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# *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 2 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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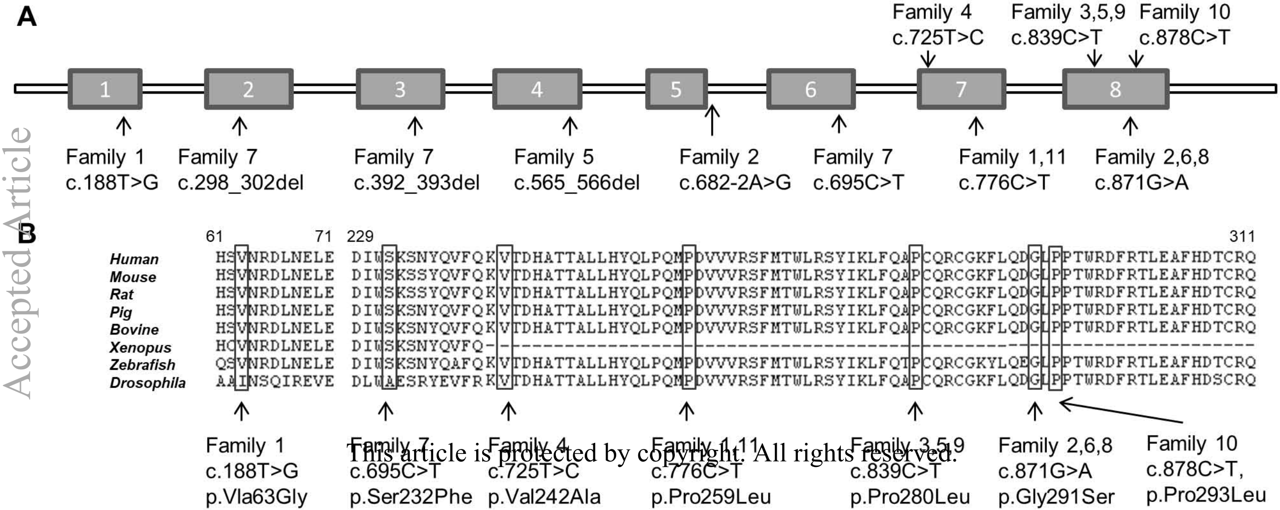
## FIGURE LEGENDS

**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.


A
Family 4
c.725T>C
Family 3,5,9
c.839C>T
Family 10
c.878C>T
1
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7
8
Family 1
c.188T>G
Family 7
c.298_302del
Family 7
c.392_393del
Family 5
c.565_566del
Family 2
c.682-2A>G
Family 7
c.695C>T
Family 1,11
c.776C>T
Family 2,6,8
c.871G>A
B
61
71
229
311
Human HSVNRDLNELE DIWSKSNYQVFQKVTDHATTALLHYQLPQMPDVVVRSFMTWLRSYIKLFQAPCQRCGKFLQDGLPPTWRDFRTLEAFHDTCRQ
Mouse HSVNRDLNELE DIWSKSSYQVFQKVTDHATTALLHYQLPQMPDVVVRSFMTWLRSYIKLFQAPCQRCGKFLQDGLPPTWRDFRTLEAFHDTCRQ
Rat HSVNRDLNELE DIWSKSSYQVFQKVTDHATTALLHYQLPQMPDVVVRSFMTWLRSYIKLFQAPCQRCGKFLQDGLPPTWRDFRTLEAFHDTCRQ
Pig HSVNRDLNELE DIWSKSNYQVFQKVTDHATTALLHYQLPQMPDVVVRSFMTWLRSYIKLFQAPCQRCGKFLQDGLPPTWRDFRTLEAFHDTCRQ
Bovine HSVNRDLNELE DIWSKSNYQVFQKVTDHATTALLHYQLPQMPDVVVRSFMTWLRSYIKLFQAPCQRCGKFLQDGLPPTWRDFRTLEAFHDTCRQ
Xenopus HCVNRDLNELE DIWSKSNYQVFQ-----------------------------------------------------------------------
Zebrafish QSVNRDLNELE DIWSKSNYQAFQKVTDHATTALLHYQLPQMPDVVVRSFMTWLRSYIKLFQTPCQRCGKYLQEGLPPTWRDFRTLEAFHDTCRQ
Drosophila AAINSQIREVE DLWAESRYEVFRKVTDHATTALLHYQLPQMPDVVVRSFMTWLRSYIKLFQAPCQRCGKFLQDGLPPTWRDFRTLEAFHDSCRQ
Family 1
c.188T>G
p.Vla63Gly
Family 7
c.695C>T
p.Ser232Phe
Family 4
c.725T>C
p.Val242Ala
Family 1,11
c.776C>T
p.Pro259Leu
Family 3,5,9
c.839C>T
p.Pro280Leu
Family 2,6,8
c.871G>A
p.Gly291Ser
Family 10
c.878C>T,
p.Pro293Leu

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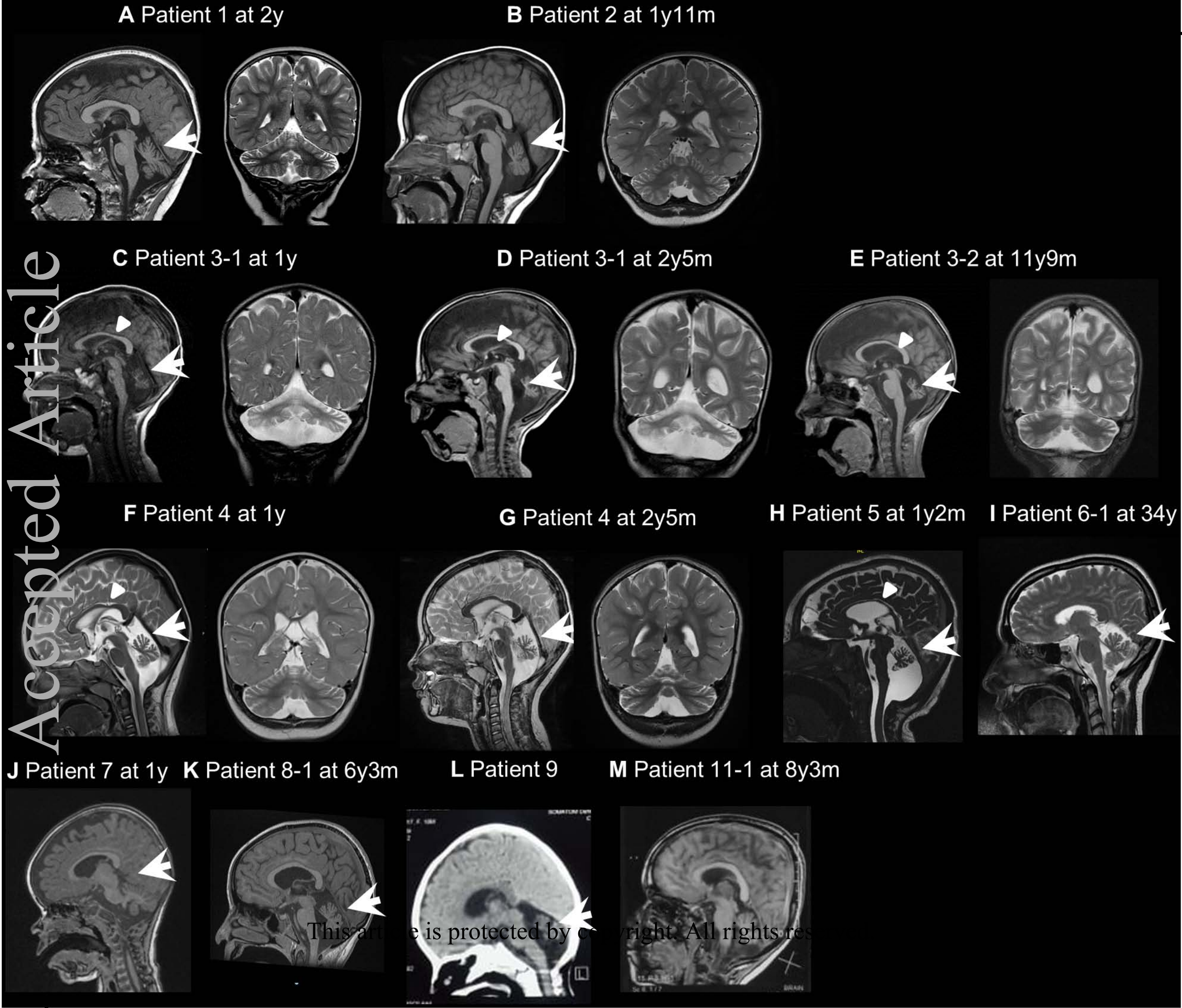

**A** Patient 1 at 2y

**B** Patient 2 at 1y11m

**C** Patient 3-1 at 1y

**D** Patient 3-1 at 2y5m

**E** Patient 3-2 at 11y9m

**F** Patient 4 at 1y

**G** Patient 4 at 2y5m

**H** Patient 5 at 1y2m

**I** Patient 6-1 at 34y

**J** Patient 7 at 1y

**K** Patient 8-1 at 6y3m

**L** Patient 9

**M** Patient 11-1 at 8y3m

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