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## 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**  
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6 2 **further delineation of the phenotype**  
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9 3 Mario Abaji\* (1,2), Cécile Mignon-Ravix\* (2), Svetlana Gorokhova (1,2), Pierre Cacciagli  
10 4 (1,3), Jérémie Mortreux (4), Florence Molinari (2), Brigitte Chabrol (5), Sabine Sigaudy  
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12 5 (1), Laurent Villard (1,2), Florence Riccardi (2,6)  
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14  
15 6  
16 7 (1) Assistance Publique Hôpitaux de Marseille, Department of Medical Genetics, La  
17 8 Timone Children's Hospital, Marseille, France  
18 9 (2) Aix Marseille Univ, INSERM, MMG, Marseille, France  
19  
20 10 (3) Biological Resources Center (CRB-TAC), Assistance Publique des Hôpitaux de  
21 11 Marseille, La Timone Children's Hospital, Marseille, France  
22  
23 12 (4) Service de Génétique, Laboratoire Eurofins Biomnis, Lyon  
24  
25 13 (5) Assistance Publique Hôpitaux de Marseille, Department of Neuropediatrics,, La  
26 14 Timone Children's Hospital, Marseille, France  
27  
28 15 (6) Centre Hospitalier Inter-Communal Toulon-La Seyne, Department of Medical  
29 16 Genetics, Sainte Musse Hospital, Toulon, France

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31 17 \*These authors equally contributed to this work.  
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34 18 Running title: Further delineation of the *TRAPPC2L*-related disorder  
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39 20 **Corresponding author:** Florence Riccardi, [florence.riccardi@ch-toulon.fr](mailto:florence.riccardi@ch-toulon.fr)  
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## 1 **ABSTRACT/SUMMARY**

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

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## 1 INTRODUCTION

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

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17 High-throughput DNA sequencing for patients with rare and severe disorders has led to  
18 the identification of pathogenic variants in eight TRAPP genes resulting in a range of  
19 human disorders named TRAPPopathies: *TRAPPC2* (3), *TRAPPC2L* (4), *TRAPPC4*  
20 (5), *TRAPPC6B* (6), *TRAPPC9* (7), *TRAPPC10* (8), *TRAPPC11* (9), and *TRAPPC12*  
21 (10). Seven of them are autosomal recessive neurodevelopmental disorders with  
22 overlapping phenotypes. Their study helped to understand the function of TRAPP  
23 subunits and demonstrated that there is no clear correlation between the phenotypes  
24 associated with specific or core subunits of these complexes.

25 The *TRAPPC2L* gene (Trafficking Protein Particle Complex 2-Like, MIM\*610970)  
26 encodes a core subunit of the TRAPP complexes. To date, two pathogenic missense  
27 variants in this gene were identified in five patients from three unrelated families (4,11).  
28 The common feature is a neurodevelopmental disorder starting in the first year of life.  
29 However, important differences were noted regarding the clinical presentation. Two  
30 patients showed regression and episodes of rhabdomyolysis during acute infectious  
31 episodes, while the other three patients had a moderate non-syndromic intellectual

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3 1 disability from the outset (**Table S.1**). Thus, a detailed description of additional patients  
4 is important to better define the phenotype, in particular muscle alteration. Here, we  
5 report two siblings in whom we identified a homozygous nonsense variant in  
6 *TRAPPC2L*, thus further clarifying the gene-disease relationship and the phenotype  
7 associated with this gene.  
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## 10 11 12 13 **MATERIALS AND METHODS**

### 14 15 **Patients**

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17 9 Two siblings with a neurodevelopmental disorder were referred to the Medical Genetics  
18 Department of La Timone Marseille Public University Hospital in 2009. Peripheral blood  
19 samples were collected from the affected siblings IV-3 and IV-4, their healthy brother IV-  
20 2, and their parents III-6 and III-7 (**Figure 1.A**). Written informed consent for genetic  
21 analyses and publication was obtained for each individual.  
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24 DNAs were prepared, stored, and delivered for research by the Biological Resource  
25 Center of La Timone Marseille Public University Hospital, according to the French  
26 regulation (CRB AP-HM Biobank; NF S96-900 & ISO 9001 v2015 Certifications).  
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### 28 29 **Genetic analyses**

#### 30 31 *Array CGH and Intellectual Disability (ID) gene panel Sequencing*

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33 19 Patient IV-3 was first analyzed with routine CGH testing using 4x180K whole-genome  
34 oligonucleotide microarray and SureSelect XT Inherited Diseases Panel sequencing  
35 (Agilent Technologies, Santa Clara, CA, USA) following the manufacturer's protocol.  
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#### 37 38 *Next-Generation Sequencing*

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40 23 Whole-exome sequencing of proband IV-3, his father (III-7), and a healthy brother (IV-2)  
41 (Figure 1.A) was performed using the SeqCap EZ MedExome sequencing kit (Roche  
42 NimbleGen, Basel, Switzerland) following standard protocols. Paired-end 2x75 base-  
43 pair (bp) sequencing was performed on a NextSeq 500 sequencing apparatus (Illumina  
44 Inc., USA). Sequencing data were analyzed according to an autosomal recessive  
45 inheritance pattern. Validation and segregation analysis of the *TRAPPC2L* variant was  
46 done by Sanger sequencing using the following primers: TRAPPC2L-F:  
47 TGTGTGTATGTGGCAAGGTC and TRAPPC2L-R: GTGGCTTTAAAACCTCTGCC. No  
48 other candidate variant has been identified.  
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## RESULTS

### Clinical findings

The patients were born after uneventful pregnancies to healthy consanguineous parents (Figure 1.A).

Patient IV-3 is a boy born at term. Birth measurements were within the normal range: weight 3.450 kg (45<sup>th</sup> percentile), height 47.5 cm (5<sup>th</sup>), and OFC 35 cm (48<sup>th</sup>). Hypotonia, poor contact, and developmental delay were noticed during the first months of life. From 2 to 6 years, growth measurements were normal. Walking and language were absent. Stereotyped hand movements and self-injurious behavior were observed. Mild dysmorphic features including oval and floppy face, strabismus, prominent nose, and a wide mouth with everted inferior lip, hyperlaxity, and OFC remained normal. At the last medical evaluation, the patient had severe intellectual disability (ID) with no walking or speech. Brain MRI scan (age 1-3 years) showed non-specific anomalies with delayed myelination and thin corpus callosum. The patient underwent surgery for hip dysplasia due to joint hypermobility (Figure 1.B).

Patient IV-4 is a girl born at term. Birth measurements were within the normal range: 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 severe developmental delay with her first assisted steps acquired at age 7-9 years. Many clinical signs were shared with her brother such as the presence of stereotyped hand movements, behavioral problems, normal OFC, hyperlaxity, mild dysmorphic features, and non-specific brain MRI abnormalities (Figure 1.C). At last medical evaluation, OFC was 51 cm (– 0.68 SD). She was able to walk without support. When standing, a hyperlordosis was noticeable. She could make vocal contact but had no clear speech.

### Metabolic screening

In patient IV-3, a full metabolic screen, including amino acid chromatography, organic acid chromatography, and acylcarnitine profile was performed in early childhood. The results were normal, and the tests were not repeated in the younger sister (patient IV-4).

### Genetic analyses

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3 1 In patient IV-3, array-CGH and ID gene panel sequencing did not reveal any candidate  
4 2 variants. Whole Exome Sequencing on the proband (IV-3), his healthy father (III-7) and  
5 3 his healthy brother (IV-2) lead to the identification of a unique homozygous variant in the  
6 4 *TRAPPC2L* gene: NC\_000016.9:g.88926373C>T(GRCh37/hg19),  
7 5 NM\_016209.3:c.367C>T, NP\_057293.1:p.(Gln123Ter). It is a protein-truncating variant  
8 6 (PTV) located in exon 4 (out of 5). Segregation analysis using Sanger sequencing  
9 7 showed that the variant is homozygous in both affected siblings (IV-3 and IV-4) and  
10 8 heterozygous in all unaffected individuals tested (III-6, III-7, and IV-2). This variant is  
11 9 absent from gnomAD (v2.1.1 and v3.1.2) and ClinVar databases. The Combined  
12 10 Annotation Dependent Depletion (CADD) score of this variant is 41.  
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### 22 12 **Additional testing in patients following the identification of the *TRAPPC2L*** 23 13 **nonsense variant**

24 14 Serum creatine kinase (CK) are molecules of 360 amino acids, formed by a dimer of 2  
25 15 subunits: M (muscular) and B (brain). There are 3 isoenzymes of CK resulting from the  
26 16 combination of these subunits: CK-BB, CK-MB and CK-MM. Only total CK and CK-MB  
27 17 are routinely measurable. An elevated MB-CK level usually reflects damage to the heart  
28 18 muscle. However, skeletal muscle contains about 7% of MB-CK. Thus, intense  
29 19 muscular exercise or chronic muscular disease can sometimes be the cause of an  
30 20 abnormal CK-MB. Both patients showed elevated levels of total CK over the course of  
31 21 2.5 years, with a maximum of 927 U/L for patient IV-3 and 2489 U/L for patient IV-4  
32 22 (**Figure S.1**). CK-MB levels were above the normal range in both patients, without any  
33 23 cardiac damage: 14.7 µg/l in IV-4 and 7.1 µg/l in IV-3, for a normal range < 4.87.  
34 24 Muscle biopsy was performed in patient IV-4, at age 8-10 years. The morphology was  
35 25 normal, without histoenzymatic alterations. The different proteins involved in the  
36 26 dystrophin membrane complex were normally expressed (dystrophins, sarcoglycans,  
37 27 dystroglycans, merosin, utrophin). In addition, the expression of caveolin-3, dysferlin,  
38 28 and emerin was also normal.  
39 29 Congenital disorders of glycosylation (CDG) were investigated by western blot and 2D  
40 30 electrophoresis of glycoproteins in patient IV-4. The results did not reveal any major  
41 31 variation in protein glycosylation, thus excluding CDG I or IIa.  
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## DISCUSSION

The TRAPPC2L protein is a core subunit of TRAPP complexes that are tethering factors ubiquitously expressed in tissues and highly conserved between species. Only 2 homozygous missense variants in the *TRAPPC2L* gene have been identified in 3 unrelated families so far. Functional studies have shown their impact on TRAPP complex assembly and membrane trafficking between the endoplasmic reticulum and the Golgi (4,11). Herein, we report 2 siblings presenting with a severe neurodevelopmental disorder and the p.(Gln123Ter) nonsense variant in the *TRAPPC2L* gene (Figure 2).

Genetic evidence is one of the main lines of evidence used to determine the link between a given gene and a phenotype. According to the Clingen clinical validity framework, the level of evidence is limited to support a gene-disease relationship for the *TRAPPC2L* gene due to the small number of patients reported (12). Thus, the formal variant ACMG/AMP classification is not possible for the identified variant (13,14). Once the gene-disease relationship is established for the *TRAPPC2L* gene, the following ACMG codes could be attributed: (1) PVS1\_strong since nonsense-mediated mRNA decay is not predicted for this nonsense variant as it is located 8bp from the end of the second-to-last-exon. However, there is no loss of function (LoF) variant in the exon 4 in population databases and this specific exon is present in all biologically-relevant transcripts. In addition, the variant is predicted to remove 12% of protein (17 amino acids); (2) PM2 since this variant is absent from the gnomAD (v2.1.1 and v3.1.2); (3) PP4 since the phenotype of the patients matches previously described patients with TRAPPC2L variants; and (4) PP1\_moderate since the variant segregates with the disease in this family. This report of two additional patients provides key genetic evidence that will be invaluable to establish the gene-disease relationship for TRAPPC2L.



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3 1 Since childhood, the two siblings have been regularly monitored and there has  
4 2 been no evidence of clinical worsening after acute infectious episodes or seizures. Both  
5 3 have slow development but no regression. This presentation is consistent with those of  
6 4 the last 3 siblings by Al-Deri et al. (11). However, it contrasts with the 2 unrelated  
7 5 patients reported by Milev et al. who had a major regression with acquired microcephaly  
8 6 after acute febrile episodes, and seizures (4) (**Table S.1**). We confirm that regression,  
9 7 seizures, and postnatal microcephaly are not constant features of the TRAPPC2L  
10 8 syndrome. Acute episodes of infection do not contribute to the neurological course of all  
11 9 patients, and therefore it is not always a progressive encephalopathy.  
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20 11 The identification of the *TRAPPC2L* variant warranted the exploration of muscle  
21 12 function in patients although there was no evidence of rhabdomyolysis or myopathy.  
22 13 Serum CK and CK-MB isoform were persistently elevated in both patients. In patient IV-  
23 14 4 who acquired walking ability, values were higher and muscle biopsy was normal.  
24 15 These results are consistent with those of Milev et al : one patient had intermittent  
25 16 elevated CK levels up to 1000 IU/l and non-specific myopathic damage on muscle  
26 17 biopsy; the other had normal intermittent CK levels and normal muscle biopsy (4). In Al-  
27 18 Deri et al. report, the CK test and muscle biopsy were not performed (11) (**Table S.1**).  
28 19 According to the European Federation of Neurological Societies (EFNS), hyperCKemia  
29 20 is defined as the presence of serum total CK values beyond 1.5 times the upper limit of  
30 21 normal in at least 2 measurements (15). Serum total CK elevation is most often due to  
31 22 myopathies and sustained elevation of CK-MB isoform raises suspicion for noncardiac  
32 23 causes, in particular muscular dystrophy. HyperCKemia values fluctuate depending on  
33 24 the degree of muscular damage, disease course, and absolute muscle mass (16,17).  
34 25 These elements may explain the variability observed between the different reports. We  
35 26 confirm that hyperCKemia is part of the clinical picture of TRAPPC2L syndrome (**Figure**  
36 27 **S.1**). Muscle biopsy does not bring any additional argument, at least in the first decades  
37 28 of life. Serum CK test will provide support in the interpretation of variants of unknown  
38 29 significance in the *TRAPPC2L* gene. Newly diagnosed patients should have regular CK  
39 30 testing and, if abnormal, be referred to a specialist of neuromuscular diseases.  
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3 1 Together, hyperCKemia associated with congenital hypotonia, joint hyperlaxity, and  
4 2 autosomal recessive inheritance suggest congenital muscular dystrophies (CMD). CMD  
5 3 are heterogeneous neuromuscular diseases that present at birth or in early childhood.  
6 4 In general, muscle biopsy is consistent with the presence of dystrophic myopathy.  
7 5 However, clear dystrophic features are not always present in the early stages. High-  
8 6 throughput DNA sequencing has led to a better characterization of CMD and the  
9 7 phenotypic spectrum is broader than originally described. Rare CMD do not fit into any  
10 8 of the classic categories (18,19). Interestingly, individuals with biallelic pathogenic  
11 9 variants in the *TRAPPC11* gene have a muscle phenotype ranging from limb-girdle  
12 10 muscular dystrophy to more severe forms of congenital muscular dystrophy (9,20). As  
13 11 for *TRAPPC2L*, affected individuals presented with motor delay, and elevated serum  
14 12 CK. Muscle biopsies from patients with *TRAPPC11* showed dystrophic changes and  
15 13 abnormalities in dystrophy associated proteins that were not identified in *TRAPPC2L*  
16 14 patients. The study of the structure of metazoan TRAPP complexes showed that  
17 15 *TRAPPC2L* interacts with *TRAPPC11* (21). We suggest that *TRAPPC2L* syndrome may  
18 16 belong to the clinical entity of rare CMD, and that the defect in association with  
19 17 *TRAPPC11* may explain the similarities in phenotypes.  
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19 This work provide provides evidence in favor of the *TRAPPC2L* gene-disease  
20 relationship and important insights into its phenotype that will help the diagnosis and  
21 management of this ultra-rare disease. To date, all reported *TRAPPC2L* variants have  
22 been associated with neurodevelopmental disorders and hyperCKemia. Further studies  
23 to investigate the specific functional effects of each variant on the TRAPP complex may  
24 explain the observed clinical heterogeneity.  
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6 4 Bioinformatics facility (GBiM), from the U 1251/Marseille Medical Genetics lab, for  
7 5 performing the NGS analysis.  
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## 10 6 **COMPETING INTERESTS**

11 7 F.R. and S.S are members of expertise centers of the French national network AnDDI-  
12 8 Rares and the European reference network ITHACA.

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14 10 A\*Midex a French “Investissements d’Avenir programme”- Institute MarMaRa AMX-19-  
15 11 IET-007” to carry out research as part of a PhD.

## 12 12 **CONTRIBUTION**

13 14 FR, MA, and CMR drafted and wrote the manuscript in consultation with all authors.  
14 15 FR, SS and BC provided clinical care. CMR, PC and FM performed research WES  
15 16 analyses. MA, FR, SS and BC performed clinical and pathological phenotyping. SG, JM,  
16 17 FM, LV, and BC contributed with interpretation and discussion of results. FR, MA and  
17 18 CMR generated figures and supplementaries. FR coordinated the study. LV provided  
18 19 financial support for the research analysis.

## 19 20 **ETHICS STATEMENT**

20 21 This study has been approved by the scientific committee of the Assistance Publique  
21 22 Hôpitaux de Marseille (PADS22-337). Each of the cases in this study has given their  
22 23 consent to participate in the study and to have their data processed for publication.  
23 24 According to the applicable legislation, this study does not require any additional  
24 25 regulatory process, therefore the solicitation of an ethics committee is not required.  
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## 26 26 **LEGENDS TO FIGURES**

27 27 **Figure 1: Family pedigree, clinical and MRI findings in the patients. A.** Family  
28 28 pedigree. Patients IV-3 et IV-4 are indicated with round and square filled in black.

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3 1 Individuals III-1, III-2, III-3 indicated with round and squares filled in gray have been  
4 diagnosed with another genetic condition. **B.** Patient IV-3. Clinical features were noted  
5 several times between 2-8 years old: oval and floppy face, strabismus, prominent nose,  
6 and a wide mouth with everted inferior lips, hand-to-mouth movements, and absence of  
7 microcephaly. Brain MRI (sagittal section) at 2-3 years-old showed non-specific  
8 anomalies with delayed myelination and thin corpus callosum. **C.** Patient IV-4. Clinical  
9 features, shared with her brother, were noted several times before her 10th: stereotyped  
10 hand-to-mouth movements, absence of microcephaly, mild dysmorphic features. Brain  
11 MRI (sagittal section) at 1-2 years-old showed thin corpus callosum and non-specific  
12 MRI abnormalities.

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**Figure 2: Scheme of *TRAPPC2L* gene showing the homozygous variants identified in published patients and those of our study.** The variant identified in patients of our study is located above the gene, and those previously reported are underneath the gene. Reference transcript: NM\_016209.3/NP\_057293.1.

## 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 years).** CK normal range is <171 U/L, represented by a dotted line. In the non-walking patient IV-3, CK levels were slightly elevated. In the walking patient IV-4, CK levels were much higher with a fluctuation over time. During this period, patient IV-3 was 12 to 14 years old, and patient IV-4 was 9 to 11 years old.

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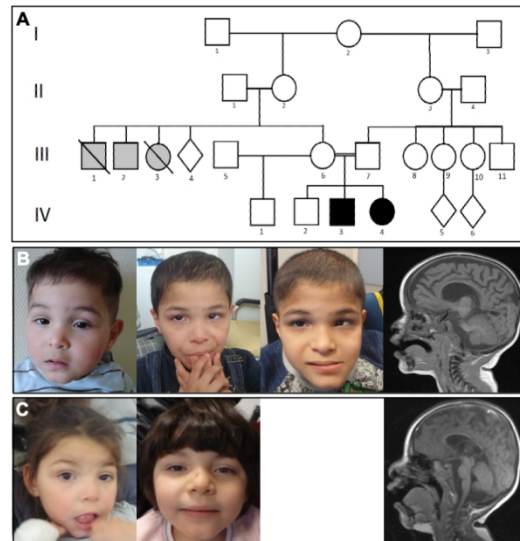


Figure 1. Family pedigree, clinical and MRI findings in the patients. A. Family pedigree. Patients IV-3 et IV-4 are indicated with round and square filled in black. Individuals III-1, III-2, III-3 indicated with round and squares filled in gray have been diagnosed with another genetic condition. B. Patient 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)



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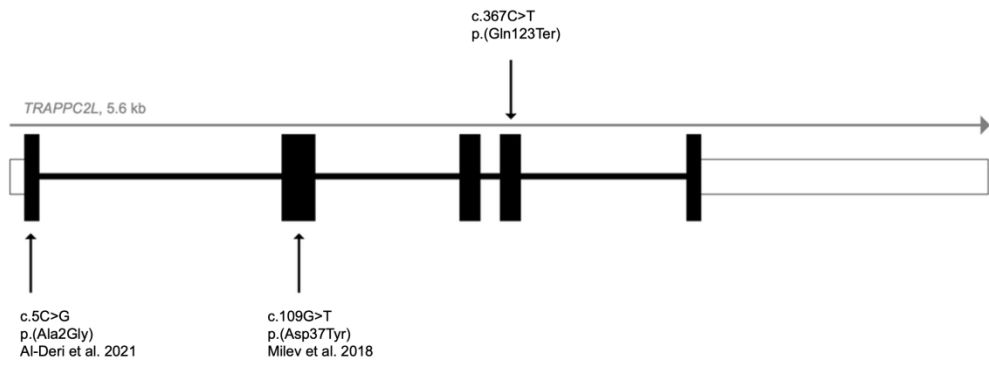


Figure 2. Scheme of TRAPPC2L gene showing the homozygous variants identified in published patients and those of our study. The variant identified in patients of our study is located above the gene, and those previously reported are underneath the gene. Reference transcript: NM\_016209.3/NP\_057293.1.

338x190mm (217 x 217 DPI)

	<b>this study</b>
<b>Individuals</b>	<b>IV.3</b>
<b>Age/sex</b>	M/ Early 10s
<b>Ancestry</b>	North African
<b>Variant (NM_016209.3/NP_057293.1)</b>	c.367C>T:(p.Gln123)
<b>Prenatal and perinatal</b>	Unremarkable
<b>DD (age of onset)</b>	+ (< 12 mo)
<b>Acute encephalopathy</b>	
<b>Regression</b>	-
<b>intermittent CK levels</b>	up to 927 IU/l
<b>intermittent CK-MB levels</b>	elevated (14.7 µg/l)
<b>Muscle biopsies</b>	NA
<b>Brain MRI (age)</b>	non-specific abnormal
<b>Motor milestones</b>	Never achieved independent walking
<b>Microcephaly</b>	-
<b>Speech</b>	Non-verbal
<b>Tetraplegia</b>	-
<b>Dystonia</b>	-
<b>Seizure</b>	-
<b>Vision anomalies</b>	strabismus
<b>Other</b>	-
<b>Genetic analyses</b>	array-CGH ID gene panel sequencing, WES

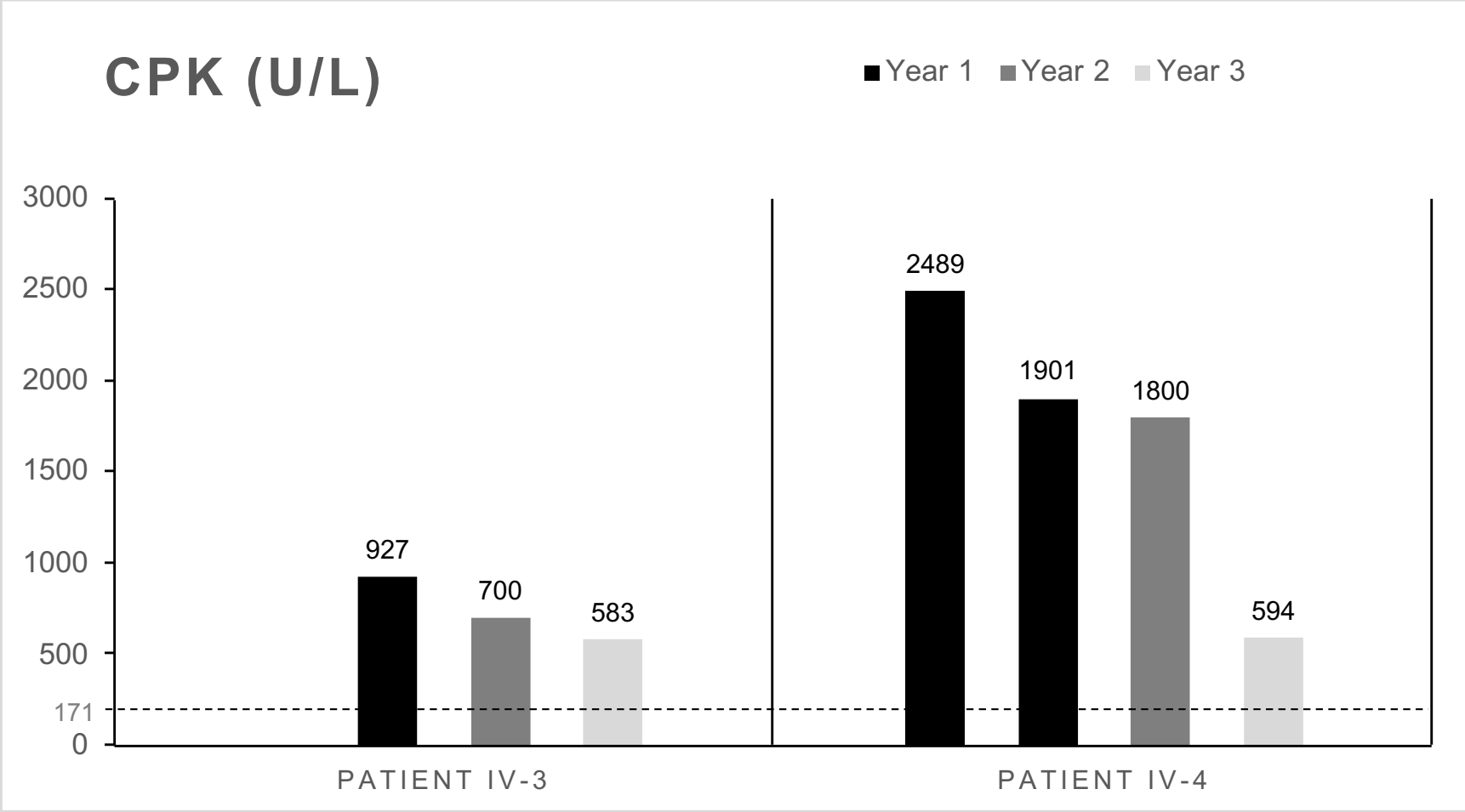
DD: developmental delay; yo: years old; mo: months old; CK: creatine kinase;

		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
Ter)			Ashkenazi Jewish c.5G>C:p.(Ala2Gly)	
			Unremarkable	
+ (< 12 mo)		+ (12 mo)	+ (4 mo)	+ (6 mo)
-		-	-	-
up to 2489 IU/l		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 walking		at 12 mo never crawled	motor delay, walked	crawled at 18 mo, wa
-		NA	NA	NA
Non-verbal		Severe expressive impairment		
-		-	-	-
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WES		chromosome analysis, Fragile-X testing and clinical exome sequencing		

NA: not available; WES: whole exome sequencing

Milev et al. 2018	
S1	S2
F/ 2-3 yo	F/ Mid 10s
Italian	Austrian
c.109G>T:p.(Asp37Tyr)	
Perinatal distress + (9 mo)	Perinatal distress, preterm labour + (9 mo)
+ (with CK level up to 16,000)	+ (with CK level up to 5,500)
+	+
up to 1000 IU/l	normal
NA	NA
unspecific myopathic damage	normal
ation, Posterior oedema, Progressive brain ;	Delayed myelination (< 1 yo)
but subsequently lost this ability, never achieved	but subsequently lost this ability, never achieved
+ (acquired)	+ (acquired)
Non-verbal	Non-verbal
+	+
+	+
+	+
cerebral visual impairment	cerebral visual impairment
Protein-losing enteropathy at 3-4 yo	cholelithiasis, recurrent pneumonia
WES	WES

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**Figure S.1: CPK measurements of IV-3 and IV-4 patients over time (period of 2.5 years).** CK normal range is <171 U/L, represented by a dotted line. In the non-walking patient IV-3, CK levels were slightly elevated. In the walking patient IV-4, CK levels were much higher with a fluctuation over time. During this period, patient IV-3 was 12 to 14 years old, and patient IV-4 was 9 to 11 years old.