica SP8 confocal microscope and processed using ImageJ.

Imaging of Fly Eyes

Images of the *Drosophila* eyes were taken using a digital camera (MicroFire; Olympus) mounted on a stereomicroscope (MZ16; Leica) using ImagePro Plus 5.0 acquisition software (Media Cybernetics). The "extend depth of field" function of the AxioVision software was used to obtain stack images by focus stacking.

Generation of Zebrafish Mutants

Zebrafish were raised according to standard protocols and in accordance with University of Oregon IACUC protocols. The zebrafish codon-optimized Cas9 plasmid was digested with XbaI, purified, and transcribed with the T3 message machine kit (Ambion). The guide RNA (gRNA) was designed (with the ZiFiT Targeter software) to the CRISPR target sequence. Templates for gRNA synthesis were prepared by PCR with a gene-specific primer. sgRNA was synthesized with the T7 MEGAscript kit (Ambion). Cas9 mRNA (300 ng/mL) and sgRNA (150 ng/mL) were mixed and injected into Oregon AB* WT zebrafish embryos at the one-cell stage with an MPPI-2 Pressure Injector with a BP-15 Back Pressure Unit (Applied Scientific Instrumentation). We confirmed CRISPR activity at the target site with a sequence analysis of pools of injected embryos at 24 h postfertilization (hpf) by using primers to amplify the region containing the target sequence. Injected founders were outcrossed to AB* and analysis of individual F1 embryos from these crosses at 24 hpf identified clutches carrying a single 8 bp deletion in exon 3, given the allele designation b1404. F1s from positive clutches were raised to adulthood and fin-clipped to identify heterozygotes that were then incrossed to obtain the F2 generation representing all three possible genotypes. Homozygous viable F2 mutants were raised to adulthood and incrossed to obtain larvae for the described experiments, alongside control larvae from homozygous wild-type F2 siblings.

Imaging of Zebrafish Skeletal Elements

Skeletal elements were stained with Alcian blue and Alizarin red as previously described.[39] Images were captured with a Leica S8APO dissecting microscope fitted with a Leica EC3 camera and LAZ EZ imaging software. Statistical analyses including the nonparametric Mann-Whitney test to measure statistical significance of the changes in size, shape, and orientation of the jaw elements were performed with GraphPad software.

D. melanogaster Cell Culture

Drosophila S2 cells were cultured in SFX-Insect media at 28°C with constant shaking at 112 rpm. To generate stable cell lines, cells were plated in 6-well plates at 2×10^6 and transfected with 2 μg of expression constructs using Effectene Transfection Reagent (-QIAGEN) in accordance with manufacturer's recommendation. Cells were selected using 250 μg/mL of hygromycin or 10 μg/mL of puromycin for 10 days and expanded to 1 l culture for complex purification. 1-l cultures were induced with 500 μM copper sulfite for 72 h and collected by centrifugation at 4,000 × *g* for 5 min.

Immunoprecipitation-Mass Spectrometry

IP-MS was performed as previously described.[32] In summary, cell pellets were resuspended in hypotonic buffer (HB) containing 10 mM Tris HCl (pH 7.5), 10 mM KCL, 1.5 mM $MgCL_2$, 1 mM DTT, 1 mM PMSF and incubated on ice for 5 min. Suspension was centrifuged at 5,000 rpm for 5 min at 4°C, and pellets were resuspended in 5 volumes of fresh HB containing protease inhibitor cocktail and homogenized using glass Dounce homogenizer. Nuclear pellets were resuspended in high salt buffer (HSB) containing 50 mM Tris HCl (pH 7.5), 300 mM KCl, 1 mM $MgCL_2$, 1 mM EDTA, 1 mM 1% NP40, 1 mM DTT, 1 mM PMSF, and protease inhibitor cocktail. Homogenate was incubated on rotator for 1 h. Homogenates then were centrifuged at 20,000 rpm (30,000 × *g*) for 1 h at 4°C using an SW32Ti rotor. Chromatin pellets were discarded and high salt nuclear extract was filtered through a 0.45 μm filter and incubated overnight with HA magnetic resin. HA beads were washed in HSB and eluted with HSB containing 1 mg/mL of HA peptide for 4 times 1.5 h each. Samples were concentrated using StrataClean beads, eluted with 1× LDS, and loaded on to SDS-PAGE gel. Samples for MS were migrated for 2 cm and stained with colloidal blue. Bands of interested were excised and submitted for MS. For gel image, samples were migrated completely and stained with silver stain (Thermo).

Results

Individuals Carrying Putatively Pathogenic Variants in *BICRA* Have Overlapping Neurodevelopmental Phenotypes

With the help of GeneMatcher[40] and the Undiagnosed Diseases Network (UDN),[41] we identified 12 individuals (subjects 1–12) in whom array-based copy number variant (CNV) analysis, whole-exome, or whole-genome sequencing identified putatively deleterious variants that affect *BICRA*. Their clinical and molecular data, including other variants observed in exome sequencing, are summarized below and in Tables 1, 2, and S1 and Supplemental Note. Throughout this report, all sequence variants are described based on reference sequence GenBank: NM_015711.3.

Subjects 1–12 have neurodevelopmental phenotypes—developmental delay (HP:0001263), intellectual disability (HP:0001249), autism spectrum disorder (HP:0000729), and/or behavioral phenotypes (HP:0000708)—and variable structural birth defects and dysmorphic features (Figure 1, Tables 2 and S1). However, only three (subjects 1, 3, and 4) had fifth digit/nail hypoplasia, a hallmark of most SSRIDDs (Table S1). Subjects 1–7 carry stop-gain or frameshift variants that may trigger nonsense mediated mRNA decay or produce truncated proteins (Table 1, Figure 2A). Subject 8 carries an approximately 126 kb deletion within chromosome band 19q13.33 (chr19: 48,030,624–48,156,194; hg19) that includes a portion of *BICRA* (Table 1). Subject 9 carries an approximately 167.8 kb deletion within chromosome band 19q13.33 (chr19:48,117,952–48,285,752; hg19) that encompasses *BICRA* (Table 1). Subject 10 carries an approximately 202 kb deletion (chr19:48,003,600–48,206,000; hg19) that contains all but the C-terminal end of *BICRA* (Table 1). Subjects 11 and 12 carry *BICRA* missense variants, c.192G>C (p.Glu64Asp) and c.4267G>A (p.Glu1423Lys), respectively. Subject 11 has macrocephaly (+3 SD), and subject 12 has a macrocerebellum not seen in any of the other subjects. These *BICRA* variants were found to be *de novo* in all cases except for subject 2, for whom paternal DNA was not available. Subject 2's variant was confirmed to be non-maternal.

Like other SSIRDDs, all 12 subjects have dysmorphic facial features (Figure 1). Four subjects have microcephaly and a fifth has borderline microcephaly with a head circumference hovering just above −2 SD (HP:0000252; Table S1). 6 of 12 subjects have frontal bossing/prominent glabella (HP:0002007/HP:0002057) and 4 have bitemporal narrowing (HP:0000341). 7 subjects have epicanthal folds (HP:0000286) and 5 have downslanting palpebral fissures (HP:0000494, Table S1). Additionally, 4 subjects have low-set ears (HP:0000369). The nose and lips are also an area of shared features, with 7 subjects having a prominent nasal tip (HP:0005274), 6 subjects with thin upper lip (HP:0000219), and 5 subjects have a rounded/bulbous nasal tip (HP:0000414) and a prominent nasal bridge (HP:0000426). These symptoms are distinct from the coarse facial features typically associated with SSRIDDs.[19]

BICRA Is Intolerant to Loss-of-Function Based upon Bioinformatic Data

To gather information about human *BICRA* and its orthologous genes in genetic model organisms, we performed an *in silico* search using MARRVEL (Model organism Aggregated Resources for Rare Variant ExpLoration).[45–47] Based on gnomAD data,[42] *BICRA* is intolerant to loss-of-function with a probability of being loss-of-function intolerant (pLI) score of 0.98 and a loss-of-function variant observed/expected (o/e) ratio of 0.14 (0.07–0.3) (Figure 2A). *BICRA* is also missense constrained with a z score of 2.54 and an o/e of 0.75 (0.7–0.8). Finally, DOMINO[43] predicts *BICRA* to be "likely dominant."

Although *BICRA* is highly intolerant to loss-of-function variants, five individuals in gnomAD[42] carry potential loss-of-function variants, one of which is flagged as low confidence. All of the stop-gain and frameshift variants we identified in subjects 1–7 are absent from HGMD,[48] Geno2MP, and gnomAD.[42] The two stop-gain variants found in subjects 3 and 7 have combined annotation-dependent depletion (CADD)[49] scores of greater than 34, placing

Table 2. Phenotypic Characteristic of *BICRA* Subjects

Subject	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	Total
Age	3 year	8 year	11 year	9 m	17 year	28 year	17 year	2 year	7 year	8 year	11 year	28 m	
Sex	F	F	M	M	M	M	M	M	F	F	M	F	
Variant	c.936delC	c.1574delG	c.1993C>T	c.2075_2078delCTCA	c.2479_2480delinsA	c.3247dupT	c.4369C>T	~126 kb loss	~167.8 kb loss	~202 kb loss	c.192G>C	c.4267G>A	
Neurodevelopmental													12/12
DD/ID	+	+	+	+	+	+	+	+	+	+	+	+	12/12
Autism spectrum disorder	–	–	+	–	–	+	–	+?	+	–	+	–	5/12
Developmental regression	–	–	–	–	–	–	–	–	–	–	+	–	1/12
Motor stereotypies	–	–	–	–	–	–	–	+	+	–	–	–	2/12
Behavioral problems	–	–	+	–	–	+	+	–	–	+	+	–	5/12
Sleep disturbance	–	–	–	–	–	+	+	–	–	–	–	–	2/12
Hypotonia	+	–	–	+	+	–	+	–	–	–	–	–	4/12
Oral motor hypotonia	–	–	–	–	–	–	–	–	–	–	–	+	1/12
Feeding difficulties	+	–	+	+	+	–	–	+	+	–	–	+	7/12
Epilepsy/seizures	–	+	–	–	–	–	–	–	+	–	–	–	2/12
CNS/PNS Anomalies													2/12
Chiari malformation	–	–	–	–	–	–	–	–	–	–	–	+	1/12
Obstructive hydrocephalus	–	–	–	–	–	–	–	–	–	–	–	+	1/12
Macrocerebellum	–	–	–	–	–	–	–	–	–	–	–	+	1/12
Facial nerve paresis	–	–	–	–	–	–	–	–	–	–	–	+	1/12
Hippocampal atrophy	–	+	–	–	–	–	–	–	–	–	–	–	1/12
Ophthalmologic													7/11
Ambliopia/strabismus	–	+	+	N/A	–	+	+	–	–	+	–	–	5/11
Myopia	–	–	+	N/A	–	–	–	–	–	–	–	–	1/11
Hyperopia	–	–	–	N/A	–	–	–	+	+	+	–	–	3/11
Auditory													1/12
Sensorineural hearing loss	–	–	–	–	–	–	+	–	–	–	–	–	1/12
Orofacial Clefting													1/12
Submucous cleft palate	–	–	–	–	–	–	+	–	–	–	–	–	1/12

(*Continued on next page*)

Table 2. ***Continued***

Subject	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	Total
Cardiac													4/12
Tatralogy of Fallot	–	–	–	–	–	–	–	–	–	+	–	–	1/12
PFO	–	–	+	–	–	–	–	–	–	–	–	–	1/12
Cardiac murmur	–	+	–	–	–	–	+	–	–	–	–	–	2/12
Gastrointestinal													7/12
Elevated liver enzymes	–	–	–	–	–	–	–	–	–	–	–	+*	1/12
Celiac disease	–	–	–	–	–	+?	–	–	–	–	–	–	1/12
GERD	+	–	–	–	–	–	–	+	–	–	–	–	2/12
Chronic constipation	–	–	–	–	+	–	+	+	+	–	–	–	4/12
Genitourinary													3/12
Horseshoe kidney	–	+	–	–	–	–	–	–	–	+	–	–	2/12
Cryptorchidism	N/A	–	+	–	–	–	–	–	N/A	N/A	–	N/A	1/8
Hypospadias	N/A	–	+	–	–	–	–	–	N/A	N/A	–	N/A	1/8
Orthopedic													5/12
Radioulnar synostosis	–	–	+	N/A	–	–	–	–	–	–	–	–	1/11
Scoliosis	–	–	–	N/A	–	–	+	–	+	-	–	–	2/11
Joint laxicity	–	–	–	–	+	–	+	–	+	–	–	–	3/12
Hip subluxation	–	–	–	–	–	–	–	+	–	–	–	–	1/12
Immunology													2/12
Recurrent infections	–	–	–	–	–	–	–	–	–	+	–	+*	2/12
Endocrine													1/12
Hypothyroidsim	–	–	–	–	–	–	–	–	–	–	–	+	1/12
Hematology													1/12
Von Willebrand disease	–	–	–	–	–	–	+	–	–	–	–	–	1/12
Growth													6/12
Short stature	–	+	–	–	+	–	–	–	–	–	–	+*	3/12
Poor weight gain	+	–	–	+	+	–	–	+	–	–	–	+*	5/12
Failure to thrive	–	–	–	–	+	–	–	+	–	–	–	+*	3/12

N/A, not applicable or required study/data not obtained; DD, developmental delay; ID, intellectual disability; PFO, patent foramen ovale; y, years; *, possibly due to cystic fibrosis with exocrine pancreatic insufficiency; ?, some uncertainty regarding this diagnosis.

Figure 1. Individuals with *BICRA* Variants Have Dysmorphic Facial Features
Individuals with variants in *BICRA* share a number of facial features including frontal bossing, epicanthal folds, low-set ears, prominent/rounded nasal tips, and thin upper lips.

them among variants predicted to be most damaging. In addition, MutationTaster[50] predicts all encountered nonsense variants to be "disease causing."

The stop-gain and frameshift variants seen in subjects 1–6 are located in areas of the gene where they are likely to trigger nonsense-mediated mRNA decay. If these variants did generate a protein product, they are predicted to disrupt the GLTSCR1 domain of *BICRA* which has been previously shown to be critical for BICRA's binding to the ncBAF complex.[32] Thus, even if these variants escape nonsense-mediated mRNA decay, they are likely to be nonfunctional. In contrast, the c.4369C>T (p.Gln1457*) variant in subject 7 is located in the final exon and would be unlikely to trigger nonsense-mediated mRNA decay. Its predicted protein product would leave the GLTSCR1 domain intact. This suggests that the C-terminal end of BICRA may play a critical functional role despite its lack of known functional domains.

In the DECIPHER database,[51] three individuals with associated phenotypic data (individuals 283094, 286478, 290625) carry <700 kb deletions that include *BICRA*. These individuals have neurodevelopmental phenotypes—delayed speech and language development, cognitive impairment, and/or intellectual disability—that overlap with those described in our subjects. This provides further evidence that haploinsufficiency of *BICRA* causes neurodevelopmental phenotypes.

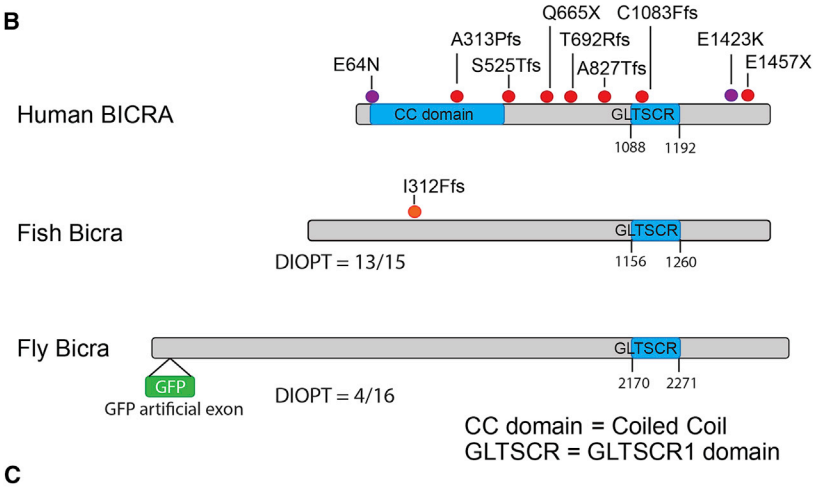
The missense variants seen in subjects 11 and 12, p.Glu64Asp and p.Glu1423Lys, respectively, are absent from HGMD,[48] Geno2MP, and gnomAD,[42] although the Glu1423 residue is found to be modified to Asn in one individual in gnomAD. The p.Glu64Asp variant has a high CADD[49] score of 24.9 and is predicted to be "damaging," "probably damaging," "deleterious," and "disease causing by M-CAP,[52] Polyphen-2,[53] SIFT,[54] and MutationTaster,[50] respectively. The p.Glu1423Lys variant has a high CADD score of 22 and is predicted to be "benign" by PolyPhen-2 and as a "polymorphism" by MutationTaster, although it is predicted to be "damaging" by M-CAP, and "deleterious" by SIFT.[50] It should be noted that PolyPhen-2 predicts the p.Glu1423Lys variant to be "probably damaging" in an alternate transcript of *BICRA*. Together, these data suggest that the variants we report are likely to compromise BICRA function.

Zebrafish *bicra* Mutants Display Craniofacial Dysmorphology

To determine whether loss of function of *BICRA* affects development in other vertebrates, we turned to zebrafish. The ortholog of *BICRA* in zebrafish is *bicra* (ZFIN ID: ZDB-GENE-031116-2). The zebrafish Bicra and human BICRA proteins are highly conserved, with a DIOPT

A

Probability of LoF intolerance (pLI)				Missense Constraint			DOMINO	
Expected	Observed	pLI score	o/e score	Expected	Observed	z score	Score	Inheritance prediction
34.5	5	0.98	0.14	823	618	2.54	0.68	Likely dominant



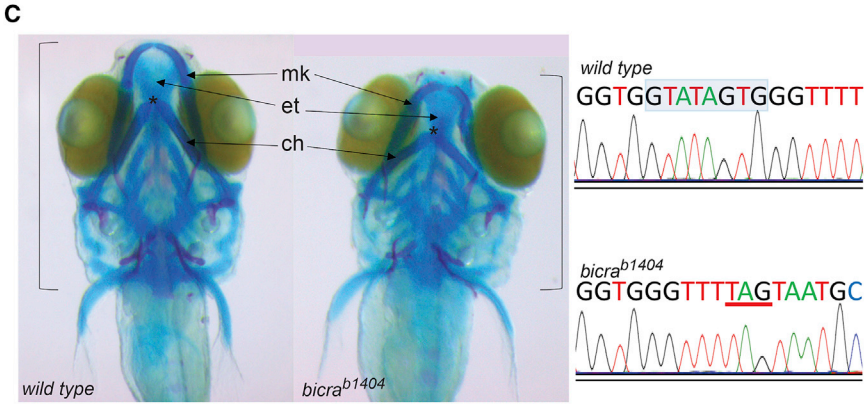


Figure 2. *BICRA* Is Loss-of-Function Constrained and Mutation of *bicra* in Zebrafish Causes Craniofacial Defects

(A) *BICRA* is variant constrained based upon the control population database gnomAD.[42] *BICRA* has a score of 0.64 in DOMINO[43] and is predicted to be "likely dominant."

(B) Protein structure of BICRA and its fish and fly orthologs. BICRA has two protein domains, a coiled-coil (CC) domain, and a GLTSCR1 domain. Variants identified in this study are indicated above the protein as dots (red, loss-of-function; purple, missense). BICRA's ortholog in zebrafish has a coiled coil domain and a GLTSCR1 domain. A frameshift mutation induced by CRISPR-Cas9 is indicated by an orange dot. BICRA's ortholog in flies has a homology score 4/16 (DIOPT v8.0[44]) and also contains a GLTSCR1 domain. The location of the internal GFP tag used in Figure 4 is shown in green.

(C) Zebrafish larvae from homozygous mutant parents exhibit craniofacial abnormalities, including anterior-posterior shortening of the head (brackets), an increased joining angle of the ceratohyal (ch) elements at the midline (82° ± 2.7° compared to 64.9° ± 0.4°; $p < .0001$), and misorientation of Meckel's cartilage (mk) relative to the ethmoid plate (et). The *bicra*^{b1410} allele is an 8 bp deletion in the 3rd exon (del c.933–40, boxed region) producing a frameshift that results in a missense mutation, p.Ile312Phe, followed immediately by a stop codon (red underline).

(DRSC Integrative Ortholog Prediction Tool) score of 13/17 (DIOPT v.8.0, Figure 2B).[44] The two proteins exhibit 38% identity and 47% similarity with 25% gaps. There is greater conservation in the GLTSCR1 domain (73% identity, Figure 2B), previously shown to be critical for BICRA to bind to other SWI/SNF complex members.[31]

To study the function of *bicra* in zebrafish, we generated a frameshift mutation, p.Ile312Phe*fs**1, leading to a premature stop codon, at a position similar to the human c.936delC (p.Ala313Profs*30) variant, which we named the *b1404* allele. We observed no phenotype in animals heterozygous for the *b1404* allele, nor in the homozygous mutant offspring of *b1404* heterozygotes. However, significant craniofacial defects were observed in the offspring of homozygous mutant parents at 7 days post fertilization (dpf) (Figure 2C). We noted that the anterior-posterior length of the heads of these *bicra* mutant fish were 14% shorter compared to wild-type controls (588 ± 73 μm versus 680 ± 45 μm; $p < .00001$), and there were notable disruptions of the jaw elements consistent with the anterior-posterior compression. The angle at which ceratohyal (ch) cartilage elements, which make up the ventral hyoid arch, converged at the midline was an average of 17 degrees broader in bicra^{b1404} mutants (82° ± 2.7°) compared to wild-type controls (64.9° ± 0.4°; $p < .0001$), and their Meckel's cartilage (mk), which normally protrudes beyond the ethmoid plate (et) at the midline, was receded. These data suggest that *bicra* functions to control craniofacial development. The absence of phenotypes in young fish derived from heterozygous parents combined with our observations from homozygous mutants suggest a role for maternal RNA or protein in regulating these early developmental events.

Bicra Binds to SWI/SNF Complex Members in *Drosophila* S2 Cells

The ortholog of *BICRA* in *Drosophila melanogaster* is *CG11873* (FlyBase ID: FBgn0039633). The gene is moderately conserved with a DIOPT score[44] of 4/16 (DIOPT v.8.0, Figure 2B). The protein encoded by *CG11873* shows

20% identity and 32% similarity across the entire protein. The fly gene is also twice the size of the human gene and hence numerous gaps appear in the alignment (32%, Figure 2B). We will refer to *CG11873* as *Bicra* throughout this manuscript.

BICRA has previously been shown to bind to SWI/SNF complex members as part of the ncBAF complex in human cells[31] but has not been functionally or biochemically studied in flies. Due to the relatively low homology of human BICRA and fly Bicra, we first asked whether Bicra binds to the ncBAF complex to confirm that it is a member of the same complex in both species. To test whether Bicra binds to other ncBAF complex members, we tagged a C-terminal region of Bicra (aa 1,973–3,003) with an HA (hemagglutinin) tag and transfected this construct into *Drosophila* S2 cells and performed immunoprecipitation-mass spectrometry (IP-MS) on the tagged protein (Figure 3A, Tables S2 and S3). Among the top 20 most abundant proteins in our precipitates were Brm (Brahma), Mor (Moira), CG7154, Bap60, and Bap55 (the fly orthologs of SMARCA2/4, SMARCC1-3, BRD7/9, SMARCD1-3, and ACTL6A/B, respectively), all of which were absent in a mock pull down (Figure 3A, Tables S2 and S3). Brm, Mor, CG7154, and Bap60 can also be clearly seen on a silver stain gel of the pull down (Figure 3A). We did not capture Snr1 or Bap111 (SMARCB1 or SMARCE1) components found in BAF and PBAF mammalian complexes, further suggesting a distinct Bicra-marked complex in flies. Further, we did not capture the d4 subunit, the ortholog of the DPF2 subunit in the canonical BAF complex. Bcl7-like and CG10555 (the orthologss of BCL7A/B/C and SS18/L1 subunits, respectively) were not detected as abundant members of our pulldown; however, this may be in part due to the limited lysine residues present in the amino acid sequences of these subunits. Bcl-7-like is however enriched in our pull down compared to the mock (Figure 3A). Further investigation is needed to confirm the presence of these proteins are present in the fly ncBAF complex. Given these data, and because we captured CG7154 a sole member of this bromodomain family of proteins in *Drosophila* and ortholog of both BRD9 and BRD7 (members of human ncBAF and PBAF complexes, respectively), we conclude that, like human BICRA, Bicra is a member of an ncBAF (ncBAP) version of the SWI/SNF complex in flies (Figure 3C).

To confirm that these interactions are ncBAF specific, we next performed IP-MS on HA-tagged Bap60, d4, and CG7154 (shared, BAF-specific, PBAF/ncBAF, respectively) (Figure 3B, Tables S4, S5, and S6). Consistent with our pull-down of Bicra, Bicra is pulled down by Bap60 and CG7154 but not d4 (Figure 3B, Tables S4, S5, and S6). Consistent with previous results, Bap60 pulls down all members of the SWI/SNF complex because it is shared between all three versions of the complex[31,55] (Figure 3B). In contrast, d4 pulls down only BAF-specific complex members such as Osa (ortholog of ARID1A) (Figure 3B). Finally, CG7154 pulls down both PBAF and ncBAF proteins as it is the sole ortholog of BRD7 and BRD9 in flies. Together these data demonstrate that all versions of the fly SWI/SNF complexes are well conserved and highly similar to the human versions.

Bicra Is Widely Expressed in the Nervous System and Localizes to the Nucleus

To determine whether fly *Bicra* could be involved in neurodevelopment, we first examined its expression pattern in the fly nervous system. We used a GFP-tagged allele of *Bicra* to investigate its expression pattern (Figures 4A and S1C). This tag was inserted as an artificial exon in the N-terminal region of the protein using the *MI00340* MiMIC allele via recombination-mediated cassette exchange[56] (Figure S1C). This allele ($Bicra^{MI03400\text{-}GFSTF.1}$) is homozygous lethal, but successfully complements a deficiency [*Df(3R) BSC806*] spanning *Bicra*. Hence, this chromosome carries a second site lethal mutation which does not interfere with our expression analysis (Figure S1A).

We first examined the expression pattern of Bicra::GFP in the larval nervous system and observed that it is broadly expressed in both neurons and glia and localizes to nuclei (Figure 4B). Bicra::GFP expression does not seem to be ubiquitous, however, and a sizeable population of neurons and glia do not express this Bicra::GFP (Figure 4B), possibly because Bicra::GFP tags only three of the four Bicra isoforms. In the adult nervous system, we observed a similar pattern, with the vast majority of neurons and glia expressing Bicra::GFP (Figure 4C). Additionally, BICRA is broadly detected in the human brain according to the Genotype-Tissue Expression[57] (GTEx) project and the Human Protein Atlas.[58] These data suggest that *Bicra* may be important for nervous system development and function.

One Copy Loss of *Bicra* in Flies Causes Position Effect Variegation

To further study the *in vivo* function of *Bicra* during development, we obtained two homozygous lethal transposon insertion alleles, $Bicra^{MB00611}$ and $Bicra^{MI14493}$ (Figure 4A).[56,59,60] Both are alleles in which *Minos* transposons are inserted into exons encoding the GLTSCR domain of *Bicra*, disrupting the four transcript isoforms (Figure 4A). To test whether the lethality of these alleles are due to loss-of-function of *Bicra*, we crossed them to a deficiency that spans *Bicra [Df(3R)BSC806]* (Figure S1A). Both alleles failed to complement the deficiency and are embryonic lethal. Flies transheterozygous for $Bicra^{MB00611}$ and $Bicra^{MI14493}$ survive to become pupae, with a small percent escaping to adulthood (Figure S1B), suggesting that these alleles are strong hypomorphs. We were also able to rescue the lethality of transheterozygous *Bicra* mutants with a genomic rescue construct that covers *Bicra* (Figure S1B). This suggests that the $Bicra^{MB00611}$ and $Bicra^{MI14493}$ alleles are indeed loss-of-function alleles.

Many chromatin remodeling proteins, including members of the SWI/SNF complex, are known to have a dominant effect on position effect variegation (PEV).[55,61–63]

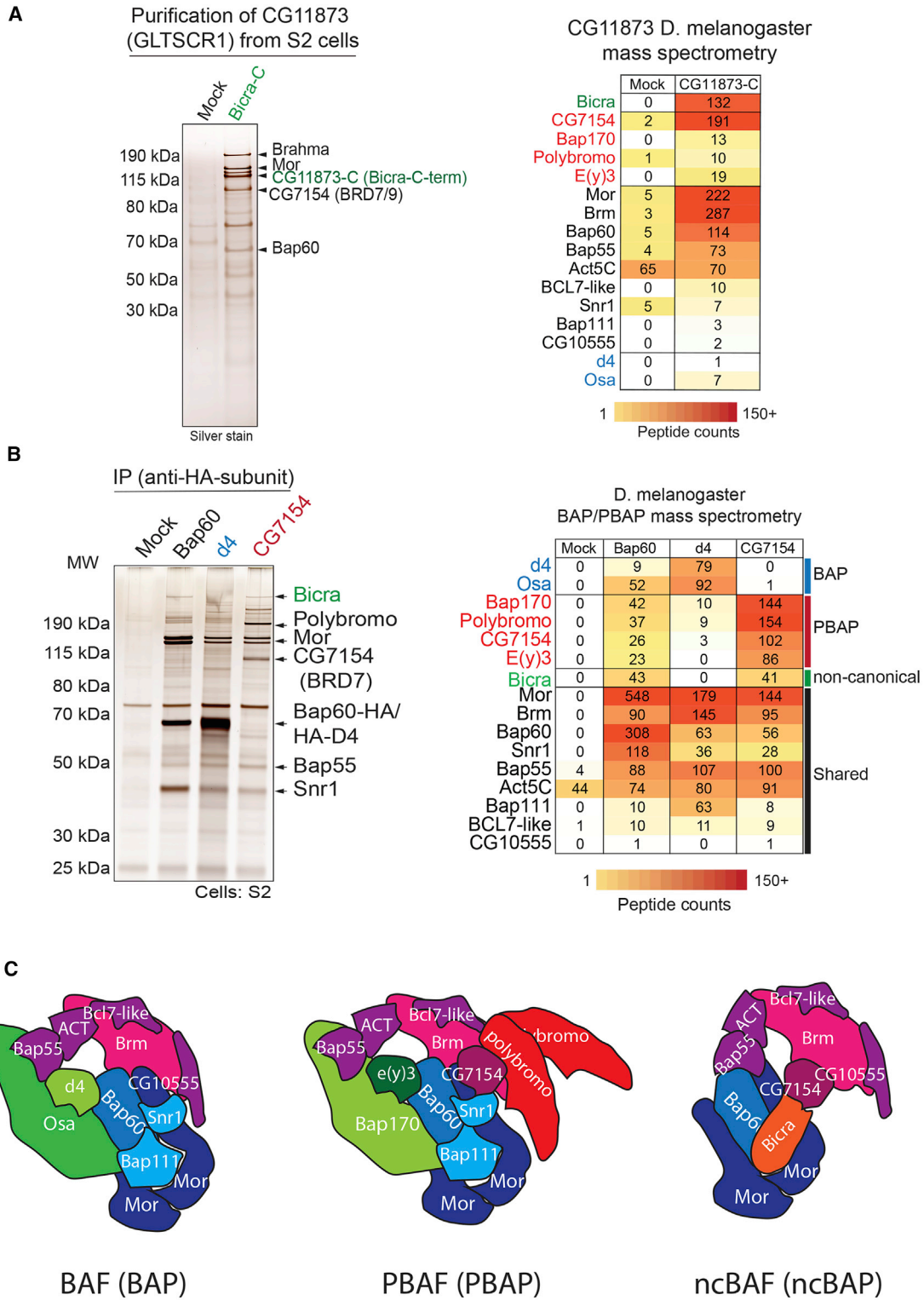


Figure 3. Bicra Binds to Other ncBAF Complex Proteins in *Drosophila* S2 Cells

(A) HA-tagged Bicra C terminus was transfected into *Drosophila* S2 and IP-MS was performed. Left, silver stain of mock and HA-Bicra purifications. Right, mass spectrometry peptide counts (see also Tables S2 and S3).

(B) Left, silver stain of mock, HA-Bap60, HA-d4, and HA-CG7154 purifications. Right, mass spectrometry peptide counts (see also Tables S4, S5, S6, and S7).

(C) Schematic depicting the fly versions of the SWI/SNF complexes based upon data from A and previously published work and proteomic mass-spectrometry results from purifications in (A) and (B).[26] The compositions of human[32] and fly complexes are very similar. Importantly, Bicra is the only ncBAF-specific member in flies.

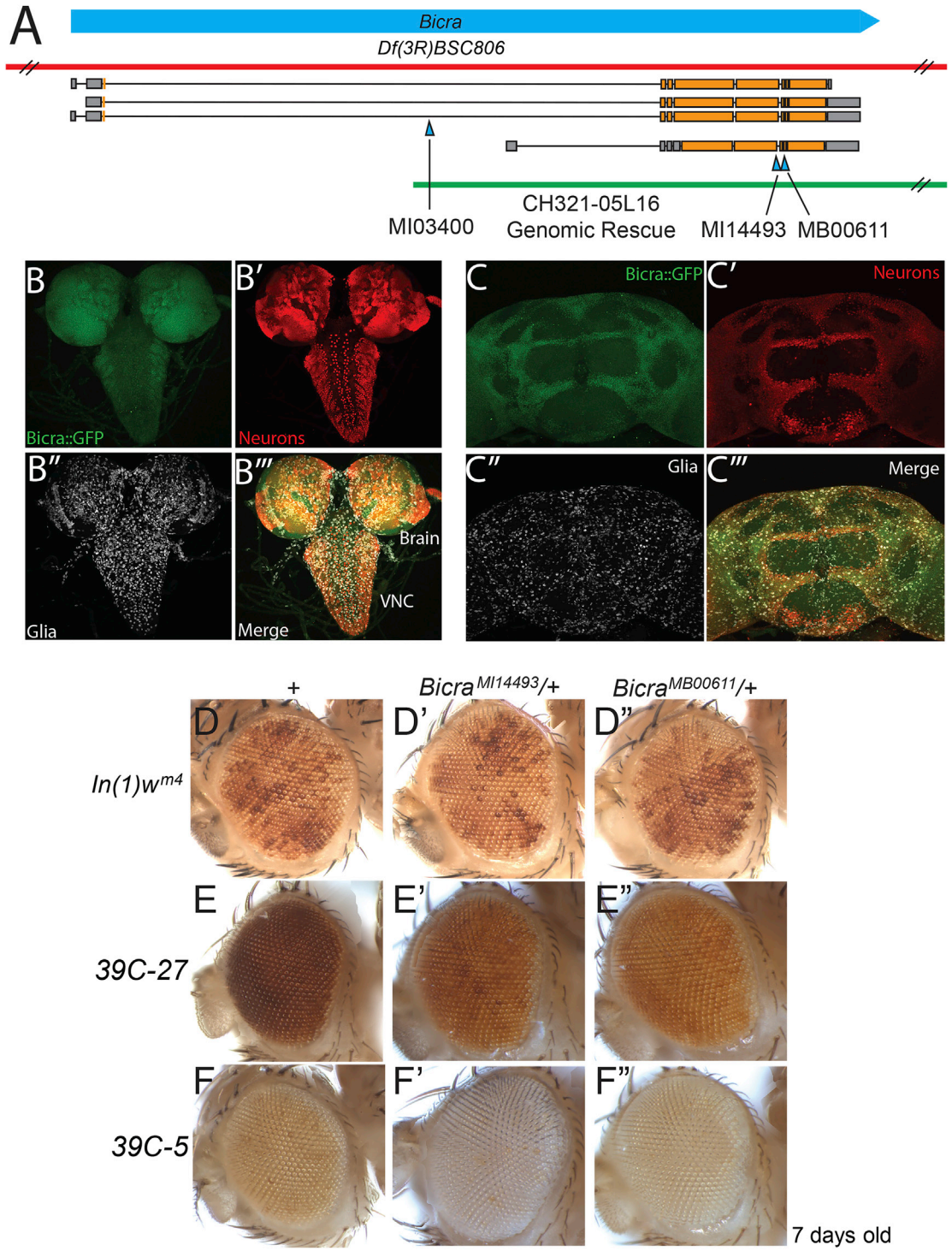


Figure 4. Bicra Is Broadly Expressed in the Brain in Flies and Controls Telomeric Position Effect Variegation

(A) Gene structure of fly *Bicra*. The gene region is shown in blue, exons in orange, introns as a black line, and untranslated regions (UTR) in gray. A deficiency and genomic rescue (GR) construct are shown in red and green, respectively. Blue arrows indicate available transposable element insertions.

(B and C) Expression of GFP-tagged Bicra in the larval (B) and adult (C) nervous systems. Bicra::GFP is shown in green, the neuronal marker Elav in red, the glial marker Repo in white. We observe broad expression of Bicra::GFP in most neurons and glia. Bicra::GFP also localizes to the nucleus as indicated by its colocalization with Elav and Repo, which are both nuclear proteins.

(D–D″) Position effect variegation (PEV) assay using the *In(1)w*^{m4} allele. One copy loss of *Bicra* with either allele does not alter variegation in the eye.

(E–E″) PEV assay using the *39C-27* w^+ insertion in the right telomere of the 2nd chromosome. Loss of *Bicra* with either allele shows a dominant lightening of eye color, suggesting that *Bicra* controls chromatin structure at this location.

(F–F″) PEV assay using the *39C-5* w^+ insertion in the left telomere of the 2nd chromosome. Loss of *Bicra* with either allele shows a dominant lightening of eye color, suggesting that *Bicra* controls chromatin structure at this location.

Variegation in flies was first described in a stock carrying an inversion of the X chromosome [*In(1)w*$^{m4\ h}$] that allows the *white* (*w*) gene to be silenced stochastically by repressive chromatin marks creating a speckled or variegated eye.[63,64] Many chromatin remodeling genes act as dominant enhancers (lightening of the eye) or suppressors (darkening of the eye).[63] Genes that encode members of the SWI/SNF complex, such as *Snr1* and *Bap60*, have been reported as suppressors of variegation.[55,61] We reasoned that if *Bicra* encodes a protein that functions in the SWI/SNF complex, it should have a similar effect on PEV as other SWI/SNF members. To test this hypothesis, we examined the effect of both *Bicra* loss-of-function alleles on the *In(1)w*$^{m4\ h}$ allele (Figure 4D). Surprisingly, we did not observe a suppression of variegation with either allele of *Bicra* (Figures 4D′ and 4D″). This leads us to conclude that *Bicra* and the ncBAF complex does not regulate chromatin structure at the *w* locus, unlike members of the BAF and PBAF complexes.

Because we did not observe any phenotype with the *In(1)w*$^{m4\ h}$ allele, we decided to examine whether *Bicra* controlled PEV in other regions of the genome. Other SWI/SNF complex members have been implicated in controlling PEV at telomeric regions based upon w^{+} transgenes inserted into the telomeres of the second chromosome.[65] Specifically, mutations in *brm, mor,* and *osa* (the orthologs of *SMARCA2/4, SMARCC1-3,* and *ARID1A/B*, respectively) were shown to cause dominant enhancement of PEV at these locations.[65] To test whether *Bicra* also controls PEV at telomeres, we crossed the *Bicra*^{MB00611} and *Bicra*^{MI14493} alleles to the *39C-5* (2L telomere) and *39C-27* (2R telomere) insertions (Figures 4E and 4F). Both mutant alleles of *Bicra* caused an obvious lightening of eye color for both insertions (Figures 4E and 4F). This suggests that *Bicra,* and the ncBAF complex, controls PEV and chromatin structure at telomeres like several other genes that encode genes of the BAF and PBAF complexes. However, Bicra seems to be unique in that it affects PEV in a much more context-specific manner compared to other genes examined to date, which suggests that ncBAF complex also functions in a more specific manner than the BAF of PBAF complexes.

Discussion

BICRA was originally identified as a gene located in a deletion of chromosome 19 that is associated with glioma tumor suppression.[35] Since then, CNVs affecting *BICRA* have been implicated in a variety of gliomas.[33,34,36,37] *BICRA*'s function *in vivo* beyond cancer, particularly in development, remains ill defined. In addition, the function of the ncBAF complex, of which BICRA is a member, is not known. The ncBAF complex has been shown to be necessary for maintenance of pluripotency in mouse embryonic stem cells[34] and in the maintenance of gene regulation and proliferative capacity in cancer cells lacking members of the canonical BAF complex;[31,66] however, all other studies have focused on understanding the composition and assembly of the complex.[31,32] This study provides evidence that *BICRA* and the ncBAF complex function in neural development in humans and flies.

Several members of the SWI/SNF complex have been shown to cause SWI/SNF related intellectual disability disorders (SSRIDDs), rare but severe neurodevelopmental disorders. Most studies have focused on genes that encode well-established members of the SWI/SNF complex such as *SMARCA2, SMARCA4*, *SMARCB1*, and *SMARCE1* among others.[17,19,21–23,67] Here we describe a recently identified member of the SWI/SNF complex, *BICRA*, whose loss of function leads to SSRIDD-like symptoms in humans. Specifically, we identified 12 individuals carrying variants in *BICRA*, 9 of whom had loss-of-function variants and 2 of whom had missense variants. All have neurodevelopmental phenotypes that include DD, particularly of speech and motor skills, ID, autism spectrum disorder, and behavioral abnormalities as well as variable structural defects and dysmorphic features. In all cases where DNA was available from both parents, the heterozygous *BICRA* variants carried by individuals described in this study were found to be *de novo*. This is consistent with a bioinformatic program DOMINO[43] predicting *BICRA* to cause a dominant disorder. Our bioinformatic analyses suggest that *BICRA* is loss-of-function intolerant. Individuals in the DECIPHER[51] database who carry relatively small deletions involving *BICRA* have neurodevelopmental phenotypes that are similar to those documented in the individuals described here. Despite this evidence, we found five individuals who carry loss-of-function variants in *BICRA* in the gnomAD database.[42] It is possible that these variants represent sequencing errors, or that these individuals also have neurodevelopmental phenotypes but represent the milder end of the phenotypic spectrum associated with *BICRA* haploinsufficiency and were included in studies that contributed their data to gnomAD.[68]

The two individuals carrying *de novo* missense variants, subjects 11 and 12, have neurodevelopmental phenotypes but also have macrocephaly (+3 SD) and a macrocerebellum, respectively. These phenotypes are not seen in subjects 1–10 who carry loss-of-function variants. This suggests that these missense variants may represent neomorphic, dominant-negative, or other types of alleles. Macrocephaly is rare in SSRIDDs but has been seen in rare cases of *ARID1B* variants.[69] Further investigation is needed to confirm the nature of these alleles and how they may function differently from loss-of-function variants and whether there is shared etiology with *ARID1B* variants.

It is notable that the individuals described here show a less severe presentation than classical Coffin-Siris syndrome (CSS), which was the first SSRIDD to be identified.[23] These individuals display mild to moderate ID and moderate DD in contrast to other cases of SSRIDDs, particularly those with mutations in *SMARCA2* and *SMARCB1*, which show more severe ID and DD.[14,19] These phenotypes are more in line with more mild SSRIDDs such as

those caused by variants in *SMARCD1, SMARCE1,* and *SMARCA4* which present with mild to moderate ID/DD.[15,19,22] In addition, the majority of individuals described here lack the fifth digit/nail hypoplasia, which is a hallmark of most SSRIDDs.[19] However, the lack of fifth digit/nail hypoplasia has been observed for a few other SWI/SNF complex members.[19] The individuals presented here also have a distinct set of facial features when compared to the coarse features typically seen in SSRIDDs[19] (Figure 1). In line with the distinct biochemical composition and chromatin targeting patterns of ncBAF complexes,[31] these physiologic features further suggest that the ncBAF complex may have different functions than the canonical versions of the complex. Identifying variants in SWI/SNF genes that have not been associated with SSRIDDs, such as *PBRM1, BICRAL, BRD7, BRD9,* and *SS18*, may lead to a better understanding of how each subunit contributes to nervous system development, particularly *BRD9*, which encodes the only other ncBAF-specific complex member.[31,32]

The craniofacial abnormalities observed in the zebrafish mutants with a truncating allele of *bicra* are consistent with the dysmorphic facial features reported in the affected individuals described here, exhibiting measurable flattening and broadening of anterior jaw elements during larval development. Although we did not observe any obvious neurological phenotypes in these animals, such as impaired swimming behavior, previously reported expression data support a potential role for fish *bicra* in the nervous system. Indeed, a large scale *in situ* hybridization study showed that *bicra* is expressed in the central nervous system and brain,[70] similar to expression data in humans and what we observe in flies. Given the established complexities of genetic compensation in zebrafish,[71,72] along with the well-studied interplay of maternal and zygotic factors driving early zebrafish development,[73,74] we hypothesize that the absence of mutant phenotypes in *bicra*^{b1404} heterozygotes or their homozygous mutant offspring is due to a combination of genetic compensation through activation of homologous, redundant, or modifier zygotic genes, in combination with rescue by maternally contributed factors. The earliest reported transcript of *bicra* in zebrafish is in the mid-gastrula stage of development,[70] but our data support a role for maternally derived *bicra* RNA or protein in the affected developmental processes.[72]

Previous reports show that one copy loss of some SWI/SNF complex genes causes a strong suppression of variegation with the *In(1)w*$^{m4\ h}$ allele and enhancement of variegation with telomeric *w+* insertions.[55,61] We show that *Bicra* does not affect variegation with the *In(1)w*$^{m4\ h}$ allele but does enhance variegation with two telomeric insertions. These data support functional subdivisions of the three versions of the SWI/SNF complex and suggest that each complex may bind to different locations in the genome. This is consistent with our observation that *BICRA* variants cause a less severe disease compared to genes in multiple complexes like *SMARCA2/4* and *SMARCC2*, whose orthologs control PEV at both genomic locations in flies. Further comparison of where each complex binds in the genome and how each member functions may reveal novel target genes and mechanisms of SWI/SNF function.

In summary, we present evidence that loss of the chromatin remodeling protein BICRA, and the ncBAF complex, leads to a neurodevelopmental disorder. This disorder is similar to but distinct from classical SSRIDDs caused by variants in other SWI/SNF complex members. The subjects described here have different facial features and mostly lack fifth nail/digit hypoplasia, suggesting functional subdivision of the SWI/SNF complex. Considering that the function of *BICRA* and its orthologs had not been explored *in vivo* prior to this study, our work also highlights how human genetics can help to inform novel biology in model organisms.

Data and Code Availability

This study did not generate datasets.

Supplemental Information

Supplemental Data can be found online at https://doi.org/10.1016/j.ajhg.2020.11.003.

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Declaration of Interests

The Department of Molecular & Human Genetics at Baylor College of Medicine receives revenue from clinical genetic testing conducted at Baylor Genetics Laboratories. C.K. is the Scientific Founder, fiduciary Board of Directors member, Scientific Advisory Board member, shareholder, and consultant of Foghorn Therapeutics, Inc. (Cambridge, MA).

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Web Resources

FlyBase, http://flybase.org/
GenBank, https://www.ncbi.nlm.nih.gov/genbank/
OMIM, https://www.omim.org/
ZFIN, http://zfin.org

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