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The Angelman Mimic: Navigating Diagnostic Uncertainty in a Genetically Diverse Landscape

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Abstract

Background: Angelman syndrome (AS) is a neurogenetic disorder primarily characterized by developmental delays, intellectual disability, and a distinct behavioral phenotype. While most AS cases are linked to pathogenic variants in the UBE3A gene, AS-like syndromes share overlapping clinical features, complicating diagnosis. This study aims to investigate the clinical and genetic characteristics of AS and expand the clinical phenotype of known neurodevelopmental disorders (NDD) genes presenting as Angelman syndrome clinical mimics.

Methods: We included children diagnosed with AS-like phenotypes and tested genetically for AS. All patients exhibited neurodevelopmental features and a behavioral phenotype marked by a happy disposition. Clinical and genetic data were analyzed using Jamovi-R, with significance considered at $p<0.05$.

Results: A total of 39 patients were classified into three groups according to molecular diagnosis: Angelman (n=22), Angelman-Like (n=8), and genetically unsolved (n=9). No significant differences were found among the groups. Clinical features such as pyramidal findings, ataxia, and speech impairments were more prominent in the Angelman group. Genetic testing revealed genetic alterations in *UBE3A* locus in Angelman patients, while eight Angelman-Like patients exhibited variants in different genes such as *ELP2*, *SLC6A8*, *AP4M1* and *P4HTM*.

Conclusions: This study highlights the diagnostic challenges of AS and AS-like syndromes, underscoring the importance of comprehensive genetic testing in achieving accurate diagnoses.

Key Message

Angelman syndrome (AS) and AS-like syndromes share overlapping clinical features, making accurate diagnosis challenging; comprehensive genetic testing is essential for differentiation.

Novel Contribution: This study systematically compares clinical and genetic profiles of AS and AS-like patients, expanding the clinical phenotype of known NDD genes (*ELP2*, *SLC6A8*, *AP4M1*, *P4HTM*) presenting as Angelman syndrome clinical mimics associated with AS-like phenotypes.

Literature Gap Addressed: While prior studies focused mainly on *UBE3A*-related AS, this research expands understanding by exploring alternative genetic etiologies in AS-like presentations.

Clinical Impact: The findings emphasize the importance of broad molecular screening panels to prevent misdiagnosis and guide personalized management strategies for neurodevelopmental disorders with AS-like features.

Scientific Impact: The study contributes to refining the clinical-genetic spectrum of Angelman and AS-like syndromes, supporting future genotype-phenotype correlation and targeted therapeutic research.

1. Background

Angelman syndrome (AS) is a rare neurogenetic disorder primarily characterized by severe developmental delay, intellectual disability, and a distinct behavioral profile. First described by Dr. Harry Angelman in 1965, AS is notable for hallmark features such as a happy demeanor, frequent inappropriate laughter, ataxia, and profound speech impairment [1]. The majority of AS cases (approximately 85–90%) are linked to dysfunction of the maternally inherited UBE3A gene located on chromosome 15q11-q13, which encodes ubiquitin protein ligase E3A, a critical regulator of protein degradation in neural development [2]. UBE3A expression in neurons is subject to genomic imprinting, where only the maternal allele is active, while the paternal allele is silenced by an antisense transcript known as UBE3A-ATS [3].

The genetic etiology of AS involves four primary mechanisms that disrupt UBE3A functionality. In individuals found to have a methylation profile consistent with AS (abnormal methylation analysis), the condition is associated with three fundamental mechanisms: i) deletion of the maternal 15q11-q13 region (including the maternal UBE3A gene); ii) UPD15 for the paternal chromosome; and iii) an imprinting center defect. The most common cause, responsible for 70–80% of cases, is the deletion of the maternal 15q11-q13 region (including maternal UBE3A gene). Paternal uniparental disomy (UPD), where both copies of chromosome 15 are inherited from the father, occurs in 3–5% of cases and leads to silencing of the maternal UBE3A gene. Imprinting center defects, also seen in 3–5% of cases, result from epigenetic changes that prevent maternal UBE3A allele expression despite its presence [2, 4]. Methylation analysis (MS-MLPA) is crucial for diagnosing AS, as aberrant methylation patterns in the 15q11-q13 region signify the lack of maternal UBE3A expression. A second diagnostic test using UBE3A gene sequence analysis is required when MS-MLPA analysis for Angelman syndrome provides normal results. Pathogenic variants within maternal UBE3A gene (with normal methylation pattern) account for 10–20% of cases, resulting in dysfunctional or absent protein. Additional diagnostic tools include fluorescence in situ hybridization (FISH) and chromosomal microarray analysis (SNP based array) to identify specific genetic mechanisms such as deletions, UPD, or imprinting defects.

In the literature, Angelman-like syndromes—which share clinical features with Angelman syndrome—appear as a broad group of disorders characterized by microdeletions/microduplications and single-gene defects. For instance, Phelan-McDermid Syndrome (Chromosome 22q13 deletions), MBD5 haploinsufficiency syndrome (2q23.1 deletions), and Koolen-de Vries Syndrome (17q21.31 deletions) all exhibit overlapping features such as intellectual disability, hypotonia, and behavioral anomalies [1, 4, 5]. Single-gene disorders, such as Pitt-Hopkins syndrome (TCF4 pathogenic variants) and Christianson syndrome (SLC9A6 pathogenic variants), further expand the spectrum of AS-like presentations. Advances in genomic technologies have identified additional genes, including ASXL3, SYNGAP1, SPTAN1, and SLC6A1, as contributors to neurodevelopmental syndromes with AS-like features [1]. These findings underscore the complexity of diagnosing AS-like syndromes and highlight the burden posed by undiagnosed or misdiagnosed cases, particularly in patients

who lack identifiable UBE3A pathogenic variants. This gap not only delays accurate diagnosis but also hinders the development of targeted interventions and personalized management strategies.

This study aims to examine the clinical and genetic findings of cases diagnosed with AS and AS-like syndromes. Specifically, it seeks to investigate the genotypic spectrum of AS-like phenotypes and elucidate the molecular mechanisms underlying these disorders. Through this effort, the study endeavors to improve diagnostic accuracy, enhance understanding of the biological mechanisms driving these conditions.

2. Methods

2.1. Study Population

The study was approved by the institutional review board of the medical center on March 29, 2023 (**decision number: 2024/010.99/9/23**). All methods were performed in accordance with the relevant guidelines and regulations and the Declaration of Helsinki. This was a retrospective study involving patients who met the inclusion and exclusion criteria. The study cohort consisted of patients presenting to the pediatric neurology clinic with clinical suspicion of Angelman syndrome (AS) who were initially evaluated using the MS-MLPA test. Cases with negative MS-MLPA results were subsequently evaluated using SNP-array and WES analyses. All diagnostic genetic tests had been performed on the patients prior to their inclusion in the study. This study included children who have neurodevelopmental phenotypes suggestive of AS including severe global developmental delay, speech impairment, seizure and a behavioral phenotype that included apparent happy disposition as the most remarkable feature and have been referred to our pediatric neurology clinic to be investigated in terms of AS and completed their whole clinical and genetic investigations. All patients presented neurodevelopmental phenotypes suggestive of AS including severe global developmental delay, speech impairment and a behavioral phenotype that included apparent happy disposition as the most remarkable feature. Exclusion criteria encompassed patients with incomplete clinical and genetic investigations or incomplete records. Patients with structural brain malformations or hypoxic injury identified on cranial MRI, as well as those with a history of extreme prematurity, were also excluded from the study. Written informed consent was obtained from the patients (or the patients' legal guardians) for publication of this study. The research has been conducted in accordance with the Declaration of Helsinki. All methods were carried out in accordance with relevant guidelines and regulations.

2.2. Clinical and Genetic Data

The demographic data (age (months), sex), clinical data (confirmed diagnosis, first seizure age, pyramidal finding ataxia, eye contact, understanding commands, seizure status (without epilepsy, epileptic encephalopathy), stereotype, speaking, anti-seizure drug usage), and genetic data for the included children were from their medical records.

Epilepsy Diagnosis and EEG Recordings

Electrodes were placed according to the international 10–20 system. All patients underwent a 20–40 minute awake-and sleep EEG recording, with activation procedures including hyperventilation, intermittent photic stimulation, and sleep deprivation. In patients who were unable to cooperate with awake recording, sleep-only EEG was performed. Epilepsy was diagnosed based on EEG findings and in accordance with the ILAE 2017 operational definition and 2022 classification framework [6, 7]

Genetic Testing Methodology

Genomic DNA was isolated from peripheral blood of all included patients that were suspected of AS using the QIAamp DNA Blood Mini QIAcube Kit (Qiagen, Hilden, Germany) as per the manufacturer’s instructions. For all patients, Methylation-specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA) assay was performed using SALSA MLPA Probemix ME028 Prader–Willi/Angelman kit (MRC Holland, Amsterdam, the Netherlands) (targeting *OCA2, SNRPN, TUBGCP5, MKRN3, APBA2, GABRB3, ATP10A, MAGEL2, NDN, NIPA1*) with 100 ng of DNA and used for DNA copy number quantification according to the manufacturer’s instructions. Data interpretations were performed using Coffalyser (MRC-Holland) software.

Patients with hypomethylation in MS-MLPA analysis underwent interphase fluorescence in situ hybridization (FISH) using a FISH DNA probe specific for the Prader Willi/Angelman region (D15S63, *SNRPN*/imprinting center) and a control probe for 15q telomere region (154P1/*UBE3A*) (Cytocell Ltd., Oxfordshire, UK) to check for a deletion in the *SNRPN* region.

Chromosomal Microarray Analysis (CMA) was carried out using the CytoScan HT-CMA 96F array SNP-oligonucleotide microarray kit (Thermo Fisher Scientific, Waltham, MA, USA) in accordance with the manufacturer’s protocol for the patients whose results were normal for MS-MLPA and FISH. The kit has a number of 1162042 probes and median intergenic and intragenic distance between probes are 4.1 Kb and 0.95 Kb, respectively. 100 ng of isolated DNA per person was used for the assay and after wet lab, chips were loaded to GeneTitan MC Fast Scan instrument (Thermo Fisher Scientific, Waltham, MA, USA). Then copy number analysis was carried out using Chromosome Analysis Suite Software (ChAS), Reproductive Health Analysis Software (RHAS) and Multi-Sample Viewer (MSV) platforms.

Lastly, all cases remaining undiagnosed were investigated with whole exome sequencing (WES). Genomic DNA was fragmented to 400 bp using QIAseq FX DNA Library Kits (Qiagen, Hilden, Germany). After fragmentation, the libraries were prepared using the QIAseq Human Exome Kit as per the manufacturer's instructions. Paired-end sequencing (150 bp) was performed on the NovaSeq6000 system according to the manufacturer's guidelines (Illumina Inc., San Diego, CA, USA). The sequencing data that was obtained from each patient was visualized through QIAGEN Clinical Insight (QCI) Interpret bioinformatic platform. Found variants evaluated via in silico prediction tools (SIFT, PROVEAN, GERP, CADD, PolyPhen2, and Mutation Taster), allele frequencies in population studies (1000 Genomes, Genome Aggregation Database, and Exome Aggregation Consortium), Franklin Genoox (https://franklin.genoox.com/clinical-db/home), HGMD (https://www.hgmd.cf.ac.uk/ac/index.php), Clinvar (https://www.ncbi.nlm.nih.gov/clinvar/) and

American College of Medical Genetics and Genomics (ACMG) criteria were used [8]. Variants were also confirmed with Sanger sequencing using the ABI 3130XL DNA Sequencer (Thermo Fisher Scientific, Waltham, MA, USA). In order to check the novelty or known pathogenicity of the variants, HGMD (https://www.hgmd.cf.ac.uk/ac/index.php) and/or ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/) databases were utilized. For the pathogenicity assessment of novel variants according to the American College of Medical Genetics and Genomics (ACMG) criteria, Franklin Genoox (https://franklin.genoox.com/clinical-db/home) and QIAGEN Clinical Insight (QCI) Interpret (https://www.qiagen.com/us/products/discovery-and-translational-research/next-generation sequencing/informatics-and-data/interpretation-content-databases/qci-interpret) platforms were used. Strategy that was followed choosing the test is summarized in Fig. 1 as a flowchart.

Data Analysis

The data were analyzed using Jamovi-R (2.3.28) statistical software package [9–11]. Descriptive statistics were presented as counts (n), percentages (%), mean ± standard deviation, median, minimum, maximum, and interquartile range values. The normality of the distribution of numerical variables was assessed using the Shapiro-Wilk normality test. The homogeneity of variances between groups was analyzed using Levene's test. For the comparison of two groups with numerical variables, an independent sample t-test was used if the data were normally distributed; otherwise, the Mann-Whitney U test was applied. For comparisons of numerical variables among more than two groups, the One-Way ANOVA (Welch's) and Kruskal-Wallis tests analysis was employed. For comparisons of categorical variables between groups, chi-square analyses (Pearson chi-square, Yates' chi-square, Fisher's exact test) were utilized. If the results of the chi-square analysis were significant, subgroup analyses were performed using the Bonferroni-corrected two-proportion Z test. The results were evaluated within a 95% confidence interval, with significance considered at $p < 0.05$.

3. Results

3.1. Baseline Characteristics and Comparisons of the Included Patients

This study included 39 patients admitted to our clinic and tested genetically for Angelman syndrome. The patients have been classified into three groups based on the results of their genetic tests (Angelman, Angelman-Like, Genetically unsolved). The mean ages across the groups were similar, with the Angelman group having a mean age of 9.1 months, the Genetically unsolved group 11.8 months, and the Angelman-Like group 7.9 months. The age at first seizure showed more variation, with the Angelman group having a mean age of 29.1 months, the Genetically unsolved group 39.5 months, and the Angelman-Like group 6.7 months. Age at diagnosis was similar across the groups (Table 1). The distribution of sex did not differ significantly across groups ($p = 0.75$), nor did the prevalence of ataxia, which was observed in all patients across groups. Pyramidal findings were more common in the Angelman group (81.8%), but differences between groups did not reach statistical significance ($p = 0.07$). Eye contact and ability to understand commands were also similar across the groups, with no significant differences ($p = 0.72$ and $p = 0.70$, respectively). Regarding seizure status, epilepsy was most common across all groups, but no significant differences were found in the proportions of patients with epilepsy or epileptic encephalopathy. Stereotypic behaviors were more prevalent in the Angelman group (36.4%) compared to the Genetically unsolved group (11.1%), with a significant difference ($p = 0.02$). Speech capabilities (**Being able to say at least three meaningful words)** were lowest in the Angelman group (72.7% non-verbal), though this difference did not reach statistical significance ($p = 0.19$). None of our patients were able to form sentences. Finally, the use of antiseizure medications was variable across groups, with no significant differences in the number of medications used ($p = 0.16$). The detailed display for each variable and the comparisons between the groups are presented in Table 1.

Table 1
Baseline characteristics of the included patients

	Group	n	Mean (SD)	P value
Age$_{(year)}$	Angelman	22	9.1 (5.20)	P = 0.18
	Genetically unsolved	9	11.8 (4.24)	
	Angelman-Like	8	7.9 (4.36)	
First Seizure Age$_{(month)}$	Angelman	22	29.1 (19.81)	P < 0.01
	Genetically unsolved	9	39.5 (26.61)	
	Angelman-Like	8	6.7 (8.08)	
Diagnosis Age$_{(month)}$	Angelman	22	46.7 (34.34)	P = 0.68
	Genetically unsolved	9	46.0 (30.75)	
	Angelman-Like	8	49.4 (58.60)	

Characteristic	**Category**	**ANGELMAN (n, %)**	**Angelman-Like (n, %)**	**Genetically unsolved (n, %)**	**P value**
Sex	Male	10 (45.5%)	3 (37.5%)	5 (55.6%)	0.75
	Female	12 (54.5%)	5 (62.5%)	4 (44.4%)	
Pyramidal Finding	No	4 (18.2%)	4 (50.0%)	5 (55.6%)	0.07
	Yes	18 (81.8%)	4 (50.0%)	4 (44.4%)	
Ataxia	Yes	22 (100.0%)	8 (100.0%)	9 (100.0%)	-
	No	0 (0%)	0 (0%)	0 (0%)	
Eye Contact	No	5 (22.7%)	2 (25.0%)	1 (11.1%)	0.72
	Yes	17 (77.3%)	6 (75.0%)	8 (88.9%)	
Understanding Commands	No	13 (59.1%)	5 (62.5%)	4 (44.4%)	0.70
	Yes	9 (40.9%)	3 (37.5%)	5 (55.6%)	
Seizure Status	Without Epilepsy	6 (27.3%)	1 (12.5%)	2 (22.2%)	0.91
	Epilepsy	10 (45.5%)	5 (62.5%)	5 (55.6%)	
	Epileptic Encephalopathy	6 (27.3%)	2 (25.0%)	2 (22.2%)	
Stereotypies	No	14 (63.6%)	2 (25.0%)	8 (88.9%)	0.02
	Yes	8 (36.4%)	6 (75.0%)	1 (11.1%)	
Speaking : Being able to say at least three meaningful words .	No	16 (72.7%)	8 (100.0%)	8 (88.9%)	0.19
	Yes	6 (27.3%)	0 (0.0%)	1 (11.1%)	
Antiseizure (n)	0	5 (22.7%)	1 (12.5%)	1 (11.1%)	0.16
	1	2 (9.1%)	3 (37.5%)	4 (44.4%)	
	2	9 (40.9%)	2 (25.0%)	0 (0.0%)	
	3	5 (22.7%)	1 (12.5%)	3 (33.3%)	
	4	1 (4.5%)	1 (12.5%)	0 (0.0%)	
	5	0 (0.0%)	0 (0.0%)	1 (11.1%)	

3.2. Genetic Testing Results

Upon testing with MS-MLPA assay, 21 out of 39 patients were seen to have loss of methylation on the 15q11 region and heterozygous copy number loss of the mentioned region was demonstrated with FISH in all patients with loss of methylation. For further investigation, remaining 18 patients were analyzed with a single nucleotide polymorphism array, in one patient, 443,008 base pairs sized copy number loss that includes parts of the TCF4 (exon 6–19) and LINC01929 genes were found. TCF4 gene is inherited autosomal dominant and its pathogenic variants are associated with Pitt-Hopkins syndrome which has many overlapping features with Angelman syndrome [12].

The remaining 17 undiagnosed patients underwent whole-exome sequencing (WES) with copy number variation analysis. WES identified explanatory variants in eight patients across eight different genes. One of these patients harbored a missense variant in *UBE3A* (c.1517G > A, p.Arg506His), the gene responsible

for Angelman syndrome. This variant has been previously reported in ClinVar, is classified as a disease-causing mutation (DM) in HGMD, and meets ACMG criteria for pathogenicity.

In the other seven patients, we detected variants in seven different genes associated with Angelman-like phenotypes (*ELP2, SLC6A8, SLC9A6, P4HTM, ZEB2, AP4M1*, and *CDKL5*). These included three X-linked (one hemizygous, two heterozygous), one autosomal dominant (heterozygous), and three autosomal recessive cases (two homozygous, one compound heterozygous). Across all eight WES-detected variants, we observed two frameshift, two nonsense, one splice-site, and three missense mutations. Four variants were *de novo* (all either nonsense or frameshift variants located in the X-linked and autosomal dominant genes), while the three autosomal recessive variants were inherited from carrier parents. Three of the eight variants are novel. Based on ACMG guidelines and databases (ClinVar, HGMD), three variants were classified as pathogenic and five as likely pathogenic.

Despite these efforts, nine patients remained undiagnosed. In summary, our cohort of 39 patients is divided into three distinct groups: 22 patients with confirmed Angelman syndrome (21 via MS-MLPA/FISH and one via WES), eight patients with Angelman-like phenotypes (One via SNP-array and seven via WES; Table 2), and nine patients whose genetic etiology remains unidentified.

Table 2
List of the genetic findings that were detected via SNP-Array and WES.

Patient	Gene	NM Number	Allelic Status	Nucleotide & Amino Acid Change	Variant type	Pattern of inheritance	Segregation	ACMG Classification & Evidence	ClinVar	HGMD
Patient 1	*AP4M1*	NM_004722.4	Homozygous	c.955T > C p.C319R	Missense	Autosomal recessive	Maternal and paternal	Likely Pathogenic PM3, PP1, PM2, PM5	P(1), LP(1), VUS(1)	DM
Patient 2	*ELP2*	NM_018255.4	Homozygous	c.1385G > A p.R462Q	Missense	Autosomal recessive	Maternal and paternal	Pathogenic PM3, PP1, PS3, PM2, PP3, PM5	P(2), LP(2)	DM
Patient 3	*P4HTM*	NM_177939.3	Compound Heterozygous	c.934G > A p.E312K c.436 + 1G > T N/A	Missense, Splicing	Autosomal recessive	Maternal and Paternal	Likely Pathogenic PM2, PP3, PM3 Likely Pathogenic PVS1, PM2	N/A N/A	DM N/A
Patient 4	*TCF4*	NM_001083962.2	Heterozygous	Exon 6–20 Deletion	N/A	Autosomal dominant	N/A	N/A	N/A	N/A
Patient 5	*UBE3A*	NM_130838.4	Heterozygous	c.1517G > A p.R506H	Missense	Autosomal dominant	Maternal	Pathogenic PS4, PP3, PM2, PM5, PP2	LP(4)	DM
Patient 6	*ZEB2*	NM_001171653.2	Heterozygous	c.1872delC p.I625fs*17	Frameshift	Autosomal dominant	De novo	Pathogenic PS4, PVS1, PM2	P(1)	DM
Patient 7	*SLC6A8*	NM_005629.4	Heterozygous	c.1685G > A p.W562*	Nonsense	X linked	De novo	Pathogenic PM2, PM6, PP5	LP(1)	DM
Patient 8	*SLC9A6*	NM_001042537.1	Hemizygous	c.184C > T p.Gln62*	Nonsense	X linked	De novo	Likely Pathogenic PVS1, PM2	N/A	N/A
Patient 9	*CDKL5*	NM_001323289.2	Heterozygous	c.1016delA p.H339fs*11	Frameshift	X Linked	De novo	Likely Pathogenic PVS1, PM2	N/A	N/A

P: Pathogenic, LP: Likely Pathogenic, DM: Disease causing Mutation, N/A: Not available

4. Discussion

This study investigated the clinical and genetic spectrum of AS and AS-like syndromes in a cohort of 39 patients. While many of the AS cases were confirmed through methylation analysis and subsequent genetic tests, a notable subset of patients with AS-like phenotypes exhibited variants in genes not

typically associated with AS. These findings highlight the diversity and complexity of genetic mechanisms underlying AS-like presentations, emphasizing the need for genetically combined clinical evaluations.

Patients with AS and negative molecular analyses are now being recognized to have a variety of Angelman-like syndromes [13, 14]. While numerous rare chromosomal microdeletions and microduplications are linked to severe developmental disabilities, as observed in AS, certain microdeletions and microduplications lead to specific AS-like presentations [13]. The overlapping clinical phenotypes observed among the diagnostic groups in our study are, in part, an expected consequence of the study design, as the Angelman-like group was defined by its phenotypic resemblance to AS. Nevertheless, the absence of statistically significant differences in clinical features across genetically distinct subgroups carries an important clinical implication: symptomatology alone is insufficient to differentiate true AS from other genetically heterogeneous conditions that mimic it. This finding underscores the diagnostic limitations of a purely clinical approach, particularly in resource-constrained settings where access to comprehensive genetic testing may be limited. Moreover, a subset of patients in our cohort presented with the full AS-like clinical phenotype yet remained genetically unexplained, highlighting that the underlying molecular landscape of this phenotypic spectrum is not yet fully characterized.

The difficulty in distinguishing AS from other conditions such as Angelman-like syndrome based on clinical symptoms alone reinforces the need for genetic confirmation to achieve diagnostic accuracy [15, 16]. For example, ataxia, stereotypic behaviors, speech impairments, and epileptic features are hallmark signs of AS but are not exclusive to it, as evidenced by their presence in Angelman-like cases and even some “Genetically unsolved” cases. This overlap may lead clinicians to prematurely conclude an AS diagnosis, potentially overlooking other genetic or neurodevelopmental conditions that might require distinct management strategies.

Our findings are consistent with prior studies reporting that almost all AS cases are linked to disruptions in the UBE3A gene, with the most common mechanism being deletions in the 15q11-q13 region [17]. This aligns with previous estimates suggesting that 70–80% of AS cases result from such deletions [18, 19]. Additionally, the discovery of variants in genes such as TCF4 (associated with Pitt-Hopkins syndrome) and SLC9A6 (linked to Christianson syndrome) supports earlier literature demonstrating significant phenotypic overlap between AS and other neurodevelopmental syndromes. Notably, the variants identified in ELP2, SLC6A8, AP4M1 and P4HTM genes further expand the genotypic spectrum of AS-like syndromes, underscoring the critical role of WES in uncovering rare and novel genetic variants.

In this study, the genes ELP2, SLC6A8, AP4M1, and P4HTM, in which variants were identified in cases classified under the AS-like syndrome group, are known NDD genes presenting as Angelman syndrome clinical mimics. Additionally, phenotypes previously associated with the AS-like phenotype in the literature were identified in four cases (CDKL5, TCF4, ZEB2, and SLC9A6). The molecular pathways most frequently associated with AS-like phenotypes in literature belong to the chromatin-remodeling disorders and synaptopathies groups [20]. The categorization of genes identified in this study based on their biological functions is summarized in Fig. 2. When classified according to their biological functions, the identified genes can be grouped as follows: transcription-regulating genes (TCF4, ZEB2, ELP2), ion transporters (SLC9A6, SLC6A8), vesicular transport genes (AP4M1), and post-translational modification genes (P4HTM). Among these genes, ZEB2 and ELP2 are classified under the chromatin-remodeling disorders group. Additionally, TCF4, ZEB2, and ELP2 have demonstrated differential gene expression in an RNA-seq study conducted on a CRISPR/Cas9-engineered rat Angelman model, suggesting that these genes may be transcriptionally regulated by UBE3A [21]. Furthermore, P4HTM gene which was AS-like phenotype-associated before plays a role in the post-translational modification process, similar to UBE3A. In light of these findings, it appears that AS-like syndrome phenotypes may share common molecular pathways with AS. Despite the wide genetic heterogeneity reported in AS-like phenotypes, identifying shared molecular pathways holds promise for the development of targeted therapeutic strategies.

Chromosomal microdeletion and microduplication syndromes, such as Phelan-McDermid Syndrome, MBD5 Haploinsufficiency Syndrome, and Koolen-de Vries Syndrome, share some clinical features with AS, including developmental delays, speech impairment, hypotonia, and seizures (see Table 3) [22–24]. However, they also present distinct differences in their phenotypic characteristics and genetic underpinnings. Phelan-McDermid Syndrome, caused by a deletion on chromosome 22q13.3, involves larger physical growth, dysmorphic features, and impaired social interactions, unlike the social engagement seen in AS [22]. MBD5 Haploinsufficiency Syndrome, caused by a deletion on chromosome 2q23.1, features motor delays, autism-like behaviors, and distinct facial dysmorphia not typically seen in AS [23]. Koolen-de Vries Syndrome, resulting from a deletion on chromosome 17q21.31, presents with developmental delays, hypotonia, friendly personality, and macrocephaly, and does not display the severe speech impairment characteristic of AS [24]. These conditions highlight the importance of chromosomal microarray testing for differential diagnosis in patients presenting with AS-like features.

Table 3
Comparison with the other chromosomal microdeletion, microduplication, and single-gene disorders syndromes

Condition	Genetic Cause	Intellectual Disability	Speech	Motor Development	Social Behavior	Seizures	Facial Features	Growth
Chromosomal microdeletion and microduplication syndromes								
Angelman Syndrome	15q11-13 deletion, paternal uniparental disomy or loss of function variations in maternally inherited *UBE3A*	Severe	Minimal to absent	Hypotonia, motor delay, more severe phenotype in deletions	Happy demeanor, inappropriate laughter	65–90%, onset < 3 years	Wide mouth, deep-set eyes, prognathism	Microcep
Phelan-McDermid Syndrome	22q13.3 deletion	Moderate-to-severe	Delayed, eventual loss of expressive language	Neonatal hypotonia, motor delays	Impaired social interactions	Common (including febrile seizures)	Large ears, hands, and dysplastic toenails	Normal o rapid phys growth
MBD5 Haploinsufficiency Syndrome	2q23.1 deletion	Severe	Variable, ranging from non-verbal to short sentences	Motor delay, ataxia in some	Autistic and maladaptive behaviors	Common, but EEG is non-specific	Broad forehead, nasal abnormalities	Microcep in most c
Koolen-de Vries Syndrome	17q21.31 deletion	Mild-to-moderate	Severe delays, but not as profound as AS	Hypotonia, feeding difficulties early	Hypersociability, friendly	Seizures common, EEG not well described	Tall/broad forehead, bulbous nose, joint laxity	Relative macrocep
Pitt–Hopkins Syndrome	*TCF4* complete/partial deletion or loss of function variations	Severe	Minimal to absent	Delayed, later walking age	Happy personality, but self-aggression	40–50%, older onset	Coarse features, full cheeks, malar prominence, abnormal ear helices	Normal to microcep
Single-gene disorders								
Angelman Syndrome	15q11-13 deletion, paternal uniparental disomy or loss of function variations in maternally inherited *UBE3A*	Severe	Minimal to absent	Hypotonia, motor delay, more severe phenotype in deletions	Happy demeanor, inappropriate laughter	65–90%, onset < 3 years	Wide mouth, deep-set eyes, prognathism	Microcep
Pitt–Hopkins Syndrome	*TCF4* complete/partial deletion or loss of function variations	Severe	Minimal to absent	Delayed, later walking age	Happy personality, but self-aggression	40–50%, older onset	Coarse features, full cheeks, malar prominence, abnormal ear helices	Normal to microcep
Christianson Syndrome	*SLC9A6* hemizygous loss of function variants	Severe	Absent	Severe delay, non-ambulatory in teens	Happy disposition, excessive drooling	Almost universal	Gaunt appearance in young adulthood	Postnatal microcep
Mowat–Wilson Syndrome	*ZEB2* haploinsufficiency	Moderate to severe	Minimal, better receptive	Delayed, broad-based gait	Happy affect but less frequent laughter	89%, focal progressing to atypical absence	Hypertelorism, flared eyebrows, uplifted earlobes, overhanging nasal tip	Gradual postnatal microcep
Kleefstra Syndrome	*EHMT1* haploinsufficiency	Moderate to severe	Minimal, better receptive	Hypotonia in childhood, delayed walking	Happy affect but less elation	30–40%	Midface retrusion, prognathism, synophrys, everted lower lip	Normal to delayed growth
MECP2 Duplication	Duplication of *MECP2*	Severe	Minimal	Hypotonia, progressive spasticity	Regressive	Common, intractable	None specific	Normal

Condition	Genetic Cause	Intellectual Disability	Speech	Motor Development	Social Behavior	Seizures	Facial Features	Growth
Chromosomal microdeletion and microduplication syndromes								
MEF2C Syndrome	*MEF2C* haploinsufficiency	Severe	Absent	Wide-based gait	Poor eye contact	Common (myoclonus, spasms)	Strabismus	Normal
ATRX Syndrome	*ATRX* loss of function variants	Variable (mild-severe)	Absent in severe	Hypotonia, drooling	Affable or labile	One-third	Microcephaly, telecanthus, everted vermilion	Normal
STXBP1 Encephalopathy	*STXBP1* loss of function variants	Severe	Absent	Hypotonia, ataxia	None specific	Early onset	None specific	Normal
ELP2-related disorder	*ELP2* bi-allelic loss of function variants	Severe	Minimal to absent	Hypotonia, spasticity, choreoathetosis	Aggressive behavior, autism spectrum disorder	Common	None specific	Short stat
Cerebral creatine deficiency syndrome 1	*SLC6A8* hemizygous loss of function variants	Severe	Severe delay	Hypotonia, motor delay, spasticity, gait abnormality	Impaired social interaction, aggressive behavior	Common	Broad forehead, midface hypoplasia, long face myopathic facies	Microcep failure to thrive
HIDEA syndrome	*P4HTM* bi-allelic loss of function variants	Severe	Minimal or absent	Hypotonia, delayed walking or inability to walk, gait abnormality	None specific	57% to 77%	Coarse facial features	Normal to microcep short stat in some patients
Spastic paraplegia 50, autosomal recessive	*AP4M1* bi-allelic loss of function variants	Severe	Absent	Neonatal hypotonia, later hypertonia, delayed motor development, loss of ambulation, spastic quadriplegia, drooling	None specific	60%	Coarse facial features, bitemporal narrowing, hypotonic face	Microcep
CDKL5 Deficiency Disorder	*CDKL5* loss of function variants	Severe	Absent	Hypotonia, motor dyspraxia, inability to walk	Poor eye contact	Almost universal, infantile onset, refractory	Prominent forehead, anteverted nares	Progressi microcep

Several single-gene disorders also can mimic AS, including Pitt–Hopkins syndrome, Christianson syndrome, Mowat–Wilson syndrome, and Kleefstra syndrome [25–29]. These conditions share overlapping features but have distinct characteristics that differentiate them from AS (see Table 3). For example, unique genetic causes, developmental profiles, seizure patterns, and physical features such as facial dysmorphic findings, social behavior, and other systemic anomalies (e.g., cardiac, gastrointestinal, or ophthalmologic abnormalities) help distinguish these syndromes.

Limitations

There are some notable limitations. A major limitation of this study is the limited sample size, which may reduce statistical power and limit the broader applicability of our conclusions. Although novel variants were identified, functional studies to confirm their pathogenicity were not performed, leaving some uncertainty regarding their clinical significance. Further research with larger cohorts and more advanced genomic techniques (genome sequencing, optical genome mapping, long read sequencing) may provide additional insights into the undiagnosed cases. The relatively small sample size of the study may have limited statistical power, increasing the risk of Type II error in subgroup comparisons

5. Conclusions

This study emphasizes the complexity of diagnosing AS and AS-like conditions, illustrating the overlapping clinical features that challenge symptom-based diagnosis. The findings underscore the importance of genetic testing in achieving accurate diagnoses, particularly in cases with clinical presentations that resemble AS but do not meet the criteria for a diagnosis of classic AS. The identification of variants in *ELP2, SLC6A8, AP4M1 and P4HTM* genes expands the

genetic spectrum of AS-like syndromes and supports the use of WES as a crucial tool in understanding rare and complex neurodevelopmental disorders. Moving forward, the continued exploration of the genetic underpinnings of these syndromes may provide more precise diagnostic markers and potential therapeutic targets for individuals with AS-like phenotypes.

Abbreviations

Angelman Syndrome (AS), Ubiquitin Protein Ligase E3A (*UBE3A*), Whole Exome Sequencing (WES), Fluorescence In Situ Hybridization (FISH), Methylation-Specific Multiplex Ligation-dependent Probe Amplification (MS-MLPA), Chromosomal Microarray Analysis (CMA), Uniparental Disomy (UPD), Single Nucleotide Polymorphism (SNP), Magnetic Resonance Imaging (MRI), Copy Number Variation (CNV), and Disease Causing Mutation (DM). Gene names include Additional Sex Combs Like 3 (*ASXL3*), Synaptic Ras GTPase Activating Protein 1 (*SYNGAP1*), Spectrin Alpha, Non-Erythrocytic 1 (*SPTAN1*), Solute Carrier Family 6 Member 1 (*SLC6A1*), Solute Carrier Family 6 Member 8 (*SLC6A8*), Elongator Acetyltransferase Complex Subunit 2 (*ELP2*), Adaptor Related Protein Complex 4 Subunit Mu 1 (*AP4M1*), and Prolyl 4-Hydroxylase Transmembrane (*P4HTM*). Bioinformatics and laboratory tools and databases include Human Gene Mutation Database (HGMD), American College of Medical Genetics and Genomics (ACMG), Chromosome Analysis Suite (ChAS), Reproductive Health Analysis Software (RHAS), Multi-Sample Viewer (MSV), and QIAGEN Clinical Insight (QCI). Predictive algorithms and scoring tools include Sorting Intolerant From Tolerant (SIFT), Protein Variation Effect Analyzer (PROVEAN), Genomic Evolutionary Rate Profiling (GERP), and Combined Annotation Dependent Depletion (CADD). CRISPR/Cas9 refers to Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated protein 9. QIAGEN is the name of a biotechnology company and not an abbreviation.

Declarations

Data Availability

All variants in this study are deposited in the Clinvar database with accession numbers SCV007108609 -- SCV007108614, SCV007109526 -- SCV007109533. Further data could be provided upon reasonable request.

Ethics approval and consent to participate: Ethics approval and consent to participate: This study was approved by the Clinical Research Ethics Committee of Kartal Dr. Lutfi Kirdar City Hospital (Reference number: 2024/010.99/9/23), and informed consent was obtained from the patients. All methods were performed in accordance with the relevant guidelines and regulations and the Declaration of Helsinki.

Consent for publication: Written informed consent was obtained from the patients (or the patient's legal guardian) for publication of this study.

Data availability Statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest Statement: The authors declare no conflicts of interest in preparing this paper.

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Author Contributions Statement: Safiye Gunes Sager conceptualized and designed the study, supervised the research process, and contributed to data interpretation and manuscript drafting. Oguzhan Demir contributed to the manuscript writing, genetic variant analysis and tables. Merve Akcay was responsible for data collection, and literature review. Ogun Bebek contributed to data collection and writing. Metin Eser assisted in data collection. Ayberk Turkyilmaz contributed to genetic data analysis and manuscript revision. Alper Han Cebi participated in clinical data acquisition and manuscript review. Ali Cansu contributed to patient recruitment, clinical evaluations, and data collection. Omar Alomari participated in all phases of the study, conducted statistical analyses, performed data analysis and interpretation, and contributed significantly to the manuscript's finalization.

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Figures

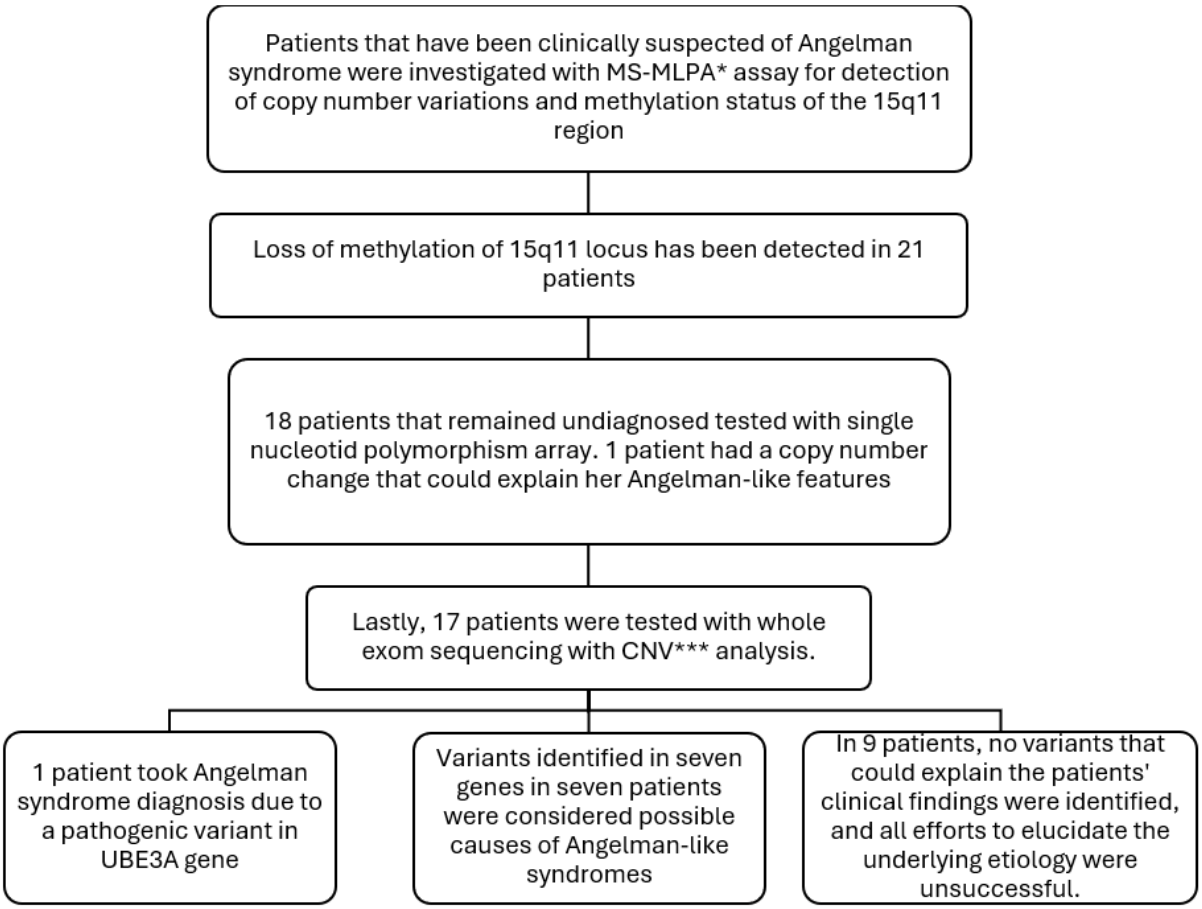


Figure 1

Scheme of diagnostic approach to patients with Angelman-like phenotype *Methylation specific Multiplex Ligation Dependent Probe Amplification **Fluorescence in-situ hybridization ***Copy number variation



Figure 2

Functional categorization of identified genes and their encoded proteins in patients presenting with Angelman-like phenotypes