

ORIGINAL ARTICLE

An Unstable ATG2A Variant Causes a Neurodegenerative Disorder via Impaired Autophagy and Proteotoxic Stress in Brain Atrophy

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ABSTRACT

Autophagy is a critical cellular process for maintaining proteostasis and neuronal health. Disruption of this pathway is increasingly recognized in pediatric neurodegenerative disorders. Here, we study a novel previously uncharacterized homozygous and autosomal recessive missense variant, c.1372G>C (p.Gly433Ala), in the autophagy gene ATG2A, identified in a 3-year-old female proband presenting with developmental regression, seizures, cerebellar ataxia, and MRI-confirmed diffuse cerebral and cerebellar atrophy. The affected residue, Gly433, is evolutionarily conserved across eukaryotes and predicted to be structurally and functionally critical. Computational modeling and molecular dynamics simulations revealed that the G433A substitution induces local β -sheet extension, increased protein flexibility, higher aggregation propensity, and global structural destabilization. Proband-derived fibroblasts expressing ATG2A-G433A showed normal transcript and protein levels, but exhibited mislocalization of ATG2A to the cytosol, reduced colocalization with LC3B, loss of autophagosome formation, and a marked increase in protein aggregates. Proteotoxic stress was further evidenced by significant accumulation of Proteostat- and SQSTM1-positive granules. Additionally, transcript levels of unfolded protein response markers (GRP78, PERK, ATF4, and CHOP) were significantly upregulated, suggesting increased ER stress signaling. Cell cycle analysis revealed a substantial increase in cell death in proband fibroblasts. Overall, our findings identify ATG2A as a potentially novel disease gene and its G433A variant as a pathogenic substitution that disrupts autophagy and proteostasis, driving neurodegeneration via aggregation-prone misfolding and autophagy failure. This work depicts the first clinical spectrum of ATG2A-related neurodegenerative disorders and highlights the importance of autophagy maintenance in pediatric neurodevelopmental processes.

1 | Introduction

Neurodegenerative disorders represent a diverse group of progressive, often fatal conditions characterized by the selective loss

of neurons in specific brain regions. Although the clinical presentations vary, including motor dysfunction, cognitive decline, seizures, and ataxia, a common pathological hallmark of many of these disorders is the accumulation of misfolded or damaged

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proteins due to impaired cellular clearance mechanisms [1]. Among the intracellular processes responsible for maintaining neuronal proteostasis, macroautophagy—a lysosome-mediated degradation pathway—plays a central role. Several studies have linked mutations in autophagy-related genes to rare pediatric neurodegenerative syndromes, with structural brain changes such as cerebral and cerebellar atrophy frequently observed on neuroimaging [2, 3].

The autophagy process is tightly regulated by a group of evolutionarily conserved proteins known as ATG (autophagy-related) proteins, which orchestrate autophagosome formation, cargo recognition, vesicle elongation, and fusion with lysosomes [4]. In neurons, autophagy is essential not only for the clearance of damaged proteins and organelles but also for neuronal survival, differentiation, axonal maintenance, and synaptic plasticity [5]. Loss or dysfunction of key ATG proteins can therefore impair neurodevelopmental processes and contribute to neuronal degeneration and death.

Among the core autophagy proteins, ATG2A plays a crucial role in autophagosome biogenesis. It functions as a lipid transporter and membrane tethering protein, bridging the endoplasmic reticulum (ER) and phagophore membranes to facilitate membrane elongation [6, 7]. Structurally, ATG2A is a large rod-shaped protein with an internal hydrophobic cavity capable of shuttling lipids between membranes. It interacts with WIPI proteins and associates with ER-autophagosome contact sites to coordinate vesicle expansion [8]. The dynamic and domain-specific interactions of ATG2A enable it to function as a molecular scaffold critical for the early stages of autophagy initiation and maturation.

Disruptions in ATG protein function, including ATG2A, can result in autophagy failure, leading to the accumulation of cytotoxic aggregates and activation of stress responses. Several ATG genes have already been implicated in neurodevelopmental and neurodegenerative syndromes. For example, biallelic mutations in ATG5 and ATG7 have been associated with early-onset ataxia and intellectual disability [2, 9]. While the precise contribution of ATG2A to human disease remains underexplored, experimental evidence in model organisms suggests that ATG2A is indispensable for autophagy-dependent survival of neurons, and its depletion leads to the accumulation of protein aggregates, ER stress, and apoptotic death. Thus, mutations affecting the structural integrity or localization of ATG2A may have profound consequences on proteostasis and neuronal viability.

In this study, we describe a novel homozygous missense variant in ATG2A (c.1372G>C; p.Gly433Ala) identified in a 3-year-old female proband with progressive neurodegeneration, seizures, and MRI-confirmed cerebral and cerebellar atrophy. Structural modeling and molecular dynamics simulations revealed that the G433A substitution destabilizes ATG2A's β -sheet architecture, enhances its aggregation propensity, and impairs its ability to associate with autophagic membranes. Functional studies in proband-derived fibroblasts demonstrated mislocalization of the mutant protein, impaired autophagosome formation, proteotoxic stress, dysregulated ER stress response, and increased cell death. Together, these findings establish ATG2A-G433A as a pathogenic variant and provide mechanistic insight into how

loss of ATG2A function contributes to neurodegenerative disease in humans.

2 | Results

2.1 | Clinical Case Presentation

The proband, 3-year-old Saudi female child born to non-consanguineous parents (Figure 1A), first presented for clinical evaluation at the age of 3 years due to concerns regarding global developmental delay and episodic neurological symptoms. She was delivered via spontaneous vaginal delivery (SVD) at term, with a birth weight of 2.12 kg. No perinatal complications were reported. At the time of initial evaluation, her weight was 10.3 kg and height was 79 cm, both below the standard growth percentiles for age, indicating failure to thrive. Developmental history revealed significant delay and signs of developmental regression, with impaired acquisition of motor, speech, and social milestones. The child exhibited mild generalized hypotonia with episodes of increased tone in the lower limbs, suggestive of evolving spasticity. Neurological assessment indicated mild dysmetria and intention tremors, further supporting cerebellar involvement. Two seizures had been documented prior to presentation. Feeding difficulties were also noted. On physical examination, the child was alert but demonstrated limited verbal expression and reduced engagement in social interaction. Reflexes were mildly brisk, and gait was unsteady with poor postural control. Family history was unremarkable, and both parents were healthy and asymptomatic, with no similar cases reported in the extended family.

MRI of the brain revealed prominent sulci, ventricles, and cisterns, indicative of diffuse cerebral atrophy (Figure 1B). Additionally, mild cerebellar atrophy was noted (Figure 1A), although the cerebellum and brainstem maintained normal signal intensity. The hippocampi and parahippocampal regions appeared normal, and myelination was appropriate for the child's age. There were no intra- or extra-axial lesions, collections, or abnormal post-contrast enhancements observed. The sellar and parasellar regions were unremarkable, and major vascular flow voids were preserved. Overall, the MRI findings support a diagnosis of neurodegeneration characterized by diffuse cerebral and mild cerebellar atrophy, correlating with the child's developmental regression, seizure activity, and motor coordination deficits.

Further genetic investigation by trio-based whole exome sequencing revealed a homozygous missense variant in the ATG2A gene, corresponding to c.1372G>C (p.Gly433Ala) in the transcript NM_015104.3 (Figure 1C). This glycine-to-alanine substitution at position 433 affects a highly conserved residue within the ATG2A protein. This specific ATG2A-G433A variant was absent in population databases, including gnomAD and ClinVar. Segregation analysis showed that both parents of the proband were heterozygous carriers, consistent with a recessive mode of inheritance.

To further assess the pathogenicity of the identified ATG2A c.1372G>C (p.Gly433Ala) variant, a detailed *in silico* analysis was performed. Glycine at position 433 of ATG2A was found to

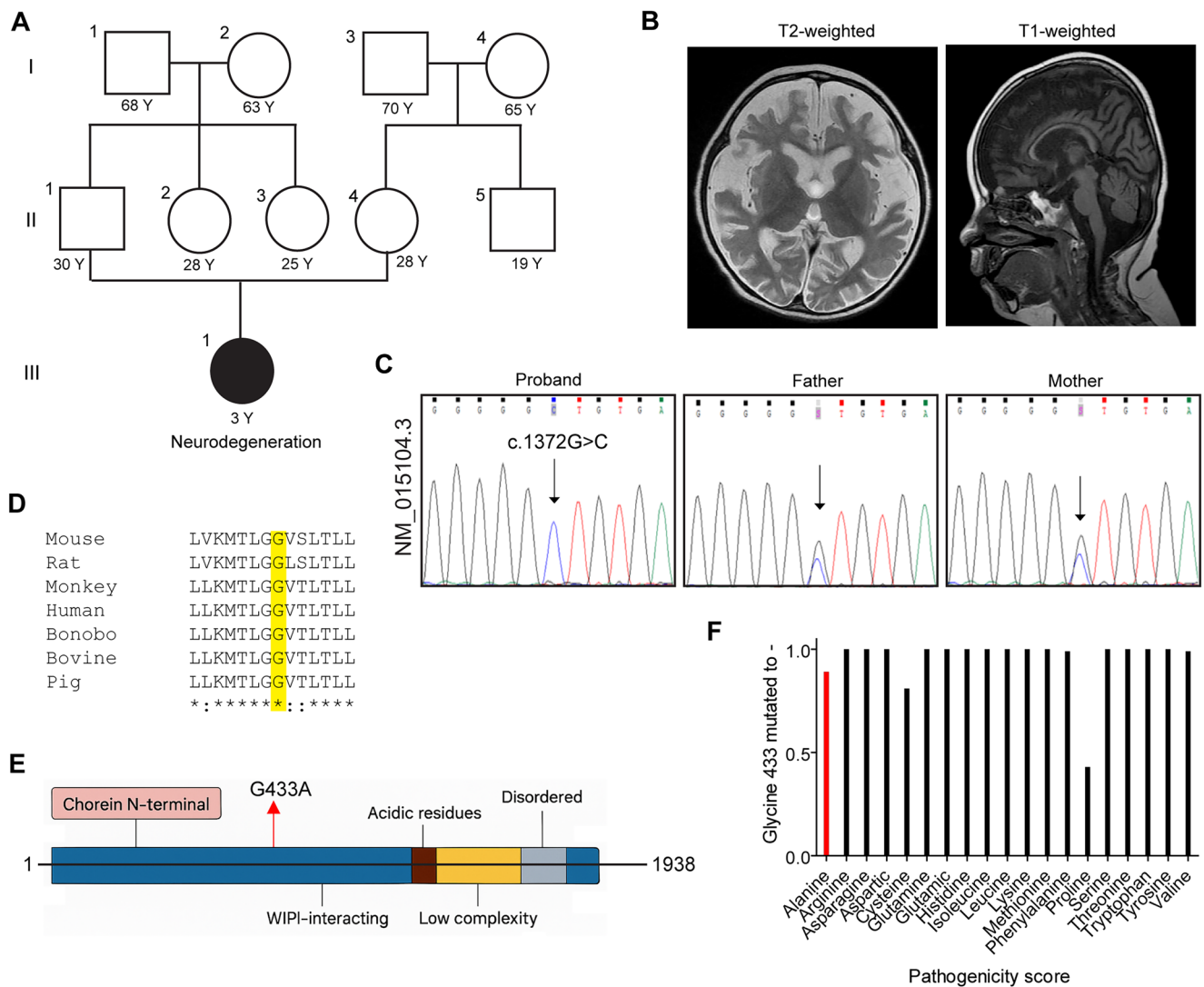


FIGURE 1 | Clinical, genetic, evolutionary, and domain-level analysis of *ATG2A*-G433A variant. (A) Pedigree of the family showing the affected proband (III-1) with neurodegeneration. The proband is a 3-year-old female born to healthy, non-consanguineous parents. No other family members are affected. (B) Brain MRI of the proband. T2-weighted (left) and T1-weighted (right) axial and sagittal images reveal marked cerebral and cerebellar atrophy, including prominent sulci and enlarged ventricles. (C) Sanger sequencing chromatograms showing a homozygous c.1372G>C variant in the proband (NM_015104.3), and heterozygous carrier status in both parents. The variant results in a missense substitution (p.Gly433Ala) in the *ATG2A* gene. (D) Multiple sequence alignment of *ATG2A* surrounding the G433 residue across diverse eukaryotic species reveals that glycine at position 433 is evolutionarily conserved. (E) Schematic representation of *ATG2A* protein structure (1–1938 amino acids) showing the position of the G433A variant (red arrow) within a β -sheet-rich region adjacent to the conserved Chorein N-terminal domain. Additional regions include disordered segments, acidic residue-rich areas, a WIPI-interacting domain, and a low-complexity segment. (F) Pathogenicity scores of substitutions at position 433 based on multiple *in silico* prediction. The G433A mutation (red bar) shows a high pathogenicity score, and all other substitutions (except G433P) are predicted to be deleterious or pathogenic. [Colour figure can be viewed at wileyonlinelibrary.com]

be highly conserved across eukaryotic species (Figure 1D,E). Pathogenicity predictions using multiple computational tools supported the deleterious nature of the p.Gly433Ala mutation. According to PolyPhen-2.0, substitution of glycine with alanine at this conserved site was classified as probably damaging, with a high score, indicating a high likelihood of functional impairment (Figure 1E). Furthermore, comparative predictions across the other 18 possible amino acid substitutions at this site revealed that all substitutions, except proline, were predicted to be damaging (Figure 1E). To validate the pathogenic potential further, additional bioinformatics tools were employed. The combined annotation dependent depletion (CADD) score for the c.1372G>C variant fell within the range typically associated

with deleterious variants. MutationTaster analysis predicted the variant to be disease-causing, while ClinPred classified the mutation as pathogenic.

2.2 | Structure of *ATG2A* Is Destabilized by G433A Mutation

To evaluate the structural consequences of the G433A substitution in *ATG2A*, a series of computational modeling and molecular dynamics simulations (MDS) were performed. Glycine 433, located at the initiation site of a β -sheet, may act as a β -sheet breaker (Figure 2A), critical for maintaining local secondary

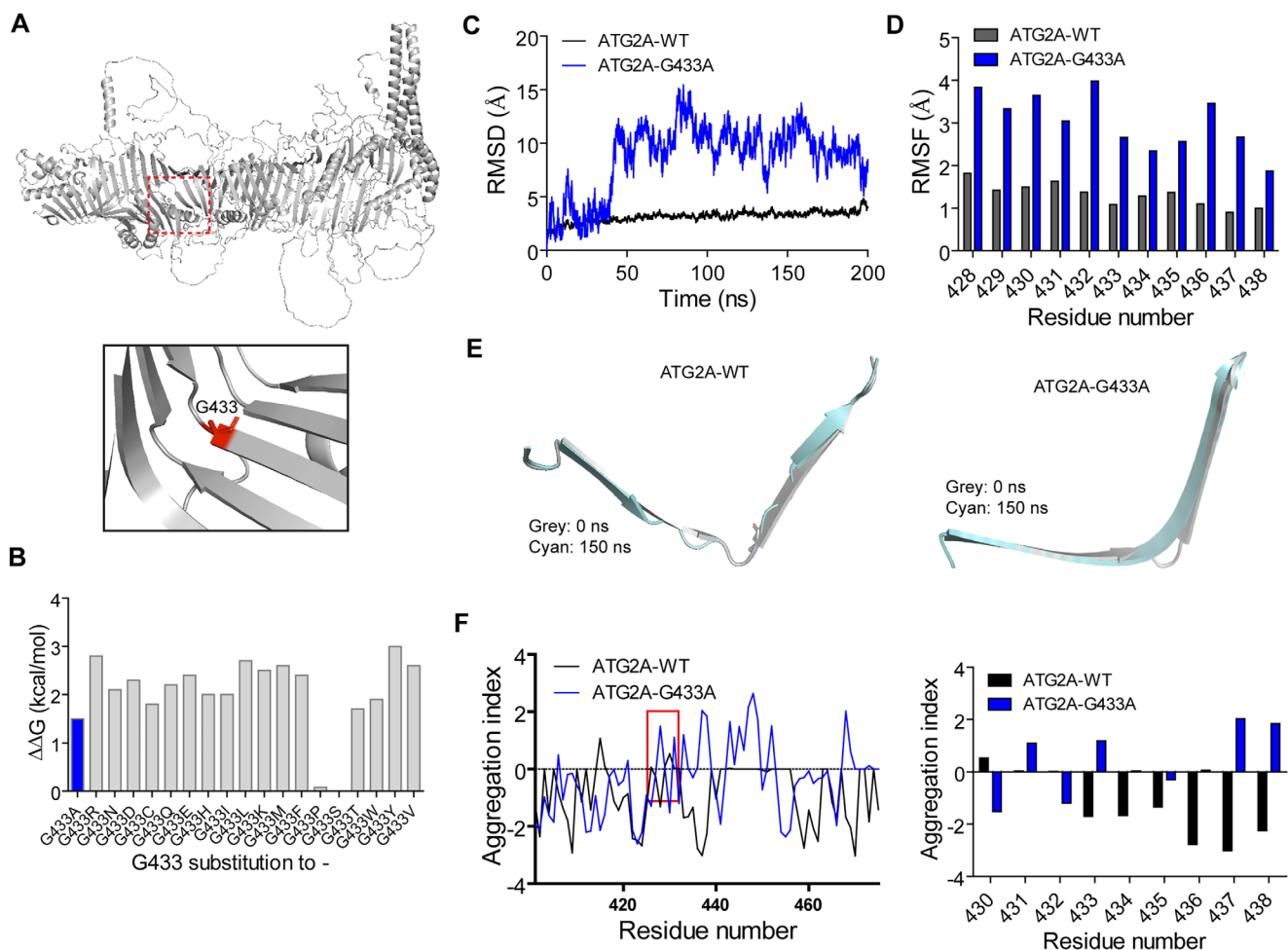


FIGURE 2 | Structural destabilization of ATG2A-G433A mutant revealed by molecular modeling and simulation. (A) Structural model of full-length ATG2A predicted by AlphaFold (AF-Q2TAZ0-F1) highlighting the location of the conserved G433 residue (red arrow) situated at the beginning of a β -sheet. Inset: Zoomed-in view of the local β -sheet structure surrounding G433. (B) Changes in folding free energy ($\Delta\Delta G$, in kcal/mol) due to substitution of G433 with all 19 other amino acids. All substitutions, including G433A (blue), are destabilizing. (C) Root mean square deviation (RMSD) plot over 200 ns molecular dynamics simulation (MDS) of wild-type ATG2A (black) and ATG2A-G433A mutant (blue). The G433A mutant shows significantly higher deviation, indicating structural instability. (D) Root mean square fluctuation (RMSF) analysis for residues 428–438 surrounding G433 and A433 in both wild-type (black bars) and mutant (blue bars) ATG2A. The G433A mutant displays markedly increased flexibility at and around the mutation site. (E) Superimposed structures of ATG2A at 0 ns (grey) and 150 ns (cyan) for wild-type (left) and G433A mutant (right). The wild-type shows a localized sheet-to-loop transition, whereas the mutant exhibits an extended, rigid β -sheet. (F) Left: Aggregation index profile (calculated using Aggrescan3D) for residues 400–475 in ATG2A-WT (black) and ATG2A-G433A (blue). The mutant shows higher aggregation propensity around G433. Right: Residue-wise aggregation index comparison for positions 430–438, indicating increased aggregation potential for each residue in the mutant. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

structure flexibility. The impact of all 19 possible substitutions at G433 on protein folding stability was assessed by calculating the $\Delta\Delta G$ (change in folding free energy) (Figure 2B). Notably, most substitutions, including G433A, were predicted to be destabilizing.

To evaluate dynamic behavior, MDS were carried out. The ATG2A-G433A mutant exhibited substantially higher root mean square (RMSD) values compared to the WT protein, indicating increased structural flexibility and conformational instability over time (Figure 2C). Complementary to this, root mean square fluctuation (RMSF) analysis revealed significantly elevated fluctuations in residues surrounding position 433 in the mutant protein compared to wild type (Figure 2D). This suggests that the local dynamics near the mutation site are enhanced in the mutant, further supporting its destabilizing effect.

A comparison of structural snapshots at different time-points of MDS (Figure 2E) showed a distinct conformational transition in the local secondary structure. In ATG2A-WT, the region surrounding G433 underwent a sheet-to-disorder transition, consistent with glycine's known role in providing flexibility and disrupting extended β -sheets. In contrast, ATG2A-G433A exhibited a continuous extension of the β -sheet across this region, suggesting that replacement with alanine abolished the β -sheet-breaking function of glycine and promoted an extended, rigid β -sheet structure, which may alter folding dynamics and interaction interfaces.

Further, aggregation propensity was analyzed across residues 400–475 (Figure 2F). The region surrounding A433 in the mutant showed a markedly higher aggregation index compared to the WT counterpart. This implies that the G433A substitution

not only perturbs structural dynamics but also promotes protein misfolding and aggregation, likely contributing to the pathogenic mechanism through proteotoxic stress.

Overall, these integrated computational analyses demonstrate that the G433A mutation destabilizes ATG2A by disrupting local secondary structure, increasing conformational dynamics, and enhancing aggregation propensity, thereby providing a structural explanation for the mutation's pathogenic role in the proband's neurodegenerative phenotype.

2.3 | ATG2A-G433A Mutant Shows Mislocalization, Aggregation, and Autophagy Impairment in Proband Fibroblasts

To examine the cellular consequences of the G433A mutation in ATG2A, we first assessed the expression of ATG2A at the transcript and protein levels in fibroblasts derived from the proband and healthy control. The transcript levels of ATG2A-G433A were comparable to wild-type (Figure 3A), indicating that the mutation does not impact mRNA expression or stability.

Similarly, ELISA-based quantification of total ATG2A protein levels revealed no significant differences between mutant and wild-type fibroblasts (Figure 3B), suggesting that the mutation does not cause protein degradation or major expression defects.

Despite comparable expression levels, immunofluorescence analysis revealed prominent differences in subcellular localization and behavior of the mutant protein (Figure 3C). In control cells expressing wild-type ATG2A, the protein localized predominantly to the ER and autophagosomes, showing a distinct reticular pattern with clear colocalization with LC3B. In contrast, the ATG2A-G433A protein in proband fibroblasts was found to be mislocalized, forming visible cytoplasmic aggregates, and showed markedly reduced colocalization with LC3B. Furthermore, the number of LC3B-positive puncta per cell—indicative of autophagosomes—was significantly reduced in the proband cells compared to controls (Figure 3D), indicating a defect in autophagosome formation. Quantitative colocalization analysis further confirmed these findings. The colocalization of ATG2A and LC3B was substantially lower in cells expressing the mutant ATG2A-G433A than in those with wild-type ATG2A (Figure 3E), demonstrating the mutant's inability to associate efficiently with autophagosomes. In addition,

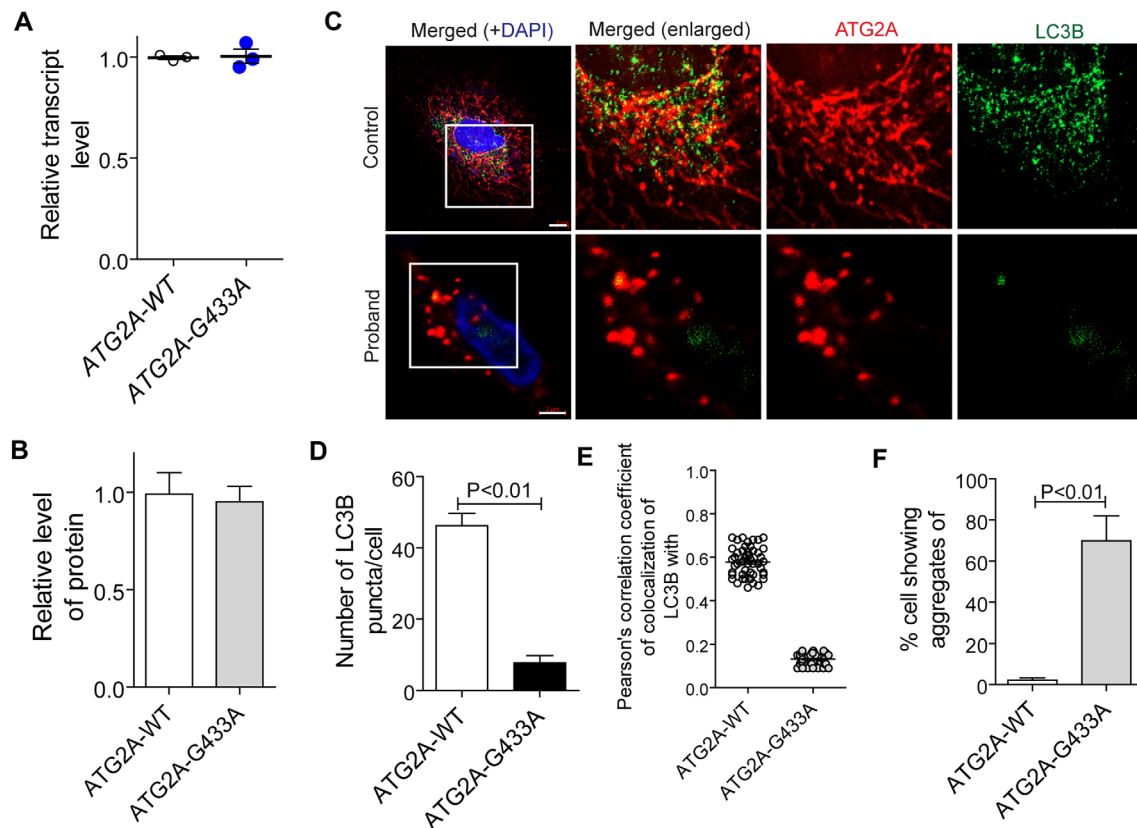


FIGURE 3 | ATG2A-G433A mutation disrupts autophagy and promotes cytosolic aggregation in proband fibroblasts. (A) Quantitative PCR analysis showing relative transcript levels of wild-type *ATG2A* and mutant *ATG2A* in fibroblasts from control and proband, respectively. (B) ELISA-based quantification of ATG2A protein in control and proband fibroblasts reveals comparable protein levels. (C) Immunofluorescence staining for ATG2A (red), LC3B (green), and DAPI (blue) in control and proband fibroblasts. Wild-type ATG2A localizes to the ER and autophagosomes, while ATG2A-G433A in the proband mislocalizes to the cytosol and forms visible aggregates. LC3B puncta, representing autophagosomes, are abundant in control cells but markedly reduced in proband cells. (D) Quantification of LC3B puncta per cell. The number of autophagosomes is significantly decreased in ATG2A-G433A fibroblasts. (E) Pearson's correlation coefficient quantifying the colocalization of ATG2A and LC3B signals. Mutant ATG2A exhibits severely reduced colocalization with LC3B, indicating impaired recruitment to autophagosomes. (F) Percentage of cells showing ATG2A-positive cytosolic aggregates. A significant increase in aggregation is observed in proband fibroblasts expressing ATG2A-G433A compared to wild-type. Scale bar in microscopy images: 5 μ m. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

the percentage of cells displaying ATG2A aggregates was significantly higher in the proband fibroblasts compared to control cells (Figure 3F), confirming that the G433A mutation induces protein misfolding and aggregation.

These findings indicate that although ATG2A-G433A is expressed at levels comparable to the wild-type protein, it is functionally impaired due to mislocalization, failure to associate with autophagosomes, loss of autophagy-related function, and a pronounced tendency to form cytosolic aggregates. This cellular phenotype supports the pathogenicity of the G433A mutation as a loss-of-function and aggregation-prone variant disrupting normal autophagic flux.

2.4 | ATG2A-G433A Mutation Induces Proteotoxic Stress and Impaired Protein Clearance to Cause Increased Cell Death in Proband Fibroblasts

To investigate whether the ATG2A-G433A mutation impairs protein homeostasis, we examined the extent of intracellular protein aggregation in proband fibroblasts. Proband fibroblasts

exhibited a marked increase in Proteostat-positive cytoplasmic protein aggregates (Figure 4A,B), compared to the control fibroblasts. This indicated an elevated proteotoxic stress, likely due to impaired clearance of misfolded proteins.

To further evaluate the autophagic degradation of ubiquitinated protein aggregates, we assessed the accumulation of SQSTM1/p62, an autophagy receptor that serves as a marker of autophagy flux impairment. SQSTM1-positive puncta were notably enriched in the cytoplasm of proband fibroblasts, in contrast to their relatively sparse presence in control cells (Figure 4C,D), indicating inefficient autophagic clearance of ubiquitinated aggregates due to the mutant ATG2A's loss of function.

In light of the observed proteostasis imbalance, we next examined the activation of the ER stress response against the misfolded proteins. qPCR analysis of key ER stress-responsive genes showed that transcript levels of *GRP78*, *PERK*, *ATF4*, and *CHOP* were significantly upregulated in proband fibroblasts but were markedly reduced in control cells expressing wild-type ATG2A (Figure 4E). This suggests an increased ER stress response in the

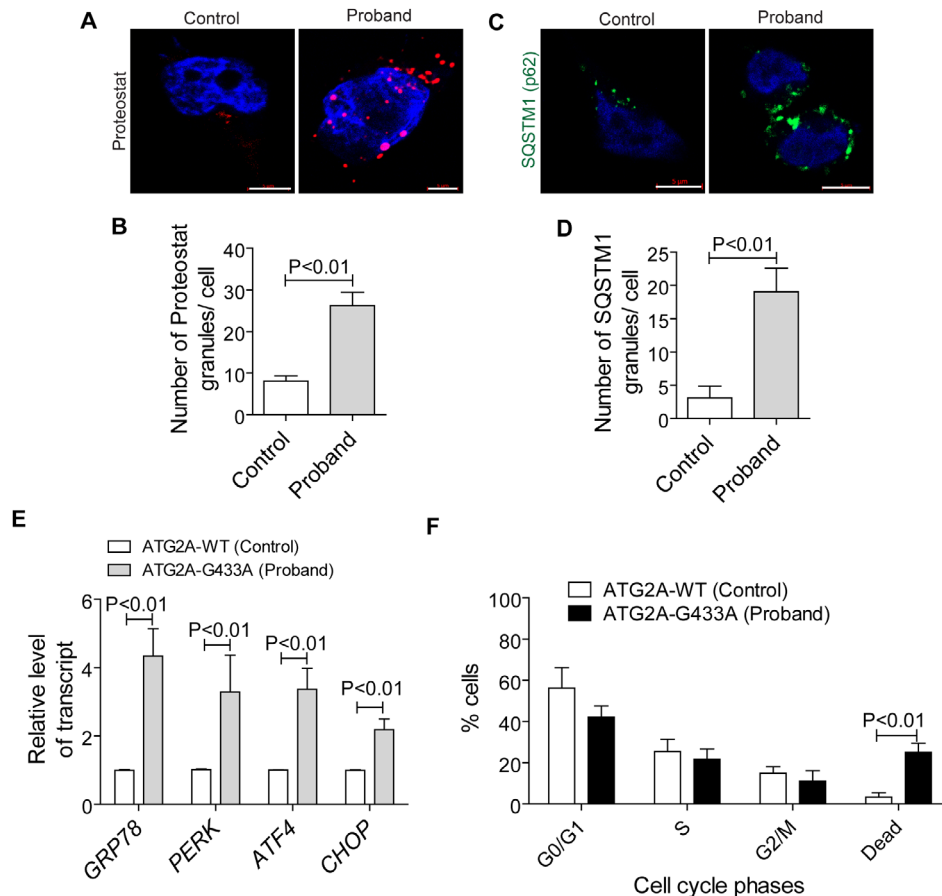


FIGURE 4 | IL3-V33I-induced proteotoxic stress, impaired proteostasis, ER stress, and increased cell death in proband fibroblasts. (A, B) Immunofluorescence staining of protein aggregates using the Proteostat dye in control and proband fibroblasts, revealing a significantly higher number of Proteostat-positive granules per cell in proband fibroblasts compared to controls. (C, D) Immunofluorescence imaging of SQSTM1/p62-positive ubiquitinated protein aggregates in control and proband fibroblasts, displaying a significant increase in p62-positive granules in proband fibroblasts. (E) Quantitative PCR-based estimation of the transcript levels of major ER stress-responsive genes—*GRP78*, *PERK*, *ATF4*, and *CHOP*—was significantly reduced in proband fibroblasts harboring the ATG2A-G433A background. (F) Flow cytometry-based cell cycle analysis showed no significant change in G0/G1, S, or G2/M phases between control and proband fibroblasts. The percentage of dead cells (sub-G1 population) was significantly higher in the proband group. Scale bar in microscopy images: 5 μ m. [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

proband cells, potentially reflecting chronic proteotoxic stress or regulatory collapse of the unfolded protein response (UPR).

Finally, to determine the impact of these cellular stresses on cell viability and proliferation, we conducted cell cycle analysis by flow cytometry (Figure 4F). While the distribution of cells in G0/G1, S, and G2/M phases showed moderate alterations between control and proband cells, a significantly higher proportion of proband fibroblasts were found in the “dead cell” fraction, indicating elevated cell death associated with the ATG2A-G433A mutation.

Collectively, these results demonstrate that the ATG2A-G433A mutation leads to severe proteostasis dysfunction, characterized by increased cytosolic protein aggregation, autophagy impairment, attenuated ER stress signaling, and ultimately increased cell death. These cellular phenotypes align with the proband's clinical presentation of neurodegeneration and support the pathogenic role of the G433A variant in disrupting cellular homeostasis and survival.

3 | Discussion

Autophagy plays an essential role in maintaining neuronal homeostasis and development by regulating the turnover of damaged proteins and organelles. Dysregulation of autophagy is increasingly implicated in early-onset neurodegenerative disorders, particularly those characterized by progressive cerebral and cerebellar atrophy [1]. Several autophagy-related potential disease gene mutations, including those in WDR45, ATG5, and ATG7, have been associated with pediatric neurodegenerative phenotypes involving developmental delay, seizures, hypotonia, and structural brain changes on MRI [2, 3]. In our study, the proband, a 3-year-old female, presented with a constellation of clinical features—developmental regression, hypotonia, spasticity, ataxia, and seizures, accompanied by MRI findings of diffuse cerebral and cerebellar atrophy—which is highly reminiscent of previously described autophagy-related neurodegenerative syndromes.

ATG proteins are evolutionarily conserved and execute distinct steps in the autophagic cascade, from phagophore nucleation to autophagosome closure and fusion with lysosomes. Among these, ATG2A is a large, rod-shaped protein involved in lipid transfer and autophagosome biogenesis [7]. It localizes predominantly at ER-autophagosome membrane contact sites, functioning as a tethering factor and lipid conduit to support autophagosome expansion [8]. Structurally, ATG2A contains a large hydrophobic cavity and β -sheet-rich domains that facilitate its membrane binding and transfer properties [6].

In this study, trio-based exome sequencing identified a homozygous missense variant in *ATG2A*, with both asymptomatic parents being heterozygous carriers. Although we did not sequence ethnically matched control DNA samples due to limited access to a sufficiently powered local cohort, the ATG2A-G433A variant was not found in major population databases, which collectively encompass diverse populations, including individuals of Middle Eastern descent. Therefore, the pathogenicity of the homozygous variant in the proband reduces the likelihood of it

being a benign population-specific polymorphism. The variant lies within a highly conserved glycine residue (G433), positioned at the beginning of a β -sheet, potentially functioning as a β -sheet breaker to maintain local folding flexibility. Substitution of glycine to alanine (G433A) was predicted to be damaging, and shown to destabilize folding energy in free energy calculations. Molecular dynamics simulations revealed that the G433A mutation led to increased RMSD and RMSF, indicating elevated structural flexibility and instability. The local β -sheet structure failed to undergo the typical sheet-to-disorder transition, instead forming an extended β -sheet. This aberrant conformation enhanced aggregation propensity in the region surrounding residue 433, as shown by aggregation index analysis. Such structural destabilization not only affects protein folding and solubility, but also potentially disrupts interactions with autophagy machinery, including LC3, WIPI proteins, and ER contact sites [10].

Interestingly, despite the structural instability, transcript, and total protein levels of ATG2A-G433A were unaltered, suggesting that the mutation does not impair mRNA stability or translation efficiency. This observation is consistent with previous findings that some destabilizing missense mutations evade nonsense-mediated decay and result in functional but misfolded proteins, which often accumulate as aggregates [11]. In proband-derived fibroblasts, ATG2A-G433A exhibited aberrant cytosolic mislocalization, forming visible aggregates and failing to localize to the ER and autophagosomes, unlike the wild-type protein. This mislocalization correlated with a dramatic reduction in autophagosome formation, as evidenced by reduced LC3B puncta and loss of colocalization between LC3B and ATG2A-G433A. These findings strongly indicate a loss of function of the mutant ATG2A in mediating autophagosome biogenesis and ER tethering.

Consequently, cells expressing ATG2A-G433A exhibited robust accumulation of protein aggregates, confirmed by increased Proteostat and p62/SQSTM1-positive granules, indicating proteotoxic stress and impaired aggregate clearance. ER stress-responsive genes, including GRP78, PERK, ATF4, and CHOP, were upregulated, suggesting an adaptive UPR and a state of chronic ER stress [12]. Cell cycle analysis revealed that mutant ATG2A-expressing cells experienced significantly increased cell death, likely due to cumulative proteotoxic stress, ER stress, and autophagy failure. Neurons, being highly reliant on autophagy for survival and non-renewable, are particularly vulnerable to such insults, offering a mechanistic basis for the observed cerebral and cerebellar atrophy in the proband. Importantly, the G433A-induced misfolding, mislocalization, aggregation, and loss of autophagy function converge into a unified pathomechanism wherein mutant ATG2A fails to scaffold autophagosomal membranes, leading to accumulation of cytotoxic aggregates, disruption of the ER-autophagy interface, and activation of neurodegenerative cascades.

Mutations in the *EPG5* gene, a key autophagy regulator involved in autophagosome-lysosome fusion, have been shown to cause Vici syndrome (VS), a severe neurodevelopmental disorder featuring developmental delay, epilepsy, and progressive neurodegeneration [13]. It is demonstrated that loss of *EPG5* in both *Drosophila* and humans leads to accumulation of protein

aggregates, proteotoxic stress, and late-onset seizures, underscoring the direct link between defective autophagic clearance and neuronal dysfunction. Similar to this effect, in our study, we describe how ATG2A-G433A disrupts autophagy and proteostasis. Although the mechanisms differ—EPG5 functions at the terminal stage of autophagosome maturation while ATG2A is involved in membrane elongation and lipid transport—both mutations converge on accumulation of autophagy substrates, ER stress, and ultimately neurodegeneration. Our proband's clinical presentation of early-onset cerebral and cerebellar atrophy, seizures, and developmental regression mirrors the phenotypes seen in EPG5-mutant patients. However, unlike the later-onset seizures observed in EPG5-associated disorders, our patient manifested neurodevelopmental deterioration and neuronal loss very early, likely due to the misfolding, aggregation, and functional inactivation of mutant ATG2A.

Together, this study identifies ATG2A-G433A as a novel neurodegeneration-linked mutation, highlighting the importance of structural integrity, autophagy function, and protein localization of ATG2A in maintaining neuronal health. Our findings reinforce the concept that loss-of-function mutations in autophagy machinery can lead to profound neurodevelopmental consequences through misfolding-driven proteotoxic stress.

4 | Methods

Detailed methods and materials are given in [Supporting Information](#).

Author Contributions

S. Rehan Ahmad: Conceptualization, study design, experimental execution, data analysis, manuscript preparation. Md. Zeyauallah: Methodology, data analysis. Abdullah M. AlShahrani: Methodology, data analysis. Danish Qavi: Methodology, data analysis. Abdelrhman A.G. Altijani: Methodology, data analysis. Mohammad Suhail Khan: Methodology, data analysis. Khursheed Muzammil: Clinical diagnosis and evaluation, methodology.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

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