De Novo Truncating Mutations in *AHDC1* in Individuals with Syndromic Expressive Language Delay, Hypotonia, and Sleep Apnea

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Clinical whole-exome sequencing (WES) for identification of mutations leading to Mendelian disease has been offered to the medical community since 2011. Clinically undiagnosed neurological disorders are the most frequent basis for test referral, and currently, approximately 25% of such cases are diagnosed at the molecular level. To date, there are approximately 4,000 "known" disease-associated loci, and many are associated with striking dysmorphic features, making genotype-phenotype correlations relatively straightforward. A significant fraction of cases, however, lack characteristic dysmorphism or clinical pathognomonic traits and are dependent upon molecular tests for definitive diagnoses. Further, many molecular diagnoses are guided by recent gene-disease association discoveries. Hence, there is a critical interplay between clinical testing and research leading to gene-disease association discovery. Here, we describe four probands, all of whom presented with hypotonia, intellectual disability, global developmental delay, and mildly dysmorphic facial features. Three of the four also had sleep apnea. Each was a simplex case without a remarkable family history. Using WES, we identified *AHDC1* de novo truncating mutations that most likely cause this genetic syndrome.

De novo pathogenic mutations are a major cause of sporadic human genetic disease.[1,2] Whole-exome sequencing (WES)[3] using next-generation-sequencing methods has proven to be a powerful tool for molecular diagnosis of mutations in genes known to underlie Mendelian disease,[1] as well as for the discovery of novel disease-associated loci.[4] Despite the rapid development of these new molecular tools, the majority of individuals who are suspected to have a genetic disease remain undiagnosed. In part, this reflects the incomplete status of the catalog of characterized Mendelian-disease-associated genes; this catalog currently includes about 4,000 entries and represents less than one-quarter of the annotated genes (~21,000) in the human genome.

We applied WES to identify de novo genetic changes in a parent-offspring trio in which the proband exhibited developmental delay, hypotonia, mild dysmorphic features, sleep apnea, and other symptoms (Figure 1; Table 1; Table S1, available online). A truncating de novo mutant allele was found in AT-hook, DNA-binding motif, containing 1 (*AHDC1* [RefSeq accession number NM_001029882.2]). We subsequently identified an additional three independent simplex cases with similar phenotypes and de novo truncating events in the same gene. This pattern of de novo variation in *AHDC1* is highly unlikely to have occurred by chance and most likely represents the underlying cause of the symptoms in these individuals.

Subject 1 was an 18-month-old female (born to unrelated parents) who presented with hypotonia, delayed motor milestones, dysmorphic features, hepatomegaly, and laryngomalacia (Figure 2). Both the healthy parents and the proband were analyzed by WES.[1] Informed consent was obtained, and all procedures were followed in accordance with the ethical standards prescribed and approved by the Baylor College of Medicine Institutional Review Board. The DNA from each of the three samples was sequenced at an average depth of coverage of greater than 120-fold, and greater than 95% of the targeted bases were covered at 20-fold or higher. The results identified de novo events, including single-nucleotide variants (SNVs) or small indel mutations in the proband, in five genes: c.415G>A (p.Glu139Lys) in *CALY* (MIM 604647; RefSeq NM_015722.3), c.1429G>A (p.Gly477Arg) in *PTPRB* (MIM 176882; RefSeq NM_001109754.2), c.1076C>A (p.Ala359Glu) in *TBCK* (RefSeq NM_033115.4), c.1093dup (p.Met365Asnfs*4) in *CCDC66* (RefSeq NM_001141947.1), and c.2373_2374 delTG (p.Cys791Trpfs*57) in *AHDC1*; all sequence coordinates are based on human reference genome hg19 (UCSC Genome Browser). A comparison of the minor allele frequencies between these variants and similar mutations in the NHLBI Exome Sequencing Project Exome Variant Server (EVS) and a local variant database (see below) eliminated three missense mutations and one putative

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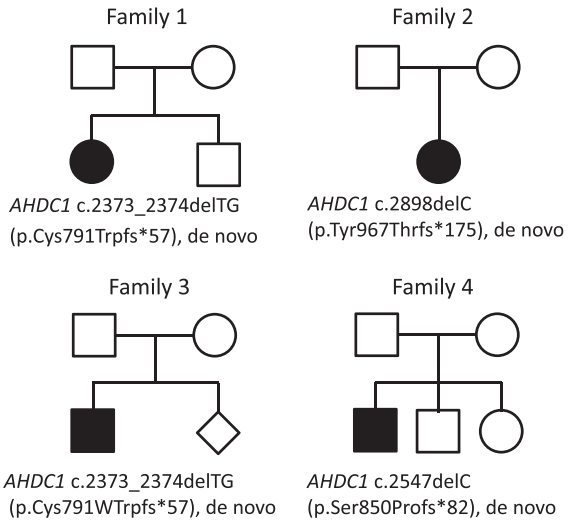

Figure 1. Pedigrees and Mutations of Four Affected Families

frameshift mutation as likely disease-causing candidates because of their occurrence in individuals without suspected developmental disorders. The remaining variant, the de novo deletion mutation in *AHDC1* (c.2373_2374delTG), results in a frameshift of the AHDC1 open reading frame, beginning at codon 791, and by conceptual translation is predicted to cause a premature termination codon (p.Cys791Trpfs*57). Predicted truncating mutations in *AHDC1* are absent from the 1000 Genomes database (healthy individuals), the EVS (about 6,500 individuals), and a database of exome data from the Atherosclerosis Risk in Communities study (approximately 8,000 community-based individuals). The absence of truncating mutations in *AHDC1* in these databases suggests that such gene perturbations are not consistent with general good health, and therefore the observed *AHDC1* mutation was considered likely to be pathogenic.

We next screened 2,000 entries of clinical WES data at the Whole Genome Laboratory at Baylor College of Medicine to identify possible additional *AHDC1* mutations. Those data consist primarily of individuals who had a sample submitted by their physician for clinical WES, as previously described.[1] The clinical WES test focuses on exome sequencing of the proband, and complete parental WES data are not routinely generated. Among 2,000 previously tested individuals, of whom 1,700 had developmental delay and/or intellectual disabilities, three were found to harbor frameshift alleles in *AHDC1* (c.2898delC [p.Tyr967Thrfs*175] at chr1: 27,875,729, c.2373_2374delTG [p.Cys791Trpfs*57] at chr1: 27,876,253–27,876,254, and c.2547delC [p.Ser850 Profs*82] at chr1: 27,876,080; Figures 1 and 3). The

Table 1. Major Clinical Features of Four Probands

	Subject			
	1	**2**	**3**	**4**
Gender	female	female	male	male
Age	18 months	4 years	8 years	11 years
Ethnicity	European descent	South Asian	European descent	European descent
Intellectual disability	NA	moderate	mild	moderate to severe
Speech delay	no words at 18 months of age	two words at 4 years of age	first words after 1 year of age, persistent speech therapy	no words, noncommunicating autism
Motor delay	no sitting at 18 months of age	sitting at 19 months of age, walking at 24 months of age	sitting at 9 months of age, walking at 18 months of age	sitting at 15 months of age, no independent ambulation
Hypotonia and failure to thrive	yes	yes	yes	yes
Dysmorphic facial features	low-set ears, esotropia, upslanting palpebral fissures, micrognathia, flat nasal bridge	protuberant ears, upslanting palpebral fissures, flat nasal bridge	protuberant low-set ears, small earlobes, hypertelorism, downslanting palpebral fissures, mild ptosis, micrognathia	upturned earlobes, hypertelorism, esotropia, flat nasal bridge
Anatomic upper-airway obstruction	laryngomalacia, obstructive sleep apnea	obstructive sleep apnea	laryngomalacia, obstructive sleep apnea	suspected tracheomalacia in infancy, history of snoring
Family history	negative, one healthy sibling	negative	negative, one healthy sibling	negative, two healthy siblings
Previous testing	MD, SMA, PWS, CMA, metabolic work-up	FX, CMA (18 Mb AOH on chromosome 5), metabolic work-up	CMA, FX, metabolic work-up	SMA, PWS, CMA, metabolic work-up

See Table S1 for additional details of clinical presentations. Abbreviations are as follows: AOH, absence of heterozygosity; CMA, chromosome microarray; FX, fragile X chromosome; MD, myotonic dystrophy; NA, not available; PWS, Prader-Willi syndrome; and SMA, spinal muscular atrophy.

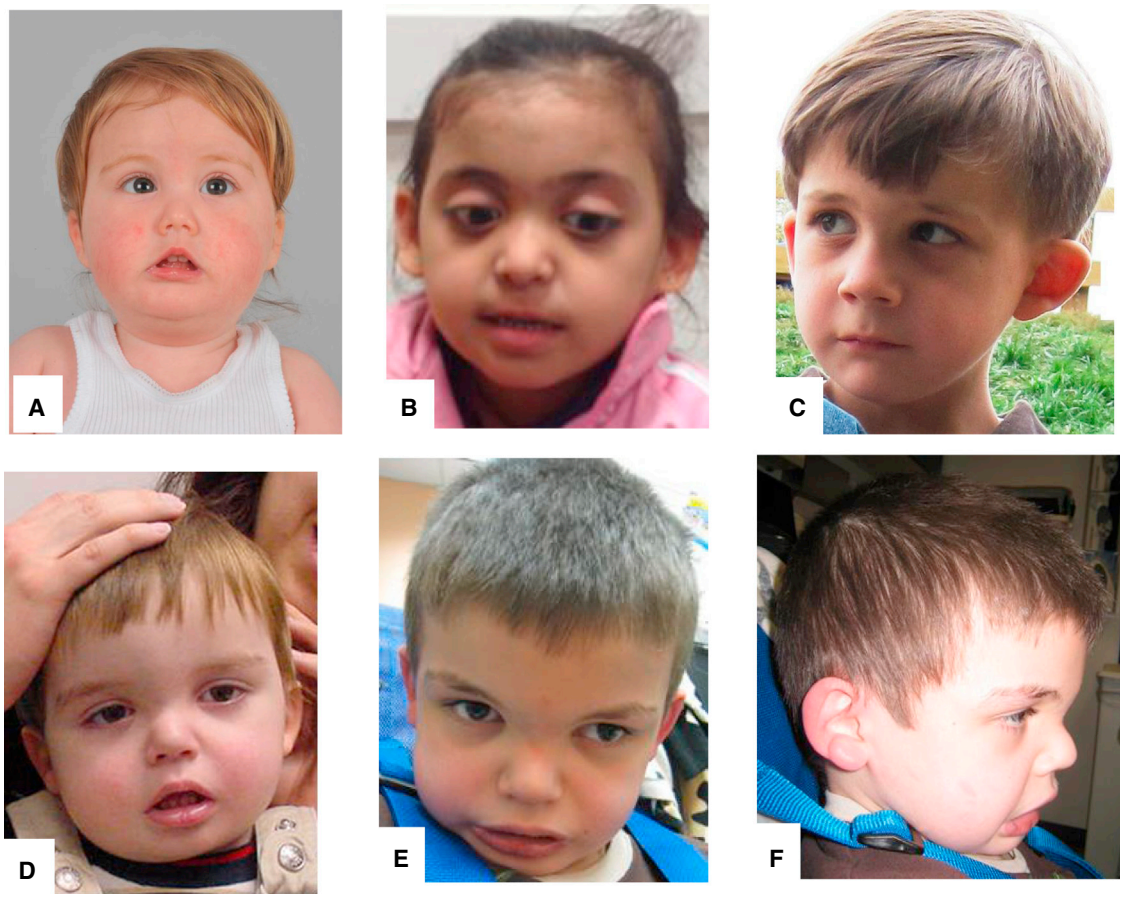

Figure 2. Facial Features of Probands
(A) Subject 1 (17 months old) with a round face, full cheeks, horizontal eyebrows, a depressed nasal bridge, anteverted nares, hypoplastic alae nasi, tented upper-lip vermillion, and microstomia.
(B) Subject 2 (4 years old) with thin eyebrows, a depressed nasal bridge, a bulbous nasal tip, and protuberant ears.
(C) Subject 3 (8 years old) with horizontal eyebrows, low-set ears, simple earlobes, and micrognathia.
(D) Subject 4 (21 months old) with a round face, full cheeks, horizontal eyebrows, a depressed nasal bridge, anteverted nares, tented upper-lip vermillion, and microstomia.
(E and F) Front (E) and side (F) views of subject 4 (9 years old) with a round face, full cheeks, horizontal eyebrows, an acute nasal angle, and fleshy pinna.

mutations in the three individuals were further demonstrated by PCR and Sanger DNA sequencing to be absent in maternal and paternal DNA and were therefore interpreted as de novo events. Interestingly, subjects 1 and 3 had the same de novo mutation (c.2373_2374delTG [p.Cys791Trpfs*57]). Except for the *AHDC1* mutations, we did not find other molecular events that could potentially explain the conditions in these probands. Therefore, the de novo mutations observed here in *AHDC1* are the most likely causes of the disease.

Clinical review of the four probands with *AHCD1* truncating mutations revealed that all had a history of congenital hypotonia and failure to thrive (Table 1; Table S1). The developmental histories were all remarkable for delayed speech, especially expressive language. All had mildly dysmorphic facial features that could be seen at a young age, and those of subject 4 persisted at an older age. Three probands also had a history of obstructive sleep apnea, potentially because of upper-airway structural abnormalities. All probands had prior brain MRI demonstrating hypoplasia of the corpus callosum. Simplification of the gyral pattern and delayed myelination were also observed. A retrocerebellar cyst was present in two of the four subjects (Figure S1).

The independent occurrence of four de novo mutational events at this locus in individuals with similar phenotypes is highly unlikely[5] (discussed in Bainbridge et al.[6]) and can be asserted as extremely strong evidence that these mutations in *ACHD1* cause this simplex disorder. To the best of our knowledge, the overall clinical presentations of these probands do not precisely match any previously known disease and, together with the statistical and molecular data, suggest a genetic syndrome defined by the mutations in *AHDC1*.

In subject 4, we also identified a de novo missense change, c.2006A>C (p.Asp669Ala), in *ANKRD11* (MIM 611192; RefSeq NM_013275.5). Haploinsufficiency of *ANKRD11* has been associated with KBG syndrome (MIM

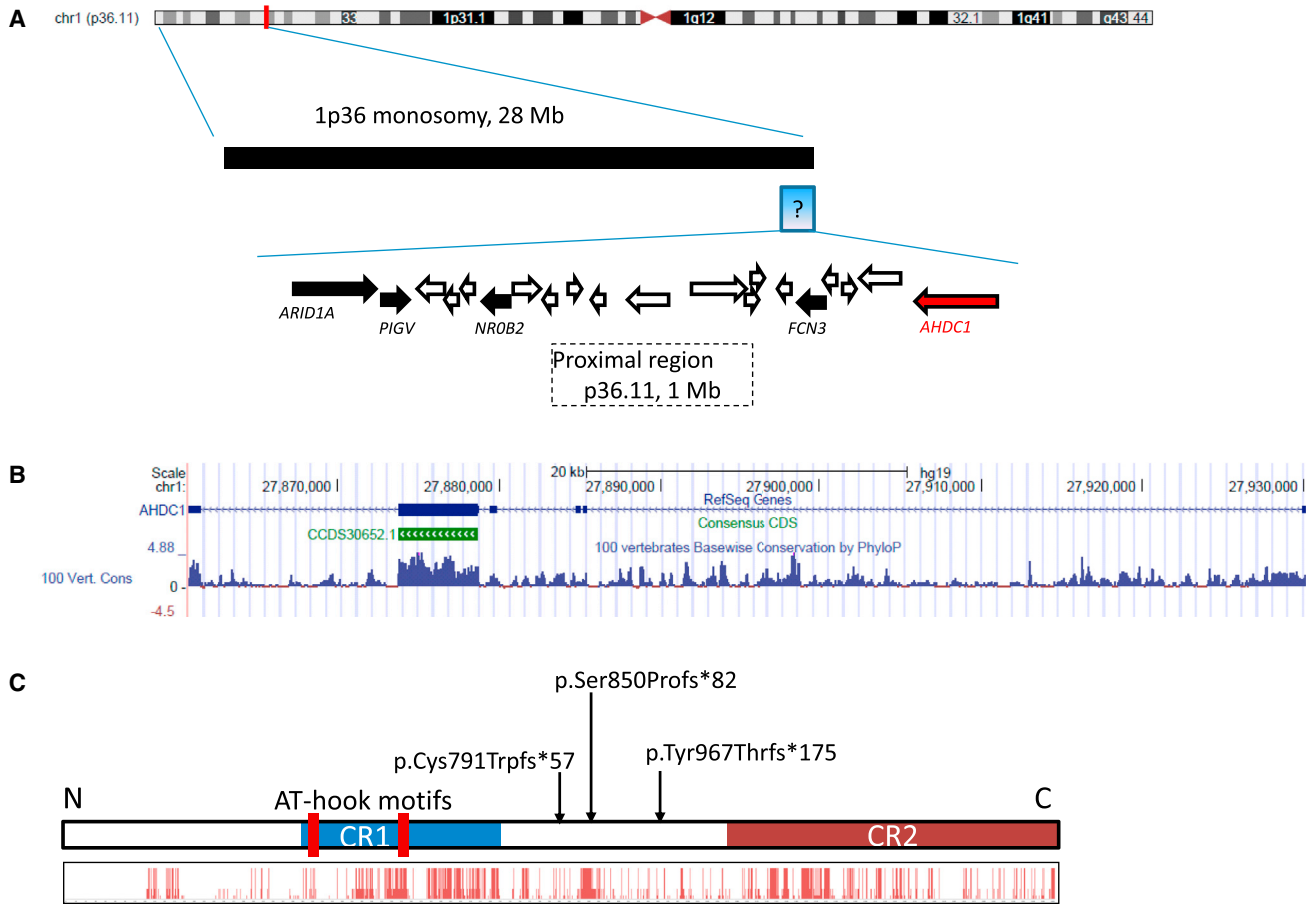

Figure 3. *AHDC1* Genomic Organization
(A) Chromosomal location of *AHDC1* and the genomic region surrounding it.
(B and C) Organization of *AHDC1* (B) and the AT-hook DNA-binding region, conserved regions, and location of the three different truncating alterations in the disorder (C). The histogram shows evolutionary conservation (see Figure S3 for details).

148050), characterized by macrodontia, variable facial dysmorphic features, mild skeletal anomalies, seizures in some individuals, and mild to moderate intellectual disability. However, the proband reported here did not have macrodontia, skeletal defects, or other features of KGB phenotypes and therefore did not meet the KBG diagnostic criteria proposed by Skjei et al.[7] Additionally, the well-characterized pathogenic mutations in *ANKRD11* are truncating.[8] Thus, the significance of *ANKRD11* missense variant c.2006A>C (p.Asp669Ala) is unclear.

AHDC1 is located on the short arm of chromosome 1 within the cytogenetic band 1p36.11, but it is more proximal than the regions identified from partial or complete monosomy of 1p36,[9] other small interstitial deletions,[10,11] and the nearby *ARID1A*, mutations in which cause autosomal-dominant Coffin-Siris syndrome[12] (MIM 135900). In the RefSeq and CCDS databases, the structural organization of *AHDC1* includes five untranslated exons upstream of a single 4,929 bp coding exon followed by a single downstream exon. This intronless coding structure is a common feature for newly evolved genes created by RNA-based retroposition.[13] Indeed, orthologs of *AHDC1* can only be found in vertebrate animals. Gaining intron structures during evolution is correlated with higher expression levels.[14] The expression level and patterns of *AHDC1* are more similar to those of the multi-intron *ARID1A* than to those of the intronless *FOXG1* (MIM 164874), two other genes associated with severe developmental disorders in humans (Figure S2). Therefore, it can be postulated that the introns of the UTRs of *AHDC1* affect and/or enhance the expression levels in various human tissues. On the nucleotide level, the single coding exon of *AHDC1* is well conserved among vertebrates (Figure 3). The 3′ untranslated exon also shows conservation levels similar to those of the coding region, suggesting a potential functional significance of this exon.

Human *AHDC1* encodes a protein of 1,603 amino acids. By aligning human AHDC1 against the protein sequences of AHDC1 orthologs in mouse, zebrafish, and western clawed frog, we found that the conserved amino acids are clustered into two regions (Figure 3C; Figure S3), suggesting two functional units. AHDC1 has two AT-hook DNA-binding motifs located at codons 396–408 and 544–556, contained in conserved region 1. AT-hook domains are DNA-binding motifs that act to fasten proteins to AT-rich sequences in DNA.[15] Although conserved, region 2

contains no known functional domains. In vitro protein-interaction assays have shown that AHDC1 interacts with a number of other nuclear proteins.[16–21] Therefore, conserved regions 1 and 2 of AHDC1 might interact with the DNA elements or protein partners. Interestingly, all of the de novo mutations found in these four probands might truncate conserved region 2 but preserve region 1. Given that each mutation identified here occurs in a single coding exon, the modified mRNA might escape nonsense-mediated decay,[22] suggesting that the autosomal-dominant mode of inheritance of these mutations is possibly due to the formation of dominant-negative proteins rather than haploinsufficiency.

Future research to better delineate the functional domains of AHDC1 is now enhanced by the phenotypic association with the truncating mutations reported here. Also, the phenotypes of the four probands are clearly similar in retrospect. However, speech delay and obstructive sleep apnea are sufficiently common conditions that it is unlikely that this syndrome would have been identified if the de novo mutations had not been uncovered first.

Supplemental Data

Supplemental Data include three figures and one table and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2014.04.006.

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Web Resources

The URLs for data presented herein are as follows:

Atherosclerosis Risk in Communities Study, http://www2.cscc.unc.edu/aric/
Baylor College of Medicine Whole Genome Laboratory, https://www.bcm.edu/research/medical-genetics-labs/wholegenomelab
CCDS, http://www.ncbi.nlm.nih.gov/CCDS/CcdsBrowse.cgi
ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/
NHLBI Exome Sequencing Project (ESP) Exome Variant Server, http://evs.gs.washington.edu/EVS/
Online Mendelian Inheritance in Man (OMIM), http://omim.org/
RefSeq, http://www.ncbi.nlm.nih.gov/refseq/

Accession Numbers

The ClinVar accession numbers for the DNA variant data reported in this paper are SCV000148377, SCV000148378, and SCV000148379.

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Supplemental Data

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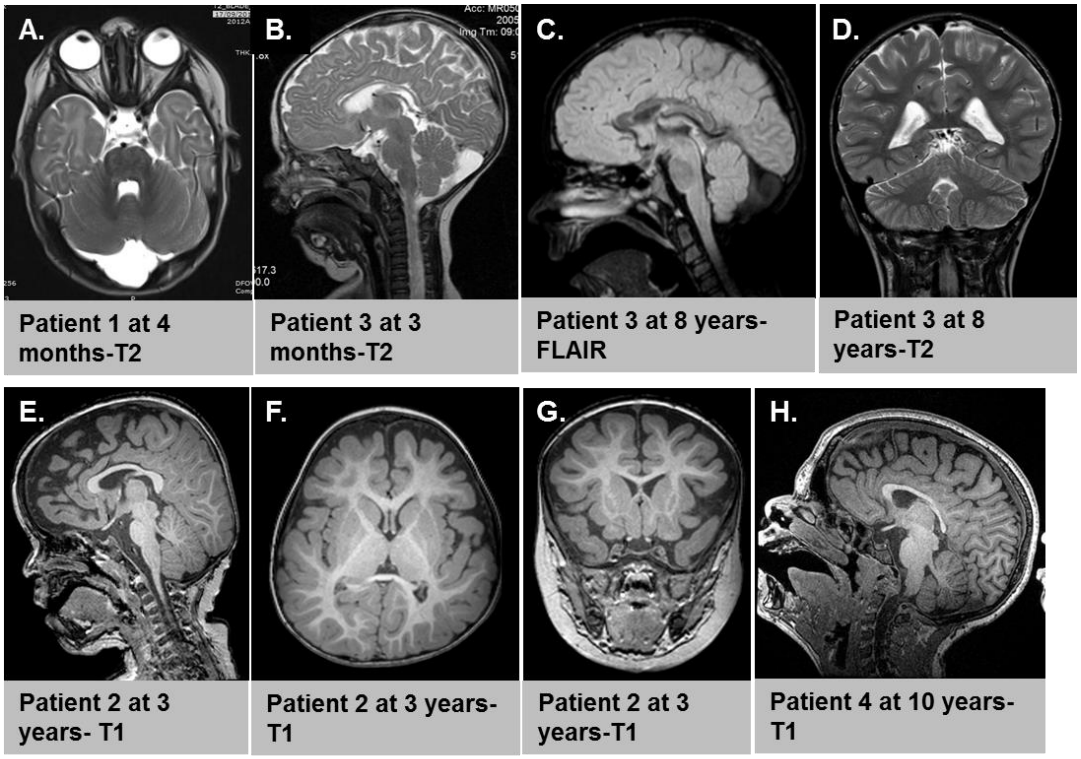


MRI Finding	Patient 1	Patient 2	Patient 3	Patient 4
Thin Corpus callosum	N.D.	+	+	+
Gyral simplification or under-operculization	+	+	+	N.D.
Cerebellar Folia simplification	N.D.	+	+	N.D.
Retrocerebellar cyst	+	-	+	-

N.D.= Not determined due to patient age (Patient 1) or image availability (Patient 4)

Figure S1: Brain MRI findings in patients with *de novo AHDC1* mutations (A) Patient 1 at 4 months- T2 demonstrates the presence of a retrocerebellar cyst. (B) Patient 3 at 3 months- T2 demonstrates thinning of the corpus callosum which can be seen in normal individuals at this age. (C) Patient 3 at 8 years- FLAIR sequence confirms thin corpus callosum, persisting at this age constituting an abnormal finding related to white-matter.(D) Patient 3 at 8 years- T2 demonstrates simplification of the cerebellar folia. (E) Patient 2 at 3 years- T1 demonstrates slumping of the posterior fossa and thinning

of the corpus callosum (F) Patient 2 at 3 years- T1 axial section demonstrates relative paucity of white matter at 3 years (G) Patient 2 at 3 years-T1 coronal sequence demonstrates thinning of the corpus callosum, gyral simplification and paucity of white matter (H) Patient 4 at 10 years- T1 sagittal section demonstrates thinning of the corpus callosum. (I) Tabulation of major MRI findings in the 4 patients, some features could not be determined due to patient age or image availability.

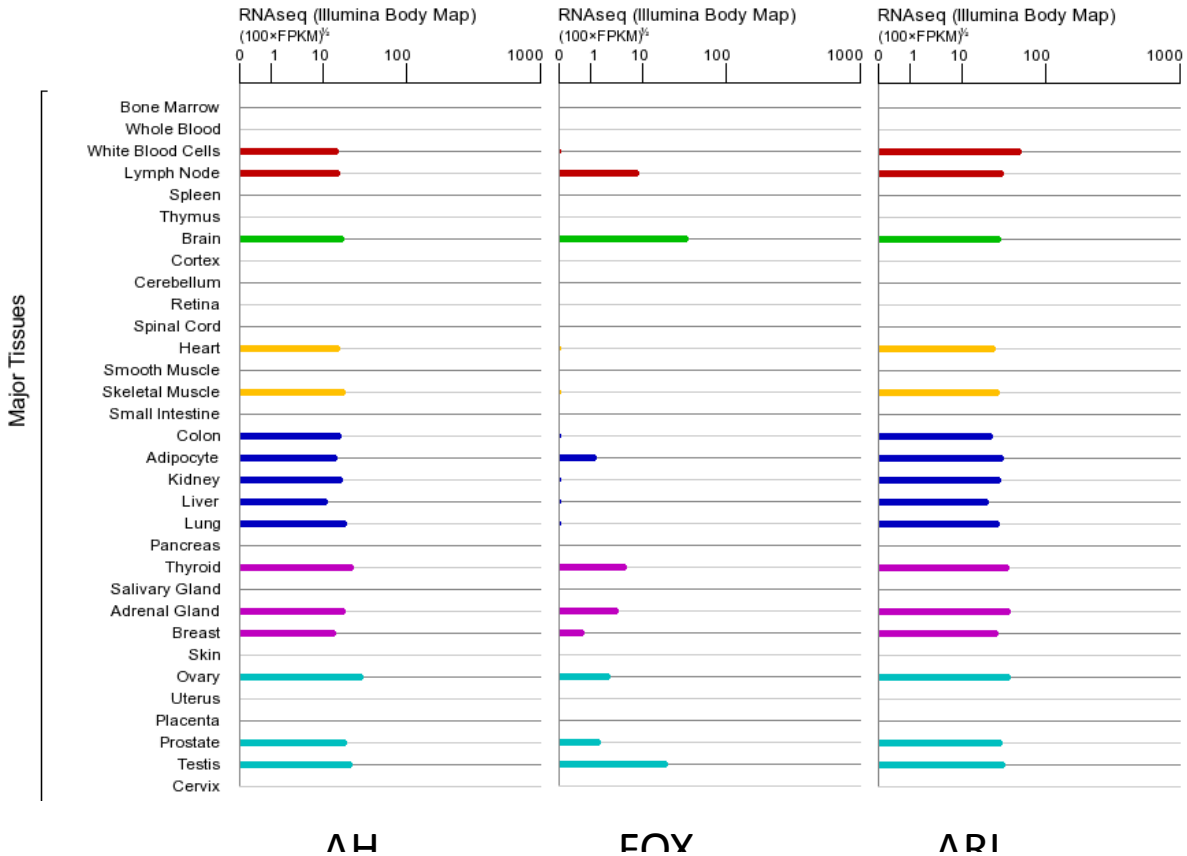
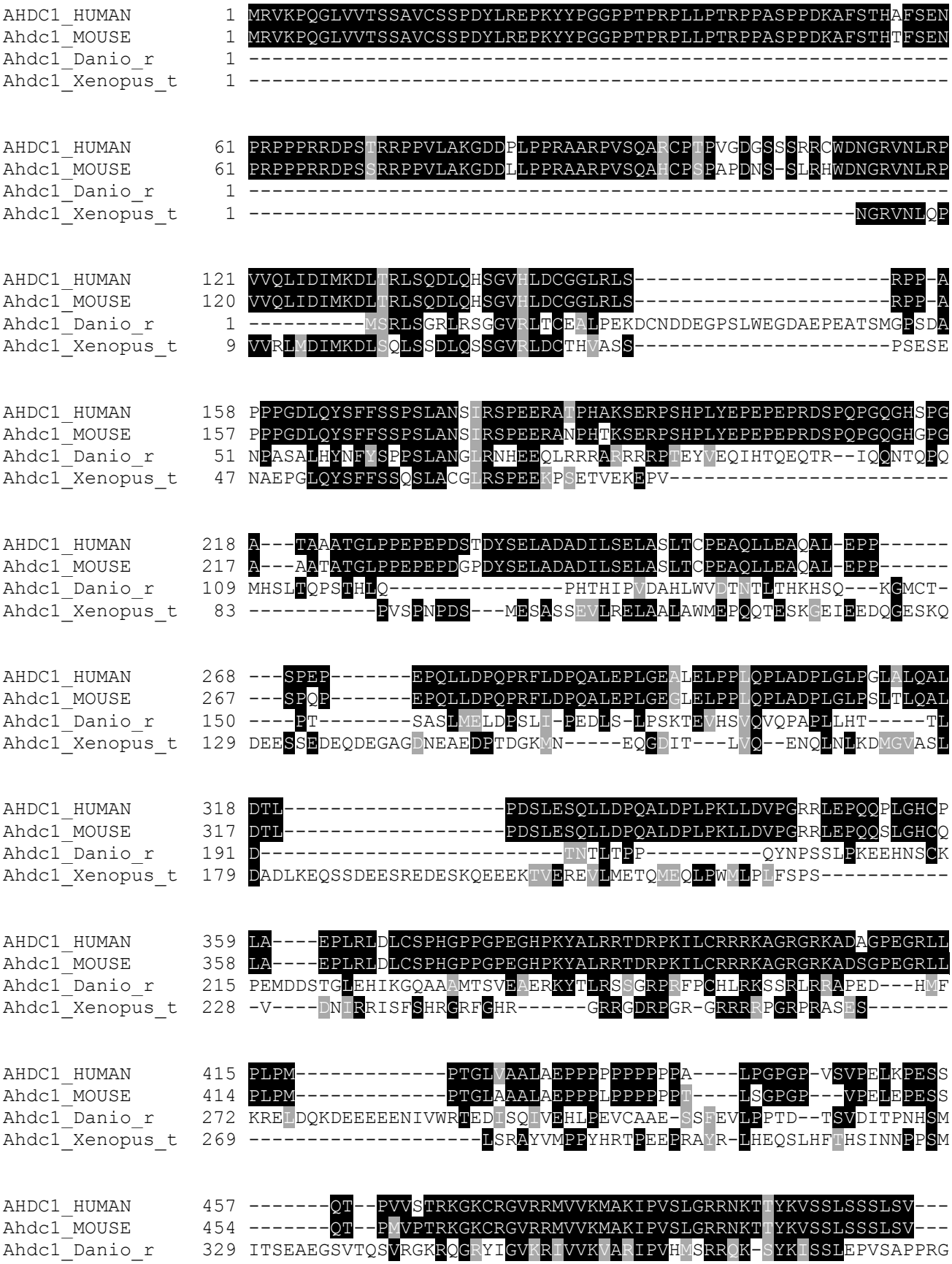


Figure S2: Expression patterns of *AHDC1*, *FOXG1* and *ARID1A* (From Genecards http://genecards.org/).


AHDC1_HUMAN 1 MRVKPQGLVVTSSAVCSSPDYLREPKYYPGGPPTPRPLLPTRPPASPPDKAFSTHAFSEN
Ahdc1_MOUSE 1 MRVKPQGLVVTSSAVCSSPDYLREPKYYPGGPPTPRPLLPTRPPASPPDKAFSTHTFSEN
Ahdc1_Danio_r 1
Ahdc1_Xenopus_t 1
AHDC1_HUMAN 61 PRPPPRRDPSTRRPPVLAKGDDPLPPRAARPVSQARCPTPVGDGSSSRRCWDNGRVNLRP
Ahdc1_MOUSE 61 PRPPPRRDPSSRRPPVLAKGDDLLPPRAARPVSQAHCPSPAPDNS-SLRHWDNGRVNLRP
Ahdc1_Danio_r 1
Ahdc1_Xenopus_t 1 NGRVNLQP
AHDC1_HUMAN 121 VVQLIDIMKDLTRLSQDLQHSGVHLDCGGLRLS RPP-A
Ahdc1_MOUSE 120 VVQLIDIMKDLTRLSQDLQHSGVHLDCGGLRLS RPP-A
Ahdc1_Danio_r 1 MSRLSGRLRSGGVRLTCEALPEKDCNDDEGPSLWEGDAEPEATSMGPSDA
Ahdc1_Xenopus_t 9 VVRLMDIMKDLSQLSSDLQSSGVRLDCTHVASS PSESE
AHDC1_HUMAN 158 PPPGDLQYSFFSSPSLANSIRSPEERATPHAKSERPSHPLYEPEPEPRDSPQPGQGHSPG
Ahdc1_MOUSE 157 PPPGDLQYSFFSSPSLANSIRSPEERANPHTKSERPSHPLYEPEPEPRDSPQPGQGHGPG
Ahdc1_Danio_r 51 NPASALHYNFYSPPSLANGLRNHEEQLRRRARRRPTEYVEQIHTQEQTR--IQQNTQPQ
Ahdc1_Xenopus_t 47 NAEPGLQYSFFSSQSLACGLRSPEEKPSETVEKEPV
AHDC1_HUMAN 218 A---TAAATGLPPEPEPDSTDYSELADADILSELASLTCPEAQLLEAQAL-EPP
Ahdc1_MOUSE 217 A---AATATGLPPEPEPDGPDYSELADADILSELASLTCPEAQLLEAQAL-EPP
Ahdc1_Danio_r 109 MHSLTQPSTHLQ PHTHIPVDAHLWVDTNTLTHKHSQ---KGMCT-
Ahdc1_Xenopus_t 83 PVSPNPDS---MESASSEVLRELAALAWMEPQQTESKGEIEEDQGESKQ
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Ahdc1_Danio_r 191 D TNTLTPP QYNPSSLPKEEHNSCK
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Ahdc1_MOUSE 358 LA----EPLRLDLCSPHGPPGPEGHPKYALRRTDRPKILCRRRKAGRGRKADSGPEGRLL
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Ahdc1_Xenopus_t 228 -V----DNIRRISFSHRGRFGHR GRRGDRPGR-GRRRRPGRPRASES
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Ahdc1_MOUSE 454 -------QT--PMVPTRKGKCRGVRRMVVKMAKIPVSLGRRNKTTYKVSSLSSSLSV---
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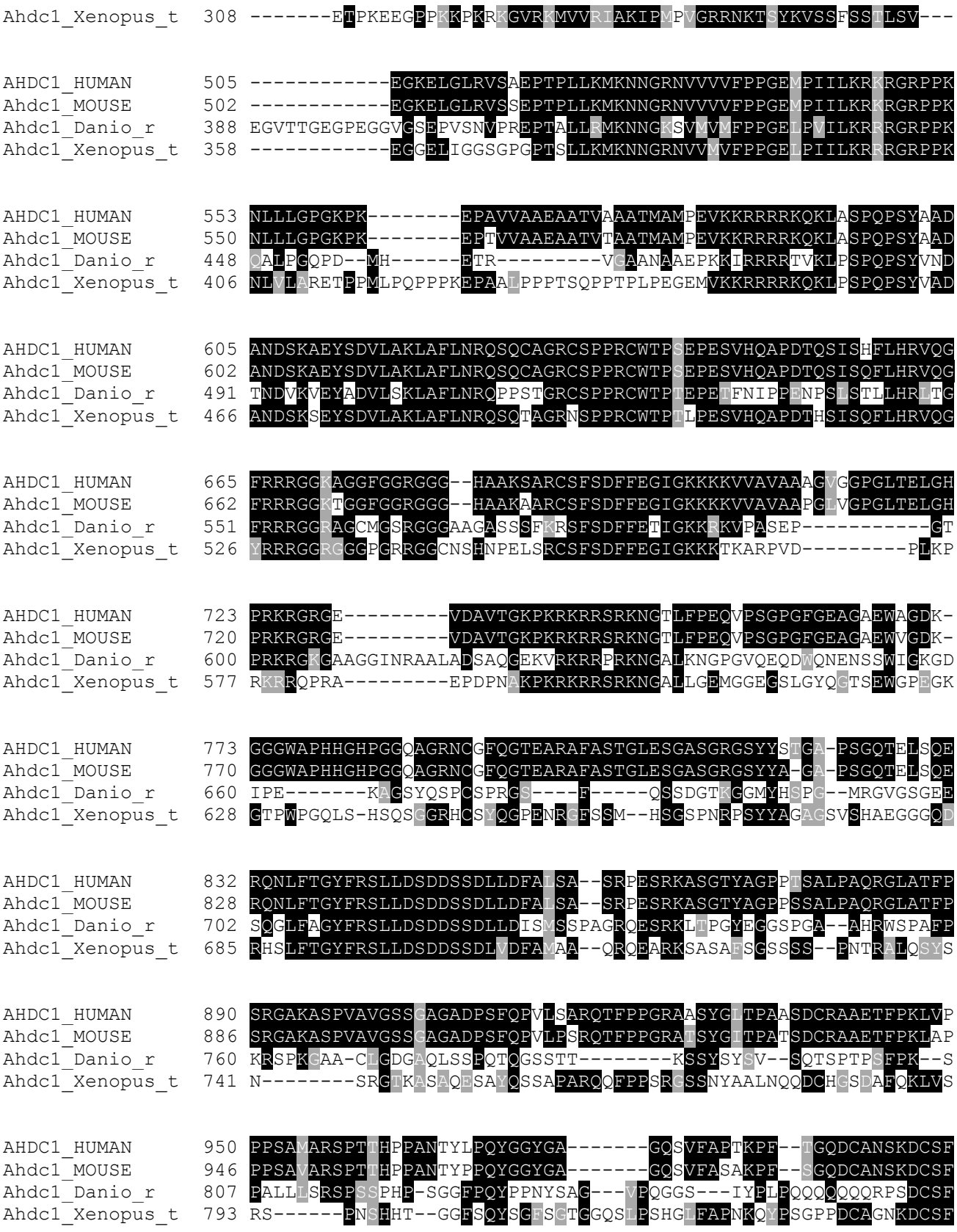
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Ahdc1_Danio_r 551 FRRRGGRAGCMGSRGGGAAGASSSFKRSFSDFFETIGKKRKVPASEP----------GT
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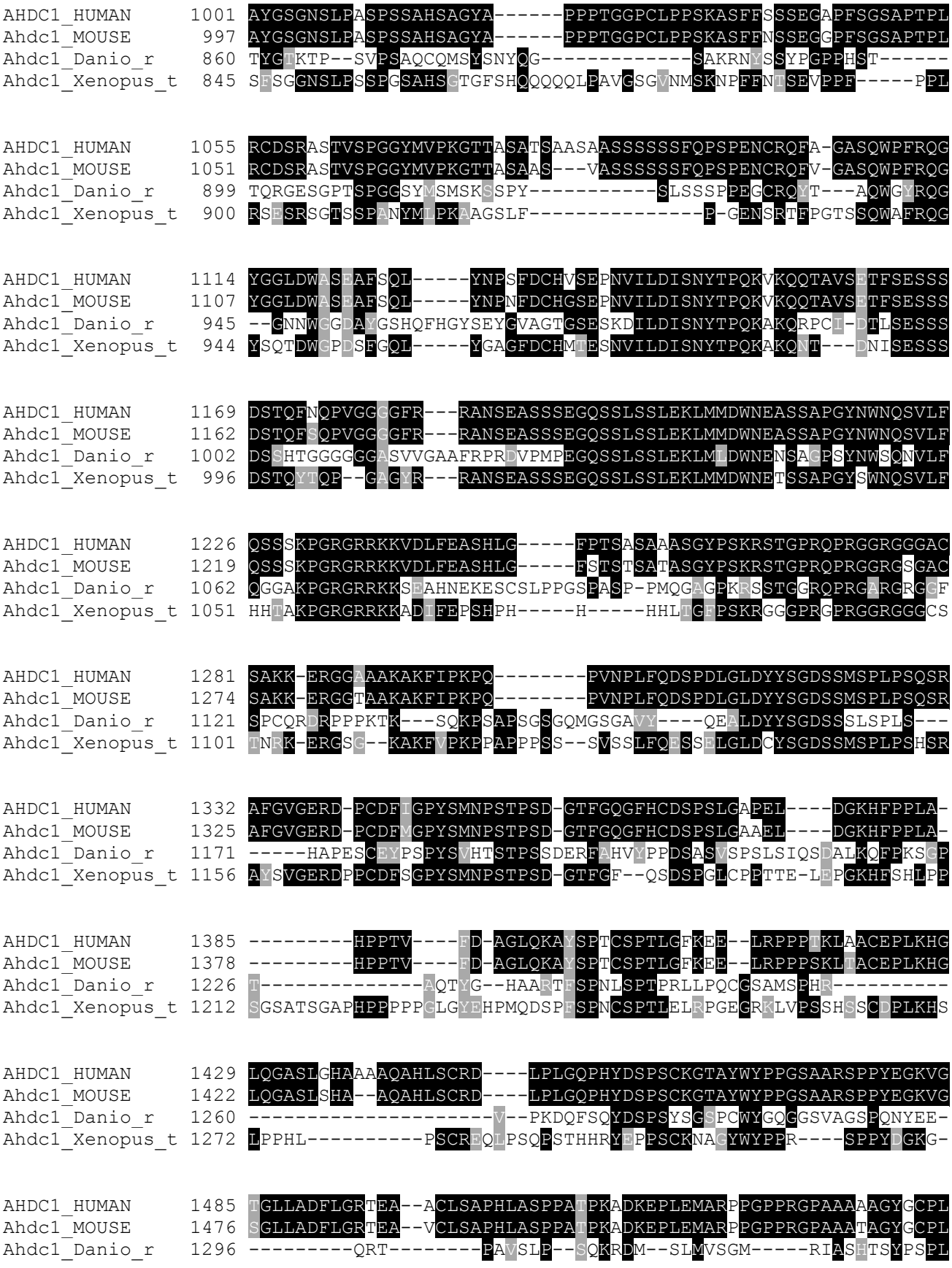
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Ahdc1_MOUSE 1051 RCDSRASTVSPGGYMVPKGTTASAAS---VASSSSSSFQPSPENCRQFV-GASQWPFRQG
Ahdc1_Danio_r 899 TQRGESGPTSPGGSYMSMSKSSPY----------SLSSSPPEGCRQYT---AQWGYRQG
Ahdc1_Xenopus_t 900 RSESRSGTSSPANYMLPKAAGSLF----------P-GENSRTFPGTSSQWAFRQG
AHDC1_HUMAN 1114 YGGLDWASEAFSQL-----YNPSFDCHVSEPNVILDISNYTPQKVKQQTAVSETFSESSS
Ahdc1_MOUSE 1107 YGGLDWASEAFSQL-----YNPNFDCHGSEPNVILDISNYTPQKVKQQTAVSETFSESSS
Ahdc1_Danio_r 945 --GNNWGGDAYGSHQFHGYSEYGVAGTGSESKDILDISNYTPQKAKQRPCI-DTLSESSS
Ahdc1_Xenopus_t 944 YSQTDWGPDSFGQL-----YGAGFDCHMIESNVILDISNYTPQKAKQNT---DNISESSS
AHDC1_HUMAN 1169 DSTQFNQPVGGGGFR---RANSEASSSEGQSSLSSLEKLMMDWNEASSAPGYNWNQSVLF
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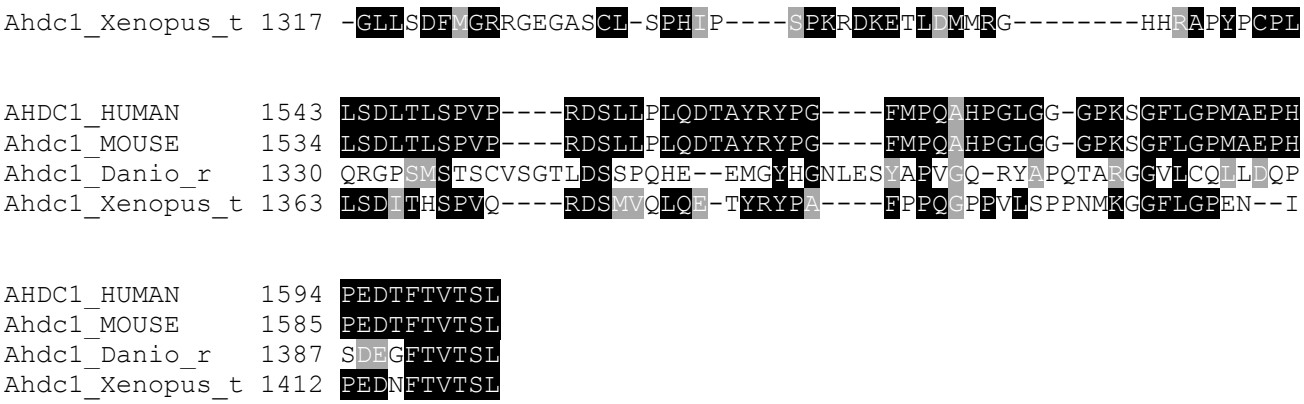

Figure S3: Sequence alignments of AHDC1 in select vertebrates.

SUBJECT	#1	#2	#3	#4
Sex	Female	Female	Male	Male
Age	18 mo	4 yo	8 yo	11 yo
Major Concern	Global developmental delay, hypotonia	Global developmental delay, hypotonia	Micrognathia, developmental delay	Autism, epilepsy, developmental delay, cortical visual impairment
Birth	Term	Term	Term	38w
Weight	<3%	25%	25%	20%
Height	10%	10%	50%	5%
HOC	50%	50%	60%	75%
Head/Face/Neck	Relative macrocephaly; anterior fontanelle not wide	Normal	Plagiocephaly, Mild synophrys	Relative macrocephaly
Ears	Low set tympanostomy tubes for middle ear effusions	Protuberant	Low set, protuberant, large helices with small/absent lobes	Left ear simple with upturned earlobe
Eyes	Intermittent esotropia, hyperopia, upslanting palpebral fissures, horizontal eyebrows, possible cortical visual impairment	Upslanting palpebral fissures	Hypertelorism, downslanting palpebral fissures. horizontal eyebrows, Mild ptosis.	Hypertelorism, esotropia, hyperopia, horizontal eyebrows,
Mouth	Tented vermillion of upper lip, micrognathia, high arched palate	No	Micrognathia requiring surgery. Small mouth. Limited jaw opening.	Tented vermillion of upper lip
Nose	Flat nasal bridge, anteverted nares	Flat nasal bridge	Normal	Flat nasal bridge, anteverted nares
Teeth	Normal eruption; teeth grinding	No	Normal	Wide spaced
Anatomic Upper Airway obstruction	Laryngomalacia	Obstructive sleep apnea	Laryngomalacia	Tracheomalacia, resolved
Gastrointestinal	Feeding problems	No	Chewing problem	G-tube fed, constipation
Genitalia	Normal external genitalia	Normal	Small penis	Normal
Hands and Feet	Clinodactyly, small hands and feet, positional talipes	Normal	Index fingers tapered with narrow nails.	Small hands and feet, persistent fetal finger

	equinovarus		Wide gap between Left index and middle fingers. Right transverse plamar crease.	pads(also on the toes), 2,3 toe syndactyly
Chest	Broad chest, wide-set nipples	Normal	Normal	Normal
Central Nervous System	Global developmental delay	Global developmental delay	Global developmental delay	Global developmental delay
Intellectual Disability	Not evaluated due the young age	Moderate	Mild	Moderate
Hypotonia	Severe	Yes	Yes	Yes
Sit	With support	19 mo	9 mo	15 mo
Walk	Not crawling	2 yo	18 mo	27 mo with walker, can now scoot, walks 15-20 feet with walker, wheelchair for long distances
Speech	Delayed; only babbling at 18 months	Delayed (2-3 words)	Delayed (First words just after 1 yo. Persistent speech therapy.)	Delayed, Vocalizes, no words
Seizures	None	None	Multiple episodes	Focal epilepsy with gelastic seizures diagnosed at 8 years, progressive
Sleep Abnormalities	Obstructive sleep apnea requiring CPAP	Obstructive sleep apnea	Obstructive sleep apnea, occasional central apnea.	Wakes for long periods at night, sleepiness during day, no history of sleep apnea
Autistic/Behaviour	Some stereotypic hand movements	No. Good eye contact	No	Yes
MRI Imaging (see also Supplementary Figure One)	Subtle hyperintensities in midbrain, pons and globus pallidi on T2 and FLAIR imaging; retro-cerebellar cyst. Gyral simplification	Gyral simplification, hypoplasia of the corpus callosum	Hypoplasia of the corpus callosum, gyral simplification, and mega cisterna magna.	Hypoplasia of the corpus collosum
Misc.	Mild self-resolving hepatomegaly	Chronic ear infections	Echocardiogram normal.	Scoliosis, echocardiogram normal
Family History	Negative; one	Negative, first	Negative, one	Negative, two

	healthy sibling	child	healthy sibling	healthy siblings
Metabolic Work up	Negative	Negative	Acylcarnitine profile, carnitine levels and lactate normal	Negative
Prior Genetic Testing (negative)	Myotonic dystrophy, SMA, PWS, CMA	FX, MECP2, CMA, AOH on chromosome 5	CMA, FX, RASopathy gene panel	SMA, PWS, SMS, Fragile X, TCF4, MED12, ATRX, CMA
***AHDC1* Mutations**	c.2373_2374delTG, p.Cys791Trpfs*57	c.2898delC, p.Tyr967Thrfs*175	c.2373_2374delTG, p.Cys791Trpfs*57	c.2547delC, p.Ser850Profs*82

Table S1: Detailed clinical features of probands.