

# TRAPPC2L-related disorder: first homozygous protein-truncating variant and further delineation of the phenotype

Mario Abaji, Cecile Mignon-Ravix, Svetlana Gorokhova, Pierre Cacciagli, Jeremie Mortreux, Florence Molinari, Brigitte Chabrol, Sabine Sigaudy, Laurent Villard, Florence Riccardi

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4	1	TRAPPC2L-related disorder: first homozygous protein-truncating variant and
5 6 7	2	further delineation of the phenotype
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33 34	18	Running title: Further delineation of the TRAPPC2L-related disorder
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#### ABSTRACT/SUMMARY

The TRAPP (TRAfficking Protein Particle) complexes are evolutionarily conserved tethering factors, involved in the intracellular transport of vesicles for secretion and autophagy processes. Pathogenic variants in eight genes (of 14) encoding TRAPP proteins are involved in ultra-rare human diseases, called TRAPPopathies. Seven of them are autosomal recessive neurodevelopmental disorders with overlapping phenotypes. Since 2018, two homozygous missense variants in TRAPPC2L have been reported in five individuals from three unrelated families with early-onset and progressive encephalopathy, with episodic rhabdomyolysis. We now describe the first pathogenic protein-truncating variant in the TRAPPC2L gene found at a homozygous state in two affected siblings. This report provides key genetic evidence invaluable to establishing the gene-disease relationship for this gene and important insights into the TRAPPC2L phenotype. Regression, seizures, and postnatal microcephaly initially described are not constant features. Acute episodes of infection do not contribute to the neurological course. HyperCKemia is part of the clinical picture. Thus, TRAPPC2L syndrome is mainly characterised by a severe neurodevelopmental disorder and a variable degree of muscle involvement, suggesting that it belongs to the clinical entity of rare congenital muscular dystrophies.

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1 INTRODUCTION

The TRAPP (Trafficking Protein Particle) complexes are tethering factors allowing initial contact between the carrier and the target membrane. They are one of the components that ensure the specificity of protein distribution in different intracellular compartments of eukaryotic cells. From yeast to humans, TRAPP heteromeric complexes are evolutionarily conserved (1). They are composed of eight core subunits and six complex-specific subunits. In humans, there are two TRAPP complexes: TRAPP II and TRAPP III. The common core is composed of TRAPPC1, TRAPPC2, TRAPPC2L, TRAPPC3, TRAPPC4, TRAPPC5, TRAPPC6A, and TRAPPC6B. In addition, TRAPP II includes TRAPPC9 and TRAPPC10 while TRAPP III includes TRAPPC8, TRAPPC11, TRAPPC12, and TRAPPC13. They act as guanine nucleotide exchange factors for Rab proteins which regulate the secretory and endocytic recycling pathways. It has been shown that the TRAPP core acts on both Rab1 and Rab11 while the TRAPP III complex is specific to Rab1, and that the additional subunits of each specific complex contribute to define Rab specificity (2).

- High-throughput DNA sequencing for patients with rare and severe disorders has led to the identification of pathogenic variants in eight TRAPP genes resulting in a range of human disorders named TRAPPopathies: TRAPPC2 (3), TRAPPC2L (4), TRAPPC4 (5), TRAPPC6B (6), TRAPPC9 (7), TRAPPC10 (8), TRAPPC11 (9), and TRAPPC12 (10). Seven of them are autosomal recessive neurodevelopmental disorders with overlapping phenotypes. Their study helped to understand the function of TRAPP subunits and demonstrated that there is no clear correlation between the phenotypes associated with specific or core subunits of theses complexes. The TRAPPC2L gene (Trafficking Protein Particle Complex 2-Like, MIM\*610970)
- encodes a core subunit of the TRAPP complexes. To date, two pathogenic missense variants in this gene were identified in five patients from three unrelated families (4,11). The common feature is a neurodevelopmental disorder starting in the first year of life. However, important differences were noted regarding the clinical presentation. Two patients showed regression and episodes of rhabdomyolysis during acute infectious episodes, while the other three patients had a moderate non-syndromic intellectual

<ul> <li>disability from the outset (Table S.1). Thus, a detailed description of additional pati</li> <li>is important to better define the phenotype, in particular muscle alteration. Here, we</li> <li>report two siblings in whom we identified a homozygous nonsense variant in</li> <li><i>TRAPPC2L</i>, thus further clarifying the gene-disease relationship and the phenotype</li> <li>associated with this gene.</li> </ul>	ents
<ul> <li>is important to better define the phenotype, in particular muscle alteration. Here, we</li> <li>report two siblings in whom we identified a homozygous nonsense variant in</li> <li><i>TRAPPC2L</i>, thus further clarifying the gene-disease relationship and the phenotype</li> <li>associated with this gene.</li> </ul>	!
<ul> <li>report two siblings in whom we identified a homozygous nonsense variant in</li> <li><i>TRAPPC2L</i>, thus further clarifying the gene-disease relationship and the phenotype</li> <li>associated with this gene.</li> </ul>	:
<ul> <li><i>TRAPPC2L</i>, thus further clarifying the gene-disease relationship and the phenotype</li> <li>associated with this gene.</li> </ul>	
10 5 associated with this gene.	
12 6	
12 0	
13 14 7 MATERIALS AND METHODS	
15 8 Patients	
17 9 Two siblings with a neurodevelopmental disorder were referred to the Medical Gen	tics
19 10 Department of La Timone Marseille Public University Hospital in 2009. Peripheral t	ood
20 21 11 samples were collected from the affected siblings IV-3 and IV-4, their healthy broth	r IV-
<sup>22</sup> 12 2, and their parents III-6 and III-7 (Figure 1.A). Written informed consent for geneti	;
13 analyses and publication was obtained for each individual.	
<sup>25</sup> <sub>26</sub> 14 DNAs were prepared, stored, and delivered for research by the Biological Resourc	÷
$\frac{27}{28}$ 15 Center of La Timone Marseille Public University Hospital, according to the French	
$^{29}_{30}$ 16 regulation (CRB AP-HM Biobank; NF S96-900 & ISO 9001 v2015 Certifications).	
17 Genetic analyses	
<sup>32</sup> 18 Array CGH and Intellectual Disability (ID) gene panel Sequencing	
$\frac{34}{35}$ 19 Patient IV-3 was first analyzed with routine CGH testing using 4x180K whole-genor	ıe
<sup>36</sup> 20 oligonucleotide microarray and SureSelect XT Inherited Diseases Panel sequencin	J
21 (Agilent Technologies, Santa Clara, CA, USA) following the manufacturer's protoco	
<sup>39</sup> <sub>40</sub> 22 Next-Generation Sequencing	
$\frac{41}{42}$ 23 Whole-exome sequencing of proband IV-3, his father (III-7), and a healthy brother	V-2)
43 24 (Figure 1.A) was performed using the SeqCap EZ MedExome sequencing kit (Rock	е
44 45 25 NimbleGen, Basel, Switzerland) following standard protocols. Paired-end 2x75 bas	<del>}</del> -
$\frac{46}{47}$ 26 pair (bp) sequencing was performed on a NextSeq 500 sequencing apparatus (Illur	nina
<sup>48</sup> 27 Inc., USA). Sequencing data were analyzed according to an autosomal recessive	
50 28 inheritance pattern. Validation and segregation analysis of the TRAPPC2L variant	/as
$_{52}^{51}$ 29 done by Sanger sequencing using the following primers: TRAPPC2L-F:	
53 30 TGTGTGTATGTGGCAAGGTC and TRAPPC2L-R: GTGGCTTTAAAACCTCTGCC	. No
55 31 other candidate variant has been identified.	
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4	1	
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9 10	4	The patients were born after uneventiul pregnancies to healthy consanguineous parents
11	5	(Figure 1.A).
12 13	6	Patient IV-3 is a boy born at term. Birth measurements were within the normal range:
14 15	7	weight 3.450 kg ( $45^{\text{th}}$ percentile), height 47.5 cm ( $5^{\text{th}}$ ), and OFC 35 cm ( $48^{\text{th}}$ ). Hypotonia,
16	8	poor contact, and developmental delay were noticed during the first months of life. From
17 18	9	2 to 6 years, growth measurements were normal. Walking and language were absent.
19	10	Stereotyped hand movements and self-injurious behavior were observed. Mild
20 21	11	dysmorphic features including oval and floppy face, strabismus, prominent nose, and a
22 23	12	wide mouth with everted inferior lip, hyperlaxity, and OFC remained normal. At the last
24	13	medical evaluation, the patient had severe intellectual disability (ID) with no walking or
25 26	14	speech. Brain MRI scan (age 1-3 years) showed non-specific anomalies with delayed
27 28	15	myelination and thin corpus callosum. The patient underwent surgery for hip dysplasia
29	16	due to joint hypermobility (Figure 1.B).
30 31	17	Patient IV-4 is a girl born at term. Birth measurements were within the normal range:
32 33	18	weight 3.660 kg (77 <sup>th</sup> percentile), height 47 cm (6 <sup>th</sup> ), and OFC 35 cm (67 <sup>th</sup> ). She had a
34 35	19	severe developmental delay with her first assisted steps acquired at age 7-9 years.
36	20	Many clinical signs were shared with her brother such as the presence of stereotyped
37 38	21	hand movements, behavioral problems, normal OFC, hyperlaxity, mild dysmorphic
39 40	22	features, and non-specific brain MRI abnormalities (Figure 1.C). At last medical
41 42	23	evaluation, OFC was 51 cm (– 0.68 SD). She was able to walk without support. When
43	24	standing, a hyperlordosis was noticeable. She could make vocal contact but had no
<ul> <li>36</li> <li>37</li> <li>38</li> <li>39</li> <li>40</li> <li>41</li> <li>42</li> <li>43</li> <li>44</li> <li>45</li> <li>46</li> <li>47</li> <li>48</li> <li>49</li> <li>50</li> <li>51</li> </ul>	25	clear speech.
46 47	26	Metabolic screening
48 40	27	In patient IV-3, a full metabolic screen, including amino acid chromatography, organic
49 50	28	acid chromatography, and acylcarnitine profile was performed in early childhood. The
51 52	29	results were normal, and the tests were not repeated in the younger sister (patient IV-4).
53 54	30	Genetic analyses
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1 2		
3 4	1	In patient IV-3, array-CGH and ID gene panel sequencing did not reveal any candidate
5	2	variants. Whole Exome Sequencing on the proband (IV-3), his healthy father (III-7) and
6 7	3	his healthy brother (IV-2) lead to the identification of a unique homozygous variant in the
8 9	4	<i>TRAPPC2L</i> gene: NC_000016.9:g.88926373C>T(GRCh37/hg19),
10	5	NM_016 <mark>209.3:</mark> c.367C>T, NP_057293.1:p.(Gln123Ter). It is a protein-truncating variant
12	6	(PTV) located in exon 4 (out of 5). Segregation analysis using Sanger sequencing
13 14	7	showed that the variant is homozygous in both affected siblings (IV-3 and IV-4) and
11 12 13 14 15 16 17 18 19 20 21 22 23	8	heterozygous in all unaffected individuals tested (III-6, III-7, and IV-2). This variant is
	9	absent from gnomAD (v2.1.1 and v3.1.2) and ClinVar databases. The Combined
18 19	10	Annotation Dependent Depletion (CADD) score of this variant is 41.
18 19 20 21 22 23 24	11	
22 23	12	Additional testing in patients following the identification of the TRAPPC2L
24	13	nonsense variant
25 26	14	Serum creatine kinase (CK) are molecules of 360 amino acids, formed by a dimer of 2
27 28	15	subunits: M (muscular) and B (brain). There are 3 isoenzymes of CK resulting from the
29 30 31	16	combination of these subunits: CK-BB, CK-MB and CK-MM. Only total CK and CK-MB
	17	are routinely measurable. An elevated MB-CK level usually reflects damage to the heart
32 33	18	muscle. However, skeletal muscle contains about 7% of MB-CK. Thus, intense
34 35	19	muscular exercise or chronic muscular disease can sometimes be the cause of an
31 32 33 34 35 36 37 38 39 40 41	20	abnormal CK-MB. Both patients showed elevated levels of total CK over the course of
	21	2.5 years, with a maximum of 927 U/L for patient IV-3 and 2489 U/L for patient IV-4
	22	(Figure S.1). CK-MB levels were above the normal range in both patients, without any
41 42	23	cardiac damage: 14.7 $\mu$ g/l in IV-4 and 7.1 $\mu$ g/l in IV-3, for a normal range < 4.87.
43	24	Muscle biopsy was performed in patient IV-4, at age 8-10 years. The morphology was
44 45	25	normal, without histoenzymatic alterations. The different proteins involved in the
46 47	26	dystrophin membrane complex were normally expressed (dystrophins, sarcoglycans,
48 49	27	dystroglycans, merosin, utrophin). In addition, the expression of caveolin-3, dysferlin,
50	28	and emerin was also normal.
50 51 52 53 54	29	Congenital disorders of glycosylation (CDG) were investigated by western blot and 2D
	30	electrophoresis of glycoproteins in patient IV-4. The results did not reveal any major
55 56	31	variation in protein glycosylation, thus excluding CDG I or IIa.
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6 7	2	DISCUSSION			
8	4				
9 10	5	The TRAPPC2L protein is a core subunit of TRAPP complexes that are tethering factor	rs		
11 12	6	ubiquitously expressed in tissues and highly conserved between species. Only 2			
13 14	7	homozygous missense variants in the TRAPPC2L gene have been identified in 3			
15 16	8	unrelated families so far. Functional studies have shown their impact on TRAPP			
17	9	complex assembly and membrane trafficking between the endoplasmic reticulum and			
18 19	10	the Golgi (4,11). Herein, we report 2 siblings presenting with a severe			
20 21	11	neurodevelopmental disorder and the p.(Gln123Ter) nonsense variant in the			
22 23	12	TRAPPC2L gene (Figure 2).			
24	13				
25 26	14	Genetic evidence is one of the main lines of evidence used to determine the link	ζ.		
27 28	15	between a given gene and a phenotype. According to the Clingen clinical validity			
29 30	16	framework, the level of evidence is limited to support a gene-disease relationship for th	e		
31	17	TRAPPC2L gene due to the small number of patients reported (12). Thus, the formal			
32 33 34 35 36 37	18	variant ACMG/AMP classification is not possible for the identified variant (13,14). Once			
	19	the gene-disease relationship is established for the TRAPPC2L gene, the following			
	20	ACMG codes could be attributed: (1) PVS1_strong since nonsense-mediated mRNA			
38	21	decay is not predicted for this nonsense variant as it is located 8bp from the end of the			
39 40	22	second-to-last-exon. However, there is no loss of function (LoF) variant in the exon 4 ir	۱		
41 42	23	population databases and this specific exon is present in all biologically-relevant			
43 44	24	transcripts. In addition, the variant is predicted to remove 12% of protein (17 amino			
45	25	acids); (2) PM2 since this variant is absent from the gnomAD (v2.1.1 and v3.1.2); (3)			
46 47	26	PP4 since the phenotype of the patients matches previously described patients with			
48 49	27	TRAPPC2L variants; and (4) PP1_moderate since the variant segregates with the			
50	28	disease in this family. This report of two additional patients provides key genetic			
51 52	29	evidence that will be invaluable to establish the gene-disease relationship for			
53 54	30	TRAPPC2L.			
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#### Journal of Medical Genetics

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Since childhood, the two siblings have been regularly monitored and there has been no evidence of clinical worsening after acute infectious episodes or seizures. Both have slow development but no regression. This presentation is consistent with those of the last 3 siblings by Al-Deri et al. (11). However, it contrasts with the 2 unrelated patients reported by Milev et al. who had a major regression with acquired microcephaly after acute febrile episodes, and seizures (4) (**Table S.1**). We confirm that regression, seizures, and postnatal microcephaly are not constant features of the TRAPPC2L syndrome. Acute episodes of infection do not contribute to the neurological course of all patients, and therefore it is not always a progressive encephalopathy.

The identification of the TRAPPC2L variant warranted the exploration of muscle function in patients although there was no evidence of rhabdomyolysis or myopathy. 2 Serum CK and CK-MB isoform were persistently elevated in both patients. In patient IVł 4 who acquired walking ability, values were higher and muscle biopsy was normal. Ł These results are consistent with those of Milev et al : one patient had intermittent 2 elevated CK levels up to 1000 IU/I and non-specific myopathic damage on muscle biopsy; the other had normal intermittent CK levels and normal muscle biopsy (4). In Al-Deri et al. report, the CK test and muscle biopsy were not performed (11) (Table S.1). 3 According to the European Federation of Neurological Societies (EFNS), hyperCKemia ) is defined as the presence of serum total CK values beyond 1.5 times the upper limit of ) normal in at least 2 measurements (15). Serum total CK elevation is most often due to myopathies and sustained elevation of CK-MB isoform raises suspicion for noncardiac causes, in particular muscular dystrophy. HyperCkemia values fluctuate depending on the degree of muscular damage, disease course, and absolute muscle mass (16,17). ł These elements may explain the variability observed between the different reports. We 5 confirm that hyperCKemia is part of the clinical picture of TRAPPC2L syndrome (Figure 5 S.1). Muscle biopsy does not bring any additional argument, at least in the first decades of life. Serum CK test will provide support in the interpretation of variants of unknown 3 significance in the TRAPPC2L gene. Newly diagnosed patients should have regular CK ) testing and, if anormal, be referred to a specialist of neuromuscular diseases. )

Together, hyperCKemia associated with congenital hypotonia, joint hyperlaxity, and autosomal recessive inheritance suggest congenital muscular dystrophies (CMD). CMD are heterogeneous neuromuscular diseases that present at birth or in early childhood. In general, muscle biopsy is consistent with the presence of dystrophic myopathy. However, clear dystrophic features are not always present in the early stages. High-throughput DNA sequencing has led to a better characterization of CMD and the phenotypic spectrum is broader than originally described. Rare CMD do not fit into any of the classic categories (18,19). Interestingly, individuals with biallelic pathogenic variants in the TRAPPC11 gene have a muscle phenotype ranging from limb-girdle muscular dystrophy to more severe forms of congenital muscular dystrophy (9.20). As for TRAPPC2L, affected individuals presented with motor delay, and elevated serum CK. Muscle biopsies from patients with TRAPPC11 showed dystrophic changes and abnormalities in dystrophy associated proteins that were not identified in TRAPPC2L patients. The study of the structure of metazoan TRAPP complexes showed that TRAPPC2L interacts with TRAPPC11 (21). We suggest that TRAPPC2L syndrome may belong to the clinical entity of rare CMD, and that the defect in association with TRAPPC11 may explain the similarities in phenotypes. This work provide provides evidence in favor of the TRAPPC2L gene-disease relationship and important insights into its phenotype that will help the diagnosis and management of this ultra-rare disease. To date, all reported TRAPPC2L variants have been associated with neurodevelopmental disorders and hyperCKemia. Further studies to investigate the specific functional effects of each variant on the TRAPP complex may explain the observed clinical heterogeneity. ACKNOWLEDGMENTS We thank the family for participating in our research program. We warmly thank Prof. Nicole Philip, now retired, who initiated the genetic investigations in her clinic and Dr. Anne-Marie Clement, pediatrician, who takes care of the children in a specialised

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### 1 institute and helped us to obtain the results of the blood tests. We thank Dr. André

- 2 Maues De Paula who performed the muscular biopsy and Dr. Marc Bartoli for his
- 3 insights into congenital muscular dystrophies. We are grateful to the Genomics and
- 4 Bioinformatics facility (GBiM), from the U 1251/Marseille Medical Genetics lab, for
- 5 performing the NGS analysis.

### 6 COMPETING INTERESTS

- F.R. and S.S are members of expertise centers of the French national network AnDDI8 Rares and the European reference network ITHACA.
- 9 F.R. has received funding from the Excellence Initiative of Aix-Marseille University -
- 10 A\*Midex a French "Investissements d'Avenir programme"- Institute MarMaRa AMX-19-
- 11 IET-007" to carry out research as part of a PhD.

### 12 CONTRIBUTION

<sup>7</sup> 13

FR, MA, and CMR drafted and wrote the manuscript in consultation with all authors.
FR, SS and BC provided clinical care. CMR, PC and FM performed research WES
analyses. MA, FR, SS and BC performed clinical and pathological phenotyping. SG, JM,
FM, LV, and BC contributed with interpretation and discussion of results. FR, MA and
CMR generated figures and supplementaries. FR coordinated the study. LV provided
financial support for the research analysis.

### 20 ETHICS STATEMENT

This study has been approved by the scientific committee of the Assistance Publique
Hôpitaux de Marseille (PADS22-337). Each of the cases in this study has given their
consent to participate in the study and to have their data processed for publication.
According to the applicable legislation, this study does not require any additional
regulatory process, therefore the solicitation of an ethics committee is not required.

26 LEGENDS TO FIGURES

Figure 1: Family pedigree, clinical and MRI findings in the patients. A. Family
pedigree. Patients IV-3 et IV-4 are indicated with round and square filled in black.

1	Individuals III-1, III-2, III-3 indicated with round and squares filled in gray have been
2	diagnosed with another genetic condition. B. Patient IV-3. Clinical features were noted
3	several times between 2-8 years old: oval and floppy face, strabismus, prominent nose,
4	and a wide mouth with everted inferior lips, hand-to-mouth movements, and absence of
5	microcephaly. Brain MRI (sagittal section) at 2-3 years-old showed non-specific
6	anomalies with delayed myelination and thin corpus callosum. <b>C</b> . Patient IV-4. Clinical
7	features, shared with her brother, were noted several times before her 10th: stereotyped
8	hand-to-mouth movements, absence of microcephaly, mild dysmorphic features. Brain
9	MRI (sagittal section) at 1-2 years-old showed thin corpus callosum and non-specific
10	MRI abnormalities.
11	Figure 2: Scheme of TRAPPC2L gene showing the homozygous variants
12	identified in published patients and those of our study. The variant identified in
13	patients of our study is located above the gene, and those previously reported are
14	underneath the gene. Reference transcript: NM_016209.3/NP_057293.1.
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16	SUPPLEMENTARY MATERIALS
17	Table S.1: clinical detail of patients and review of the literature.
18	Figure S.1: CPK measurements of IV-3 and IV-4 patients over time (period of 2.5
19	<b>years).</b> CK normal range is <171 U/L, represented by a dotted line. In the non-walking
20	patient IV-3, CK levels were slightly elevated. In the walking patient IV-4, CK levels
21	were much higher with a fluctuation over time. During this period, patient IV-3 was 12 to
22	14 years old, and patient IV-4 was 9 to 11 years old.
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Figure 1. Family pedigree, clinical and MRI findings in the patients. A. Family pedigree. Patients IV-3 et IV-4 are indicated with round and square filled in black. Individuals III-1, III-2, III-3 indicated with round and squares filled in gray have been diagnosed with another genetic condition. B. Patient IV-3. Clinical features were noted several times between 2-8 years old: oval and floppy face, strabismus, prominent nose, and a wide mouth with everted inferior lips, hand-to-mouth movements, and absence of microcephaly. Brain MRI (sagittal section) at 2-3 years-old showed non-specific anomalies with delayed myelination and thin corpus callosum. C. Patient IV-4. Clinical features, shared with her brother, were noted several times before her 10th: stereotyped hand-to-mouth movements, absence of microcephaly, mild dysmorphic features. Brain MRI (sagittal section) at 1-2 years-old showed thin corpus callosum and non-specific MRI abnormalities.

338x190mm (117 x 117 DPI)



1 2		this study				
3	Individuals	IV.3				
4	Age/sex	M/ Early 10s				
с 5	Ancestry	North African				
7	- Variant (NM 016209.3/NP 057293.1)	c.367C>T:(p.Gln123				
8	Prenatal and perinatal	Unremarkable				
9	DD (age of onset)	+ (< 12 mo)				
10	Acute encentral on a thy	. (* 12 110)				
11 12	Pogrossion					
13	intermittent CK levels	- up to 027     /				
14						
15	Intermittent CK-MB levels					
16 17		NA				
17 18	Brain MRI (age)	non-specific anorma				
19	Motor milestones	Never achieved independent walking				
20	Microcephaly	-				
21	Speech	Non-verbal				
22	Tetraplegia	-				
3 1	Dystonia	-				
+ 5	Seizure	-				
6	Vision anomalies	strabismus				
7	Other	_				
8	Genetic analyses	rray-CGH ID gene nanel sequencing WE				
9	Conotic unarysos	may-oon to gene parter sequencing, WE				
U 1	DD: dovelopmental delay: yes years	ald: ma: manthe ald: CK: graating kingso:				
2	טט: developmental delay; yo: years	DD: developmental delay; yo: years old; mo: months old; CK: creatine kinase;				
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	Al-deri et al. 2021		
IV.4	II.2	II.3	II.4
F/ Before 10th	F/ Late 30s	F/ Late 30s	M/ Early 30s
		Ashkenazi Jewish	,
Ter)	c.5G>C:p.(Ala2Glv)		
,		I Inremarkable	
$+(-12m_{0})$	$\pm (12 \text{ mo})$	$\pm (4 \text{ mo})$	+ (6 mo)
r (< 12 mo)	· (12 mo)	· (4 mo)	· (0 110)
-	-	-	-
up to 2489 IU/I	NA	NA	NA
elevated (7.1 µg/l)	NA	NA	NA
normal	NA	NA	NA
lies	NA	NA	normal (1-2 yo)
steps at 8, independent walki	ntly at 12 mo never crawle	dd motor delay, walked	d crawled at 18 mo, wa
-	NA	NA	NA
Non-verbal	Seve	re expressive impairme	ent
-	-	· · ·	-
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_	_	_	_
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-	-	-	-
-	- 	- N to a the second all all a	-
WES	chromosome analysis, Fra	aglie-X testing and clini	cal exome sequencing
NA. HOL AVAIIADIE, WES. WI	tole exome sequencing		

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