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DIPLOMA THESIS

Mechanisms of immune dysregulation leading to inflammatory bowel disease

Abstract

Inflammatory bowel disease (IBD) is a complex disorder characterized by chronic inflammation of the gastrointestinal tract. Classical IBD is a multifactorial disease with adulthood or later-childhood onset. However, children with very early onset IBD (VEO-IBD, before 6 years of age) are a specific cohort, whose pathology can be caused by severe genetic defects in genes connected to immune homeostasis in the gut.

We aimed to identify the causal genetic variants in 20 pediatric patients diagnosed with IBD (age of onset from 3 to 154 months) using whole exome sequencing (WES). We evaluated several bioinformatical approaches for WES data analysis. This included a comparison of two methods of variant identification using VarScan2 or GATK4-based tools. Furthermore, we compared 4 gene lists ("virtual panels") for variant filtering, one of which was compiled purposefully for this thesis.

We identified and validated via segregation analysis 5 causal variants in 4 genes (*DUOX2* compound heterozygote, *FOXP3*, *NLRP3* and *NOD2*) accounting for 20 % of the cohort. NOD2 (p.A755V) variant has already been reported in IBD cases, while *DUOX2* (p.R1216W + p.A1131T), *FOXP3* (p.H400L) and *NLRP3* (p.V200M) were newly discovered variants in this context. Moreover, we suggested 6 more variants in 5 genes for further validation, bringing new insight into genetics of VEO-IBD. Finding the same success rate of causal variant calling and filtering, we proved the suitability of the newly developed GATK4-based pipeline for variant analysis in a clinical setting. We found that the most adequate approach for variant filtering in VEO-IBD cases is using virtual panels of genes related to primary immunodeficiencies and up-to-date reported VEO-IBD cases.

In conclusion, we strongly support the hypothesis of many VEO-IBD cases being primary immunodeficiency with gastrointestinal manifestation. However, additional functional tests and research into genetic and non-genetic causes are required to understand molecular mechanisms of the VEO-IBD pathogenesis.

Key words: whole exome sequencing, inflammatory bowel disease, primary immunodeficiency, immune dysregulation