CXCR4 Variant Landscape in WHIM Syndrome: Variant Interpretation Using Clinical and Functional Data

Introduction

- Warts, Hypogammaglobulinemia, Infections, Myelokathexis (WHIM) syndrome is a rare immunodeficiency disease primarily caused by gain-of-function variants in the C-terminus of CXCR4 chemokine receptor 4 (CXCR4)\(^1\)
- Individuals with WHIM syndrome can present with heterogeneous clinical manifestations\(^2\)
- Due to variable clinical presentations, diagnosis of WHIM syndrome can be challenging
- Genetic testing can expedite and support the clinical diagnosis of WHIM syndrome\(^3\)
- CXCR4 variants can be classified as pathogenic (P), likely pathogenic (LP), or variant of uncertain significance (VUS)
- WHIM syndrome is predominantly caused by gain of function, resulting in a frameshift or nonsense variant
- Herein, we aimed to expand knowledge of the genetic landscape in WHIM syndrome by incorporating results from in vitro functional testing with Invitae’s Sherloc variant classification framework, a refined version of the 2015 American College of Medical Genetics and the Association for Molecular Pathology guidelines for interpretation of sequence variants\(^4\)

Aim

To evaluate all known CXCR4 variants and identify potential disease-causing variants using the Sherloc variant classification framework.

Methods

- Literature, databases (Invitae, gnomAD), and a genetic testing program (Invitae/PATHWARD) were used to identify and collect information on CXCR4 variants observed in people with WHIM syndrome
- Variants were classified by Invitae using the Sherloc variant classification framework, which used evidence derived from a combination of clinical and functional data
- CXCR4-chimeric ligand (CXCL12)-induced internalization of CXCR4 receptor in identified in vitro assays using CXCR4 variant-expressing cells, to assess 1 aspect of pathogenicity

Results

- As of July 2023, 36 CXCR4 variants (resulting in 34 distinct protein variants) in people with WHIM syndrome had been identified via publications, ClinVar, and the Invitae/PATHWARD genetic screening initiative (Figure 1)
- Of those, only 22 CXCR4 variants were classified as P or LP by Invitae, leaving potentially disease-causing variants categorized as VUS
- Variants were classified as VUS due to the lack of clinical data, or based on predominately caused by gain of function

In vitro functional testing of 32/34 identified CXCR4 protein variants showed that all 32 exhibited substantially impaired internalization across a range of CXCL12 concentrations.

CXCR4 C-terminus Variants

- The 36 identified CXCR4 variants were reclassified in collaboration with Invitae using the Sherloc variant classification framework (Figure 2, Figure 3, Table 1): Absence in the general population (per gnomAD), segregation with disease, and multiple unrelated cases were factors that confirmed the most pathogenic points for CXCR4 variant classification

A total of 31/36 CXCR4 variants were reclassified using integrated genetic, clinical, and functional data

<table>
<thead>
<tr>
<th>CXCR4 variants</th>
<th>Clinical criteria</th>
<th>Functional testing</th>
<th>Interpretation</th>
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<tbody>
<tr>
<td>Frameshift, nonsense, frameshift, P</td>
<td>No change</td>
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<td>P</td>
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CXC4 receptor internalization

Conclusions

- As of July 2023, 36 variants in the CXCR4 C-terminus were identified in people with WHIM syndrome in publications, databases (gnomAD, Invitae), and a genetic testing program (Invitae/PATHWARD)

- Using results from in vitro functional testing together with data from published clinical cases of WHIM syndrome, 27 variants were reclassified from VUS to LP and 4 from LP to P, resulting in a total of 36 CXCR4 variants currently being recognized as LP or P

- The current body of evidence allows to make a prediction that any novel truncating variant (nonsense or frameshift) between aa 317 and 346 will likely be a pathogenic variant for WHIM syndrome

- We also showed the value of in vitro testing and detailed variant analysis in resolving the pathogenic potential of variants, especially where clinical information is insufficient to confidently variant interpretation

- These data provide the most complete overview of the CXCR4 variant landscape in WHIM syndrome to date to enhance our understanding of the genetic factors underlying WHIM syndrome

- Further characterization and classification of novel CXCR4 variants are warranted to expand our knowledge of the CXCR4 variant landscape in WHIM syndrome and inform future best practice medicine approaches

Acknowledgements

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References

- AB, M, SP, KN, and MV are former employees of X4 Pharmaceuticals and/or have equity ownership of X4 Pharmaceuticals.
- OD, MA, LM, AO, KN, and MV are current employees and/or have equity ownership of X4 Pharmaceuticals.
- MS is current employee of Invitae

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Table 1. CXCR4 variants identified in patients with WHIM syndrome, including the variant’s assigned interpretation and segregation based on the Sherloc variant classification framework.**<sup>1</sup>** De novo segregation with WHIM syndrome.**<sup>2</sup>** Absent or low frequency variants.

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