***MED27* variants cause Developmental Delay, Dystonia, and Cerebellar Hypoplasia**

Running head: *MED27* associated with novel neurodevelopmental syndrome

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ABSTRACT

The Mediator multiprotein complex functions as a regulator of RNA polymerase II-catalyzed gene transcription. In this study, exome sequencing (ES) detected biallelic putative disease-causing variants in *MED27*, encoding Mediator Complex Subunit 27, in sixteen patients from eleven families with a novel neurodevelopmental syndrome. Patient phenotypes are highly homogeneous including global developmental delay, intellectual disability, axial hypotonia with distal spasticity, dystonic movements, and cerebellar hypoplasia. Seizures and cataracts were noted in severely affected individuals. Identification of multiple patients with biallelic *MED27* variants supports the critical role of MED27 in normal human neural development, particularly for the cerebellum.

INTRODUCTION

The Mediator complex acts as a bridge between transcription factors at enhancers and the basal transcriptional machinery at specific promoters, thereby stabilizing the preinitiation complex and stimulating promoter release [1, 2]. It is also involved in additional aspects of transcriptional regulation, including mRNA and noncoding RNA processing, chromatin remodeling and epigenetic regulation[3]. *MED12* was the first human disease gene discovered in the Mediator complex, associated with Opitz-Kaveggia syndrome (MIM: 305450) and Lujan-Fryns syndrome (MIM: 309520) [4, 5]. Since then, a total of six genes in the complex have been reported as disease genes mostly in association with neurodevelopmental disorders. However, the majority of the Mediator genes remain without disease associations.

Here, we report a novel neurodevelopmental syndrome with a unique phenotype of global developmental delay, axial hypotonia with appendicular spasticity, dystonic movements, cerebellar hypoplasia, epilepsy, and cataracts. Exome sequencing detected biallelic disease-causing variants in *MED27* (MIM: 605044), a eukaryotic specific subunit thought to represent an ortholog of budding yeast Med3 [6, 7].

PATIENTS AND METHODS

Patients were identified through GeneMatcher and all subjects were examined by the referring physicians[8]. Clinical information including medical notes, facial photos, and MRI images were collected, critically reviewed and compared. Informed consents were obtained from the legal guardians of all subjects. The study was performed in accordance with the guidelines specified by the Institutional Review Boards and Ethics Committees at each institution.

Exome sequencing (ES) on each patient was performed in commercial or academic laboratories per each laboratory's protocol. Targeted Sanger sequencing was performed in probands and

available relatives to validate variant segregation. Variant filtering and prioritization were performed by assessing variant characteristics including general population frequency, variant severity, *in silico* prediction, inheritance modeling, pathway analysis, and family segregation analysis. *MED27* variants were annotated on reference sequence NM_004269.3.

RESULTS

Prior to ES analysis, most reported patients had extensive clinical, metabolic, and genetic investigations, yet had not received a molecular diagnosis. ES analysis followed by targeted Sanger sequencing revealed biallelic variants, either compound heterozygous or homozygous alleles, in *MED27* in sixteen affected individuals (Supplementary Table 1). A total of eleven unique *MED27* variants were identified, including frameshift (3/11), canonical splice-site (1/11), and missense variants (7/11) (Fig 1, Supplementary Table 2). Three recurrent variants [c.776C>T (p.Pro259Leu), c.839C>T (p.Pro280Leu), and c.871G>A (p.Gly291Ser)] affecting CpG sites were identified in multiple families with different ethnic backgrounds. All *MED27* variants are either absent or rare in the Genome Aggregation Database (gnomAD v2). The variants occurred at residues that are extremely conserved from human to drosophila with GERP++ RS score greater than 5.0 [9]. Multiple *in silico* programs including PolyPhen2, SIFT[1], Mutation Taster and CADD (Combined Annotation Dependent Depletion) support the deleterious effect of these variants (Supplementary Table 2). [10,11,12,13] Notably, six out of seven missense variants are located in close proximity near the C-terminal end of the protein (Fig 1).

The clinical phenotypes of each subject are summarized in Table 1 and Supplementary Table 3. The reported cohort consists of 13 pediatric patients (ages 0 to 13 years) and three adult patients (ages 26, 36, and 42 years). Consanguinity was noted by historical report in five of the eleven families. Except for one subject born preterm (11-2), all were born full term with no perinatal complications. Patient 10-2, who was previously published in a large arthrogryposis

cohort study, had a dual molecular diagnosis with homozygous pathogenic variants in both *MED27* and *COG6*, the latter of which is associated with a congenital disorder of glycosylation type III (MIM: 614576).[14] The severe clinical symptoms and early death of this individual were most likely attributable to the *COG6* variant and may have masked the *MED27*-related phenotype. Therefore, this individual was excluded from clinical analysis.

Global developmental delay, ranging from mild to profound, and intellectual disability were observed in all patients. Eight patients were severely affected and were non-verbal and unable to sit or walk independently. Among them, two patients (2, 3-2) also had motor regression. Five patients were moderately delayed developmentally but achieved ambulation and some expressive language. Two siblings (6-1, 6-2) were reported to have normal development until 8-9 years old when both demonstrated progressive difficulties with ambulation, speech articulation, writing, and school performance. Motor and cognitive symptoms progressed until the late teenage years and recent neurocognitive testing demonstrated moderate intellectual disability.

Axial hypotonia was noted in 93.3% (14/15) of patients. Appendicular spasticity and dystonic movements were seen in 86.7% (13/15). These symptoms were especially prominent in two siblings (6-1, 6-2) who experienced generalized dystonia and moderate dysarthria due to involvement of the oromandibular muscles.

Brain MRI demonstrated cerebellar hypoplasia involving the vermis more than the cerebellar hemispheres in 86.7% of patients (Fig 2). Multiple severely affected patients (3-1, 3-2, 4, 5) had strikingly severe vermian hypoplasia. In some patients, additional brain abnormalities were observed, including hypomyelination (5), cerebral atrophy (3-1, 3-2), thin corpus callosum (3-1, 3-2, 5), and enlarged ventricles (5, 7). Progressive atrophy involving the cerebrum, cerebellum

and/or basal ganglia was seen in four patients (2, 3-1, 4 and 5). Microcephaly was present in 28.6% of patients (4/14, 2, 3-1, 3-2, 5).

Epilepsy was present in 60.0% (9/15) of patients with an age of onset ranging from 20 days to 5 years. Reported seizure types were varied and included focal motor seizures (3/7; 43%), generalized tonic-clonic seizures (2/7; 29%), hemifacial clonic seizures (2/7; 29%), generalized myoclonic seizures (1/7; 14%), epileptic (infantile) spasms (1/7; 14%), atonic seizures (1/7; 14%) and atypical absence seizures (1/7; 14%). Epilepsy was drug-resistant in 3/9 (33%) and drug-responsive in 5/9 (56%). One patient with seizures was not treated with anti-epileptic drugs (AEDs). Two of the subjects with drug-resistant epilepsy experienced multiple seizures daily. AEDs that were trialed included valproate (5/9), levetiracetam (3/9), clobazam (2/9), gabapentin (1/9), carbamazepine (1/9), phenobarbital (1/9), topiramate (1/9), and vigabatrin (1/9). The combination of valproate and levetiracetam or clobazam was reported to be effective in three patients (8-1, 8-2, 11-1).

Cataracts were present in 66.7% (10/15) of patients. Four reported mature cataracts and two had posterior cataracts. Feeding difficulties were present in seven patients, with one individual (2) requiring G-tube placement. Dysmorphic features were reported in some patients (Supplementary Table 1 and 3), though no recognizable facial gestalt or pattern was appreciated.

DISCUSSION

We report sixteen patients with a novel autosomal recessive disease due to pathogenic variants in *MED27* consisting of global developmental delay, axial hypotonia, spastic tetraplegia, dystonia, cerebellar hypoplasia, seizures, and cataracts (Table 1). Missense variants were more commonly identified than frameshift and splicing variants. Three missense variants associated

with milder phenotypes [c.188T>G (p.Val63Gly), c.776C>T (p.Pro259Leu), and c.878C>T (p.Pro293Leu)]. The c.188T>G variant is distinct as it is the only missense variant located outside the C-terminal region where all other missense variants clustered (Fig 1).

The Mediator complex is composed of 25 (yeast) or 30 (human) subunits that form four modules: head, middle, tail, and CDK8 kinase [2, 15]. MED27 is a metazoan-specific Mediator subunit sitting at the junction of the head and tail modules of the complex[15]. *Med27/Crsp34* loss-of-function in zebrafish disrupts dopaminergic amacrine cells and serotonergic neurons at 2.5 dpf (days post-fertilization) [16]. Mutant embryos had a reduction of head, eye, and jaw size, and died around 6 dpf. In flies, *Med27* knockout caused lethality at the pupal stage [17, 18]. Similarly, chickens carrying homozygous *MED27* insertional truncating mutations were born at less than expected Mendelian ratios, suggesting partial embryonic lethality in homozygotes[19]. Although the specific biological function of MED27 remains unknown, it clearly plays an essential role in early embryonic and neuronal development.

Similar to the effect of knockout mutations, homozygous C-terminal Flag-tagged *Med27* mutations were also lethal in fruit flies, suggesting a critical role of the MED27 C-terminal domain.[18] In the patients reported here, 6/7 missense variants clustered near the C-terminus of the MED27 protein. One study indicated MED27 C-terminal domain has a C2-H2 zinc finger motif [6]. MED27 interacts extensively with multiple subunits in the head module, including MED17 [20]. Cryo-electron microscopy of the *S. pombe* head module reveals that Med27 connects Med18/20 with Med17 [15]. Interestingly, compared with other Mediator complex-associated diseases, *MED27* and *MED17*-related diseases are most similar. Both are autosomal recessive and characterized by psychomotor developmental delay, spasticity, seizures, progressive microcephaly and cerebellar atrophy (Supplementary Table 4). One intriguing hypothesis would be that variants in MED27 disrupt its interaction with other Mediator

complex subunits, such as MED17, leading to similar disease phenotypes. Additional studies on the functional consequence of *MED27* variants are needed to further address the molecular mechanisms underlying the disease.

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Author contributions

L.M. and Y.Y. contributed to study conception and design. L.M. drafted the manuscript text and prepared the figures. All authors contributed to patient clinical data and exome sequencing data acquisition and analysis, and manuscript review and revision.

Potential Conflicts of Interest:

L.M. is employee of Baylor Genetics, in which exome sequencing of patient 1 was performed. The Department of Molecular and Human Genetics at Baylor College of Medicine receives revenue from clinical genetic testing conducted at Baylor Genetics (BG) Laboratories. Other authors have no potential conflicts to report.

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FIGURES

Figure 1 Nucleotide and amino acid changes in patients with *MED27* biallelic variants.

(A) Schematic representation of *MED27* gene structure (not drawn to scale) and nucleotide position of eleven variants identified in eleven families. (B) Alignment of human MED27 protein sequence with other model organisms including mouse, rat, pig, bovine, Xenopus, zebrafish and Drosophila.

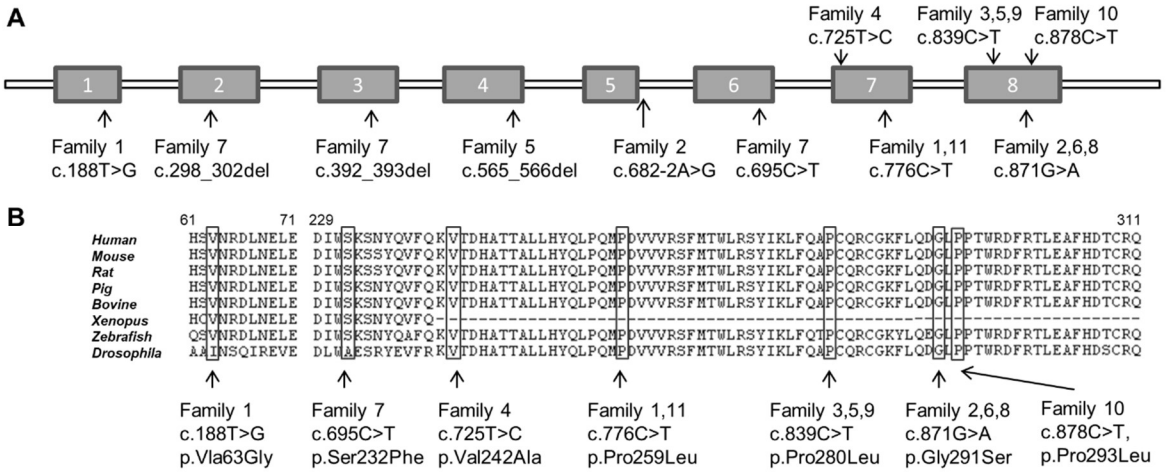


Figure 2 Brain imaging of patients with MED27 biallelic variants.

Brain imaging from ten patients. Sagittal T1 +/- coronal T2 brain MRI imaging of nine patients (A-K, M). Sagittal brain computed tomography (CT) imaging is provided for patient 9 (L). (A) Patient 1 (2 years) showing mild cerebellar vermian hypoplasia (ARROW). (B) Patient 2 (2 years 11 months) showing normal-appearing corpus callosum and cerebellar vermian hypoplasia (ARROW). (C) Patient 3-1 (1 year) showing thin corpus callosum (ARROWHEAD) and severe cerebellar hypoplasia (ARROW). (D) Patient 3-1 (2 years 5 months), showing thin corpus callosum (ARROWHEAD) and progressive cerebellar atrophy (ARROW). (E) Patient 3-2 (11 years 9 months) showing thin corpus callosum (ARROWHEAD) and cerebellar vermian hypoplasia (ARROW) (F) Patient 4 (1 year), showing cerebellar vermian hypoplasia (ARROW).

(G) Patient 4 (2 years 5 months) showing progressive cerebellar atrophy (ARROW) and cortical gyral simplification. (H) Patient 5 (1 year 2 months), showing thin corpus callosum (ARROWHEAD), severe cerebellar hypoplasia (ARROW) with flattening of the pons and hypomyelination. (I) Patient 6-1 (34 years) showing mild cerebellar vermian hypoplasia (ARROW). (J) Patient 7 (1 year) showing cerebellar vermis hypoplasia (ARROW). (K) Patient 8-1 (6 years 3 months) showing cerebellar vermian hypoplasia (ARROW). (L) Patient 9 showing cerebellar hypoplasia (ARROW) (M) Patient 11-1 showing cerebral atrophy and normal cerebellum.

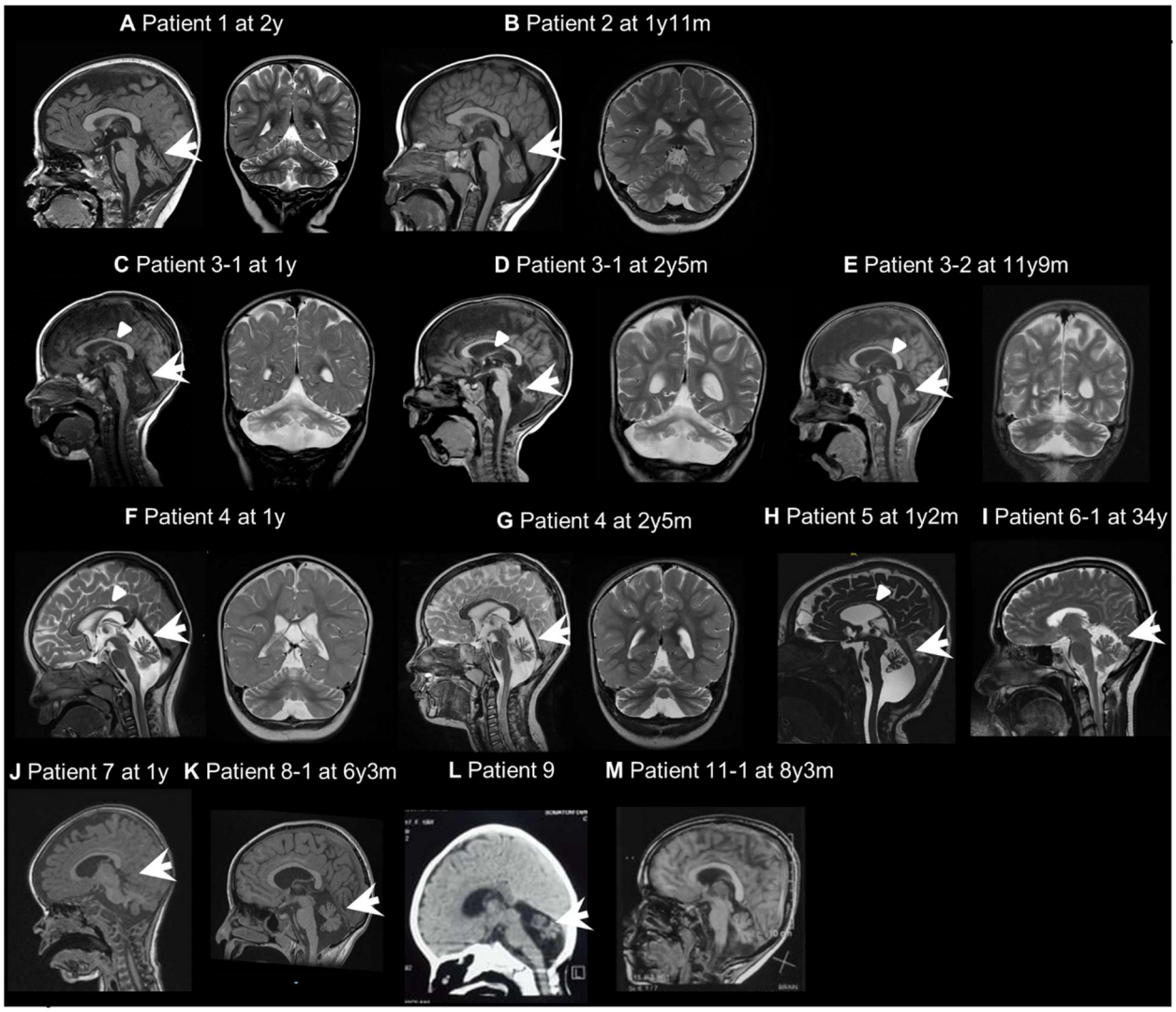


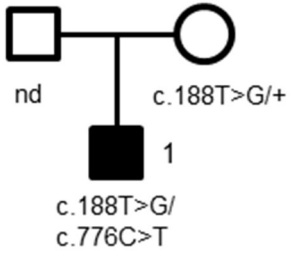
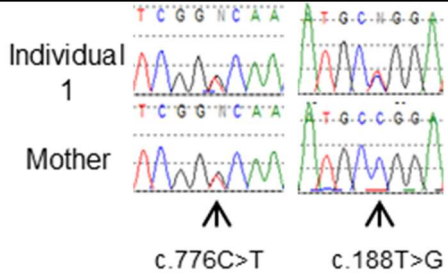
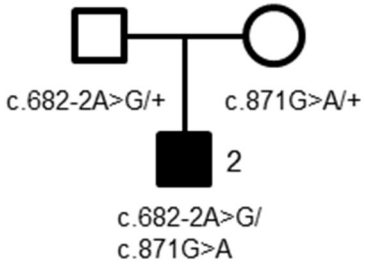
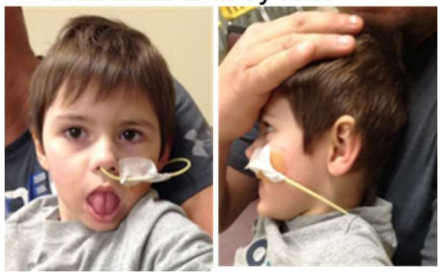
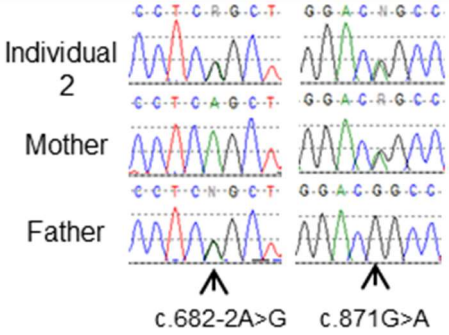
Table 1 Clinical features of patients with biallelic *MED27* variants

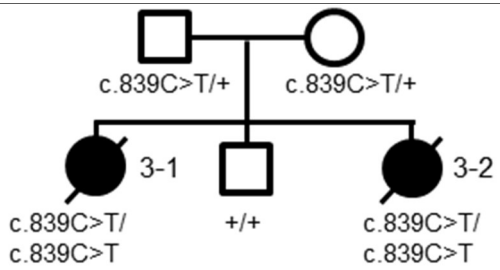
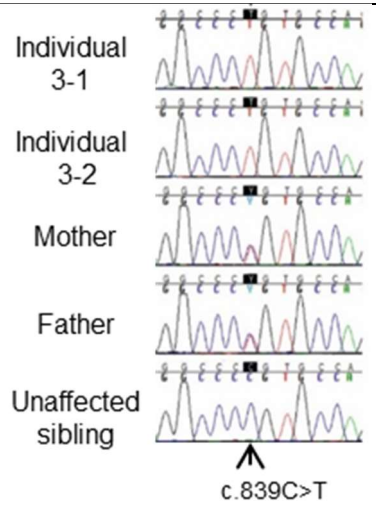
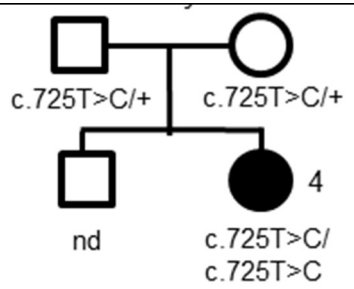
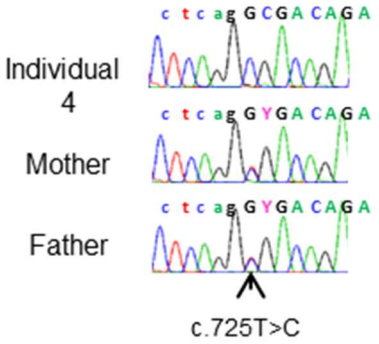
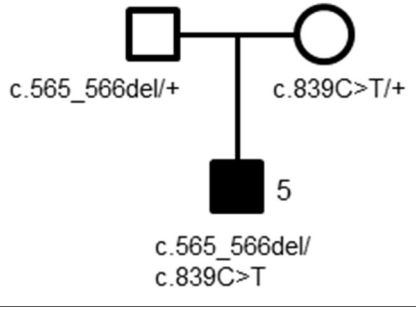
Clinical findings	Severe (n=8)	Moderate (n=5)	Family 6 (n=2)	Overall*
Gender				5M/10F
Intellectual disability	100% (7/7)	100% (5/5)	100% (2/2)	100% (14/14)
Central hypotonia	100% (8/8)	80.0% (4/5)	100% (2/2)	93.3% (14/15)
Distal limb spasticity/dystonic movement	100% (8/8)	60.0% (3/5)	100% (2/2)	86.7% (13/15)
Delayed motor/speech development	100% (8/8)	100% (5/5)	0% (0/2)	86.7% (13/15)
Cerebellar hypoplasia	100% (8/8)	75.0% (3/4)	50.0% (1/2)	85.7% (12/14)
Cataracts	87.5% (7/8)	20.0% (1/5)	100% (2/2)	66.7% (10/15)
Epilepsy	87.5% (7/8)	40.0% (2/5)	0% (0/2)	60.0% (9/15)
Feeding issues	42.8% (3/7)	60.0% (3/5)	100% (2/2)	57.1% (8/14)
Microcephaly	50.0% (4/8)	0.0% (0/4)	0% (0/2)	28.6% (4/14)
Developmental regression	25.0% (2/8)	20.0% (1/5)	100% (2/2)	33.3% (5/15)

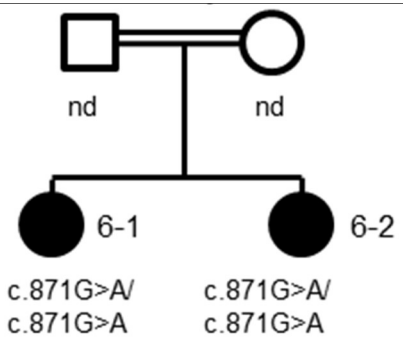
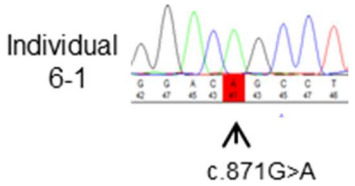
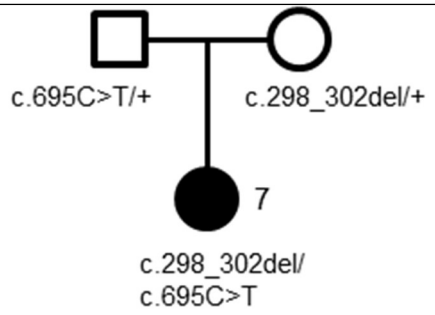
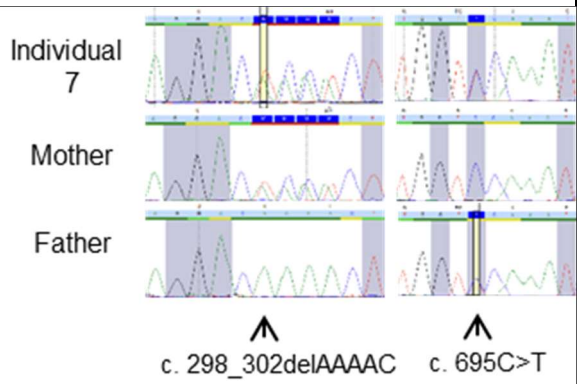
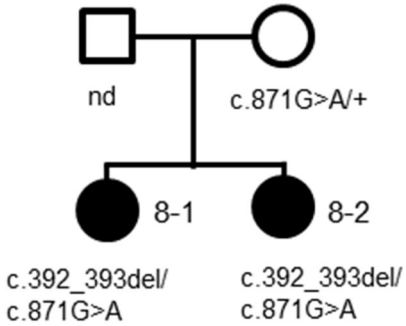
*Patient 10-2 had dual diagnosis with variants in both *MED27* and *COG6*. Therefore the case is excluded in this summary of *MED27*-associated phenotypes.

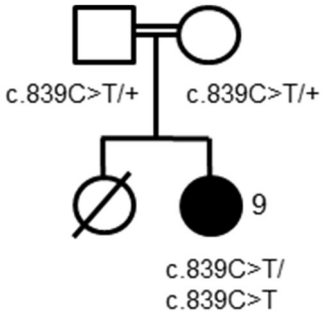
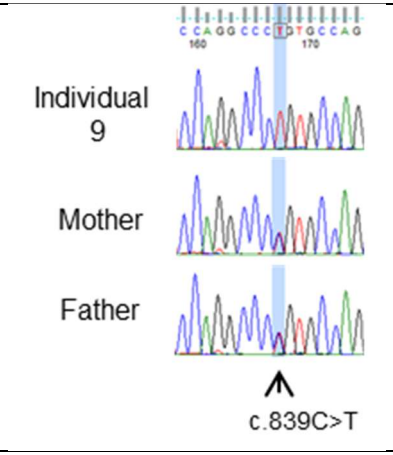
Key: M, male; F, female

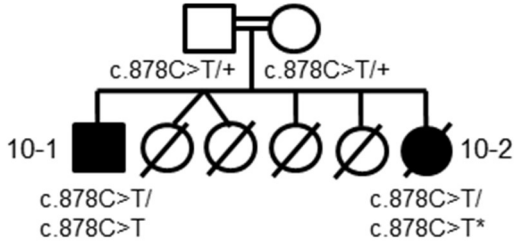
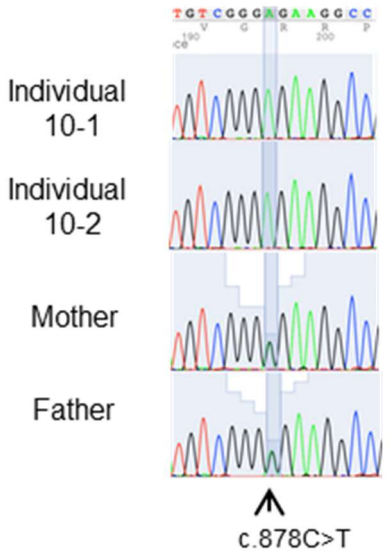
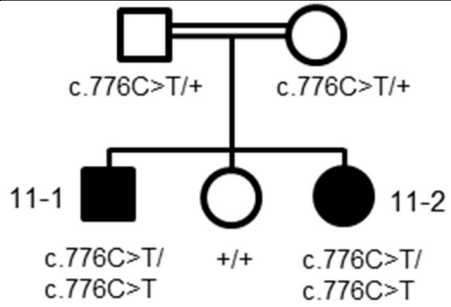
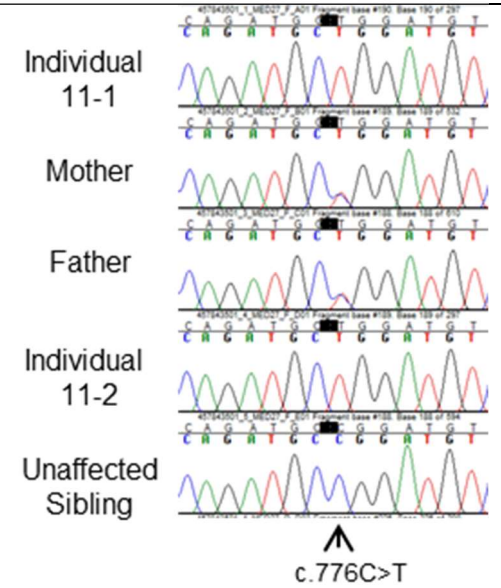
Supplementary Table 1 Family pedigrees, facial images of individuals with biallelic *MED27* variants and Sanger sequencing data

Family	Pedigree	Facial photos	*MED27* variants (Sanger sequencing)
1		Individual 1 at 7y6m 	
2		Individual 2 at 3y10m 	

3			
4			
5		Individual 5 at 1y2m 	

6			
7		Individual 7 at 2y3m 	
8		Individual 8-1 at 8y  Individual 8-2 at 6y	

			
9	 c.839C>T/+ c.839C>T/+ 9 c.839C>T/ c.839C>T		 Individual 9 Mother Father c.839C>T

10		Individual 10-1 	
11		Individual 11-1 at 9y5m  Individual 11-2 at 6y9m 	

Patients and/or legal guardians have consented for publication of facial images.

Supplementary Table 2 *MED27* Variant characteristics identified in this study

Family	Exon/Intron	cDNA	Protein	Variant type	GERP++_RS	PolyPhen2	SIFT	Mutation Taster	CADD Phred score	gnomAD database
1	Exon 1	c.188T>G	p.Val63Gly	Missense	5.07	D	D	D	28.2	2/245554
7	Exon 2	c.298_302del	p.Lys100Serfs*4	Frameshift	NA	NA	NA	NA	NA	0
8	Exon 3	c.392_393del	p.Gln131Leufs*7	Frameshift	NA	NA	NA	NA	NA	0
5	Exon 4	c.565_566del	p.M189Afs*21	Frameshift	NA	NA	NA	NA	NA	0
2	Intron 5	c.682-2A>G	N/A	Splice site	5.62	NA	NA	NA	34	2/239080
7	Exon 6	c.695C>T	p.Ser232Phe	Missense	5.62	P	D	D	31	0
4	Exon 7	c.725T>C	p.Val242Ala	Missense	5.52	B	D	D	26.3	0
1,11	Exon 7	c.776C>T	p.Pro259Leu	Missense	5.52	D	D	D	34	0
3,5,9	Exon 8	c.839C>T	p.Pro280Leu	Missense	5.3	P	D	D	34	14/241660
2,6,8	Exon 8	c.871G>A	p.Gly291Ser	Missense	5.3	D	D	D	27.5	3/235830
10	Exon 8	c.878C>T	p.Pro293Leu	Missense	5.46	D	D	D	29.9	0

Reference sequence: NM_004269.3; D: probably damaging; P: possibly damaging; B: benign

Supplementary Table 3 Summary of clinical features in patients with biallelic *MED27* variants.

Individual	**1**	**2**	**3-1**	**3-2**	**4**	**5**	**6-1**	**6-2**
Disease severity	Moderate	Severe	Severe	Severe	Severe	Severe	Family 6	Family 6
Gender	Male	Male	Female	Female	Female	Male	Female	Female
Ethnicity	Hispanic	Western European	Finnish	Finnish	Turkish/Syrian	European, French Canadian	Kuwaiti	Kuwaiti
Consanguinity	No	No	No	No	No	No	Yes	Yes
Birth history	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Age at measurement	10y 7m	5y 10m	11y, (passed at 13y)	8m (passed at 5y)	2y 11m	20m	36y	42y
Height	23%ile (-0.8SD)	52%ile (-0.1SD)	<1%ile (-3SD)	20%ile (-0.84SD)	49%ile (-0.03SD)	35%ile (-0.39SD)	NA	<1%ile (-2.8 SD)
Weight	14%ile (-1.1SD)	12%ile (-1.3SD)	43%ile (-0.18SD)	18%ile (-0.92SD)	27%ile (-0.61SD)	33%ile (-0.44SD)	NA	23%ile (-0.74SD)
FOC	2%ile (-1.99SD)	<1%ile (-3.3SD) (at 33m)	<1%ile (-5SD)	<1%ile (-3.5SD)	5%ile (-1.61SD)	<1%ile (-4.6SD)	Normocephalic	Normocephalic
Microcephaly	-	+	+	+	-	+	-	-
Feeding issues	Difficult swallowing	Swallowing issue, G-tube placement	-	-	severe swallowing issue	Dysphagia	Occasional choking	Occasional choking
Development assessment	Chronological age 10.8y, intelligence <4y, expressive language 23m, receptive language 3y, fine motor 3.5y, visual perceptual ability 4y	NA	NA	NA	NA, felt to be 6m at chronological age of 6y	NA	NA	NA
Motor delay	+, delayed, able to walk	+, cannot sit/walk	+, cannot sit/walk	+, achieved walking but regressed and lost ability to move	+, sit with support, cannot walk	+, poor head control, cannot sit/walk	-	-
Language delay	+, delayed, 10 words at 40 m	+, non-verbal	+, non-verbal	+, non-verbal	+, non-verbal	+, non-verbal (early)	-, progressive dysarthria since age 8	-, progressive dysarthria since age 5
Intellectual disability	Moderate	Profound	Severe	Severe	Profound	NA (early)	Moderate	Moderate

Developmental regression	-	+, at 7y, motor function is worsening	+, onset around 6y	-	-	-	+	+
Central hypotonia	-	+	+	+	+	+	Mild	Mild
Distal spasticity	-	+	+	+	+	+	+	+
Dystonic movement	-, Unsteady gait	+, tongue protrusion	+	+	+, hands	+, tongue protrusion	+, tongue, mandibular, dysarthria, posture	+, tongue, mandibular, dysarthria, posture
Epilepsy	-, seizure like activity in infancy, normal EEG	+, onset 2y	+, onset 3y	+, onset 2y	+, onset 2-3y,	+, onset 8m, abnormal EEG, consistent with modified hypsarrhythmia	-	-
History of Drug-resistant epilepsy	NA	-	+ (at 6 y then became seizure free on AEDs at 11y)	-	+	+	NA	NA
Seizure type(s) and frequency	NA	Generalized tonic-clonic seizure	N/A	N/A	Myoclonic during sleep, atypical absences during daytime; multiple daily.	Focal motor (tonic), absence, epileptic (infantile) spasms; multiple daily.	NA	NA
AEDs	NA	GBP and low dose LEV	VPA, TPM and PB	VPA	N/A	Steroids, ACTH, Vigabatrin, LEV and CBZ	NA	NA
Brain MRI cerebellar atrophy	mild	+	+	+	+, progressive	+	mild	-
Brain MRI others	-	progression of diffuse cerebral atrophy and progression of abnormal signal and atrophy in basal ganglia	cerebral atrophy, thin corpus callosum	cerebral atrophy, thin corpus callosum	cortical gyral simplification	hypomyelination with a thin corpus callosum, progressive *ex vacuo* ventriculomegaly	-	-
Cataracts	-	+, left mature cataract, right incomplete cataract	+, congenital, operated at 7y, type NA	+, congenital, operated at 5m, type NA	+, mature	+, developed at 23m, type not specified	+, mature	+, mature
Dysmorphic features	Large cupped ears with	Prominent eyebrows	-	-	narrow forehead, frontal upsweep	Large ears, slightly upturned	-	-

	thickened helices, long eyelashes, frontal bossing, upswept frontal hair				of hair, synophrys, highly arched broad eyebrows, large eyes, blue sclerae, prominent nasal bridge, anteverted nares, exaggerated cupid bow, widely spaced teeth, long eyelashes, long hyperextensible and broad fingers, hypertrichosis	nose, long eyelashes, full lips		
Other issues	Mild obstructive sleep apnea; Significant drooling, behavioral concerns; Dental concern	Born with loud breathing and diagnosed with laryngomalacia as infant	Peripheral neuropathy both in lower and upper limbs	N/A	Drooling	Severe central sleep apnea, moderate obstructive sleep apnea, laryngomalacia, milk protein allergy, constipation, bilateral hearing loss	Urinary urgency with frequent episodes of incontinence	Urinary urgency with frequent episodes of incontinence
MED27 (NM_004269.3)	c.776C>T (p.Pro259Leu) presumed paternal; c.188T>G (p.Val63Gly) maternal	c.682-2A>G paternal; c.871G>A (p.Gly291Ser) maternal	Homozygous c.839C>T (p.Pro280Leu)	Homozygous c.839C>T (p.Pro280Leu)	Homozygous c.725T>C (p.Val242Ala)	c.565_566del(p.Met189Alafs*21) paternal; c.839C>T (p.Pro280Leu) maternal	Homozygous c.871G>A (p.Gly291Ser)	Homozygous c.871G>A (p.Gly291Ser)
Additional diagnosis								

Individual	7	8-1	8-2	9	10-1	10-2*	11-1	11-2	Total
Disease severity	Severe	Severe	Severe	Moderate	Moderate	NA	Moderate	Moderate	8 severe; 5 moderate
Gender	Female	Female	Female	Female	Male	Female	Male	Female	5M/11F
Ethnicity	North-western European	Algerian	Algerian	Iraqi	Turkish	Turkish	Pakistani	Pakistani	
Consanguinity	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	
Birth history	Normal	Normal	Normal	Normal	Normal	Polyhydramnios, SGA, respiratory distress	Normal	Preterm	
Age at measurement	2y 3m	7y 4m	5y 11m	10m	20 y	16 d, (passed at 3m)	9y 5m	6yr 9mo	
Height	2%ile (-2.04SD)	18%ile (-0.3SD)	20%ile (-0.82SD)	NA	25%ile (-0.67SD)	50%ile (0SD)	44%ile (-0.16SD)	21%ile (-0.82SD)	
Weight	41%ile (-0.24SD)	14%ile (-0.65SD)	29%ile (-0.57SD)	>99%ile (>=+3.0SD)	3%ile (-1.88SD)	25%ile (-0.67SD)	75%ile (+0.67SD)	25%ile (-0.68SD)	
FOC	18%ile (-0.92SD)	12%ile (-1.16SD)	13%ile (-1.12SD)	NA	<3%ile, <-1.88SD	<3%ile, <-1.88SD	53%ile (+0.08SD)	27%ile (-0.61SD)	4/14
Microcephaly	-	-	-	-	-	-	-	-	4/14
Feeding issues	NA	-	-	+	-	+	-	Mild	8/14
Development assessment	Bailey scale: chronological age 16m, cognition 7m	NA	NA	NA	NA, felt to be 5-6y level at chronological age of 20y	NA	Protage guide: Chronological age 9.5y, Cognition 66.4m, Socialization 57.8m, Self-help 61.5m, Motor 61.6m, Language 48m	Protage guide: Chronological age 7.1y, Cognition 67m, Socialization 59m, Self-help 68m, Motor 66m, Language 59m	
Motor delay	+, cannot sit/walk	+	+, cruising	+, walk with support	+, able to walk	NA	+, delayed, able to walk	+, delayed, able to walk	13/15
Language delay	+, non-verbal	+, delayed	+, non-verbal	+, delayed, one word at 22m	+, delayed, able to read	NA	+, delayed	+, delayed	13/15
Intellectual disability	Severe	Profound	Profound	Moderate	Moderate	N/A	Moderate	Mild	14/14

Developmental regression	-	-	-	-	+	NA	-	-	5/15
Central hypotonia	+	+	+	+	+	+	Mild	Mild	14/15
Distal spasticity	+	+	+	+	-	-	+	mild	13/15
Dystonic movement	+, tongue protrusion, stereotypical movements	+	+	+	-	NA	+, tongue fasciculations	+, tongue fasciculations	13/15
Epilepsy	-, disturbed EEG	+, onset 5y, abnormal EEG	+, onset 5y, abnormal EEG	+, onset at 18m; normal EEG	-	-	+, onset at 20d	-	9/15
History of Drug-resistant epilepsy	NA	-	-	Unable to assess	NA	NA	-	NA	3/15
Seizure type(s) and frequency	NA	Hemifacial clonic seizures; none while on AEDs	Hemifacial clonic; none while on AEDs	Focal motor with impaired awareness	NA	NA	Atonic and right- sided focal; once every week till 6Y when he experienced a prolonged GTC; Last seizure was 7Y on AEDs	NA	
Current AEDs	NA	VPA and CLB effective; LEV previously used and only partially effective	VPA and CLB effective; LEV previously used and only partially effective	none	NA	NA	VPA and LEV for last 3yrs (effective)	NA	
Brain MRI cerebellar atrophy	mild	+	+	+	mild	mild	-	NA	12/14
Brain MRI others	-	-	-	-	-	-	cerebral atrophy with ischemic demyelination in parietal and occipital white matter	-	
Cataracts	-	+, congenital	+, congenital	-	+, posterior cataracts	+, posterior cataracts	-	-	10/15

Dysmorphic features	frontal bossing, sparse medial eyebrow, wide nasal bridge, hypoplasia of the primary teeth, Deep palmar creases	no (long narrow face)	-	-	micrognathia	-	-	Hypertelorism, flat nasal bridge, low set ears, broad nose, convergent squint	
Other issues	Drooling	Drooling	Drooling	NA	Knee and wrist contractures, dysmetria	Hand and feet contractures	Micropenis with normal testosterone levels, mild drooling	Drooling	
MED27 (NM_004269.3)	c.695C>T (p.Ser232Phe) paternal; c.298_302del (p.Lys100Serfs*4) maternal	c.871G>A (p.Gly291Ser) maternal; c.392_393del(p.Gln131Leufs Ter7) presumed paternal	c.871G>A (p.Gly291Ser) maternal; c.392_393del(p.Gln131Leufs Ter7) presumed paternal	Homozygous c.839C>T (p.Pro280Leu)	Homozygous c.878C>T: p.Pro293Leu; both parents are heterozygous	Homozygous c.878C>T: p.Pro293Leu; both parents are heterozygous	Homozygous c.776C>T, p.Pro259Leu	Homozygous c.776C>T, p.Pro259Leu	
Additional diagnosis						Homozygous COG6 c.726del (p.Cys242Trpfs*7)			

*Patient has dual diagnosis with variants in both *MED27* and *COG6*, not counted towards phenotype summary in Table 1

AED: antiepileptic drug; CBZ: carbamazepine; CLB: clobazam; EMG: electroneuromyography; GBP: gabapentin; LEV: levetiracetam; NA: not available; NCS: nerve conduction studies; PB: phenobarbital; SSEP: somato-sensory evoked potentials; TPM: topiramate; VPA: valproate

Supplementary Table 4 Summary of Mediator subunits known to be associated with human diseases

Gene	Module	Inheritance	Disease	Phenotype description
MED12	Kinase	XL	Lujan-Fryns syndrome [MIM: 309520]; Ohdo syndrome, X-linked [MIM: 300895]; Opitz-Kaveggia syndrome [MIM: 305450]	dysmorphic features, intellectual disability, relative macrocephaly, hypotonia, and constipation, partial agenesis of the corpus callosum
MED13	Kinase	AD	Intellectual developmental disorder 61 [MIM: 618009]	intellectual disability and/or developmental delays, including speech delays or disorders, autism
MED13L	Kinase	AD	Mental retardation and distinctive facial features with or without cardiac defects (MIM: 616789); Transposition of the great arteries, dextro-looped 1 (MIM: 608808)	delayed psychomotor development, poor speech acquisition, distinctive dysmorphic facial features, variable penetrance of cardiac malformations
MED17	Head	AR	Microcephaly, postnatal progressive, with seizures and brain atrophy (MIM: 613668)	microcephaly, postnatal progressive, with seizures and brain atrophy
MED23	Tail	AR	Mental retardation, autosomal recessive 18 (MIM: 614249)	mild to moderate ID, no dysmorphic
MED25	Unassigned	AR	Basel-Vanagait-Smirin-Yosef syndrome (MIM616449)	severely delayed psychomotor development resulting in intellectual disability, as well as variable eye, brain, cardiac, and palatal abnormalities.
MED27	Head/ Tail	AR	Current report	global developmental delay, central hypotonia, spasticity, dystonic movements, cerebellar hypoplasia, epilepsy, and cataract

AD, autosomal dominant; AR, autosomal recessive; XL, X-linked