Table S1. Phenotype of individuals with *AFF3* variants

Individual	Proband 1	Proband 2	Proband 3	Proband 4	Proband 5	Proband 6	Proband 7	Proband 8	Proband 9	Proband 10	Deletion proband*
Age [years]	17	3 years	18	21	5	9	8	8	8	11	4 months
Sex	M	F	M	F	F	F	F	F	M	M	F
AFF3 variant	p.(A258S)	p.(A258S)	p.(A258T)	p.(A258T)	p.(A258T)	p.(A258T)	p.(A258T)	p.(A258T)	p.(A258V)	p.(V260G)	500kb deletion
Inheritance	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*	*De novo*
Neurodevelopmental anomalies											
Severe DD/ID	+	+	+	+	+	+	+	+	+	+	+
Epilepsy	Generalized tonic-clonic seizures (onset at 5 years), nocturnal, treatment with Keppra and Micropakine	Multifocal epileptiform discharges in bilateral posterior quadrant, no clinical seizures.	Generalized, tonic-clonic seizures (onset at 5 months), initially controlled with phenobarbital, developed drug-resistance, partially controlled with carbamazepine, clobazam, nitrazepam and phenobarbital	Generalized, tonic-clonic seizures	Generalized, tonic-clonic seizures	Complex partial with secondary generalization	Generalized, tonic-clonic seizures	Generalized (onset at 3 years), tonic-clonic seizures, inefficient treatments but remission since 6 years 9 months	-	Generalized, tonic-clonic seizures	Myoclonic jerks, convulsions
Muscle tone	Limb hypertonia, spastic tetraparesis	Generalized hypotonia	Axial hypotonia, peripheric hypertonia	Limb hypertonia	Hypotonia	Axial hypotonia, peripheric hypertonia	Hypotonia	Hypotonia	Normal	Hypotonia	N/A
Vision and hearing	Normal	Cortical visual impairment, hyperopic refractive error and small angle intermittent strabismus	Normal	Myopia, strabismus	Strabismus	Central vision loss due to occipital impairment, central progressive hearing loss	Normal	Strabismus inconstant	Conductive hearing loss (resolved), vision not yet formally assessed	Strabismus	N/A
Brain MRI	Enlargement of the ventricular system and peri-cerebral spaces, thin and irregular appearance of the corpus callosum	Partial agenesis of the corpus callosum, forshortened and undersulcated frontal lobes, small cerebellar vermis with mega cisterna magna and wide sylvian fissures	Pachygyria of frontal lobes, abnormal opercularization of insulae with thicker insular cortices, wide Sylvian fissures, cerebral atrophy, brainstem hypoplasia, increased volume of the trigones and occipital horns of the lateral ventricles	N/A	Cerebral atrophy with possible brainstem hypoplasia	Prominence of CSF spaces	Cerebral atrophy	Cerebral atrophy, pachygyria of frontal lobes	Normal	N/A	Brain atrophy, ventriculomegaly
Other	Stereotypic movements	-	-	-	-	-	-	-	Autism and ADHD	-	-

Craniofacial features											
Microcephaly	+ (plagiocephaly)	-	+	-	+	- (brachycephaly)	+	+	+	+	-(dolicocephaly)
Nose	Small nose, anteverted nares	Prominent columella	Large nose with bulbous nasal tip and low hanging columella	Large nose with bulbous nasal tip and low hanging columella	Small nasal tip	Bulbous nasal tip, low hanging columella with low insertion	Large nose with bulbous nasal tip and low hanging columella	Large nose with bulbous nasal tip and low hanging columella	Normal	N/A	N/A
Short philtrum	-	-	+	+	-	+ (smooth)	+	+	Smooth philtrum	N/A	N/A
Mouth	Wide mouth with square upper lip	Wide mouth, downturned corners, thin upper lip	Wide mouth with square upper lip	Wide mouth with square upper lip	Wide mouth with square upper lip	Wide mouth with downturned corners, thick lower lip vermillion	Prominent upper lip	Wide mouth	Thin upper vermillion, mild ankyloglossia (snipped), high arched palate	Ankyloglossia	N/A
Teeth and gum abnormalities	Small, gingival hypertrophy	Small, widely spaced, bruxism	+	+	Small, widely spaced	+	-	Widely spaced	Hypomineralisation	+	N/A
Chin	Prognathism	Normal	Micrognathia	Triangular chin	Normal	Micrognathia	Mild micrognathia	Prognathism	Micrognathia	N/A	N/A
Synophrys and hypertrichosis	+	-	+	+	+	+	+	+	+	N/A	N/A
Other	Hypertelorism, short neck	Full cheeks, mild facial asymmetry	-	-	-	Long palpebral fissures, low-set and posteriorly rotated large ears with a simple helix, facial asymmetry	Long palpebral fissures	Long palpebral fissures, low set ears, mild facial asymmetry, gingival hyperplasia diabetes	Slight metopic prominence	-	Short palpebral fissures, low set ears, short neck
Skeletal abnormalities											
Mesomelic dysplasia	-	Lower limbs	4 limbs	4 limbs	4 limbs	4 limbs	4 limbs	4 limbs	Mild lower limbs	Lower limbs	4 limbs
Arms and legs	Bilateral elbow dislocation	Bilateral fibular agenesis, short and curved tibia, bilateral Syme amputations with resection of cartilaginous fibular anlage and bilateral tibial osteotomies for angular deformity correction, fitted with bilateral lower extremity prosthetics at 2 years 3 months.	Madelung deformity, slender limb bones, fibular hypoplasia/agenesis	Short ulna and radius, radial head dislocation/subluxation, styloid process of ulna on radius, carpal coalition, hypoplastic femora, short and curved tibia with metaphyseal flaring, mid tibial dimples, deviated knees, hypoplastic and gracile fibula	Short and thick ulna, slightly shortened radius with convex distal end bilaterally, dislocation of right radial head, short and curved tibia, extremely short rectangular fibula	Short fibula, discoid meniscus, limited knee extension	Limited supination, radial head dislocation/subluxation, hypoplastic fibula	Short humerus, hypoplastic short fibula	Mild lateral bowing of both radii, normal bone age	Bowed radii, unilateral bowed ulna, shortened ulna, abnormal radial diaphysis, bowed and angulated tibias, hypoplastic fibula	Radial head dislocation/subluxation, slightly short radius and ulna, short and dysplastic triangular tibias, fibular agenesis
Hands and feet	Limited pronosupination, bilateral camptodactyly, edema of back-hands and -feet, bilateral simian creases, tapered fingers, dorsum pedis edema, small toes, hypoplasia of	Right single transverse and left bridged palmar crease, bilateral hypoplastic 4th metatarsals, absence of the 5th ray and phalanges of lateral toes, 4 splayed toes	Hypoplastic talipes, fusion of tarsal bones	Carpal coalition, small feet, hypoplastic left 5th, metatarsal synostosis	Talus ossified in hindfoot, one ossified bone in midfoot (cuneiform), missing one lateral ray in left foot, 4th-5th right metatarsal synostosis	Soft tissue syndactyly of fingers 3rd-4th, small feet, pes planus, 2nd toe overlapping hallux bilaterally	Limited supination, pes planus, broad toe tips	Transverse palmar crease, limited pronosupination, proximal deviation of thumbs, small feet, absent calcanei, broad 1th toes, polydactyly, cutaneous	Very broad feet. Bilateral hind foot varus deformity, mild metatarsus adductus. misshaped and large talus, cuboid and calcaneum, mild shortening and Y	Wide distal radial metaphyses, oligodactyly: 2 tarsal bones on each foot, absent/hypoplastic calcanei, 3 metatarsals, 3 associated phalanges, 1 phalanx not	Small equinovalgus feet, oligodactyly: 4 toes on 1 foot, 5 on the other, abnormally spaced

	distal phalanges, ungual hypoplasia, neonatal arthrogryposis							process on the side of the 5th finger and cutaneous syndactyly 3th-6th toes on left foot, four metatarsals and partial syndactyly 3th-4th toes on right foot	shaped fusion of the left 4th and 5th metatarsals, hyperplastic/dysplastic toenails, hand Xrays normal	associated with a metatarsal bone	
Ribs and spine	Scoliosis	13 rib-bearing thoracic-type vertebrae and 5 lumbar type vertebrae, hypoplastic L1 with focal kyphosis	Severe scoliosis, C2-C3 vertebral fusion, L5-S1 vertebral cleft	Scoliosis	Bilateral cervical ribs	Scoliosis, incomplete coronal cleft of T9 and T12 vertebrae, low lying spinal cord, termination of conus medullaris at upper border of L3	Pectus excavatum	Scoliosis, fusion 1th-2th ribs, sacral sinus	6 lumbar vertebrae, 13 pairs of ribs, sacral dimple	Scoliosis, cervical ribs, anterior superior vertebral notching, tethered cord	Sacral sinus
Hips and pelvis	Bilateral coxa valga, dislocation of the hips	Normal	Normal	Bilateral coxa valga with hypoplastic ilia, hip dislocation	Coxa valga	Bilateral coxa valga	Hip dislocation	Coxa valga, hip dysplasia	Normal	Coxa valga, unilateral hip dysplasia	N/A
Osteopenia	N/A	+	+ (osteoporosis)	+	-	+	+ (osteoporosis)	-	-	+	N/A
Additional features											
Horseshoe kidney	-	+	-	+	+	+	-	+	+	+	+
Gastro-intestinal symptoms	N/A	GERD, dysphagia, gastrostomy tube dependent, concerns for esophageal dysmotility ± abnormal gastric accommodation, abnormal gastric emptying with no evidence of small intestinal dysmotility	GERD, constipation	GERD, constipation	Constipation, swallowing difficulties, percutaneous endoscopic gastrostomy	GERD, gastrojejunostomy tube dependent, chronic constipation, hiatal hernia, pancreatitis	Constipation	Constipation, anal dystopia	Constipation, feeding difficulties due to floppy larynx and adenotonsillar hypertrophy, frequent obstruction	GERD, constipation	Colon malrotation
Failure to thrive	+	-	+	+	+	+	+	+	+	+	+
Respiratory problems	Apnea	Multicompartmental respiratory disease (upper airway obstruction, lower airway obstruction, ineffective mucociliary clearance, restrictive lung disease, aspiration and pneumonia), moderate to	Neonatal respiratory distress, recurrent pneumonia, frequent hiccups improved with carbamazepine, respiratory arrest leading to death at 21 years	-	Nightly desaturations treated with CPAP from 3 years of age	-	-	-	Severe obstructive sleep apnea, adenotonsillectomy at 3 years	-	Recurrent apnea, respiratory arrest leading to death at 4 months

		severe mixed sleep apnea, severe laryngomalacia status post supraglottoplasty at 18 months, cough assist and inhaled steroid and bronchodilator and supplemental oxygen with sleep, tonsillectomy and adenoidectomy planned			
Other	Bilateral cryptorchidism, bicuspid aortic valve	History of bilateral vesicoureteral reflux, grade II	Menstrual cycle perturbations	Popliteal pterygium	Short stature

Footnote: AFF3 variants are described according to RefSeq NP_002276.2. ***Steichen-Gersdorf *et al* (2008)**[15]
Abbreviations: DD= developmental delay, ID= intellectual disability, N/A = not available, ADHD= Attention deficit hyperactivity disorder, GERD= gastroesophageal reflux disease