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

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Introduction

- Warts, Hypogammaglobulinemia, Infections, Myelokathexis (WHIM) syndrome is a rare immunodeficiency disease predominately caused by gain-of-function variants in the C-terminus of C-X-C chemokine receptor 4 (CXCR4)[1-3]
- Individuals with WHIM syndrome can present with heterogenous clinical manifestations[1,4-7]
- Due to variable clinical presentations, diagnosis of WHIM syndrome can be challenging. Genetic testing can expedite and support the clinical diagnosis of WHIM syndrome[4,8]
- *CXCR4* variants can be classified as pathogenic (P), likely pathogenic (LP), or variant of uncertain significance (VUS). However, VUS are not informative for clinical decision-making and therefore should not be used to support clinical diagnosis of WHIM syndrome[9,10]
- Herein, we aimed to expand knowledge of the genetic landscape in WHIM syndrome by incorporating results from *in vitro* functional testing into 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[9,11]

Aim

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

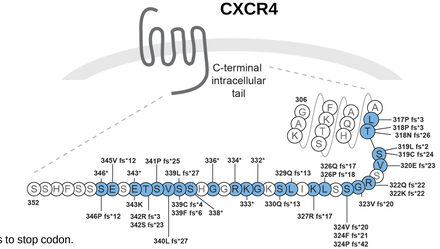
Methods

- Literature, databases (ClinVar, gnomAD), and a genetic testing program (Invitae/PATH4WARD) 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
- C-X-C chemokine ligand 12 (CXCL12)-induced internalization of CXCR4 receptor in identified *CXCR4* variants were investigated in *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/PATH4WARD genetic screening initiative (**Figure 1**)
- Of those, only 9 *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 Invitae's method of variant classification

CXCR4 C-terminus Variants



*Refers to stop codon.

Figure 1. CXCR4 protein variants identified in people with WHIM syndrome that are localized in the C-terminal intracellular tail of the receptor. The figure indicates protein variants identified to date and positions at which they alter the WT sequence of the CXCR4 protein. The C-terminus is visualized from amino acid 306 to 352. Adapted from GPCRdb.org. WT, wild-type.

Results (cont'd)

- 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, *de novo* occurrence, and reports of multiple unrelated cases were factors that conferred the most pathogenic points for *CXCR4* variant classification

Clinical criteria	**Population data** Absent or low frequency in gnomAD	**Observations in well-characterized individuals** Segregation with disease *De novo* in affected individual Multiple case reports
Functional criteria	**Variant type** Frameshift, nonsense, missense	**Expected consequence for gene product** Disruption of C-terminus Escape NMD
		Experimental data Impaired CXCR4 internalization

Figure 2. Summary of clinical and functional criteria most frequently applicable for CXCR4 variant classification. gnomAD, genome aggregation database; NMD, nonsense-mediated decay.

***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, in line with previous reports of known pathogenic *CXCR4* variants[12,13]**

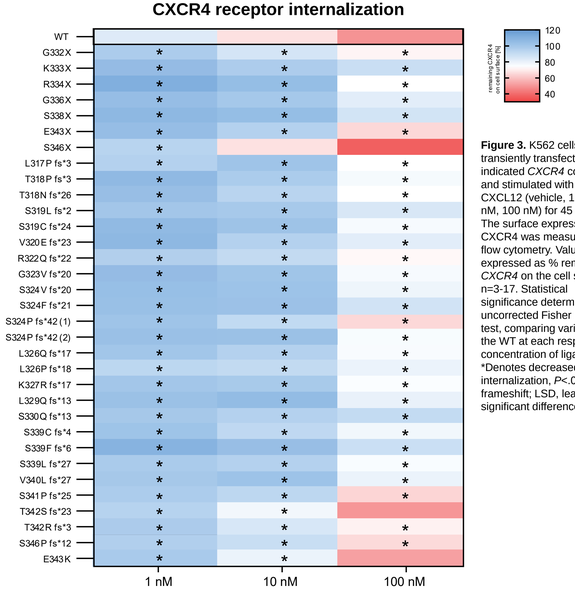


Figure 3. K562 cells were transiently transfected with indicated *CXCR4* constructs and stimulated with CXCL12 (vehicle, 1 nM, 10 nM, 100 nM) for 45 minutes. The surface expression of CXCR4 was measured by flow cytometry. Values are expressed as % remaining *CXCR4* on the cell surface, n=3-17. Statistical significance determined by uncorrected Fisher LSD test, comparing variants to the WT at each respective concentration of ligand. *Denotes decreased internalization, *P*<.033. fs, frameshift; LSD, least significant difference.

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

HGVS c-name	HGVS p-name	Impact	Source / Reference	Original Interpretation	Sherloc interpretation
c.1027G>A	p.Glu343Lys	missense	PMID: 22596258	LP	No change
c.994 G>T	p.Gly332*	nonsense	ClinVar variation ID: 574352	LP	P
c.997A>T	p.Lys333*	nonsense	PMID: 24139496	VUS	LP
c.1000C>T	p.Arg334*	nonsense	PMID: 12692554	P	No change
c.1006G>T	p.Gly336*	nonsense	PMID: 15026312	P	No change
c.1013C>G	p.Ser338*	nonsense	PMID: 15536153	P	No change
c.1013C>A	p.Ser338*	nonsense	PMID: 35947323	VUS	P
c.1027G>T	p.Glu343*	nonsense	PMID: 12692554	VUS	P
c.1037_1040del	p.Ser346*	nonsense	PMID: 35947323	VUS	P
c.950_953del	p.Leu317Pro fs*3	frameshift	PMID: 32499645	VUS	P
c.951del	p.Thr318Pro fs*3	frameshift	PMID: 36883568	VUS	P
c.952dup	p.Thr318Asn fs*26	frameshift	PMID: 35947323	VUS	LP
c.954del	p.Ser319Leu fs*2	frameshift	PMID: 35947323	VUS	P
c.956_957del	p.Ser319Cys fs*24	frameshift	PMID: 27484033	VUS	P
c.959_960del	p.Val320Glu fs*23	frameshift	PMID: 31942606	LP	P
c.963dup	p.Arg322Gln fs*22	frameshift	PMID: 35947323	VUS	P
c.964dup	p.Arg322Lys fs*22	frameshift	Invitae	VUS	LP
c.966_967del	p.Gly323Val fs*20	frameshift	PMID: 28643496	VUS	P
c.969_970insG	p.Ser324Val fs*20	frameshift	PMID: 23009155	VUS	P
c.970_971insTCCT	p.Ser324Phe fs*21	frameshift	PMID: 35947323	VUS	P
c.969del	p.Ser324Pro fs*42 (1)	frameshift	PMID: 35947323	VUS	P
c.970del	p.Ser324Pro fs*42 (2)	frameshift	PMID: 32870250	VUS	P
c.976dup	p.Leu326Pro fs*18	frameshift	PMID: 35947323	VUS	P
c.977_978del	p.Leu326Gln fs*17	frameshift	PMID: 35947323	VUS	P
c.979_980insG	p.Lys327Arg fs*17	frameshift	PMID: 35947323	VUS	P
c.986_990del	p.Leu329Gln fs*13	frameshift	PMID: 27059040	LP	P
c.988_989del	p.Ser330Gln fs*13	frameshift	ClinVar variation ID: 1163801	VUS	LP
c.1016_1017del	p.Ser339Cys fs*4	frameshift	PMID: 12692554	P	No change
C.1012_1015dup	p.Ser339Phe fs*6	frameshift	PMID: 34973340	VUS	P
c.1014del	p.Ser339Leufs*27	frameshift	PMID: 35947323	VUS	P
c.1016_1017dup	p.Val340Leufs*27	frameshift	PMID: 35947323	VUS	P
c.1021del	p.Ser341Profs*25	frameshift	PMID: 19321197	VUS	P
c.1025_1028del	p.Thr342Ser fs *23	frameshift	ClinVar variation ID: 1319371	VUS	P
c.1025_1026del	p.Thr342Arg fs*3	frameshift	ClinVar variation ID: 1494228	VUS	LP
c.1032_1033del	p.Glu345Val fs*12	frameshift	PMID: 35493524	VUS	P
c.893_1034dup	p.Ser346Pro fs*12	frameshift	PMID: 35947323	LP	P

Table 1. *CXCR4* variants identified in patients with WHIM syndrome, including the variants' current interpretation and interpretation based on the Sherloc variant classification framework.[7,9,10] HGVS, Human Genome Variation Society.

- Thirty-one *CXCR4* variants were reclassified: 22 from VUS to P, 5 from VUS to LP, and 4 from LP to P, resulting in 36 variants being recognized as LP or P for WHIM syndrome (**Table 1**)

Conclusions

- As of July 2023, 36 variants in the CXCR4 C-terminus were identified in people with WHIM syndrome in publications, databases (ClinVar, gnomAD), and a genetic testing program (Invitae/PATH4WARD)
- 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/P 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 functional *in vitro* testing and detailed variant analysis in resolving the pathogenic potential of variants, especially in cases where clinical information is insufficient for confident 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 precision medicine approaches

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Disclosures

AB, SM-M, SP, and **IW** are former employees of X4 Pharmaceuticals and/or have equity ownership of X4 Pharmaceuticals. **GD, BM, JM, LN, AGT,** and **KZ** are current employees and/or have equity ownership of X4 Pharmaceuticals. **KN** is a current employee and stockholder of Invitae.

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