Appendix 1 Supplementary clinical data (*Table S2*)

Individual 1—NM_015100.4*: *c.1180_1181del*, *het*, *de novo*, *novel

The male patient was two years and nine months old at first presentation. He was transferred to our pediatric development clinic with speech dyspraxia and general developmental delay. He was full-term born by vaginal delivery, G1P1, with birth weight 3,400 g and unremarkable perinatal history. After birth, he presented feeding difficulty and poor sensory integration ability gradually. He sat at 6 months and walked at 26 months, had gait instability, and spoke less than 20 words until 33 months, yet he could not reliably use sentences. He exhibited autism-like behaviors, such as poor eye contact, repetitive language, stereotyped behaviors, and hindered social development. Physical examination revealed his height was 86.4 cm (less than 3 centiles for his age), weight was 13.1 kg (within the 10 to 25 centiles for his age), and head circumference in the normal range. Distinctive facial features were noticed, including low-set ears, bilateral nostril valgus, wide and depressed nasal bridge, short philtrum, thin vermilion border, and short chin. Hypermetropia and astigmatism were found in version examination. No significant abnormalities were detected via hearing examination, electroencephalogram (EEG), and brain magnetic resonance imaging (MRI). The biochemical parameters were all within the normal range, including the serum or plasma levels of lactic acid, cysteine, very long chain fatty acids, amino acids, blood lipids, cholesterol, and hormones including TSH, FT4, LH, FSH. Analysis of urinary organic acid did not reveal abnormal metabolites.

Individual 2—NM_015100.4*: *c.1185+6 T>A (splicing)*, *het*, *de novo*, *novel

The male patient was five years and three months old when he was transferred to our pediatric development clinic with speech dyspraxia and general developmental delay. He was born by vaginal delivery at 35 weeks of gestation, G1P1, with birth weight of 2,100 g. His perinatal history was unremarkable except for the preterm delivery. He walked at 36 months. He started babbling at age 12–14 months and spoke his first words after age 3 years, yet he could not reliably use sentences. He exhibited repetitive behavior, limited sharing behaviors, and eye contact during speech and body language communication in a consultation room. Physical examination revealed her height was 109.8 cm (within the 25 to 50 centiles for his age), and weight was 17.6 kg (within the 25 to 50 centiles for his age), head circumference was in the normal range. Distinctive facial features were noticed, including low-set ears, bilateral nostril valgus, wide and depressed nasal bridge, short philtrum, thin vermilion border, and short chin. Astigmatism and myopia were found in version examination. Hearing disorder was detected via hearing examination. Long T2 abnormal signal shadow in the left lateral ventricle and the widened perivascular space were reported in brain MRI. EEG examination was normal. The biochemical parameters were all within the normal range, including the serum or plasma levels of lactic acid, cysteine, very long chain fatty acids, amino acids, blood lipids, cholesterol, and hormones including TSH, FT4, LH, FSH. Analysis of urinary organic acid did not reveal abnormal metabolites.

Individual 3—NM_015100.4*: *c.3715G>A*, *het*, *novel

The patient is three years and two months old boy diagnosed with ASD. He exhibited poor eye contact and communication, repetitive behavior, and language. His DQ score was absent because he did not do the DQ test. His parents reported no other abnormality during pregnancy. He showed speech delay or motor delay during development. The parents reported no special facial dimorphisms, and other growth parameters were within normal range except short stature. No abnormality was found in the brain MRI. The patient has no epilepsy and no abnormality in EEG. No abnormality was found in blood examination, including biochemical, hematological, endocrine, and hormone tests. The patient has no other psychiatric or physical disorders.

https://dx.doi.org/10.21037/tp-2025-477

Individual 4—NM_015100.4: c.1427G>A, het, inherited, reported

The patient is three years and three months old boy diagnosed with ASD and general developmental delay. His parents reported no other abnormality during pregnancy. He exhibited speech delay or motor delay during development. He also showed limited social communication and repetitive behaviors and no family history of psychiatric or physical disorders. The parents reported no special facial dimorphisms, and all growth parameters were within the normal range. No abnormality was found in the brain MRI. The patient has no epilepsy and no abnormality in EEG. No abnormality was found in blood examination, including biochemical, hematological, endocrine, and hormone tests. The patient has no other psychiatric or physical disorders. His mother does not have a job. Unfortunately, the mother carrying the variant gene did not undergo a formal neuropsychological assessment.

Individual 5—NM_015100.4: c.1375G>A, het, inherited, reported

The patient is 1 years and 8 months old girl diagnosed with speech delay. She is the first child born to healthy nonconsanguineous parents. Her perinatal history was unremarkable. She walked at 12 months but had significant expressive language impairment. He also showed limited social communication but no repetitive behaviors. A hearing and visual screening test was not done and no family history of psychiatric or physical disorders. Brain MRI and EEG were not done in this patient. No abnormality was found in blood examination, including biochemical, hematological, endocrine, and hormone tests. The patient has no other psychiatric or physical disorders. Her mother showed no abnormal clinical symptoms, but there was no formal neuropsychological assessments for her.

Table S1 Effect of the variants (NM_015100.4) were predicted based on the classification of ACMG guidelines

Individuals	Variant in *POGZ* gene	Matched ACMG guidelines
Individual 1	c.1180_1181del; p.Met394ValfsTer9	PVS1: null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene where loss-of-function is a known mechanism of disease
		PS2: de novo (both maternity and paternity confirmed) in a patient with the disease and no family history
		PM2: absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
Individual 2	c.1185+6 T>A(splicing)	PS2: de novo (both maternity and paternity confirmed) in a patient with the disease and no family history
		PM1: located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation
		PM2: absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium
		PP3: multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.)
Individual 3	c.3715G>A p.Val1239Ile	PM1: located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation
		PM2: absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium. Absent in population databases
		PM6: assumed de novo, but without confirmation of paternity and maternity
		BP1: missense variant in a gene for which primarily truncating variants are known to cause disease
		BP4: multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)
Individual 4	c.1427G>A; p.Arg476Gln	BS1: allele frequency is greater than expected for disorder
		BP1: missense variant in a gene for which primarily truncating variants are known to cause disease.
		BP4: multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)
Individual 5	c.1375G>A; p.Asp459Asn	PM5: novel missense change at an amino acid residue where a different missense change determined to be pathogenic has been seen before
		BS1: allele frequency is greater than expected for disorder
		BS2: observed in a healthy adult individual for a recessive (homozygous), dominant (heterozygous), or X-linked (hemizygous) disorder, with full penetrance expected at an early age
		BP4: multiple lines of computational evidence suggest no impact on gene or gene product (conservation, evolutionary, splicing impact, etc.)

https://dx.doi.org/10.21037/tp-2025-477

Table S2 Summary of clinical features for the five participants

Individuals	Individual 1	Individual 2	Individual 3	Individual 4	Individual 5
Age at assessment	3Y1M	5Y2M	3Y2M	4Y8M	1Y8M
Sex	M	M	M	M	F
Mutation (NM_015100.4)	c.1180_1181del; p.Met394ValfsTer9	c.1185+6 T>A(splicing)	c.3715G>A; p.Val1239Ile	c.1427G>A; p.Arg476Gln	c.1375G>A; p.Asp459Asn
DQ	48	36	ND	62	71
Gross motor	60	34	ND	86	98
Fine motor	48	24	ND	65	68
Language	40	34	ND	38	55
Personal-social behavior	45	36	ND	71	65
Adaptive behavior	46	53	ND	52	68
Autism screen test					
M-Chat	+	+	+	+	-
ABC	+	+	+	+	-
Autism diagnostic criteria*	+	+	+	+	-

*, be assessed by at least one experienced developmental-behavioral pediatricians whether meet the diagnostic criteria of ASD from the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). M, male; F, female; DQ, developmental quotient; M-Chat, Modified Checklist for Autism in Toddlers; ABC, Autism Behavior Checklist; ASD, autism spectrum disorder; ND, no data.

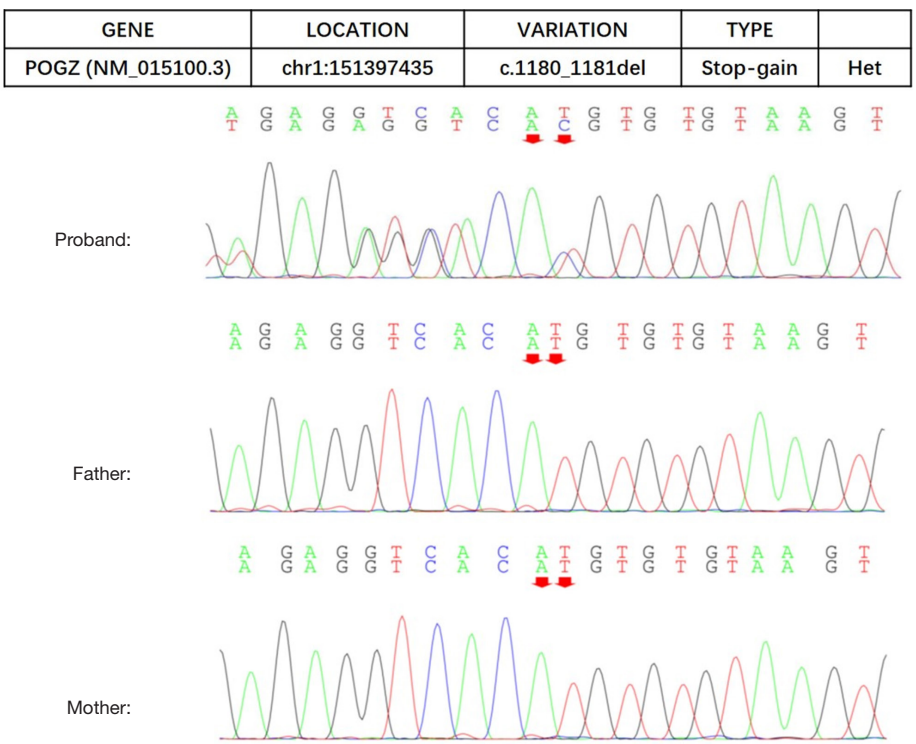

Figure S1 Individual 1: sanger sequencing of the *POGZ* gene of c.1180_1181del in the 8 exon. The proband was heterozygous of the stop-gain mutated gene, while her father and mother were normal.

https://dx.doi.org/10.21037/tp-2025-477

GENE	LOCATION	VARIATION	TYPE	
POGZ (NM_015100.3)	chr1:151397425	c.1185+6T>A	Splicing	Het

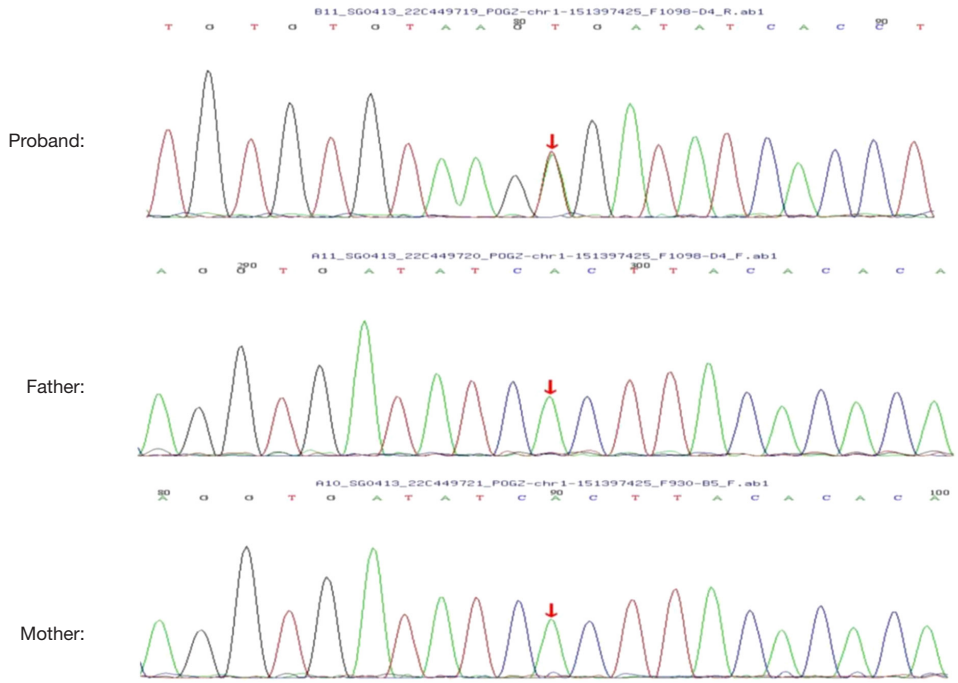

Figure S2 Individual 2: sanger sequencing of the *POGZ* gene of c. 1185+6 T>A. The proband was heterozygous of the splicing mutated gene, while her father and mother were normal.

GENE	LOCATION	VARIATION	TYPE	
POGZ (NM_015100.3)	chr1:151377796	c.3715G>A	Missense	Het

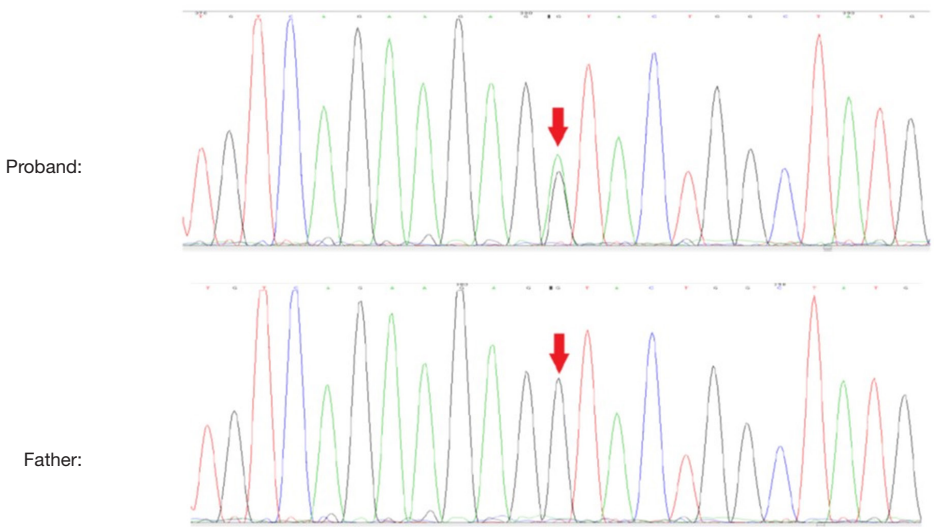

Figure S3 Individual 3: sanger sequencing of the *POGZ* gene of c. 3715G>A. The proband was heterozygous of the missense mutated gene, while her father was normal.

https://dx.doi.org/10.21037/tp-2025-477

GENE	LOCATION	VARIATION	TYPE	
POGZ (NM_015100.3)	chr1:151396521	c.1427G>A	Missense	Het

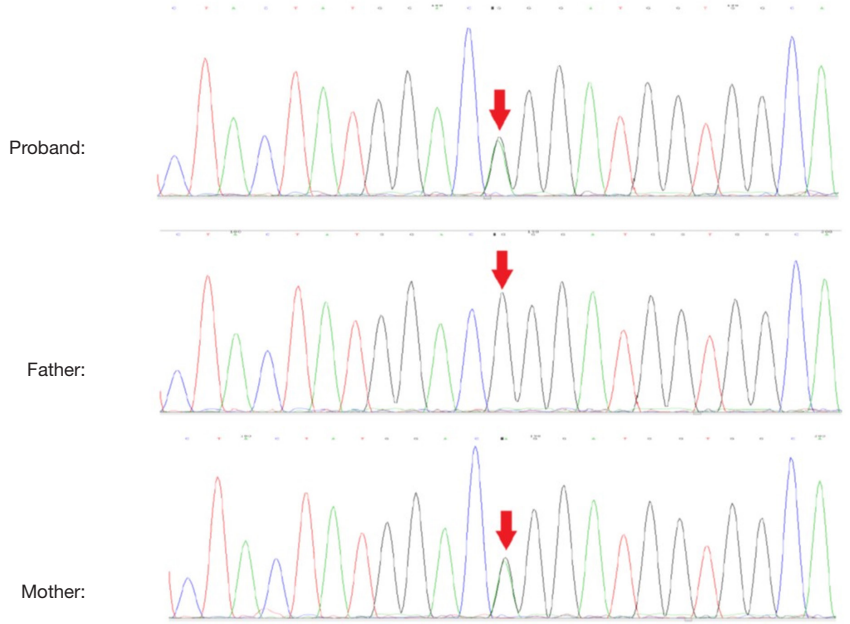

Figure S4 Individual 4 and 5: sanger sequencing of the *POGZ* gene of c.1427G>A. The proband was heterozygous of the missense mutated gene, his mother was asymptomatic carrier, while her father was normal.

GENE	LOCATION	VARIATION	TYPE	
POGZ (NM_015100.3)	chr1:151396573	c.1375G>A	Missense	Het

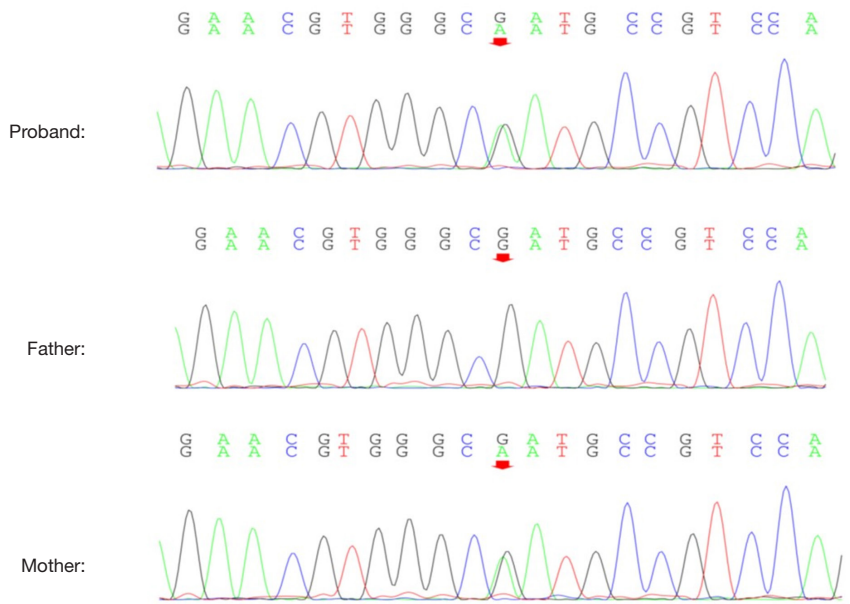

Figure S5 Individual 6 and 7: sanger sequencing of the *POGZ* gene of c.1375G>A. The proband was heterozygous of the missense mutated gene, his mother was asymptomatic carrier, while her father was normal.

https://dx.doi.org/10.21037/tp-2025-477