Case Study: A Novel CXCR4 p.Ser346Profs*12 Variant in a Child With WHIM Syndrome

Poster #314

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Background

- Warts, Hypogammaglobulinemia, Infections, Myelokathexis (WHIM) syndrome is a rare primary immunodeficiency caused by gain-of-function (GOF) genetic variants in *CXCR4*, leading to neutropenia, lymphopenia, and monocytopenia owing to impaired leukocyte trafficking[1-3]
- Nearly all WHIM syndrome cases have been linked to heterozygous GOF variants predominately in the C-terminus of CXCR4 that result in increased receptor signaling[1-3]
- The clinical presentation of patients with WHIM syndrome varies, even in familial forms; most penetrant phenotypes include myelokathexis, hypogammaglobulinemia, and recurrent infections. Hence, diagnosis of WHIM syndrome is typically confirmed by genetic testing for pathogenic variants in *CXCR4*[1,3-8]
- Here, we describe a case of a female presenting with symptoms of WHIM syndrome since age 3 years and harboring a novel heterozygous and likely pathogenic *CXCR4* variant, c.893_1034dup (p.Ser346Profs*12)

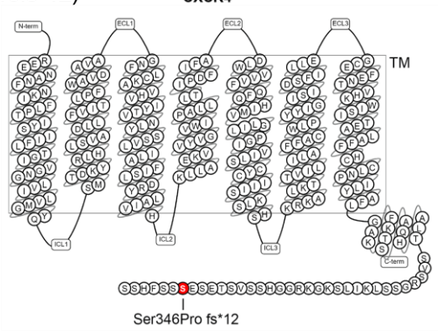

Figure 1. Schematic representation of CXCR4 protein with highlighted amino acid residues. The S346Pfs*12 variant is localized in the C-terminus cytoplasmic tail of CXCR4. TM, transmembrane.

Methods

- Peripheral blood mononuclear cells (PBMCs) were isolated from a healthy donor and from the patient harboring CXCR4^{S346Pfs*12}
- CXCR4-negative K562 cell line was transfected to express CXCR4^{WT} and CXCR4^{S346Pfs*12}
- For *in vitro* functional characterization, PBMCs from the patient and transfected K562 cells expressing CXCR4^{S346Pfs*12} were analyzed to assess the functional effect of the variant on receptor internalization and chemotaxis

Results

A novel heterozygous *CXCR4* variant in a patient with WHIM syndrome phenotype

- The patient is a female aged 8 years diagnosed with autoimmune neutropenia at age 3 years
- She presented with a history of persistent severe leukopenia, including neutropenia
- Medical history showed profound history of recurrent bacterial and viral infections since age 4 months. After age 2 years, she was managed on an outpatient basis
- She has no history of warts
- Assessment for mutations associated with congenital neutropenia revealed patient did not harbor variants in *ELANE, HAX-1, G6PC3, or JAGN 1* genes

Table 1. Blood Tests

Measure	2014	2016	2018	2021	2022	Normal Range[9]
WBC (× 10^9/L)	2.40	1.40	1.08	0.90	4.54	3.80–10.40
ANC (× 10^9/L)	0.24	0.37	0.28	0.08	N/A	1.50–6.50
ALC (× 10^9/L)	2.08	0.75	0.75	0.70	0.21	1.40–3.90
AMC (× 10^9/L)	0.08	0.23	0.03	0.08	0.07	0.20–0.80

ALC, absolute lymphocyte count; AMC, absolute monocyte count; ANC, absolute neutrophil count; N/A, not available; WBC, white blood cell.

Table 2. Immunophenotyping and Antibody Testing

Measure	2016	2018	2021	2022	Normal Range[10-12]
CD3+ (%/abs)	75.4/550	74.3/676	83/582	77.3%/555	55.0–78.0/700–4200
CD4+ (%/abs)	47.4/331	44.8/408	49.4/346	49.9%/358	27.0–53.0/300–2000
CD8+ (%/abs)	13/91	12.8/116	12.6/88	12.6%/90	19.0–34.0/300–1800
CD19+ (%/abs)	6.1/46	9.3/85	5/35	7.9%/57	10.0–31.0/200–1600
CD56+ (%/abs)	16.6/126	15.1/137	10.8/76	14.5%/104	4.0–26.05/90–900
IgG (mg/dL)	690	741.7	831	-	462–1682
IgA (mg/dL)	80	56.1	82.7	-	34–274
IgM (mg/dL)	57.8	125.4	131.6	-	38–251
IgE (IU/mL)	-	-	36.5	-	≤403

abs, absolute; CD, cluster of differentiation; Ig, immunoglobulin.

- **Genetic testing** revealed c.893_1034dup (p.S346Pfs*12); neither parents carried the *CXCR4* variant
- **Bone marrow findings** indicative of myelokathexis
- Acute treatment with 1 mcg/kg/d filgrastim [granulocyte colony-stimulating factor (G-CSF)] led to marked improvement in leukocyte counts and ANC in peripheral blood over 1 week. Of note, chronic use of G-CSF maybe associated with debilitating side effects, such as bone pain, that have negative impact on quality of life[13-15]

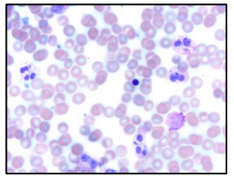

Figure 2. Bone marrow showed neutrophil hyper segmentation and connection of nuclear lobes with fine chromatin filaments and frequent cytoplasmic vacuolization, indicative of myelokathexis.

Cells expressing CXCR4^{S346Pfs*12} exhibited impaired receptor internalization following CXCL12 binding

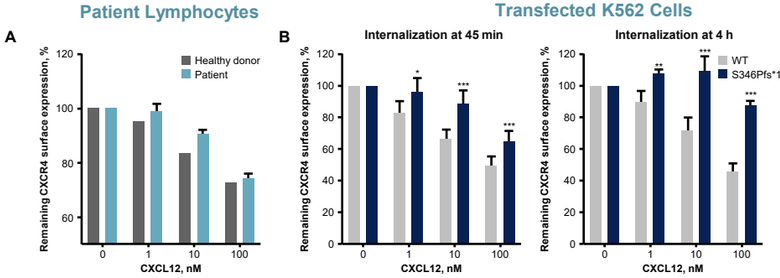

Figure 3. Primary PBMCs isolated from a patient (p.S346Pfs*12) and a healthy donor **(A)** or transiently transfected K562 cells expressing CXCR4 (WT) and S346Pfs*12 variant **(B)** were stimulated *in vitro* with CXCL12. Cell surface expression of CXCR4 was measured by flow cytometry. Cells were gated based on forward and side scatter and isotype control, and the mean fluorescence intensity of the lymphocyte population was analyzed. Values are expressed as % remaining CXCR4 compared to vehicle-treated cells. Statistical significance was determined by unpaired 2-tailed *t* test as follows: **P*<.05; ***P*<.01; ****P*<.001 comparing the variant to the WT in **(B)**. PBMC, peripheral blood mononuclear cell; WT, wild-type.

Cells expressing CXCR4^{S346Pfs*12} exhibited enhanced chemotaxis toward CXCL12

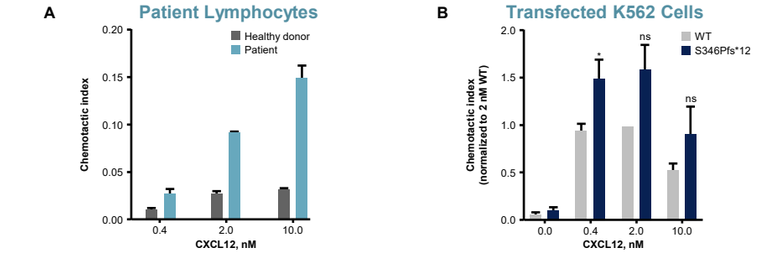

Figure 4. Primary PBMCs isolated from a patient (p.S346Pfs*12) and a healthy donor **(A)** or transiently transfected K562 cells expressing CXCR4 (WT) and S346Pfs*12 variant **(B)** were allowed to migrate toward varying concentrations of CXCL12 in transwell plates. Migrated cells were harvested and counted by flow cytometry to determine the level of chemotaxis in each cell condition. Lymphocyte population was gated based on forward and side scatter. Statistical significance was determined by unpaired 2-tailed *t* test as follows: **P*<.05; ns, not significant comparing the variant to the WT in **(B)**. PBMC, peripheral blood mononuclear cell; WT, wild-type.

Conclusions

- A novel and likely pathogenic *CXCR4* variant, *CXCR4*^{S346Pfs*12}, was identified in a patient clinically confirmed with WHIM syndrome
- S346Pfs*12 is the most distal C-terminal CXCR4 frameshift variant observed to date, with a truncation of only the last 7 amino acids
- Patient PBMCs and recombinant K562 cells harboring CXCR4^{S346Pfs*12} showed impaired CXCR4 receptor internalization and enhanced chemotaxis in response to CXCL12, typical hallmarks of *CXCR4*^{WHIM} variants

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Disclosures

KZ, SMM, and **AGT** are current employees of X4 Pharmaceuticals and/or have equity ownership. **JB, CG, MY, PEN, JW,** and **MC** are consultants for X4. **SP** and **IW** are former employees of X4 Pharmaceuticals and have equity ownership. This study was funded by X4 Pharmaceuticals.

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