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LETTER TO EDITOR

Compound Heterozygous Mutations of IL2-Inducible T cell Kinase in a Swedish Patient: the Importance of Early Genetic Diagnosis

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To the Editor:

Human primary immunodeficiency diseases (PIDs) comprise a group of highly heterogeneous defects in the immune system and more than 350 genes associated with PIDs have been identified to date [1, 2]. The most common presentation of PIDs is recurrent and chronic infections. Some of the PIDs are life-threatening and manifest in childhood and also result in an enhanced susceptibility to infectious complications, autoimmunity, and malignancy. However, a selected group of patients have a mild and gradual progression due to hypomorphic mutations, presenting late with atypical manifestations.

Effective therapeutic options are available to treat PIDs or preventing infections, such as hematopoietic stem cell transplantation, which cures the underlying immune defect; cellular therapies, which have been developed recently; cytokine treatment; and gammaglobulin substitution, as well as gene therapy, once the underlying molecular defect is identified [3].

The development of next generation sequencing has revolutionized the identification of the genetic etiology of PID and provides a possibility for precision medicine. Early genetic diagnosis, particularly in those with a mild presentation, could markedly improve the clinical outcome. Recent studies have shown that a correct genetic diagnosis allows differential management and treatment for half of the patients with common variable immunodeficiency (CVID) [4]. Here, we studied a female patient who suffered from an early onset CVID and utilized next generation sequencing technology to unravel the causative mutant gene. She showed to be the first PID patient found in Sweden to be due to compound heterozygous mutations of the interleukin 2 inducible T cell kinase (*ITK*).

The patient was born in 1981 of non-consanguineous parents and died from a highly malignant lymphoma in 2017. At the age of 18, she started suffering from recurrent upper and lower respiratory tract infections. In 2003, a first immunologic consultation was performed and serum immunoglobulin levels were found to be normal, except for a slightly reduced level of IgG2 (0.7 g/l). Between the period of 2003 and 2005, she suffered from recurrent upper respiratory infections (~ 20 episodes) where cultures showed growth of *Pneumococcus pneumonie* or *Haemophilus influenzae*, and X-ray confirmed three pneumonias. In 2005, she started to have periods of intermittent B symptoms (fever, night sweats, and weight loss). Multiple enlarged lymph nodes (up to 15 mm in diameter) in the chest and axilla, in addition to a slightly enlarged spleen, were identified on CT. IgG antibodies against EBV

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VCA were noted (whereas IgM antibodies were negative) and the number of EBV copies was elevated (44,500 copies/ml). EBV serology measured already in 2003 showed the same antibody pattern. Based on PAD from an extirpated lymph node in the axilla, an anaplastic diffuse large B cell lymphoma (DLBCL), positive for CD20 and CD30 but negative for CD15, was diagnosed (WHO classification C83.3 stage IVB). She was successfully treated with Rituximab and CHOEP-14 × 6, followed by a dose Cytosar as CNS prophylaxis (Rituximab 630 mg on day 1, Vincristine 2 mg, Cyclophosphamide 1250 mg, Doxorubicine 85 mg, Etoposide 225 mg, and Deltisone 75 mg/day on days 1–5, followed by Etoposide 250 mg on day 2 and 3 (slightly reduced dose due to a low albumin level). After treatment, CT showed a complete remission.

In 2006, she suffered from pneumococcal septicemia. At that time, a very low level of serum IgG was noted (1.9 g/l) which was deemed not to be due to the lymphoma and subcutaneous immunoglobulin therapy (30 ml/week) was initiated. Further immunological investigations in 2008 and after chemotherapy resulted in a diagnosis of CVID (IgM 0.22 g/l, IgG 9.20 g/l (during substitution), and IgA 0.37 g/l). Surface markers on T lymphocytes were markedly aberrant (showing a normal total level of T cells but a reduced proportion of CD4 positive cells and a substantial proportion (> 10%) of $\gamma\delta^+$ $CD3^+$ double negative cells)). The B cell profile showed a normal proportion of B lymphocytes but a high level of naïve cells, suggesting a maturational defect. The specific response to specific antigen (influenza vaccine, Pandemrix) was also reduced.

In 2012, she had a miscarriage. In the following year, she gave birth to a son and a daughter in 2016 (Fig. 1a). During pregnancy, her dose of subcutaneous immunoglobulin therapy was adjusted to 50 ml/week which, after delivery, was changed back to the normal dose of 30 ml/week. Her IgG-through level was 6.5 g/L in late pregnancy and 8–9 g/L after delivery. The infectious control was good with only one X-ray confirmed pneumococcal pneumonia in 2012 and a bacterial sinusitis in 2015 due to *Haemophilus influenzae*. However, she developed atopic manifestations, including allergy to penicillin and urticaria.

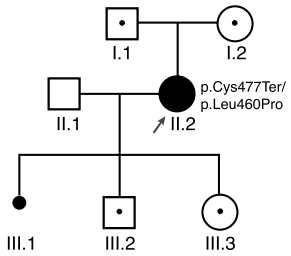
In May 2017, she developed recurrent fever, dry cough, dyspnea, and increasing fatigue. Physical examination suggested an enlarged liver, confirmed by CT (which also showed an increased size of the spleen) and multiple enlarged lymph nodes in the upper abdomen and retroperitoneally. Ascites and pleural effusion was also present. Liver biopsy showed infiltration of proliferating CD30 positive, CD79A positive, CD20 and CD15 negative, and BCL2 positive B cells classified as a DLBCL. Levels of EBV were markedly elevated (1,200,000 copies/ml). Due to the suspected reappearance of her lymphoma (possibly a subclone as the CD pattern was slightly different), she was started on cytostatic treatment (Carboplatine, Gemzar, and Adcetris (Brentuximab vedotin)). She subsequently developed hemophagocytic lymphohistiocytosis (HLH) with fever, splenomegaly, thrombocytopenia, raised levels of ferritin, triglycerides, and sCD25R, and received treatment with Etoposide, Rituximab, and Simulect (Basiliximab, a chimeric antibody directed against the alpha chain of the IL-2 receptor), followed by IKE (Ifosphamide, Carboplatine and Etoposide). In September 2017, she was EBV negative and deemed to be in complete metabolic remission and was being selected for stem cell transplantation. However, within a few weeks, she again experienced fever with return of her disease (1,100,000 EBV copies/ml) and passed away after one additional month.

Fig. 1 Identification of compound heterozygous *ITK* mutations in a Swedish patient. **a** Pedigree of the Swedish ITK deficiency patient with one miscarriage and two normal children. **b** Allelic segregation of two adjacent variants in the ITK deficient patient. **c** Confirmatory Sanger sequencing validation of the two identified *ITK* mutations. **d**. Comparison of allele frequency of the two identified *ITK* variants and the CADD score distribution of all known variants. **e**. Structure analysis of the missense mutation (p. Leu460Pro) showing, using Chimera, that the mutant is possibly disrupting the secondary structure of ITK. **f**. The frequency of variants found in *ITK* in the general population and a summary of mutations in ITK deficient patients. Mutations found in the Swedish patient are in red, the compound heterozygous in the Italian/Greek patient are in purple whereas black color represents the homozygous variants found previously

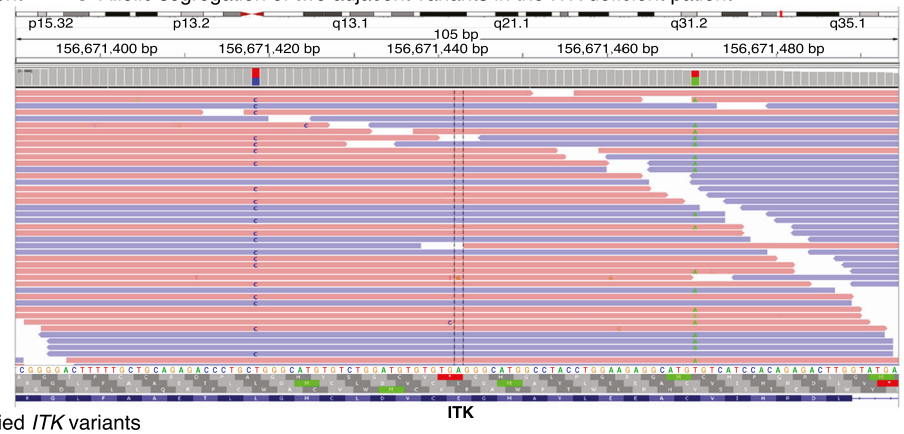
The underlying genetic cause of the clinical features was elucidated by using an in-house target region sequencing with a panel containing 219 PID genes. Using the previously published pipeline [5], two compound heterozygous mutations in *ITK* were found, a missense variant c.1379T>C/p.Leu460Pro and a nonsense mutation c.1431T>A/p.Cys477Ter, both of them are novel in our in-house database and very rare in normal individuals based on the gnomAD database (http://gnomad.broadinstitute.org), and only found in European populations with a frequency of 0.00001219 and 0.000008147, respectively. Sanger sequencing validation confirmed the mutations (Fig. 1b, c) and in silico predictors suggest both to be deleterious (Fig. 1d, e).

While 17 PIDs patients have been reported with ITK deficiency worldwide, this patient is the first identified case in Sweden (P18). She is also the second reported PID patient with compound heterozygous variants, being the first case of combination of a missense and a nonsense mutation [6–10] (Fig. 1f). Interestingly a minority of ITK deficient patients carrying missense variants have a slightly later onset of disease (7 years vs. 5 years) compared to the majority of cases with nonsense mutations, usually with less severe complications. Hematopoietic stem cell transplantation (HSCT) is less frequent in the former (28% vs. 45%) with a higher graft failure rate (50% vs. 20%). Of note, the rate of malignancy

a Pedigree of the Swedish ITK deficient patient

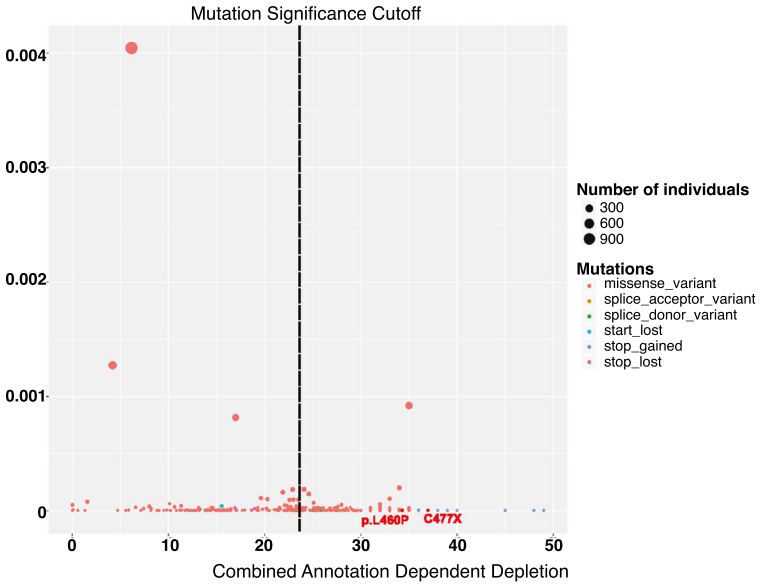


b Allelic segregation of two adjacent variants in the ITK deficient patient

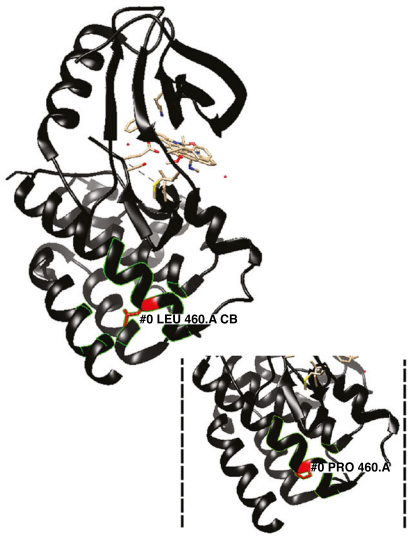


c Confirmatory Sanger sequencing of identified *ITK* variants

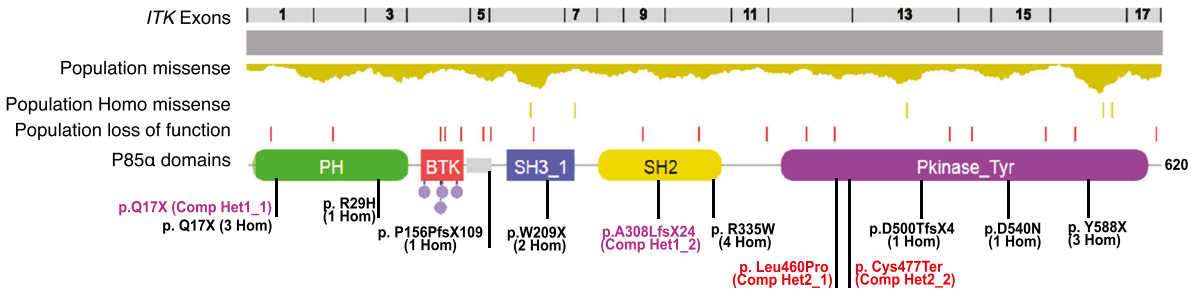
d Comparative of allele frequency of the two identified *ITK* variants



e Structural analysis of the p.L460P missense mutation



f Frequency of mutations in *ITK* in the general population



and autoimmunity are also increased in the patients with missense mutations. Although the median age at death in patients with missense mutations is slightly higher (10 years vs. 7 years) the majority die shortly after diagnosis (71.4% vs. 36.3%). Our patient differs slightly from previously described patients (summarized in [6, 9–11]) by carrying novel mutations, a comparatively high age at onset of the lymphoma and an uncommon histological type but otherwise clinically similar (frequent relapses, induction of hypogammaglobulinemia, high numbers of viral copies, and a high rate of mortality).

ITK (OMIM 186973), being an important analogoue of Bruton's tyrosine kinase [8], is a Tec family non-receptor tyrosine kinase expressed by T cells, and plays a critical role in T cell differentiation and function, T cell repertoire signaling, and cytokine release. ITK deficiency results in an autosomal recessive PID (Lymphoproliferative syndrome-1, OMIM 613011) characterized by early onset of EBV-associated lymphoproliferative disease, lymphoma, lymphomatoid granulomatosis, HLH, Hodgkin's disease, hypogammaglobulinemia, and, occasionally, autoimmune disorders.

Already when the diagnosis of CVID was made in our patient, almost 10 years ago, it was noted that this was not a "typical" case of the disease. However, no additional follow-up was made at the time. In a late stage of her disease, the patient contacted us for a second opinion and a genetic investigation was initiated. However, when the result was available, the patient had already passed away due to her aggressive B cell lymphoma. Correct diagnosis in patients with CVID has previously been shown to alter treatment or care in half of the individuals [4]. Our patient should, had her mutation been known, been stem cell transplanted instead of merely being given gammaglobulin substitution. This adds to the growing amount of evidence suggesting that genetic investigations into the etiology of PID (CVID) should be initiated at an early stage of the patient's disease.

Author's Contribution M.F. and X.L. performed target region sequencing and bioinformatic analysis; L.H. and E.S. provided clinical care of the patient and provided clinical and immunological assessment; H.A. performed Sanger sequencing validation experiments and revised the paper; M.F., Q. P-H., and L.H. wrote the paper.

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Data Availability The data reported in this study are available in the CNGB Nucleotide Sequence Archive (CNSA: https://db.cngb.org/cnsa; accession number CNP0000214).

Compliance with Ethical Standards

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

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