BICRA, a SWI/SNF complex member, is associated with a novel BAFopathy

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ABSTRACT

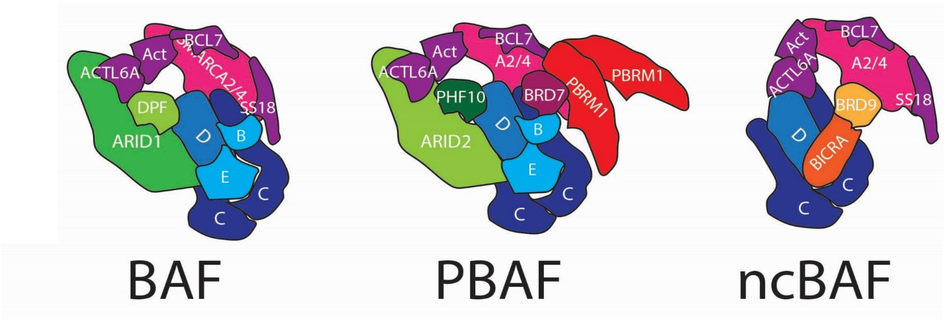
SWI/SNF-related intellectual disability disorders (SSRIDDs) are rare but severe neurodevelopmental disorders that are characterized by developmental disability, hypoplasia of the fifth digit, and coarse facial features. Variants in the members of the SWI/SNF chromatin remodeling complex cause SSRIDDs. Through the Undiagnosed Disease Network (UDN) and GeneMatcher we identified 12 individuals with symptoms similar to CSS, who carries a *de novo* frameshift allele in the gene BRD4 Interacting Chromatin Remodeling Complex Associated protein (BICRA), a non-canonical member of the SWI/SNF complex, not previously connected to disease. Here we present the first functional characterization of the *Drosophila* homolog of BICRA, *bicra,* and correspondingly the first functional characterization the non-canonical SWI/SNF complex *in vivo*. We show that Bicra binds to other non-canonical SWI/SNF complex members and is broadly expressed in neurons and glia in the nervous system. We demonstrate that, unlike other SWI/SNF complex members, loss of *bicra* is a dominant enhancer of position effect variegation at telomeres. *bicra* mutants also exhibit climbing defects at day 1 and live only for one week, both of which can be rescued by the genomic locus. Finally, we show that *bicra* plays a critical role in glia via RNAi knockdown. Together our data show that BICRA is a SWI/SNF complex member whose loss leads to a novel SSRIDD.

BACKGROUND

Chromatin modifying proteins have been connected to a large variety of cancers as well as neurological diseases like Weaver Syndrome, Ataxia-Telangiectasia, Coffin-Siris Syndrome, and Nicolaides-Bariatser Syndrome[1]. Understanding the genetic and molecular mechanisms of these diseases will be critical to developing potential treatments to these severe diseases.

Several members of the SWI/SNF complex have been associated with SSRIDDs, a series of severe neurodevelopmental disorder that are characterized by developmental disability, hypoplasia of the fifth digit, coarse facial features, and a variety of other rarer symptoms[5]. The Swith/Sucrose Non-Fermentalbe (SWI/SNF) complex is a chromatin remodeling complex that shifts the position of nucleosomes along DNA strands and thereby alters what regulatory elements are accessible to be bound by DNA binding factors[2]. In fruit flies, members of the SWI/SNF complex are part of the Trithorax group of proteins and are required for the maintenance of Hox gene expression[3]. Where else these factors bind in the genome and how they regulate nervous system development is still poorly understood. The complex can be further divided into three subcomplexes, the BAF, PBAF, and the recently identified ncBAF complex[4]. Very little is known about the similarities and differences between the function of each complex and the function of the ncBAF complex is entirely unknown.

Here we describe multiple patients with *de novo* heterozygous variants in *BICRA* (aka *GLTSCR1*), a core component of ncBAF complex, that have Coffin-Siris-like symptoms, as well as the first functional study of the ncBAF complex. Loss of *bicra* flies, leads to defects in telomeric chromatin structure, lifespan, and climbing ability. Bicra also binds to other SWI/SNF complex members in S2 cells. Together these data suggest that loss of *BICRA* causes a a novel SSRIDD.



Adapted from Mashtalir et al.[4] Structure of the three versions of the SWI/SNF complex. Only BICRA and BRD9 are unique the ncBAF complex and neither has been functionally studied. Act=Actin, A2/4=SMARCA2/4, B=SMARCB1, C=SMARCC1/2/3, D=SMARCD1/2/3, E=SMARCE1, DPF=DPF1/2/3, BCL7=BCL7A/B/C, SS18=SS18/L, ARID1=ARID1A/B, BICRA=BICRA/L

Subjects with *BICRA* LOF variant have SSRIDD-like symptoms

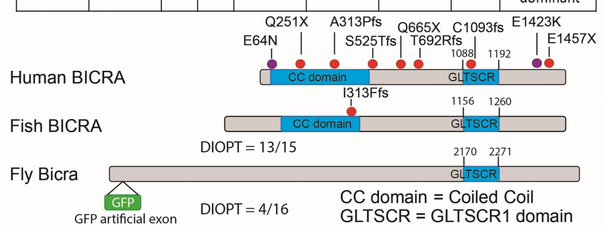
Age, sex		2 yo female	5 yo male	11 yo male	9 mo male	25 yo male	15 yo male	2 yo male	2 yo female		11 yo male	2 mo female
Variant classification	De novo	De novo		De novo	De novo	De novo	De novo	De novo	De novo		De novo	De novo
Amino acid change	Q251X	A313Pfs	S525Tfs	Q665X	T692Rfs	C1093fs	E1457X	126kb deletion	200kb deletion	Frame-shift	E64D	E1423K
Intellectual disability	+	+	+	+/-	N/A	+	+				+	+
Developmental delay	+	+	+	+	+	+/-	+	+	+	+	+	+
Microcephaly	+	-	+	-	-	+/-	+/-		+		-	-
Enlarged brain/region	-	-	-	-	-	-	-		-	+/-	+	+
Behavioral abnormalities	+	+	-	+		+	+		+/-		+	-
Autism		-	-	-	N/A	+	-		-	+	+	-
Failure to thrive	+	+	-	-	+/-	-	-	+	-		-	+
Hearing loss		-	-	+	-	-	+		-		-	-
Cleft Palate	-	-	-	-	-	-	+		-		-	-
Dysmorphic facial features	+	+	+/-	+	+	+	+		+		-	+
Sparse scalp hair		+	-	-	-	-	-		+/-			-

Description of patient phenotypes. We identified 12 patients with overlapping symptoms that carry variants in *BICRA.* 10 patients carry loss-of-function variants, a frameshift and a stop gain respectively, with two carrying missense variants. All 12 patients have neurological phenotypes similar to that of SSRIDDs but lack the hypoplasia of the fifth digit.

A

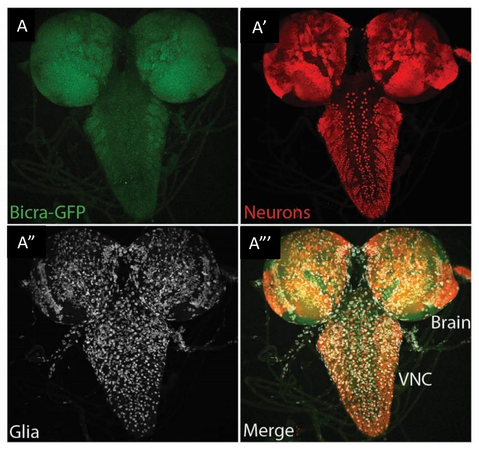
Probability of LoF intolerance (pLI)				Missense Constraint			DOMINO	
Expected	Observed	pLI score	o/e score	Expected	Observed	z score	Score	Inheritance prediction
34.5	5	0.98	0.14	823	618	2.54	0.68	Likely dominant

B



(A) Population genomic data from gnomAD[6] predict *BICRA* to be highly intolerant to loss-of-function (pLI=0.98, o/e=0.14) and missense constrained (z=2.54). (B) Structure of human and *Drosophila* BICRA. Both loss-of-function alleles delete the GLTSCR domain of BICRA. The fly homolog of BICRA has a DIOPT[7] score of 4/16 and is roughly twice the size of the human protein.

Fly Bicra is expressed in the CNS and binds to the ncBAF complex in S2 cells

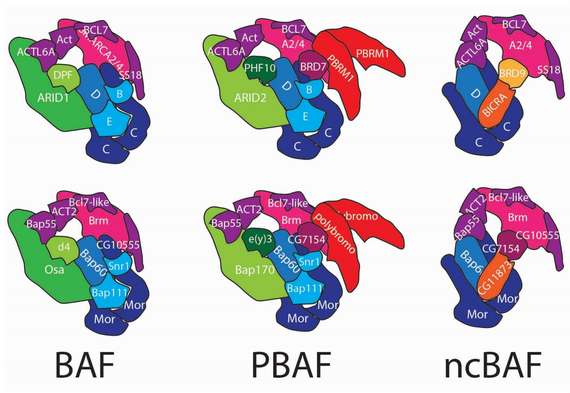


Unique	Total	reference	Gene Sym	MWT(kDa)	AVG	Human Homolog
116	287	P25439_B	brm	184.97	2.894	SMARCA2/4
91	222	Q9VF03_	mor	131.28	3.0233	SMARCC2
60	132	Q9VAP9_	CG11873	319.19	3.3799	BICRA
57	191	Q9VLX2_	CG7154	95.86	3.0779	BRD7/9
46	49	Q0E9E2_Q	PCB	132.59	3.1346	
44	58	P22700_A	Ca-P60A	111.63	3.0875	
42	114	Q9VYG2_	Bap60	58.13	2.8801	SMARCD1
42	48	P13607_A	Atpalpha	115.53	3.0986	
36	43	Q8IQV9_	Nup205	235	2.9827	
33	49	P29844_H	Hsc70-3	72.22	3.0568	
32	64	Q05825_A	ATPsyn-b	54.07	3.4444	
32	35	Q7KUT2_L	Lon	114.96	3.1407	
30	73	Q7K012_Q	Bap55	47.29	3.1217	ACT6L
28	54	P35381_A	blw	59.38	2.8156	
26	48	P11147_H	Hsc70-4	71.09	3.0585	
26	34	Q24560_T	betaTub5	50.12	3.5138	
26	28	P91875_R	RpI1	185.29	2.8316	
25	27	Q9VEX6_	bor	68.32	3.0934	
24	70	P10987_A	Act5C	41.79	3.0141	

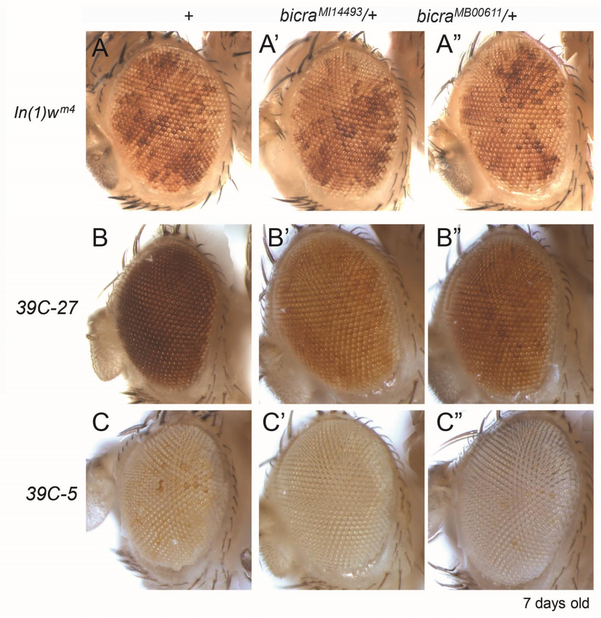
Bicra is expressed in neurons and glia in the larval brain and is localized to the nucleus. We tagged Bicra with an artificial exon containing a splice acceptor-GFP-splice donor using MIMiC based Recombination Mediated Cassette Exchange (RMCE)[8]. This cassette is located in the N-terminal region of Bicra. This allele successfully complements a deficiency that spans the *CG11873* locus and therefore does not affect the function of the protein. We observed Bicra protein was localized to nuclei and was expressed in neurons (labeled by Elav, A', A'') and glia (labeled by Repo, A", A'").

IP-Mass Spec data from S2 cells shows that Bicra binds to other SWI/SNF complex members. V5-tagged CG11873 was expressed in S2 cells and IP-Mass Spectrometry was then performed. We found that Brm (SMARCA2/4), Mor (SMARCC1/2/3), CG7154 (BRD7/9), Bap60 (SMARCD1/2/3), and Bap55 (ACTL6), all members of the ncBAF complex as elucidated in humans4, were among the top 20 unfiltered hits. This suggests that the ncBAF complex is similarly constituted in flies and humans and that CG11873 is a member of the ncBAF complex in culture.

bicra controls telomeric position effect variegation and chromatin

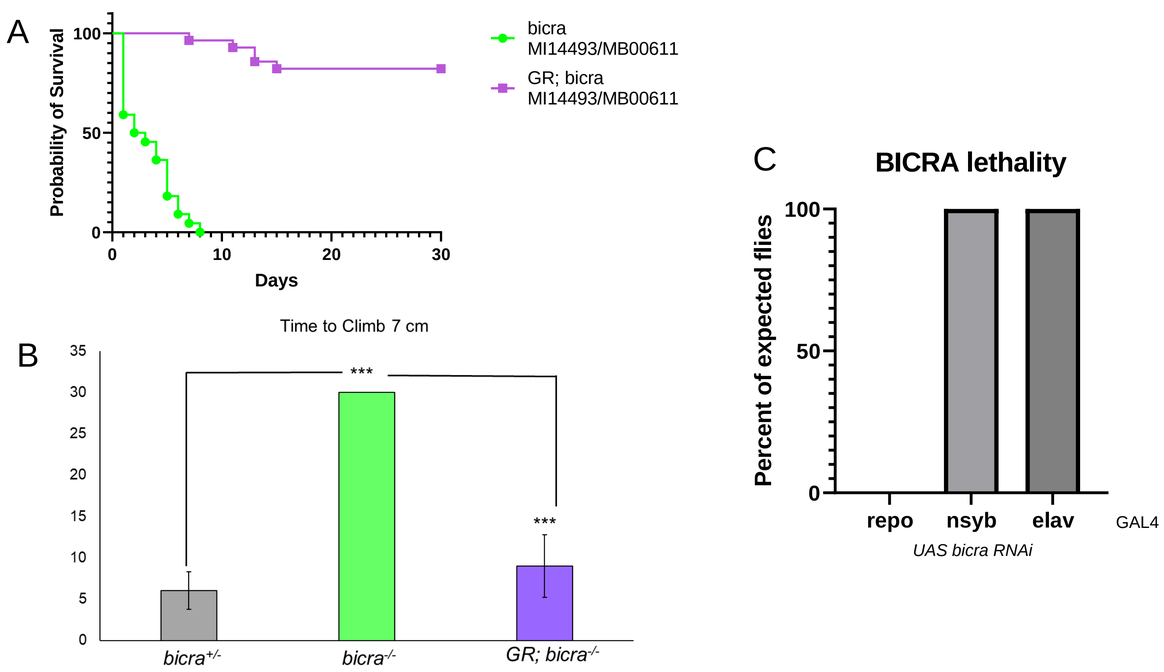


Left: structure of the known versions of the SWI/SNF complex in humans and flies[4]. CG11873 is the only member of the complex that is unique to the ncBAF complex in flies. Act=Actin, A2/4=SMARCA2/4, B=SMARCB1, C=SMARCC1/2/3, D=SMARCD1/2/3, E=SMARCE1, DPF=DPF1/2/3, BCL7=BCL7A/B/C, SS18=SS18/L, ARID1=ARID1A/B, BICRA=BICRA/L



Right: Position Effect Variegation (PEV) assay shows that *bicra* control telomeric chromatin structure. (A-A") PEV assay using the *ln(1)w*^{m4} allele[9] shows that one copy loss of *bicra* shows do effect on variegation suggesting that the ncBAF complex does not effect chromatin at this locus. (B-B") PEV assay with *39C-27 w*$^{+}$ allele[9] inserted into the telomere of 2R shows an enhancement of variegation (lightening of the eye color) upon one copy loss of *bicra*. (C-C") PEV assay with *39C-5 w*$^{+}$ allele[9] inserted into the telomere of 2L shows an enhancement of variegation (lightening of the eye color) upon one copy loss of *bicra*. These data suggests that the ncBAF complex controls telomeric chromatin structure.

bicra mutants have severe lifespan and climbing defects



Because we observed changes to telomeric chromatin in the previous assay, we next tested *bicra* transheterozygous mutants for lifespan defects (A). We observe that these mutants have a severely shortened lifespan (green line, 50% of flies are dead on day 2). Introduction of and 80kb PacMan clone covering the genomic locus of *bicra* was able to restore lifespan (purple line). (B) We next tested the flies for neurological defects and observed that at day 1 flies are unable to climb 7 cm in a vial. They do retain mobility but cannot climb. The genomic locus is able to rescue this phenotype to near wildtype levels. (C) We next explored in which cell types of the nervous system Bicra functions. To do this we knocked down *bicra* using glial (repo) and neuronal (nsyb and elav) specific GAL4 drivers. Knockdown in glia caused significant lethality at the pupal stage with no flies surviving to adulthood. Knockdown in neurons on the other hand did not cause any lethality, suggesting that *bicra* plays more important role in glia than in neurons.

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Conclusions and Future Directions

Here we present three patients with loss-of-function or missense variants in *BICRA*, who have overlapping systems. All patients have developmental delay, intellectual disability, and dysmorphic facial features, similar to SSRIDDs. When we examined fly Bicra, we found that it was localized to the nucleus of both neurons and glia in the CNS. Further, it was able to bind to other SWI/SNF complex members in culture. We also show that one copy loss of *bicra* leads to an enhancement of telomeric PEV. Correspondingly, *bicra* transheterozygous mutants have a dramatically reduced lifespan as well as climbing defects in 1-day old flies. Knockdown of *bicra* in glia caused pupal lethality suggesting a key role in glial development. In the future we plan to investigate where Bicra binds in the genome and how it affects global chromatin structure. We also plan to investigate the differences and similarities between members of the SWI/SNF complex in their expression patterns, and molecular and biological functions.