P793 SCREENING OF NATURALLY OCCURRING CXCR4 VARIANTS FOR IDENTIFICATION OF NOVEL PATHOGENIC MUTATIONS FOR WHIM SYNDROME

Topic: 11. Bone marrow failure syndromes incl. PNH - Biology & Translational Research

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Background: WHIM (Warts, Hypogammaglobulinemia, Infections, Myelokathexis) syndrome is a rare, autosomal-dominant primary immunodeficiency marked by neutropenia and lymphopenia. Classically, WHIM syndrome pathogenesis is causally linked to a variety of heterozygous gain-of-function mutations in the C-terminus of chemokine receptor CXCR4, a master regulator of immune cell trafficking and homeostasis. As of February 2022, 18 variants in CXCR4 have been implicated in WHIM syndrome, while a substantial number remain unexplored for their association with the disease. Using in vitro functional assays, we previously found that impaired receptor internalization and enhanced chemotaxis are conserved in all CXCR4WHIM variants and that defective internalization correlates with neutropenia, the most penetrant phenotype of patients with WHIM syndrome. We used this approach to confirm functional defects in primary cells isolated from patients harboring a novel variant, suggesting that the in vitro profiling approach can potentially drive identification of pathogenic variants.

Aims: We aimed to functionally characterize multiple previously uncharacterized variants of CXCR4 using in vitro assays. We also examined allele frequencies of these novel variants to estimate the potential number of individuals harboring these variants with a long-term goal of determining the actual prevalence of WHIM syndrome.

Methods: The CXCR4-negative K562 cell line was used as a model system to express 53 novel CXCR4 variants identified in patient and population databases (ClinVar, Ensembl, CentoMD, GnomAD) and genetic screening initiatives (Invitae PATH4WARD) found in the C-terminus (hotspot of CXCR4WHIM mutations) as well as throughout the protein. The effects of these mutations on CXCR4 internalization and chemotaxis were studied in cells stimulated with the C-X-C chemokine ligand 12 (CXCL12). The in vitro functional parameters were compared with known pathogenic CXCR4 variants and were used to assign a cumulative score (impaired internalization + enhanced chemotaxis) of functional defect severity.

Results: Out of 53 selected variants, 42 were missense (ms), 6 were frameshift (fs), 3 were nonsense (ns), and 2 were transcript variants (tv). Eighteen of the screened variants led to a decreased internalization of CXCR4 in comparison to the wild-type (WT) receptor upon stimulation with CXCL12 while 20 variants demonstrated enhanced chemotaxis of cells toward CXCL12. Overall, 34 variants showed defects in at least one CXCR4-dependent marker. Correlation analysis and scoring of the functional parameters in cells expressing mutated or CXCR4WT revealed 17 variants (9 ms, 5 fs, 2 ns, 1 tv) that co-segregated with the known pathogenic WHIM variants. Finally, white blood cell counts in 3 patients harboring novel variants showed that CXCR4 internalization defects in vitro correlated with the severity of neutropenia.

Summary/Conclusion: This is the first study characterizing functional impairments in naturally occurring CXCR4 variants via a pipeline of assays intended to predict pathogenicity. Seventeen CXCR4 variants were identified (several outside the C-terminus of CXCR4) that caused in vitro functional defects resembling those exhibited by known WHIM variants. Many (10/17) of these newly identified variants are present at high frequencies (4.4 × 10^-4–8.0 × 10^-6) in genomic databases. Potential association of these variants with WHIM syndrome is under investigation and is likely to expand current estimates of WHIM syndrome prevalence.