Title
Loss of the neural-specific BAF subunit ACTL6B relieves repression of early response genes and causes recessive autism.

Permalink
https://escholarship.org/uc/item/65w0b3xr

Journal
Proceedings of the National Academy of Sciences of the United States of America, 117(18)

ISSN
1091-6490

Authors
Wenderski, Wendy
Wang, Lu
Krokhotin, Andrey
et al.

Publication Date
2020-05-01

DOI
10.1073/pnas.1908238117

Peer reviewed
Loss of the neural-specific BAF subunit ACTL6B relieves repression of early response genes and causes recessive autism

Wendy Wenderskiab,c,d, Lu Wange,f,g,1, Andrey Krokhinab,c,d,1, Jessica J. Walshh, Hongjie Lid,i, Hirotaka Shojij, Shereen Ghoshf,g,1, Renee D. Georgef,g,1, Erik L. Millerab,c,d,1, Laura Elisaab,c,d,1, Mark A. Gillespief, Esther Y. Sonab,c,d,1, Brett T. Staahlab,c,d, Seung Tae Baekf,g,1, Valentia Stanelyf,g,1, Cynthia Moncadaab,c,d, Zohar Shiponyj,1, Sara B. Linkera, Maria C. N. Marchettol, Fred H. Gagef,g,1, Dillon Chenf,g,1, Tipu Sultamt, Maha S. Zakin, Jeffrey A. Ranishh, Tsuyoshi Miyakawai, Ligun Luod, Robert C. Malenkana, Gerald R. Crabtreeab,c,d,2, and Joseph G. Gleesona,e,f,g,1

*Department of Pathology, Stanford Medical School, Palo Alto, CA 94305; 3Department of Genetics, Stanford Medical School, Palo Alto, CA 94305; 3Department of Developmental Biology, Stanford Medical School, Palo Alto, CA 94305; 3Howard Hughes Medical Institute, Stanford University, Palo Alto, CA 94305; 3Department of Neuroscience, University of California San Diego, La Jolla, CA 92037; 3Howard Hughes Medical Institute, University of California San Diego, La Jolla, CA 92037; 3Nancy Priztkker Laboratory, Department of Psychiatry and Behavioral Sciences, Stanford Medical School, Palo Alto, CA 94305; 3Department of Biology, Stanford University, Palo Alto, CA 94305; 3Division of Systems Medical Science, Institute for Comprehensive Medical Science, Fujita Health University, 470-1192 Toyoake, Aichi, Japan; 3Institute for Systems Biology, Seattle, WA 98109; 3Laboratory of Genetics, The Salk Institute for Biological Studies, La Jolla, CA 92037; 3Department of Pediatric Neurology, Institute of Child Health, Children Hospital Lahore, 54000 Lahore, Pakistan; and 3Clinical Genetics Department, Human Genetics and Genome Research Division, National Research Centre, 12311 Cairo, Egypt

Synaptic activity in neurons leads to the rapid activation of genes involved in mammalian behavior. ATP-dependent chromatin remodelers such as the BAF complex contribute to these responses and are generally thought to activate transcription. However, the mechanisms keeping such “early activation” genes silent have been a mystery. In the course of investigating Mendelian recessive autism, we identified six families with segregating loss-of-function mutations in the neuronal BAF (nBAF) subunit ACTL6B (originally named BAF53b). Accordingly, ACTL6B was the most significantly mutated gene in the Simons Recessive Autism Cohort. At least 14 subunits of the nBAF complex are mutated in autism, collectively making it a major contributor to autism spectrum disorder (ASD). Patient mutations destabilized ACTL6B protein in neurons and rerouted dendrites to the wrong glomerulus in the fly olfactory system. Humans and mice lacking ACTL6B showed corpus callosum hypoplasia, indicating a conserved role for ACTL6B in facilitating neural connectivity. Actl6b knockout mice on two genetic backgrounds exhibited ASD-related behaviors, including social and memory impairments, repetitive behaviors, and hyperactivity. Surprisingly, mutation of Actl6b relieved repression of early response genes including AP1 transcription factors (Fos, Fosl2, Fosb, and Junb), increased chromatin accessibility at AP1 binding sites, and transcriptional changes in late response genes associated with early response transcription factor activity. ACTL6B loss is thus an important cause of recessive ASD, with impaired neuron-specific chromatin repression indicated as a potential mechanism.

Although present in all cells, BAF complexes orchestrate cell type-specific functions through combinatorial assembly of ~15 subunits from the products of 29 genes (5). During neural

Significance

Autism is a complex neurodevelopmental disorder whose causative mechanisms are unclear. Taking advantage of a unique cohort with recessively inherited autism, we identified six families with biallelic mutation of the neuronal-specific subunit of the BAF complex, ACTL6B (also known as BAF53b). Relative to all other genes, ACTL6B was the most statistically significant mutated gene in the recessive autism cohort. We describe autism-relevant phenotypes in human brain organoids and in mouse and fly models. We foresee the outcomes from this study will be the following: 1) a link between neuronal activity-dependent transcriptional repression and autism; 2) a characterization of mouse and fly models to study ACTL6B mutant autism; and 3) an understanding of the role of ACTL6B and nBAF complexes in neuronal transcriptional regulation.

Edited by Arthur L. Beaudet, Baylor College of Medicine, Houston, TX, and approved March 9, 2020 (received for review May 20, 2019)
development, exit from the cell cycle is accompanied by BAF subunit exchange: neural progenitor (np) subunits ACTL6A (BAF53a), DPF2/PHF10 (BAF45a/d), and SS18 are exchanged for neuron-specific (n) subunits ACTL6b (BAF53b), DPFI/3 (BAF45b/c), and SS18L1 (CREST), respectively (10–13). Subunit exchange is critical for neuronal function, as genetic deletion of either ACTL6b or SS18L1 impairs activity-dependent dendritic arborization (14, 15). Furthermore, expression of the two microRNAs, miR9* and miR124, which control BAF subunit switching, is sufficient to convert fibroblasts into neurons (13).

Recent findings link mutations in nearly every constitutive member of the BAF complex to ASD or intellectual disability (ID), including syndromic forms such as Coffin–Siris and Nicolas–Baraitser syndromes (16–18). Implicated subunits include the following (protein/gene): BAF250a/ARID1B, BAF250a/ARID1A, BAF200/ARID2, BCL11A/BCL11A, BRG1/SMARCA4, BRM/SMARCA2, BAF155/SMARCC1, BAF170/SMARCC2, BAF45a/PHF10, BAF45d/DPF2 BAF47/SMARCB1, BAF57/SMARCE1, BAF53a/ACTL6A, BAF53b/ACTL6b, BAF66a/SMARCD1, and β-actin/ACTB (17, 19–29). BAF mutant forms of ASD share overlapping clinical features such as corpus callosal hypoplasia, epilepsy, ID, lack of speech, craniofacial abnormalities, developmental delays, and fifth-digit shortening (17). The mechanisms through which BAF subunit mutations give rise to ASD are unclear.

Results

Biallelic Inherited Mutations in ACTL6B Cause Recessive Autism.

Phenotypic and genotypic heterogeneity in ASD make this a challenging disorder to study at the molecular level. Distinct molecular mechanisms may underlie social deficits and repetitive behaviors, as well as ID, epilepsy, sleep and mood disorders, hyperactivity, and systemic issues that are frequently comorbid with autism (30). Because autism mutations are predominantly de novo and can occur in genes that function in a variety of tissues during development (4, 16, 21, 31), it can also be difficult to define the relevant developmental and cellular contexts in which to study ASD mechanisms. Finally, many ASD mutations increase susceptibility but do not consistently cause autism phenotypes in humans or in animal models (32, 33).

One strategy to uncover causative mechanisms in ASD is to study families with recessively inherited autism, since it is rare for two copies (homozygous) of the same gene to segregate perfectly with recessive autism by chance. Thus, we studied 135 ASD probands from consanguineous marriages recruited for the Simons Recessive Autism Cohort (SRAC) (34–37). Genomic DNA underwent whole-exome sequencing and were compared with a cohort of 256 controls with recessive neurodevelopmental disease (NDD) without ASD (SI Appendix, Supplementary Text). To identify significantly mutated genes, we generated quartile–quartile (Q–Q) plots comparing the observed to expected number of damaging recessive genotypes (deletion, frameshift/stop, or damaging missense) in the SRAC vs. non-ASD recessive NDD cohorts. When considering all coding genes, only ACTL6b, a subunit of the nBAF complex, and CD36, a fatty acid translocase and scavenger receptor (38), showed genome-wide significance for mutations in the SRAC (Fig. 1 A and C). Limiting to just loss of function (LoF)-intolerant genes (i.e., those with pLI ≥ 0.9) left only ACTL6b with genome-wide significance (P < 10−16) (SI Appendix, Fig. S1A). A similar statistical analysis was conducted on the non-ASD NDD cohort of 256 individuals of similar genetic background, matched for consanguinity. The non-ASD NDD cohort showed enrichment for genes previously implicated in non-ASD NDD (39) but not ACTL6b (Fig. 1B and SI Appendix, Fig. S1B). To test whether ACTL6b mutations were enriched in a genetically distinct recessive autism cohort, we ranked ordered 409 genes with recessive missense variants in the Autism Sequencing Consortium by their RAFT P value and found that ACTL6b was the sixth most significantly mutated gene (SI Appendix, Fig. S1C) (25). Individuals with recessive ASD-like phenotypes linked to biallelic ACTL6b mutations have also been identified in recent case studies (29, 40, 41). Cumulatively, these findings implicate mutations in ACTL6b as an important cause of recessive autism.

Focusing on ACTL6b, we more closely examined the patient phenotype and sought to understand how mutations in this gene give rise to autism. Six families representing 4.4% of the SRAC cohort demonstrated homozygous variants in ACTL6b with fully penetrant recessive inheritance (Fig. 1 D and SI Appendix, Table S1). Variants were not present in ExAC or gnomAD databases, nor in ethnically matched controls from the Greater Middle East Variome or the cohort of 256 controls (42, 43), indicating that they are rare variants. Subjects exhibited nonverbal autism with stereotypes, as well as ID, developmental delay, hyperactivity, and mild spasticity (SI Appendix, Table S2 and Movie S1). There were no syndromic features that would have identified these patients as unique a priori from other SRAC subjects (SI Appendix, Table S2 and Supplementary Text). Epilepsy was seen in all subjects, presenting at 2 wk to 5 y of age, and generally responded to anticonvulsant medication. Males and females were similarly affected, consistent with fully penetrant inheritance. While the SRAC patients were of Middle Eastern descent, similar phenotypes have been reported for ACTL6b mutant individuals of French-Canadian, Sicilian, and Finnish descent (29, 40, 41, 44, 45). The high penetrance and perfect segregation of mutant alleles with ASD indicate a causal relationship between ACTL6b mutation and autism. This distinguishes ACTL6b from most other autism-associated genes, which substantially increase risk when mutated, but alone may not be sufficient to cause disease.

Patient Mutations Destabilize ACTL6b and Reduce Its Incorporation into the BAF Complex.

Consistent with the recessive mode of inheritance, patient variants were protein truncating, frame shifting, or missense for highly conserved residues (SI Appendix, Fig. S2 A–C). Variants occurred throughout the open reading frame, arguing against dominant-negative effects. Gibbs free energy calculations revealed that the missense variants likely disrupt protein folding, in line with PolyPhen-2 “damaging” predictions (SI Appendix, Fig. S2D). To visualize how the patient mutations are oriented in relation to the BAF complex, we generated a protein model of human ACTL6b binding to the conserved HSA domain of SMARCA4. This revealed two patient missense variants (L154F and G393R) located in residues that are critical for stabilizing the hydrophobic binding interface between ACTL6b and SMARCA4 (SI Appendix, Fig. S2E), consistent with a LoF disease mechanism.

To test these predictions in vitro, we assessed ACTL6b missense mutant protein expression and incorporation into BAF complexes in the HEK293T cell line, human embryonic stem cells, and Actl6b−/− primary mouse neurons. To rule out the effects of species or cellular context, we also derived induced pluripotent stem cells (iPSCs) from control or affected humans in family 2703 (ACTL6b154F/154F) and conducted neural differentiation over 8 wk. RNA sequencing (RNA-seq) of (n = 3) control individuals confirmed that the switch of progenitor BAF subunits for nBAF subunits (11) was conserved in humans, as indicated from down-regulation of nonneuronal ACTL6A and up-regulation of ACTL6b (SI Appendix, Fig. S3 A and B). In each cellular context, ACTL6b mutant protein expression was dramatically reduced relative to wild type, with little to no mutant protein incorporated into neuronal or nonneuronal BAF (Fig. 2 A–D and SI Appendix, Fig. S4 A–E). These data suggest that the patient mutations destabilize ACTL6b protein and result in the formation of nBAF complexes that lack ACTL6b.
Patient Mutations Produce LoF “Perfect Dendritic Retargeting” Phenotype in Fly Brain. Social communication is a key defect in ASD (30). In insects, social communication is dependent upon olfactory cues (46) and specifically olfactory projection neurons, which transmit signals to specific glomeruli in a stereotyped manner. Previously, human ACTL6B was shown to genetically complement the fly ortholog Bap55 and rescue a LoF “perfect dendritic retargeting” phenotype in the fly olfactory system, where dendritic trees project cell autonomously to the wrong glomerulus with 100% expressivity (47). This results in a switch in synaptic specificity that may affect how the fly interprets chemical cues. Using the mosaic analysis with a repressible cell marker (MARCM) method (48), we simultaneously replaced the fly ortholog with human wild-type or ASD mutant ACTL6B and expressed GFP in single neurons or neuroblast clones (SI Appendix, Fig. S5A). We found that wild type but not patient ACTL6B missense alleles quantitatively rescued targeting (Fig. 2 E and F and SI Appendix, S5 B and C), confirming that patient mutations show LoF in vivo and suggesting that altered synaptic specificity may contribute to the ACTL6B autism phenotype.

Defects in Callosal Anatomy in Humans and Mice Lacking ACTL6B. Perfect dendritic retargeting in ACTL6B mutant flies may be mechanistically related to a distinctive clinical feature of BAF mutant ASD: reduced or absent corpus callosum (17, 49). This defect involves a failure of axons to cross to the opposite cerebral hemisphere and is often associated with mutations in guidance molecules such as semaphorins, which also direct dendritic and axonal targeting in flies (50–52). Hypogenesis of the corpus callosum may further reflect altered functional connectivity, as has been detected by resting-state functional MRI in some ASD patients (53, 54). We compared available clinical brain MRIs (axial and midline sagittal) to identify evidence of altered brain anatomy in autistic patients with ACTL6B mutations. Compared with a healthy control, affected individuals showed reduced cerebral white matter volume and most showed corpus callosum hypoplasia (Fig. 3 A and D). 

Aktl6b knockout mice have thinner myelin sheaths (14), consistent with the reduction in white matter in ACTL6B patients. This prompted us to examine Aktl6b knockout mice for evidence of reduced or absent corpus callosum. We immunostained coronal sections of adult mouse brain for neurofilament and

Fig. 1. Biallelic mutations in ACTL6B cause recessive autism. (A) Q-Q plot showing the observed/expected number of mutations for all coding genes in the Simons Recessive Autism Cohort (n = 135) and CD36 were significantly mutated. (B) Q-Q plot showing the observed/expected number of mutations for all coding genes in a genetically matched non-ASD recessive neurodevelopmental cohort, where ACTL6B was not found to be enriched. (C) ACTL6B encodes a tissue-restricted subunit of the neuronal (nBAF) BAF complex. Representation of the multisubunit nBAF complex containing ubiquitously expressed subunits (gray), a core ATPase subunit (dark blue), and neuronal-specific subunits (yellow) including ACTL6B in bold. The balls on a string represent nucleosomes. (D) Recessive ASD inheritance with ACTL6B mutations in six independent consanguineous families. Double lines, first cousin status; squares, males; circles, females; slash-through, mortality; black fill, ASD. Missense variants (green) and truncating variants (blue). Obligate carriers depicted with dot at center of symbol.
Thus, like other BAF subunits, differences in cortical thickness were observed (Fig. 3) indicating that this is a recessive phenotype in mice. No significant changes in weight or body mass were observed (SI Appendix, Fig. S5 A and B). (C) Patient missense mutant proteins were less stable than wild-type ACTL6B when expressed in HEK293T cells; Patient mutations destabilize ACTL6B protein and cause a loss-of-function phenotype.

**Mice Exhibit Autism-Related Behaviors in Two Genetic Backgrounds.** Because the patient mutations were LoF and Actl6b−/− mice showed no difference in cortical thickness, indicating that this is a recessive phenotype in mice. No significant differences in cortical thickness were observed (Fig. 3D). Reduced corpus callosum volume has also been reported in mice lacking one copy of the ASD-related BAF subunit Arid1b (55). Thus, like other BAF subunits, ACTL6B is required for corpus callosum formation in humans and mice.

We assessed social behavior, a hallmark of autism, using a juvenile interaction test and three-chamber sociability assay (Fig. 4 A and B). The juvenile interaction test measured the amount of time 7–8-week-old test mice spent interacting with a 4-week-old juvenile wild-type mouse of the same sex. This revealed significant and gene dosage-dependent decreases in social interaction in both male and female C57BL/6 mice (P < 0.0001 for male or female wild type vs. Actl6b−/− or Actl6b+/-; P = 0.011 for Actl6b−/− vs. Actl6b−/− males, P = 0.0047 for Actl6b−/− vs. Actl6b−/− females by one-way ANOVA; Fig. 4C). These results were specific for social defects, because replacing the juvenile mouse with a novel toy mouse revealed no differences in interaction across genotypes (Fig. 4D). Similarly, male knockout mice on C57BL/6 × 129SvEv background showed significantly reduced social interactions with an adult mouse of the same sex and genotype (P = 0.0208, Student’s t test; SI Appendix, Fig. S6 A and B), suggesting a role for Actl6b in murine social interaction that is independent of genetic background.

In the three-chamber assay for sociability, animals were given the choice of inhabiting a chamber containing a novel juvenile mouse in a cage or a novel, empty cage (Fig. 4B). Social preference scores, calculated from the ratio of time spent in each chamber, indicated that Actl6b−/− and Actl6b+/− mice were less sociable compared to wild types (for males, P = 0.025 for wild type vs. Actl6b+/− and P = 0.048 for wild type vs. Actl6b−/−; for females, P = 0.038 for wild type vs. Actl6b−/− and P = 0.0037 for wild type vs. Actl6b−/− by one-way ANOVA; Fig. 4E). Female
mice exhibited a trend toward increasing social avoidance with decreasing Actl6b gene dosage. These results suggest a direct role for Actl6b in social interaction and sociability.

Patients with autism also display restricted or stereotypic (repeated) behaviors. We thus assessed stereotypy in the open-field test in male littermates (Actl6b+/−, Actl6b−/−, C57BL/6 F1 × Actl6b+/−, C57BL/6 F1 cross) using repeated breaks of the same photobeam (stereotypic counts). Actl6b+/− mice showed significantly elevated stereotypic counts, consistent with increased repetitive behaviors (P < 0.0001, Student’s t test; Fig. 4F).

ID affects 45% of all autism patients (30), is a consistent feature of BAF mutant ASD (17), and was present in affected individuals with ACTL6B mutations. We therefore conducted the Barnes maze test for spatial memory (Fig. 4G) and the T-maze forced alternation test for working memory (SI Appendix, Fig. S6C). These forms of memory depend upon neural circuits involving the hippocampus and prefrontal cortex, respectively (56, 57). Both tests revealed significant memory impairment in male Actl6b−/− mice (129S6/SvEv × C57BL/6 F1 cross) (P = 0.0003 for Barnes maze and P < 0.0001 for T-maze forced alternation, Student’s t test; Fig. 4G and SI Appendix, Fig. S6 D and E), consistent with previous studies showing impaired hippocampal memory consolidation, striatum-dependent cocaine-conditioned place preference, and amygdala-based fear learning in heterozygous Actl6b+−/− mice (58–60). We conclude that Actl6b is required for memory formation in multiple neuronal systems.

Hyperactivity and anxiety are common comorbidities in ASD that show bias toward males and females, respectively (30). However, all affected individuals with ACTL6B mutations showed hyperactivity. We recorded the location and distance mice traveled in an open field to assess anxiety and activity levels (Fig. 4A and SI Appendix, Fig. S6F). Male Actl6b−/− mice showed little clear trend in anxiety as indicated from relative time spent in the open field center; however, female Actl6b−/− were more anxious than their heterozygous or wild-type sisters (P = 0.0017 for Actl6b−/− vs. Actl6b+/− and P = 0.064 for Actl6b−/− vs. wild type, one-way ANOVA; SI Appendix, Fig. S6 G and H). Total distance traveled in the open field indicated that Actl6b−/− mice of both cohorts and sexes traveled more than three times greater distance than their littermates, suggestive of a recessive hyperactive phenotype (P < 0.001 in all comparisons to Actl6b+/− for each sex, one-way ANOVA; Fig. 4H, SI Appendix, Fig. S6 I–L, and Movies S2 and S3). We conclude that Actl6b−/− mice robustly model general autism-related behaviors and behaviors that are characteristic of ACTL6B mutant ASD.

Actl6b−/− nBAF Complexes Retain the Nonneuronal Variant Actl6a and Have Reduced Affinity for Neuronal Chromatin. Having established conserved roles for ACTL6B in mammalian social behavior and neural circuit formation, we sought to uncover the molecular function of ACTL6B and explore disease-relevant mechanisms. In mouse and human, ACTL6B expression is restricted to neurons, with lower expression in testes (10, 61). Previous studies have indicated that the majority of ACTL6B protein is likely associated with the nBAF complex (11, 12, 14). Because ACTL6B mutant proteins were not incorporated into nBAF complexes, we considered that the assembly of nBAF might be affected by the loss of Actl6b. We immunoprecipitated Actl6b+/− or wild-type nBAF complexes with the J1 antibody, which recognizes both Smarca2 and Smarca4 (62), and then digested and labeled peptides with “heavy” Δ4 (+13C15N) or “light” Δ0 (12C14N) mTRAQ reagent, respectively. Peptides from each complex were pooled 1:1 and analyzed by mass spectrometry, where +4-Da shifted peaks indicated Actl6b−/− peptides (SI Appendix, Fig. S7A). Normalized automated statistical analysis of protein (ASAP) ratios of heavy knockout to light wild-type peptides were generated (Protein Prophét Probability cutoff > 0.9) such that values <1 indicated reduced interactions and >1 indicated stabilized interactions in Actl6b−/− nBAF complexes (SI Appendix, Fig. S7 B–D). The total number of peptides from the pool of wild-type and knockout complexes was also recorded. Because BAF complexes are highly abundant in the nucleus...
shown. (B) Social preference scores were calculated from time spent in each zone using the formula social preference = time (Interaction zone - Control zone) / time (Interaction zone + Control zone)

** Fig. 4. Actl6b−/− mice exhibit autism-related behaviors.** (A) Social interaction and control tests: relative interaction time between the adult test mouse and a juvenile mouse (3–5 wk) or novel object. Open-field test measured activity over 20 min as total distance traveled. (B) Schematic of three-chamber sociability assay: Test mice may enter the zone with a novel object or the zone with a novel juvenile mouse. Social preference scores were calculated from time spent in each zone using the formula shown. (C) Box plots showing male and female littermates of Actl6b−/− × Actl6b−/− crosses with gene dosage-dependent impairment in social interaction with a juvenile mouse but not with (D) a control novel object. (E) Actl6b−/− and Actl6b−/+ mice of both sexes showed defects in sociability, which were most severe in female knockouts. (F) Single, repeated photobeam breaks or “stereotypic counts” represented by a small red box in an open field. Male Actl6b−/− mice showed repetitive movements indicated from increased stereotypic counts over 120 min. (G) Diagram of Barnes maze test of spatial memory: an elevated, white, circular open field containing 12 holes, with one “target” escape hole leading to a comfortable cage. Visual cues provided the mouse with a frame of reference for the location of the target hole. (H) Social preference = time (Interaction zone - Control zone) / time (Interaction zone + Control zone)
(300,000 per cell), its interactions with low abundance proteins (e.g., transcription factors) of <10,000 per cell are difficult to detect. Thus, we included proteins recovered as only a single peptide (62) as a resource, but with cautious interpretation.

Proteomic analysis of Actl6β−/− nBAF complexes indexed complete assembly of nBAF subunits, with peptides specific to the nonneuronal homolog Actl6a recovered in place of Actl6b. The Actl6b ASAP ratio ~2 and nearly equivalent peptide counts for Actl6a (in knockout) and Actl6b (in wild type) suggested that Actl6β−/− nBAF complexes fully retain Actl6a (SI Appendix, Fig. S7B). Mutant nBAF complexes showed reduced interaction with the abundant chromatin protein histone H1 and with autism-related proteins including Kat6a/Myt3 and Adnp, both confirmed BAF interacting proteins (63, 64) (SI Appendix, Fig. S7C). Several proteins exhibited stronger interactions with mutant than wild-type complexes (SI Appendix, Fig. S7D), including Wiz, a promoter binding protein previously linked to abnormal social behavior in mouse (65). The proteomic data indicate that Actl6b loss results in the formation of an abnormal nBAF complex containing Actl6a with reduced interactions with established binding partners and gained interactions with novel partners. Actl6a and Actl6b proteins share 95% sequence similarity except in the 43-residue subdomain 2, which shows only 53% sequence similarity (66). We previously showed that Actl6a cannot rescue neuronal function of nBAF in Actl6β−/− cells, and neither can a chimeric Actl6b containing the subdomain 2 of Actl6a (14). To confirm misincorporation of Actl6a into nBAF in the absence of Actl6b, we assessed Actl6a expression and incorporation into nBAF complexes in postnatal day 0 (P0) brain tissues from wild-type, Actl6a−/−, Actl6b−/−, and Actl6b+/− mouse littersmates. We also confirmed Actl6b deletion in Actl6b−/− neurons (SI Appendix, Fig. S9C).

To learn whether ACTL6A was transcriptionally up-regulated in affected human neurons, we measured expression in brain organoids, an improved culture method for modeling human brain development (67). We first confirmed that human brain organoids express ACTL6A and ACTL6B during maturation in controls at day 69 (SI Appendix, Fig. S8A). Brain organoids from unaffected (ACTL6B+/−),−/− or two affected (ACTL6B−/−,−/−) individuals of family 2703 were cultured to day 28 and harvested for RNA or immunostained for developmental markers of neural progenitors (Sox2) and immature neurons (Tuj1). No gross differences in development were observed between unaffected and affected brain organoids (SI Appendix, Fig. S8B). However, brain organoids from two affected individuals displayed about a three-fold increase in ACTL6A transcript compared to the unaffected control (SI Appendix, Fig. S7H). These data support a conserved mechanism in which ACTL6A expression is increased transcriptionally in the absence of functional ACTL6B, leading to the formation of an abnormal nBAF complex.

The incorporation of Actl6a into Actl6β−/− nBAF was associated with reduced interactions between mutant complexes and histone H1 (SI Appendix, Fig. S7C), suggesting that mutant nBAF complexes may have abnormal interactions with chromatin. To test this, we fractionated chromatin from embryonic day 18.5 (E18.5)/day in vitro 7 (DIV7) wild-type vs. Actl6β−/− cortical neurons using increasing concentrations of salt (300–700 mM NaCl) and blotted for nBAF in the resulting soluble nuclear or chromatin fractions. We found that Actl6β−/− nBAF complexes were not as stably associated with chromatin as wild type across a wide range of salt concentrations (68) (SI Appendix, Fig. S7J), suggesting impaired BAF–chromatin interactions as a result of altered nBAF candidacy.

ACTL6B Suppresses the Activity-Responsive Transcriptional Program in Resting Neurons. Actl6b is required in neurons for activity-dependent dendritic outgrowth and long-term potentiation (14, 58), processes that require transcription and facilitate learning and memory (69). Targeted chromatin immunoprecipitation experiments in neurons have suggested that nBAF complexes are bound to the promoter of the immediate early gene Fos and may regulate its expression (70). However, genome-wide expression studies in postmitotic neurons have yielded inconsistent insights into the molecular function of Actl6b and the neuronal BAF complex (58, 71).

To clarify the role of ACTL6B in transcriptional regulation, we conducted RNA-seq and ATAC sequencing (ATAC-seq) on primary cortical neurons from wild-type or Actl6b+/− E16.5 embryos cultured for 7 DIV (72). Neurons from n = 5 wild-type and n = 7 knockout biological replicates (littermates) were collected after 2-h treatment with TTX/APV, which silenced network activity in order to reflect the resting state (73). RNA libraries were prepared from a fraction of the neurons in each biological sample, while another fraction was transposed with Tn5 to cut and tag accessible chromatin for sequencing (ATAC-seq) (74) (Fig. 5A and Datasets S1 and S2). The quality of collected data were confirmed by principal-component analysis, which demonstrated separation of biological samples by genotype in both RNA-seq and ATAC-seq datasets (SI Appendix, Fig. S9 A and B). We also confirmed Actl6b deletion in Actl6b−/− neurons (SI Appendix, Fig. S9C).

Comparing mRNA expression in wild-type vs. Actl6b−/− neurons, we identified 503 genes with increased expression and 383 genes with reduced expression in the absence of Actl6b (false-discovery rate [FDR] < 5%; absolute log fold change > 0.5) (Fig. S8B). Gene coexpression analysis revealed that down-regulated genes frequently coexpress with ZNF821 (Fig. S5C), a transcription factor that was previously linked to methamphetamine-associated psychosis (75). Up-regulated genes in Actl6b−/− frequently coexpress with early response transcription factors Nrl4a2 and members of the API family including Fosb and Jun. This was surprising because early response transcription factors are normally expressed at very low levels in resting neurons. During neural activity, they are rapidly induced and subsequently regulate the expression of “late response” genes, which encode proteins that support neural plasticity (76).

The results from coexpression analysis prompted us to explore a connection between transcriptional changes in Actl6β−/− and transcriptional changes due to neural activity. To define activity-responsive genes, we conducted RNA-seq on wild-type E16.5/DIV7 cortical cultures that were silenced with TTX/APV for 1 h and then stimulated for 1 or 6 h with 55 mM KCl to model neural activity (n = 5 biological replicates each for 1-h KCl and 1-h control, n = 3 for 6-h KCl, and n = 4 for 6 h control). In wild-type mice, we identified 534 up-regulated and 237 down-regulated “early response” genes at 1-h KCl; and 2,603 up-regulated and 2,287 down-regulated late response genes at 6-h KCl (FDR < 5%, absolute log fold change > 0.5) (SI Appendix, Fig. S10 A and B). Over 40% of differentially expressed genes in Actl6β−/− neurons could be classified as “activity responsive” (Fig. 5D). Of the 66 early response genes that were differentially expressed in resting Actl6β−/− neurons, 94% followed a pattern of expression expected during neural activity (Spearman correlation
**Neuronal & stimulus-responsive genes**

Neurons that were stimulated for 1 or 6 h with 55 mM KCl. Early response genes showed altered expression after 1-h KCl stimulation in wild type (KO; $|\text{log}_2 \text{fold change}| > 10062$) from ($\text{WT}$) coexpress with early response transcription factors such as Pou5f1, Fos, Jun, and their late response target gene Gdf5. (A) Experimental design: Primary E16.5 DIV7 cortical cultures from ($n = 5$) wild-type or ($n = 7$) Actl6b−/− littermates were treated for 2 h with TTX/APV to silence action potentials and represent the “resting” neuronal state. RNA was collected to measure transcription by RNA-seq and DNA was transposed with Tn5 to measure chromatin accessibility by ATAC-seq. (B) MA plot of transcriptional changes in resting Actl6b−/− (KO) neurons relative to wild type (WT). Differentially expressed genes showing FDR < 5% and absolute log2 fold change >0.5 were defined as “significant” and highlighted in red. Gene names are shown for the top 30 most significant genes. (C) Transcription factor coexpression analysis was performed on the significantly up- or down-regulated genes in resting Actl6b−/− (KO) neurons. Up-regulated genes commonly coexpress with early response transcription factors such as Jun, N4a2, and Fosb, indicating possible activity-dependent transcription factor activity. (D) Heat maps showing log2 fold changes for genes that were both significantly differentially expressed in resting Actl6b−/− (KO) neurons and in wild-type (WT) neurons that were stimulated for 1 or 6 h with 55 mM KCl. Early response genes showed altered expression after 1-h KCl stimulation in wild type (SI Appendix, Figs. S10A) and late response genes showed altered expression after 6-h KCl stimulation in wild type (SI Appendix, Fig. S10B). Representative genes from each group are labeled. Transcriptional changes in resting Actl6b−/− neurons significantly correlated with activity-induced responses in wild type. (E) mRNA expression of activity-responsive genes in the biological samples used for RNA-seq, measured by RT-qPCR. AP1 transcription factors Fos, Fosb, and their late response gene Vgf were significantly increased in resting Actl6b−/− neurons. (F) mRNA expression of activity-responsive genes in 28-d-old cerebral brain organoids cultured from induced pluripotent cells of an unaffected father (ACTL6BL154F/+) and his two affected children (ACTL6BL154F/L154F) in family 2703, measured by RT-qPCR; n = 3 technical replicates per individual. AP1 transcription factors FOS, FOSB, and their late response target gene VGF were significantly increased in affected human brain organoids. (G) Chromatin accessibility was assayed by ATAC-seq as described in A, and HOMER de novo motif analysis was performed on the significantly increased or decreased sites in Actl6b−/− (KO) neurons. Sites with increased chromatin accessibility were selectively enriched for the AP1 transcription factor binding motif, indicated by FRA1. (H) Summary model: Autism mutant ACTL6B “B” proteins are unstable and rapidly degraded, leading to retention of the nonneuronal homolog ACTL6A “A” in the nBAF complex. The loss of ACTL6B relieves transcriptional repression on early response transcription factors but increases repression on repetitive elements in resting neurons. mRNAs encoding early response transcription factors, particularly those in the AP1 family, are translated into proteins that regulate the expression of late response neuronal genes. Multisubunit nBAF complexes containing ubiquitously expressed subunits (gray), a core ATPase subunit (dark blue), neuronal-specific subunits (yellow), and neural-progenitor subunit ACTL6A (purple). AP1 transcription factor proteins are shown in pink. Balls on a string indicate nucleosomes. The arrow represents transcriptional activation; T represents transcriptional repression. Dashed lines in H indicate that the mechanism of repression may be direct or indirect. Significance was calculated by Spearman rank correlation in D, individual Student’s t tests in E, and two-way analysis of variance (ANOVA) in F. Error bars indicate SEM. *P < 0.05; **P < 0.01; ***P < 0.001.
KCl stimulation, repetitive element transcripts were strongly de-repressed while repetitive elements were more silenced in the absence of Actl6b.

Since activity-dependent transcription is required for long-term memory formation (69, 76), the inappropriate activation of activity responsive genes in resting Actl6b−/− neurons is a plausible explanation for impaired memory in Actl6b mutant mice (Fig. 4G and SI Appendix, Fig. S6 C–E) (58–60, 79) and in affected humans (SI Appendix, Table S1) (29, 40, 41, 44, 45, 80–82). However, it is unclear whether this mechanism can account for other aspects of the ACTL6B patient phenotype, such as social deficits or corpus callosum hypogenesis. To gain deeper insight into the molecular changes in Actl6b−/− neurons and how these relate to aspects of the ACTL6B mutant phenotype, we used the Enrichr (83, 84) tool to perform a set of enrichment analyses on up-regulated and down-regulated genes in mutant neurons. The most enriched term for molecular functions of up-regulated genes in Actl6b−/− neurons was transcription factor activity, consistent with the DNA binding activity of many early response genes (SI Appendix, Fig. S13A). Enrichment analysis for down-regulated genes highlighted terms related to the nucleosome remodeling and histone deacetylase (NuRD) complex, semaphorin signaling, and genes involved in ion balance. These terms are consistent with altered chromatin repression, impaired axon guidance, and seizure activity, respectively. Biological pathway analysis revealed that up-regulated genes in Actl6b−/− neurons were involved in neuroactive ligand–receptor interactions and in serotonin and anxiety-related events (SI Appendix, Fig. S13 B and C). Genes associated with these terms included stress-related corticotropin releasing hormone (Cnrh, also known as CRF) and its receptor Cnrh1, as well as serotonin receptors 1b (Htr1b), 2c (Htr2c), and 3a (Htr3a). CRF administration can induce seizures in rats (85), and both CRF and serotonin have been implicated in memory and social behaviors (86, 87). Pathway analysis of down-regulated genes identified roles in axon guidance and fatty acid biosynthesis, in line with callosal defects and loss of white matter observed in ACTL6B mutant humans and mice (Fig. 3 A–C). Enrichment analysis thus revealed potential mechanisms underlying the corpus callosum defects, seizures, memory, and social impairments in ACTL6B mutant humans and mice.

Discussion

We identified ACTL6B as the most significantly mutated gene in the SRAC, indicating that mutations in this gene may be a relatively common cause of recessive autism. In line with this finding, ACTL6B was the sixth most significantly mutated gene out of 409 genes with recessive missense variants in the Autism Sequencing Consortium (25), and recent case studies have identified individuals with biallelic ACTL6B variants showing similar recessive neurodevelopmental phenotypes to the patients we describe. Severe epilepsy, ID, lack of speech, developmental delays, feeding difficulties, microcephaly, hypomyelination, and corpus callosum hypogenesis were consistent clinical features associated with ACTL6B LoF (29, 40, 41, 44, 45, 82). One patient was diagnosed with Rett syndrome (82). Several patients passed away before 10 y of age (29, 41). Patients were not uniformly diagnosed with autism, possibly due to the severity of the phenotype or to differences in diagnostic criteria. ACTL6B mutations segregated perfectly with recessive phenotypes in a variety of genetic backgrounds (Middle Eastern, French Canadian, Finnish, Sicilian) and in children of both consanguineous and nonconsanguineous parents, affirming high penetrance.

Interestingly, two dominant ACTL6B alleles (G343R or D77G) were recently identified in patients with autism who did not experience seizures but otherwise exhibited characteristics of the recessive ACTL6B patients (29). In a separate study of genetic causes of ID, two affected individuals were found to have inherited the G343R mutation from their intellectually disabled carrier parents.

Wenderski et al. PNAS | May 5, 2020 | vol. 117 | no. 18 | 10063
father (80). The dominant G343R mutation was also identified as a de novo mutation in large screens for genetic causes of developmental delays, ID, and seizures (81, 88, 89). These dominant alleles may act on the wild-type copy protein or may poison the function of the nBAF complex, although further research is needed to explore these possibilities. In addition, one patient diagnosed with ASD was found to have a postzygotic mosaic, synonymous mutation in ACTL6B (90). Including the present study, there are at least 38 recessively affected and 19 dominantly affected individuals documented with ASD-related phenotypes caused by mutations in ACTL6B (25, 29, 40, 41, 44, 45, 80–82, 90, 91).

Deletion of the fly ortholog of ACTL6B in olfactory projection neurons resulted in cell-autonomous rewiring of the olfactory system in a manner that might cause the fly to misinterpret chemical social cues. Altered semaphorin signaling may underlie this defect, as deletion of SemalA in fly olfactory neurons results in similar but less specific dendritic rewiring to the olfactory system, increasing while the severity of social behavior deficits increased with decreased peractivity and callosal defects showed recessive inheritance, suggesting a connection with the callosal hypogenes we observed in humans and mice lack ACTL6B.

We also found that Actl6b knockout mice exhibit similar behaviors to ACTL6B patients, including social behavior deficits, increased repetitive movements, memory impairments, and hyperactivity. Autism mouse models 1p11.2, Shank3, Ctnnap2, Ptnc1, Tsc1, En2, and Gabrb3, show similar defects to Actl6b knockout mice in the juvenile interaction and three-chamber assays (32, 87, 94), but unlike some of these models, behaviors in Actl6b−/− mice were robust on two genetic backgrounds. Hyperactivity and callusial defects showed recessive inheritance, while the severity of social behavior deficits increased with decreasing Actl6b gene dosage. Previous studies showed memory defects in heterozygous mice (58–60), suggesting dosage sensitivity; however, the heterozygous relatives of patients here showed no overt symptoms. Approximately 40% of gene expression changes in electrically silenced semaphorin receptors reflected transcriptional responses to neural activity, indicating that the de-repression of early response transcription factors like API may be a primary defect in ACTL6B mutant neurons (Fig. 5H). Similarly, heterozygous mutations in the BAF ATPase SMARC4 that cause autism-related Nicolaides–Baraitser syndrome were found to de-repress FOSL2 and lead to API pioneer factor activity and BAF recruitment to enhancers in human neural progenitor cells (95). That mutation of either ACTL6B or SMARC4 results in elevated API expression suggests that the mechanism for de-repression involves a functional breakdown of the BAF complex. This is consistent with the fact that BAF mutations cause similar forms of ASD (23, 29, 96). We speculate that abnormal API activation may be a common mechanism in BAF mutant ASD.

In summary, we show that LoF mutations in ACTL6B cause recessive autism in humans, patient-related phenotypes in mice and flies, and abnormal expression of early response genes, repetitive elements, semaphorins, CRF, and serotonin receptors in mouse neurons. The mechanism may involve the formation of nBAF complexes containing the nonneuronal homolog Actl6a, which have reduced affinity for neuronal chromatin. The genetic background and gender dependence of many genes implicated in autism have made biochemical analysis difficult because one would expect that any biochemical event underlying behavior would be subject to undetermined variables. In contrast, the genetic and gender background independence of ACTL6B mutations in both humans and mice should provide a powerful model for analysis of the role of chromatin regulation in autism.

Materials and Methods

This study was approved by the institutional review boards of University of California, San Diego, for human subjects, and by the animal review board of Stanford University and Fujita Health University. Blood, skin punch, and clinical data were obtained from research subjects after obtaining informed consent. Detailed description of the patient clinical history, generation of iPSCs, methods for study of Bap55 fly model using MARCM, behavioral assessment of mouse Actl6b mutants, primary neuronal cultures, transcriptional and proteome profiling, and assessment of chromatin are provided in SI Appendix, Supplementary Text.

Data Availability. Exome-sequencing data from individuals in this study have been deposited to the Database of Genotypes and Phenotypes (dbGaP) under accession number phs000288.v2.p2. BAF53b and ACTL6B are used interchangeably. The GenBank accession ID is NR_134539.1. Raw RNA- and ATAC-seq data have been deposited in the Gene Expression Omnibus under GSE147056. The processed RNA-seq dataset is included as Dataset 51 and ATAC-seq as Dataset 52. Bap55 mutant flies expressing actl6b mutants for MARCM, patient-derived iP cells, ACTL6B mutant plasmids, a-Actl6A/B antibodies, and other resources developed or used in the paper are available upon request.

ACKNOWLEDGMENTS. We thank Lei Chen, Alex Valdeifera, and Claire M. Ellis for their help with mouse breeding. We thank Kyle Loh, Lay Teng Ang, Alon Goren, and Ian Maze for thoughtful discussion. This work was supported by Simons Foundation for Autism Research Grant 514863 (to J.G.G. and G.R.C.); National Institutes of Health Grant NS046789 (to G.R.C.); Grant R01NS048453 and Qatar National Research Fund National Priorities Research Program Grant 6-1463-3-351 (to J.G.G. and T.B.-O.); Grants F13FM116588-01 and 2T32GM007970-38 (to W.W.); Grant ST32GM008666 (to S.G.); and Grants U54HG003067 (to the Broad Institute) and U54HG006504 (to the Yale Center for Mendelian Disorders). Behavior studies were supported by grants from the National Institutes of Health (to J.G.G. and G.R.C.); National Institutes of Health Grant 5P50CA093379 (to S.G.); the National Institute of Neurological Disorders and Stroke Grants R01NS089166, R01NS094853, and U54HG006504 (to S.G.); and Grant R01NS094853 (to V.I.). This study was approved by the institutional review boards of Stanford University and Fujita Health University. Blood, skin punch, and clinical data were obtained from research subjects after obtaining informed consent. Detailed description of the patient clinical history, generation of iPSCs, methods for study of Bap55 fly model using MARCM, behavioral assessment of mouse Actl6b mutants, primary neuronal cultures, transcriptional and proteome profiling, and assessment of chromatin are provided in SI Appendix, Supplementary Text.

1. J. Bai et al., Prevalence of autism spectrum disorder among children aged 8 years—autism and developmental disabilities monitoring network, 11 sites, United States, 2014. MMWR Surveill. Summ. 67, 1–23 (2018).
2. M. Elshabbag et al., Global prevalence of autism and other pervasive developmental disorders. Autism Res. 5, 160–179 (2012).
3. D. Bai et al., Association of genetic and environmental factors with autism in a 5-country cohort. JAMA Psychiatry. 101,1031/5 (2014).1911 (2019).
4. B. S. Abrahams et al., SfARI gene 2.0: A community-driven knowledgebase for the autism spectrum disorders (ASDs). Mol. Autism. 4, 36 (2013).
5. C. Hedges, J. G. Kirkland, G. R. Crabtree, The many roles of BAF (mSWi/SNF) and PBAF complexes in cancer. Cold Spring Harb. Perspect. Med. 6, a026930 (2016).
6. E. L. Miller et al., TOP2C synergizes with BAF chromatin reorganization for both resolution and formation of facultative heterochromatin. Nat. Struct. Mol. Biol. 24, 344–352 (2017).
7. C. Kadotoh, G. R. Crabtree, Reversible disruption of mSWi/SNF (BAF) complexes by the SMARCA2-SS18-SSX oncogenic fusion in synovial sarcoma. Cell 152, 71–85 (2013).
8. C. Kadotoh et al., Dynamics of BAF-Polycomb complex opposition on heterochromatin in normal and oncogenic states. Nat. Genet. 49, 213–222 (2017).
29. S. Bell et al., Neurogranin null mutant mice display performance deficits on spatial learning tasks with anxiety related components. Hippocampus 11, 763–775 (2001).

30. N. C. Bramsweig et al., Heterozygosity for ARID2 loss-of-function mutations in individuals with a Coffin-Siris syndrome-like phenotype. Hum. Genet. 136, 297–305 (2017).

31. C. Dias et al., DDD Study, BCO2 haploinsufficiency causes an intellectual disability syndrome and dysregulates transcription. Am. J. Hum. Genet. 99, 253–274 (2016).

32. E. T. Lim et al., Autism Sequencing Consortium, Rates, distribution and implications of postzygotic mosaic mutations in autism spectrum disorder. Nat. Neurosci. 20, 1217–1224 (2017).

33. D. C. Tarlungeanu et al., Patterns and rates of exonic de novo mutations in autism spectrum disorders. Nature 485, 242–245 (2012).

34. V. Vasileiou et al., Deciphering Developmental Disorders Study, Mutations in the BAF-complex subunit DP2 are associated with Coffin-Siris syndrome. Am. J. Hum. Genet. 102, 468–479 (2018).

35. K. C. Johnson et al., DDD Study, A syndromic neurodevelopmental disorder caused by mutations in SMARCD1, a core SWI/SNF subunit needed for context-dependent neuronal gene regulation in flies. Am. J. Hum. Genet. 104, 596–610 (2019).

36. R. N. Doan et al., Autism Sequencing Consortium, Recessive gene disruptions in autism spectrum disorder. Nat. Genet. 51, 1092–1098 (2019).

37. D. Wieczorek et al., A comprehensive molecular study on Coffin-Siris and Nalidixic-Barrier syndromes identifies a broad molecular and cellular spectrum converging on altered chromatin remodeling. Hum. Mol. Genet. 22, 5121–5135 (2013).

38. R. Marom et al., Heterozygous variants in ACTL6A, encoding a component of the BAF complex, are associated with intellectual disability. Hum. Mutat. 38, 1365–1371 (2017).

39. S. Cuvierotto et al., DDD Study, ACTB loss-of-function mutations result in a pleiotropic syndrome. Am. J. Hum. Genet. 101, 1021–1033 (2017).

40. S. L. Neale et al., Mutations in ACTL6B cause neurodevelopmental deficits and epilepsy and lead to loss of dendrites in human neurons. Am. J. Hum. Genet. 104, 815–834 (2019).

41. S. S. Jeste, D. H. Geschwind, Disentangling the heterogeneity of autism spectrum disorder through genetic findings. Nat. Rev. Neurosci. 10, 74–81 (2014).

42. A. J. Lopez, M. A. Wood, Role of nucleosome remodeling in neurodevelopmental and intellectual disability disorders. Front. Behav. Neurosci. 9, 100 (2015).

43. J. Ellegeod, J. N. Crawley, Behavioral and neuroanatomical phenotypes in mouse models of autism. Neurotherapeutics 12, 521–533 (2015).

44. L. Rylaarsdam, A. Guemez-Gamboa, Genetic causes and modifiers of autism spectrum disorder. Front. Cell. Neurosci. 13, 385 (2019).

45. D. C. Turlengerau et al., Impaired amino acid transport at the blood brain barrier is a cause of autism spectrum disorder. Cell 167, 1481–1494.e18 (2016).

46. G. Novarino et al., Mutations in BCKD-KIN lead to a potentially treatable form of autism with epilepsy. Science 338, 394–397 (2012).

47. A. Johansen et al., Mutations in MBOAT7, encoding lysophosphatidylinositoyl acyltransferase I, lead to intellectual disability accompanied by epilepsy and autonomic dysfunction. Am. J. Hum. Genet. 99, 512–516 (2016).

48. L. Wang et al., Whole exome and whole genome sequencing of human families with recessive autism. Database of Genotypes and Phenotypes (dbGaP). https://www.ncbi.nlm.nih.gov/projects/gpap/cvbi/studystudy?study_id=ph000288.v2.p2. Deposited 22 June 2010.

49. R. L. Silverstein, M. Febbraio, CD36, a scavenger receptor involved in immunity, metabolism, angiogenesis, and behavior. Sci. Signal. 2, re3 (2009).

50. I. Faber et al., SPG1 mutations cause widespread white matter and basal ganglia abnormalities, but restricted cortical damage. Neuroimage Clin. 19, 848–857 (2018).

51. Z. Yüksel, M. Yazel, E. Gümüs, Pathogenic homozygous variations in ACTL6B cause DECAM syndrome: Developmental delay, epileptic encephalopathy, cerebral atrophy, and abnormal myelination. Am. J. Med. Genet. A. 179, 1603–1608 (2019).

52. M. Fichera et al., Mutations in ACTL6B, coding for a subunit of the neuron-specific BAF (SWI/SNF) complexes in neural development and disorders. Front. Mol. Neurosci. 10, 243 (2017).

53. L. Ho et al., An embryonic stem cell chromatin remodeling complex, eSBF, is essential for embryonic stem cell self-renewal and pluripotency. Proc. Natl. Acad. Sci. U.S.A. 106, 5184–5186 (2009).

54. V. A. Arboleda et al., UCLA Clinical Genomics Center, De novo nonsense mutations in KAT6A, a lysine acetyl-transferase gene, cause a syndrome including microcephaly and global developmental delay. Am. J. Hum. Genet. 96, 498–506 (2015).

55. G. Vandeyken et al., The transcriptional regulator ADRN links the BAF (SWI/SNF) complexes with autism. Am. J. Med. Genet. C Semin. Med. Genet. 166C, 315–326 (2014).

56. L. Isbel et al., Wiz binds active promoters and CTCF-binding sites and is required for normal behaviour in the mouse. eLife 5, e15082 (2016).

57. J. Muller et al., Sequence and comparative genomic analysis of actin-related proteins. Mol. Biol. Cell. 16, 5736–5748 (2005).

58. P. Arlotta, Organoids required! A new path to understanding human brain development and disease. Nat. Rev. Neurosci. 17, 207–218 (2016).

59. R. T. Nakayama et al., SMARCB1 is required for widespread BAF complex-mediated activation of enhancers and bivalent promoters. Nat. Genet. 49, 1613–1623 (2017).

60. C. M. Alberni, E. R. Kandel, The regulation of transcription in memory consolidation. Cold Spring Harb. Perspect. Biol. 7, a021741 (2015).

61. Z. Qiu, A. Ghosh, A calcium-dependent switch in a CREST-BRG1 complex regulates activity-dependent gene expression. Neuroreport 60, 775–787 (2008).

62. Z. Zhang et al., Autism-associated chromatin regulator Brg1/marc4kA is required for synapse development and myocyte enhancer factor 2-mediated synapse remodeling. Mol. Cell Biol. 36, 70–83 (2016).

63. W. Wenderski, A. Krokhotin, and G. R. Crabtree. Loss of the neuro-specific BAF subunit ACTL6B relieves repression of early response genes and causes recessive autism. Gene Expression Omnibus. https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc= GSE44056. Deposited 17 May 2006.

64. I. Maze et al., Critical role of histone turnover in neuronal transcription and plasticity. Neuron 87, 77–94 (2015).

65. J. D. Buenrostro, P. G. Giresi, L. C. Zaba, H. Y. Chang, W. J. Greenleaf, Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome positioning. Nat. Methods 10, 1213–1218 (2013).

66. M. S. Breen et al., Candidate gene networks and blood biomarkers of methamphetamine-associated psychosis: An integrative RNA-sequencing report. Transl. Psychiatry 6, e802 (2016).

67. E. Y. Yap, M. E. Greenberg, Activity-regulated transcription: Bridging the gap between neural activity and behavior. Neuroreport 100, 330–348 (2018).

68. P. J. Skene et al., Neuronal MeCP2 is expressed at near histone-octamer levels and regulates the chromosomal organization of neurexin-1. Mol. Cell. 77, 457–468 (2010).

69. K. M. Noh et al., ATRX tolerates activity-dependent histone H3 methyllys switch to maintain repetitive element silencing in neurons. Proc. Natl. Acad. Sci. U.S.A. 112, 6820–6827 (2015).

70. T. Zhu et al., Histone methyltransferase Ash1l mediates activity-dependent reprogramming of neuroxin-1a. Sci. Rep. 6, 26597 (2016).

71. F. Leocci et al., Variant recurrence in neurodevelopmental disorders: The use of publicly available genomic data identifies clinically relevant pathogenic missense variants. Genet. Med. 21, 2504–2511 (2019).

72. S. H. Lelieveld et al., Spatial clustering of de novo missense mutations identifies candidate neurodevelopmental disorder-associated genes. Am. J. Hum. Genet. 101, 478–484 (2017).

73. S. A. Sajan et al., Enrichment of mutations in chromatin regulators in people with Rett syndrome lacking mutations in MECP2. Ann. Neurol. 79, 13–19 (2017).

74. E. Y. Chen et al., Enrich: Interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics 14, 128 (2013).
84. M. V. Kuleshov et al., Enrichr: A comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 44, W90–W97 (2016).
85. S. R. Weiss et al., CRF-induced seizures and behavior: Interaction with amygdala kindling. Brain Res. 372, 345–351 (1986).
86. S. Hupalo, C. W. Berridge, Working memory impairing actions of corticotropin-releasing factor (CRF) neurotransmission in the prefrontal cortex. Neuropsychopharmacology 41, 2733–2740 (2016).
87. J. J. Walsh et al., 5-HT release in nucleus accumbens rescues social deficits in mouse autism model. Nature 560, 589–594 (2018).
88. H. O. Heyne et al.; EuroEPINOMICS RES Consortium, De novo variants in neurodevelopmental disorders with epilepsy. Nat. Genet. 50, 1048–1053 (2018).
89. Deciphering Developmental Disorders Study, Prevalence and architecture of de novo mutations in developmental disorders. Nature 542, 433–438 (2017).
90. D. R. Krupp et al., Exonic mosaic mutations contribute risk for autism spectrum disorder. Am. J. Hum. Genet. 101, 369–390 (2017).
91. Deciphering Developmental Disorders Study, Large-scale discovery of novel genetic causes of developmental disorders. Nature 519, 223–228 (2015).
92. M. Belya, S. J. Kraft, S. Brown; Pediatric Imaging, Neurocognition and Genetics Study, PlexinA polymorphisms mediate the developmental trajectory of human corpus callosum microstructure. J. Hum. Genet. 60, 147–150 (2015).
93. N. Daviaud, K. Chen, Y. Huang, R. H. Friedel, H. Zou, Impaired cortical neurogenesis in plexin-B1 and -B2 double deletion mutant. Dev. Neurobiol. 76, 882–899 (2016).
94. A. L. Bey, Y. H. Jiang, Overview of mouse models of autism spectrum disorders. Curr. Protoc. Pharmacol. 66, 5.66.1-5.66.26 (2014).
95. F. Gao et al., Heterozygous mutations in SMARCA2 reprogram the enhancer landscape by global retargeting of SMARCA4. Mol. Cell 75, 891–904.e7 (2019).
96. Y. Tsurusaki et al., Mutations affecting components of the SWI/SNF complex cause Coffin-Siris syndrome. Nat. Genet. 44, 376–378 (2012).