Clinical Genetics

WILEY

CLINICAL GENETICS

SHORT REPORT

Novel *RORA* Variants Reveal Genotype–Phenotype Diversity and Variable Expressivity in Neurodevelopmental Disorders

Gul Unsel-Bolat[1] | Hilmi Bolat[2] | Senol Citli[3] | Ozlem Ozdemir[4] | Ibrahim Baris[5]

[1]Department of Child and Adolescent Psychiatry, Faculty of Medicine, Balıkesir University, Balıkesir, Turkey | [2]Department of Medical Genetics, Faculty of Medicine, Balıkesir University, Balıkesir, Turkey | [3]Department of Medical Genetics, Faculty of Medicine, Recep Tayyip Erdogan University, Rize, Turkey | [4]Private Clinics of Child Neurology, Bursa, Turkey | [5]Department of Molecular Biology and Genetics, Koç University, Istanbul, Turkey

Correspondence: Ibrahim Baris (ibaris@ku.edu.tr)

Received: 1 November 2025 | **Revised:** 22 November 2025 | **Accepted:** 25 November 2025

Keywords: neurodevelopmental disorder | RORA | WES

ABSTRACT

The RAR-related orphan receptor alpha (*RORA*) gene encodes a nuclear receptor involved in transcriptional regulation, circadian rhythm, and neurodevelopment. Dominant *RORA* variants are associated with intellectual developmental disorder with or without epilepsy or cerebellar ataxia, yet the phenotypic spectrum remains poorly defined. We performed comprehensive genetic and clinical analyses in four individuals with *RORA* variants from three unrelated families, using whole exome sequencing and chromosomal microarray analysis. Identified variants were confirmed by Sanger sequencing. Genetic analyses revealed three distinct *RORA* variants: a 15q21.2–q22.2 deletion encompassing *RORA*, a de novo nonsense variant c.499C>T (p.Gln167*), and a novel heterozygous frameshift variant c.683_686del (p.Glu228Valfs*78) segregating within a family. Clinical findings ranged from severe neurodevelopmental delay and epilepsy to mild intellectual disability and behavioral abnormalities, demonstrating marked intrafamilial variability. Notably, the same frameshift variant presented with differing phenotypes in the family, indicating variable expressivity—the first such observation reported in *RORA*-related disorders. Our findings broaden the genotypic and phenotypic spectrum of *RORA*-related neurodevelopmental disorders. The observed intrafamilial variability highlights the complexity of RORA-associated pathogenesis and underscores the importance of considering variable expressivity in future genotype–phenotype studies.

1 | Introduction

Advances in genomic technologies, particularly whole exome sequencing (WES) [1] and chromosomal microarray analysis (CMA) [2], have markedly increased the detection of rare genetic variants associated with neurodevelopmental disorders.

Retinoic acid receptor-related orphan receptor-α (RORα), a member of the orphan nuclear receptor family, functions as a transcriptional activator influenced by circadian rhythms [3, 4]. While RORα is known for its role in regulating circadian clock mechanisms, it is also implicated in carcinogenesis, inflammation, and lipid homeostasis [5, 6]. However, the precise molecular mechanisms underlying its mode of transcriptional regulation remain unclear. High levels of RORα are found in cerebellar Purkinje cells, retinal ganglion cells, and thalamic nuclei. The *RORA* gene, located on human chromosome 15q22.2, is expressed in various tissues, including the liver, skin, lungs, adipose tissue, brain, and muscle [7].

Dominant *RORA* variants are associated with intellectual developmental disorder with or without epilepsy or cerebellar ataxia (OMIM: 600825). However, reported phenotypes range widely, including Intellectual Disability (ID), epileptic seizures, autism spectrum disorders (ASD), and cerebellar ataxia. Guissart et al. classified these phenotypes into two

distinct groups under the framework of "dual molecular effects of dominant *RORA* variants causing two variants of syndromic intellectual disability with either autism or cerebellar ataxia" [8]. So, *RORA* gene variants provide a complex impact on the phenotypic features.

In this study, we aimed to present genetic and clinical findings of probands with novel *RORA* gene variants and to evaluate how the clinical phenotypes of these variants differ in these probands.

2 | Methods

Ethical approval was obtained by the Balıkesir University Ethics Committee on May 24, 2024, with decision number 2024/102. Written informed consent was obtained from the patient's legal guardians.

2.1 | DNA Extraction

Genomic DNA was extracted from peripheral venous blood using the Exgene Blood SV kit (GeneAll, Korea). Genomic DNA was extracted from the patient and available family members, including both parents (and siblings when available).

2.2 | Whole Exome Sequencing

WES was performed using the Illumina SureSelect V6 capture kit and sequenced on the Illumina HiSeq4000 platform (100 bp paired-end reads; ~100× mean coverage). Data processing, including quality control, alignment to the GRCh38 reference genome, and variant calling, followed standard GATK best-practice pipelines. Annotation of detected variants was performed using Franklin (https://franklin.genoox.com/), VarSome (https://varsome.com/), ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), OMIM, and the Human Genome Mutation Database (HGMD, http://www.hgmd.cf.ac.uk/ac/index.php). Variants with a frequency higher than 0.5% were filtered out. dbNSFP (which contains SIFT, PolyPhen-2, LRT, and Mutation Taster) was used to predict the pathogenicity of the deleteriousness of variants.

2.3 | Sanger Sequencing

Candidate variants were validated by bidirectional Sanger sequencing using standard procedures, and chromatograms were evaluated with SeqScape v2.5.

2.4 | Chromosomal Microarray Analysis

DNA isolated from the patient's sample was analyzed using the GenetiSure Cyto CGH Microarray Kit, 8×60 K (Agilent). GRCh38 Genome build names were used. The data obtained from the analyses were searched in Genomic Variants Databases (DGV, http://dgv.tcag.ca/dgv/app/home), DECIPHER (https://www.deciphergenomics.org/), OMIM, and other relevant databases. The LogR value of the microarray data obtained from the study was determined to be 0.15 (< 0.2), and the median call rate value was 0.98 (0.98–1).

3 | Results

Clinical and genetic findings of four probands were summarized in Table 1. Genetic findings of probands were presented in Figure S1 as follows: (a) Array CGH result showing loss in the 15q21.22 region; (b) Sequencing electrogram showing the c.499C>T (p.Gln167*) variant in Proband 2; and (c) Sequencing electrogram showing the position of the c.683_686del (p.Glu228Valfs*78) variant in Probands 3 and 4 in the forward and reverse directions.

3.1 | Family 1

A 5-year-old girl was born at 40 weeks of gestation with a birth weight of 3800 g and a length of 54 cm. Although no records of occipitofrontal circumference (OFC) at birth were available, the family reported it to be within the normal range. The pregnancy was unremarkable, and delivery via cesarean section was performed due to breech presentation. The first symptoms observed by the family were hypotonia and delayed motor milestones. Her seizures began at 10 months of age. She achieved sitting with support at 9 months and sitting independently by the age of 2.

Brain magnetic resonance imaging (MRI) revealed increased subarachnoid cerebrospinal fluid (CSF) spaces in the bilateral frontotemporal region, cortical atrophy in the right hemisphere (notably in the frontotemporal region), hyperintensities at the centrum semiovale, and a thin corpus callosum.

At her most recent evaluation at age 5, she exhibited severe developmental delay, with no meaningful speech or ability to walk. Her height was 105 cm (SD: −1.02), weight was 17.5 kg (SD: −0.44), and OFC was 53.5 cm (SD: 2.11), consistent with macrocephaly.

Chromosomal microarray analysis identified a de novo 9.8 Mb heterozygous deletion in the 15q21.22 region (arr[GRCh38] 15q21.2q22.2(51196236_61012458)x1). This region includes multiple OMIM genes, including *RORA*, which is associated with monoallelic haploinsufficiency, and *TCF12* (OMIM: 600480), which is implicated in neurodevelopmental disorders. Exome sequencing did not reveal any additional variants associated with her phenotype.

3.2 | Family 2

A 5-year-old boy was born at 40 weeks of gestation with a birth weight of 2700 g. No significant complications were reported during pregnancy or delivery. He achieved his first word and walking at 18 months of age. The family initially sought medical attention due to concerns about developmental delay. The patient was monitored for mild developmental delay but did not experience seizures. Brain MRI findings were normal.

At his most recent evaluation at age 5, he demonstrated the ability to construct sentences of up to four words. His height was

TABLE 1 | Clinical and genetic findings of probands included in this study.

	Family 1	Family 2	Family 3	
	Proband-1	**Proband-2**	**Proband-3**	**Proband-4**
Sex	Female	Male	Male	Male
Age	5 years	5 years	9 years	8 years
Variant type	Gross deletion	Nonsense	Small deletion (frameshift)	
Variant (GRCh38)	15q21.2-q22.2 (51422365_61274230)x1	NM_134261.3: c.499C>T (p.Gln167Ter)	NM_134261.3: c.683_686del (p.Glu228Valfs*78)	
Inheritance	de novo	de novo	Maternal	
Birth weight (Grams/SD)	3800 g (SD: 0.9)	2700 g (SD: −1.56)	4500 g (SD: 2.5)	3500 g (SD: 0.22)
Birth height (cm/SD)	54 cm (SD: 1.82)	–	50 cm (SD: 0)	50 cm (SD: 0)
Height at last investigation (cm/SD)	105 cm (SD: −1.02)	121 (SD: 2.22)	124 cm (SD: −1.46)	127 cm (SD: 0.02)
Weight at last investigation (kg/SD)	17.5 kg (SD: −0.44)	26 (SD: 2.34)	34 kg (SD: 0.93)	25 kg (SD: 0.29)
Head Circumference at last investigation	53.5 cm (SD: 2.11)	53.5 (SD: 1.27)	55 cm (SD: 1.4)	54 cm (SD: 1.06)
Age of walking	None	18 months	1 year	1 year
Age of first words	None	18 months	18 months	None
Intellectual Disability	Severe	Mild	Moderate	Moderate
Current language ability	None	Sentences consisting of max four words	Several words	None
Behavioral anomalies	–	ADHD, articulation disorder	ASD	ASD
Seizure	+	None	+	+
Other neurological findings	Hypotonia	None	None	None
Brain Imaging	Brain MRI: increased subarachnoid cerebrospinal fluid (CSF) spaces in the bilateral frontotemporal region, cortical atrophy in the right hemisphere (notably in the frontotemporal region), hyperintensities at the centrum semiovale, and a thin corpus callosum.	Brain MRI: No pathological findings	Brain CT: No pathological findings	Brain MRI: corpus callosum hypoplasia
Eye anomalies	None	None	None	None
Other clinical findings	None	None	None	Bilaterally operated polydactyly, operated heart anomaly (great vessel transposition)

121 cm (SD: 2.22), his weight was 26 kg (SD: 2.34), and OFC was 53.5 cm (SD: 1.27). While he displayed hyperactivity as a behavioral concern, there were no indications of autism spectrum disorder.

Clinical exome sequencing identified a heterozygous NM_134261.3: c.499C>T (p.Gln167*) nonsense variant in the *RORA* gene. Sanger sequencing confirmed the de novo state of the variant. The variant is predicted to cause a premature

termination at position 167 of the Nuclear receptor ROR-alpha protein. As a result of this variant, a 357-amino-acid region, including the ligand-binding domain, is expected to be lost, and thereby impairing truncated protein function (RORA_HUMAN, UniProt ID: P35398). This variant has been reported in the ClinVar database as pathogenic (Variation ID: 3024237). This variant was classified as pathogenic according to ACMG criteria (PVS1, PM2, and PP5).

3.3 | Family 3

Proband 3, a 9-year-old boy, presented with moderate ID, ASD and epilepsy to our Genetics outpatient clinic. During the clinical evaluation, the family disclosed information about another son (Proband 4), who had been diagnosed with moderate ID, ASD and epilepsy. The family was advised to seek genetic evaluation for Proband 4 at the outpatient clinic.

Proband 3 was born weighing 4500 g and measuring 50 cm in length. Although OFC records at birth were unavailable, the family reported it as larger than average. He began walking at 1 year of age and spoke his first word at 18 months, but his language development did not progress further. At 9 years old, he could only say "mom" and "dad" and was unable to form sentences. His height was 124 cm (SD: −1.46), weight was 34 kg (SD: 0.93), and OFC was 55 cm (SD: 1.4). His epileptic seizures began at the age of 1 and continue as generalized tonic–clonic seizures despite treatment.

Whole exome sequencing identified a novel NM_134261.3: c.683_686del (p.Glu228Valfs*78) frameshift variant in the

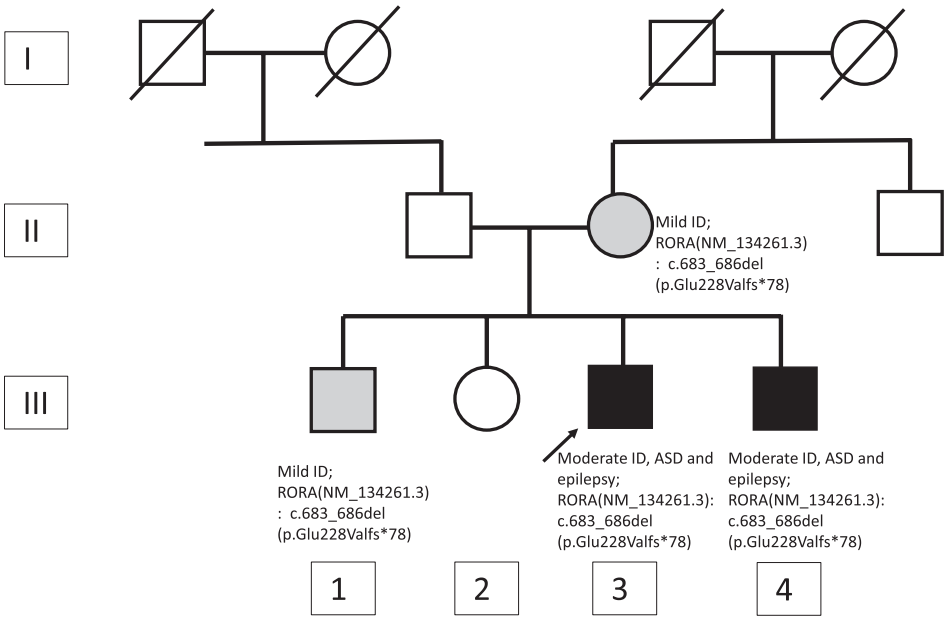

FIGURE 1 | The pedigree of family three presents variable expressivity among family members with the *RORA* variant.

TABLE 2 | Clinical features of patients with RORA variant in the present report and previous reports.

Clinical features	Previous reports ([9]; [8]; [10])	This study (Proband-1)	This study (Proband-2)	This study (Proband-3)	This study (Proband-4)
Male/female	32/28	Female	Male	Male	Male
Developmental delay/ intellectual disability	51/57	+	+	+	+
Autistic features	13/54	−	−	+	+
Hypotonia	18/43	+	−	−	−
Tremor	15/42	−	−	−	−
Seizure	32/58	+	−	+	+
Brain MRI abnormalities	21/41	+	−	N/A	+
Strabismus	9/17	−	−	−	−
Cerebellar ataxia	24/42	+	−	−	−

RORA gene in both Proband 3 (III-3) and Proband 4 (III-2). The variant is predicted to cause a frameshift starting at position 228, leading to premature termination after 78 residues. As a result of this variant, a 295-amino-acid region, including the ligand-binding domain, is expected to be lost, thereby impairing truncated protein function (RORA_HUMAN, UniProt ID: P35398). This variant has not been reported in the literature; however, its predicted premature termination and absence from gnomAD suggest that it may have clinical significance like other published pathogenic variants. This variant was classified as likely pathogenic according to ACMG criteria (PVS1, PM2).

Segregation analysis revealed the same variant in the mother and an additional sibling (III-1). Clinical evaluation indicated that both the mother and sibling III-1 exhibit mild intellectual disability. Sibling III-1 also had a history of low academic performance. The family pedigree is presented in Figure 1.

4 | Discussion

In this study, we presented four probands with *RORA* variants from three families. To date, the Human Gene Mutation Database (HGMD) Professional 2024.4 has reported 27 *RORA* variants, while the ClinVar dataset lists 53 pathogenic or likely pathogenic variants. These include single nucleotide variants (SNVs) (primarily nonsense or frameshift variants) and copy number variants (CNVs) (15q22 deletions and duplications). According to the HGMD, reported *RORA* variants included 12 missense/nonsense variants, 2 splice site variants, 3 small deletions, 2 small insertions, 6 gross deletions, 1 complex variant, and 1 gross insertion. Despite these records, only a limited number of variants have been characterized in published case reports, restricting current insights into the genotype–phenotype correlations of *RORA*-related neurodevelopmental disorders. We summarized the clinical findings of published cases in addition to our four probands in Table 2.

Early evidence included reports of ID in individuals with microdeletions at the 15q22.2 chromosomal region, which encompasses the *RORA* gene [9, 11]. Yamamoto et al. further narrowed the candidate genes within this region to *NARG2* and *RORA* by analyzing genotype–phenotype correlations from five patients across four international centers [9]. Guissart et al. [8] expanded the understanding of *RORA* gene variants by presenting clinical and genetic data from 16 individuals across 13 families.

Guissart et al. [8] described two distinct neurodevelopmental phenotypes linked to *RORA* variants: (1) Severe ID, epilepsy, and motor impairments associated with cerebellar degeneration, and (2) behavioral abnormalities, including ASD and attention deficit hyperactivity disorder (ADHD), accompanied by mild ID or normal cognition, often with epilepsy. Group 1 ("cognitive, motor and cerebellar" phenotype) is characterized by moderate-to-severe developmental delay or intellectual disability, hypotonia, cerebellar ataxia, tremor, and high rates of epilepsy. These presentations were associated with missense variants, particularly those affecting the DNA-binding domain (DBD). Group 2 ("cognitive, behavioral, ASD-linked" phenotype) involves individuals with mild intellectual disability and predominant behavioral features, including ASD and ADHD. Epilepsy is also reported in this group, but tends to be less severe and often occurs without cerebellar structural abnormalities. These cases were associated with haploinsufficiency variants located in the ligand-binding domain (LBD). Talarico et al. reported *RORA*-related cerebellar symptoms in 25 of 34 individuals and structural cerebellar abnormalities, including cerebellar hypoplasia and atrophy, in 16 of 25 cases [10]. As highlighted, these abnormalities were strongly enriched among individuals carrying DBD missense variants, consistent with the earlier Group 1 phenotype characterized by cerebellar involvement.

The clinical features of our probands align with this classification. Proband 2, 3, and 4 displayed Group 2 features, such as behavioral disorders and ID. Proband 2, 3, and 4 (all male) did not exhibit significant motor impairments. Notably, the variable expressivity observed in these cases highlights the complexity of *RORA*-related disorders. Interestingly, the c.683_686del (p.Glu228Valfs*78) variant in Proband 3 and 4 resulted in severe symptoms, whereas the carrier mother and another brother displayed only mild intellectual disability. This suggests variable expressivity of the variant, marking the first report of such variability in *RORA*-related disorders. Proband 1 with 15q21.2q22.2(51196236_61012458)x1 deletion exhibited Group 1 symptoms, including severe ID, epilepsy, and motor impairments. Although the proband's clinical presentation is broadly consistent with the gene–phenotype relationships reported in the literature, it remains important to approach this interpretation with caution. The deleted region encompasses multiple OMIM genes.

As a conclusion, clinical findings in our study ranged from severe neurodevelopmental delay and epilepsy to mild intellectual disability and behavioral abnormalities, demonstrating marked intrafamilial variability. Notably, the same frameshift variant presented with differing phenotypes in the family, indicating variable expressivity—the first such observation reported in *RORA*-related disorders. The observed intrafamilial variability highlights the complexity of RORA-associated pathogenesis and underscores the importance of considering variable expressivity in future genotype–phenotype studies.

Funding

The authors have nothing to report.

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.

References

1. H. Bolat, G. Ünsel-Bolat, H. Derin, A. Şen, and S. Ceylaner, "Distinct Autism Spectrum Disorder Phenotype and Hand-Flapping Stereotypes: Two Siblings With Novel Homozygous Mutation in TRAPPC9 Gene and Literature Review," *Molecular Syndromology* 13, no. 4 (2022): 263–269.

2. G. Ünsel Bolat and H. Bolat, "The Role of Copy Number Variations and FHIT Gene on Phenotypic Characteristics of Cases Diagnosed With Autism Spectrum Disorder," *Molecular Syndromology* 12, no. 1 (2021): 12–19.

3. G. B. Atkins, X. Hu, M. G. Guenther, C. Rachez, L. P. Freedman, and M. A. Lazar, "Coactivators for the Orphan Nuclear Receptor RORalpha," *Molecular Endocrinology* 13, no. 9 (1999): 1550–1557.

4. T. Hirose, R. J. Smith, and A. M. Jetten, "ROR-Gamma: The Third Member of ROR/RZR Orphan Receptor Subfamily That is Highly Expressed in Skeletal Muscle," *Biochemical and Biophysical Research Communications* 205 (1994): 1976–1983.

5. A. M. Jetten, "Retinoid-Related Orphan Receptors (RORs): Critical Roles in Development, Immunity, Circadian Rhythm, and Cellular Metabolism," *Nuclear Receptor Signaling* 7 (2009): e003.

6. J. M. Lee, H. Kim, and S. H. Baek, "Unraveling the Physiological Roles of Retinoic Acid Receptor-Related Orphan Receptor α," *Experimental & Molecular Medicine* 53, no. 9 (2021): 1278–1286.

7. E. André, K. Gawlas, and M. Becker-André, "A Novel Isoform of the Orphan Nuclear Receptor RORbeta is Specifically Expressed in Pineal Gland and Retina," *Gene* 216, no. 2 (1998): 277–283.

8. C. Guissart, X. Latypova, P. Rollier, et al., "Dual Molecular Effects of Dominant RORA Mutations Cause Two Variants of Syndromic Intellectual Disability With Either Autism or Cerebellar Ataxia," *American Journal of Human Genetics* 102, no. 5 (2018): 744–759.

9. T. Yamamoto, M. A. Mencarelli, C. Di Marco, et al., "Overlapping Microdeletions Involving 15q22.2 Narrow the Critical Region for Intellectual Disability to NARG2 and RORA," *European Journal of Medical Genetics* 57 (2014): 163–168.

10. M. Talarico, J. de Bellescize, M. De Wachter, et al., "RORA-Neurodevelopmental Disorder: A Unique Triad of Developmental Disabilities, Cerebellar Anomalies, and Myoclonic Seizures," *Genetics in Medicine* 27 (2025): 101347, https://doi.org/10.1016/j.gim.2024.101347.

11. S. R. Lalani, T. Sahoo, M. E. Sanders, S. U. Peters, and B. A. Bejjani, "Coarctation of the Aorta and Mild to Moderate Developmental Delay in a Child With a de novo Deletion of Chromosome 15 (q21.1q22.2)," *BMC Medical Genetics* 7, no. 1 (2006): 1–6.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Genetic findings of probands. (a) Array CGH result showing loss in the 15q21.22 region; (b) Sequencing electrogram showing the c.499C>T (p.Gln167*) variant in proband 2, and (c) Sequencing electrogram showing the position of the c.683_686del (p.Glu228Valfs*78) variant in probands 3 and 4 in the forward and reverse directions.