



## Case Report

## PPFIA4 mutation: A second hit in POLG related disease?

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## ARTICLE INFO

## Article history:

Received 25 February 2021

Revised 26 April 2021

Accepted 1 May 2021

Available online 07 May 2021

## Keywords:

Epilepsy

Case report

PPFIA4

POLG

Mitochondria

## ABSTRACT

Epilepsy in *POLG* related disease usually involves biallelic recessive mutations causing chronic neuronal loss and neuronal death. However, monoallelic *POLG* mutations have been reported in patients with neurological features such as seizures [1]. In these patients a second allele/gene was anticipated but not identified. The genetic etiology in epilepsy can contribute to better treatment strategies. For example, valproic acid (VPA) should be avoided in patients with *POLG* related epilepsy due to possible hepatotoxicity. We report a 12-year old boy with initially drug-resistant focal onset epilepsy, a mild developmental delay and behavioral issues. He carries potential pathogenic variants in the DNA polymerase gamma (*POLG*) gene (from asymptomatic mother) and in the liprin-alpha-4 (*PPFIA4*) gene (from asymptomatic father). This latter gene has never been related to (neurological) disorders, although its gene product interacts with several genes that play a role in excitatory neurotransmission and epileptogenesis. Hence, we hypothesize that the phenotype of our patient could be due to combination of detrimental effects to the neurons by the two aforementioned pathogenic variants. Nonetheless, we cannot exclude another undetected *POLG* mutation. In essence, genetic research should be aware that unexplained neurological disease can be caused by an oligogenic, rather than a monogenic, etiology.

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## 1. Introduction

Epilepsy in *POLG* related disease usually involves biallelic recessive mutations causing chronic neuronal loss and neuronal death. However, monoallelic *POLG* mutations have been reported in patients with neurological features such as seizures [1]. In these patients a second allele/gene was anticipated but not identified. The genetic etiology in epilepsy can contribute to better treatment strategies. For example, valproic acid (VPA) should be avoided in patients with *POLG* related epilepsy due to possible hepatotoxicity.

We report a 12-year old boy with initially drug-resistant focal onset epilepsy, a mild developmental delay and behavioral issues. He carries potential pathogenic variants in the DNA polymerase gamma (*POLG*) gene (from asymptomatic mother) and in the liprin-alpha-4 (*PPFIA4*) gene (from asymptomatic father). This latter gene has never been related to (neurological) disorders, although its gene product interacts with several genes that play a role in excitatory neurotransmission and epileptogenesis. Hence, we hypothesize that the phenotype of our patient could be due to combination of detrimental effects to the neurons by the two

aforementioned pathogenic variants. Nonetheless, we cannot exclude another undetected *POLG* mutation.

In essence, genetic research should be aware that unexplained neurological disease can be caused by an oligogenic, rather than a monogenic, etiology.

## 2. Case report

A 12-year old boy with a history of developmental delay, aggressive behavior and focal epilepsy was transferred to our hospital (University Hospitals Leuven) at the age of six years due to drug-resistant seizures.

He was born at term after an uncomplicated pregnancy. The parents were non-consanguineous and no health problems have been reported in them. During early childhood delay in language and fine motor skills was noted as well as behavioral problems. Clinical examination was normal. There was a family history of epilepsy since two nieces (from mother's side) had epilepsy that started during puberty.

Our patient experienced his first, focal seizure when he turned six years of age and reported to "feel sick" and started vomiting. This was followed by a few minutes of staring, head and eye deviation to the right. After a third seizure with similar semiology, he was initially treated with VPA during which no hepatotoxicity

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was noted. Since seizure frequency did not decrease and problems of fatigue and behavior were noticed, VPA was stopped after down-titration. Subsequently, our patient also failed carbamazepine (CBZ) and lamotrigine (LMT). LMT treatment led to increased seizure frequency and a different semiology. He experienced at least four seizures per week and they consisted out of eye blinking and facial jerks, lasting for about five minutes. Current treatment is monotherapy oxcarbazepine (OXC) due to which he recently obtained seizure freedom after uptitration to maximum tolerated doses (27 mg/kg/day). Our patient never showed any liver dysfunction clinically or by laboratory testing. A brain MRI did not show any anomalies. EEG showed generalized epileptiform activity, with maximal amplitudes in the left hemisphere (Fig. 1).

Karyotype analysis and a chromosomal microarray did not show any anomalies. Gene panel analyses (targeted next generation sequencing; IPG institute, Charleroi) revealed two variants of unknown significance (VUS): c.3313C>G (p.Gln1105Glu) substitution in the *PPFIA4* gene (inherited from the father) and c.2993C>T (p.Ser998Leu) substitution in the *POLG* gene (inherited from the mother). These substitutions in the *PPFIA4* and *POLG* gene are not present in the control population (ExAC). InterVar (<http://winter-var.wglab.org/>; last accessed on 19th of February 2021) classified the *POLG* variant as likely pathogenic and the *PPFIA4* variant as a pathogenic variant by MetaSVM, which is thought to be one of the most accurate predictors to date [3]. InterVar generated an output of four distinct analyses (Table S1): (1) Combined Annotation Dependent Depletion (CADD), (2) Genomic Evolutionary Rate Profiling (GERP++), (3) Sorting Intolerant From Tolerant (SIFT), and (4) a tool using Ensemble scores based on a Support Vector Machine (MetaSVM). CADD scores show the impact of human single nucleotide (DNA) substitutions based on 63 variant annotations from Ensemble, the ENCODE project and UCSC genome browser [4]. GERP++ indicates the level of conservation by multiple DNA sequence alignments [5]. SIFT analyzes the amino acid (AA) substitution and predicts if this affects the protein function based on

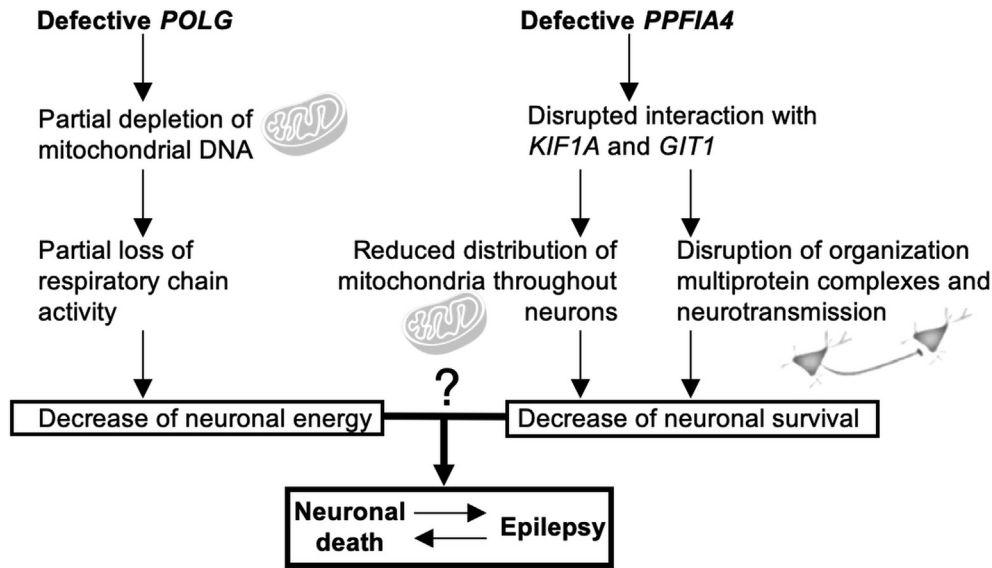
homology data and physical properties of the AA [6]. MetaSVM is one of the three most accurate predictors and combines the output of ample individual methods to obtain a variant interpretation, also using Ensemble [3]. This quantitative analysis (Table S1) and qualitative analysis (Table S2) suggest that both variants could be pathogenic and the combination of these variants may explain our patient's phenotype (Fig. 2).

### 3. Discussion

Epilepsy in *POLG* related disease involves biallelic recessive mutations in the catalytic subunit of polymerase gamma (*POLG*) that affects the mitochondrial DNA (mtDNA). Research has shown that about 40% of the mtDNA gets depleted leading to a progressive loss of respiratory chain activity and thereby a significant decrease of neuronal survival [7]. In our patient there was a presumably pathogenic variant of the *POLG* gene, inherited from the mother, that could not explain the epileptic phenotype since it was a monoallelic mutation. However, Tang et al. suggested that patients with one mutant allele of the *POLG* gene can exhibit neurological disorders. They identified *POLG* mutations in 136/2697 (5%) unrelated patients with a broad range of clinical features suggestive for *POLG* related disease; such as seizures, ataxia, developmental delay and peripheral neuropathy [1]. Of importance, our case was a child when the seizures started to occur. Seizures were accompanied by vomiting and there was a good response to OXC treatment. These findings could be suggestive for *POLG* related epilepsy and treatment, although the phenomenology can be very diverse [7,8]. Of interest we did see a worsening of the epilepsy after the start of LMT, already reported in *POLG*-related epilepsy [7]. Moreover, there was no improvement while on VPA and this antiseizure medication should be avoided in *POLG*-related disease due to the possibility of accelerating and/or precipitating hepatotoxicity [7,8]. So, the genetic etiology could lead to proper choices regarding epilepsy treatment [9].



Fig. 1. Representative EEG traces of 10 seconds, showing generalized spike wave complexes (3 Hz). Maximal in amplitude in temporal regions (300 μV), represented by the black arrow.



**Fig. 2.** Second hit hypothesis of *PPFIA4* and *POLG*, adjusted from Hikmat et al., 2017. The proposed, digenic model is likely the result of additive heterozygous effects in two functionally remotely linked genes.

The aforementioned research of Tang and colleagues showed that 41/136 (30%) had one heterozygous *POLG* mutation with a second mutant allele or gene unidentified [1]. Our patient also carries a second, potentially pathogenic variant of the *PPFIA4* gene, inherited from the father. This gene, encoding for a protein-tyrosine phosphatase receptor-type F polypeptide-interacting protein alpha 4, has a dominant expression in the brain [10] and has not been related to any (neurological) disease yet (<https://www.omim.org/entry/603145>; last accessed on 5th of April 2021). Nonetheless, six research centers (Finland, the Netherlands, France, Switzerland, the United Kingdom and the United States of America) have submitted variants of *PPFIA* to GeneMatcher [11], of which at least three are focusing on epilepsy genetics. Previous research has shown that the protein encoded by *PPFIA4* forms homodimers and heterodimers with liprins-alpha and liprins-beta and thereby interact with genes such as *KIF1A* [12] and *GIT1* [13]. These two genes play an important role in the excitatory neurotransmission and neuronal junctions (synapses), respectively. Moreover, variants of the *GIT1* gene have been reported in patients with *TBC1D24* mutations who experience epilepsy and intellectual disability disorder (IDD) [14]. It has been shown that *KIF1A* plays a key role in the transport of mitochondria along the neurons [15] and *KIF1A* dysfunction could be involved in epileptogenesis [16]. Therefore, a pathogenic variant of the *PPFIA4* gene could interfere with the normal function of *GIT1* and *KIF1A*, resultantly disturbing the neuronal function.

Thus, we believe that neurons are partially affected by the *POLG* gene mutation and partially by the *PPFIA4* gene mutation, leading to a significant decrease of functional neurons (Fig. 2). In spite of the aforementioned hypothesis, we acknowledge some limitations to our report: (1) another undetected *POLG* mutation cannot be excluded and whole genome sequencing, in combination with RNAsequencing, could address this limitation; (2) we have not performed muscle tissue analysis of respiratory chain dysfunction to indirectly verify the involvement of *POLG*; and (3) we did not examine *PPFIA4* and *POLG* variants using animal models. For example, zebrafish would be ideal to examine an epileptic phenotype since the orthologous zebrafish genes (*ppfia4* and *polg*) share high sequence similarity with human genes. Therefore, CRISPR/Cas9 techniques [17] could be used to induce mutations in these genes and evaluate a possible epileptic phenotype.

#### 4. Conclusion

We report a potential role of *PPFIA4* in epilepsy. We speculate the cause of our patient's initially drug-resistant epilepsy, behavioral problems and mild IDD could be due to the combination of mutations in the *POLG* and *PPFIA4* gene, since the mother and father (carrier of the *POLG* and *PPFIA4* variant, respectively) do not exhibit any neurological features. Future research should consider unexplained neurological disease in patients with multiple variants in two or more genes could be due to the combined effects of gene variants, especially if they fulfill crucial roles in the development and function of the central or peripheral nervous system.

#### Ethical statements and informed consent

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. Highest ethical standard was maintained during the study. Parents' informed consent was taken. All procedures performed in our study involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

#### CRediT authorship contribution statement

**Jo Sourbron:** Conceptualization, Methodology, Formal analysis, Investigation, Resources, Data curation, Writing - original draft, Writing - review & editing, Visualization. **Katrien Jansen:** Conceptualization, Writing - review & editing, Supervision. **Nele Aerts:** Formal analysis. **Lieven Lagae:** Conceptualization, Investigation, Writing - review & editing, Supervision.

#### Acknowledgements

The authors of this paper would like to thank the patient and its family for cooperating in the study, and for allowing their data to be used. We did not receive any financial support for the research, authorship, and/or publication of this article. We would like to

thank Aleksandra Siekierska for her input regarding possible zebrafish research.

### Conflict of interest

LL received grants, and is a consultant and/or speaker for Zogenix; LivaNova, UCB, Shire, Eisai, Novartis, Takeda/Ovid, NEL, Epihunter. LL has a patent for ZX008 (fenfluramine) for the treatment of Dravet syndrome and infantile epilepsies assigned to his institution and licensed to Zogenix.

The remaining authors have no conflict of interest to disclose.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ebr.2021.100455>.

### References

- [1] Tang S, Wang J, Lee N-C, Milone M, Halberg MC, Schmitt ES, et al. Mitochondrial DNA polymerase gamma mutations: an ever expanding molecular and clinical spectrum. *J Med Genet* 2011;48:669–81. <https://doi.org/10.1136/jmedgenet-2011-100222>.
- [2] Li Q, Wang K. InterVar: Clinical Interpretation of Genetic Variants by the 2015 ACMG-AMP Guidelines. *Am J Hum Genet* 2017;100:267–80. <https://doi.org/10.1016/j.ajhg.2017.01.004>.
- [3] Anderson D, Lassmann T. A phenotype centric benchmark of variant prioritisation tools. *NPJ Genomic Med* 2018;3:5. <https://doi.org/10.1038/s41525-018-0044-9>.
- [4] Suybeng V, Koepfel F, Harlé A, Rouleau E. Comparison of pathogenicity prediction tools on somatic variants. *J Mol Diagn* 2020;22:1383–92. <https://doi.org/10.1016/j.jmoldx.2020.08.007>.
- [5] Dong C, Wei P, Jian X, Gibbs R, Boerwinkle E, Wang K, et al. Comparison and integration of deleteriousness prediction methods for nonsynonymous SNVs in whole exome sequencing studies. *Hum Mol Genet* 2015;24:2125–37. <https://doi.org/10.1093/hmg/ddu733>.
- [6] Sim N-L, Kumar P, Hu J, Henikoff S, Schneider G, Ng PC. SIFT web server: predicting effects of amino acid substitutions on proteins. *Nucleic Acids Res* 2012;40:W452–7. <https://doi.org/10.1093/nar/gks539>.
- [7] Hikmat O, Eichele T, Tzoulis C, Bindoff L. Understanding the epilepsy in POLG related disease. *Int J Mol Sci* 2017;18. <https://doi.org/10.3390/ijms18091845>.
- [8] Cohen BH, Chinnery PF CW. POLG-Related Disorders. Adam MP, Ardinger HH, Pagon RA, Al, Ed GeneReviews® [Internet] Available from <https://www.ncbi.nlm.nih.gov/books/NBK26471/> 2018.
- [9] Nabbout R, Kuchenbuch M. Impact of predictive, preventive and precision medicine strategies in epilepsy. *Nat Rev Neurol* 2020;16:674–88. <https://doi.org/10.1038/s41582-020-0409-4>.
- [10] Serra-Pagès C, Medley QG, Tang M, Hart A, Streuli M. Liprins, a family of LAR transmembrane protein-tyrosine phosphatase-interacting proteins. *J Biol Chem* 1998;273:15611–20. <https://doi.org/10.1074/jbc.273.25.15611>.
- [11] Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. *Hum Mutat* 2015;36:928–30. <https://doi.org/10.1002/humu.22844>.
- [12] Shin H, Wyszynski M, Huh K-H, Valtschanoff JG, Lee J-R, Ko J, et al. Association of the kinesin motor KIF1A with the multimodular protein liprin-alpha. *J Biol Chem* 2003;278:11393–401. <https://doi.org/10.1074/jbc.M211874200>.
- [13] Ko J, Kim S, Valtschanoff JG, Shin H, Lee J-R, Sheng M, et al. Interaction between liprin-alpha and GIT1 is required for AMPA receptor targeting. *J Neurosci* 2003;23:1667–77. <https://doi.org/10.1523/JNEUROSCI.23-05-01667.2003>.
- [14] Lin L, Lyu Q, Kwan P-Y, Zhao J, Fan R, Chai A, et al. The epilepsy and intellectual disability-associated protein TBC1D24 regulates the maintenance of excitatory synapses and animal behaviors. *PLoS Genet* 2020;16:e1008587. <https://doi.org/10.1371/journal.pgen.1008587>.
- [15] Gabrych DR, Lau VZ, Niwa S, Silverman MA. Going too far is the same as falling short: kinesin-3 family members in hereditary spastic paraplegia. *Front Cell Neurosci* 2019;13:419.
- [16] Guo Y, Chen Y, Yang M, Xu X, Lin Z, Ma J, et al. A rare KIF1A missense mutation enhances synaptic function and increases seizure activity. *Front Genet* 2020;11. <https://doi.org/10.3389/fgene.2020.00061>.
- [17] Siekierska A, Stamberger H, Deconinck T, Opreescu SN, Partoens M, Zhang Y, et al. Biallelic VARS variants cause developmental encephalopathy with microcephaly that is recapitulated in vars knockout zebrafish. *Nat Commun* 2019;10. <https://doi.org/10.1038/s41467-018-07953-w>.