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Citation

Erdogan, E. N., Cheng, C. V., Caraffi, S. G., Ivanovski, I., Piatelli, G., Errichiello, E., ... Aldinger, K. A. (2025). Further delineation of the AUTS2 HX repeat domain-related phenotype. *The American Journal Of Medical Genetics - Part A*, 197(9).
doi:10.1002/ajmg.a.64093

Version: Publisher's Version

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Downloaded from: <https://hdl.handle.net/1887/4298676>

Note: To cite this publication please use the final published version (if applicable).

ORIGINAL ARTICLE

Further Delineation of the AUTS2 HX Repeat Domain-Related Phenotype

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Received: 20 December 2024 | **Revised:** 11 February 2025 | **Accepted:** 8 April 2025

Funding: This work was supported by National Institutes of Health grant 1R01NS050375 (to W.B.D.) and the Wellcome Trust [# WT223718/Z/21/Z].

Keywords: AUTS2 | genotype-phenotype | human genetics

ABSTRACT

Haploinsufficiency of *AUTS2* is associated with a neurodevelopmental disorder characterized by intellectual disability, autistic features, and spasticity. *AUTS2* protein interacts with p300, encoded by *EP300*, through the HX repeat domain of *AUTS2*, thereby activating transcription. We previously reported two de novo variants in the HX repeat domain of *AUTS2*. These variants disrupt the *AUTS2*-P300 interaction, resulting in a phenotype resembling Rubinstein-Taybi Syndrome (RSTS) associated with variants in *EP300/CREBBP*. Here, we expand beyond the initial clinical description to delineate the HX domain-associated phenotype and compare it to the *AUTS2*-haploinsufficient phenotype. We reviewed clinical data, photographs, and neuroimaging studies to examine genotype-phenotype relationships. Our review of 80 individuals included 14 individuals we present here

and 66 individuals with *AUTS2* variants presented in the literature. The clinical features for individuals with variants in the HX repeat domain include severe intellectual disability, severe language disability, distinct craniofacial and skeletal dysmorphic features, and neuroimaging findings. Facial dysmorphisms include wide and prominent nasal bridges with complex nasal shapes and dysmorphic eyebrows. Dysmorphisms include digit anomalies: Symphalangism and hypoplasia of distal phalanges, exclusive to the HX domain variant group. Cerebellar anomalies not seen with other *AUTS2* variants are seen within this group. Our report delineates a distinct and severe clinical phenotype associated with variants in the *AUTS2* HX domain, including an in-depth comparison with the *AUTS2* haploinsufficiency phenotype features.

1 | Introduction

Activator of transcription and developmental regulator *AUTS2* (HGNC:14262) spans 1.2 Mb on chromosome 7q11.22 and encodes 19 exons that produce a full-length isoform and at least one smaller C-terminal isoform in humans (Yamashiro et al. 2020; Beunders et al. 2013; Hori et al. 2014; Gao et al. 2014). The *AUTS2*-related neurodevelopmental disorder (OMIM #615834) is mainly associated with heterozygous single or multiple exonic deletions as well as structural variations and a variable phenotype associated with haploinsufficiency (Beunders et al. 2016). Clinical findings include a prominent behavioral phenotype, mild to moderate intellectual disability (ID), microcephaly, cerebral palsy, facial dysmorphic features, and variable autistic features (Beunders et al. 2016; Sengun et al. 2016; Fan et al. 2016). *AUTS2* is implicated in several neurological and non-neurological disorders beyond the neurodevelopmental phenotype (Hori et al. 2014; Gao et al. 2014; Denk et al. 2012). It has a wide array of functions including transcriptional activation, RNA metabolism regulation, actin cytoskeleton organization, neuronal migration, and synaptogenesis in the developing human brain (Yamashiro et al. 2020; Beunders et al. 2013; Hori et al. 2014; Gao et al. 2014).

Polycomb repressive complexes (PRC) are large protein complexes that regulate gene expression by modifying histones (Gao et al. 2014). Full-length human *AUTS2* binds directly to the PRC1.3 and PRC1.5 subgroups of mammalian PRC1 that include Polycomb group proteins PCGF3/5, RING1A/B, RYBP or its homolog YAF2, and the heterotetrameric holoenzyme Casein Kinase 2 (CK2) (Gao et al. 2014; Liu et al. 2021). PRC1 acts canonically to repress transcription (Gao et al. 2014). However, PRC1 binding to CK2 represses this inhibitory action, while recruitment of p300 (encoded by *EP300*) by *AUTS2* turns the complex into a transcriptional activator (Gao et al. 2014). Recent work showed that p300 is recruited to PRC1 by the HX repeat domain in exon 9 of *AUTS2* (Liu et al. 2021). The HX repeat domain is a short and highly conserved histidine-rich motif with repeating sequences of histidine (H) and another amino acid (X) (Liu et al. 2021). The specific HX repeat domain of *AUTS2* encodes a HXHQHQHQHQTHQHHT amino acid sequence at positions 525 to 542 (Liu et al. 2021).

Several PRC1 subunits, including *AUTS2*, CK2 components CSNK2A1 and CSNK2B, RING1, and YAF2, are reported as causative or strong candidates for neurodevelopmental disorders. FBRSL1, which shares high sequence homology with *AUTS2*, interacts with PRC1.3 and PRC1.5 and causes a neurodevelopmental disorder (Pauli et al. 2021; Bukvic et al. 2024). The human *EP300* gene and its structural and functional twin *CREBBP* are associated with Rubinstein-Taybi syndrome (RSTS) (OMIM

#180849), a neurodevelopmental condition characterized by recognizable facial dysmorphisms, broad thumbs and halluces, short stature, and ID. Variants in the HX repeat in exon 9 in *AUTS2*, a missense (p.Thr534Pro) and a small in-frame deletion (p.His535_Thr542del), disrupt binding to p300 in PRC1 and produce a phenotype that overlaps with classic RSTS (Liu et al. 2021). This is striking as the phenotype associated with these variants appears to be more severe than the syndrome associated with the *AUTS2* haploinsufficiency (Liu et al. 2021). However, the initial report that delineated these findings included scant clinical detail, necessitating the identification of additional patients and description of clinical features.

The interference of the missense and small in-frame deletions of the HX-repeat with protein function emphasizes the importance of accurately classifying such variants that may be overlooked in genes that are associated with loss-of-function. The pathogenicity of *AUTS2* variants in the context of small amino acid changes has not yet been clarified, and patient findings have not been adequately detailed to aid clinical decision-making and correct grouping of patients. Additionally, nonsense variants causing haploinsufficiency are reported less frequently, while most of the *AUTS2* variants reported so far are associated with exon deletions or structural variations.

Here, we present a more comprehensive delineation of the severe *AUTS2*-related phenotype associated with missense and in-frame variants in the HX repeat domain in exon 9, initially defined by (Liu et al. 2021). Our analysis highlights the differences between the haploinsufficiency-related phenotype and the HX repeat-related phenotype—the latter referred to as *AUTS2*/HX-NDD for distinction. Additionally, we expand the phenotypes associated with variants causing haploinsufficiency by reporting deletion, nonsense, and splice variants across the gene. The HX-repeat domain variants produce structural brain defects, especially cerebellar malformations, not seen with other variants in *AUTS2*. Additional skeletal and facial dysmorphic features are first described here in detail. The analysis concludes with a review of the pathogenicity of *AUTS2* variants and regional constraint analysis.

2 | Materials and Methods

A total of 80 individuals with variants in *AUTS2* (RefSeq: NM_015570.4) were ascertained through national and international collaborations and the literature. Ten individuals were included in the *AUTS2*/HX-NDD group: five individuals (LR05-007, LR15-003, LR18-404, LR19-314, LR19-506) were briefly discussed by (Liu et al. 2021). Another individual (LR23-017) was studied in a brain organoid system by (Fair et al. 2023), with

limited clinical details presented. Additionally, two new patients are reported in this article (LR- CG-1, LR23-023). These eight Individuals with HX repeat variants were analyzed with two other previously published case reports describing patients with variants in the *AUTS2* HX repeat domain (Palumbo et al. 2021; Martinez-Delgado et al. 2021).

Seventy individuals were included in the *AUTS2*-haploinsufficient variant group: two individuals (LR15-004, LR15-097) discussed by Liu et al. 2021; four new patients reported in this article (LR19-321, LR19-315, LR15-081, LR-ER-2) and 64 additional cases identified after literature review with heterozygous variants and sufficient phenotypic details to describe the haploinsufficiency related phenotype as compared to the HX-repeat related phenotype (Beunders et al. 2013, 2016; Sengun et al. 2016; Fan et al. 2016; Kalscheuer et al. 2007; Bakkaloglu et al. 2008; Huang et al. 2010; Sanchez-Jimeno et al. 2021; Nagamani et al. 2013; Jolley et al. 2013; Amarillo et al. 2014; Liu et al. 2015; Saeki et al. 2019; Ziats et al. 2020; Gault et al. 2021; Sultana et al. 2002; Schneider et al. 2015; Asadollahi et al. 2014; Gieldon et al. 2021; Stojanovic et al. 2020). Clinical details for LR15-004 were excluded from the main analysis and discussed separately in the supplement, due to the variant's presence in All of Us and gnomAD databases (Karczewski et al. 2020; Bick et al. 2024). Her clinical data was included in Table S3 for a complete update to the *AUTS2*-related phenotypes as she did not have any other diagnosis to explain her clinical presentation.

Previously published patients are openly cross-referenced in Table S2, and their updated and expanded clinical information with facial photographs and MRIs was included for a complete demonstration of the *AUTS2* phenotypic spectrum along with the patients from the literature. Clinical details were obtained on all individuals through a standardized datasheet, including photographs and original neuroimaging files of the main cohort, and used to construct a database of features. Phenotyping was led by an experienced child neurologist and clinical geneticist (W.B.D.) based on the principles of Elements of Morphology (Carey et al. 2012). A complete medical history for all *AUTS2*/HX-NDD patients is included in the Supplement in the form of a case series. Each individual was re-evaluated based on their clinical characteristics and *AUTS2* variant.

Written informed consent was obtained from all participants through protocols approved by the Institutional Review Boards at Seattle Children's Hospital or the local institutional review board at each site.

All variants were identified by clinical or research exome sequencing. One individual's (LR-ER-2) variant was detected using exome-based CNV analysis. Genomic data is presented in Table S3 for the main cohort and Table S1 for patients from the literature. All variants were heterozygous. Our cohort included 13 individuals with de novo variants and one with paternal inheritance (LR-ER-2). Two *AUTS2*/HX-NDD cases from the literature were de novo, while the rest of the literature included 42 de novo, 2 paternal, and 6 maternal cases. An additional 14 cases from the literature were of unknown inheritance, 4 of these being nonmaternal and 1 of these being nonpaternal. Variants reported here were verified using VariantValidator and

are reported according to HGVS Nomenclature (den Dunnen et al. 2016; Freeman et al. 2018).

3 | Results

We detailed the phenotypic information of 13 unrelated individuals with heterozygous, *de novo* variants in *AUTS2* (NM_015570.4), one with paternal inheritance (Table S3), and 66 from the literature.

A summary of the medical history of the index case (LR05-007) and the remaining *AUTS2*/HX-NDD cases is provided in the [Supporting Information](#), and their clinical data together with the four new *AUTS2*-haploinsufficient cases reported in this article and the case LR15-097 described by (Liu et al. 2021) are summarized in Table S3.

3.1 | *AUTS2* Haploinsufficiency

An analysis of the clinical features of 69 patients with *AUTS2*-haploinsufficient variants (LR15-004 is not included) revealed high frequencies of ID (96%) and autistic features (63%). These patients had mild to moderate ID and delayed language and motor development. They also presented with spasticity (32%) and ADHD (32%) which defined the main features of this phenotype. They had varying degrees of microcephaly (56%, usually mild) and facial dysmorphisms (81% of the patients had periorbital dysmorphology, 59% had nose and philtrum dysmorphology, 59% had lips, mouth and oral region dysmorphology, 44% had hand and feet dysmorphology, 35% had ear dysmorphology). (Figure 1, Table S1) Additional clinical information for these individuals is found in the [Supporting Information](#).

Included in this group are five individuals we present in detail (Table S3), four of them reported here for the first time, each carrying a heterozygous *AUTS2* variant predicted to cause loss-of-function. This group demonstrated features typical of haploinsufficiency-related *AUTS2*-neurodevelopmental disorder. ID (moderate to severe in our cohort), autistic features, generalized hypotonia, feeding difficulties, spasticity, and variable but mild dysmorphic facial features were seen. Brain MRIs were all normal with no structural anomalies in the main group. The total frequency of structural brain defects, including the literature, was only 9%. 5% of those were corpus callosum malformations with nonspecific MRI changes without any cerebellar anomalies.

3.2 | Exon 9: *AUTS2*/HX-NDD

This group of 10 individuals had a distinct phenotype characterized by severe ID, striking dysmorphic facial features, and structural brain defects not seen with variants outside this domain (Figure 1). A wide and prominent nasal bridge with complex nasal shapes, down-slanting palpebral fissures, and dysmorphic eyebrows are observed (Figure 2). Dysmorphisms also include digit anomalies that are seen only in this group (Figure S1). The brain malformations observed include hypoplasia of the cerebellum, corpus callosum, and posterior fossa, making this

AUTS2/HX-NDD	p.His529Pro	p.His533Tyr	p.Thr534Pro	p.His535_Thr542del	p.His535_Thr542del Palumbo et al.	p.Gln532_His541del Martinez-Delgado et al.	Total (n=10)	AUTS2/HI	Total (n=69)	p values (HX-vs-HI, Fisher's Exact)
Short Stature							4/10 40%	Short Stature	26/63 41%	1
Microcephaly							2/10 20%	Microcephaly	37/66 56%	0.0443
Intellectual disability/ GDD							10/10 100%	Intellectual disability/ GDD	66/69 96%	1
Autistic features							6/8 75%	Autistic features	40/63 63%	0.7
Epilepsy							4/10 40%	Epilepsy	7/63 11%	0.038
Spasticity							2/9 22%	Spasticity	20/63 32%	0.71
ADHD							2/8 25%	ADHD	20/63 32%	1
Structural brain anomaly							9/10 90%	Structural brain anomaly	4/44 9%	<0.00001
Corpus callosum malformation							6/10 60%	Corpus callosum malformation	2/44 5%	0.0002
Cerebellar malformation							7/9 78%	Cerebellar malformation	0/44 0%	<0.00001
Periorbital dysmorphism							10/10 100%	Periorbital dysmorphism	51/63 81%	0.2
Nose and philtrum dysmorphism							10/10 100%	Nose and philtrum dysmorphism	37/63 59%	0.011
Lips, mouth and oral region dysmorphism							10/10 100%	Lips, mouth and oral region	37/63 59%	0.011
Hand and feet dysmorphism							8/10 80%	Hand and feet dysmorphism	28/64 44%	0.043
Ear dysmorphism							10/10 100%	Ear dysmorphism	22/63 35%	0.0001

FIGURE 1 | Tabulated clinical information of individuals with variants in the HX repeat (AUTS2/HX-NDD, including patients reported by Palumbo et al. [1 individual] and Martinez-Delgado et al. [1 individual]) compared to individuals with variants reported throughout the gene (AUTS2/HI, HI: Haploinsufficiency, including the patients we report [5 individuals] and patients from the literature [64 individuals]). Colors on the heatmap denote present features, while black denotes absent, and gray denotes unknown features. Each box represents one individual. The frequency of certain features, such as dysmorphisms and structural brain malformations, differed between the two groups. Two groups are compared with a Fisher's Exact test for each category, and p scores below <0.5 are shown in bold.

phenotype consistently more severe than the phenotype observed in individuals with AUTS2-haploinsufficient variants (Figures 1 and 3). This group of patients consistently had severe language deficiencies. Three of the main group (LR23-023, LR05-007, LR15-003) never developed speech, and the rest were severely delayed.

3.2.1 | AUTS2/HX-NDD Missense (p.His529Pro, p.His533Tyr and p.Thr534Pro)

All (LR05-007, LR-CG-1, LR23-023, LR23-017) had low-set eyebrows that were mildly horizontal and sparse, with a high and broad nasal bridge and malformed ears. They had unique facial features exclusive to the missense group. These features include mild blepharophimosis and a narrow nasal ridge in two (LR23-023, LR23-017 with p.Thr534Pro variants) and a sharp nasal ridge in one (LR23-023, p.His533Tyr), a very thin vermilion of the upper lip, and deep-set eyes in all four individuals.

Both individuals with the p.Thr534Pro variant and the individual with the p.His529Pro variant had mildly broad halluces. Two also had kyphoscoliosis and tight heel cords. Three individuals with missense variants (LR05-007, LR23-023, LR23-017) had abnormal fingers, including variable hypoplasia of the distal phalanges and occasionally the middle phalanges, and variable symphalangism with absence or fusion of the proximal interphalangeal (PIP) joint (Figure S1). This striking digit phenomenon did not occur with other reported AUTS2 variants. Brain MRI features of three individuals were strikingly similar. All three had hypoplasia of the corpus callosum, and two (p.(Thr534Pro)) also had hypoplasia of the cerebellum and small posterior fossae. Interestingly, neuroimaging of one individual in this group (LR-CG-1) was normal.

3.2.2 | AUTS2/HX-NDD In-Frame Deletion (p.His535_Thr542del)

Individuals with the HX-repeat in-frame deletion (p.His535_Thr542del) had typical features of AUTS2/HX-NDD, including

feeding difficulties in infancy, severe developmental delays most pronounced in speech, and severe intellectual disability with autistic features. Individuals were not spastic, and ADHD was only seen in one individual (LR19-314). Neuroimaging features in the 4 individuals with recurrent p.His535_Thr542del in the distal HX motif were similar but less severe. Two had mild hypoplasia of the corpus callosum, while all four had mild vermispredominant cerebellar hypoplasia. It was noticeable that in corpus callosum defects, the middle part was most affected. Other neuroimaging findings are discussed in detail in Table S3 and Figure 3.

3.3 | Predicted Pathogenicity of AUTS2 Variants

AUTS2 is predicted to be intolerant to loss of function based on two primary metrics: oe (observed/expected ratio)/LOEUF (Loss of function Observed/Expected Upper bound Fraction) and the probability of being loss-of-function intolerant (pLI) (Lek et al. 2016). Oe metric in gnomAD based on the canonical AUTS2 transcript (ENST00000342771.10, gnomAD v4) (Chen et al. 2024) demonstrates (oe = 0.15 [0.1–0.23] and LOEUF = 0.232) strong selection against loss of function variants possibly associated with haploinsufficiency. Similarly, the pLI for AUTS2 is 1.0, while for any loss-of-function causing variants, pLI > 0.9 implies an extremely intolerant transcript (Lek et al. 2016). In contrast, AUTS2 is predicted to possibly tolerate missense variants based on two main metrics: missense oe/Z-score (Chen et al. 2024) and AlphaMissense (Cheng et al. 2023) scores. In contrast to the loss of function metrics, the missense oe metric (oe = 0.87 [0.84–0.91]) demonstrates a lack of constraint to missense variants. A missense Z score of 1.86 was calculated based on the expected rare variants per transcript (minor allele frequency, %0.1 ENST00000342771.10, based on gnomAD v4), which is less than the expected missense Z-score (constrained if Z > 3.09, p < 0.001) for a highly constrained gene. AlphaMissense predicts the effects of missense variants by giving a pathogenicity score between 0 and 1 (0: least likely to be pathogenic, 1: most likely to be pathogenic) and a prediction for every possible amino acid substitution in the proteome building on the AlphaFold protein visualization (Cheng et al. 2023). The



FIGURE 2 | Legend on next page.

FIGURE 2 | Facial appearance in 10 individuals with *AUTS2* variants. A, B: LR-CG-1 (p.His529Pro); C, D: LR23-023 (p.His533Tyr); E, F: LR05-007 (p.Thr534Pro); G, H: LR23-017 (p.Thr534Pro); I, J: LR15-003 (p.His535_Thr542del); K, L: LR18-404 (p.His535_Thr542del). Individuals with variants in the HX domain (A–L) all have striking dysmorphic facial features with deep-set eyes and abnormal eyebrows always prominent. M, N: LR19-321; O, P: LR19-315; Q, R: LR15-097; S, T: LR15-081. Minimal to no dysmorphic features were seen in these (M–T) individuals with variants outside of the HX repeat.

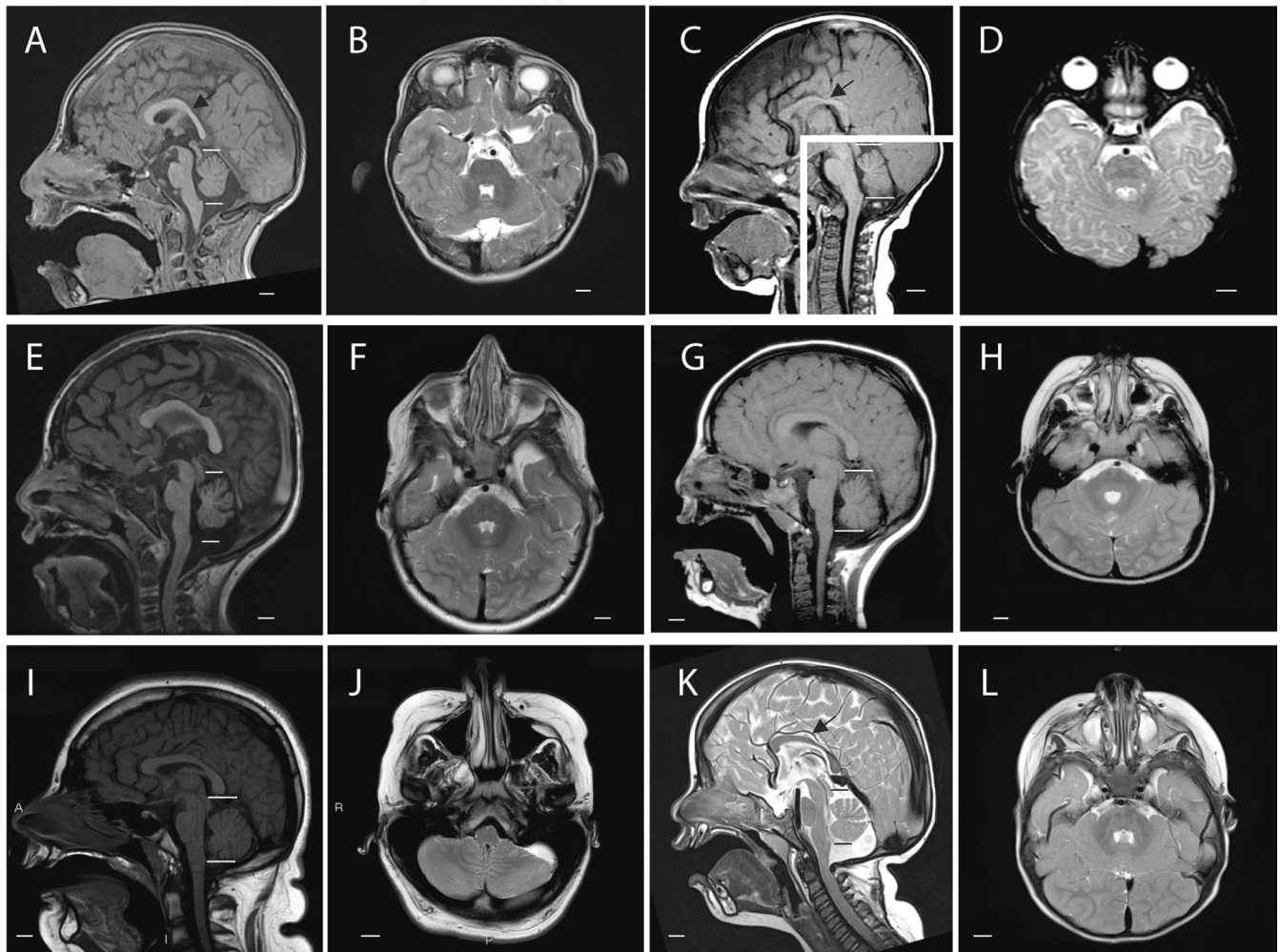


FIGURE 3 | Brain magnetic resonance images in 6 individuals with variants in the HX domain including LR05-007 (A, B), LR18-404 (C, D), LR23-017 (E, F), LR15-003 (G, H), LR19-314 (I, J), LR19-314 (K, L). Midline sagittal images (A, C, E, G, I, K) show mildly small and dysplastic corpora callosa (black arrows, A, C, E, G, I, K) and mildly small vermis in all individuals. The vermis typical extends from about the mid-tectum to the obex, marked here with short horizontal white lines. The small vermis and hemispheres can be seen on the axial image in LR05-007 (B) but not the others.

mean predicted pathogenicity per amino acid substitution for *AUTS2* calculated by the AlphaMissense algorithm was 0.437, demonstrating the presence of benign variation that potentially does not affect the protein.

In contrast, genomic constraint analysis of a smaller region within *AUTS2* that includes the HX repeat domain (1 kb, chr7:70766000–70767000) resulted in a Z-score of 4.84 (gnomAD, constrained if $Z > 3.09$, $p < 0.001$) which shows that the area is highly constrained for missense variants (minor allele frequency, %0.1 ENST00000342771.10, based on gnomAD v4) (Figure 4). A larger local regional genomic constraint Z score (40 kb, chr7: 70746234–70786234) showed that the neighboring Z scores ranged between -0.84 and 2.76 . This is striking

as the 1 kb region encompassing exon 9, including the HX repeat, was the only constrained part within a larger part of *AUTS2* (40 kb, from the end of intron 6 to beginning of the intron 16). While the mean predicted pathogenicity per amino acid substitution (AlphaMissense) score for the *AUTS2* gene was 0.437, the mean score for the HX repeat domain was 0.97, the highest among known *AUTS2* domains (Figure 4). AlphaMissense also evaluated the patient variants presented here as pathogenic.

Methods and algorithms mentioned here support the pathogenicity of reported *AUTS2* variants, and they may be used for additional variant scrutiny. These results, together with our genotype–phenotype analysis, suggest that *AUTS2* is likely

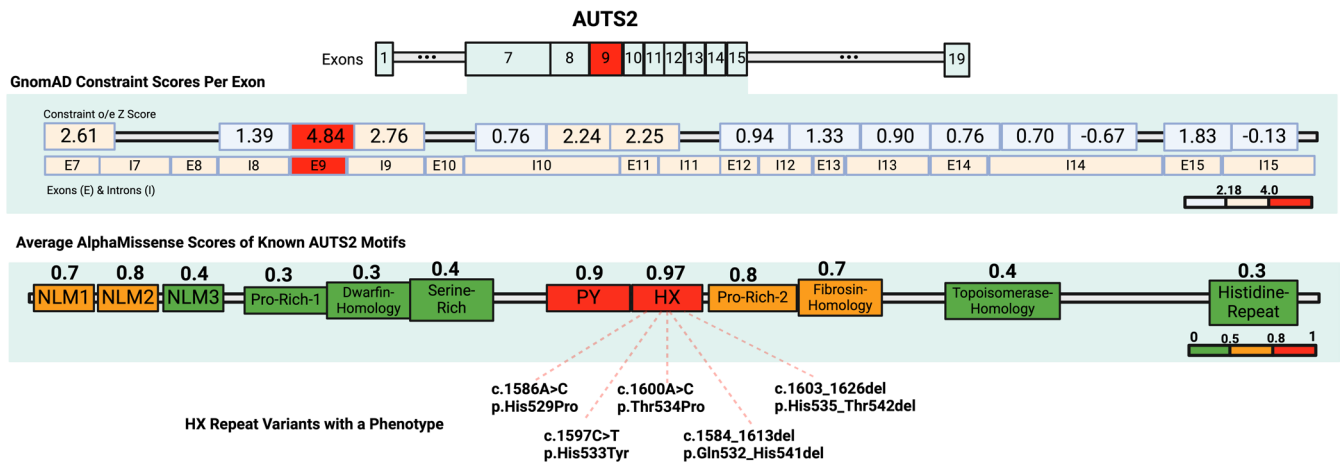


FIGURE 4 | AUTS2 gene is shown with exon numbers 1–19 specified. Local genomic constraint analysis of a region in exon 9 (1 kb, chr7:70766000–70,767,000) show a high Z score of 4.84. The neighboring Z scores ranged between –0.67 and 2.76. Average AlphaMissense scores for each known AUTS2 motif are shown on the gene diagram below. Highest average scores belonged to the PY domain in exon 9 and the HX repeat domain. HX-Repeat variants associated with a phenotype are shown below, including the c.1584_1613del p.Gln532_His541del variant reported by Martinez-Delgado et al.

disrupted by missense variants in the HX repeat domain in exon 9.

4 | Discussion

The key features of AUTS2/HX-NDD included multiple comorbidities: a combination of ID with severe language deficit, motor delay, prominent facial dysmorphism, and skeletal especially—digit anomalies. We demonstrate that the AUTS2/HX-NDD phenotype overlaps with RSTS, as it was suggested by (Liu et al. 2021), but is not close enough to define a subtype of RSTS. The most distinctive features of RSTS—the low-hanging columella and broad thumbs/halluces—are mild in all individuals. However, clinical diagnosis of RSTS was suggested prior to genetic testing in three individuals (LR05-007, LR15-003 and LR-CG-1). While AUTS2 is generally not highly constrained for missense variants, we show that exon 9 and the HX domain are highly constrained. Our data also suggest that the three individuals with missense variants in the middle of the HX domain (positions 533–534) had more severe features than the four individuals with the more terminal deletion (positions 535–542) and the single individual with an amino acid change in the beginning of the repeat (position 529).

Structural brain malformations have frequently been reported in neurodevelopmental disorders associated with other PRC1.3/5 components but not with AUTS2 haploinsufficiency (Figure S2). Abnormal brain MRIs were described in 14 of 25 (56%) individuals with CSNK2A1-related NDD (Owen et al. 2018; Okur et al. 2016; Trinh et al. 2017; Wu et al. 2021; Colavito et al. 2018; Nakashima et al. 2019; Jafari Khamirani et al. 2022; Akahira-Azuma et al. 2018; Martinez-Monseny et al. 2020; Chiu et al. 2018; Xu et al. 2020). However, the findings were variable, and only two had hypoplasia of the corpus callosum, one of those also having cerebellar vermis hypoplasia. Similarly, abnormal brain MRI findings were reported in 8 of 26 (31%) individuals with CSNK2B-related NDD, another component of PRC1.3/5, one with microcephaly and typical secondary

features such as a thin corpus callosum, two with cerebellar vermis hypoplasia, one with undefined “mega-cisterna magna” one with Chiari malformation and syringomyelia, and three with other findings (Nakashima et al. 2019; Orsini et al. 2022; Wilke et al. 2022; Poirier et al. 2017; Selvam et al. 2021; Bonanni et al. 2021; Sakaguchi et al. 2017; Li et al. 2019). RSTS (EP300/CREBBP) is also associated with agenesis or hypoplasia of the corpus callosum and cerebellar hypoplasia (Ajmone et al. 2018). These structural brain defects, including the ones we report here, hint at earlier arrest in brain development that is common among these disorders. The analysis of the specific functional interactions between Polycomb proteins and further phenotypic data may uncover other key features shared between Polycomb component-related disorders. A list of all PRC1 related disorders is found in the Supporting Information (Table S4).

Seven genes containing HX repeats longer than 8 residues have been identified (Palmer et al. 2019). Among those, *AUTS2*, *ATN1*, *FBRSL1*, and *RERE* are associated with neurodevelopmental disorders. De novo missense or insertion variants in the HX repeat have been reported in ATN1-related CHEDDA syndrome (congenital hypotonia, epilepsy, neurodevelopmental delay, digit abnormalities) (Palmer et al. 2021). CHEDDA (OMIM #618494) is non-progressive and differs markedly from the previously described ATN1-related dentatorubral-pallidoluysian atrophy (DRPLA, OMIM #125370) caused by gain of function due to repeat expansion. Therefore, CHEDDA and DRPLA are examples of two distinct phenotypes associated with two different mechanisms in which a gene harbors an HX repeat. The role of HX repeats in the context of human disorders needs to be investigated further.

Interestingly, there are individuals with in-frame indels encompassing the HX repeat domain reported in gnomAD (c.1588_1599del p.Gln530_His533del and c.1594_1599del p.Gln532_His533del) who have higher allele frequencies (allele count:13, allele frequency: 0.00008055 and allele count: 73, allele frequency: 0.00004523, respectively). The phenotype information for these individuals is not provided. Therefore, it is

unclear whether these variants are associated with a phenotype. The HX repeat may include or border other regulatory elements. Hence, the specific amino deletions may have unique properties in their effect on the AUTS2/HX-P300 interaction that do not apply to every in-frame HX repeat deletion.

It was previously suggested that the variability of the phenotype associated with AUTS2 haploinsufficiency differed based on whether a shorter AUTS2 transcript starting in exon 9 was affected (C terminus vs. N terminus) (Beunders et al. 2013). Our literature review and cohort revealed milder phenotypes with exonic deletions encompassing exons 1–5 but not a more severe presentation associated with variants in and beyond exon 9 (Table S1). Notably, there is a higher frequency of pathogenic variation seen with variants affecting exon 6, with somewhat more severe features. Functional analysis of variants may reveal unique properties of exon 6 and may predict which variants encompassing the HX repeat may result in the deleterious features we report. Further analysis of AUTS2 interactors and downstream targets is needed to understand other diverse functions of AUTS2 and their contributions to clinical phenotypes.

In conclusion, our analysis further delineates a distinct and severe phenotype associated with HX repeat variants in AUTS2 compared to the patients we report and previously published reports of AUTS2 haploinsufficiency. The brain malformations and severe features seen with missense or small in-frame deletions warrant further variant scrutiny in genes associated with loss of function, such as *AUTS2*. We believe this work will inform clinicians and researchers about the distinct genetic effects of AUTS2 variants on phenotypic presentation.

Author Contributions

All authors designed and contributed to the work that led to submission. E.N.E. led the project and W.B.D. and K.A.A. supervised. S.G.C., I.I., G.P., E.E., O.Z., A.S.P., G.V., A.R., S.E.H., D.C.K., M.C.S., J.P., N.D.D., T.L., M.S.P., J.H., J.R., S.B., H.M., M.C.K., A.S., A.W., C.L., L.G., A.H., C.A.L.R., M.P., B.P., and P.Y.B.A. provided clinical and genetic data and supported K.A.A., W.B.D., and E.N.E. in the conceptualization of the project. S.K. provided genetic data and a review of the classification of variants. E.N.E. led the analysis. E.N.E., C.V.C., W.B.D., and K.A.A. initially drafted the manuscript that was reviewed and edited by all authors. All authors approved the final version.

Acknowledgments

The authors wish to thank all of the families involved, several of whom have worked with us for many years. This work was supported by NIH grant 1R01NS050375 (to W.B.D.).

The study also uses data generated by the DECIPHER community. A full list of centers that contributed to the generation of the data is available from: <https://deciphergenomics.org/about/stats> and by email from contact@deciphergenomics.org. DECIPHER is hosted by EMBL-EBI and funding for the DECIPHER project was provided by the Wellcome Trust [# WT223718/Z/21/Z].

Ethics Statement

This research was conducted in accordance with the Declaration of Helsinki and/or the Belmont report. Written informed consent was obtained from all participants through protocols approved by the

Institutional Review Boards at Seattle Children's Hospital or the local institutional review board at each site. For three individuals, we were sent only de-identified data. We obtained written informed consent to publish images for all the patients whose photographs were used. We did not use patient images in the cases where patient consent did not explicitly include the use of images.

Conflicts of Interest

S.K. is an employee of and may own stock in GeneDx LLC. Authors disclose no conflicts of interest relevant to this topic. This work was supported by NIH grant 1R01NS050375 (to W.B.D.).

Data Availability Statement

Any data not available in the Supporting Information are available upon request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.