Clinical and genetic diagnosis of warts, hypogammaglobulinemia, infections, and myelokathexis syndrome in 10 patients

To the Editor:

The syndrome of warts, hypogammaglobulinemia, infections, and myelokathexis (WHIM; OMIM no. 193670) is a rare primary immunodeficiency that is usually inherited as an autosomal dominant trait. Leukopenia is a common hematologic manifestation of the disease and is associated with the pathologic retention of neutrophils in the bone marrow, also known as myelokathexis.[1,2] Most patients with WHIM syndrome carry heterozygous mutations of the gene encoding the chemokine receptor CXCR4. Typically, mutations result in premature termination or frameshift in the region that encodes the cytoplasmic tail of the protein.[3-6]

The objective of our study was the characterization of the natural history of WHIM syndrome and the definition of the diagnostic criteria for timely identification of patients with incomplete manifestations of the disease by means of retrospective analysis of patients referred for neutropenia to our center. Although a positive family history might facilitate the diagnosis of WHIM, sporadic cases are usually correctly diagnosed only when the complete spectrum of clinical and laboratory manifestations is present. To better define the clinical spectrum of sporadic cases of WHIM syndrome, we have performed genetic analysis of the *CXCR4* (RefSeq NC_000002.10) gene in 53 patients referred for severe/moderate chronic neutropenia regardless of age and of the presence of additional manifestations and in whom mutations in other neutropenia-causing genes (eg, *ELA2*, *Gfi1*, and *HAX1*) had been ruled out.

Using this strategy, we have identified 9 patients with previously reported heterozygous mutations of *CXCR4* that result in a C-terminus domain–truncated protein lacking 14 to 19 amino acid residues, whereas patient P5 carried a novel heterozygous frameshift mutation resulting in an abnormal elongation of the C-terminal tail (Table I and see Fig E1 in this article's Online Repository at www.jacionlione.org). In these patients we have performed analysis of immunoglobulin levels, T-cell subpopulations, CXCR4 expression, and chemotaxis in response to CXCL12.

In all of the patients, leukopenia was observed already at the onset of the disease (median leukocyte count, 1361 ± 302), and neutropenia was particularly prominent (median neutrophil count, 213 ± 127). Bone marrow aspiration was performed in 8 patients and disclosed myelokathexis in 7 of them, although not in patient P6 (Table I). Clinical and genetic diagnoses of WHIM syndrome were delayed in the majority of patients, and the diagnosis was missed in 1 patient (P8) who died at 54 years of age because of B-cell lymphoma and was identified postmortem as affected with WHIM syndrome only after the same disease was recognized in her daughter (P7).

Because of chronic neutropenia, 5 patients (P1, P4, P6, P7, and P9) were treated with subcutaneous injections of recombinant granulocyte colony-stimulating factor at doses starting from 3 to 5 μg/kg/d. This treatment resulted in a significant increase in the neutrophil count from 208 ± 137 (mean ± SD) to 2966 ± 1003 cells/μL. Four of these patients (P4, P6, P7, and P9), have continued to receive granulocyte colony-stimulating factor, whereas this treatment was discontinued after 6 months in patient P1 (Table I). Moderate lymphopenia was observed in all patients except P5, whereas analysis of lymphocyte proliferation in response to PHA in patients P1 and P5 was found to be normal, as previously reported.[4]

Hypogammaglobulinemia was observed in patients P2, P6, and P9, whereas another 4 patients (P1, P3, P4, and P5) showed borderline levels. Another 3 patients (P7, P8, and P10) presented with normal values of IgG, IgA, and IgM (see Table E1 in this article's Online Repository at www.jacionlione.org). Moreover, 2 patients (P1 and P6) mounted protective antibody responses after immunization with tetanus toxoid, but their titers of specific antibodies decrease rapidly, and anti–tetanus toxoid antibodies were no longer present 1 year after immunization. Warts were observed in 7 subjects, whereas in the remaining patients the typical cutaneous manifestation of WHIM syndrome was never observed.

Because impaired CXCL12-induced CXCR4 internalization was described in T lymphocytes from patients with WHIM, we evaluated the effect of CXCL12 on CXCR4 expression in activated T lymphocytes from healthy subjects and patients P1, P2, P4, P6, P7, and P10. CXCL12 induced a significant downregulation of cell-surface expression of CXCR4 in healthy subjects' cells, whereas it was impaired in the cells of patients with WHIM (Fig 1, *A*). Analysis of CXCL12-mediated internalization in the COS7 cell line transfected with either wild-type CXCR4 ($CXCR4^{WT}$) or mutated CXCR4 receptors ($CXCR4^{336}$, $CXCR4^{338}$, and $CXCR4^{del341}$) confirmed that the loss of the C-tail prevents receptor internalization (Fig 1, *B*).

We and other investigators showed that neutrophils and activated T cells from patients with WHIM display increased chemotactic responses to CXCL12.[4,5,7] On the basis of experiments, Balabanian et al[5] proposed that the enhanced chemotactic response described in patients with WHIM was dependent on impaired desensitization of CXCR4 in response to CXCL12, as measured based on calcium mobilization. Preincubating the cells of patients with WHIM with CXCL12 at a concentration of 200 nmol/L did not affect the subsequent chemotactic response to the same chemokine, whereas preincubation of normal T cells with CXCL12 resulted in 60% inhibition of CXCL12-induced chemotaxis (Fig 1, *C*).

To date, all the studies describing patients with WHIM have reported just 6 mutations in the *CXCR4* gene, including 4 nonsense and 2 frameshift mutations, also including a previously unreported mutation of this study (eg, S341fsX365), without finding a genotype-phenotype correlation (see Table E2 in this article's Online Repository at www.jacionlione.org).

Definition of the molecular basis of WHIM syndrome has now permitted the identification of patients with incomplete manifestations of the disease. We observed that leukocyte cell numbers were consistently reduced in all patients with WHIM, whereas lymphocyte counts were reduced to a lower extent, supporting the notion that neutropenia and leukopenia represent the hallmarks of WHIM syndrome, even in patients with partial manifestations of the disease.[1,6] In addition, this study confirms the extreme variability in the susceptibility to human papillomavirus infections: although some patients have relatively few or no warts, others have intractable cutaneous verrucosis or genital condyloma acuminata associated with genital dysplasia.[1,2,5] Analysis of serum

TABLE I. Main clinical and laboratory features of patients with WHIM

	P1	P2*	P3*	P4	P5	P6	P7*	P8*	P9	P10
Warts	−	+	+	−	+	−	+	+	+	+
Myelokathexis	+	+	+	+	+	+/−†	+	NA	+	NA
Neutropenia	+	+	+	+	+	+	+	+	+	+
Lymphopenia	+	+	+	+	−	+	+	NA	+	+
Hypogammaglobulinemia	−	+	−	−	−	+	−	−	+	NA
Age at onset (y)	1.9	0.3	2	0.3	3	0.3	1.7	NA	1.5	9
Age at clinical diagnosis (y)	4	0.8	23	14	15	4.5	27	NA	25	9
Age at molecular diagnosis (y)	7	17	36	14	15	4.5	27	Postmortem	25	9
Onset	Recurrent respiratory tract infections	Meningitis, recurrent respiratory tract infections	Recurrent respiratory tract infections	Bronchopneumonia	Fever, osteitis	Enteritis, recurrent respiratory tract infections	Pneumonia	NA	Protracted enteritis	Otitis media, bronchopneumonia, varicella, recurrent respiratory tract infections
G-CSF (y)	4 (suspended)	−	−	9.5	−	0.8	27	NA	25	−
Antibiotic	+ (prophylaxis; suspended)	+ (prophylaxis)	−	−	−	− (sporadically)	−	NA	− (sporadically)	+
IVIG (y)	−	1	−	11-12	3-6	2	−	NA	(Subcutaneous immunoglobulin)	−
Other symptoms	Tetralogy of Fallot	−	−	−	−	Idiopathic mental retardation	Genital condyloma acuminata	Lymphoma	−	−
Mutation (DNA)	1000C>T/wt	1006G>T/wt	1006G>T/wt	1000C>T/wt	1021delT/wt	1000C>T/wt	1013C>G/wt	1013C>G/wt	1016-17delCT/wt	1000C>T/wt
Mutation (protein)	R334X/wt	G336X/wt	G336X/wt	R334X/wt	S341fsX365/wt	R334X/wt	S338X/wt	S338X/wt	S339fsX342/wt	R334X/wt

wt, Wild-type.

*Family history showed autosomal inheritance in kindreds of patients P2/P3 and P7/P8.

†Myelokathexis was not recognized in bone marrow aspirate, despite the presence of numerous mature neutrophils with abnormal morphology.

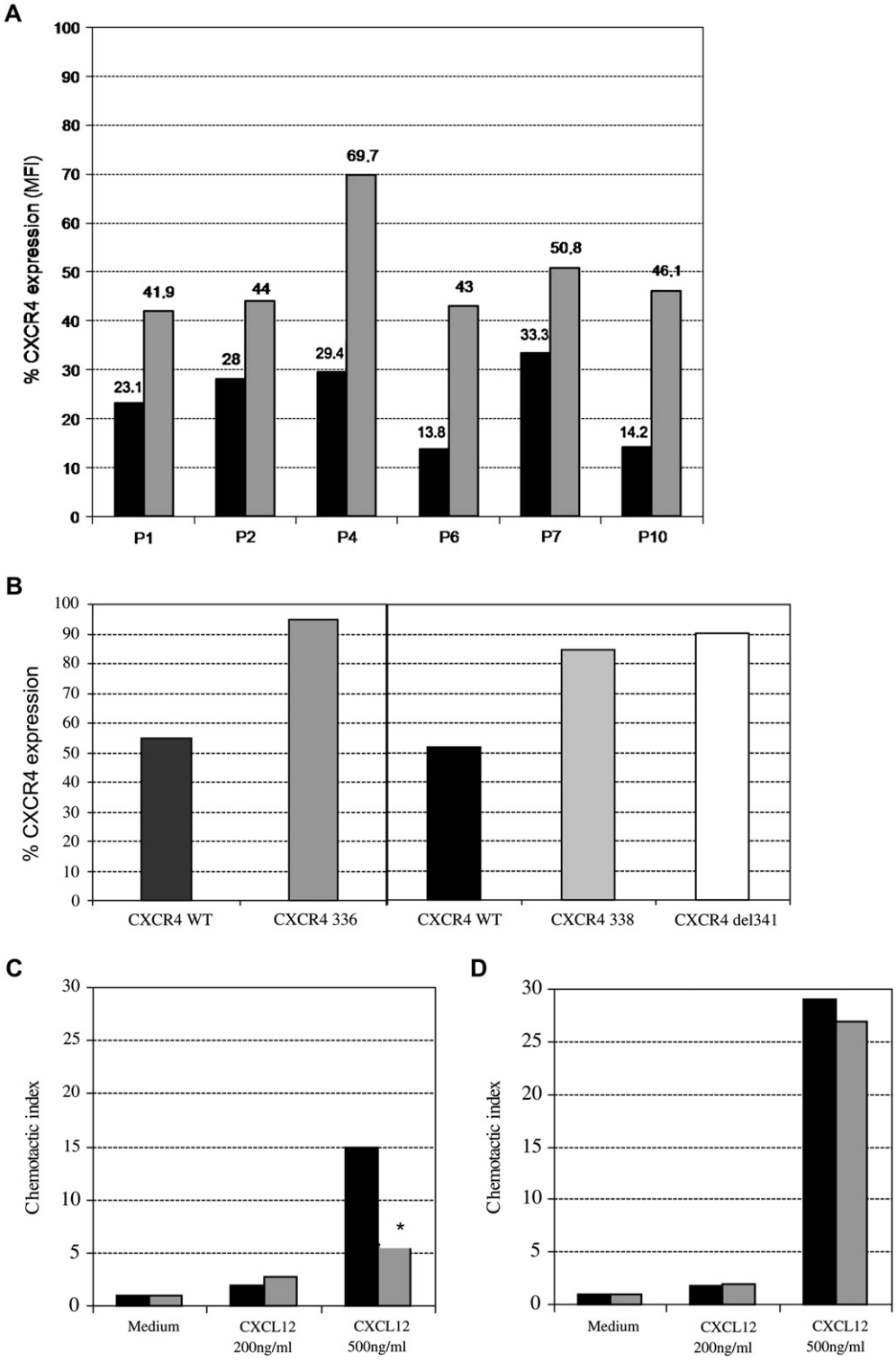

FIG 1. CXCR4 internalization and impaired chemotaxis desensitization in patients with WHIM syndrome. **A**, CXCR4 cell-surface expression in CXCL12 (200 nmol/L) treated T cells from control subjects *(black columns)* or patients with WHIM syndrome *(gray columns)* compared with untreated cells. **B**, CXCR4 expression in wild-type *CXCR4* and mutated *CXCR4*-transfected COS7 cells compared with that seen in untreated cells. **C** and **D**, Control (Fig 1, *C*) or P10 activated T cells (Fig 1, *D*) were subjected to chemotaxis assay after preincubation with CXCL12 (200 nmol/L) *(gray columns)* or left untreated *(black columns)* and thereafter stimulated with CXCL12. The experiment was done in duplicate. *Asterisks* indicate significant inhibition of chemotactic response to CXCL12 (500 ng/mL) after preincubation with the chemokine in cells of control subjects but not of patients with WHIM syndrome, as assessed by using the Wilcoxon comparison test ($P < .05$).

immunoglobulin concentrations showed significant variability, with some patients with WHIM syndrome having normal immunoglobulin levels. Accordingly, hypogammaglobulinemia should not be considered a consistent feature of the disease.

Myelokathexis was not recognized in 1 patient (P6) not displaying all the aspects of WHIM syndrome in bone marrow. On the basis of our study, we propose that *CXCR4* genetic analysis might be considered in patients with chronic neutropenia and retention of mature neutrophils in bone marrow, despite lack of hypogammaglobulinemia and severe warts. Although WHIM syndrome is considered to be a benign disorder and many patients are given diagnoses in adulthood, several malignancies were reported in patients with WHIM syndrome.[6,8,9] Nonetheless, it is likely that the diagnosis of WHIM syndrome might often be missed, even in patients with recurrent infections, as shown here by the case of patient P8 and also by the recent report of a patient who had bronchiectasis before the diagnosis of WHIM syndrome was established.[10] Therefore development of an international disease-specific registry with prospective enrollment of patients is needed to better define long-term clinical outcome and the efficacy of available therapeutic modalities in patients with WHIM.

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A survey of allergists regarding the association of thyroid autoimmunity with chronic urticaria

To the Editor:

The Urticaria and Angioedema Committee of American Academy of Allergy, Asthma & Immunology (AAAAI) developed a questionnaire to survey the opinions of the members of the AAAAI regarding the possible association of chronic urticaria (CU) and thyroid autoimmunity. The objectives of this preliminary survey were to determine whether there was consensus or variability in the opinions of interested AAAAI members about the link between thyroid autoimmunity and CU and whether there was consensus or variability regarding treatment of such patients with thyroxine. The questionnaire was created *de novo* and has not been previously validated. Although it is currently known that thyroid autoantibodies are present at a relatively high frequency in patients with CU, there has been considerable controversy about whether there is a causative link and whether treatment with thyroxine might benefit the patient. We hypothesized that a survey of the opinions of AAAAI members with interest in the treatment of CU would be of benefit to readers.

Two e-mail messages were sent to AAAAI physician members with registered e-mail addresses in February and March 2007, encouraging them to click the link to the 8-question Zoomerang survey and submit their opinions. The survey was sent to the 3388 physician members of the AAAAI, with 828 responses (24.5% response rate, assuming no individual completed the survey more than once). We recognize that the responders might well represent a self-selected subpopulation of the AAAAI membership who are particularly interested in the clinical management of urticaria. The results of our study cannot be generalized to the entire AAAAI membership without a relatively high likelihood of introducing bias.[1] A similar survey initiated by the Immunotherapy and Allergy Diagnostics Committee of the AAAAI has been previously published.[2] The design of these surveys does not allow for statistically powered

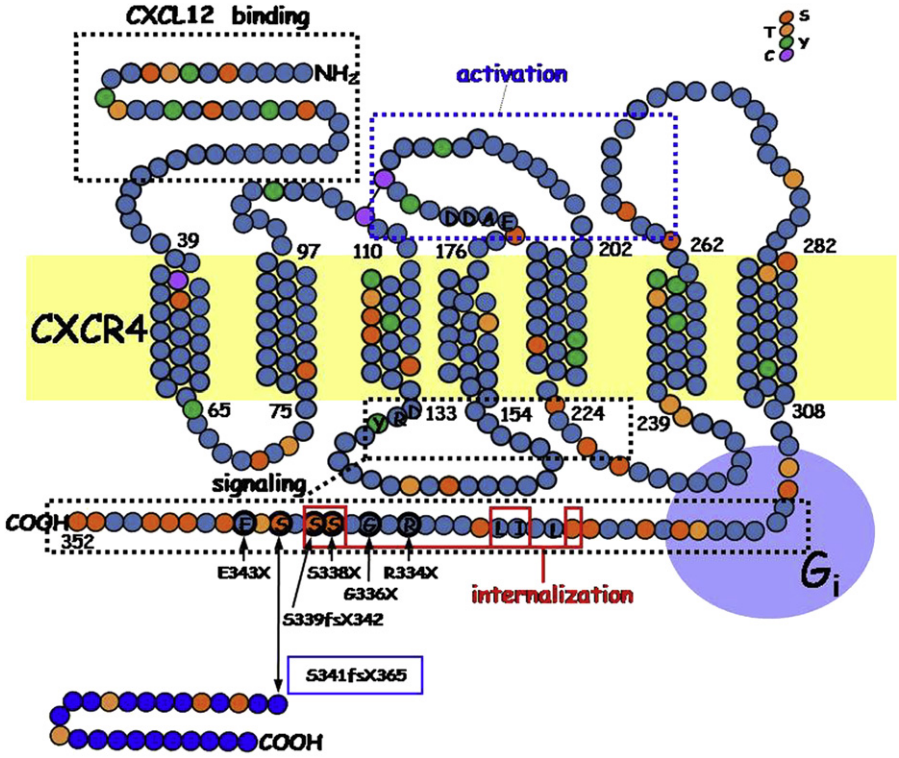

FIG E1. *CXCR 4* mutations of patients with WHIM syndrome. *CXCR4* mutations affect the last 10 to 19 amino acid residues of *CXCR4*.

TABLE E1. Immunologic features of the patients with WHIM syndrome

	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10
WBC (cells/μL)	1500	400	1300	720	2900	1490	800	NA	510	1120
Neutrophils (cells/μL)	300	32	416	79	377	75	208	NA	150	232
Lymphocytes (cells/μL)	1005	312	793	482	2320	1222	401	NA	300	726
CD3 % (cells/μL)	55 (553)	90 (281)	70 (555)	77 (371)	72 (1670)	65 (794)	NA	NA	49 (280)	72 (523)
CD4 % (cells/μL)	42 (422)	45 (140)	47 (373)	59 (284)	55 (1276)	58 (709)	20 (80)	NA	35 (200)	61 (443)
CD8 % (cells/μL)	6 (60)	38 (119)	14 (111)	13 (63)	19 (441)	9 (110)	30 (120)	NA	12 (70)	26 (189)
CD16 % (cells/μL)	31 (312)	5 (16)	17 (135)	30 (145)	NA	35 (428)	NA	NA	41 (240)	8 (58)
CD19 % (cells/μL)	2.4 (24)	1.8 (6)	3.8 (30)	2.1 (10)	1.8 (42)	5 (61)	NA	NA	4 (20)	4 (29)
IgG	415	174	438	597	375	192	1137	955	NA	790
IgA (mg/dL)	12	15	25	135	42	8	243	288	65	130
IgM	33	125	42	60	87	50	256	86	162	160

WBC, White blood cell count; *NA*, not applicable; *G-CSF*, granulocyte colony-stimulating factor; *IVIG*, intravenous immunoglobulin.

TABLE E2. *CXCR4* gene mutations reported in patients with WHIM syndrome

	Nucleotide change	Mutation	Amino acid change	Reference
1	g.1000C>T	Nonsense	p.R334X	Hernandez et al, 2003; Gulino et al, 2004; Tarzi et al, 2005; Taniuchi et al, 2005; Hagan and Nguyen, 2007
2	g.1006G>T	Nonsense	p.G336X	Gulino et al, 2004
3	g.1013C>G	Nonsense	p.S338X	Gulino et al, 2004; Alapi et al, 2007; Vinurel et al, 2008, present study
4	g.1016-17delCT	Deletion	p.S339fsX342	Hernandez et al, 2003; Sanmun et al, 2006; present study
5	g.1021delT	Deletion	p.S341fsX365	Present study
6	g.1027G>T	Nonsense	p.E343X	Hernandez et al, 2003

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