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# Novel de Novo Nonsense Variants in *AGO3* and *KHSRP*: Insights into Global Developmental Delay and Autism Spectrum Disorders through Whole Genome Analysis

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Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

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**Conflict of interest:** None declared

**Patient:** Female, 6-year-old

**Final Diagnosis:** De novo likely pathogenic variants in *AGO3* and *KHSRP* in association with global developmental delay and autistic features • developmental delay

**Symptoms:** Autistic features • global developmental delay • hypotonia

**Clinical Procedure:** —

**Specialty:** Genetics • Neurology • Pediatrics and Neonatology

**Objective:** Rare disease

**Background:** Neurodevelopmental disorders (NDD) are umbrella disorders that encompass global developmental delay (GDD), intellectual disability, autism spectrum disorders, motor developmental disorders, and sleep disorders. Both GDD and autism spectrum disorder are common and yet clinically and genetically heterogeneous disorders. Despite their high prevalence and the advent of sequencing detection methods, the genomic etiology of GDD and autism spectrum disorder in most patients is largely unknown.

**Case Report:** In this study, we describe a 6-year-old girl with GDD, autistic features, and structural brain abnormalities, including a moderate reduction in periventricular white matter and bilateral optic nerve hypoplasia, Chiari malformation type I with normal myelinization. A comprehensive joint whole-genome analysis (WGS) of the proband and her unaffected parents was performed. The trio-WGS analysis identified novel de novo nonsense variants *AGO3*: c.1324C>T (p.Gln442*) and *KHSRP*: c.1573C>T (p.Gln525*). These variants have not been reported in gnomAD and published literature. *AGO3* and *KHSRP* are not currently associated with a known phenotype in the Online Mendelian Inheritance in Man (OMIM); however, they may be involved in neuronal development.

**Conclusions:** This report highlights the utility of joint WGS analysis in identifying novel de novo genomic alterations in a patient with the spectrum of phenotypes of GDD and neurodevelopmental disorders. The role of these variants and genes in GDD requires further studies.

**Keywords:** Autism Spectrum Disorder • AGO3 Protein, Human • KHSRP Protein, Human • Developmental Disabilities

**Full-text PDF:** https://www.amjcaserep.com/abstract/index/idArt/943641





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## Introduction

Neurodevelopmental disorders (NDD) are complex disorders with a broad spectrum of clinical and genetic heterogeneity. NDD may present with global developmental delay (GDD), intellectual disability, autism spectrum disorders, communication and learning disorders, motor developmental disorders, epilepsy, sleep disorders, and behavioral disorders. NDD affects 1-3% of the population [1], while GDD is a common clinical finding with an estimated prevalence of 3% among preschool children [2]. Without large-scale advanced genome analysis methods, the underlying etiology of these conditions often remains unknown [3].

De novo causative variants have been reported in an estimated 1% of NDD patients [4,5], and an estimated 1 in 200-400 children with DD are reportedly positive for de novo causative variants (DD) [6]. This number is expected to increase as more causative variants are discovered [3,7]. Next-generation sequencing is an effective first-tier test in patients with DD [3,8]. Trio joint WGS is particularly effective in the evaluation of NDD, as it enables comprehensive genomic and phasing analysis. Herein, we present the phenotypic spectrum and joint WGS analysis of a patient with GDD, autistic features, and hypotonia.

## Case Report

A 6-year-old girl and her unaffected mother and father (**Figure 1**) consented to participate in the CROseq genome program at the Department of Pediatrics, University Hospital Centre Zagreb. The CROseq Genome Program is a collaborative research program between Brigham and Women's Hospital (BWH) (Boston, USA) and the Department of Pediatrics, University Hospital Centre Zagreb (Zagreb, Croatia), supported by the Mila Za Sve Foundation (Rijeka, Croatia). Participants were enrolled in the study at the University Hospital Center Zagreb.

The proband presented with GDD, hypotonia, and autistic features. She was the second child from healthy and non-consanguineous parents with South European ancestry. The family history was negative for GDD, hypotonia, and autistic features. She was conceived by in vitro fertilization and born after an uneventful pregnancy, except for weaker fetal movements in comparison to the mother's previous pregnancy. At 2 months old, the patient presented generalized hypotonia and poor head control. Despite her muscle hypotonia, her deep tendon reflexes were normal. She also had right hip dysplasia and left hip subluxation. During infancy, she had feeding difficulties, failure to thrive, absence of a social smile, and poor eye contact, fixation, and tracking of faces and objects.

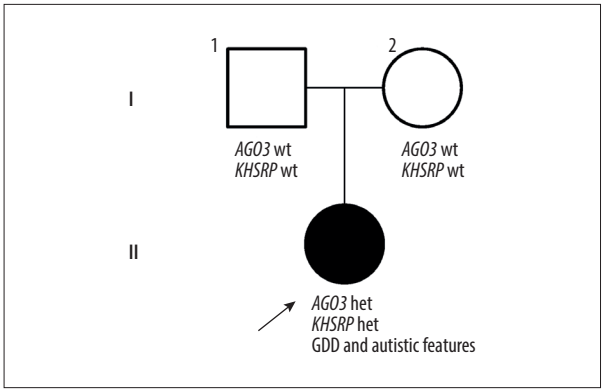


**Figure 1.** The pedigree of a family with the proband affected by global developmental delay and autistic features. Circles and squares represent female and male family members, respectively. The proband is indicated by the arrow. GDD – global developmental delay; het – heterozygous; wt – wild type.

At 6 and 8 months she could hold her head up and roll her head, respectively. She could independently sit at 15 months and walk at 30 months. She had delayed visual maturation and alternating exotropia. At 2 years of age, a visual evoked potential study revealed cortical visual impairment. At 3 years old, a brain MRI scan showed a moderate reduction in periventricular white matter, which was more prominent in the frontal lobe (**Figure 2**). Subsequently, the frontal horns of the lateral ventricles were enlarged. The brain MRI also displayed posterior corpus callosum and bilateral optic nerve hypoplasia. Chiari malformation type I with normal myelinization was also notable in MRI. Later, electroencephalography (Nihon Kohden, Neurofax EEG-1200K; Zagreb, Croatia) was performed to assess the development of brain activity and monitor possible changes. EEG signals were recorded at a sampling rate of 200-10 000 Hz. The patient's forehead was cleaned with alcohol-soaked cotton to keep the impedance of each channel electrode at less than 5 kΩ. The electrodes were placed according to FPz-F9 and FPz-AF7 of the international 10-20 system, encompassing the brain in the same hemisphere. EEG had showed epileptiform activity (**Figure 3**). However, there was no seizure history. Metabolic workup was within normal limits.

At 7 years of age, the patient's weight and height measured at 15 kg (Weight SDS -3.49; Weight for Height SDS -2.25; BMI SDS 12.86, standard deviations according to Centers for Disease Control and Prevention) and 108 cm (Height SDS (CDC) -2.67;). Dysmorphic features were present: high forehead/frontal bossing, broad nasal bridge, epicanthic folds, low-set ears, macrotia, overfolded helix, short nose, and thin upper lip vermilion (**Figure 4**). She had enlarged frontal tubers, epicanthus, and upper lip frenulum (**Figure 4**). She also exhibited moderate neurodevelopmental delay, including cognitive impairment and learning difficulties. Although she could understand simple, familiar,



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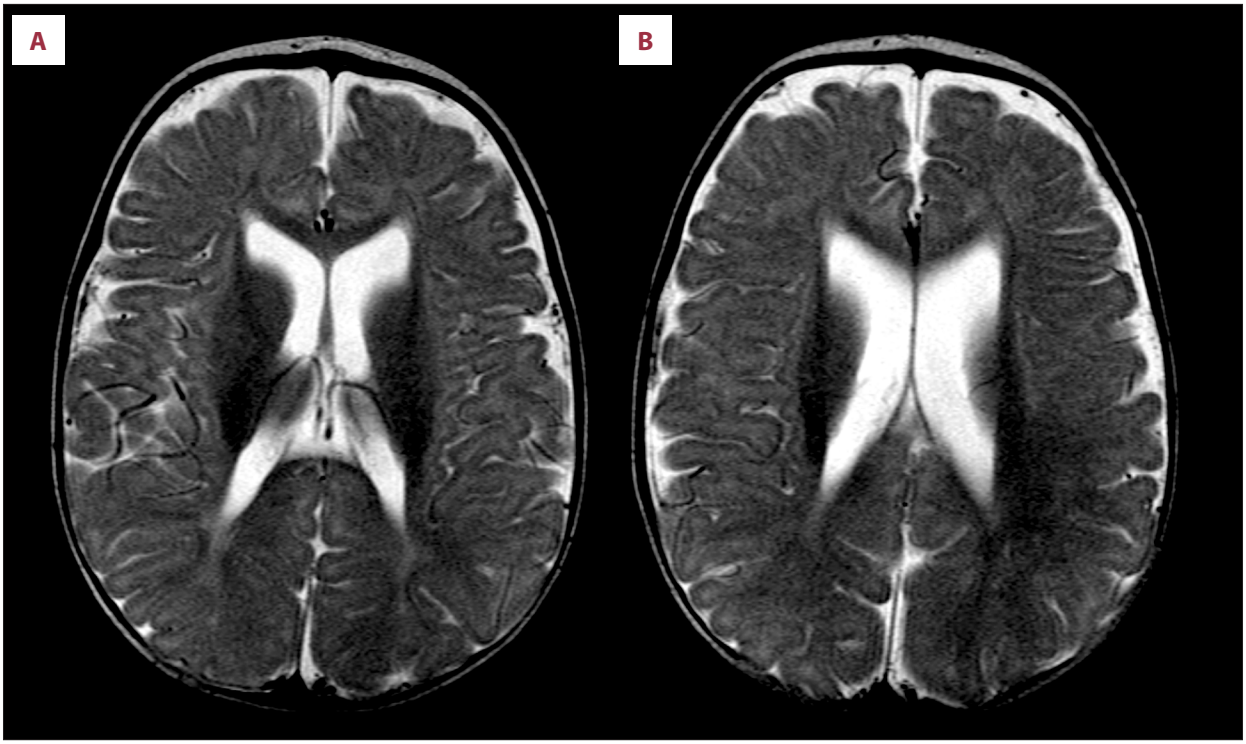


**Figure 2.** Brain MRI of the patient. At 7 months of age, axial T2-weighted images of 2 contiguous slices: (**A**) at the level of the lateral ventricles and basal ganglia and (**B**) immediately superior to that, at the level of corona radiata showed a reduced volume of periventricular white matter, with subsequently slightly *ex vacuo* enlarged lateral ventricles, especially frontal horns. Also, there was unfinished myelination, which was within the normal limits for the chronological age of 7 months at the time of scan.

and situationally supported commands, she had not developed speech or verbal communication by the age of 7 years. She could only communicate by smiling, crying, and gesturing. Her eye contact was poor and short. Visual attention was also easily distracted; however, she was particularly interested in objects that produce sound. She had visual-spatial perception difficulties. She was unable to climb stairs independently and often crawled upstairs. Her fine motor skills were poor, she required feeding assistance, and she did not have control over her sphincter muscles. She had sleep disturbances, including early-morning awakening with difficulty in falling back to sleep.

The karyotype analysis and CMA were normal. Therefore, she was enrolled in the CROseq genome program. The trio-WGS joint analysis was performed at BWH based on the phenotypical features of the proband. The following Human Phenotype Ontology (HPO) terms were used for analysis: generalized hypotonia (HP: 0001290), motor delay (HP: 0001270), delayed speech and language development (HP: 0000750), cognitive impairment (HP: 0100543), global developmental delay (HP: 0001263), autistic behavior (HP: 0000729), EEG with focal epileptiform discharges (HP: 0011185), Arnold-Chiari type I malformation (HP: 0007099), cerebral white matter atrophy (HP: 0012762), and ventriculomegaly (HP: 0002119).

There were no disease-causing variants in the OMIM genes which were associated with these phenotypical findings. However, 2 de novo nonsense heterozygous variants were identified, and Sanger confirmed (**Tables 1, 2; Figure 5**). The variant *AGO3* (NM_024852.4): c.1324C>T (p.Gln442*) is in exon 11, and *KHSRP* (NM_001366299.1): c.1573C>T (p.Gln525*) is located at exon 15. The gene constraint metrics for *AGO3* and *KHSRP* are pLI=1, o/e = 0.15, and pLI=1, o/e=0.17, respectively; therefore, both genes are predicted to be loss of function intolerant. A knock-out mouse study showed that loss of function in the *KHSRP* gene leads to impairment in neuronal development [9]. Therefore, loss of function may be a mechanism of disease for *KHSRP* in the context of the GDD and autistic features. It is not clear whether the *AGO3* variant acts in the loss of function manner for the GDD phenotype. The variants were absent in the gnomAD database. They have not been reported in the ClinVar database or any published literature. Because it is not clear whether loss of function serves as the mechanism of disease associated with these genes, the identified variants are considered as presumably deleterious.





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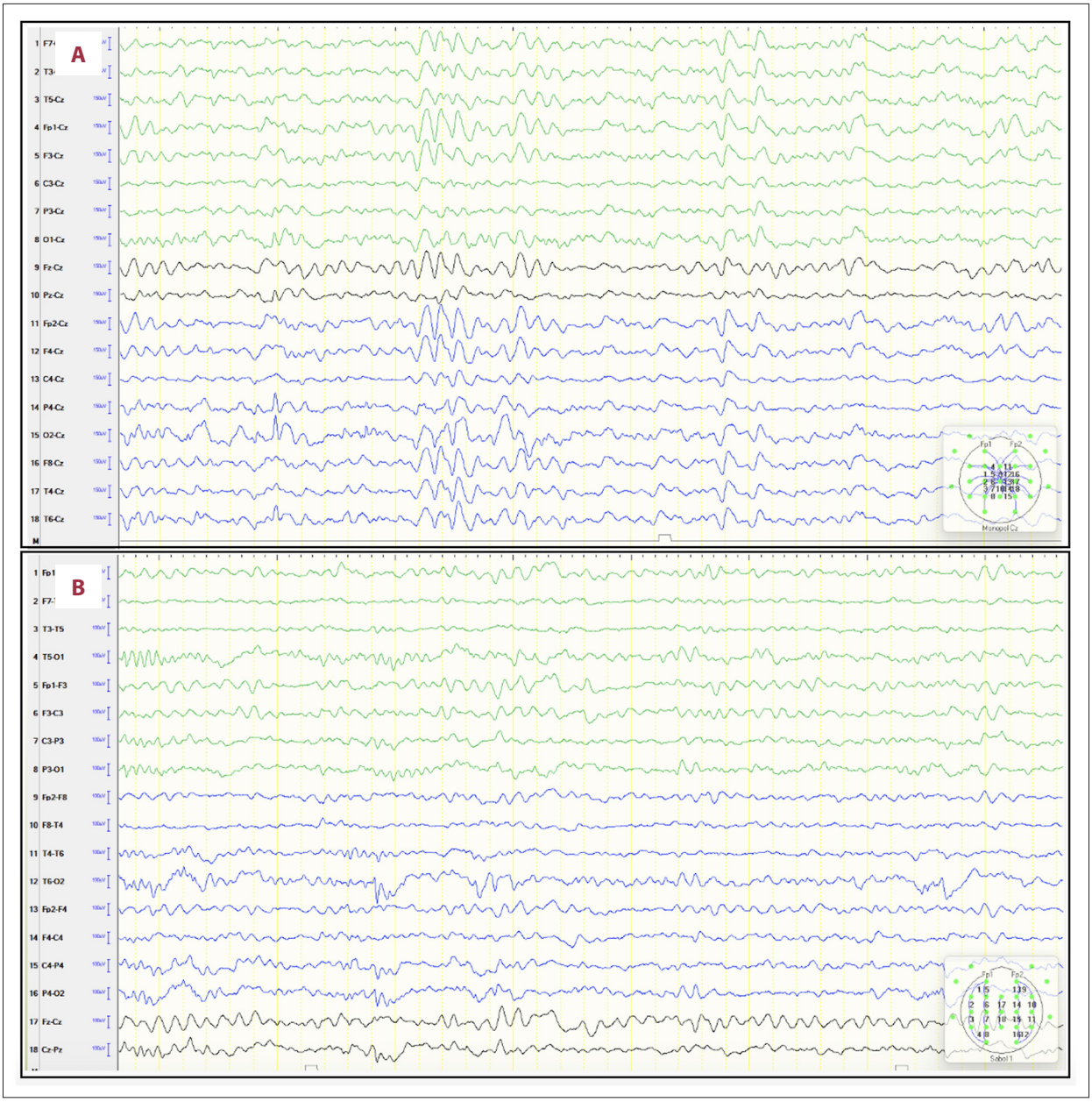


**Figure 3.** EEG of the patient. At the age of 3 years, EEG showed mild right parietooccipital focal epileptogenic discharges. (**A**) Monopolar montage; (**B**) Bipolar longitudinal montage.

## Discussion

Joint WGS assessment is a valuable tool for investigation of complex disorders by allowing comprehensive analysis of whole-genomic data and simultaneous phasing investigation of the genome. In this study, trio-WGS joint analysis identified two de novo nonsense variants in a patient with GDD and hypotonia.

*The* NM_024852.4: c.1324C>T variant is in the *AGO3* gene, located on chromosome 1p34.3, and is involved in miRNA processing [10,11], and negative mRNA regulation via a de-capping mechanism [12]. The encoded protein is part of an RNA-induced silencing complex (RISC) but appears to have no function in mRNA cleavage [10,11]. The *AGO3* gene is currently not known to be associated with a syndrome in OMIM. However, other members of the Argonaute protein family, *AGO1* and *AGO2,* are OMIM genes with phenotypes similar to those in the proband in our study. *AGO1* gene is associated with an autosomal dominant condition called neurodevelopmental disorder with language delay and behavioral abnormalities, with or without seizures (NEDLBAS, MIM # 620292) [13,14]]. It is typically associated with hypotonia, feeding difficulties, and subtle facial





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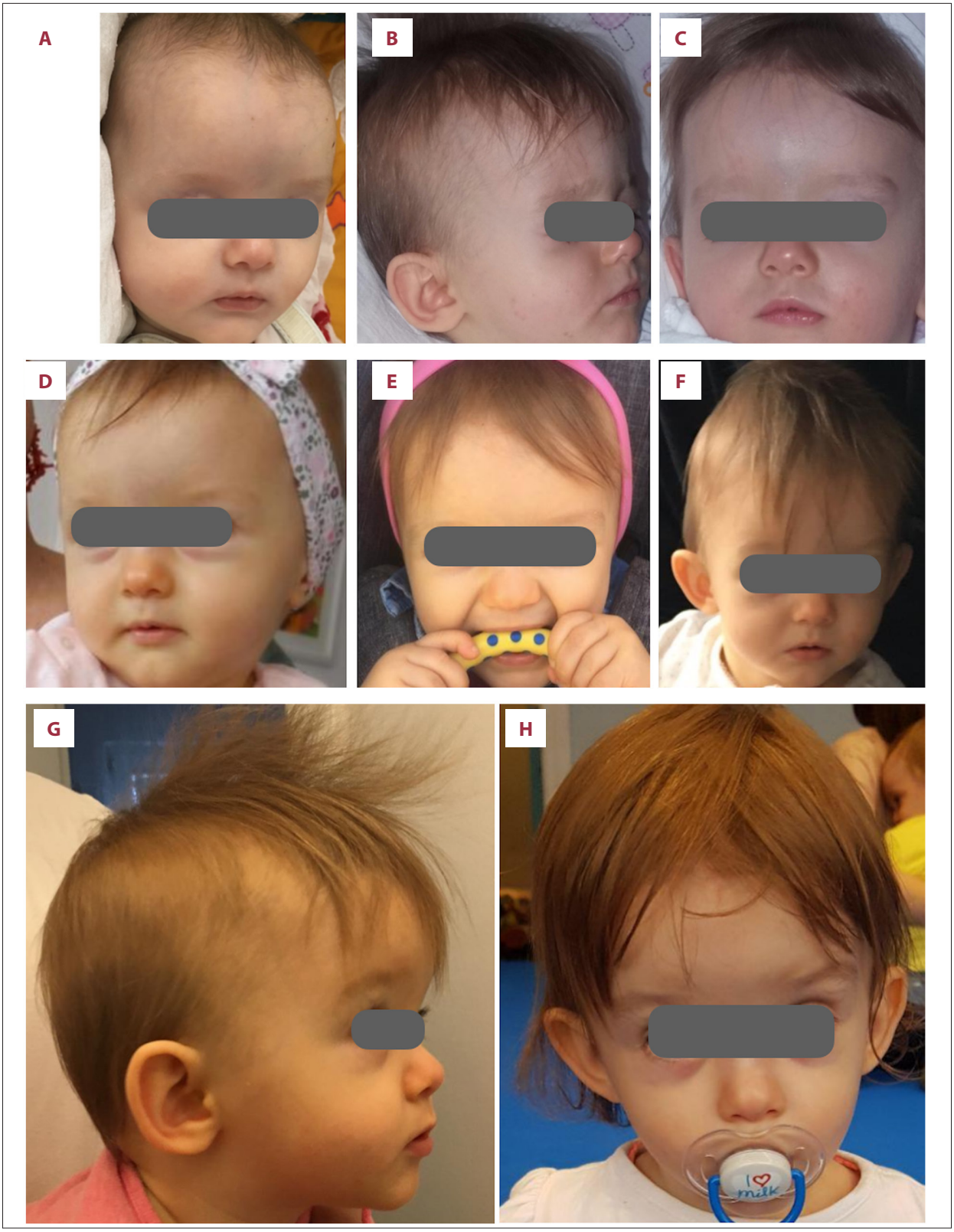


**Figure 4.** Dysmorphic features of the proband. From 0-3 years of age. (**A-C**) dysmorphic features in the first year of life: high forehead/frontal bossing, broad nasal bridge, epicanthic folds, low-set ears, macrotia, overfolded helix, short nose, thin upper lip vermilion; (**D-F**) dysmorphic features during the second year of life; (**G, H**) dysmorphic features during the third year of life.





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**Table 1.** De novo likely pathogenic variants identified in the proband.

| Gene | Transcript | Chromosome | Coordinate | c.DNA | Protein | gnomAD frequency | Classification | ACMG criteria | Variant depth |
|---|---|---|---|---|---|---|---|---|---|
| *AGO3* | NM_024852.4 | 1 | 36479567 | c.1324C>T | p.Gln442* | N/A | Presumably deleterious | PM2, Presumably PVS1 | 21 |
| *KHSRP* | NM_001366299.1 | 19 | 6416334 | c.1573C>T | p.Gln525* | N/A | Presumably deleterious | PM2, Presumably PVS1 | 14 |

**Table 2.** Primer for Sanger sequencing *AGO3* and *KHSRP*.

| Primer sequence | Position |
|---|---|
| Ago3_F: 5'-GAGTATGGGACATGCGAGGG -3' | exon 11 |
| Ago3_R: 5'-TGCTGGAACACAGAAGGTCTTT -3' | exon 13/14 junction |
| Khsrp_F: 5'-ATAAACCAGCAGACGGGAG -3' | exon 14 |
| Khsrp_R: 5'-GGCTGCTGGTAGTAGTGTGA -3' | exon 17 |

dysmorphism. The *AGO2* is associated with autosomal dominant Lessel-Kreienkamp syndrome (LESKRES, MIM#619149) [15], characterized by an NDD, GDD, cognitive deficits, speech and language delay, behavioral problems, hypotonia, gait abnormalities, visual defects, and nonspecific dysmorphic facial features. Both *AGO1*- and *AGO2*- associated phenotypes share similarities with our proband, particularly *AGO2*, which includes visual defects, suggesting possible overlapping features with *AGO3*. We did not observe any phenotype in our *AGO3*-positive patient that was not present on reported *AGO1* and *AGO2*-associated conditions.

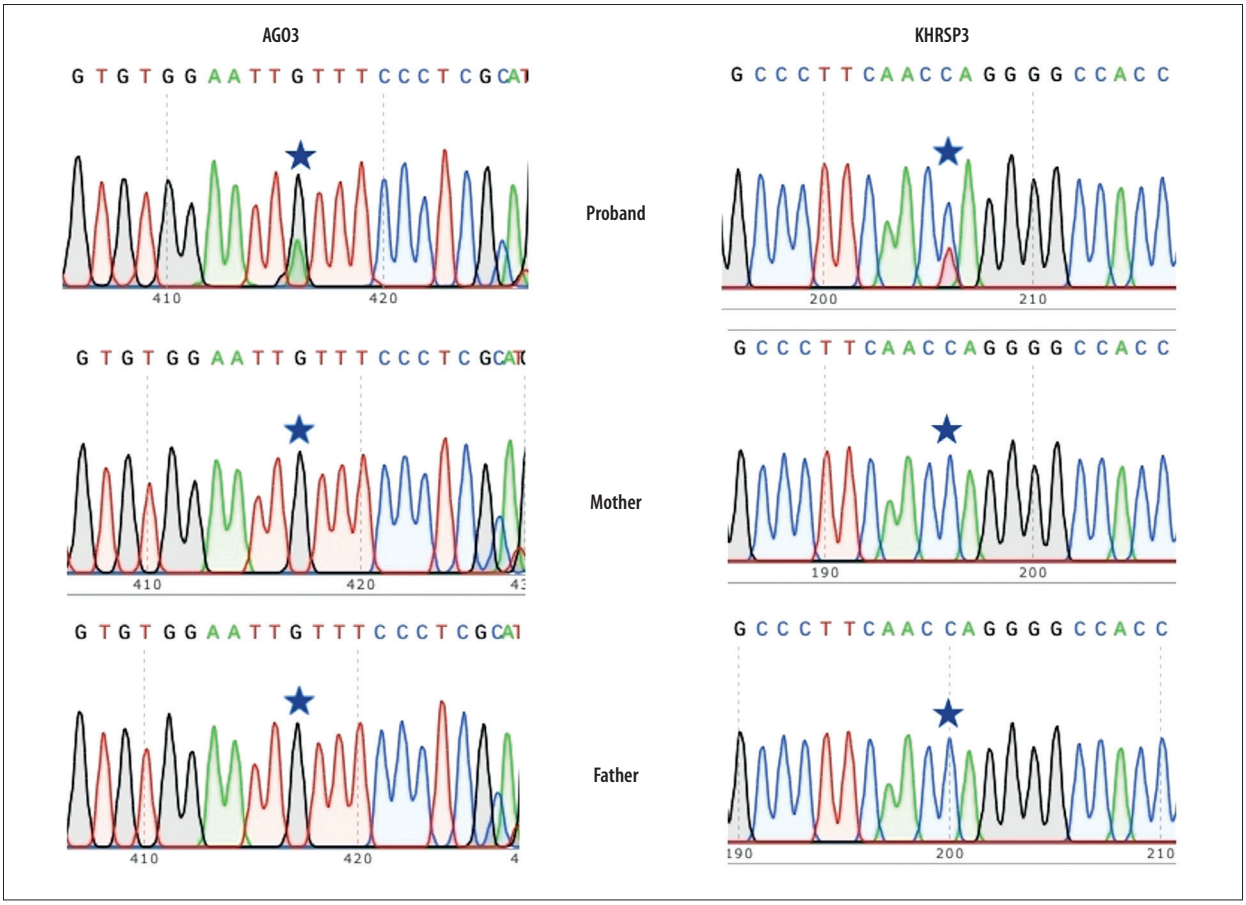


**Figure 5.** Results of Sanger sequencing of *AGO3* (**left**) and *KHSRP* (**right**) cDNA in the proband and her parents. Heterozygote variants are shown on the electropherogram in the proband, and wild-type sequences in her parents. Variant sites are marked with stars.





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A clinical case series described 5 patients with GDD and the potential role of *AGO3* in the neurodevelopmental findings [16]. The patients in that study had a deletion of 1p34.3 region encompassing contiguous *AGO1* and *AGO3* genes. The authors hypothesized that haploinsufficiency of *AGO1* and *AGO3* genes accounted for the neurocognitive phenotypes, including motor and verbal delay, hypotonia, and mild-to-moderate intellectual disability, as well as dysmorphic features [16]. These phenotypic features in that case series were also present in our proband in this study. Furthermore, 2 patients from this case series had cortical brain atrophy, similar to our patient. Joint laxity was another common feature, as our patient had hip dislocation. These observations suggest a potential role of *AGO3* in these common phenotypes. More case collection and experimental studies are required to elucidate the role of *AGO3* in disease.

The *KHSRP*: c.1573C>T is in chromosome 19p13.3. The *KHSRP* gene encodes an RNA-binding protein that promotes the degradation of a subset of mRNAs [17]. This gene binds the G-rich terminal of the precursor miRNA, which leads to increased precursor miRNA cleavage for maturation [18]. The *KHSRP* gene is not yet known to be associated with a Mendelian human disease. Experimental studies have shown that *KHSRP* is expressed in neural tissues, colocalizes with the Dicer complex in axon endings, and impedes axon growth [19]. Loss of *KHSRP* function in mouse knock-out models can increase axon and dendrite growth [9]. Additionally, *KHSRP* reportedly regulates genes involved in autism spectrum disorders, such as *AUTS2* and *GRIN2A* [20], which also exhibit increased dendrite density [21]. Based on the findings of these studies on *KHSRP,* it could be postulated that *KHSRP* haploinsufficiency may play a role in the autistic features observed in the patient in our study. Comprehensive functional studies are needed to investigate the precise role of *KHSRP* in disease.

Several genes and molecular pathways are involved in the pathogenesis of GDD and autism [22,23]. Both *KHSRP* and *AGO3* play a role in miRNA metabolism by interacting with *DROSHA* [18,24,25]. However, their role in GDD etiology, either separately or possibly together, is not known. Experimental studies are needed to investigate the potential role of these genes in neuronal development and disease pathology.

## Conclusions

In summary, a comprehensive trio-WGS analysis of a patient with GDD, hypotonia, and autistic features identified a novel and de novo likely pathogenic nonsense variant in each *AGO3* and *KHSRP*. The exact roles of these genes and variants in disease, both separately and possibly together, are not known. It is also not clear whether other genetics and non-genetic modifiers influence the phenotype. Further experimental studies and larger cohorts with detailed clinical descriptions are needed to delineate the functional role of these variants. Sharing reports of these rare conditions is important to communicate these genomic findings in the literature with a larger community and potentially identify similar cases.

### Ethics Statement

The Institutional Review Board at BWH and University Hospital Centre Zagreb approved this study (Class: 8.1-21/6-2; Reg. No.: 02/21 AG). Written informed consent was obtained from all the participants at the University Hospital Centre Zagreb, Croatia. This study is compliant with the General Data Protection Regulation (GDPR), approved by BWH, Mila Za Sve Foundation, and the University Hospital Centre Zagreb, Croatia.

### Acknowledgments

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### Declaration of Figures' Authenticity

All figures submitted have been created by the authors who confirm that the images are original with no duplication and have not been previously published in whole or in part.

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