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# Heterozygous variants in *ACTL6A*, encoding a component of the BAF complex, are associated with intellectual disability

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## Abstract

Pathogenic variants in genes encoding components of the BAF chromatin remodeling complex have been associated with intellectual disability syndromes. We identified heterozygous, novel variants in *ACTL6A*, a gene encoding a component of the BAF complex, in three subjects with varying degrees of intellectual disability. Two subjects have missense variants affecting highly conserved amino acid residues within the actin-like domain. Missense mutations in the homologous region in yeast actin were previously reported to be dominant lethal and were associated with impaired binding of the human ACTL6A to β-actin and BRG1. A third subject has a splicing variant that creates an in-frame deletion. Our findings suggest that the variants identified in our subjects may have a deleterious effect on the function of the protein by disturbing the integrity of the BAF complex. Thus, *ACTL6A* gene mutation analysis should be considered in patients with intellectual disability, learning disabilities or developmental language disorder.

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**Conflicts of Interest**

The Department of Molecular and Human Genetics at Baylor College of Medicine receives revenue from clinical testing done at Baylor Genetics Laboratories.

**Keywords**

*ACTL6A*; BAF complex; speech delay; intellectual disability

Heterozygous pathogenic variants in genes encoding components of the BRG1-associated factor (BAF) complex, are causally related to syndromic and non-syndromic conditions with intellectual disability (Kosho, et al., 2014; López and Wood, 2015; Santen, et al., 2012b). Coffin-Siris syndrome (CSS [MIM# 135900, 614607, 614608, 614609, 616938]), characterized by developmental delay, coarse facial features, and hypoplastic fifth phalanges (Coffin and Siris, 1970), is associated with pathogenic variants in genes encoding *ARID1B* (MIM# 614556), *ARID1A* (MIM# 603024), *SMARCB1* (MIM# 601607), *SMARCA4* (MIM# 603254), *SMARCE1* (MIM# 603111), and *SOX11* (MIM# 600898) (Santen, et al., 2012a; Tsurusaki, et al., 2014; Tsurusaki, et al., 2012). Mutations in *SMARCA2* (MIM# 600014) are thought to result in a phenotype better described as Nicolaides-Baraitser syndrome (Nicolaides and Baraitser, 1993), with overlapping clinical features (Mari, et al., 2015; Van Houdt, et al., 2012; Wolff, et al., 2012). Haploinsufficiency and loss-of-function variants in *ARID1B* are also found in 1% of individuals with intellectual disability without distinguishing physical features (Hoyer, et al., 2012). More recently, pathogenic variants in other chromatin remodeling proteins, or factors that closely interact with the BAF complex were implicated in autism and intellectual disability, including *ADNP* (MIM# 611386) (Helsmoortel, et al., 2014), *ARID2* (MIM# 609539)(Shang, et al., 2015), *DYRK1A* (MIM# 600855) (Courcet, et al., 2012; Ji, et al., 2015; van Bon, et al., 2016), *BCL11A* (MIM# 606557)(Dias, et al., 2016) and *BAZ1A* (MIM# 605680)(Zaghlool, et al., 2016). With the advent of next-generation sequencing new intellectual disability genes are being discovered, and many of these genes play a role in neurodevelopment via epigenetic regulation of gene expression (Casanova, et al., 2016; López and Wood, 2015; Son and Crabtree, 2014).

We present clinical and molecular data from three unrelated subjects with developmental disability primarily affecting language. Whole exome sequencing (WES) identified pathogenic variants in *ACTL6A* (MIM# 604958), which encodes a member of the BAF complex that has not been previously associated with intellectual disability syndromes. A summary of the clinical and molecular findings in all three subjects is provided in Figure 1 and in the Supp. Table S1.

Subject 1 is a 15-year-old female that was described in detail in a prior publication (Brautbar, et al., 2009), although she did not have an identified genetic etiology. Briefly, she presented with developmental delay that involved speech/language difficulties and inattention problems. She had to repeat first grade and continued to have significant learning difficulties in school. She had atrial septal defect and cleft mitral valve that required surgical repair. Her medical history was otherwise significant for torticollis, gastro-esophageal reflux, recurrent ear infections and urinary bladder irritability – all of which resolved during childhood. She also underwent a surgical repair of bilateral inguinal and umbilical hernias. She was evaluated for suspected autonomic dysfunction due to chronic fatigue, exercise intolerance and episodes of syncope. She was small for age at birth (weight and length at the 5$^{th}$ centile), but her current growth parameters are within the normal limits. She has

dysmorphic features that include coarse facies, bushy eyebrows, prominent ears and broad nasal tip (Figure 1). She has hypoplastic nails on multiple digits, unusually broad thumbs and mildly persistent fetal fingertip pads. Neurological examination was non-focal. The patient's chromosome analysis and chromosome microarray analysis were unremarkable. Clinically, her diagnosis was considered to be a potential mild CSS versus brachymorphism-onychodysplasia-dysphalangism syndrome (MIM# 113477) (Brautbar, et al., 2009). WES analysis revealed a *de novo*, heterozygous variant, NC_000003.11:g. 179304340C>T, in exon 13 of *ACTL6A*. This variant results in amino acid substitution at position 377 (p.Arg377Trp, Figure 1).

Subject 2 was identified via review of the Baylor Genetics WES database. This is a 6-year-old male with developmental delay, primarily affecting speech, and attention deficit hyperactivity disorder (ADHD) with behavioral problems (aggressive behavior, impulsiveness and tantrums, and sleep problems). Pregnancy was complicated by intrauterine growth restriction. He was born prematurely at 31 weeks gestation, and birth weight was 820 grams ($2^{nd}$ centile). He walked at 15 months. He said his first words at 12 months but started using phrases at 3 years of age and had articulation problems. He has poor fine motor skills and sensory integration deficits. Psychological evaluation at 5 years of age revealed borderline intelligence, with both receptive and expressive language skills below the mean for age. He has a history of hyperopia, recurrent respiratory infections, asthma and allergies. Early in life he had failure to thrive, gastro-esophageal reflux and constipation which resolved. He underwent bilateral inguinal hernia and umbilical hernia repair. The family history is significant for learning disabilities in both parents. His growth parameters on most recent exam were within the normal limits. The physical examination is notable for dysmorphic features, which include narrow face with prominent forehead, long eyelashes and poorly developed philtrum ridge. He has $5^{th}$ finger clinodactyly, partial 3–4 finger syndactyly and 2–3 toe syndactyly. Neurological exam was normal. The patient's chromosome analysis, SNP array and fragile X (MIM# 300624) testing were normal. He had negative testing for Russell-Silver syndrome (MIM# 180860). Transferrin isoelectric focusing screen for congenital disorders of glycosylation was normal. Newborn screen and other metabolic work up was normal, including plasma amino acids, urine organic acids, urine acylglycines, acylcarnitine analysis, and urine & plasma levels of creatine/guanidinoacetate. Brain MRI was normal. Clinical WES was done at Baylor Genetics. Research analysis of the data revealed a heterozygous variant, NC_000003.11:g. 179294612G>C, in exon 8 of *ACTL6A* that results in amino acid substitution at position 227 (p.Glu227Gln, Figure 1). The mother tested negative for this variant. The father, who is not available for testing, has a history of ADHD and learning disabilities – thus this variant could be potentially *de novo*, or inherited from an affected parent.

Subject 3 was identified via GeneMatcher search (https://genematcher.org) (Sobreira, et al., 2015). This is a 6-year-old male with cognitive and language delay. He was born prematurely at 35 weeks gestation, and birth weight was 2635 grams ($60^{th}$ centile). He had prenatal diagnosis of bilateral hydronephrosis due to urethral valve obstruction for which he underwent a surgical repair after birth. He was also noted to have glandular hypospadias and laryngomalacia. Perinatal course was complicated by feeding difficulties, and he was hospitalized during the first 4 weeks of life. As for his development, he started walking at

the age of 15 months and spoke his first words at 12 months. However, he required speech therapy for expressive and receptive language deficits. He had neurodevelopmental evaluation at 6 years of age and was found to have moderate cognitive impairment (IQ 56). In school, he is attending special education classes. He had extensive neuropediatric evaluation due to exercise intolerance (leg pain after minor exercise), which remained unexplained. Remarkably, he was noted to have a high pain threshold. The family history is significant for learning disabilities in the mother. His older brother and sister had some speech delay initially but do not have cognitive delays and attend regular education classes. His older sister also had epilepsy. Physical examination at the age of 6 years showed normal growth parameters, and dysmorphic facial features that include a prominent high forehead, low set everted ears, narrow eyelids with mild hypertelorism, small chin and a normal palate (Figure 1). He had flat feet with overriding 2$^{nd}$ toes and clinodactyly of the 3$^{rd}$, 4$^{th}$ and 5$^{th}$ toes. He had joint hypermobility (Beighton score 6 out of 8), hypospadias with normally descended testes and multiple café-au-lait macules on his skin but without freckling in axillary and inguinal regions. He had a normal EEG. Several genetic tests were performed, including metabolic work up, SNP array, and DNA analysis of *FMR1*, *NF1* and *SPRED1*, which all appeared normal. WES analysis revealed a *de novo*, heterozygous variant in *ACTL6A*. This variant, NC_000003.11 (NM_004301.3): c.1209+1G>C in intron 13, is predicted to affect splicing (Figure 1). To study the effect of the splicing variant on the *ACTL6A* transcript, total RNA was isolated from subject 3 and control lymphoblastoid cells, PCR-amplified, cloned and sequenced (Figure 2 A). Subject 3 cDNA sequencing results were aligned with control and reference *ACTL6A* mRNA sequence (UCSC genome browser, https://genome.ucsc.edu/). Sequencing revealed abnormal splicing that creates an in-frame deletion of exon 13. Western blot analysis detected decreased amount of the ACTL6A protein in subject 3 cell lysate, supporting a loss-of-function, or haploinsufficiency mechanism of this variant (Figure 2 B). Silencing of *ACTL6A* in HeLa cells resulted in decreased stability of the BAF complex (Nishimoto, et al., 2012). To evaluate the effect of ACTL6A deficiency on the BAF complex formation in subject 3 cells, we studied the interaction of ACTL6A and BRG1 by co-immunoprecipitation. The results demonstrate reduced binding of ACTL6A and BRG1 in subject 3 lymphoblasts, compared to control cells (Figure 2C).

Mutations in genes encoding components of the BAF complex, or interacting proteins, have been associated with CSS (Santen, et al., 2012a; Tsurusaki, et al.), and intellectual disability disorders (Hoyer, et al., 2012; Son and Crabtree, 2014). In the present report, we review clinical information and molecular data from three subjects with speech delay and varying degrees of developmental disability. We identified novel, heterozygous variants in *ACTL6A*, a gene encoding a component of the BAF complex that has not been previously associated with intellectual disability. The variants that were found in *ACTL6A* include two amino acid substitutions that involve highly conserved residues, and a splice variant (Figure 1). Two of the variants were confirmed to be *de novo*, and in one patient inheritance is unknown (due to unavailability of paternal testing). None of the variants has been previously reported in the Exome Aggregation Consortium database (ExAC, Cambridge, MA, http://exac.broadinstitute.org/) (Lek, et al., 2016) and the 1000 Genomes database (http://browser.1000genomes.org/) (Auton, et al., 2015). The variant found in subject 2 (p.Glu227Gln) was

reported once in gnomAD database (GnomAD, http://gnomad.broadinstitute.org) (Lek, et al., 2016) with an allele frequency of 4.06e-6. Since Trio WES was not performed, we were unable to identify other *de novo* variants which may explain the phenotype seen in these subjects. We therefore cannot state beyond doubt that the *ACTL6A* variants are disease-causing vs. incidental findings in these patients. Importantly, WES analysis did not detect pathogenic variants in other genes that are known to be associated with intellectual disability. We reviewed DECIPHER (https://decipher.sanger.ac.uk) (Firth, et al., 2009) cases that involve *ACTL6A*. These include 13 gains and only 1 case with a deletion, all larger than 5 Mb and include 20 genes or more. Thus, data is lacking to directly support the contribution of *ACTL6A* solely in the pathogenicity of these copy number variants.

ACTL6A (actin-like 6a, also known as BAF53a/INO80K/Arp4) is a scaffold component of the BAF complex. It appears essential in maintaining the undifferentiated status of embryonic stem cells (Lu, et al., 2015) and epidermal progenitor cells (Bao, et al., 2013), and is implicated in neuronal and hematopoietic development (Krasteva, et al., 2012; Staahl, et al., 2013). Homozygous deletion of *Actl6a* in mice results in early embryonic lethality (Krasteva, et al., 2012). Conditional knock out of the gene within the hematopoietic lineage caused bone marrow failure and early death at about 3 weeks post deletion (Krasteva, et al., 2012). Knockdown of *ACTL6A* by siRNA in cells affected histone methylation and induced transcription of several cell cycle regulators (including MDM2, p21 and cyclin D1), resulting in cell cycle arrest (Lee, et al., 2007). We preformed cell cycle study in subject 3 and control lymphoblastoid cell lines. We observed a trend of cells from subject 3 to be retained in G1/S phase, with a smaller fraction of cells residing in G2 phase compared to control (9–10% of subject 3 cells in G2 phase, compared to 15–16.8% of control cells in G2 phase in two independent experiments, Figure 2 D). This finding is consistent with previous observation in ACTL6A-depleted cells (Lee, et al., 2007), and supports a mechanism of loss of function in our patient.

During epidermal differentiation, *ACTL6A* is significantly down-regulated, and conditional deletion is associated with loss of functional progenitor cells and tissue hypoplasia (Bao, et al., 2013). In neural development, ACTL6A substitution with ACTL6B (MIM# 612458) dictates a switch from neural progenitor npBAF complex to neural specific nBAF complex, to allow proper mitotic exit and neuronal differentiation (Staahl, et al., 2013). Altogether, these findings suggest that during development ACTL6A serves as an important regulator of progenitor/stem cell function.

ACTL6A was found to be highly homologous to actin; however, unlike actin it does not have ATPase activity and does not form actin-like filaments (Fenn, et al., 2011; Zhao, et al., 1998). Actin and actin-related proteins are involved in epigenetic regulation of transcription and chromatin dynamics (Chen and Shen, 2007; Olave, et al., 2002; Shen, et al., 2003). *De novo* heterozygous pathogenic variants in actin genes *ACTB* (MIM# 102630) and *ACTG1* (MIM# 102560) are associated with Baraitser-Winter syndrome (MIM# 243310) (Baraitser and Winter, 1988), characterized by microcephaly, ocular and brain malformations, and intellectual disability (Di Donato, et al., 2014; Johnston, et al., 2013; Rivière, et al., 2012). ACTL6A directly interacts with beta actin (ACTB) and SMARCA4/BRG1 while enhancing

chromatin binding and ATPase activity of the BAF complex (Nishimoto, et al., 2012; Zhao, et al., 1998).

WES identified three heterozygous variants in *ACTL6A*, two of which were confirmed to be *de novo* in our patients. The two missense variants: p.Glu227Gln and p.Arg377Trp, involve highly conserved amino acid residues and are predicted to be damaging by Polyphen2 and SIFT. In addition to these findings, analysis of the variants via Mutation Taster program, PhyloP score and the Cadd score, supports a deleterious effect of the identified variants (variant scores are detailed in Supp. Table S2). These two amino acids reside in a region with structural similarity to actin, where induced missense mutations were found to be dominant lethal in yeast (Wertman, et al., 1992). Interestingly, a previously published study in cells transiently transfected with human *ACTL6A* showed that introducing mutations into these same two residues found in our subjects impaired binding of the ACTL6A protein to ACTB, to SMARCA4/BRG1 (Nishimoto, et al., 2012), and to the TIP60 (MIM# 601409) chromatin remodeling complex (Lu, et al., 2015; Nishimoto, et al., 2012). A third subject was found to have a *de novo* splicing variant in intron 13. RNA and protein studies using a lymphoblastoid cell line derived from this subject showed abnormal splicing that creates an in-frame deletion of exon 13 (Figure 2A), resulting in reduced amount of ACTL6A protein (Figure 2B). Since all pathogenic variants are heterozygous and are predicted to result in loss of function, we hypothesize that haploinsufficiency is the mechanism underlying this disorder. The high haploinsufficiency score (HI index=3.57) reported in DECIPHER database (Firth, et al., 2009; Huang, et al., 2010) for *ACTL6A* supports this prediction.

Clinically, our subjects share a number of phenotypic features (Supp. Table S1). They all presented with developmental delay and variable degree of learning disabilities. Subjects 1 and 2 on the milder end of the spectrum, had inattention and learning problems with neurocognitive assessment at the low-normal range. Consistent with this observation, the variant that was identified in subject 2 was reported once in gnomAD database, which includes sequencing results from individuals that are not affected by severe pediatric disorders. On the other hand, subject 3 had a splicing variant causing a deletion of the full length of exon 13 that likely leads to loss of function of the mutated allele as demonstrated by western blot (Figure 2B), and presented with more severe cognitive impairment of moderate degree, compared to the other two subjects with mild intellectual disability and missense variants. The probability of loss-of-function intolerance (pLI) score reported in ExAC database is 0.99, predicting that *ACTL6A* is highly intolerant of loss-of-function variation. Potentially, variants that have less severe functional effects may give rise to the relatively milder neurocognitive phenotype, and may lead to under-ascertainment. In addition to delayed development, speech delay and learning disabilities, a number of organ systems were affected in some of the subjects, with cardiac defects in 1/3 subjects, genitourinary anomalies in 2/3 subjects, hernias in 2/3 subjects and finger and toe abnormalities in 3/3 subjects. Our subjects were born small for age or had failure to thrive early in life, but current growth parameters are within the normal limits. To further delineate the clinical spectrum associated with pathogenic variants of *ACTL6A* we established the website: www.humandiseasegenes.com/ACTL6A to collect detailed clinical information of additional individuals that will be identified over time.

In conclusion, we report here heterozygous pathogenic variants in *ACTL6A* in three subjects with varying degrees of intellectual disability. Subjects exhibit common physical features including genitourinary and skeletal defects. *ACTL6A* is a BAF-related gene that plays an essential role in neurodevelopment. Two of three variants localize to exon 13, shown here and in previous functional studies to affect protein-protein interaction at the BAF complex. We suggest that sequencing of *ACTL6A* should be considered in the diagnostic work-up of developmental delay and learning disabilities.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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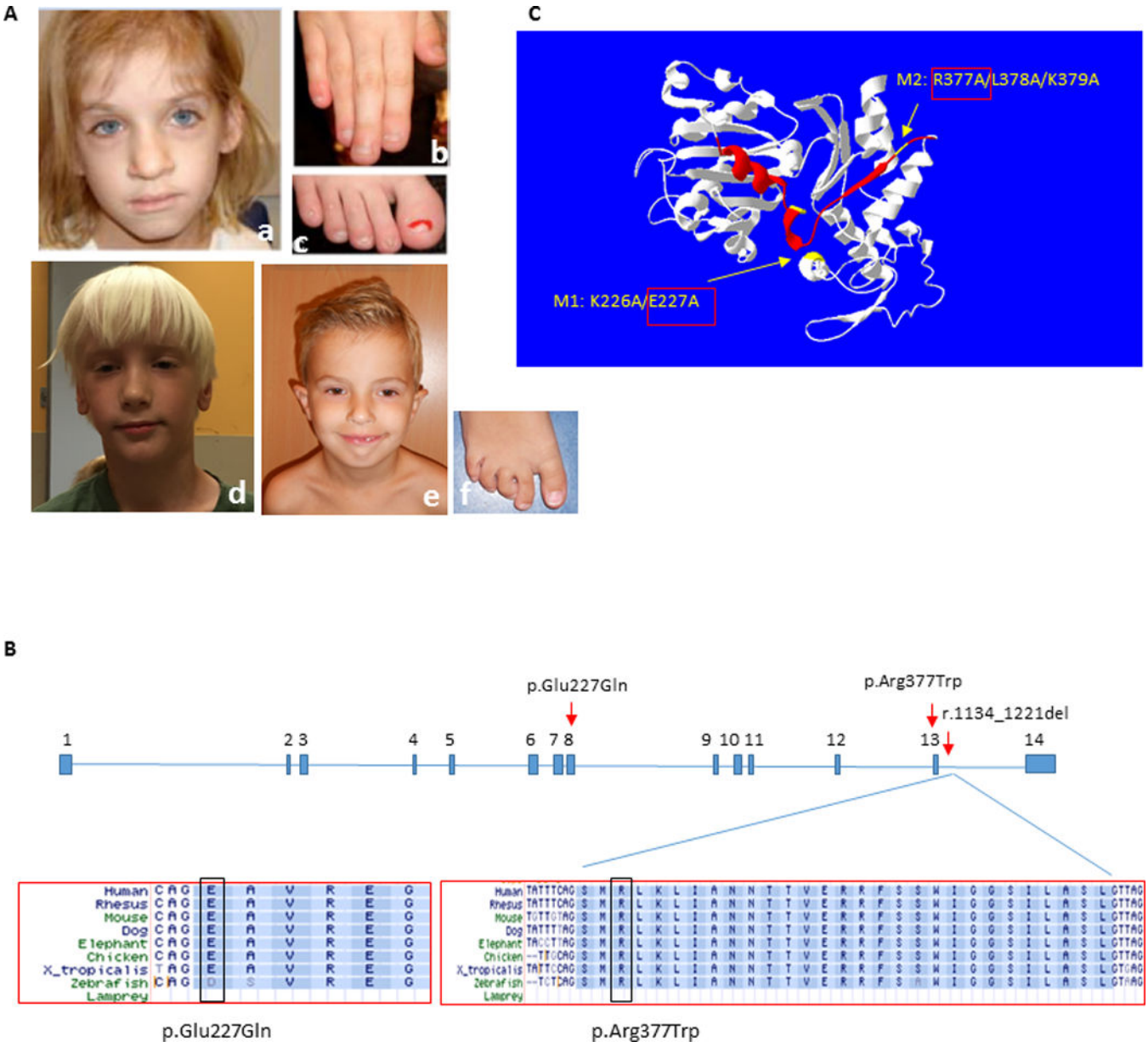


**Figure 1. Clinical photographs of subjects and summary of ACTL6A pathogenic variants**

(A) Subject 1 at age 7 years, demonstrating coarse facial features with broad nasal tip (a), broad fingers and toes with dystrophic nails, and short distal phalanges (b, c); Subject 2 at age 6 years, demonstrating elongated face with large forehead, narrow eyelids, broad nasal tip and poorly developed philtrum ridge (d); and Subject 3 at age 6 years, showing prominent high forehead, low set everted ears, narrow eyelids, broad nasal tip, and small chin (e). Note digital anomalies, including overriding second toe, clinodactyly of 3rd–5th toes and sandal gap (f). *The photographs of Subject 1 were reproduced with permission from the American Journal of Medical Genetics, Part A (Brautbar, et al., 2009).*

(B) Annotation of the two amino acid residues in exons 8 and 13 affected in Subjects 1 and 2, and the splicing variant causing in-frame deletion of exon 13 in Subject 3. Conservation across species is shown for the three variants.

(C) ACTL6A protein structure model as predicted by I-TASSER server (http://zhanglab.ccmb.med.umich.edu/I-TASSER) (Zhang, 2008) and visualized using Swiss-Pdb viewer (http://spdbv.vital-it.ch/) (Guex and Peitsch, 1997), showing localization of the mutated amino acids in Subjects 1 and 2 (yellow arrows), and the deleted exon 13 in Subject 3 (red ribbon). Previous study (Nishimoto, et al., 2012) demonstrated that M1 and M2 mutants of human ACTL6A exhibit impaired binding capacity to beta actin (ACTB) and BRG1/SMARCA4, and thus disrupt ACTL6A recruitment to the BAF complex. This data supports a deleterious effect for p.Glu227Gln (E227Q/M1) and p.Arg377Trp (R377W/M2) on ACTL6A protein function.

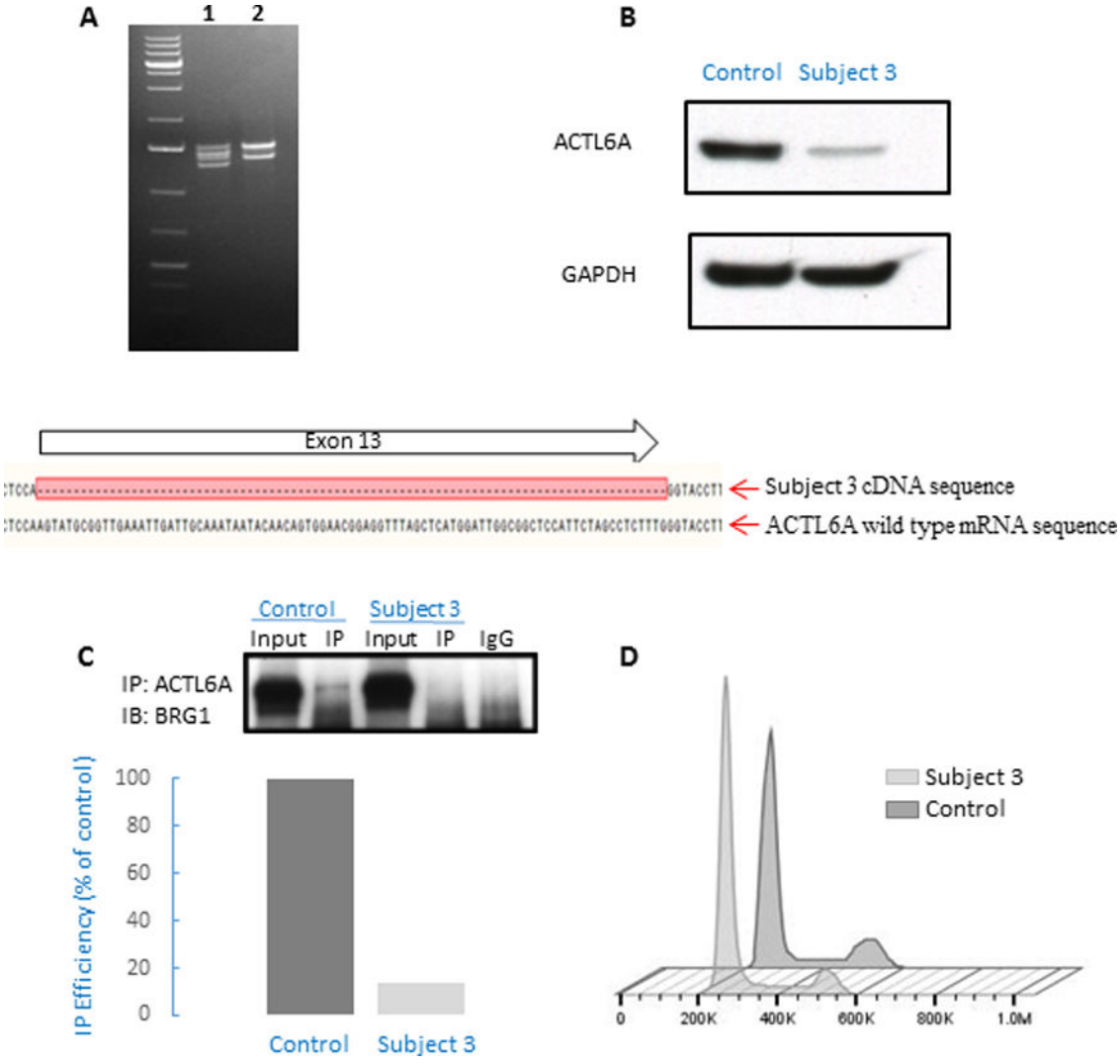


**Figure 2. RNA, protein and cell cycle studies in Subject 3 lymphoblasts encompassing a heterozygous *ACTL6A* splicing variant**

(A) RT-PCR amplification of the full-length cDNA from control sample (2) shows two transcripts due to alternative splicing at *ACTL6A* exon 1, while amplification of Subject 3 cDNA (1) shows four transcripts. PCR-cloning and sequencing, followed by alignment of the sequence results with *ACTL6A* mRNA sequence demonstrates that two of the four transcripts in Subject 3 correspond to expression of the two isoforms of wild-type allele, and two transcripts correspond to expression of the two isoforms of mutated allele containing an in-frame deletion of the full length of exon 13. (B) Western blot analysis of ACTL6A protein expression in Subject 3 and control cells, revealing reduced protein expression in Subject 3 cells that is suggestive of a haploinsufficiency mechanism.

(C) Immunoprecipitation (IP) with anti-ACTL6A antibody, showing decreased interaction with BRG1 in subject cells (Subject 3) compared to unrelated healthy control (Control). There was no binding to negative control (rabbit IgG). Immunoblot (IB) was quantified (using ImageJ program), showing 85% reduction in co-IP efficiency, presented here as % of control. The reduction of co-IP efficiency was repeated in two other independent

experiments (showing 70% and 20% reduction, respectively). (D) Cell cycle analysis of cells from Subject 3 and control cells. As compared to control, a smaller fraction of cells from subject 3 resided in G2 phase (10.5 % vs. 16.8 % in control samples). This trend was observed in two independent experiments and is consistent with previous reports describing cell cycle perturbation in ACTL6A-depleted cells (Lee, et al., 2007).