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# POLD3 deficiency is associated with severe combined immunodeficiency, neurodevelopmental delay, and hearing impairment

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## ABSTRACT

Combined immunodeficiency diseases (CID) represent the most severe forms of inborn errors of immunity. Defective T cell development and/or function, leading to an impairment in adaptive immunity are responsible for these diseases. The DNA polymerase  $\delta$  complex is important for genome duplication and maintenance and consists of the catalytic subunit POLD1, and the accessory subunits POLD2 and POLD3 which stabilizes the complex. Mutations in *POLD1* and *POLD2* have been recently shown to be associated with a syndromic CID characterized by T cell lymphopenia with or without intellectual deficiency and sensorineural hearing loss. Here we report a homozygous *POLD3* variant (NM\_006591.3; p.Ile10Thr) in a Lebanese patient, the product of a consanguineous family, presenting with a syndromic severe combined immunodeficiency (SCID) with neurodevelopmental delay and hearing loss. The homozygous *POLD3*<sup>Ile10Thr</sup> variant abolishes POLD3 as well as POLD1 and POLD2 expression. Our findings implicate POLD3 deficiency as a novel cause of syndromic SCID.

## 1. Introduction

Inborn errors of immunity (IEI) are a heterogeneous group of >485 different diseases that alter the normal immune response. Patients with IEI are prone to recurrent or opportunistic infections that can occur with additional manifestations such as autoimmunity, auto-inflammatory disease, allergy, bone-marrow failure, and/or malignancy [1]. Among IEI, combined immunodeficiency disorders (CIDs), represent the most severe forms that are characterized by defective T cell development and/or function, leading to a complete impairment in cellular and humoral immunity [2,3]. Depending on the specific genetic defect, B and NK cell

lymphopenia may also be present. The 2022 update on the classification from the International Union of Immunological Societies (IUIS) expert committee recognizes 135 CID entity. Of these, 19 are severe combined immunodeficiency diseases (SCID) characterized by a drastic T-cell lymphopenia (CD3+ T cell count lower than 300 cells/uL) [4]; and 69 are syndromic or associated with extra-immune presentations [1]. Genetic causes of SCID include defects affecting signaling through the T-lymphocyte antigen receptor, Variable, Diversity and Joining (VDJ) recombination defects; and defects impairing lymphocyte survival [5]. Less severe defects affect disruption of key signaling steps, activation and proliferation of T and B lymphocytes, causing combined

**Abbreviations:** CID, Combined Immunodeficiency Disorder; IEI, Inborn errors of immunity; SCID, Severe Combined Immunodeficiency; WES, Whole Exome Sequencing..

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immunodeficiency [6]. CID with associated or syndromic features include the following 8 main groups: immunodeficiency with congenital thrombocytopenia, DNA repair defects, thymic defects with additional congenital anomalies, immune-osseous dysplasias, hyper-IgE syndromes, defects of vitamin B12 and folate metabolism in addition to anhidrotic epidermolytic immunodeficiency [1].

Here we report a homozygous variant in *POLD3* in a Lebanese family with syndromic severe combined immunodeficiency (SCID,  $T^{lo}B^{+}NK^{lo}$ ) including neurodevelopmental delay and hearing impairment.

## 2. Material and methods

### 2.1. Study subjects

The proband is a 5-year-old male child of first-cousin consanguineous Lebanese parents. Syndromic primary immunodeficiency including neurological manifestations and hearing loss was detected in him and in three of his older siblings (Fig. 1A). He underwent a work-up including clinical evaluation, molecular analyses, total body X-rays, brain MRI, immune screen tests, hearing assessment, and routine blood tests (complete blood count, serum electrolytes, blood glucose levels, cholesterol, thyroid, liver and renal function tests, and phosphatase alkaline levels). Written informed consent from the patient's parents and assent from the patient and his unaffected siblings were obtained.

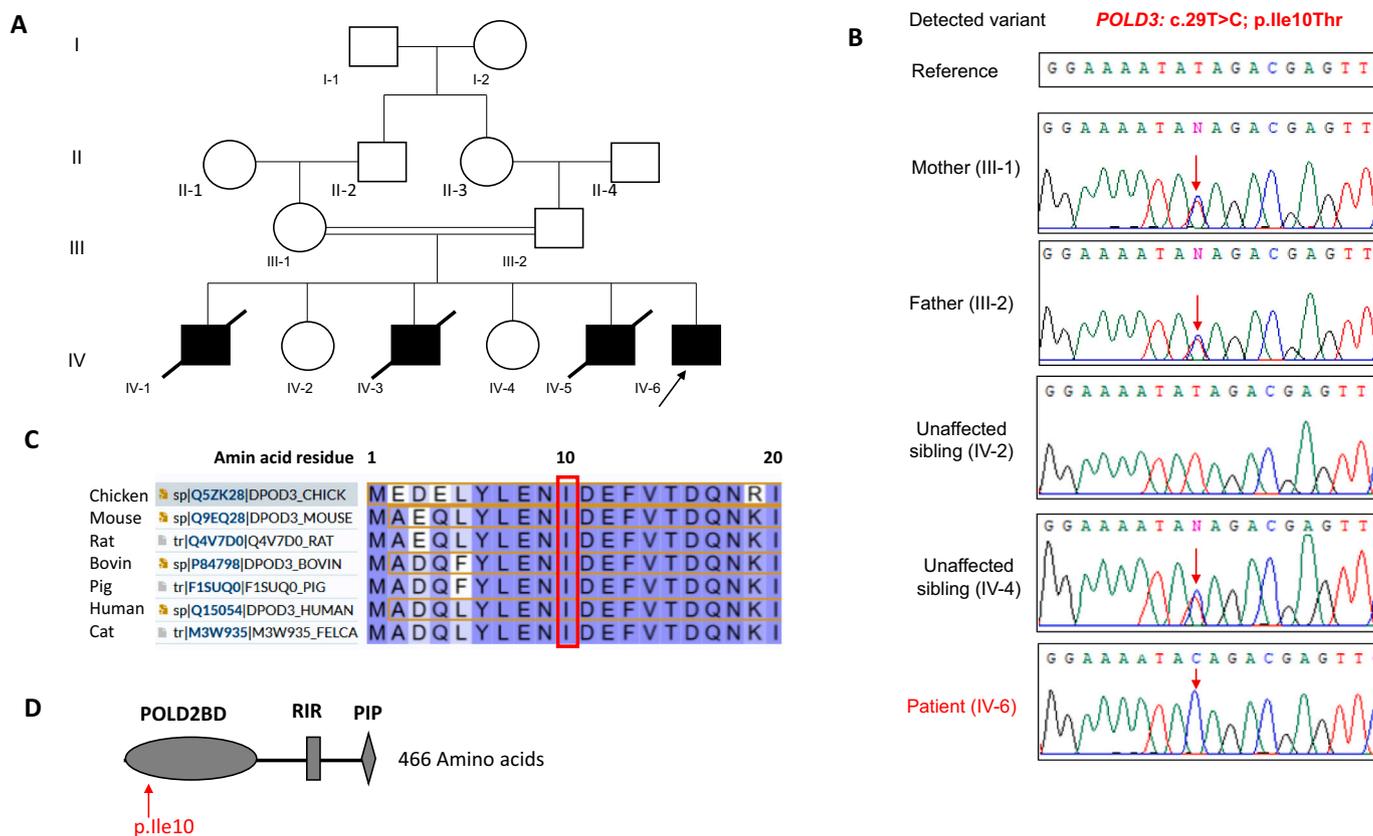
### 2.2. Whole exome sequencing (WES)

EDTA blood samples from all members of the family were collected for genetic studies. Genomic DNA was isolated from peripheral blood by standard salt-precipitation methods [7].

WES was carried-out in the patient. Exome was captured and enriched using the solution Agilent SureSelect Human All Exon kit version 5.0 and samples were then multiplexed and subjected to sequencing on an Illumina HiSeq 2500 PE100–125. Reads files (FASTQ) were generated from the sequencing platform via the manufacturer's proprietary software. Reads were aligned to the hg19/b37 reference genome using the Burrows-Wheeler Aligner (BWA) package version 0.7.11 [8]. Variant calling was subsequently performed using the Genome Analysis Tool Kit (GATK) version 3.3 [9]. Variants were called using high stringency settings and annotated with VarAFT software 1.61 [10] containing information from dbSNP147 and the Genome Aggregation database (gnomAD, <http://gnomad.broadinstitute.org>). Only nonsynonymous coding and splicing variants found in the patient were considered. Variant filtering was performed according to the mode of transmission of the disease in the family, the frequency of the variant in the gnomAD database ( $<0.01\%$  and  $<50$  heterozygous carriers or  $<5$  homo-/hemizygous carriers), and in our in-house database ( $<1$  homozygous occurrence).

### 2.3. Sanger sequencing

The selected variant was studied in the proband, his parents and two



**Fig. 1.** A homozygous variant in *POLD3* causes a syndromic severe combined immunodeficiency including neurological manifestations and hearing loss. **A:** Pedigree of the family included in this study. **B:** Sanger sequencing confirming the segregation of the variant with the disease in the family. The red arrow pinpoints the c.29T>C variant in *POLD3* that is heterozygous in parents and in one unaffected sibling (IV-4), and is homozygous in the proband (IV-6). The variant is not carried by the unaffected sibling IV-2. **N:** heterozygous peak. **C:** Alignment of *POLD3* protein sequences between several mammalian species (Human, Chicken, Mouse, Rat, Bovine, Pig, Cat), using UniProt alignment tool. The red box highlights the position of the residue Ile10 that is affected in the proband, and is highly conserved among several mammalian species. **D:** *POLD3* protein structure and domains. POLD2BD: POLD2 binding domain; RIR: Rev1-interacting regions; PIP: PCNA-interacting protein.

of his unaffected siblings by Sanger sequencing. Genomic and cDNA sequences of *POLD3* were obtained from UCSC Genomic Browser (NM\_006591). Primers used for PCR amplification were designed using Primer3 software (<http://frodo.wi.mit.edu>) to amplify the region surrounding the mutation detected by exome sequencing. PCR products were purified by exonuclease I/Shrimp Alkaline Phosphatase treatment (ExoSAP-IT; Fisher Scientific SAS, Illkirch, France) according to the manufacturer's instructions and both strands were sequenced using the Big Dye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems). Sequence reactions were purified on Sephadex G50 (Amersham Pharmacia Biotech, Foster City, CA) and capillary electrophoresis was performed on Genetic Analyser 3100 (Applied Biosystems). Electropherograms were analyzed on the Sequence Analysis Software version 5.2 (Applied Biosystems) and aligned with the wild-type *POLD3* gene sequence using ChromasPro version 1.22 (Technelysium, Queensland, Australia).

#### 2.4. Cell culture

Human PBMCs were isolated from heparinized blood obtained from the patient, his father and healthy controls using Ficoll-Hypaque (GE Healthcare).

#### 2.5. Immunoblot analysis

Total protein was extracted from PBMCs from the patient, his father and two healthy controls. Cell lysates were separated by SDS-PAGE, transferred onto nitrocellulose membranes. Non-specific binding was blocked by incubating membranes 1 h at room temperature in 5% non-fat dry milk in Tris Buffered Saline-Tween. Blots were then probed with primary antibodies, 2 h at room temperature. Primary antibodies used are anti-POLD1 (1:200; sc-17776, Santa Cruz Biotechnology); anti-POLD2 (1:500; HPA026745, MilliporeSigma); anti-POLD3 (1:1000; A301-244A-M, Bethyl Laboratories) and anti-GAPDH (1:200; sc-166574, Santa Cruz Biotechnology). Secondary antibody used are: peroxidase affini-pure goat anti-mouse IgG (1:10000; 115-035-003, Jackson immunoresearch) or peroxidase affini-pure goat anti-rabbit IgG (1:10000; 111-035-003, Jackson immunoresearch). Antibody binding was detected by western lightning plus-ECL, enhanced chemiluminescence substrate (PerkinElmer). Specific protein expression was first normalized to endogenous GAPDH expression, in each individual. The relative expression of the normalized protein was then assessed in each individual, in comparison to the average expression in the two healthy controls.

### 3. Results

#### 3.1. 3.1. Identification of a homozygous variant in *POLD3* in a syndromic combined immunodeficiency

The proband is the youngest child of a healthy 32-year-old G6P6A0 mother and a 34-year-old father. He was born to a first degree consanguineous Lebanese family with a history of syndromic SCID, manifesting in three of his older brothers (IV-1, IV-3 and IV-5) (Fig. 1A). The affected siblings had a severe developmental delay, absence of ambulation, intellectual disability, hearing impairment, hepatosplenomegaly, and repetitive upper respiratory tract infections accompanied with fever and diarrhea that began at around 7 months of age and progressed to severe pulmonary insufficiency. The patients IV-1, IV-3 and IV-5 (Fig. 1A) died at age 3, 5, and 5 years old respectively, from heart arrest after an infection episode.

The proband (IV-6; Fig. 1A) was born at term at 39 weeks of gestation after an unremarkable pregnancy. His birth weight was 3270 g (65th percentile), his length was 50 cm (50th percentile) and his head circumference was 35 cm (65th percentile). He started to experience recurrent respiratory infections and bronchiectasis since the age of 7

months old. Developmental milestones were slightly delayed. He could sit alone at 10 months and started to walk at 13 months. During the following year, the parents noticed that the child's walk was unstable and that he was not normally reaching the developmental milestones. At the age of 2 years, as the child was not reacting to voices and sounds, an auditory brainstem response test (ABR) was performed and showed severe bilateral sensorineural hearing loss.

He presented to us at the age of 4 years and a half. Height was 93 cm, weight 10 kg, and head circumference 47.5 cm (all measures below the 3rd percentile). Developmental delay was present as he was not yet toilet trained, was not responding correctly to simple orders even from his parents, and was quickly passing from a calm and cuddly state to banging his head against the floor or the wall. Clinical examination revealed the absence of dysmorphic facial features, an unsteady gait, normal osteotendinous reflexes, a dry skin, very small tonsils, and hepatosplenomegaly. Skeletal survey, echocardiography, and brain MRI showed no abnormalities. Ophthalmological evaluation was normal. Results of serum electrolytes, blood glucose levels, triglycerides, cholesterol, amino acid studies of plasma and urine, urinary screening for organic acids, urinalysis, thyroid, liver and renal function tests, CPK, P, Ca were all unremarkable.

Lymphocyte phenotyping performed on peripheral blood showed a low absolute lymphocyte numbers with severe depletion of CD3<sup>+</sup> T cells and decreased numbers of CD3<sup>+</sup> CD56<sup>+</sup> CD16<sup>+</sup>, NK cells. There was a drastic decrease in the percentage of CD45RA<sup>+</sup> CCR7<sup>+</sup> naïve CD4<sup>+</sup> and CD8<sup>+</sup> T cells, indicating very poor thymic output, and a drastic increase in the percentage of CD45RA<sup>-</sup> CCR7<sup>+</sup> CD4<sup>+</sup> and CD45RA<sup>-</sup> CCR7<sup>+</sup> CD8<sup>+</sup> cells, raising the possibility that these memory T cells could be of maternal origin (Table 1). T cell functional testing was not possible because of the unavailability of patient samples as parents declined to provide further blood samples. The absolute number of CD19<sup>+</sup> B cells and the percentages of CD27<sup>-</sup> naïve B cells and CD27<sup>+</sup> memory B cells were normal (Table 1). Serum immunoglobulin levels showed decreased IgA and normal IgG and IgM levels in the absence of immunoglobulin replacement therapy (Table 1). Specific antibody response to vaccines was not assessed because the patient was never vaccinated.

**Table 1**

Immune phenotyping of the patient's lymphocytes. Bolded values are outside the normal range.

Age at the time of testing (years)	P1
4	
<b>Hemogram (normal range)</b>	
Hemoglobin, g/dL	12.1 (11–14)
WBCs, 10 <sup>3</sup> cells/μL	7.4 (5–15)
Neutrophils, 10 <sup>3</sup> cells/μL	5.07 (1.9–8)
Lymphocytes, 10 <sup>3</sup> cells/μL	<b>0.61</b> (0.9–7)
Monocytes, 10 <sup>3</sup> cells/μL	0.80 (0.16–1)
Platelets, 10 <sup>3</sup> cells/μL	376 (150–400)
<b>Lymphocyte subsets</b>	
CD3 <sup>+</sup> , 10 <sup>3</sup> cells/μL	<b>158</b> (850–4300)
CD3 <sup>+</sup> CD4 <sup>+</sup> , 10 <sup>3</sup> cells/μL	<b>19</b> (500–2700)
CD45RA <sup>+</sup> CCR7 <sup>+</sup> naïve, % CD4 <sup>+</sup>	<b>5.3</b> (45.56–75.28)
CD45RA <sup>-</sup> CCR7 <sup>-</sup> Effector memory, % CD4 <sup>+</sup>	5.3 (2.08–8.78)
CD45RA <sup>-</sup> CCR7 <sup>+</sup> Central memory, % CD4 <sup>+</sup>	<b>94.1</b> (22.06–46.46)
CD45RA <sup>+</sup> CCR7 <sup>-</sup> Effector, % CD4 <sup>+</sup>	<b>5.3</b> (0.00–1.06)
CD3 <sup>+</sup> CD8 <sup>+</sup> , 10 <sup>3</sup> cells/μL	<b>25</b> (200–1800)
CD45RA <sup>+</sup> CCR7 <sup>+</sup> naïve, % CD8 <sup>+</sup>	<b>4.2</b> (41.58–77.90)
CD45RA <sup>-</sup> CCR7 <sup>-</sup> effector memory, % CD8 <sup>+</sup>	4.2 (1.58–13.18)
CD45RA <sup>-</sup> CCR7 <sup>+</sup> central memory, % CD8 <sup>+</sup>	<b>87.4</b> (12.08–30.54)
CD45RA <sup>+</sup> CCR7 <sup>-</sup> TEMRA, % CD8 <sup>+</sup>	4.2 (1.70–24.62)
CD19 <sup>+</sup> , 10 <sup>3</sup> cells/μL	296 (200–2100)
CD27 <sup>-</sup> naïve, % CD19 <sup>+</sup>	85.5 (61.4–101.6)
CD27 <sup>+</sup> Memory, % CD19 <sup>+</sup>	14.5 (8.1–33.3)
CD3 <sup>-</sup> CD56 <sup>+</sup> CD16 <sup>+</sup> , 10 <sup>3</sup> cells/μL	<b>44</b> (100–1000)
<b>Immunoglobulins</b>	
IgG, g/L	11.4 (5.53–13.07)
IgM, g/L	0.64 (0.563–2.184)
IgA, g/L	<b>0.28</b> (0.328–1.799)
IgE, IU/mL	17 (<60)

Clinical examination of the father was unremarkable. His lymphocyte phenotyping was also normal (data not shown). Audiometry testing showed that he has a hearing impairment at high frequencies that is mild to moderate in the right ear and moderate to severe in the left ear (Supplementary Fig. 1). The mother was diagnosed with a left muscle tissue neck rhabdomyosarcoma at age 16, treated with chemotherapy and radiation therapy, with a current relapse. The mother refused donating blood for immunologic studies.

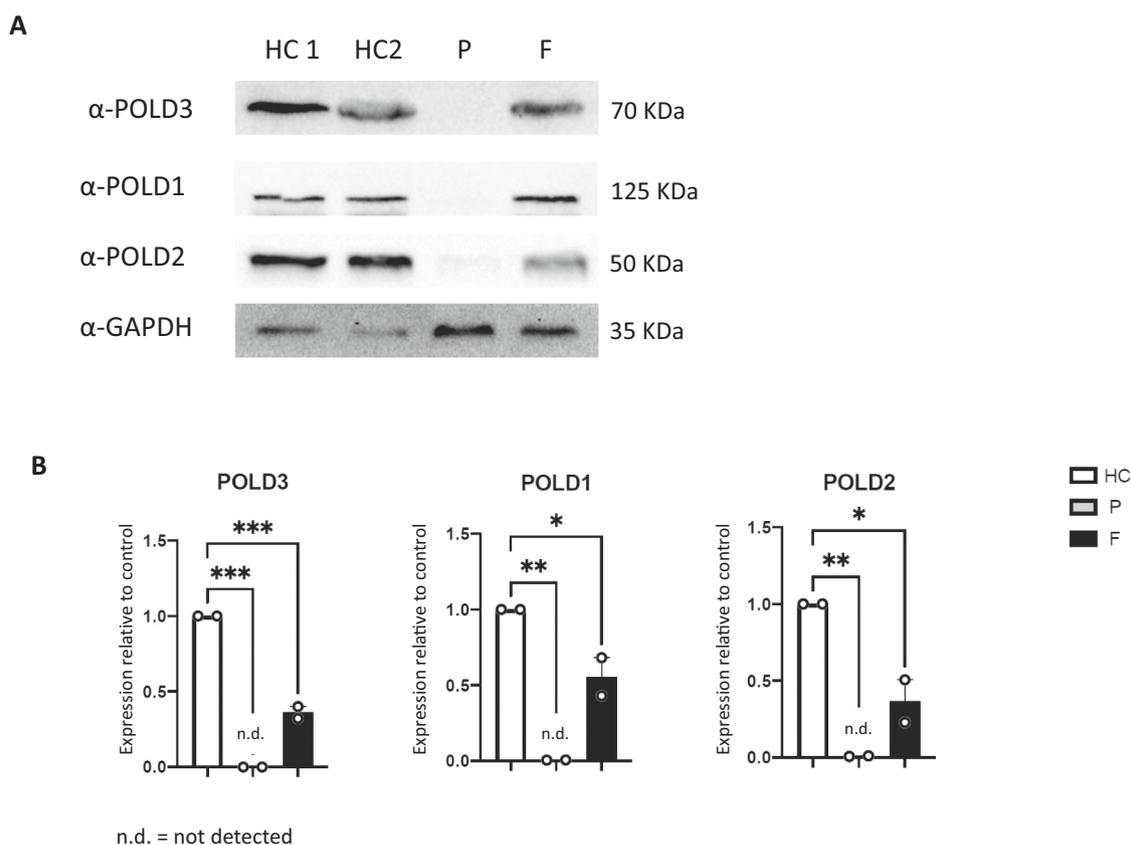
WES was performed in the patient and led to the identification of 98,340 variants. Considering the recessive inheritance of the disease in the pedigree and consanguinity, we assumed identity by descent and thus selected variants homozygous in the patient. This led to the selection of 35,809 variants. Additional filtering was then performed to exclude all non-genic, non-splice site, and intronic variants as well as all frequent variants (present at a frequency of >1% in databases). This filtering strategy led to the selection of a list of 185 variants. A supplementary step consisting of excluding all variants predicted to be benign or likely benign according to different prediction software led to the selection of 58 variants that were all variants of unknown significance (Supplementary Table 1) that were thoroughly studied in order to select the potential disease-causing variant. One homozygous variant, the p.Ile10Thr (c.29 T > C) in the first exon of *POLD3* (NM\_006591.3), was considered as the only candidate variant based on the gene function. *POLD3* encodes the accessory subunit 3 of the DNA polymerase  $\delta$  complex that is involved in DNA replication and repair. Sanger sequencing confirmed the presence of the identified variant in a homozygous state in the affected patient (IV-6) and in a heterozygous state in both parents (III-1 and III-2). It was found to be absent in the oldest live healthy sibling (IV-2) and was carried at heterozygous state by the second unaffected sibling (IV-4) (Fig. 1B). Unfortunately, in the absence of Guthrie

cards (or any other sample) from the deceased siblings, their testing for the candidate variant was not possible. The identified variant in the *POLD3* gene is absent in our local database and in public databases and affects an amino acid residue that is highly conserved among species (Fig. 1C). It is located in the N-terminal domain of the protein that is known to interact with *POLD2* (domain known as *POLD2* binding domain) (Fig. 1C) [11]. The p.Ile10Thr variant is predicted to be pathogenic by the following 6 prediction tools: BayesDel\_addAF, BayesDel\_noAF, MetaRNN, LRT, MutPred, PrimateAI, versus Variant of Unknown Significance (VUS) by the 12 prediction tools: REVEL, BLOSUM, DANN, DEOGEN2, EIGEN, EIGEN\_PC, LIST-S2, Mutation Assessor, Mutation Taster, PROVEAN, SIFT and SIFT4G. Six prediction tools classified it as Benign: MetaLR, MetaSVM, FATHMM-MKL, FATHMM-XF, M-CAP, and MVP. The variant has a CADD score of 25.7; and the pLI score of the gene retrieved from gnomAD is 0.998, indicating intolerance to protein-truncating variations.

In order to verify that no other candidate variants were missed, WES and coverage data were then checked for a panel of genes implicated in IEs. Analysis showed that the selected genes were well covered (at a minimum of 20 $\times$ ); which helped us exclude the presence of known pathogenic variants involved in known IEs.

### 3.2. The p.Ile10Thr variant in *POLD3* abolishes the expression of *POLD3* as well as *POLD1* and *POLD2*

To assess the impact of the p.Ile10Thr variant on the expression of the *POLD3* protein, immunoblotting was performed on PBMC lysates from the patient, his heterozygous father and two healthy donors. *POLD3* expression was absent in the patient and significantly decreased to ~ half normal level in the heterozygous father compared to healthy



**Fig. 2.** *POLD3*<sup>Thr10Ile</sup> alters the expression of *POLD1*, *POLD2* and *POLD3* proteins. A: Immunoblots analysis of *POLD1*, *POLD2* and *POLD3* in PBMCs from the patient (P), his father (F) and two healthy controls (HC). B: Quantitation of *POLD1*, *POLD2* and *POLD3* protein expression in PBMCs of P, F and two HCs. *POLD* protein expression was normalized to GAPDH, and expressed relative to the mean ratios of two healthy controls. Results represent means  $\pm$  S.E.M. \*\* $p$  < 0.01; \*\*\* $p$  < 0.001; \*\*\*\* $p$  < 0.0001, by one-way ANOVA. n.d.: not detectable.

controls (Fig. 2).

*Pold3* deletion in mice was shown to destabilize the Pol $\delta$  complex [12]. The levels of POLD1 and POLD2 were examined in the patient PBMCs, his father and two healthy controls. Expression of both POLD1 and POLD2 was almost completely abolished in the patient; and significantly to ~ half normal level in the heterozygous father (Fig. 2).

#### 4. Discussion

Here we report a homozygous mutation in *POLD3* (NM\_006591.3: c.29T>C; p.Ile10Thr) in the offspring of a consanguineous Lebanese family with syndromic T<sup>+</sup>B<sup>+</sup>NK<sup>-</sup> SCID, including neurodevelopmental delay and profound hearing loss.

*POLD3* encodes an accessory subunit of the DNA polymerase  $\delta$  and the DNA polymerase  $\zeta$  complexes. It plays a crucial role in DNA replication and repair. DNA replication in eukaryotes is regulated by 3 B-family DNA polymerase complexes: polymerase  $\alpha$ , polymerase  $\delta$  and polymerase  $\epsilon$ . While polymerase  $\alpha$  has a primase activity, polymerase  $\delta$  and polymerase  $\epsilon$  are involved in the synthesis of the leading and lagging strand, respectively [13,14]; with recent studies linking polymerase  $\delta$  complex to the leading-strand DNA synthesis as well [15]. Each of these complexes comprises a catalytic subunit associated with several accessory subunits. The polymerase  $\delta$  complex is composed of POLD1 bearing the catalytic activity, in addition to POLD2, POLD3 and POLD4 that are accessory/structural subunits responsible for the stabilization of the complex [13]. Beyond DNA replication, polymerase  $\delta$  has a role in DNA double-strand break (DSB) repair, DNA repair synthesis, and in common fragile site instability [16]. The involvement of POLD3 in the DNA damage response is not restricted to its contribution to POLD1 function. It is also mediated through the DNA polymerase  $\zeta$  complex that is involved in translesion DNA synthesis, and that includes REV3L as a catalytic subunit, in addition to REV7, POLD2 and POLD3 as accessory proteins [17].

The POLD3<sup>Ile10Thr</sup> variant detected in the studied family was shown to virtually abolish the expression of POLD3, as well as POLD1 and POLD2 proteins. This allows the classification of the variant as pathogenic; and suggests a link between the loss of POLD3 and the syndromic SCID in the patient and likely in his deceased brothers. Interestingly, biallelic *POLD1* and *POLD2* variants have been recently linked to syndromic combined immunodeficiency disorders associated with T cell lymphopenia with or without intellectual deficiency and sensorineural hearing loss [18,19]. While T-cell lymphopenia was detected in all reported patients with biallelic *POLD1* or *POLD2* mutations (5 patients), sensorineural hearing loss was detected in 3 cases; and intellectual disability in 3 cases; with hearing impairment and intellectual disability occurring together in 2 cases [18,19]. A heterozygous de novo *POLD1* variant was also detected by neonatal screening in a case with non-SCID T-cell lymphopenia [20].

In comparison to POLD1 and POLD2 deficiencies [18,19], POLD3 deficiency is likely more severe whereby the patient herein studied suffers from a striking T-cell depletion, that was also reported to have been detected in his siblings who died by 5 years of age. This is not surprising, since *Pold3* deletion destabilizes the DNA polymerase  $\delta$  complex and leads to embryonic lethality in mice [12]. This suggests possible redundancy in some of the functions of POLD3 in human, compared to mice; similar to the case of POLD2 deficiency that is also embryonically lethal in mice [21].

In contrast to the patients reported by Conde et al., with replicative stress, neurodevelopmental abnormalities, and immunodeficiency [18], B cell lymphopenia was not detected in the patient herein studied; nor in two of the patients described by Cui et al., with a combined immunodeficiency disorder associated with T cell lymphopenia, CD8+ T cell oligoclonality and TCR repertoire restriction [19]. Redundancy in the role of the DNA polymerase complex  $\delta$  or its different components in human B cells may be possible. Further studies are needed to explore this hypothesis and to uncover the role of the DNA polymerase complex  $\delta$  in

DNA replication and proliferation in human B cells.

The involvement of polymerases in syndromic inborn errors of immunity is not restricted to polymerase  $\delta$  components, as mutations in *POLE1* and *POLE2*, encoding components of the polymerase  $\epsilon$  complex have been reported in patients with primary immunodeficiency disease with dysmorphic features [22,23]. Our report further highlights the importance of DNA polymerase complexes in the modulation of immune cells proliferation and normal functioning. The severity of the clinical presentation of the patient herein reported with POLD3 deficiency, suggests that a bone marrow transplant would likely be beneficial. The involvement of the polymerase  $\delta$  complex in the DNA damage response and its role in non-immune cells should be taken into consideration when choosing conditioning regimens to prepare the patient for the transplantation. Given the ubiquitous expression of the DNA polymerase  $\delta$ , its role in the DSB repair mechanism and in the maintenance of the genomic stability [24], and the history of cancer in the patient's mother, surveillance for malignancy would also be required.

Besides the immune phenotype of the patients with polymerase  $\delta$  deficiencies, sensorineural hearing loss was observed in some of the cases. *POLD1* variants leading to reduced polymerase activity have also been linked to sensorineural hearing loss without syndromic features [25]; thus indicating an important role of the DNA polymerase complex  $\delta$  in maintaining the integrity of the inner ear and the auditory nerve. Interestingly, hearing impairment at high frequencies was detected in the father of the proband, who is haploinsufficient for POLD3. Reporting and investigating further cases with *POLD3* variants is needed to better understand the involvement of the gene in deafness.

In summary, we report POLD3 deficiency as a novel potential cause of syndromic SCID. Reporting additional patients with *POLD* pathogenic variants is essential to further delineate the spectrum of polymerase  $\delta$  deficiencies and to assess the possible outcome of therapeutic approaches in the affected patients. This report should prompt the investigation of *POLD3* variants as a cause in patients with SCID to better understand the clinical spectrum of POLD3 deficiency.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.clim.2023.109326>.

#### Authorship contributions

CM, EC and AM designed the study, performed genetic and functional studies, interpreted data and wrote the manuscript.

SAA and WS contributed to technical work.

GL, MM and VD contributed to the interpretation of clinical and genetic data.

IM contributed to the interpretation of clinical laboratory data.

All authors approved the manuscript.

#### Ethics approval and consents

Approval to conduct the study was obtained from the IRB of the Lebanese American University, Lebanon (LAU.SOM.CM1.30/Jan/2023). Written informed consent for analysis, and data publication was obtained from the family in compliance with national ethics regulation.

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#### Declaration of Competing Interest

None.

## Data availability

Data is available from the corresponding author upon request.

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