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ARTICLE

De novo variants in *GATAD2A* in individuals with a neurodevelopmental disorder: GATAD2A-related neurodevelopmental disorder

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Summary

GATA zinc finger domain containing 2A (GATAD2A) is a subunit of the nucleosome remodeling and deacetylase (NuRD) complex. NuRD is known to regulate gene expression during neural development and other processes. The NuRD complex modulates chromatin status through histone deacetylation and ATP-dependent chromatin remodeling activities. Several neurodevelopmental disorders (NDDs) have been previously linked to variants in other components of NuRD's chromatin remodeling subcomplex (NuRDopathies). We identified five individuals with features of an NDD that possessed *de novo* autosomal dominant variants in *GATAD2A*. Core features in affected individuals include global developmental delay, structural brain defects, and craniofacial dysmorphology. These *GATAD2A* variants are predicted to affect protein dosage and/or interactions with other NuRD chromatin remodeling subunits. We provide evidence that a *GATAD2A* missense variant disrupts interactions of GATAD2A with CHD3, CHD4, and CHD5. Our findings expand the list of NuRDopathies and provide evidence that *GATAD2A* variants are the genetic basis of a previously uncharacterized developmental disorder.

Introduction

Chromatin modifiers and remodelers have been recently implicated in a variety of neurodevelopmental disorders (NDDs).[1–8] The nucleosome remodeling and deacetylase (NuRD) complex has been linked to four NDDs with overlapping phenotypes as a result of dominant variants in several paralogous subunits of the complex (NuRDopathies).[5–9] NuRD regulates a variety of cellular processes including cell-cycle progression, genome integrity, and cellular differentiation.[10–12] The NuRD complex consists of several different subunits, each with a set of paralogous proteins.[9] The holoenzyme complex can be divided into two subcomplexes: the chromatin remodeling subcomplex (CRS) and a histone deacetylation (HDAC) subcomplex, or HDAC core (Figure 1A). The HDAC core consists of three subunits in multiple copies comprised of different paralogs that include four retinoblastoma-binding protein (RBBP4/7) subunits, two metastasis-associated protein (MTA1/2/3) subunits, and two histone deacetylase (HDAC1/2) subunits.[10,12] By contrast, the CRS is composed of three monomeric paralogous subunits in series: a methyl-binding domain protein (MBD2/3), a GATAD2 protein (GATAD2A/B, previously known as p66α/β), and a chromodomain helicase DNA-binding protein (CHD3/4/5). A CDK2AP1 protein serves as the final member of the CRS and interacts with GATAD2 and CHD paralogs (not shown in diagram).[10,12]

Notably, the various paralogs enable a wide variety of NuRD subtypes, each with the potential to provide unique functions. For example, CHD3-, CHD4-, and CHD5-possessing NuRD subtypes (CHD3-NuRD, CHD4-NuRD, etc.) are differentially expressed during corticogenesis. CHD4-NuRD subtypes activate expression of a specific set of genes in neural progenitor cells, which are subsequently repressed in cortical neurons by CHD3-NuRD.[13,14] Interestingly, *CHD3*, *CHD4*, and *CHD5* are all associated with dominant NDDs with overlapping phenotypes (*CHD3*-related syndrome [CHD3RS/Snijders-Blok-Campeau syndrome;

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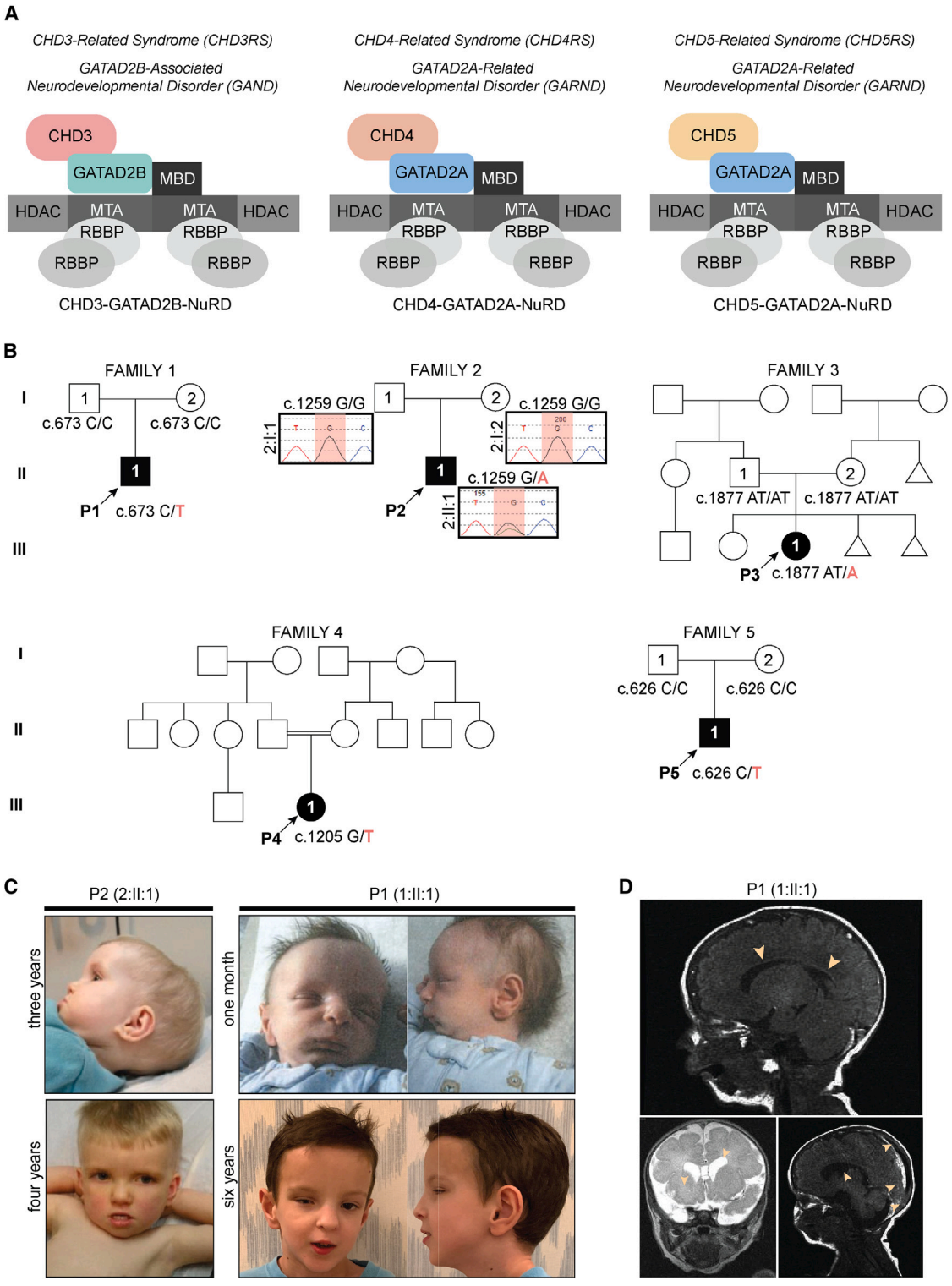

Figure 1. Individuals with monoallelic variants in *GATAD2A*

(A) Schematic of different NuRD subtypes and associated developmental disorders. NuRD subtypes can be defined by the presence of one paralog (CHD3-NuRD) or multiple paralogs (MBD3-GATAD2A-CHD4-NuRD). The disorders would be due to a deficiency of any particular NuRD paralog and all of its associated subtypes (e.g., CHD3-NuRD deficiency = CHD3RS).

(B) Pedigrees of five families with *GATAD2A* variants segregating with developmental disorders and the genotypes of sequenced individuals. Squares represent males; circles represent females; triangles represent pregnancies not carried to term; clear shapes indicate unaffected status; solid shapes indicate individuals with GARND.

(C) Facial photos across development showing subtle overlapping craniofacial dysmorphology in P1 and P2, including prominent or broad forehead, deep-set eyes, and broad nasal root. Macrocephaly is also seen in P2.

(D) Sagittal and coronal MRI of P1 at 4 days old. Yellow arrows highlighting thin corpus callosum (top), slightly enlarged ventricles (bottom left), and parieto-occipital subdural hematoma (bottom right).

MIM: 618205], *CHD4*-related syndrome [CHD4RS/Sifrim-Hitz-Weiss syndrome; MIM: 617159], and *CHD5*-related syndrome [CHD5RS/Parenti-Mignot neurodevelopmental syndrome, MIM: 619873]) (Figure 1A).[6–8,15] Of note, dominant variants in *GATAD2B*, which tethers the CHD paralogs to the rest of the complex in GATAD2B-NuRD (2B-NuRD) subtypes, have been associated with GATAD2B-associated neurodevelopmental disorder (GAND) (MIM: 615074), a NuRDopathy whose phenotypes encompass nearly all features of CHD3RS, CHD4RS, and CHD5RS combined (Figure 1A).[16] To date, GATAD2A has not been associated with a NuRD-related NDD; however, GATAD2A deficiency has been linked to increased expression of fetal hemoglobin with a nonsense *GATAD2A* variant (c.19C>T, p.R7*),[17,18] and regulatory variants in *GATAD2A* are significantly associated with schizophrenia and bipolar disorder.[19,20]

The degree to which GATAD2A-NuRD (2A-NuRD) and 2B-NuRD subtypes functionally overlap or diverge is unclear, although some research has suggested non-redundant functions in certain cell types.[10,17,21,22] GATAD2A possesses proline-rich PPPLφ motifs (absent in GATAD2B) that allow for interaction with MYND domains in proteins like ZMYND8.[21] GATAD2A also seems to have a non-redundant role in early stem cell differentiation and its ablation enhances pluripotent reprogramming.[22] Interestingly, *GATAD2A* is highly expressed during early neural development,[23] which is consistent with early embryonic lethality and variable developmental defects in *Gatad2a* knockout mice.[24]

We report the identification of five novel *de novo* heterozygous variants in *GATAD2A* in five unrelated individuals with NDD phenotypes. Despite variable expressivity of phenotypes, shared clinical features in affected individuals include global developmental delay (GDD), structural brain defects, and craniofacial anomalies. Observed clinical phenotypes overlap with other NuRDopathies, suggesting that NuRD paralog deficiencies may converge on similar mechanisms during development. We also demonstrate that one missense variant (c.1259 G>A, p.C420Y) disrupts known interactions between GATAD2A and CHD paralogs. We hypothesize that these *GATAD2A* variants likely act through a loss-of-function (LoF) haploinsufficiency mechanism. Together, we provide evidence for a GATAD2A-related neurodevelopmental disorder that we have termed GARND (Figure 1A).

Materials and methods

Research subjects

All subjects and parents or guardians provided informed consent and were enrolled in institutional review board (IRB)-approved research studies. Consenting was performed in accordance with the ethical standards of the respective IRB committees on human research subjects and in keeping with international standards. Probands (P) 2–5 were identified through multiple nodes in the MatchMaker Exchange, including GeneMatcher and MyGene2.[25,26] Participants were recruited at the following institutions: the University of Michigan Pediatrics Genetics Clinic at C.S. Mott Children's Hospital (P1), Department of Medical Genetics at the Alberta Children's Hospital, University of Calgary (P2), St. Louis Children's Hospital in St. Louis, Missouri (P3), Saint Peter's University Hospital (P4), and the Ludwig Maximilian University of Munich Dr. von Hauner Children's Hospital (P5).

Exome sequencing and analysis

Genomic DNA for each participant were extracted using the DNA Genotek Prep-IT L2P (no. PT-L2P) or QIAGEN DNeasy Blood & Tissue Kit (no. 69504), quantified with the Life Technologies Quant-iT PicoGreen dsDNA Assay Kit (no. P7589), and 3 μg were submitted for exome sequencing on an Illumina HiSeq instrument at the following institutions: the University of Washington Center for Mendelian Genomics, the Department of Medical Genetics at the University of Calgary, GeneDx, and the Institute of Human Genetics at the Technical University of Munich School of Medicine. Reads were aligned to the hg38 reference genome (GRCh38.p13) using Burrows-Wheeler Aligner. Read and alignment quality was assessed for each sample using PLINK (v.1.90b2m), and kinship was confirmed with KING v.1.4.0.

Variant calling and annotation

Variant calling of single-nucleotide variants (SNVs) and copy number variants was performed using GATK and CONIFER, respectively. The data were filtered and annotated using GEMINI v.0.19.1 Variant Effect Predictor (VEP). Variants were also filtered against public databases including the 1000 Genomes Project phase 311, Genome Aggregate Database (gnomAD), and NHLBI Exome Sequencing Project (ESP) 6500. Those with a minor allele frequency >0.005 were excluded. In addition, variants flagged as low impact, low quality, or putative false positives (Phred quality score <20) were excluded from the analysis. Variants in genes known to be associated with NDD were selected and prioritized based on predicted pathogenicity.

The *de novo* status of *GATAD2A* variants in P1, P3, and P5 was reported based on trio exome sequencing results, and by Sanger sequencing confirmation in participants P2 and P3 and respective parents (Figure 1B). Pathogenicity of variants was assessed according to American College of Medical Genetics (ACMG) guidelines and using the Franklin Genoox online classification tool. All variants were submitted to Database: ClinVar (accession no. VCV001705818.2, VCV001705819.2, VCV001705820.2, VCV001705821.2, and VCV001705822.2).

Protein conservation, structure, and *in silico* analyses

NCBI HomoloGene tool was used to obtain aligned amino acid sequences of GATAD2A across species at affected residues and flanking regions. Protein alignment was performed on GATAD2A and GATAD2B sequences using Geneious Prime v.2022.1.1 global alignment with free end gaps and a Blosum62 cost matrix. PDB files for GATAD2A (AF-Q86YP4) were downloaded and extracted from the AlphaFold Protein Structure Database's reference *Homo sapiens* proteome file no. UP000005640. The structure was edited in PyMOL v.2.5.2. *In silico* prediction of the functional impact of GATAD2A variants was performed using Polymorphism Phenotyping (PolyPhen-2) v.2.2.3r406 using the HumDiv model, Sorting Intolerant From Tolerant (SIFT), varSEAK, and MutationTaster2021.[27–29] Combined annotation-dependent depletion (CADD) Phred scores were obtained for each variant using

CADD v.1.6 against GRCh38[30] MetaDome analysis was performed on NM_017660.3 transcript using the online tool.[31]

Plasmids

Full-length cDNA sequence for human *GATAD2A* (GenBank: NM_017660.5) as well as partial cDNA sequences for the C-terminal domain (CTD) of CHD3 (encoding residues 1246–1944 [GenBank: NM_001005273.3]), CHD4 (residues 1230–1912 [GenBank: NM_001273.5]), and CHD5 (residues 1218–1954 [GenBank: NM_015557.3]) were each cloned into the pcDNA3.1 expression vector to generate the following plasmids: HA-tagged GATAD2A (HA-GATAD2A^{WT}), FLAG-tagged CHD3-CTD (FLAG-CHD3_CTD), FLAG-tagged CHD4-CTD (FLAG-CHD4_CTD), and FLAG-tagged CHD5-CTD (FLAG-CHD5_CTD). Site-directed mutagenesis was performed on HA-GATAD2A^{WT} to introduce the p.C420Y missense variant (HA-GATAD2A^{C420Y}).

Western blotting and co-immunoprecipitation

Immunoprecipitation assays were performed as previously described.[16] In brief, HA-GATAD2A^{WT} and HA-GATAD2A^{C420Y} proteins were independently co-expressed with each of the three FLAG-CHD-CTD fusion proteins in rabbit reticulocyte lysates for *in vitro* translation (IVT). Expressed FLAG-CHD-CTD proteins together with HA-GATAD2A proteins were immobilized on anti-FLAG resin, washed, and eluted with 3X-FLAG peptide. In parallel, IVT lysates expressing only an HA-GATAD2A or FLAG-CHD-CTD protein, as well as lysates with no expressed protein, were run as negative controls. Immunoprecipitation inputs and eluates were loaded and run with sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by transfer to a polyvinylidene fluoride membrane. Immunoblots were probed with anti-HA-HRP (1:20,000, Cell Signaling Technology, no.2999S) followed by probing with anti-FLAG-HRP (1:80,000, Sigma Aldrich, no. A8592).

Results

Individuals with heterozygous *GATAD2A* variants exhibit features of developmental disorders

Through research exome-based sequencing, we identified a *de novo* variant of uncertain significance in the *GATAD2A* gene (NM_017660.5) in a child (P1; c.673C>T, p.R225*) who presented with multiple congenital anomalies at age 1 month (Figures 1B–1D). He was diagnosed with imperforate anus, moderate membranous ventricular septal defect, patent ductus arteriosus, right optic nerve coloboma, microphthalmia, and bilateral hydronephrosis. Craniofacial dysmorphisms include choanal atresia, prominent or broad forehead, deep-set eyes, and broad nasal root (Figure 1C). He had GDD with a brain MRI at 4 days old that showed pyriform aperture stenosis, small optic nerves, bilateral cerebellar hematomas, parietooccipital hematoma, enlarged ventricles, and a thin corpus callosum (Figure 1D).

Normal testing results from chromosomal microarray and a CHARGE syndrome sequencing panel necessitated exome sequencing. A *de novo* nonsense *GATAD2A* variant (c.673C>T, p.R225*) was identified by trio exome sequencing that is absent in control populations (gnomAD v.2.1.1), with *in silico* analysis supporting a deleterious effect (Table 1). After identifying individual P1, we subsequently identified four additional unrelated individuals with novel *de novo* variants in *GATAD2A* through international variant-sharing efforts and have summarized their phenotypes below as well as in Table 1 (Figure 1B).

Individual P2 was a 7-year-old male who presented with mild short stature, chronic otitis media and associated hearing loss, hypotonia, and borderline macrocephaly. He had feeding difficulties, mild GDD, and speech delay although he continued to make developmental progress. Echocardiogram identified mild atrial enlargement. Head ultrasound revealed mildly asymmetric ventricles. Craniofacial features include prominent forehead, deep-set eyes, midface hypoplasia, and a broad nasal root (Figure 1C). Exome sequencing identified a *de novo* heterozygous missense *GATAD2A* variant (c.1259G>A, p.C420Y) in P2, with inheritance confirmed by subsequent trio Sanger sequencing.

Individual P3 was an 8-year-old female who presented with mild hemihyperplasia, horseshoe kidney, and bilateral Wilms tumor. She had normal development, and neuroimaging was not performed. Craniofacial dysmorphisms included hypertelorism, prognathism, and broad nasal tip. Trio exome sequencing identified a *de novo* heterozygous frameshift *GATAD2A* variant (c.1877delT, p.I627Tfs) in P3, which was confirmed by Sanger sequencing.

Individual P4 was a 4-year-old female who presented with right-sided hemihyperplasia. She had GDD, speech delay, and autistic features. Neuroimaging was not performed. No craniofacial dysmorphisms were noted. Exome sequencing identified a heterozygous *GATAD2A* missense variant (c.1205G>T, p.G402V) in P4 of unknown inheritance.

Individual P5 was a 4-year-old male who presented with GDD. No structural brain anomalies were observed by brain MRI and no craniofacial dysmorphisms were noted. Trio exome sequencing identified a *de novo* heterozygous *GATAD2A* missense variant (c.626C>T, p.T209I) in P5.

In summary, the shared clinical features with variable expressivity include GDD, hemihyperplasia, craniofacial dysmorphism, and structural brain defects (Table 1). Nearly all (4/5) individuals in our cohort presented with developmental and growth defects. Three of the five individuals exhibited craniofacial dysmorphology. Musculoskeletal anomalies were also observed in three of the five individuals. Although unlikely, variants of uncertain significance (VUSs) were identified in other genes in P1 and P2 that may also be contributing to the observed clinical phenotypes (Table 1).[32,33]

GATAD2A heterozygous variants predicted to disrupt NuRD interactions correlate with neurodevelopmental features

Both GATAD2 proteins possess two highly conserved domains: conserved region 1 (CR1) and conserved region 2 (CR2).[34] CR1 is more N-terminal and encodes a coiled-coil domain that interacts with a similar domain in

Table 1. Clinical summary of individuals with monoallelic variants in *GATAD2A* in this study

Individual	P1	P2	P3	P4	P5
***GATAD2A* variant information**					
cDNA (GenBank: NM_017660.5)	c.673C>T	c.1259G>A	c.1877delT	c.1205G>T	c.626C>T
Protein consequence	p.R225*	p.C420Y	p.I627Tfs	p.G402V	p.T209I
Genotype	heterozygous	heterozygous	heterozygous	heterozygous	heterozygous
Inheritance	*de novo*	*de novo*	*de novo*	N/A	*de novo*
Sequencing method	research-based trio exome	research-based trio exome, Sanger confirmed	research-based trio exome, Sanger confirmed	clinical exome	research-based trio exome
gnomAD v.2.1.1 frequency	0	0	0	0	0
ACMG pathogenicity classification	VUS	VUS	likely pathogenic	VUS	VUS
CADD Phred score (GRCh38-v1.6)	40	29.9	33	36	16.67
SIFT (GRCh38-v1.6)		deleterious (0.00)		deleterious (0.00)	tolerated (0.28)
PolyPhen-2 (GRCh38-v1.6)		probably damaging (1.000)		possibly damaging (0.930)	benign (0.001)
Other genetic findings	hemizygous variant in *PLXNA3* (NM_017514: c.1093C>G, p.L365V)	hemizygous variant in *SLC9A7* (NM_001257291.1: c.1363del, p.L455Wfs*29)			
Clinical features					
Developmental/growth defects	+	+	N/A	+	+
Structural brain anomalies	+	+	N/A	N/A	–
Vision/hearing defects	+	+	N/A	N/A	–
Craniofacial dysmorphisms	+	+	+	–	–
Cardiovascular anomalies	+	+	N/A	N/A	N/A
Gastrointestinal/renal defects	+	–	+	N/A	N/A
Musculoskeletal anomalies	+	–	+	+	–

VUS, variant of uncertain significance; CADD, combined annotation dependent depletion; +, present; –, absent; N/A, not available.

MBD-proteins for coiled-coil binding; by contrast, CR2 is downstream and possesses GATA-type zinc finger domains shown to interact with CHD paralogs (Figure 2A).[5,16] CR1 is thought to tether the MBD-HDAC core unit to GATAD2 proteins, whereas CR2 tethers GATAD2 proteins to the CHD paralogs. CDK2AP1 has also been shown to interact with CR2.[35] Pairwise alignment indicates that GATAD2A has protein sequence homology (40.841%) with its paralog GATAD2B, predominantly around the CR1 and CR2 domains. Of note, in *GATAD2B*, LoF variants were identified across most of the coding sequence, while missense variants only localized to CR1 and CR2 domains.[5,16,36]

Among the identified *GATAD2A* variants in our cohort, three were missense (c.1205G>T, G402V; c.1259 G>A, p.C420Y; c.626C>T, p.T209I), one was nonsense (c.673C>T, p.R225*), and one was frameshift (c.1877delT, p.I627Tfs) at the extreme C terminus (Figure 2A). Following ACMG standards and guidelines, one of the five *GATAD2A* variants was classified as likely pathogenic (p.I627Tfs), whereas the other four were VUSs (p.T209I, p.R225*, p.G402V, p.C420Y) (Table 1).[37] All discovered variants were absent in public databases (gnomAD v.2.1.1), where *GATAD2A* demonstrated both high probability of LoF intolerance (pLI) (observed/expected SNVs [o/e] = 0.06; pLI = 1) and high intolerance to missense variation (o/e = 0.83; Z = 1.27). In light of the difficulty of interpreting variants of uncertain significance, we used the MetaDome web server, which pools data from gnomAD and the Human Gene Mutation Database to provide intolerance profiles for missense variants at amino acid-level resolution.[31] From this analysis, there is uneven intolerance across *GATAD2A*, with notable and predictable hotspots of intolerance concentrated around the CR1 and CR2 regions (Figure 2B). Importantly, missense variants identified in our cohort lie at predicted intolerant residues in MetaDome. All *GATAD2A* variants affect conserved residues localized throughout GATAD2A (Figure 2C). Except p.T209I, all variants have CADD scores above 20 (Table 1). Missense variants p.G402V and p.C420Y are predicted to be possibly or probably damaging by PolyPhen-2, and deleterious by SIFT (Table 1). These missense variants affect homologous residues that are conserved in GATAD2B (Figure 2C). Notably, the p.G402V

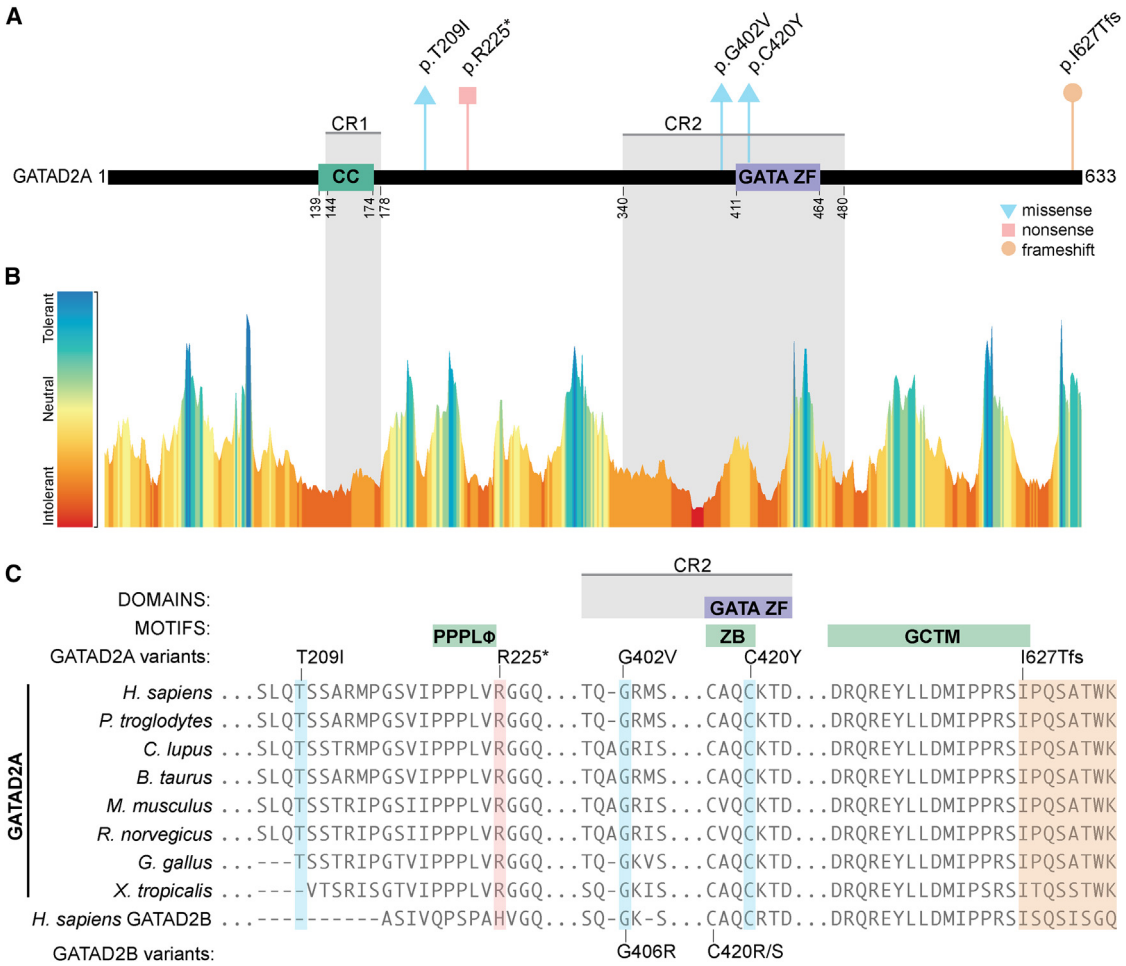

Figure 2. Features of *GATAD2A* variants

(A) Linear GATAD2A protein map with missense (blue triangle), nonsense (red square), and frameshift (orange circle) variants identified in present study. CR1, conserved region domain 1; CR2, conserved region domain 2; CC, coiled-coil domain; GATA ZF, GATA-type zinc finger domain.

(B) Predicted tolerance landscape of GATAD2A by MetaDome analysis.

(C) GATAD2A protein alignment showing cross-species conservation at affected residues, and alignment with human GATAD2B (bottom) showing conserved regions at affected residues within the GATA ZF domain and the previously unreported conserved C-terminal motif. The homologous human GATAD2B variants are shown below. The PPPLφ motif near R225* is also shown. ZB, zinc-binding motif; GCTM, GATAD2 C-terminal motif.

missense variant affects a homologous residue to the GATAD2B p.G406 residue, where a pathogenic variant was previously identified in GAND,[16] as well as in two previously unreported individuals with GAND (variants p.G406S and p.G406C, data not shown) (Figure 2C). These findings in several individuals with GAND indicate that the GATAD2B p.G406 residue has functional importance and the homologous residue p.G402 in GATAD2A is likely to have a similar effect. Previous studies have shown that GATAD2B p.G406R does not disrupt GATAD2B interactions with CHD proteins, suggesting that its pathogenicity may be associated with disruption of other protein interaction(s). Alternatively, both the GATAD2A (c.1205G>T, p.G402V) and GATAD2B (c.1216G>C, p.G406R) changes lie at exon-intron boundaries, with the GATAD2A variant predicted to have a LoF effect on the 3′ splice site of intron 8, which may result in use of a cryptic splice three nucleotides upstream (varSEAK, class 4). GATAD2B variants also provide additional evidence for the pathogenicity of the GATAD2A p.C420Y variant. Missense variants in *GATAD2B* affecting homologous zinc-binding cysteines were present in multiple individuals with GAND and are expected to have the same effect on GATAD2A.[16] Finally, our GATAD2 paralog protein alignment revealed that the p.I627Tfs variant lies within a C-terminal motif that is highly conserved across species as well as with GATAD2B, with the residues flanking the motif being widely divergent between the two paralogs (Figure 2C). Conversely, the p.R225* nonsense and the p.T209I missense variants lie near PPPLφ motifs that are absent in GATAD2B (Figure 2C). Together, these computational and population genetics analyses provide additional evidence for the negative functional consequence of these *GATAD2A* heterozygous variants.

Of note, variants that might alter protein dosage (p.R225* and p.G402V) and/or structure of the CR2

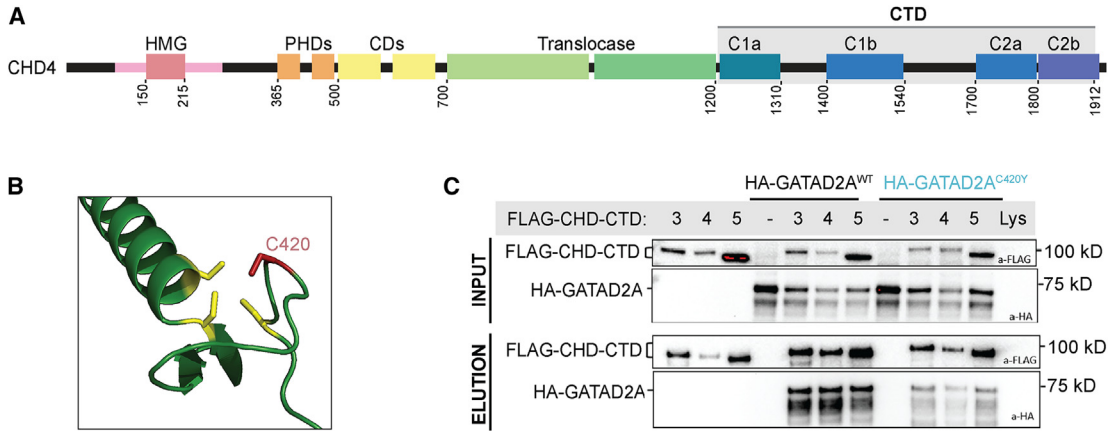

Figure 3. Modeling *GATAD2A* missense variant effect on NuRD complex interactions

(A) Domain architecture of human CHD4. CTD indicates the region required for GATAD2 binding.[38]

(B) Structural prediction of the GATAD2A zinc finger region (AlphaFold2). The four highlighted cysteines in yellow and red (C420) are essential for zinc coordination.

(C) Western blots of co-immunoprecipitation of FLAG-CHD3-CTD, FLAG-CHD4-CTD, or FLAG-CHD5-CTD co-expressed with either HA-GATAD2A^{WT} or HA-GATAD2A^{C420Y} in IVT lysates, along with negative controls. Inputs are on top and immunoprecipitated eluates are on the bottom.

domain (p.G402V and p.C420Y), and result in a functional haploinsufficiency, are present among individuals with neurodevelopmental defects. Among these, P1 and P2 (p.R225* and p.C420Y, respectively), present with structural brain defects including enlarged or asymmetric ventricles, thin corpus callosum, and macrocephaly. This evidence suggests that disruption and/or decreased dosage of the GATAD2A-CHD paralog interactions may be important for neural development.

GATAD2A interaction with NuRD components is disrupted by missense variant p.C420Y

Previous work has shown that the CTD of NuRD complex members CHD3, CHD4, or CHD5 is sufficient for GATAD2 protein interactions (Figure 3A).[5,16] The GATAD2 proteins possess GATA-type zinc finger domains within CR2 that are required for this interaction.[16] Given that the p.C420 of GATAD2A residue is one of four cysteines that coordinate the zinc ion, we hypothesized that the p.C420Y missense variant may disrupt CR2 zinc finger folding and its subsequent interaction with CHD paralogs. This was previously seen for *GATAD2B* CR2 zinc-binding cysteine residue variants (p.C420R and p.C420S), which resulted in a GAND diagnosis (Figure 3B).[5,16] To investigate the impact of the p.C420Y missense variant on interactions with CHD3, CHD4, and CHD5, we performed IVT in rabbit reticulocyte lysates to co-express the GATAD2A^{C420Y} protein with each CHD-CTD FLAG-tagged fusion protein. Immunoprecipitation was performed using the FLAG-tagged CHD-CTD fusion proteins as bait followed by pull-down with an anti-FLAG antibody. Compared with GATAD2A^{WT} protein, there was a marked reduction in GATAD2A^{C420Y} binding to all three CHD paralogs (Figure 3C). These findings provide evidence that the p.C420Y missense variant perturbs interactions within the CRS of the NuRD complex.

Discussion

We report *de novo* heterozygous dominant variants in *GATAD2A* as a genetic basis of a developmental disorder that we abbreviate as *GATAD2A*-related neurodevelopmental disorder (GARND). Five distinct *GATAD2A* variants were identified in five unrelated individuals whose prior genetic testing did not reveal other pathogenic or structural variants in genes that could explain the full array of clinical presentations. Many of the individuals in our cohort presented with overlapping developmental defects. *In silico* and functional analyses, along with evidence from homologous variants in *GATAD2B*, support a deleterious effect of the identified *GATAD2A* nonsense, frameshift, and missense variants. Our immunoprecipitation studies also show a disruption of the interaction between the GATAD2A^{C420Y} and the CHD paralogs within the CRS. Further investigation will be required to determine if this results in a LoF haploinsufficiency mechanism of disease or a dominant-negative disorder due to sequestration of HDAC-MBD-GATAD2A^{C420Y} partial complex from the CHD paralogs.

Despite some shared clinical features, our clinical findings indicate a range of phenotypic findings in GARND including craniofacial dysmorphisms, musculoskeletal anomalies, cerebral malformations, cardiovascular anomalies, and ophthalmological abnormalities. Little information has been known about *GATAD2A* variants in disease. One previous report of a nonsense variant in *GATAD2A* (c.19C>T, p.R7*) linked it to elevated levels of fetal hemoglobin, but made no mention of neurodevelopmental status.[18] The report also identified several predicted benign *GATAD2A* missense variants with only one present within CR2 (p.N382S). None of these were associated with changes in fetal hemoglobin levels and no neurodevelopmental data

was reported. Whether individuals in our cohort have elevated fetal hemoglobin is unknown.

The array of clinical phenotypes in our GARND cohort show moderate overlap with other dominant NuRDopathies (CHD3RS, CHD4RS, CHD5RS, and GAND).[5–8,16] In all five disorders, GDD, hypotonia, and dysmorphic craniofacial features (broad forehead, hypertelorism, wide nasal bridge) have been noted. For all disorders except CHD5RS, macrocephaly and ventriculomegaly have been observed, although it is more common in GAND than in CHD3RS and CHD4RS.[5] The phenotypes of GARND, GAND, CHD3RS, and CHD5RS all include speech deficits, whereas CHD4RS, GARND, and GAND phenotypes include congenital cardiac defects. GARND, GAND, and CHD3RS also share neonatal feeding difficulties. Unlike GAND's relatively consistent phenotype across affected individuals, the GARND phenotypes reported here were more variable across individuals. Whether this was the result of the cohort's unique variant makeup (and lack of redundancy) or GARND itself needs to be determined. As more cases of GARND are defined, it will be important to evaluate the frequency of divergent phenotypic NuRDopathy features such as macrocephaly, kidney disease, and hearing impairment, and the pattern of shared features between GARND and other NuRDopathies.

Our findings provide evidence in support of a hypothesis wherein pathogenic heterozygous variations in *GATAD2A* act through a LoF haploinsufficiency mechanism in affected individuals. The variability in clinical phenotypes in our cohort, coupled with the variable predicted intolerance across *GATAD2A*, may reflect the importance of 2A-NuRD during development and/or a polygenic effect based on genetic background. Furthermore, pleiotropic functions of *GATAD2A* in tissues may reflect cell-type variation in GATAD2 and NuRD paralog redundancy. Of note, we observed neurodevelopmental features in individuals with *GATAD2A* variants that could cause haploinsufficiency of CR2 function, which are predicted to disrupt interactions with the CTD region of CHD paralogs. These findings suggest that neural development is particularly susceptible to impairment of the chromatin remodeling activity of 2A-NuRD and 2B-NuRD complexes. Our co-immunoprecipitation findings confirm diminished GATAD2A-CHD interaction in the presence of the CR2-localized p.C420Y missense variant (similar to a number of patient variants in GAND).[5,16] We showed that the GATAD2A p.C420Y affects a cysteine within a zinc-binding motif, which is also disrupted by previously reported GATAD2B p.C420 variants in individuals with GAND (Figure 2C).[5,16] We also found that the affected glycine of GATAD2A p.G402V is homologous to that of the previously identified GATAD2B p.G406R.[5,16] Together, these findings provide evidence for the pathogenicity of GATAD2A p.G402V and p.C420Y. We hypothesize different mechanisms for dysfunction for the p.T209I and p.I627Tfs variants, which do not localize to or disrupt CR1 or CR2 interaction domains. For instance, the p.T209I variant lies within a region containing three PPPLφ motifs, which are important for GATAD2A interactions with ZMYND8 and the subsequent recruitment of 2A-NuRD to sites of DNA damage for assisting in repair.[21] It remains unclear if there are other specific functions of the three PPPLφ motifs in neural development. The C-terminal p.I627Tfs variant, which lies within a previously unreported GATAD2 motif, may disrupt an important interaction with other as yet unknown proteins involved in 2A-NuRD (and likely 2B-NuRD) function. Alternatively, although less likely given its extreme C-terminal location, the frameshift variant may trigger mRNA or protein degradation, resulting in haploinsufficiency. Of course, this variant could also represent a benign change in an individual with a different developmental disorder. Additional cases will help to confirm GATAD2A-related pathogenicity in development and refine the GARND clinical spectrum.

Our human genetics findings suggest distinct but overlapping pathogenic mechanisms of variants in *GATAD2* paralogs. It remains unclear if GATAD2A and GATAD2B provide full, partial, or no redundancy in NuRD function during development, and whether their functions are cell-type specific with minimal overlap of expression. In prior studies, GATAD2B overexpression failed to rescue a GATAD2A-related phenotype in GATAD2A-depleted induced pluripotent stem cells, potentially indicating non-redundancy with GATAD2B.[22] GATAD2B non-redundancy is also demonstrated by the unique GATAD2A interaction with ZMYND8 through PPPLφ domains, which are absent in GATAD2B.[21] To date, there are no specific assays of GATAD2B function and therefore there are no data to determine if GATAD2A could compensate for its deficiency. Additional work is necessary to assess the degree to which, if any, GATAD2B and GATAD2A compensatory activity mediates variable expressivity in or between GARND and/or GAND.

In summary, we report five unrelated individuals with heterozygous variants in *GATAD2A* and a neurodevelopmental disorder characterized by GDD, structural brain defects, and craniofacial dysmorphism. Discovery of additional affected individuals will provide further insight into the breadth of pathogenic genetic variation and constellation of clinical features associated with GARND.

Data and code availability

All data relevant to the study are included in the report. Identified variants have been submitted to ClinVar (accession nos. VCV001705818.2, VCV001705819.2, VCV001705820.2, VCV001705821.2, and VCV001705822.2).

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Declaration of interests

A.M.I., M.J.B., and J.X.C. serve in a voluntary capacity as members of the Human Genetics and Genomics Advances (HGG-A) Editorial Board.

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Web resources

Genome Aggregation Database, https://gnomad.broadinstitute.org/

NHLBI Exome Sequencing Project, https://evs.gs.washington.edu/EVS/

Online Mendelian Inheritance in Man, http://www.omim.org

Franklin Genoox, https://franklin.genoox.com/clinical-db/home

ClinVar, https://www.ncbi.nlm.nih.gov/clinvar/

NCBI HomoloGene tool, https://www.ncbi.nlm.nih.gov/homologene

UniProt, https://www.uniprot.org/

AlphaFold Protein Structure Database, https://alphafold.ebi.ac.uk/

Combined Annotation Dependent Depletion Tool, https://cadd.gs.washington.edu/

Polymorphism Phenotyping v.2, http://genetics.bwh.harvard.edu/pph2/

Sorting Intolerant From Tolerant, https://sift.bii.a-star.edu.sg/

varSEAK, varseak.bio

MetaDome, stuart.radboudumc.nl/metadome

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