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ORIGINAL ARTICLE

Overlap of polymicrogyria, hydrocephalus, and Joubert syndrome in a family with novel truncating mutations in *ADGRG1/GPR56* and *KIAA0556*

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Abstract

Genetic mutations associated with brain malformations can lead to a spectrum of severity and it is often difficult to determine whether there are additional pathogenic variants contributing to the phenotype. Here, we present a family affected by a severe brain malformation including bilateral polymicrogyria, hydrocephalus, patchy white matter signal changes, and cerebellar and pontine hypoplasia with elongated cerebellar peduncles leading to the molar tooth sign. While the malformation is reminiscent of bilateral frontoparietal polymicrogyria (BFPP), the phenotype is more severe than previously reported and also includes features of Joubert syndrome (JBTS). Via exome sequencing, we identified homozygous truncating mutations in both *ADGRG1/GPR56* and *KIAA0556*, which are known to cause BFPP and mild brain-specific JBTS, respectively. This study shows how two independent mutations can interact leading to complex brain malformations.

Keywords Polymicrogyria · Hydrocephalus · Joubert syndrome · Lissencephaly

Introduction

Brain malformations are often caused by mutations in genes regulating neurogenesis and neuronal and glial differentiation [1] . In many cases, mutations in the same gene can lead to a combination of malformations with variable severities. However, genotype/phenotype correlation can be difficult to determine due to additional environmental or genetic modifiers. The presence of digenetic or oligo-genetic causes has been suggested in ciliopathies, a group of highly heterogeneous disorders affecting the brain, eyes, kidneys, and multiple other systems in different combinations depending on the genes involved [2]. Joubert syndrome (JBTS; MIM 213300) is caused by mutations in more than 30 genes leading to hypoplasia of the cerebellar vermis linked to a characteristic elongation of the cerebellar peduncles on magnetic resonance imaging (MRI), termed molar

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tooth sign, developmental delay, and variable retinal and renal abnormalities [3]. It has been proposed that in cases with recessive mutations in *NPHP1* (MIM 607100), more severe JBTS phenotypes including renal involvement could be caused by epistatic heterozygous variants in other JBTS genes, *NPHP6* (MIM 610142), and *AHI1* (MIM 608894) [4]. While genetic interactions have been found in mouse models where heterozygous loss of *Ahi1* worsens the phenotype of *Nphp1* knockout animals [5], the true impact of oligogenicity has been difficult to measure [6].

Here, we present a family where multiple siblings are affected by severe psychomotor delay, intellectual disability, and seizures, with severe brain malformations. In addition to cerebellar hypoplasia and molar tooth sign, MRI scans show bilateral polymicrogyria (multiple small cortical gyri), diffuse white matter alterations, and enlarged ventricles (hydrocephalus) reminiscent of bilateral frontoparietal polymicrogyria (BFPP, MIM 606854) and cobblestone cortex. BFPP has a distinctive malformation pattern characterized by bilateral polymicrogyria with more pronounced frontal involvement, areas of cortical dysplasia where neurons both under and over migrate, patchy white matter signal changes indicating dysmyelination, and variable hypoplasia of the cerebellar vermis and pons [7, 8]. BFPP is caused by mutations in *ADGRG1/GPR56* (MIM 604110) [7–9], a G protein-coupled receptor which interacts will extracellular matrix proteins and regulates the proliferation of both neuronal progenitors and glial cells [10–12].

However, the presentation in the proband was more severe than previously reported BFPP or JBTS cases. Whole exome sequencing revealed two homozygous truncating variants, one in *ADGRG1/GPR56* and one in *KIAA0556* (MIM 616650), a gene found mutated in a mild form of JBTS restricted to the brain (JBTS26, MIM 616784) [13, 14]. We propose this to be a case where two disorders leading to forebrain and midbrain malformations interact to generate profound disruptions in neurogenesis and gliogenesis.

Patients and methods

Patients

We studied a consanguineous Sudanese family with three children affected by global developmental delay, intellectual disability, and seizures. The cases were examined by the Neuropediatrics team at Soba University Hospital in May 2015 and the Neurogenetics team at the Faculty of Medicine, University of Khartoum in May 2018. The Internal Review Board of the George Washington University and the Ethical Committee of the University of Khartoum Medical Campus approved this study. Written informed consent was obtained from each patient and healthy family member before participation in the study. For participants under the age of 18, their father provided written informed consents.

Whole exome sequencing

We collected saliva samples from the patients, a healthy sibling and their parents using Oragene•DNA collection kit then extracted their DNA following the prepIT-L2P (DNA Genotek, Canada) extraction protocol. DNA from two affected siblings and their parents was sequenced at the Broad Institute Genomic Services (Broad Institute, Cambridge, MA) through their Germline Exome pipeline. Sequencing reads were aligned to a reference genome (UCSC hg19) using Burrows Wheeler Aligner [15]. Aligned reads were sorted and duplicates marked using Picard Tools. The Genome Analysis Toolkit was used to call and recalibrate variants using the best practices protocol for variant analysis [16, 17]. Variants were annotated in Annovar [18] and loaded into an SQL database, where custom queries were used to filter variants shared between the two affected individuals. Variants were filtered for frequency lower than 1% in the total and African population in the Genome Aggregation Database [19] and in the African subgroup of the Greater Middle East Variome Project [20] and for coding changes resulting in missense, truncating, splicing changes, or small deletions and duplications. Candidate variants were then tested for frequency in the sequencing batch to remove sequencing errors and for recessive inheritance in the parental exomes. Regions of homozygosity were determined by analyzing stretches of homozygous variants in the exome data and borders were defined by the appearance of multiple heterozygous variants. Sanger sequencing validation was performed on the affected individuals, parents, and unaffected sibling at Genewiz (South Plainfield, NJ) and sequencing primers are available upon request.

Results

Clinical presentation

The family presented in this report consisted of unaffected consanguineous parents who had four healthy boys, three affected siblings, and one previous miscarriage (Fig.1a). There were no similar cases in the other branches of the family. The proband is a 6-year-old boy with severe global developmental delay, severe intellectual disability, and seizures. He was born after an uneventful pregnancy and cesarean section. He appeared unaffected at birth, but presented with psychomotor developmental delay by 4 months of age. He has never been able to control his head or sit unsupported. Although he smiled at the age of 3 months, he never achieved direct eye contact or showed genuine interest in his surroundings. He reached for objects at the age of 4 months, but his pincer grip

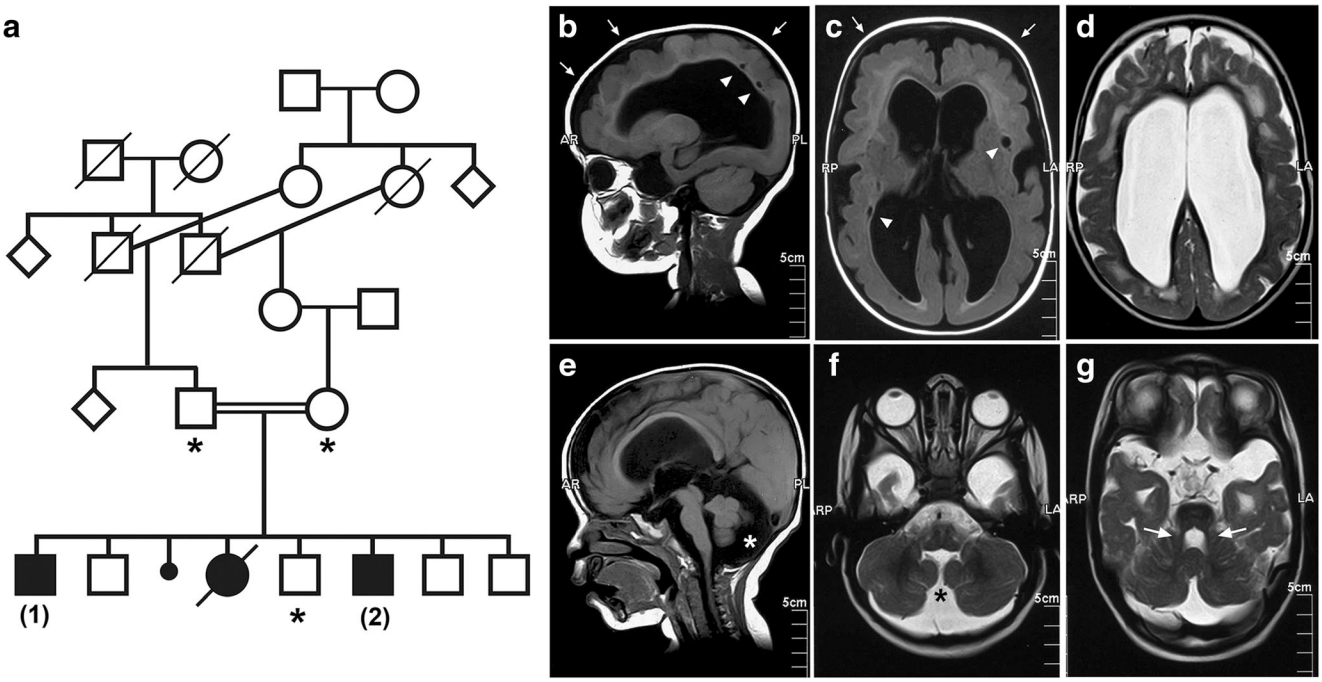

Fig. 1 Complex brain malformation with features of bilateral fontoparietal polymicrogyria (BFPP) and Joubert syndrome (JBTS). **a** Pedigree of the family. **b**, **c** MRI imaging of the proband (case 2 in the pedigree) showing thickened cortical plate characteristic of polymicrogyria (arrows), enlarged ventricles, and multiple subcortical cysts (arrowheads). **d** Patchy white matter signal (high T2 signal) both frontally and posteriorly. **e**–**g** Cerebellar vermis and brainstem hypoplasia are evident with posterior fossa enlargement (asterisk in **e** and **f**). **g** A mild molar tooth sign with elongated cerebellar peduncles highlighted by arrows and an enlargement of the interpeduncular fossa

remains immature to date. He crawled at 8 months of age, yet never walked. At no point of his development he could speak; his hearing appeared normal and his parents stated that he is irritated by loud noises. He developed myoclonic jerks at 4 months which are well controlled with valproic acid.

We examined the proband twice, in 2015 and 2018. During the most recent clinical examination he scored at Level V (most severe) on the Gross Motor Function Classification System (GMFC) scale [21] presenting with generalized poor body tone with brisk deep tendons reflexes. He has neither progressed nor regressed in his abilities with time. Weight and height were below average: weight was 10 kg at initial examination at the age of 2 years 9 months. His orbitofrontal circumference (OFC) was unchanged in the past 3 years and remained at 51 cm at 6 years of age. While there are no OFC charts available for this specific population, these numbers fall in the ~ 30th centile in existing OFC charts [22]. The ophthalmologic examination was normal, and he showed no signs of cerebellar ataxia or extrapyramidal manifestations.

Magnetic resonance imaging (MRI) was performed at 2 months of age showing enlarged lateral ventricles and areas of diffuse bilateral polymicrogyria with more prominent frontal involvement, and areas resembling cobblestone lissencephaly (Fig. 2b, c). Multiple cysts were evident in the white matter. High T2 signal was found in the white matter both frontally and occipitally, showing patchy white matter signal changes (Fig.2d). The corpus callosum was thin with an arched body, and the pons, brain stem, and cerebellar vermis were hypoplastic (Fig. 2e, f). Overall, the findings were consistent with a severe case of blateral frontoparietal polymicrogyria (BFPP). However, elongated cerebellar peduncles and enlarged interpeduncular cistern showed a mild molar tooth sign (Fig.2g), suggesting a possible diagnosis of Joubert syndrome, though no abnormalities were observed in the eyes or kidneys.

Similar to his brother, the second patient (currently 20 years old), was normal at birth apart from an obvious dysconjugate gaze. His initial presentation was with delayed psychomotor development at 4 months of age. He smiled at 3 months, made eye contact at 4 months, and developed head control at 11 months. At 9 months of age, hydrocephalus was suspected and he had surgery for a cerebrospinal shunt. He has severe intellectual disability with limited language. He spoke his first words at 3 years of age but never formed complete sentences. At 3 year old, he also started having generalized tonic-clonic seizures which were controlled with valproic acid.

During the most recent clinical examination, he scored at Level III on the GMFC scale with generalized muscle wasting, upper and lower limb spasticity, and hyperreflexia. Weight was significantly below the 3rd centile with reduced muscle bulk. His motor strength score on the Medical Research Council Manual Muscle Testing scale was 4 in the upper limbs and 2 in the lower limbs and the plantar reflex was down going. He never walked, though, similar to his brother, he could crawl. He had mild back kyphoscoliosis, bilaterally

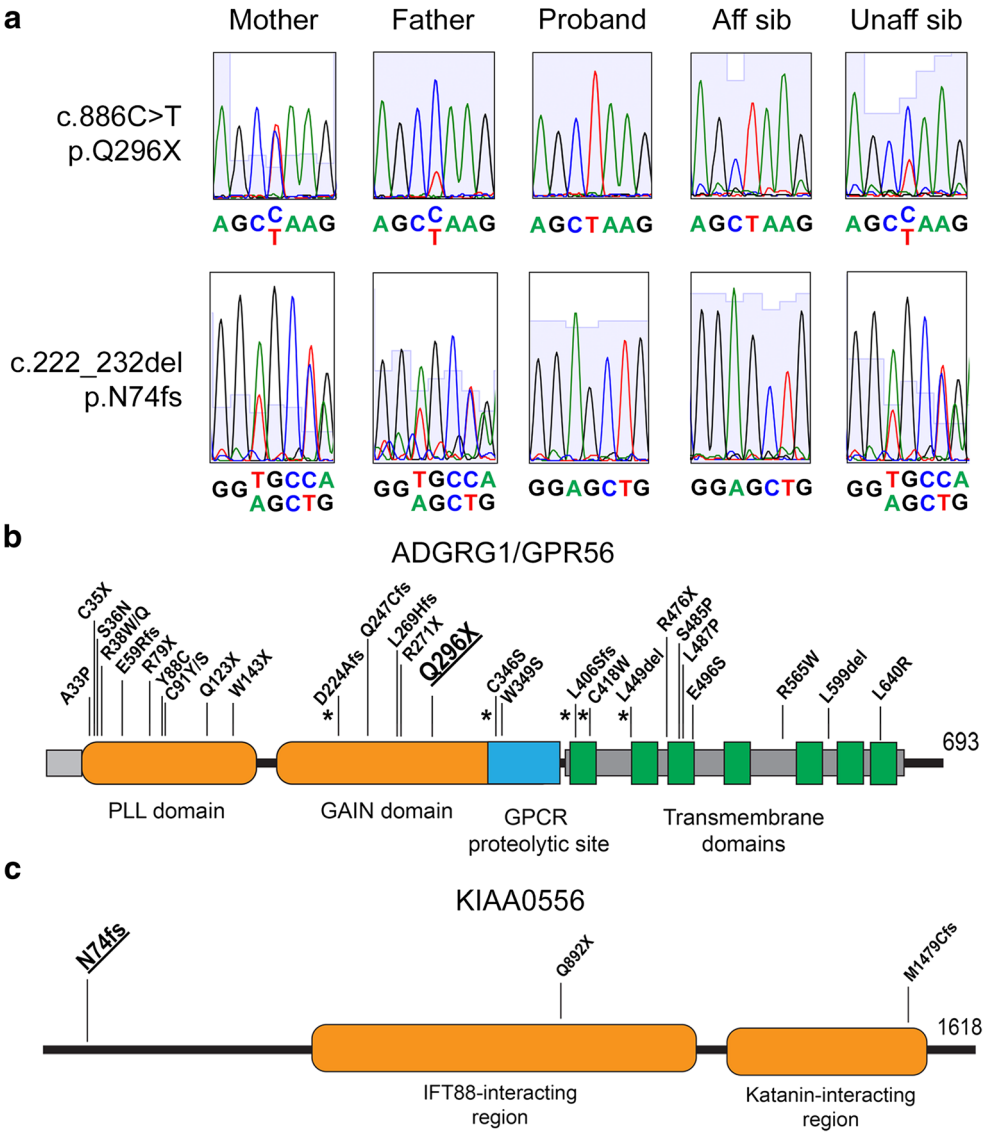

Fig. 2 Two truncating mutations in *ADGRG1/GPR56* and *KIAA0556*. **a** Sanger sequencing confirmation of mutation inheritance in *ADGRG1/GPR56* (top) and *KIAA0556* (bottom) in the parents, affected individuals (Proband and Aff sib), and one unaffected sibling (Unaff sib). **b** Mutations distribution on the ADGRG1/GPR56 protein. Variants reported to lead the most severe presentation are labeled with an asterisk (*). **c** Known mutations in KIAA0556. Newly identified truncating variants in both genes (p.Q296X in ADGRG1 and p.N74fs in KIAA0556) are underlined

adducted hips, fixed flexion deformity, tight Achilles tendon, and mild pes cavus and talipes. The ophthalmologic examination showed left-sided ptosis, left-sided lateral ophthalmoplegia, and bilateral vertical ophthalmoplegia that were more marked on the left eye. His OFC was 52 cm at 17 years and 53 cm at 20 years of age. These numbers fall below the 3rd centile in existing OFC charts [22], suggesting microcephaly. There were no signs of cerebellar ataxia or extrapyramidal manifestations.

The parents also described an affected daughter who died at 12 years of age of unknown causes. Her condition was most similar to the oldest brother, with complete head control by 9 months. She never walked but could stand supported by the bed at 1 year and developed a mature pincer grip at about the same time. She spoke her first words when she was 21 months old, but never progressed to form complete sentences. None of the three affected siblings developed sphincter control, and their mother described a compulsive behavior that they all shared of inserting their index fingers in their lower eyelids. The family has four healthy boys and they have had one previous miscarriage. There are no similar cases in the other branches of the family.

Identification of both *ADGRG1/GPR56* and *KIAA0556* truncating mutations

Whole exome sequencing was performed on both affected brothers and their unaffected parents. Due to the consanguinity in the family, we filtered for homozygous variants that were rare in the African control populations in both the Genome Aggregation Database [19] and in the Greater Middle East Variome Project [20] and that were leading to a protein sequence change (missense, truncating, or splicing variants) in both brothers. Variants were also filtered for autosomal recessive inheritance in the parental exomes. This approach left five homozygous autosomal variants (Table 1). The most severe variants were both in large regions of homozygosity on

chromosome 16 that were defined by stretches of homozygous variants. We identified an 11 bp deletion in *KIAA0556* [NG_046731.1:c.222_232del, p.(Asn74GlufsTer11)] in a 1.5 Mb region (chr16: 26,847,683-28,352,630), and a nonsense variant in *ADGRG1/GPR56* (NG_011643.1:c.886C>T, p.Gln296Ter) in a 15 Mb region (chr16: 46,432,831-61,761,418). *KIAA0556* had previously been found mutated in mild cases of JBTS [13, 14], and mutations in *ADGRG1/GPR56* cause BFPP [9]. Both variants were absent in control databases and dbSNP, and to our knowledge have not been reported before in the literature or in ClinVar. The variants were validated in the affected individuals and the parents and an unaffected brother via Sanger sequencing (Fig. 2a). Both variants lead to early truncations in the respective proteins (Fig.2b, c).

Discussion

We presented a case of severe BFPP caused by a truncating mutation in *ADGRG1* complicated by an additional truncating mutation in *KIAA0556* which has been found mutated in JBTS with short stature. The severe phenotype in the proband can be explained by the additive effect of the two mutations causing a combination of forebrain, midbrain, and hindbrain malformations. Diffuse polymicrogyria and patchy white matter signal typical of BFPP are accompanied by hydrocephalus more severe than in any previously reported cases [7, 8]. Cerebellar vermis, pons, and brain stem hypoplasia can be associated with severe BFPP, but the cerebellar peduncle elongation leading to the molar tooth sign is likely due to the early *KIAA0556* truncation.

KIAA0556 mutations have only been reported in two families affected by mild JBTS [13, 14]. JBTS is often associated to retinal dystrophy, renal cysts, and breathing dysregulation, but none of these findings have been reported in the family we describe or in other cases with *KIAA0556* mutations, suggesting that the effects of loss of this gene are restricted to the brain, though short stature was also reported in one family [13]. While the previously reported cases showed no hydrocephalus, mice where *Kiaa0556* is removed had significant enlargement of the ventricles due to a blockage of the cerebral aqueduct, suggesting that the *KIAA0556* mutation may be responsible for exacerbating hydrocephalus in our cases. However, no cerebellar phenotype was reported in the mouse model [13].

The KIAA0556 protein is not well studied, but initial reports show that it may be involved in microtubule stability and cilia regulation through interaction with intercellular microtubules, microtubule-severing katanin complexes, and potentially other microtubule regulating proteins at the ciliary base [13]. The 11 bp deletion in *KIAA0556* identified in the present study is predicted to impact protein function through either early truncation of the protein (p.Asn74GlufsTer11/p.N74fs)

Table 1 Homozygous variants showing recessive inheritance in the family

Chr	bp	Ref	Alt	Gene	DNA change	Aa change	gnomAD All (%)	gnomAD Afr (%)	GME Afr (%)	CADD	Polyphen	SIFT
1	32042841	T	C	TINAGL1	c.T92C	p.L31P	0.00	0.00	0.00	28.1	1.000	0.058
1	110231873	C	T	GSTM1	c.C286T	p.R96C	0.00	0.00	0.00	23.3	0.997	0.006
16	27640063	CGGTGCCAATT	–	KIAA0556	c.222_232del	p.N74fs	0.00	0.00	0.00			
16	30119704	C	G	GDPD3	c.G571C	p.V191L	0.01	0.00	0.05	0.268	0.000	1.000
16	57689428	C	T	GPR56	c.C886T	p.Q296X	0.00	0.00	0.00	47	–	0.000

Chr, chromosome; *bp*, base pair; *Ref*, reference base; *Alt*, alternative base; *Aa*, aminoacid; *Afr*, African

or complete loss of the transcript from nonsense mediated decay. In either case, the mutation likely produces a null allele as all known functional domains are lost.

ADGRG1/GPR56 function is better understood. It is an adhesion GPCR consisting of a long extracellular N-terminal, a transmembrane C-terminal, and a GPCR proteolysis site that post-translationally cleaves the two terminal ends into two fragments that remain non-covalently attached [23]. The nonsense mutation p.Gln296Ter identified in this study falls just upstream of the proteolysis site (p.Leu382) and the truncated protein product would preserve the large majority of the extracellular N-terminal fragment. The N-terminal fragment attachment to the C-terminal fragment is believed to inhibit the functions of the C-terminal domains, thereby regulating ADGRG1 function [23–25]. The extracellular N-terminal fragment contains ligand binding sites for multiple proteins, including the extracellular matrix (ECM) component collagen III [10] and ligand binding has been hypothesized to separate the two fragments, enabling downstream signaling via a tethered agonist mechanism [24, 26, 27]. Collagen III binding of ADGRG1 activates the RhoA pathway through the seven-transmembrane domain and it is believed to play a critical role in neuronal migration in the cerebral cortex during development, particularly in frontal cortical regions affected in BFPP [10]. Biallelic mutations in COL3A1 lead to brain malformations including polymicrogyria and cobblestone cortex [28, 29], and homozygous knockout mice of *Gpr56* or *Col3a1* display abnormal cortical migration [12, 30]. ADGRG1 also plays a critical role in white matter development. In mice, the N-terminal fragment binds to microglia derived transglutaminase-2 enhancing oligodendrocyte precursor cell proliferation and consequently myelination in the corpus callosum and optic nerve, most likely through activation of the RhoA pathway [11, 31]. From these studies, mouse knockout models of either *Gpr56* or *Tg2* show oligodendrocyte and myelination deficits consistent with white matter defects in human cases of BFPP [11, 32, 33].

We reviewed the existing literature on *ADGRG1* mutations [7–9, 34, 35] to determine whether the *ADGRG1* mutation alone could be responsible for the observed phenotype. ADGRG1 has multiple protein isoforms, most notably a short isoform (S4 isoform) that is missing the entire Pentraxin/Laminin/neurexin/sex-hormone-binding-globulin-Like (PLL) domain which includes the collagen binding site and displays higher basal activity [24, 25]. A cluster of severe mutations appears at the first and second transmembrane domain [8] (Fig.2b), but the variant in our family would truncate all known isoforms. Either an N-terminal fragment of the long isoforms could be generated, or the protein could be lost via nonsense mediated decay of the truncated mRNA. In both cases, the C-terminal fragment functions are eliminated, specifically the activation of the RhoA pathway. However, the p.Gln296Ter mutation still displays a much more severe phenotype of other neighboring truncations (Fig.2b).

The presence of potentially null alleles in the two neurodevelopmental disease genes, *ADGRG1/GPR56* and *KIAA0556* likely has an additive effect towards worsening the presentation of the patients. As outlined above, *ADGRG1/GPR56* plays a critical role in neural and glial cell migration and development particularly in the cortex and posterior fossa. *KIAA0556* is important in microtubule stability and organization particularly in cilia and is needed for development of the posterior fossa and possibly other areas of the central nervous system yet to be elucidated. It is possible that a disruption of *KIAA0556* in a cell population already disrupted by loss of *ADGRG1/GPR56* produces a phenotype summing their effects on development. Such a genetic interaction has been shown in multiple cases of ciliopathy where additional heterozygous mutations or copy number variants have acted as modifiers contributing to a worsening phenotype [4, 5, 36]. A novel heterozygous *ADGRG1* missense mutation has been described as a modifier in a microcephaly patient with loss of *WDR62* and a severe presentation with additional characteristics of BFPP [37]. It remains unclear if the two mutations interact to increase the severity of cerebellar vermis, pons, and brain stem hypoplasia, as these can be severe in BFPP. Further studies in animal models will be able to define how the two genes contribute to cerebellar development.

Overall, this study supports the importance of determining whether the combination of distinct deleterious mutations lead to severe neurodevelopmental conditions with complicated brain malformations. Digenic mutations leading to a presentation with features of multiple disorders have been identified in a few cases [38–40]. However, it is often unclear how these genetic changes interact, in particular, if they are missense variants. Thus, following gene identification of the most likely candidate gene, reporting homozygous, and truncating heterozygous changes in other disease genes could be important to highlight the possible contribution of modifiers leading to future studies.

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