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The phenotypic and genetic spectrum of *AKT3*-related neurodevelopmental condition

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This study was undertaken to expand the phenotypic and genetic spectrum of *AKT3*-related neurodevelopmental disorders and to investigate genotype–phenotype correlations. To date, more than 200 patients with *AKT3*-related disorders have been identified, including those with *AKT3* single nucleotide variants and copy number variations affecting the *AKT3* gene. Adding our three newly diagnosed patients, the total number of patients with *AKT3* single nucleotide variant-related neurodevelopmental disorders is now 61. A total of 20 distinct *AKT3* variants have been identified, with p.E17K and p.R465W being potential mutation "hotspots". Approximately 77% (47/61) of the patients experienced macrocephaly, and 81.9% (50/61) had megalencephaly. Seizures were present in 62.3% (38/61) of individuals, and 29.5% (18/61) of patients displayed a thick corpus callosum. In addition, 57 patients with pathogenic or likely pathogenic *AKT3* duplications and 175 patients with *AKT3* deletions were also reviewed. Among the 68 patients with *AKT3* deletions and detailed information reported previously, 97% (66/68) have microcephaly, 72% (49/68) have agenesis or hypoplasia of the corpus callosum, and 63.2% (43/68) suffer from epilepsy. In the 5 patients with pure *AKT3* deletion, 100% have microcephaly, while none suffer from epilepsy or abnormal corpus callosum. Patients with *AKT3* gain-of-function variants typically present with megalencephaly and structural brain abnormalities. In contrast, *AKT3* loss-of-function variants may have a stronger correlation with microcephaly.

Keywords *AKT3*, 1q43q44 duplication, 1q43q44 deletion, Neurodevelopmental disorder

The phosphoinositide 3-kinase (PI3K)-Akt-mammalian target of rapamycin (mTOR) pathway (PI3K-Akt-mTOR pathway), vital for intracellular signaling, is associated with various cellular functions[1,2]. Mutations in multiple genes within the PI3K-AKT-mTOR pathway are widely recognized as leading factors of brain overgrowth (megalencephaly) as well as segmental cortical dysplasia, including hemimegalencephaly, focal cortical dysplasia and polymicrogyria[3–6]. The serine/threonine kinase AKT, also known as PKB, plays a central role in this pathway by regulating several downstream pathways. *AKT3* is one of the three closely related serine/threonine-protein kinases (AKT1, AKT2 and AKT3), collectively referred to as the AKT kinase, which regulate many processes[7,8]. The *AKT3* gene, located on human chromosome 1 (q43-44), plays an important role in brain development. *AKT3* protein is broadly expressed across tissues, and highly expressed in brain, endocrine tissues, respiratory system, female reproductive system, intestine, and more. *AKT3* is the most abundant *AKT* homolog found in the brain during CNS neurogenesis and is highly expressed in neuronal cells[9–11].

Mutations in the *AKT3* gene have been found to underlie autosomal dominant megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome 2 (MPPH-2) (OMIM #611,223). With the expansion of massive parallel sequencing, an increasing number of variants in the AKT3 gene have been reported. Typical clinical symptoms include macrocephaly, vascular malformations, polydactyly, dermal abnormalities, and a range of neurological symptoms such as intellectual disability, hydrocephalus, hemimegalencephaly, polymicrogyria, and seizures[12,13]. Patients with a 1q43q44 duplication that contains the *AKT3* gene often exhibit symptoms similar to those of patients with *AKT3* gene variants, such as macrocephaly, developmental delay, and seizures[14,15]. Additionally, patients with a distal 1q43q44 deletion may also experience neurodevelopmental disorders[16,17]. In this study, we aim to provide a comprehensive overview of the phenotypic and genotypic spectrum of *AKT3*-related neurodevelopmental disorders, including three previously unreported cases, as well as all published cases of *AKT3*-related neurodevelopmental disorders and those uploaded to the ClinVar and DECIPHER databases. To our knowledge, this study is the first to comprehensively describe the clinical and genetic characteristics of

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patients with *AKT3* single nucleotide variants and copy number variants. It may provide guidance for future clinical practice by offering a comprehensive overview of *AKT3*-related neurodevelopmental conditions.

Results

Identification of *AKT3* variants in three patients

In this study, we identified three patients with *de* novo *AKT3* gene constitutional variant, including *AKT3* p.R465W, p.V268A, p.W79C. All the three patients exhibited macrocephaly and megalencephaly (MEG). Vascular abnormalities, connective tissue dysplasia (CONN), and polydactyly were not observed in any of the three patients. Patient 1 with *AKT3* p.R465W variant suffered from drug-refractory epilepsy. Seizures first occurred 9 days after birth, initially presenting as focal seizures, while infantile spasms occurred at 4 months of age. Electroencephalogram (EEG) shows diffused fast waves and slow wave in left temporal region, along with epileptic spasms. Patient 3 with *AKT3* p.W79C also experienced epileptic spasms, which began at 10 months old. Both patient 1 and patient 3 experienced severe developmental delays. Patient 2 with the p.V268A variant didn't have epilepsy and exhibited mild hypoplasia. Her development continues to progress. Currently, at 6 years old, she is able to attend school, sing children's songs, and climb stairs, although there is slight coordination impairment. The molecular and clinical data of our 3 patients are summarized in Table 1. All three *AKT3* variants reported in this series were identified through clinical whole exome sequencing (WES) on the proband, followed by targeted parental variant analysis using Sanger sequencing. These three different variants have been reported previously. The variants were detected in peripheral blood-derived DNA and were confirmed to be de novo. Detailed clinical data, EEG results, and neuroimaging features of the three patients are provided in Supplementary Information 1.

Probands	Patient 1	Patient 2	Patient 3
Gender	Male	Female	Female
Age last assessed (years)	4y	6y	1y4m
cDNA change	c.1393C > T (NM_005465)	c.803 T > C (NM_005465)	c.237G > T (NM_005465)
Amino acid change	p.Arg465Trp	p.Val268Ala	p.Trp79Cys
Inheritance	De novo	De novo	De novo
Mutation type	Constitutional	Constitutional	Constitutional
ACMG category	P	LP	LP
Diagnosis	MEG	MEG/PMG	MEG/PMG
Birth OFC-SD	40 cm > 3SD	41 cm > 3SD	40 cm > 3SD
Last OFC (age)	54 cm (4y)	56 cm (5y)	51 cm (1y4m)
Developmental milestones	Delay	Slightly delay	Delay
Epilepsy	+	-	+
Seizure onset age	9d	-	10 m
Seizure type	Focal seizure; Epileptic spasm	-	Epileptic spasm
Drug refractory epilepsy	+	-	+
MEG	+	+	+
PMG	-	+	+
PNH	-	-	-
VMEG/HYD	-	-	-
CC	Normal	Normal	Thick
EEG	Diffused fast waves; slow wave in left temporal region; Epileptic spasm	Normal	Diffused fast waves, significant in the right temporal region; Epileptic spasm
Tone	Normal	Hypotonia	High muscle tone in the left limb
Vascular abnormalities	-	-	-
CONN	-	-	-
POLY (H/F)	-	-	-
Other features	Multiple irregular café-au-lait spots on the skin; Through palm	Café-au-lait spots on right hip	Hearing impairment

Table 1. Clinical and molecular findings of patients with *AKT3* gene mutation in this study. Abbreviations: *OFC* occipitofrontal circumference, *MEG* megalencephaly, *PMG* polymicrogyria, *PNH* periventricular nodular heterotopia, *VMEG/HYD* ventriculomegaly or hydrocephalus, *CC* callosal abnormalities, *EEG* electroencephalogram, *CONN* connective tissue dysplasia, *POLY (H/F)*, polydactyly (hand/foot).

Phenotypic and genetics spectrum of patients with *AKT3* single nucleotide variants

Along with our three patients, a total of 61 individuals with *AKT3*-related neurodevelopmental disorders have been reported[3–5,13,18–39]. However, one patient was reported without available mutation site information. Patients' clinical features are summarized in Table 2, and detailed information of patients enrolled is shown in Supplementary Table1. Among the 61 patients, approximately 77% (47/61) of the patients experienced macrocephaly, with about 13 patients without head circumference information. Seizures were present in 62.3% (38/61) of individuals, with at least 55.3% (21/38) of the patients suffering from refractory seizures. Among the patients with seizure, 63.2% (24/38) developed seizures within the first year of life, and 19 patients experiencing seizures within the first month of life. Focal seizure (FS) (15/38), epileptic spasms (9/38) and generalized tonic–clonic seizure (GTCS) (4/38) are common seizure types among these patients. Epileptic spasms mainly occurred in patients with the somatic variant of p.E17K (4/9), as well as in patients with the germline variant of p.F54Y (1/9), p.V183D (1/9), p.W79C (1/9) and p.R465W (2/9). 62.3% (38/61) of these patients experienced varying degrees of development delay or intellectual disability (DD/ID). Patients with *AKT3* germinal variants may experience more serious development problems, especially those with the p.R465W variant (12/16). Additionally, 29.5% (18/61) of patients with *AKT3* variants had vascular abnormalities, 21.3% (13/61) exhibited connective tissue dysplasia, and 11.5% (7/61) presented with hypoglycemia. Since some patients lack detailed information, the actual percentage is likely higher. The physical examination revealed a higher proportion of hypotonia in patients with germline variants (23/42) compared to those with somatic variants (5/19).

Patients with *AKT3* single nucleotide variants exhibit typical brain magnetic resonance imaging (MRI) abnormalities. Among these patients, 81.9% (50/61) had megalencephaly (MEG), 50.8% (31/61) had polymicrogyria (PMG), 13.1% (18/61) had hydrocephalus or hemimegalencephaly (HYD/VMEG), 21.3% (13/61) had hemimegalencephaly (HMEG), and 8.2% (5/61) had cerebellar tonsillar ectopia (CBTE). Furthermore, we observed that an abnormal corpus callosum is a common feature in *AKT3* single nucleotide variants-related neurodevelopmental diseases. Approximately 36% (22/61) of patients displayed an abnormal corpus callosum (CC), with 81.9% (18/22) of these cases exhibiting a thickened corpus callosum.

AKT3 pathogenetic variants identified so far in 61 cases, with 19 carrying somatic variants and 42 carrying germinal variants. However, mutation site information was not available for one patient. A total of 21 distinct *AKT3* variants have been identified (Supplementary information 2), including 4 somatic mutation and 17 germinal mutations. Most *AKT3* variants localize to the pleckstrin homology domain (PH) or the catalytic kinase domain (KD), including 10 that located in the PH domain, 10 located in the KD domains, and only p.R465W is located in the C-terminal domains (CTD). Among the somatic mutations, 84.2% (16/19) were identified as p.E17K. The most common germinal mutation was p.R465W, accounting for 38.1% (16/42) of cases. Therefore, the *AKT3* p.E17K is a mutation "hotspot" among somatic mutations, while p.R465W may be a mutation "hotspot" among the germinal mutations. Figure 1 shows a schematic overview of the mutation spectrum of the *AKT3* gene and its protein domains. Most of the *AKT3* variants, including p.N53K, p.F54Y, p.E17K, p.N229S, p.V268A, p.V183D, p.D322N, p.R465W exhibit elevated phospholipid binding activity resulting from a gain-of-function (GOF) in the brain compared to the wild type[3].

Phenotypic and genetic spectrum of patients with distal 1q dupplication affecting the *AKT3* gene

57 patients with a duplication in the distal 1q region, which includes the *AKT3* gene, were reviewed in this study (Supplementary Table 2). Among them, 6 patients with detailed clinical information were analyzed. 5 patients had germline SNV duplication variants (del(1)(q43q44) or del(1)(q44)), and one patient had a somatic SNV duplication variant (del(1)(q21.1-q44)). Except for the patient with the del(1)(q21.1-q44) somatic duplication variant, 83.3% (5/6) of the patients had a documented history of macrocephaly. With the exception of one patient who was reported at three months, all patients had developmental delay or intellectual disability (DD/ID), with distinct speech delays observed in these patients. Fifty percent (3/6) of the patients suffered from seizures, with one patient presenting with epileptic spasms, who became seizure-free after surgery. The minimum size of the duplication region is 1.06 Mb, which includes disease-associated *AKT3* and *SDCCAG8* genes, or *AKT3*, *SDCCAG8*, and *ZETB18* genes.

Phenotypic and genetic spectrum of patients with distal 1q deletion affecting the AKT3 gene

175 patients with a deletion in the distal 1q region, including the *AKT3* gene, were reviewed in this study (Supplementary Table 3). Among them, 68 patients with detailed clinical information were further analyzed. Of the 68 patients, 97% (66/68) had microcephaly, 97% (66/68) had varying degrees of ID/DD, and 72.1% (49/68) had agenesis or hypoplasia of the corpus callosum (ACC/HCC). Approximately 63.2% (43/68) suffered from epilepsy. Among patients with seizures, 48.8% (21/43) had seizure onset before the first year of life. Based on the limited data available, among the 17 patients with seizure prognosis data, approximately 10 became seizure-free. Hypotonia was present in about 42.6% (29/68) of patients, and 61.7% (42/68) of these patients had facial deformities, while 27.9% (19/68) had cardiac defects. Additionally, among 5 patients with a pure *AKT3* deletion, all had microcephaly, 3 had facial deformities, but none suffered from epilepsy or had abnormal corpus callosum findings. In almost all patients, the brain MRI was generally normal (Table 3).

Genotype–phenotype correlations of *AKT3*-related diseases

Both gain-of-function and loss-of-function variants of the *AKT3* gene can lead to neurodevelopmental disorders. Gain-of-function variants, including *AKT3* single nucleotide variants and *AKT3* duplications, always lead to megalencephaly and typical structural abnormalities in the brain, such as HMEG, PMG, CBTE, and others. The majority of patients experience varying degrees of developmental delay and seizures, with an abnormal corpus callosum being common among these patients. In addition, patients with a distal 1q deletion containing

Variant	Case number (M,F)	MEG	PMG	VMEG/ HYD	CBTE	HMEG	abnormal CC	Seizure	Epileptic spasms	refractory epilepsy	DD/ID	Hypot-onia	Hypogly-cemia	Vascular abnormalities	CONN
Somatic	Total 19														
p.E17K	16 (10, 6)	14/16	6/16	0/16	0/16	9/16	1/16 thin CC 1/16 thick CC	14/16	4/16	12/16	5/16	4/16	1/16	6/16	2/16
p.Q78K	1 (1,0)	1/1	1/1	0/1	0/1	0/1	ND	1/1	0/1	ND	1/1	ND	ND	ND	0/1
p.T81dup	1 (1,0)	1/1	1/1	0/1	1/1	1/1	1/1 thick CC	1/1	0/1	0/1	1/1	0/1	ND	1/1	0/1
p.T288I	1(0,1)	1/1	1/1	1/1	0/1	0/1	1/2 thick CC	1/1	ND	1/1	1/1	1/1	0/1	1/1	0/1
Germline	Total 42 (with one variant site unavailable)														
p.E40K	1 (1,0)	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	1/1	1/1	0/1	1/1
p.N53K	1 (0,1)	1/1	0/1	0/1	0/1	0/1	1/1 thick CC	1/1	0/1	0/1	1/1	1/1	0/1	0/1	0/1
p.F54Y	1 (0,1)	1/1	1/1	1/1	0/1	0/1	1/1 thick CC	1/1	1/1	0/1	1/1	1/1	0/1	0/1	0/1
P.L77H	1 (ND)	ND	ND	ND	ND	ND	ND	1/1	ND	ND	1/1	ND	ND	ND	ND
p.W79C	4 (2, 1, ND)	3/4	1/4	1/4	0/4	0/4	1/4 thick CC	1/4	1/4	1/4	3/4	2/4	0/4	2/4	1/4
p.E84K	1 (0,1)	1/1	0/1	1/1	1/1	0/1	0/1	0/1	0/1	0/1	1/1	1/1	1/1	0/1	0/1
P.K161Q	1 (ND)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
P.K180E	1 (ND)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
p.V183D	2 (ND, 1)	2/2	2/2	2/2	0/2	0/2	1/2 hick CC	1/2	1/2	1/2	1/2	2/2	1/2	0/2	2/2
p.N229S	4 (2, 2)	4/4	4/4	3/4	0/4	1/4	1/4 thin CC 1/4 thick CC	2/4	0/4	0/4	2/4	3/4	ND	2/4	2/4
P.N229H	1 (ND)	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
p.V268A	2 (0, 2)	2/2	2/2	1/2	1/2	1/2	1/2 thick CC	1/2	0/2	1/2	2/2	1/2	1/2	1/2	1/2
P.T288I	1 (1,0)	1/1	1/1	1/1	0/1	1/1	1/1 dysplastic CC	1/1	0/1	1/1	1/1	0/1	0/1	0/1	0/1
P.N321K	1 (1,0)	1/1	1/1	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1
p.D322N	2 (0, 2)	2/2	2/2	0/2	0/2	0/2	2/2 thick CC	0/2	0/2	0/2	2/2	2/2	0/2	1/2	0/2
p.D322Y	1 (1,0)	1/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	0/1	1/1	1/1	0/1	1/1	0/1

Table 2. Clinical details of patients with *AKT3* variants-related neurological diseases. Abbreviations: *M* male, *F* female, *MEG*,megalencephaly, *PMG*,polymicrogyria, *VMEG* ventriculomegaly, *HYD* hydrocephalus, *HMEG* hemimegalencephaly, CBTE, *CC* Corpus callosum, *DD/ID* Development delay/ Intellectual disability, *CONN*, connective tissue dysplasia, *ND*, no data.

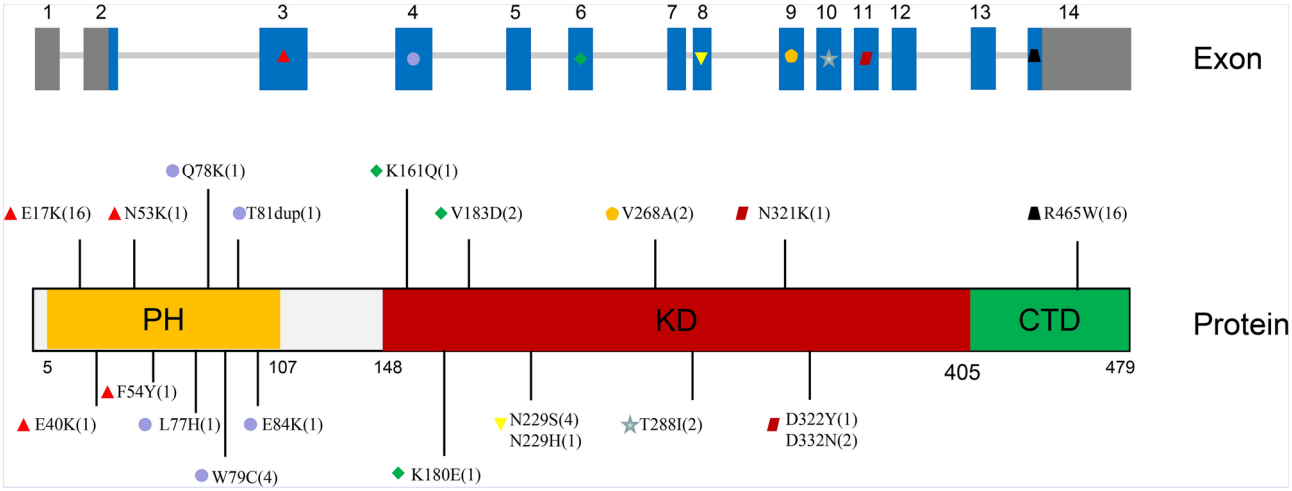

Fig. 1. Schematic overview of the mutation spectrum of the *AKT3* gene and the protein domains. The pleckstrin homology (PH), kinase domains (KD) and the C-terminal domains (CTD) are indicated. Pathogenic variants identified to date are shown. Different shapes represent variants located in the same exon. The numbers in brackets represent the number of patients with each variant.

the *AKT3* gene exhibit loss of function of *AKT3*. The majority of these patients have microcephaly, ID/DD, epilepsy, ACC or HCC, and facial deformities. A thick corpus callosum is common in patients with *AKT3* gain-of-function variants, while agenesis or hypoplasia of the corpus callosum is affected in the majority of patients with *AKT3* deletions. However, all patients with a pure *AKT3* gene deletion have microcephaly, but none suffer from epilepsy or have an abnormal corpus callosum. Therefore, it is possible that *AKT3* deletion may have a stronger correlation with microcephaly. The clinical characteristics of *AKT3*-related conditions are summarized in Table 4.

Discussion

A growing spectrum of cortical development malformations is now known to be caused by germline or mosaic variants of genes within the PI3K-AKT-MTOR signal network[4,21]. The *AKT3* gene plays a crucial role in regulating various cellular processes, including growth, survival, metabolism, and proliferation, particularly in the development and function of the brain[40]. Mutations in the *AKT3* gene are associated with various neurodevelopmental disorders. Gain-of-function (GOF) mutations in *AKT3* have been reported in patients with autosomal dominant inheritance, brain structure abnormalities, epilepsy, developmental delays, and other malformations[3,41]. These mutations can lead to macrocephaly and megalencephaly, where the brain becomes abnormally large. This is often accompanied by structural brain abnormalities, such as polymicrogyria, heterotopia, and cortical dysplasia. In contrast, a loss of function in *AKT3* leads to disrupted signaling, impairing the normal processes of cell growth, proliferation, and survival. Loss-of-function mutations in *AKT3*, often due to 1q deletions affecting the *AKT3* gene, can result in microcephaly, intellectual disability, and other developmental delays[42–45].

The *AKT3* gene has been linked to MPPH-2 syndrom in the OMIM database (OMIM #611,223)[8]. Brain structural abnormalities are a typical manifestation in patients with *AKT3* gain-of-function variants. Patients with *AKT3* mosaic variants often exhibit more severe brain structural abnormalities than those with germline variants. HYD/VMEG and CBTE are more commonly observed in patients with *AKT3* germline variants, while patients with *AKT3* deletions often have normal or nonspecific MRI findings. Epilepsy is a common clinical manifestation in patients with *AKT3* gene variants, always with early seizure onset, focal seizures, GTCS and epileptic spasms being prevalent. In patients with *AKT3* mosaic variants, the epilepsy rate is higher than patients with germline variants. Among the 13 patients with *AKT3* mosaic variants and recorded epilepsy surgery data, 7 achieved seizure freedom after surgery. Surgery is a viable option for patients with AKT3 variant-related refractory epilepsy.

The *AKT3* gene is crucial for proper neuronal growth and development. Variants of the *AKT3* gene can disrupt normal brain development pathways. GOF variants of *AKT3* activate the PI3K/AKT/mTOR pathway, leading to excessive cell growth and proliferation in the brain, particularly affecting neurons. This results in the overgrowth of brain tissue and enlargement of the head. Patients with *AKT3* GOF mutations typically present with macrocephaly or megalencephaly. Conversely, when *AKT3* is deleted or nonfunctional, it leads to reduced neurogenesis (the formation of new neurons), impairing brain development and resulting in a smaller-than-normal brain, characteristic of microcephaly. In this study, 77% of patients with *AKT3* single nucleotide variants experienced macrocephaly, and 81.9% had megalencephaly. The proportion may be higher, as some patients lack detailed information. Approximately 97% of patients with *AKT3* gene deletion variants exhibited microcephaly. *Akt3* knockout mice also displayed reductions in brain size[46]. Therefore, it's possible that *AKT3* deletion may have a stronger correlation with microcephaly. In addition, the majority of patients with *AKT3* gene variants

Number	Variation	deletion size	Gender	age at report	Seizures	ID/DD	Hypot-onia	Abnormal CC	Microce-phaly	Other brain malformation	Facial deformity	Vascular abnormalities	Reference PMID
1	Chr1q43-44 (243,766,972–243,869,687)	102 Kb	F	11 m	-	+	-	-	+	-	+	+	21,934,713
2	del(1)q44 (243,800,911–243,933,961)	133 Kb	M	2.3y	-	ND	ND	-	+	Displacement of posterior pituitary with Rathke cleft cyst	-	ND	21,800,092
3	del(1)q44 (243,831,018–244,043,589)	213 Kb	F	2.8y	-	ND	ND	-	+	small white matter lesion	+	ND	21,800,092
4	Chr1q44 (243,786,019–244,182,939)	396 Kb	M	5.5y	-	+	+	-	+	normal	+	-	25,424,989
5	Chr1q44 (243,734,763–243,827,143)	92.38 Kb	M	ND	-	+	-	-	+	-	ND	ND	Decipher: 317,423

Table 3. Clinical data of patients with pure *AKT3* gene deletion. Abbreviations: *M* male, *F* female, *DD/ID* development delay/ Intellectual disability, *CC* corpus callosum, *ND* no data.

Characteristics	*AKT3* variant (N = 61)	*AKT3* Dup (N = 57, 6 patients with detailed information)	*AKT3* Del (N = 175, 68 patients with detailed information)
male	30/61	3/6	34/68
Head size			
Macrocephaly	47/61	5/6	0/68
Microcephaly	0/61	0/6	66/68
Neuroimaging features			
MEG	50/61		
PMG	31/61		
HYD/VMEG	18/61		
CBTE	5/61		
HMEG	13/61		
Abnormal CC			
Thick CC	18/61	0/6	0/68
ACC/HCC	4/61	0/6	49/68
DD/ID	38/61	5/6	66/68
Epilepsy	38/61	3/6	43//68
Epilepsy onset age			
< 1y	24/38	1/3	21/68
> 1y	7/38	1/3	11/68
Epilepsy type			
Focal seizure	15/38	0/3	2/43
GTCS	4/38	0/3	8/43
epileptic spasm	9/38	1/3	0/43
Hypotonia	28/61	2/6	29/68
Hypoglycemia	7/61	0/6	0/68
Vascular abnormalities	18/61	0/6	19/68
CONN	13/61	0/6	ND

Table4. Clinical characteristics of *AKT3*-related conditions. Abbreviations: *MEG* megalencephaly, *PMG* polymicrogyria, *VMEG* ventriculomegaly, *HYD* hydrocephalus, *HMEG* hemimegalencephaly, *CBTE*, ; *CC* corpus callosum, *DD/ID* development delay/ Intellectual disability, *GTCS* generalized tonic–clonic seizure, *CONN* connective tissue dysplasia, *ND* no data.

experienced DD/ID. These variations in brain size highlight the importance of the *AKT3* gene in regulating brain growth and neural development.

Abnormal corpus callosum is a major clinical feature in patients with *AKT3* variants. Mutations or dysregulation of *AKT3* can affect the development of the corpus callosum, the structure that connects the left and right hemispheres of the brain. Patients with *AKT3* GOF variants have a high proportion of thick corpus callosum. While patients with *AKT3* deletion variants always experience agenesis or hypoplasia of the corpus callosum. 29.5% of patients with single nucleotide variants have a thick corpus callosum, while 72% of patients with 1q deletion affecting *AKT3* gene experienced agenesis or hypoplasia of the corpus callosum. The proportion may be higher, as detailed clinical information was unavailable for some patients. These abnormalities in the corpus callosum may contribute to neurological conditions such as developmental delays, intellectual disability, and other cognitive impairments. However, in the five patients with pure *AKT3* gene deletions, none exhibited abnormalities in the corpus callosum or seizures. So, the relationship between *AKT3* deletion and agenesis or hypoplasia of the corpus callosum may require further validation through a larger cohort of patients.

At present, there is no specific treatment for neurodevelopmental diseases related to the *AKT3* variants. The majority of melanomas exhibit elevated *Akt3* expression and activity. Studies have shown that siRNA-mediated knockdown of *Akt3* reduces downstream phosphorylated PRAS40 levels and sensitizes melanoma cells to apoptosis[47]. The PI3K/AKT/mTOR signaling pathway plays a vital role in cell survival, cell growth, and cell cycle progression. The dysregulation of signal transduction can predispose to various human cancers and *AKT3* inhibitors may develop as a novel cancer therapy that efficiently suppresses the PI3K-AKT-mTOR pathway[48]. For GOF *AKT3* variants, potential therapeutic approaches may include the use of *AKT3* inhibitors or the silencing of the *AKT3* gene. Since *AKT3* GOF variants can upregulate mTOR expression, mTOR inhibitors may also be a viable treatment strategy. However, LOF variants of the *AKT3* gene also cause diseases, so it's a challenge to balance the potential dual impact. According to the literature review, there is no recommended anti-seizure medications, epilepsy surgery is a choice for patients with refractory epilepsy. With the advantage of gene therapy technology, targeted *AKT3* gene editing therapy emerges as a promising avenue for patient treatment.

In conclusion, this study reports three new Chinese patients with de novo *AKT3* gene variants and provides an overview of all patients with *AKT3*-related neurodevelopmental disorders, including those with *AKT3* gene single nucleotide variations, duplications, and deletions. We highlight the phenotypic differences between patients with germline versus somatic *AKT3* mutations, as well as the distinctions between *AKT3* gain-of-

function and loss-of-function variants, offering guidance for clinical practice. Further elucidation of disease mechanisms may facilitate the development of targeted treatments.

Materials and methods

Patients

Three subjects from three unrelated families with macrocephaly and developmental delay with unknown etiology were analyzed. All patients were clinically evaluated by experienced pediatric neurologists and underwent whole exome sequencing (WES). This study was approved by the Ethics Committee of Xiangya Hospital of Central South University (Human Study/Protocol #202,310,892) and was conducted in accordance with the ethical standards outlines in the Declaration of Helsinki. Written informed consent was obtained from the parents.

Sequencing and analysis

Peripheral blood samples were obtained from the patients and his/her biological parents. DNA extraction, sequencing, and data analyses were conducted as previously described[49]. The pathogenicity of variants was predicted by VarCards2 (http://www.genemed.tech/varcards2/)[50], including PolyPhen-2, SIFT, CADD, Mutation Taster, and others. Allele frequency in population were evaluated by the 1000 Genomes, ExAC, and gnomAD databases. The identified variant was validated by Sanger sequencing. The pathogenicity of the novel variant was evaluated according to the American College of Medical Genetics (ACMG) standard guidelines[51].

Systematic literature review

We conducted a systematic literature review focused on *AKT3* variant-related neurodevelopmental diseases by searching the PubMed and Chinese medical databases using the keywords "AKT3" "1q43q44 duplication", "1q43q44 deletion" and "neurology." All publications were reviewed for case series or cohort studies containing clinical data on patients with *AKT3* single nucleotide variants, *AKT3* duplications, and *AKT3* deletions. Individuals with central nervous system involvement were included. In addition, we included patients with pathogenic or likely pathogenic *AKT3* variants uploaded to the ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) and DECIPHER (https://www.deciphergenomics.org/gene/AKT3) databases, along with phenotype information. We performed a reassessment of all variants, aligning nucleotide and amino acid numbering to the *AKT3* reference transcript NM_005465 (reference protein NP_005456.1). We conducted a comprehensive review of the detailed clinical and molecular genetics of patients with *AKT3*-related neurodevelopmental disorders, aiming to expand the phenotypic and genetic spectrum of *AKT3*-related neurodevelopmental conditions and evaluate genotype–phenotype correlations.

Data availability

Data is provided within the manuscript or supplementary information files. The datasets generated and analyzed during the current study are available in the [Clinvar] repository (https://www.ncbi.nlm.nih.gov/clinvar/) ID: SUB15093593, SUB15093627, SUB15093645.

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Author contributions
XW conducted data collection, data analysis and drafted the manuscript; ZZ performed data collection and data analysis; PP performed data collection; JP supervised and writing-reviewed and edited. All authors read and approved the final manuscript.

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Declarations

Competing interests
The authors declare no competing interests.

Ethics approval and consent to participate
This study was approved by the Ethics Committee of Xiangya Hospital of Central South University (Human Study/Protocol #202310892) and was conducted in accordance with the ethical standards outlines in the Declaration of Helsinki. Written informed consent was obtained from the participants' legal guardian.

Consent to publication
We obtained the written consent for publication from the guardian of the patient.

Additional information
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