

Abstract

Primary immunodeficiencies (PID) are genetic disorders characterized by increased susceptibility to infections and various degrees of immune dysregulation. With the expansion of massive parallel sequencing, an increasing number of defects in immune-related genes is being identified in PID. However, the biological impact of the found mutations is often unknown. It is necessary to devise methods to clarify their causality for disease development, which may also aid therapeutic decisions.

One of the novel discoveries are gain-of-function mutations in *STAT1* gene, resulting in chronic mucocutaneous candidiasis. Candidiasis may be ameliorated with antimycotics or with targeted JAK-STAT inhibitor, ruxolitinib. For our patient with a novel mutation in *STAT1*, we developed a simple test for the detection of phospho-STAT molecules in peripheral blood lymphocytes. The test confirmed the gain-of-function character of the identified mutation and was used to monitor ruxolitinib treatment efficacy. In the second patient, who presented with lymphadenopathy and immunodeficiency, the as yet undescribed mutation in *CASP8* was found. We proved its loss-of-function property expressed as reduced caspase-8 and caspase-3 cleavage, impaired cellular apoptosis, and decreased NFκB-related signaling. The third patient who suffered from pulmonary fibrosis and vasculitis carries a remarkable mutation in Src-family kinase *HCK*. We found a mutated HCK protein in the patient's cells and proved its activation character, detected as hyperphosphorylation of HCK, increased expression of adhesion molecules, and enhanced release of cytokines IL-1β and TNFα.

In conclusion, we established correlations between novel *STAT1*, *CASP8*, and *HCK* mutations and altered cellular functions by using a serie of functional tests.