



Research Letter

## PLCG2 Mutation in a Patient Presenting with Type 2 Autoimmune Hepatitis



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Received: April 8, 2024 | Revised: May 6, 2024 | Accepted: May 13, 2024 | Published online: May 28, 2024

**Citation of this article:** Gardin A, Gaigne L, Magérus A, Rieux-Laucat F, Jacquemin E. *PLCG2* Mutation in a Patient Presenting with Type 2 Autoimmune Hepatitis. *J Clin Transl Hepatol* 2024. doi: 10.14218/JCTH.2024.00124.

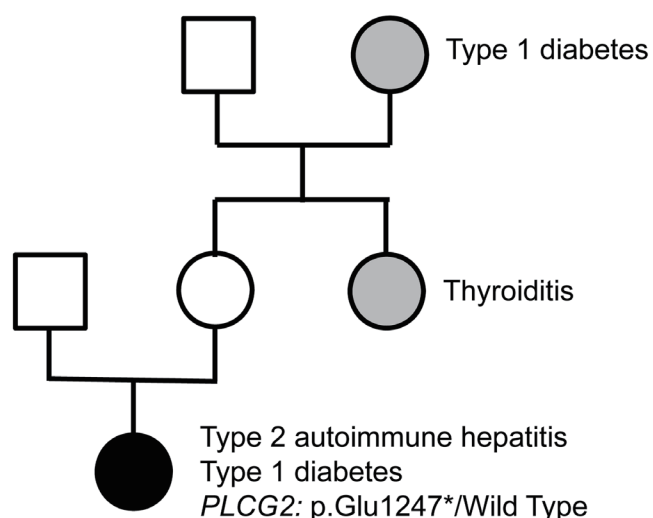
The combination of defective immune tolerance and external triggers is thought to underlie the pathophysiology of autoimmune hepatitis.<sup>1,2</sup> Some polymorphisms associated with susceptibility to autoimmune hepatitis have been reported, and some cases have been described in patients with monogenic primary immune deficiencies or systemic diseases.<sup>1-4</sup> Nevertheless, causative genetic defects involved in defective immune regulation remain unidentified in the vast majority of patients, especially in children and adults with isolated autoimmune hepatitis, namely in the absence of primary immune deficiency or systemic disease. Using whole exome sequencing analysis, we searched for causative genetic defects in children with autoimmune hepatitis. Data were analyzed with in-house software and in silico tools, and findings were confirmed by Sanger sequencing.

In a now adult female diagnosed with type 2 autoimmune hepatitis at one year of age, the molecular analysis identified a heterozygous nonsense variation (NM\_002661; c.3739G>T; p.Glu1247\*; ACMG classification: class 5; phred-scaled CADD score: 52; gnomAD allele frequency: 0) located in the last exon of the *PLCG2* gene - encoding Phospholipase C gamma 2 (PLC $\gamma$ 2) and in the post-C2 part of PLC $\gamma$ 2, 19 amino acids upstream of the regular stop codon. Analysis of family medical history revealed that the maternal aunt and grandmother of the *propositus* suffered from thyroiditis and type 1 diabetes, respectively, as shown in Figure 1. Unfortunately, DNA samples were not available from other family members. At diagnosis, clinical examination showed moderate hepatomegaly and jaundice. Serum liver tests were abnormal (ASAT=410 UI/L, N<40; ALAT=539 UI/L, N<40; GGT=276 UI/L, N<35; ALP=256 UI/L; total bilirubin=126  $\mu$ mol/L, N $\leq$ 17; prothrombin time=57%, N $\geq$ 70%). Ultrasonography of the liver was normal, and liver histology showed inflammation and fibrosis typical of autoimmune hepatitis. The concentration of serum gammaglobulins was at the upper limit of normal value, and blood cell counts were normal. Anti-liver-kidney microsome type 1 antibodies were detected in the sera using an indirect immunofluorescence method at an antibody titer of 1/2,500. According to the International Autoimmune Hepatitis Group and the European Society of Pediatric Gastroenterology Hepatology and Nutrition scoring systems, the diagnosis of autoimmune hepatitis was deemed definite in the patient.<sup>5,6</sup> All other classical causes of acute or chronic liver disease were excluded, including hepatitis C virus infection. The search for other antibodies and markers of other autoimmune diseases was negative except for the presence of anti-Langerhans islet antibodies (value of 5, N<3) in the absence of diabetes. Beyond autoimmunity, the patient exhibited none of the clinical phenotypes associated with *PLCG2* variants in the literature.<sup>7</sup> The patient was treated with azathioprine and prednisone since the diagnosis. She was in remission with normal serum liver tests five years after the initiation of the treatment. An attempt to stop treatment was unsuccessful, and the treatment was resumed, normalizing serum liver tests. At the age of 10, the patient developed type 1 diabetes. Prednisone was stopped, and cyclosporine was introduced. At the last follow-up, the patient, now 21 years old, is doing well. She is treated with azathioprine and low doses of cyclosporine and has displayed normal serum liver tests. So far, no other autoimmune diseases and no new clinical phenotypes have been observed in this patient.

Pathogenic variants of *PLCG2* cause two related forms of autosomal-dominant immune dysregulation: PLC $\gamma$ 2-associated antibody deficiency and immune dysregulation (PLAID) and autoinflammatory PLAID (APLAID).<sup>7</sup> These diseases comprise different clinical presentations related to functional classes of *PLCG2* variants, including cold-induced urticaria, humoral immune deficiency, recurrent or atypical infections, atopy, cytopenia, autoinflammation, and autoimmunity. The p.Glu1247\* variant of the *PLCG2* gene harbored by our patient was recently reported and functionally characterized *in vitro*, but no precise clinical phenotypic data linked to this variant was provided.<sup>7</sup> When overexpressed in a *Plcg2*-deficient DT-40 chicken B cell line, this variant led

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**Fig. 1. Pedigree of the family.** An Empty symbol indicates a normal phenotype. *PLCG2* sequence analysis was performed in the *propositus* after obtaining informed consent, following protocols for human studies approved by our ethics committee. DNA samples were not available from other family members.

to the decreased extracellular signal-regulated kinase (ERK) phosphorylation following stimulation and was classified as a *PLCG2* loss-of-function variant (hypomorphic: 75% of wild-type function). These data suggest the pathogenic effect of this variant. This partial loss of function is likely due to the fact that a nonsense mutation within the last exon cannot activate nonsense-mediated mRNA decay, yielding a stable mRNA that directs the synthesis of a C-terminally truncated protein with impaired function.<sup>8</sup> The ERK pathway, a major signaling cassette of the mitogen-activated protein kinase signaling pathway, contributes to the regulation of numerous cellular processes, involved in the regulation of cell proliferation such as T-cell activation.<sup>9</sup> The patient reported here had familial and personal medical histories of isolated autoimmune diseases without clinical features of PLAID/APLAID. Unfortunately, the segregation of p.Glu1247\* variant within the family could not be studied (Fig. 1). Nevertheless, considering the family pedigree, it can be hypothesized that the variant was transmitted in an autosomal dominant mode with variable penetrance, although a *de novo* occurrence of the mutation in the *propositus* could not be excluded. The classification of the *PLCG2* loss-of-function variant as hypomorphic, leading to a moderate decrease in function, might explain why our patient initially manifested with isolated autoimmune hepatitis without the classical signs described in patients with PLAID/APLAID in the literature. Further studies are required to precisely characterize the mechanisms linking heterozygous loss of *PLCG2* function to the development of autoimmune hepatitis. Additionally, future confirmation that *PLCG2* plays a role in autoimmune hepatitis pathogenesis is needed and deserves consideration. *PLCG2* could represent a candidate gene for patients with autoimmune hepatitis, at least for those with type 2 autoimmune hepatitis. While type 2 autoimmune hepatitis mainly affects children, it may also present in adults, sometimes with an insidious clinical course.<sup>1,2,10</sup> We believe that the search for *PLCG2* variations in pediatric or adult patients with autoimmune hepatitis warrants consideration.

#### Acknowledgments

We thank AMFE (Association Maladie Foie Enfants, Malakoff,

France), Association "Pour Louis 1000 Foie Merci" (Fournet-Luisans, France), and Fondation Rumsey-Cartier (Genève, Switzerland) for their support.

#### Funding

This work was supported by the Institut National de la Santé et de la Recherche Médicale (INSERM) and by government grants managed by the Agence Nationale de la Recherche as part of the "Investment for the Future" program (Institut Hospitalo-Universitaire Imagine, grant ANR-10-IAHU-01, Recherche Hospitalo-Universitaire, grant ANR-18-RHUS-0010), the Centre de Référence Déficiences Immunitaires Héritaires (CEREDIH), the Agence National de la Recherche (ANR-14-CE14-0026-01 "Lumugene"; ANR-18-CE17-0001 "Action"; ANR-22-CE15-0047-02 « BREAK-ITP »), the Fondation pour la recherche Médicale (FRM: EQU202103012670).

#### Conflict of interest

The authors have no conflict of interests related to this publication.

#### Author contributions

Data collection (AG), patient recruitment (AG, EJ), study initiation (EJ), project design and supervision (EJ, FRL, AM), exome analysis (AM, LG). All authors analyzed the data, provided intellectual guidance, and wrote and reviewed the manuscript.

#### Ethical statement

The study was conducted in accordance with the guidelines of the 1975 Declaration of Helsinki and in compliance with French regulatory authorities for data handling and processing. The study received approval from the Ile de France ethics committee (CPP IDF2 DC-2014-22722015-03-03 AF) and the sample collection was declared to the French ministry of research with the reference DC 2014-2272. DNA samples were obtained from the Centre de Ressources Biologiques of Imagine Institute. Informed consent was obtained from the patient/their family.

#### Data sharing statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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