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AP2M1 Is a Candidate Gene for Microcephaly and Intellectual Disability in 3q27.1 Deletions

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ABSTRACT

Deletions of the 3q26.33q27.2 region appear to correlate with a distinct phenotype, although there are few reported cases. Here, we present seven previously unreported individuals carrying de novo 3q27 deletions (under 5 Mb), which include the *AP2M1* (adaptor-related protein complex 2, mu-1 subunit) gene and summarize data from 12 previously reported cases from the literature. The overall cohort of 19 individuals demonstrates almost universal intrauterine growth restriction, intellectual disability, and post-natal microcephaly, along with common features of hypotonia, post-natal short stature, and facial dysmorphisms. Newly identified features include bicuspid aortic valve, atrial septal defect, congenital malformation of the mesentery, and metopic craniosynostosis, present in a subset of individuals. These seven newly identified individuals allow narrowing of the previously reported smallest region of overlap to 430 kb at 3q27.1. This region includes 20 protein coding genes. We propose *AP2M1* as the most likely contributor to the neurodevelopmental phenotype, based on its predicted intolerance to haploinsufficiency, functional evidence in murine models, and similar phenotypes associated with other adaptor-protein-complex-family members. Furthermore, we report the first individual with a de novo loss-of-function nonsense single nucleotide variant in *AP2M1* with neurodevelopmental features including severe epilepsy. We discuss the implications of this finding in the context of previously reported epileptic encephalopathy in individuals with the recurrent p.Arg170Trp variant in *AP2M1*. In conclusion, our study expands the phenotypic spectrum of 3q27 microdeletions and highlights the potential importance of *AP2M1* in its clinical presentation.

1 | Introduction

Deletions of the 3q26.33q27.2 region are extremely rare, but a shared phenotype is emerging. Consistent phenotypic features previously reported in the literature include intra-uterine growth restriction (IUGR), developmental delay (DD) and/or intellectual disability (ID) and microcephaly. Frequent features include neonatal feeding difficulties, hypotonia, post-natal short stature, dysmorphic facial features, dental anomalies, and thrombocytopenia (Barua et al. 2022; Õunap et al. 2016).

To date, there are 12 reported overlapping deletions of the 3q26.33q27.2 genomic region (Barua et al. 2022; Bouman et al. 2015; Dasouki et al. 2014; Mandrile et al. 2013; Õunap et al. 2016; Robilliard and Caylan 2020; Şahin et al. 2014; Zarate et al. 2013; Tolezano et al. 2024). The deletions range in size from 1.9 to 8.4 Mb, and the smallest region of overlap (SRO) was recently defined as a 1.2 Mb segment, encompassing 46 protein-coding genes (Barua et al. 2022).

A number of genes in this SRO have been proposed to account for specific phenotypic features: *LAMP3* (lysosome-associated membrane protein 3, OMIM 605883) for dental anomalies; *THPO* (thrombopoietin, OMIM 600044) for thrombocytopenia (Dasouki et al. 2014); *PARL* (presenilin-associated rhomboid-like protein, OMIM 607858) for short stature; and *CHRD* (chordin, OMIM 603475) for structural cardiac anomalies (Õunap et al. 2016). Most recently, haploinsufficiency of *DVL3* (disheveled 3, OMIM 601368) has been proposed as a likely contributor to the overall gestalt, based on phenotypic overlap with *DVL3*-related Robinow syndrome (White et al. 2016). The *AP2M1* gene (adaptor-related protein complex 2, mu-1 subunit; OMIM 601024) has also been suggested as a gene of interest (Barua et al. 2022), based on a recurrent gain-of-function missense variant causing epileptic encephalopathy and the prediction that *AP2M1* is highly intolerant to loss-of-function (pLI = 1; LOEUF = 0.19; pHaplo = 0.94).

Our study describes seven previously unreported individuals with overlapping deletions restricted to 3q27.1 including *AP2M1*: two with detailed clinical information (individuals 1 and 2) from a single center, and an additional five cases identified through collaborative efforts, thereby increasing the number of known cases to 19 unrelated individuals. This expanded cohort confirms several previously described features and introduces new phenotypic features. Our data narrows the SRO from 1.2 Mb down to 430 kb, considerably reducing the number of protein-coding genes from 46 to 20. Using multiple lines of evidence, we propose that within this SRO the *AP2M1* gene is the strongest candidate for the neurodevelopmental features associated with this deletion syndrome. We also review the evidence for the contributions of some of the previously proposed gene candidates.

2 | Materials and Methods

2.1 | Case Identification

Individuals 1 and 2 were identified through standard clinical care at the Victorian Clinical Genetics Service, Melbourne, Australia.

We subsequently identified individuals reported in the DECIPHER database (Firth et al. 2009) carrying deletions that included *AP2M1*. We restricted our search to deletions less than 5 Mb (freeze on July 1, 2023) to reduce the phenotypic complexity associated with larger deletions. We also entered *AP2M1* in Matchmaker Exchange via GeneMatcher (Philippakis et al. 2015; Sobreira et al. 2015) seeking to identify any unpublished *AP2M1* single nucleotide variants (SNVs) causing haploinsufficiency. Submitting clinicians were contacted, and clinical data was shared. Informed consent for publication of clinical information, including photographs, was obtained from the parent/guardian of each participant by the local clinicians according to the Declaration of Helsinki and in accordance with our local institutional Research, Governance and Ethics guidelines. Growth parameters were compared to the Centers for Disease Control and Prevention growth charts (Kuczmarski et al. 2002).

2.2 | Molecular Cytogenetic Analysis

Individuals 1 and 2 had saliva DNA samples extracted and analyzed on the lllumina Infinium GSA-24 v1.0 SNP microarray (Illumina Inc., San Diego, CA, USA). All other individuals had CNV data obtained using either single nucleotide polymorphism (SNP) microarray or array-based Comparative Genomic Hybridization (aCGH), based on current practice at their relevant institution (Table S1). All genomic coordinates were converted to the hg38 human genome assembly. The SRO and associated protein coding genes were established by comparing all deletions in the UCSC Browser, using the Reference Sequence (RefSeq) database at the National Center for Biotechnology Information (O'Leary et al. 2016). For individual 20, additional genome analysis was undertaken, as previously described (Wojcik et al. 2024). Briefly, filters were designed to comprehensively evaluate suspected de novo dominant and recessive conditions through the evaluation of variants that segregated appropriately for each inheritance pattern, including rare single nucleotide variants or indels that impact coding regions, essential and extended splice sites, noncoding exons, transcription factor binding sites, or regulatory regions; or large structural variants that were predicted to lead to loss-of-function or impacted introns, UTRs, or promoter regions. Additional overrides were implemented to return variants that have a SpliceAI score > 0.1, and variants below 5% minor allele frequency in gnomAD that are classified as Uncertain Significance, Likely Pathogenic, or Pathogenic in ClinVar. For this family, we also screened all rare variants in a panel of 713 genes previously associated with epilepsy.

3 | Results

3.1 | Individual 1

Individual 1 is a 16-year-old female, the first child of healthy, non-consanguineous Caucasian parents. There is no family history of neurodevelopmental disorders.

The pregnancy was uncomplicated; however, IUGR was noted on the ultrasound scan. She was delivered by spontaneous vaginal delivery at 41 weeks gestation with a birth weight of 2100 g

(1st centile), a length of 46 cm (7th centile), and an occipito-frontal circumference (OFC) of 32 cm (4th centile). Her post-natal growth has followed the 50th centile for height, 15th centile for weight, and 1st centile for OFC.

As a neonate, she was hypotonic with feeding difficulties and failure to thrive. She was admitted to the Special Care Nursery and experienced jaundice requiring phototherapy. She remained physically healthy through infancy and childhood.

She exhibited global developmental delay and hypotonia from infancy. She never crawled but walked independently at 18 months and has ongoing coordination difficulties and fatigues easily; she has difficulties with fine motor activities such as using cutlery and doing zippers or buttons. She had early speech delay and by the age of 16 years speaks in short sentences, often displaying echolalia. She has never had developmental regression nor seizures. She attends a special developmental school and requires assistance with activities of daily living. Her Full-Scale Intelligence Quotient (FSIQ) was 48 when assessed at 12 years, consistent with moderate intellectual disability and she was also diagnosed with autism spectrum disorder.

At 14 years, she developed a severe volvulus, secondary to a small bowel herniation through a large congenital defect of the colonic mesentery, identified at emergency laparotomy. There was no fixation of the caecum, transverse, or descending colon. She required repair of the mesentery and a Brookes ileostomy that was closed uneventfully after 18 months.

She has hypermetropia, and normal hearing. Menarche occurred at 13 years, and she has mild dysmenorrhea with heavy regular periods.

Examination at 14 years identified craniofacial features of broad eyebrows, minor epicanthic folds, a full nasal bridge and fleshy nasal tip, a short philtrum, downturned corners of the mouth, prominent gums, and irregular teeth. She has relatively short fifth fingers. She tends to maintain a slightly hunched posture with lordosis but no scoliosis. There is generalized hypotonia and mild joint hypermobility. There are no focal neurological features.

Fragile X test results were normal. SNP microarray identified two deletions. The first was a 1.0 Mb novel deletion in the long arm of one chromosome 3 (arr[GRCh 37]3q27.1q27.2(183600625_184554595)x1). The second was a recurrent 1.3 Mb deletion of 17p12 (arr[GRCh37] 17p12(14098277_15441486)x1), causative for 17p12-associated hereditary neuropathy with pressure palsies (OMIM #162500). Parental testing confirmed both deletions were de novo.

3.2 | Individual 2

Individual 2 is a two-and-a-half-year-old male. He is the third child for his healthy non-consanguineous Caucasian parents. His father has a history of literacy problems, and his paternal half-brother is reported to have developmental concerns, but further details are unknown. There is a history of learning difficulties in a maternal half-brother, and speech delay in the proband's biological brother.

During the pregnancy, IUGR was identified at 28 weeks leading to frequent monitoring and an induction of labor at 37 weeks, with a subsequent emergency caesarian section due to fetal distress. He was briefly admitted to the Special Care Nursery for observation. Birth parameters were in the low normal range: weight 2410 g (3rd centile), length 47 cm (13th centile), and OFC 31.5 cm (3rd centile). By 10 months of age his OFC was 40.5 cm (<< 0.01 centile). He continued to have poor weight gain and short stature: at 26 months his height was 78.9 cm (< 1st centile) and weight was 10.5 kg (2nd centile).

He was admitted to intensive care with pneumonia at 6 weeks of age, following pertussis infection at 3 weeks. He was treated with intravenous antibiotics and non-invasive ventilation with supplemental oxygen. He continues to experience frequent upper respiratory tract infections and gastroesophageal reflux.

Due to evolving microcephaly and trigonocephaly, a computed tomography (CT) scan was conducted at 12 months which identified metopic craniosynostosis. At 18 months he underwent a bifrontal orbital advancement (BFOA) procedure which was uncomplicated.

He has not had seizures nor developmental regression at the last examination at 26 months. Ophthalmology examination was normal at 20 months and audiology results confirmed middle ear effusions.

Developmentally he demonstrates mild delays, most significantly with language. His first words were at 13 months, with approximately five word-approximations at 22 months; comprehension and capacity to follow single step instructions are variable. He rolled independently at 6 months, crawled at 12 months, and walked independently at 18 months, with physiotherapy intervention from 11 months. His fine motor skills are in the low-normal range.

Examination at 26 months identified minor epicanthic folds, mild hypertelorism, and a full, squared nasal tip. He has fine eyebrows that are medially sparse and slightly flared. He has mild brachydactyly with broad thumbs and great toes (Figure 1). There is generalized mild hypotonia. Cardiovascular, abdominal, and genital examination was normal.

SNP microarray identified a 0.4 Mb novel deletion in the long arm of one chromosome 3 (arr[GRCh37]3q27.1(183696387_184124294)x1). Parental testing revealed this to be a de novo deletion.

3.3 | Additional Individuals

DECIPHER (Firth et al. 2009) contained 10 individuals (accessed April 2023) meeting the inclusion criteria of the current study (Firth et al. 2009). One was our index case (DECIPHER 409458), another (DECIPHER 257773) was previously reported by Mandrile et al. (2013) (patient 1); and another (DECIPHER

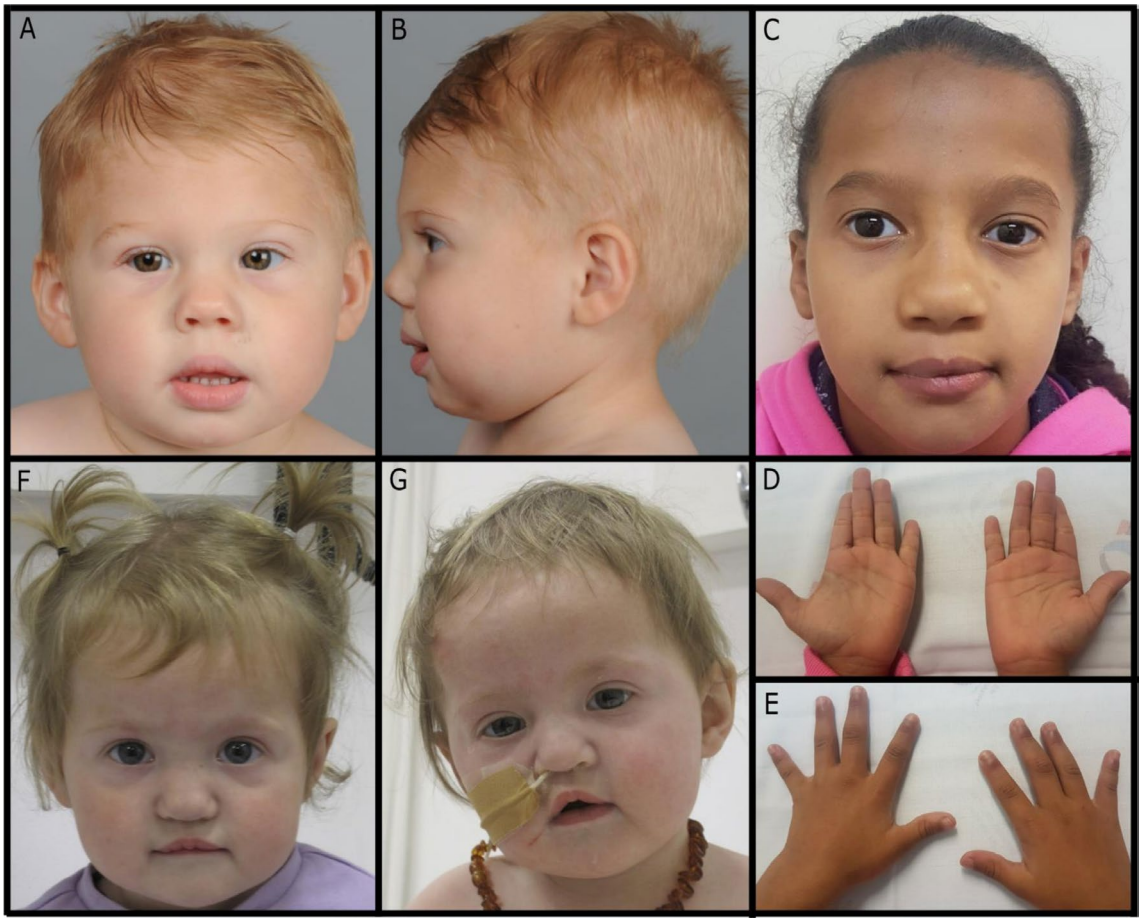

FIGURE 1 | Clinical photographs of individuals with 3q27.1 deletions. Individual 2 is shown in panels (A) and (B), individual 19 is shown in panels (C), (D), and (E), and individual 6 is shown in panels (F) and (G). Shared facial features include: Medially sparse eyebrows (A, C, F), smooth philtrum (A, C, E, G), short philtrum (F, G), full/fleshy nasal tip (A, C, F, G). Mild brachydactyly is evident in individual 19, shown in panels (D) and (E).

421069) was previously reported by Tolezano et al. (2024). Two submitters were unable to be contacted (DECIPHER 317983, 303788). Therefore, we were able to include the remaining five individuals in this study. Individual 7 was a fetal case and therefore phenotypic data is limited. Two loss-of-function sequence variants in *AP2M1* were identified through Matchmaker Exchange (Pais et al. 2022; Philippakis et al. 2015; Sobreira et al. 2015), however only one individual provided consent for inclusion in this publication.

3.4 | Clinical Phenotype

Table 1 describes the phenotypic features of the 19 individuals with deletions: 12 previously reported and seven new cases. The most common traits include IUGR (18/18), postnatal microcephaly (13/13), and DD and/or ID (16/16)—which was mild in six, moderate in four, severe in three, and not specified or not available for the two prenatal cases and one case under 6 months of age.

Although not all cases displayed distinctive facial characteristics, common features include a flat, broad nasal tip and epicanthic folds and less commonly a short philtrum, upturned nares, and broad or medially sparse eyebrows (Figure 1).

Previously described features that are present in the majority of these new cases include postnatal growth restriction (10/14), hypotonia (10/15), neonatal feeding problems (11/14), and dental anomalies (9/10). Thrombocytopenia is described in one new case and three previously reported cases.

Novel phenotypic features include: a congenital defect of the colonic mesentery in individual 1, metopic craniosynostosis in individual 2, bicuspid aortic valve in individuals 4 and 6, aqueduct stenosis and hydrocephalus in individual 7, and an atrial septal defect in individual 19.

Individual 20, identified through Matchmaker Exchange, is a 13-year-old girl with a de novo heterozygous nonsense variant in *AP2M1* (NM_004068.4(*AP2M1*):c.757C>T (p.Arg253Ter)). This variant was the most compelling candidate identified and was submitted to Matchmaker Exchange. All other returned variants either fell within genes with no clear association to the phenotype, had uncertain impact on protein function, or represented "single hits" in recessive genes with no likely disease-causing variants on the opposite allele. Clinical information is limited; however, she had normal height and weight at 13 years and normal head circumference at 10 years (62nd percentile). Her Full Scale IQ is 73, and she has features of attention deficit disorder and autism spectrum disorder. She developed seizures

TABLE 1 | Phenotypic summary of new and previously reported individuals with deletions of the 3q26.33q27.2 region (hg38).

Case	Age (years)	Sex	Del (Mb)	IUGR	Microcephaly	Short stature	Distinctive facial features	Dental anomalies	Cardiac anomalies	Ocular anomalies	Digital anomalies	Scoliosis or kyphosis	Hypotonia	Neonatal feeding problems	Thrombocytopenia	ID/DD
1	15	F	1.0	+	+	−	+	+	−	+	+	+	+	+	−	Mod
2	1	M	0.4	+	+	−	+	NR	−	−	−	−	−	−	−	Mild
3	25	F	2.3	+	+	+	−	−	−	−	+	−	−	−	−	Mild
4	1	M	1.8	+	NR	NR	NR	NR	+	+	+	−	−	+	−	Speech
5	20	F	2.0	+	+	−	NR	−	−	+	+	+	+	+	−	Mild
6	9	F	2.7	+	+	−	+	+	+	+	+	−	+	+	−	Mild
7	Fetal	F	5.0	NR	NR	NR	+	NR	NR	NR	NR	NR	NR	NR	NR	NR
8[a]	6	M	4.1	+	+	+	+	+	+	+	NR	+	+	+	+	Severe
9[a]	17	M	4.3	+	+	+	+	+	−	+	NR	−	+	+	−	Severe
10[a]	12	F	2.1	+	+	+	+	+	−	+	NR	−	+	+	+	Mild
11[b]	16	F	5.0	+	+	+	+	+	−	−	NR	−	+	+	−	Severe
12[c]	10	M	2.0	+	+	+	+	+	−	−	NR	−	+	−	+	+
13[d]	7	F	4.3	+	+	+	+	−	+	−	−	−	−	−	−	Mod
14[e]	Fetal	F	6.2	+	+	+	+	NR	+	NR	NR	NR	NR	NR	NR	NR
15[f]	16	F	8.4	+	+	−	+	+	−	+	NR	+	+	+	−	Mild
16[g]	0.4	M	3.8	+	NR	NR	+	NR	+	+	+	−	−	+	−	NR
17[h]	8	M	4.9	+	+	+	+	+	−	NR	+	−	−	+	−	Mod
18[h]	3	F	2.4	+	+	+	+	−	−	NR	−	−	−	+	−	Mild
19[i]	12	F	3.7	+	+	+	+	−	+	+	+	+	−	+	+	Mod
Total (%)		12F (63)		18/18 (100)	16/16 (100)	11/16 (69)	16/17 (94)	9/14 (64)	7/18 (39)	10/15 (67)	8/11 (73)	5/17 (29)	9/17 (53)	13/17 (76)	4/17 (24)	16/16 (100)

Note: $n = 19$; denominator reflects number of cases with feature recorded.
Abbreviations: + = present; − = not identified; AVG = average; DD = developmental delay; Del = deletion size; ID = intellectual disability; IUGR = in utero growth restriction; Mb = megabases; NR = not recorded or too young to assess.
[a]Mandrile et al. (2013).
[b]Zarate et al. (2013).
[c]Dasouki et al. (2014).
[d]Şahin et al. (2014).
[e]Bouman et al. (2015).
[f]Õunap et al. (2016).
[g]Robilliard and Caylan (2020).
[h]Barua et al. (2022).
[i]Tolezano et al. (2024).

from age 10 years with a clinical diagnosis of Lennox–Gastaut syndrome (seizure types: atypical absence, tonic, atonic, myoclonic, and generalized tonic–clonic) and generalized abnormalities on EEG.

3.5 | Smallest Region of Overlap

Deletions in the seven newly described individuals range in size from 0.43 to 4.96 Mb (Table 1, Table S1, Figure 2), and parental testing demonstrated all of these deletions were de novo. An additional six published cases also had parental testing, all of which also supported de novo status of the deletion (Table S1).

Analysis of these seven new cases and the 12 previously reported cases reveals a SRO of approximately 430 kb, correlating with individual 2's deletion (chr3: 183978599-184406506, hg38) (Figure 2). This segment lies entirely within the 3q27.1 chromosomal band, and includes 20 protein-coding genes: [*ABCC5, HTR3D, HTR3C, HTR3E, EIF2B5, DVL3, AP2M1, ABCF3, VWA5B2, ALG3, EEF1AKMT4, ECE2, CAMK2N2, PSMD2, EIF4G1, FAM131A, CLCN2, POLR2H, THPO, CHRD*].

Applying the classification criteria for Copy Number Variation (CNV) from the American College of Medical Genetics (ACMG) (Riggs et al. 2020) classifies this new SRO as a variant of uncertain significance (VUS), with a score of 0.6 points based on the following:

- 1A: contains protein-coding or other known functionally important elements (0 points);
- 2H: Two or more haploinsufficiency predictors suggest that at least one gene in the interval is haploinsufficient (0.15 points);
- 3A: Contains 0–24 genes (0 points);
- Section 4: Not applicable as there have been no reports associating either the CNV or any genes within the CNV with human phenotypes caused by loss of function (LOF) or copy number loss;
- 5A: Observed copy number loss is de novo (0.45 points).

Previously, the larger SRO was classified by Barua et al. as "pathogenic" due to application of the additional criteria 4C (SRO overlap with four previously reported cases that were assumed de novo, 0.4 points) and 3B (containing 26 protein coding genes, 0.45 points), in addition to 2H and 5A (total 1.1 points).

4 | Discussion

We present clinical and molecular data from seven new cases with 3q27.1 deletions including the *AP2M1* gene, bringing the total number of reported individuals with microdeletions of this region to 19. We confirm near universal phenotypic features of intra-uterine growth retardation, DD and/or ID, post-natal microcephaly, and dysmorphic facial features, and these data support the existence of a recognizable 3q27.1 deletion syndrome. Other frequent features including hypotonia, neonatal feeding problems, dental anomalies, thrombocytopenia, and post-natal short stature are also confirmed. Newly described phenotypic features include bicuspid aortic valve, atrial septal defect, congenital malformation of the mesentery and metopic craniosynostosis.

Our two new individuals (1 and 2) with the smallest deletions have mild and moderate ID respectively, while individuals with larger deletions (8, 9, 11) have severe ID, suggesting that loss of other genes, beyond the SRO, may influence neurodevelopment. However, the largest deletion (individual 15) has mild ID at the age of 16 years, so we cannot draw a direct

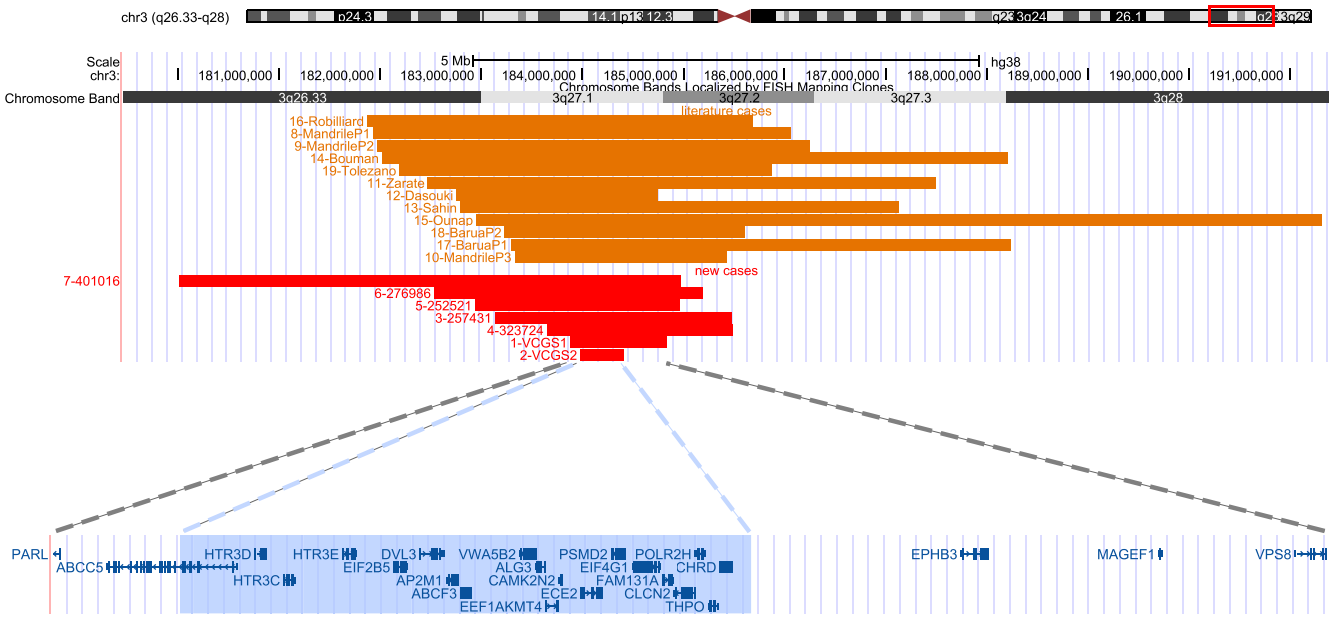

FIGURE 2 | Schematic representation of overlapping 3q26.33q27.2 deletions, and genes involved. Smallest region of overlap (light blue) is 0.43 Mb, encompassing 20 protein-coding genes including *AP2M1*.

correlation between deletion size and level of intellectual impairment.

Considering individual 20, with the p.Arg253Ter variant, phenotypic information is somewhat limited and therefore it is challenging to draw conclusions about similarities or differences with our deletion cohort. However, she does not have microcephaly, and has severe epilepsy, and thus is more similar in fact to the individuals reported with the recurrent missense variant p.Arg170Trp reported by Helbig et al. (2019). Understanding if there are consistent phenotypic differences between individuals with chromosomal deletions involving *AP2M1* (along with additional genes) and *AP2M1* SNVs leading to nonsense mediated decay will require additional individuals with SNVs to be reported.

The new 430 kb SRO (Figure 2) lies entirely within the 3q27.1 chromosomal band, which represents a significant narrowing from the 1.2 Mb SRO previously delimited by Barua et al. (2022). This reduced SRO no longer meets the ACMG CNV criteria (Riggs et al. 2020) for pathogenicity (as compared to the *pathogenic* status of Barua et al.'s SRO), since we cannot apply ACMG criteria 3B or 3C, due to the reduced number of affected protein coding genes. Furthermore, we cannot apply criterion 4, since there are no similar or smaller deletions currently reported in the literature or relevant databases. While the ACMG criteria have been very helpful in providing guidelines for curating CNVs, very small deletions that do not meet pathogenicity criteria can still be disease causing depending on their position and precise gene content. For the reasons outlined, and the phenotypic overlap of our cases, we contend that this new 430 kb SRO is likely pathogenic in nature, even if it fails to meet the ACMG criteria.

Of the 20 protein coding genes within the SRO, only three are predicted to be highly intolerant to loss-of-function (pLI score > 0.90; LOEUF = 0.19; pHaplo = 0.94), highlighting them as plausible candidate genes: *PSMD2* (proteasome 26S subunit, non-ATPase, 2; OMIM 606223), *EIF4G1* (eukaryotic translation initiation factor 4G; OMIM 600495) and *AP2M1*.

PSMD2 is a multi-catalytic proteinase complex involved in the degradation of proteins to maintain cellular homeostasis (Rechsteiner 2013). It has not yet been linked definitively to any human disease. *EIF4G1* is a heterotrimeric protein complex that promotes eukaryotic translation initiation by binding the 5′ cap of messenger RNAs (Huttenlocher et al. 2015). Rare variants within *EIF4G1* have been suggested as contributing to Parkinson disease, possibly due to dominant-negative loss-of-function (Chartier-Harlin et al. 2011). However, evidence is limited and this proposed link has been recently refuted (Saini et al. 2021). While LOF variants in both genes could potentially influence the phenotype of individuals with 3q27.1 deletions, insufficient evidence currently exists to postulate a pathological role for either gene.

In contrast, we propose *AP2M1* as a plausible candidate gene to cause the neurodevelopmental phenotype in this cohort, based on direct and indirect observations. *AP2M1* encodes a hetero-tetramer subunit of the adaptor protein complex 2 (AP-2). This complex controls mammalian clathrin-mediated endocytosis of synaptic vesicles (Kononenko et al. 2014) to regulate excitatory and inhibitory neurotransmission through pre-synaptic vesicle and post-synaptic ion channel mediation (Kittler et al. 2005). It belongs to a family of evolutionarily conserved adaptor protein complexes (Behne et al. 2020) and it is highly expressed in the central nervous system (Carithers et al. 2015).

AP2M1 is predicted to be intolerant to LOF, exhibiting a pLI score of 0.99 (Karczewski et al. 2020). Although potential *AP2M1* SNV/indel LOF variants have been identified, their validity and impact are difficult to verify. Two SNV/indel LOF variants were ascertained via Matchmaker Exchange (Pais et al. 2022; Philippakis et al. 2015; Sobreira et al. 2015), however, only one individual, with limited phenotypic data, provided consent for inclusion in this manuscript. ClinVar (Landrum et al. 2018) contains three predicted loss-of-function variants ((p.Phe265Leufs*11), (p.Val19Glufs*71), (p.Asn9Ilefs*80))—all classified as being of uncertain clinical significance and without any phenotypic information to aid interpretation. Furthermore, since the *AP2M1* gene has not formally been linked to any human phenotype, application of ACMG criteria are limited, and further review of these three variants confirms they remain of uncertain significance. These variants do not assist in advancing understanding at this time due to limited information. GnomAD v4 (Karczewski et al. 2020) includes 12 specific predicted loss-of-function variants (using MANE select transcript ENST00000292807.9) in a total of 67 individuals. However, only two individuals were identified in genome data; the remaining 65 are from earlier exome data. Two variants, identified 17 and 18 times respectively, are located within the same highly repetitive region and may be artefactual. Eight of the twelve variants are predicted to interfere with splice donor sites with unknown consequences, and therefore interpretation of the significance of these is difficult.

Downregulating *Ap2m1* in murine models leads to reduced dendritic branching in developing cortical neurons, which is postulated to hinder developmental morphological changes in neurons (Yasumura et al. 2021), and *Ap2m1* knockout is embryonically lethal in mice (Mitsunari et al. 2005).

The recurrent *AP2M1* gain-of-function missense variant c.508C>T (p.Arg170Trp) causes a developmental and epileptic encephalopathy, emphasizing the important role of this gene in neurodevelopment. Functional work has demonstrated the cause is likely due to an alteration of the AP-2 complex, leading to the impairment of clathrin-mediated endocytosis of transferrin (Helbig et al. 2019). Individual 20, who has a de novo p.Arg253Ter variant that is predicted to undergo nonsense mediated decay, is more phenotypically similar to these cases, in that she developed epilepsy at age 10, requiring a vagal nerve stimulator at 13 years, and she is normocephalic. Severe seizure onset in the four individuals with the p.Arg170Trp variant was prior to the age of 4 years. However, seizures are rare in our deletion cohort, reported in only 2/19 individuals.

Several other members of the adaptor protein complex family, which regulate clathrin-coated vesicle assembly and trafficking of vesicles between organelles, are also associated with neurodevelopmental phenotypes. For example, Pettigrew syndrome is an X-linked recessive condition caused by loss of function in *AP1S2* (adaptor-related protein complex 1, sigma-2 subunit, OMIM #300629) (Cacciagli et al. 2014); biallelic loss of function

of *AP4M1* (adaptor-related protein complex 4, mu-1 subunit, OMIM #602296) is associated with intellectual disability and microcephaly (Najmabadi et al. 2011; Verkerk et al. 2009); MEDNIK syndrome is an autosomal-recessive multisystem intellectual-disability syndrome due to biallelic loss-of-function variants in *AP1S1* (adaptor-related protein complex 1, sigma-1 subunit, OMIM 603531) (Martinelli and Dionisi-Vici 2014) and a similar MEDNIK-like syndrome is associated with *AP1B1* (adaptor-related protein complex 1, beta-1 subunit, OMIM 600157) (Alsaif et al. 2019).

Interestingly, disease causing variants in *AP2M1* appear to operate in a dominant manner, while other adaptor protein complex genes, such as *AP4M1*, *AP1S1* and *AP1B1* follow autosomal recessive inheritance, and *AP1S2* follows X linked recessive inheritance. The disease-causing mechanism for *AP4M1*, *AP1S1*, *AP1B1*, and *AP1S2* is biallelic or hemizygous loss of function, respectively, with heterozygous carriers typically unaffected. However, heterozygous recurrent missense variants at position Arg15 in *AP2S1* cause familial hypocalciuric hypercalcaemia type 3 (FHH3) (Hannan et al. 2015), while five different heterozygous missense variants in *AP2S1* have recently been reported in 26 individuals with neurodevelopmental disorders including epilepsy but no evidence of hypercalcaemia (Stevenson et al. 2024). Functional studies confirmed various impacts including decreased cell viability, reduced clathrin-mediated endocytotic transferrin uptake and disrupting interactions with other AP2 complex subunits. This is analogous to the impairment of clathrin-mediated endocytic transferrin uptake demonstrated by Helbig et al. (2019) in their cohort of individuals with the p.Arg170Trp variant in *AP2M1*.

Clathrin-mediated endocytosis (CME) is a critical pathway involved in the uptake of cell surface receptors and their bound ligands, playing a crucial role in cell–cell signaling, intercellular signaling, and cellular homeostasis (Mettlen et al. 2018). The process is tightly controlled, and there is some redundancy in the system, with many of the AP subunits and their interacting partners having multiple isoforms and many proteins sharing high sequence homology (Skeldal et al. 2023). However, the AP2 subunits have relatively fewer isoforms compared to the AP1 and AP3 subunits; specifically, the medium μ subunit encoded by *AP2M1* is unique (Shin et al. 2021). Furthermore, the AP2 complex is fundamental to the initiation and stabilization of clathrin-coated pits, the key first step in CME (Mettlen et al. 2018).

The precise reason for the different inheritance patterns described remains unclear, but it may relate to the fact that the threshold for loss of function specifically for *AP2M1* is much lower compared to the other genes.

A number of other genes within the previous SRO have been proposed as possibly contributing to specific phenotypic characteristics. Two of these genes now sit outside of the SRO—*LAMP3* and *PARL* (Õunap et al. 2016).

LAMP3 was proposed by Dasouki et al. (2014) as a potential cause for dental anomalies due to its role in amelogenin degradation. Of the nine cases described that have delayed or dysplastic dentition, three (individuals 1, 11, and 18) have deletions that do not include *LAMP3*. This new data suggests that dental anomalies may be due to multiple factors that may or may not include the contribution of *LAMP3*. Of the six remaining cases that do not mention dental anomalies (excluding the fetal and neonatal cases), four include *LAMP3* and two do not (individuals 2 and 4) however, these cases are under the age of 3 years and therefore information regarding dental phenotype is incomplete.

PARL was proposed by Õunap et al. (2016) as a possible cause of IUGR and short stature, since *PARL* knockout mice display growth retardation, cachexia and atrophy of muscle, spleen and thymus in the context of mitochondrial dysfunction (Cipolat et al. 2006). There is no currently known gene-disease association. Of the 19 deletions reported here, only one does not include *PARL*—individual two, who had IUGR and subsequently displays post-natal short stature (< 1st centile). We are not able to confirm or refute the role of *PARL* haploinsufficiency contributing to poor growth. The deletion of *PARL* therefore remains a potential contributor to postnatal short stature in addition to the influence of other genetic and non-genetic factors.

Three remaining candidate genes from previous publications (*CHRD*, *THPO* and *DVL3*) remain within the SRO.

Õunap et al. (2016) proposed haploinsufficiency of *CHRD* as a potential contributor to cardiac anomalies, based on a chordin-null mouse phenotype affecting multiple mid-line structures including the heart, albeit noting complex variability and penetrance based on genetic background. Three further cardiac anomalies in our newly described cases (individuals 4, 6, and 7), including bicuspid aortic valve and atrial septal defect, support the hypothesis that *CHRD* may contribute to cardiac anomalies, albeit with incomplete penetrance.

Dasouki et al. (2014) proposed haploinsufficiency of *THPO* may cause thrombocytopenia, based on a family that displayed mild thrombocytopenia in loss-of-function *THPO* heterozygous family members and aplastic anemia in homozygous members. Conversely, a gain-of-function missense variant has been shown to increase the efficiency of thrombopoietin with subsequent thrombocythemia (Prouzet-Mauléon et al. 2020). One new case (individual seven) with thrombocytopenia in this cohort supports the hypothesis that *THPO* haploinsufficiency may be responsible, albeit with incomplete penetrance or this feature may be unrecognized in other cases.

Finally, *DVL3* was proposed by Barua et al. (2022) as a possible contributor to the overall phenotype based on some of the overlapping features of 3q27.1 deletions with the autosomal dominant type III Robinow syndrome (OMIM #616894). However, the mutational mechanism in *DVL3*-related Robinow syndrome appears to be a dominant negative effect, with reported truncating *DVL3* pathogenic variants escaping nonsense-mediated decay (White et al. 2015). If haploinsufficiency of *DVL3* is in fact responsible for the 3q27.1 deletion phenotype, it would be via an alternative mutational mechanism. Furthermore, the pLI score of 0.39 (Karczewski et al. 2020) suggests that loss of function is tolerated in the healthy population. We agree with the conclusions from Barua et al. that the precise contribution of haploinsufficiency of *DVL3* to the 3q27.1 deletion phenotype remains unclear.

Of the remaining 13 genes contained within the SRO, two have been associated with human disease, both autosomal recessive in nature: *CLCN2* (chloride voltage-gated channel 2; OMIM 600570) and *ALG3* (alpha-1,3-mannosyltranferase; OMIM 60875). *CLCN2* encodes for a transmembrane voltage-gated protein that regulates chloride homeostasis in various cells. It is associated with autosomal recessive leukoencephalopathy with ataxia and familial hyperaldosteronism type II. Its role in autosomal dominant susceptibility to epilepsy remains controversial, it has not been confirmed in more recent studies, and is considered refuted for epilepsy by ClinGen (Niemeyer et al. 2010; Rehm et al. 2015). *ALG3* is involved in mannose metabolism, and it is associated with autosomal recessive congenital disorder of glycosylation, type Id. Both genes have pLI scores of 0.00 (Karczewski et al. 2020) with many loss-of-function heterozygous variants seen in the gnomAD cohort. Thus haploinsufficiency of these two genes is unlikely to be a major contributor to phenotype in this case series.

One limitation of our study is that no individuals in the deletion cohort have undergone genomic sequencing to investigate the possibility of alternative or additional diagnoses. Nevertheless, the phenotypic similarity of these individuals supports the pathogenicity of the shared deleted region.

The significance of isolated *AP2M1* haploinsufficiency due to an SNV is difficult to determine based on currently available evidence. The similarity in epilepsy phenotype between our single case and the four individuals with the p.Arg170Trp variant raises the question of whether neurodevelopmental features and epilepsy severity in individuals with disease-causing *AP2M1* variants occur on a gradient, from early-onset seizures in the dominant negative p.Arg170Trp variant to later-onset epilepsy associated with haploinsufficiency in the p.Arg253Ter variant in individual 20.

5 | Conclusion

Our report confirms a common phenotype for 3q27.1 deletions and introduces newly described features including metopic craniosynostosis, bicuspid aortic valve, atrial septal defect, and congenital malformation of the colonic mesentery. The combined analysis of 12 previously reported and seven newly identified individuals narrows the SRO to 430 kb, reducing the number of protein-coding genes to 20. We propose *AP2M1* as the strongest candidate gene for the neurodevelopmental features of this cohort, including post-natal microcephaly, and add supportive evidence for the contributions of *THPO* and *CHRD* to thrombocytopaenia and congenital heart disease, respectively. The precise contribution of haploinsufficiency of *PARL*, *LAMP3*, and *DVL3* remains uncertain. Identification of more individuals with loss-of-function SNVs in *AP2M1* would allow clarification of the consequences of such variants and improve understanding of the phenotypic impact on neurodevelopment.

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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.