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ORIGINAL ARTICLE

# PID in Disguise: Molecular Diagnosis of IRAK-4 Deficiency in an Adult Previously Misdiagnosed With Autosomal Dominant Hyper IgE Syndrome

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**Abstract** Autosomal recessive IL-1R-associated kinase 4 (IRAK-4) deficiency is a rare cause of recurrent pyogenic infections with limited inflammatory responses. We describe an adult female patient with severe lung disease who was phenotypically diagnosed as suffering from autosomal dominant Hyper IgE syndrome (AD HIES) because of recurrent skin infections with *Staphylococcus aureus*, recurrent pneumonia and elevated serum IgE levels. In contrast to findings in AD HIES patients, no abnormalities were found in the Th17 and circulating follicular helper T cell subsets. A panel-based sequencing approach led to the identification of a homozygous *IRAK4* stop mutation (c.877C>T, p.Gln293*).

**Keywords** Primary immunodeficiency · IRAK-4 · hyper IgE syndrome · next-generation sequencing

## Introduction

Autosomal recessive IL-1R-associated kinase 4 (IRAK-4) deficiency was first described by Picard et al. as a cause of recurrent pyogenic infection with limited inflammatory responses in 3 unrelated children [1]. IRAK-4 is a serine threonine kinase that acts downstream of MyD88, a cytosolic adaptor providing a bridge from Toll-like receptors (TLRs) and the interleukin-1 receptors (IL-1R) to the IRAK complex. This complex consists of 2 active kinases (IRAK-1 and IRAK-4) and 2 noncatalytic subunits (IRAK-2 and IRAK-3). It forms an essential link between receptor stimulation and downstream activation of the NF-κB and MAPK pathways.

IRAK-4 deficiency is considered a phenocopy of MyD88-deficiency [2]. Patients display impaired Toll-IL-1R immune responses and have an increased susceptibility to bacterial infections, mostly caused by *Streptococcus pneumoniae* and *Staphylococcus aureus* [1–3]. MyD88- and IRAK-4-

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dependent signaling is vital during early childhood but its importance declines with increasing age, as no deaths have been reported in these patients beyond the age of 8 and no invasive infections have been documented after the age of 14 [3, 4]. In IRAK-4 deficiency, loss of $IgM^{+}IgD^{+}CD27^{+}$ memory B-cells due to inadequate TLR-mediated proliferation has been shown to lead to diminished T-independent IgM responses which, in combination with age-related impairment of anti-carbohydrate IgG, may underlie increased susceptibility to pyogenic infections [5, 6]. There have been no reports on invasive fungal infection in IRAK-4 deficiency [3].

Here we describe a patient in whom a phenotypical diagnosis of autosomal dominant (AD) Hyper IgE syndrome (HIES) was refuted by molecular diagnosis of IRAK-4 deficiency in adulthood. Interestingly, this patient continued to suffer from recurrent pyogenic infections (esp. pneumonia) with ensuing chronic lung disease and colonization with *Aspergillus fumigatus*.

## Methods

### Clinical Investigation and HIES Scoring

To investigate a possible diagnosis of HIES, the National Institutes of Health (NIH) scoring system was applied [7, 8]. Alternatively, the scoring method proposed by Woellner et al. for the diagnosis of STAT3-deficient HIES, was used [9–12].

### Genetic Investigations

An in-solution NimbleGen SeqCap EZ capture was designed in order to cover all exons of 174 primary immunodeficiency (PID) related genes. The probe design covered approximately 1386 kb, corresponding to 91 % of the targeted region. The list of genes is available upon request. 1 μg of genomic DNA was fragmented by sonication and libraries were prepared by use of the TruSeq DNA Sample Preparation Kit from Illumina. For each capture reaction, 6 libraries were pooled. Sequencing was done on the Hiseq 2500 rapid mode (Illumina) as 150 bp paired-end reads. Mapping of the reads was performed against the reference genome build 19. Further bio-informatics analysis was done with an in-house pipeline (base-calling, alignment and variant calling were performed using the GATK package). Variant annotation and classification of variants was performed within the Cartagenia BENCHlab NGS module (Cartagenia v3.2). Filtering steps included exonic nonsynonymous and surrounding intronic variants (+− 20 bp) with a maximum minor allele frequency (MAF) of 2 % if present in the different population databases. Sanger sequencing was applied to confirm the presence of single-nucleotide variants found by the PID capture.

### Routine Immunological Assays

Serum immunoglobulin concentrations, complement activation, dihydrorhodamine oxidation, and leukocyte subsets were measured in the clinical laboratory of the University Hospitals Leuven. Results were compared with age-related reference values.

### In Vitro Evaluation of NF-κB Function

Peripheral blood mononuclear cells (PBMCs) were prepared from heparinized peripheral blood by means of centrifugation trough Ficoll. PBMCs were seeded immediately at $2 \times 10^6$ cells/ml in a 96-well culture plate and stimulated for 24 h with different TLR ligands and IL-1β: $Pam_3CSK_4$ (TLR1/2, 100 ng/ml, Invivogen), Poly I:C (TLR3, 12.5 μg/ml, Invivogen), LPS (TLR4, 10 μg/ml, Sigma-Aldrich), R-848 (TLR7/8, 3 μg/ml, Invivogen), IL-1β (IL-1R, 50 ng/ml, eBioscience), and heat-inactivated *Streptococcus pneumonia* serotype 3 ($1.2 \times 10^8$ CFU/ml). Primary skin fibroblasts were derived from skin biopsies performed on the index patient and 6 healthy adult volunteers. Fibroblast cell lines were prepared in the Department of Human Genetics at the University Hospitals Leuven. Cells were seeded at a concentration of $2 \times 10^4$cells/stimulation in 96-well culture plates and stimulated for 24 h with IL-1β (1 ng/ml) and TNF-α (10 ng/ml). After stimulation, cells were pelleted by centrifugation and the supernatants were frozen at −20 °C. IL-6 was measured by a commercial ELISA kit, according to the manufacturer's instructions (BD). Data was visualized with GraphPad Prism (version 3.02, GraphPad).

### Flow Cytometry: Additional B/T Cell Subsets

In addition to the routine leukocyte subsets, transitional B cells, naïve B cells, plasmablasts, memory T cells, circulating follicular helper T ($cT_{FH}$), Th1, and Th17 cells were quantified in separate experiments on frozen PBMCs [12, 13]. The list of employed antibodies is available upon request. Data acquisition was performed on a FACSCanto II flow cytometer (BD). Data was analyzed using FlowJo (version 10, Treestar).

## Results

The patient was born term with normal healing of the umbilical cord. There was no neonatal rash. At age 6 months, she was hospitalized for a liver abscess caused by *Staphylococcus aureus*. During hospitalization and antibiotic treatment, the patient acquired meningitis for which no microbiological cause was identified. During her childhood, she suffered from mild eczema and had several skin abscesses (mostly with

*Staphylococcus aureus).* At age 18 years, she suffered from *Neisseria meningitides* meningitis and recovered without sequelae. In the same year an episode of reactive arthritis occurred after immunization with Hepatitis B vaccine. Thereafter, the patient had recurrent pneumonia (11 episodes) mostly located in the right lung. Isolates included *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae* and *Pseudomonas aeruginosa.* Additional treatment with amphotericin B and later voriconazole was started when sputum culture grew *Aspergillus fumigatus* and when her clinical course was complicated. Differential diagnosis included allergic bronchopulmonary aspergillosis (ABPA), albeit in the absence of asthma, and invasive aspergillosis. Despite intensive treatment, a right pneumonectomy was needed due to complete destruction and chronic infection (Fig. 1). Histological investigation of the explanted tissue revealed honeycombing and fibrotic destruction of the entire right lung. Pneumonectomy was complicated by infection of the lung cavity and purulent pericarditis with *Staphylococcus aureus* and *Prevotella* sp requiring thoracoscopic debridement. One year after thoracoscopy, infection of the thoracic cavity recurred, again requiring thoracotomy and a quadriceps muscle flap into the cavity. To date, at age 32, she continues to suffer from recurrent respiratory tract infections (mostly caused by Gram negative bacteria) and chronic infection with *Aspergillus fumigatus,* with cessation of systemic antifungal treatment resulting in respiratory exacerbation. Her baseline FVC and FEV1 values are at 2.13 L (3.72 L predicted) and 1.74 L (3.24 L predicted), respectively. The patient and her pediatrician reported that she was able to mount fever and produce high levels of CRP only relatively late in the course of invasive infections (empyema, abscess). Acute phase responses were absent or diminished in the early time course of infections. For instance, malaise and subfebrillitas prevailed at the time of pneumonia in adulthood; neutrophil count was not higher than $4.4 \times 10^9$/L (normal range: 2.5–$7.8 \times 10^9$/L) and CRP was normal (5.6 mg/L (normal range 0.0–7.0 mg/L). In response to respiratory exacerbation due to *Aspergillus fumigatus* and due to viral infection, acute phase responses were low throughout.

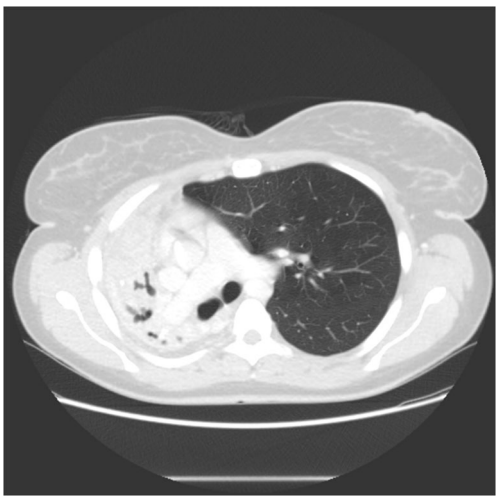

**Fig. 1** CT scan of the chest showing abcedation with complete obliteration of the right lung

Routine laboratory evaluation revealed high serum IgE and IgG4 levels (Table 1). The absolute eosinophil count was normal. Lymphopenia with a decrease in total $CD3^+$ and $CD3^+CD4^+$ cells was found. In the B cell subsets, the $CD27^+IgM^+IgD^+$ and $CD24^{++}CD38^{++}$ B cell count was low compared to values found in healthy adult controls [14]. No abnormalities were found in Th17 and $cT_{FH}$ cell subsets. IgG antibodies against pneumococcus before and after vaccination with the conjugate vaccine could be detected although no increase of the antibody titer was seen (28 U/L vs. 20 U/L, laboratory cutoff>19 U/L).

At the age of 21, she had been diagnosed with HIES due to the presence of skin infections, severe lung infections, and elevated IgE levels. However, a molecular diagnosis was never pursued. Upon referral to the Department of Pediatric Immunology at the University Hospitals Leuven at the age of 31, several features were found unusual for HIES (absence of *Candida* infections, presence of liver abscess, and absence of non-immunological features of AD HIES) reflected by NIH HIES and STAT3 HIES scores of only 39 and 20, respectively [7–12]. Chronic granulomatous disease (CGD), TLR defects, complement defects, and hypomorphic SCID mutations were also considered. Therefore, she was included in an in-house panel-based sequencing approach targeting 174 PID related genes. In total, we obtained 295 variants. After filtering as described in Material and Methods, 1 homozygous missense variant in *IRAK4* and 1 heterozygous missense variant in *AIRE* were found. In addition, 10 intronic variants were obtained. The homozygous variant c.877C>T in the *IRAK4* gene leads to a p.Q293* stop mutation, resulting in a truncated kinase domain [15]. The other variants were analyzed and classified as likely benign. Simultaneously, functional evaluation of NF-κB function revealed severely impaired IL-6 production after stimulation with TLR and IL-1R ligands in patients' PBMCs and skin-derived fibroblasts (Fig. 2). More importantly, the *STAT3* gene showed no mutation in the capture array. CGD was also excluded by sequencing and a negative dihydrorhodamine oxidation assay. Complement assays were normal.

## Discussion

The c.877C>T (p.Gln293*) mutation is frequent in IRAK-4 deficiency as 22 of 48 previously reported patients carry the mutation in a homozygous or compound heterozygous state

**Table 1** Laboratory evaluation of Ig levels and lymphocyte subsets

| | At age 31 | Normal values |
|---|---|---|
| Immunoglobulins | | |
| IgG, g/L | 14.10 | 7.51–15.60 |
| IgG1, g/L | 7.83 | 4.90–11.40 |
| IgG2, g/L | 4.47 | 1.50–6.40 |
| IgG3, g/L | 0.22 | 0.20–1.10 |
| IgG4, g/L | **5.16** | 0.08–1.40 |
| IgA, g/L | 2.54 | 0.82–4.53 |
| IgM, g/L | 1.59 | 0.46–3.04 |
| IgE, kU/L | **1392** | ≤114 |
| Differential WBC count, $10^9$ cells/L | | |
| Neutrophils | 5.9 | 2.5–7.8 |
| Eosinophils | 0.1 | ≤0.4 |
| Basophils | 0.0 | ≤0.1 |
| Lymphocytes | **1.1** | 1.2–3.6 |
| Monocytes | 0.6 | 0.2–0.8 |
| Total lymphocytes, $10^9$ cells/L | **1.072** | 1.208–3.586 |
| $CD19^+$, $10^9$ cells/L | 0.127 | 0.082–0.476 |
| B cell subsets, % of $CD19^+$ | | |
| $CD27^+IgM^+IgD^+$ | **6.6** | 13.4–21.4[A] |
| $CD27^+IgM^-IgD^-$ | **19.0** | 9.2–18.9[A] |
| $CD27^-IgM^+IgD^+$ | 61.5 | 58.0–72.1[A] |
| $CD24^{++}CD38^{++}$ | **0.43** | 1.0–3.6[A] |
| $CD24^-CD38^{++}$ | 1.32 | 0.6–1.6[A] |
| Total $CD3^+$, $10^9$ cells/L | **0.760** | 0.798–2.823 |
| $CD3^+CD4^+$, $10^9$ cells/L | **0.429** | 0.455–1.885 |
| $CD3^+CD8^+$, $10^9$ cells/L | 0.293 | 0.219–1.124 |
| T cell subsets, % of $CD3^+$ cells | | |
| $CD4^+$ T cell subsets, % of $CD4^+$ | 40.0 | 33.0–62.0 |
| $CD45RO^+$ | **49,3** | 12.7–47.6 |
| $CD3^+CD4^+CD45RO^+CXCR5^+$[B] | 4.17 | 2.31–7.54 |
| $CD4^+$ T cell subsets, % of $CD4^+CD45RA^-$ | | |
| Th1 (IFN-γ) | 2.40 | 1.01–7.27 |
| Th17 (IL-17A) | 0,59 | 0.38–0.81 |
| $CD8^+$ T cells | 27.3 | 13.5–42.4 |
| $CD56^+$ T cells | 14.9 | ≤20.0 |
| HLA-$DR^+$ T cells | 8.7 | ≤18.0 |
| $CD3^-CD16^+/CD56^+$, $10^9$ cells/L | 0.177 | 0.066–0.745 |

Abnormal values are shown in bold.

[A] Reference values (interquartile ranges) for the B cell subsets were adapted from Morbach et al. [14]

[B] Circulating follicular helper T cells in peripheral blood [13]

[3]. Gln293 is located in exon 8 and is part of the central kinase domain essential for downstream NF-κB signaling. The c.877C>T substitution leads to a p.Gln293* stop mutation resulting in a truncated kinase domain [1]. Defective expression of full-length *IRAK4* mRNA by Northern blotting was demonstrated in lymphoblastoid B cells of patients carrying the same homozygous mutation [1]. The IRAK-4 protein was also not expressed in the affected cell lines [1, 4]. The $CD27^+IgM^+IgD^+$ memory B cell count was low in our patient, while the $CD27^+IgM^-IgD^-$ memory B cell count was high. This confirms previous research on the role for IRAK-4 in the maintenance and survival of the $CD27^+IgM^+IgD^+$ memory B subset [6]. We also found low numbers of transitional B cells in our patient, in contrast with earlier reports of IRAK-4 deficient patients [6]. The amount of $cT_{FH}$ cells, implicated in establishing B cell memory in germinal centers [16], was normal suggesting no impairment of $cT_{FH}$ cell numbers in IRAK-4 deficiency.

Although this patient has the most frequently described stop mutation in the *IRAK4* gene, she shows a peculiar disease course with an uncomplicated adolescence yet exacerbation and persistence of invasive pyogenic infections into adulthood, finally leading to a destroyed lung and pneumonectomy. The first invasive infection was a *Staphylococcus aureus* liver abscess at the age of 6 months. Effectively, 79.2 % of patients with IRAK-4 deficiency have their first invasive bacterial infection before the age of 2 [3]. Her second invasive infection was a meningococcal meningitis when she was 18 years old. Although *Neisseria meningitides* infections are not exceptional in IRAK-4 deficient patients, the age of presentation is noteworthy. To our knowledge, there has been no previous description of invasive infections after the age of 14 in IRAK-4 deficiency [3]. Skin infections are the most frequent noninvasive infections in IRAK-4 deficiency, as was the case in this patient. While only 18.8 % of IRAK-4 cases had one or more pneumonia episodes and no reports of chronic pulmonary disease have yet been made [3], this adult patient suffered from severe lung pathology requiring pneumonectomy.

Literature indicates that in IRAK-4 deficiency, most patients suffer from recurrent infections in early childhood, necessitating antibiotic prophylaxis and/or immunoglobulin treatment up to the age of 14 years approximately. The hypothesis is that development of the adaptive immune system renders the function of the innate immune system redundant in the defense against pyogenic microbes after adolescence [1, 5]. This case report highlights the need for judicious follow-up of any patient with suspected PID, even in the absence of a molecular diagnosis, in order to optimize quality of life and prevent life-shortening irreversible end organ damage. In this patient no antibiotic prophylaxis was instituted, despite strong suspicion of PID, which possibly contributed to the development of structural lung damage and chronic lung infection. The patient also suffered from infection and colonization with *Aspergillus fumigatus*. Reported IRAK-4 deficient patients do not show increased susceptibility to this fungus [3]. In our case, APBA was deemed unlikely due to the absence of asthma, likewise no evidence was found for invasive aspergillosis on histological examination of the explant lung. Nevertheless, culture of *Aspergillus fumigatus* in the sputum always coincided with a significant increase in dyspnea, mucous

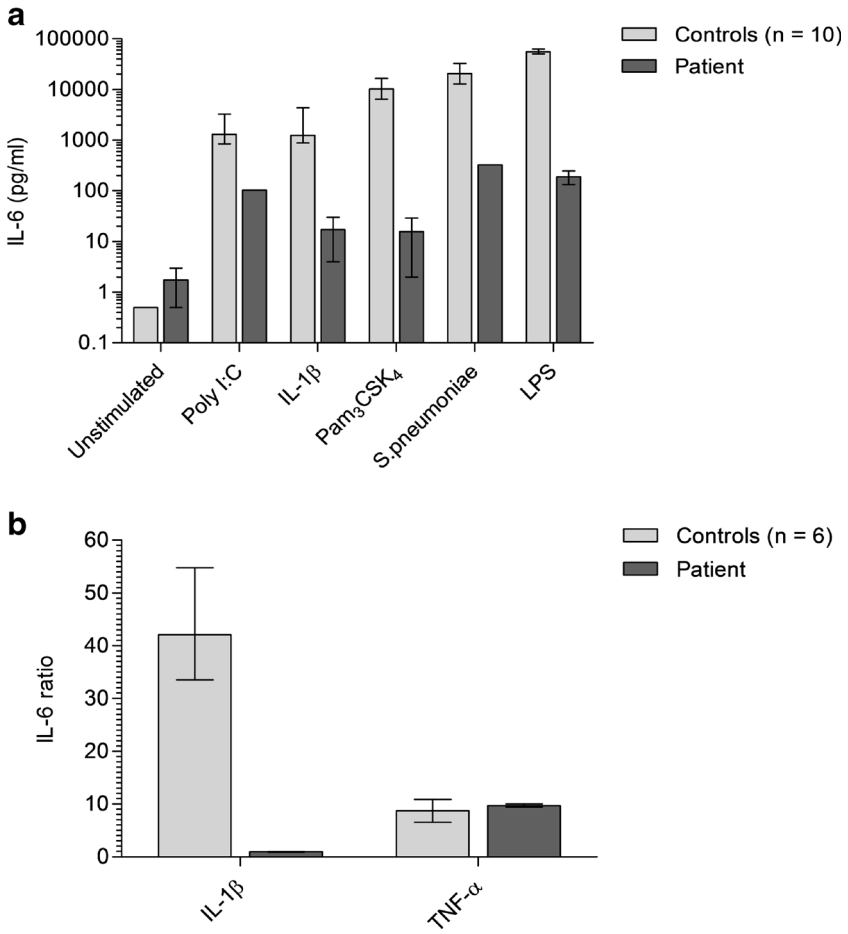


**Fig. 2** **a** IL-6 production of PBMCs isolated from the patient compared to values obtained in 10 healthy controls (median±IQR). Two independent experiments were performed in the patient for $Pam_3CSK_4$, LPS, and IL-1β. For *S. pneumoniae* and Poly I:C only one experiment was performed. Medians are displayed for the experiments performed twice. **b** IL-6 production of patient skin-derived fibroblasts compared to 6 healthy adult controls after 24 h stimulation with IL-1β and TNF-α (median±IQR). The following ratio was calculated: $IL\text{-}6^{stimulated}/IL\text{-}6^{unstimulated}$. Samples were evaluated in triplicate

plugging, and sputum production. Only antifungal therapy (voriconazole) was efficacious in improving the condition. It is clear that in the presence of structural lung damage, patients can be prone to ABPA but also to invasive fungal infection if the immune system is permissive. Therefore, in any patient with IRAK-4 deficiency and chronic lung disease, fungal cultures of sputum or broncho-alveolar lavage are mandatory to initiate adequate treatment. Moreover, patients and treating physicians are to be educated about the necessity of early initiation of empiric parenteral antibiotic treatment as soon as a bacterial infection is suspected, even in the absence of fever, leukocytosis and elevated CRP [1, 3].

This case highlights the importance of molecular diagnosis. Routine laboratory evaluation revealed high serum IgE levels (Table 1). Although elevated IgE is an important feature of AD HIES, the main characteristic of this syndrome is a deficiency in Th17 cell mediated immunity. In adults, the use of the NIH score is a reliable tool for diagnosing AD HIES as the sensitivity runs up to 97 % with a score of 49,5 [7–12]. Our patient had a score of 39 but no mutation in STAT3 was demonstrated and no impairment of the Th17 subsets was found [17]. The use of a targeted next-generation sequencing (NGS) approach identified the IRAK-4 stop mutation. This confirms the effectiveness of NGS panels in screening for disease-causing PID mutations [18–20]. The finding also had important implications for genetic counseling. The patient is the mother of two children. The molecular diagnosis of an autosomal recessive disease as opposed to an autosomal dominant one was a relief to the patient and her family. Moreover, the fear of vascular accidents, as in AD HIES, had been a burden to the family.

Elevated IgE levels are not uncommon in IRAK-4 deficiency, as 70 % of patients display this finding [3]. There have also been descriptions of single nucleotide polymorphisms in IRAK-4 that are associated with increased IgE levels in chronic rhinosinusitis and asthma patients, although this finding could not be replicated in patients with allergic rhinitis [21, 22]. Although IgE levels in IRAK-4 deficiency remain modest compared to those found in HIES, this case demonstrates that differential diagnosis remains difficult. A possible explanation for the higher IgE levels could be found in decreased IFN-γ and IL-10 production by IRAK-4 deficient T cells and the subsequent dysregulation of IL-4 stimulatory effects on IgE producing naïve B cells [4, 23]. It would be interesting for future research to further investigate this connection.

## Conclusion

We diagnosed IRAK-4 deficiency in an adult with severe lung disease previously diagnosed with AD HIES. This case highlights several points. First, the importance of judicious follow-up and treatment into adulthood of a patient with suspected PID cannot be overstressed to prevent end organ damage. Second, fungal infections and colonization can be important in patients with IRAK-4 deficiency and structural lung anomaly. Finally, molecular diagnosis is crucial for counseling and optimal management.

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### Compliance with ethical standards

**Author contributions** GF drafted the manuscript and conducted experiments. LM conducted experiments and aided in preparing the manuscript and the HIES work-up. GW conducted the TLR testing. RS, BB, HS, and LD participated in the clinical care of the patient. XB supervised the routine laboratory immunology work-up and TLR testing. AC performed the genetic analysis and established the genetic diagnosis. IM drafted and finalized the manuscript, characterized the immune deficiency and is coordinating clinical care for the patient. Each author has critically revised the final version of the manuscript and has read and approved the final manuscript.

**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval** The study was performed in accordance with the 1964 Helsinki declaration and its later amendments. Informed consent was obtained for genetic analysis and report of the case. The study was approved by the Ethics Committee of UZ Leuven.

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