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Polymorphisms in the cytotoxic T lymphocyte-associated protein-4 immune regulatory gene and their impact on inhibitor development in patients with hemophilia A

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Abstract

Objective: The development of inhibitors against infused factor VIII represents the most severe complication of substitution therapy in hemophilia A (HA) patients. Data on risk factors for inhibitor formation in Iraqi Kurdish patients with HA are unavailable. This study aimed to evaluate the impact of two single nucleotide polymorphisms (SNPs) in an immune regulatory gene in the emergence of inhibitors.

Methods: We focused on 126 patients with either severe or mild/moderate HA presenting with and without inhibitors. We analyzed the frequency of two polymorphisms in the cytotoxic T lymphocyte-associated protein-4 gene (*CTLA-4*; CTLA-4-318C > T and CTLA-4 + 49A > G). Genotyping was performed using restriction fragment length polymorphism–PCR and direct sequencing.

Results: We found no significant correlation between the CTLA-4-318 C > T T allele and inhibitor development among patients with severe or mild/moderate HA. However, a significantly high inhibitor risk was detected for the CTLA-4 + 49 A > G G allele (odds ratio [OR] = 3.1, 95% confidence interval [CI] = 1.383–7.024) and (OR = 4, 95% CI = 1.719–9.437) among patients with severe and mild/moderate HA, respectively.

Conclusion: We conclude that the CTLA-4 +49 A > G SNP plays a substantial role as a potential risk determinant for inhibitor formation in Iraqi Kurdish patients with HA.

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Hemophilia A, inhibitors, CTLA4, immune regulatory gene, single nucleotide polymorphism, risk factors

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Introduction

Hemophilia A (HA) is an X-linked recessive bleeding disorder caused by a quantitative or qualitative deficiency in the factor VIII (FVIII) protein.[1] HA is treated by replacing the deficient FVIII protein, but this therapy is often ineffective following the manifestation of neutralizing antibodies (inhibitors) against the infused FVIII protein; this is therefore the most burdensome complication of hemophilia management.[2–4] In unselected patients with hemophilia, the prevalence of inhibitors is 5% to 7%, while its incidence is 25% to 35% in patients with severe disease and 3% to 13% in patients with mild/moderate disease.[2]

As a typical multifactorial trait, risk factors for inhibitor formation in HA patients are classified into two main groups: modifiable (environmental factors) and non-modifiable (genetic factors).[5–7] Environmental risk factors include treatment-related factors and immune system challenges.[5] The main genetic predisposition for inhibitor development in HA patients is the causative FVIII genotype,[8] but it also includes a group of auxiliary risk factors that are weaker than the FVIII genotype such as a family history of inhibitors, ethnicity, human leucocyte antigen haplotype, and polymorphisms of immune system-related genes including interleukin-10, tumor necrosis factor-α, and cytotoxic T lymphocyte-associated protein-4 (*CTLA-4* on chromosome 2q33).[9–11] Human genetic data support the hypothesis that predisposing factors associated with autosomal genes, including those mentioned above, are ethnically divergent and thus not constant among populations worldwide.[12]

HA is caused by several known gene defects, which accounts for the heterogeneity of disease phenotypes and inhibitor production.[13] Patients with severe molecular defects (e.g. large deletions, inversions, and nonsense mutations) that result in the complete lack of the clotting protein appear to have a higher propensity to develop inhibitors than those with milder defects (e.g. missense and splice site mutations) in which some remnant FVIII antigen is present.[8,14] Nevertheless, the discordance in inhibitor production observed in patients or siblings with similar mutations indicates that other genetic factors potentially function as modifiers.[14]

The production of inhibitors to the infused FVIII protein is mediated by a T helper (T_H) cell-dependent process that also incorporates antigen-presenting cells (APCs) and B lymphocytes.[5] Major histocompatibility complex class II molecules expressed on APCs present peptides of the infused factor to the T cell receptor expressed on T_H cells. However, a second co-stimulatory signal is needed to completely evoke the immune response. This signal is produced by the interaction between B7 (CD80/86) molecules on APCs and CD28 on T_H cells. CTLA-4 is a receptor primarily expressed on activated T cells, which competes with CD28 for the interaction with B7 molecules, leading to a decrease in T cell activity. Accordingly, blockade of this

interaction by CTLA-4-antibodies enhances T cell proliferation and B cell activity.[15,16]

Two single nucleotide polymorphisms (SNPs) in *CTLA-4* (CTLA-4-318 C > T in the promoter region and CTLA-4 + 49 A > G in coding sequence 1 encoding a threonine to alanine substitution in the leader peptide) have been found to activate the immune response in patients with antibody-mediated autoimmune diseases such as Graves' disease, systemic lupus erythematosus, Hashimoto's thyroiditis, Wegener's granulomatosis, and multiple sclerosis.[17–21] Furthermore, these two polymorphisms have also been shown to modify the propensity of HA patients to produce inhibitors.[9,12]

Of the 5.7 million Iraqi Kurds, approximately 450 registered patients with HA have been identified. The frequency of patients with severe, moderate, and mild HA is 35.6%, 51.1%, and 13.3%, respectively. The current study aimed to evaluate whether the two SNPs also influence the risk of inhibitor development in a case-controlled study of 126 Iraqi Kurd patients subdivided into those with severe disease and those with mild/moderate HA presenting with and without a history of inhibitor development.

Patients and methods

This study was conducted according to the principles of the Declaration of Helsinki and with the approval of the local institutional ethical committee (College of Medicine, University of Sulaemaniyah approval no. 55; September 7 2017). All patients with hemophilia A (HA) are registered in local hemophilia treatment centers belonging to the Iraqi Society of Hemophilia, and written informed consent was obtained from all participants. Patients were diagnosed with HA according to World Federation of Hemophilia guidelines.[4] All patients exhibited a prolonged activated partial thromboplastin time (aPTT) and reduced FVIII activity.

We performed a case–control study of inhibitor risk associated with two SNPs (CTLA-4-318 C > T and CTLA-4 + 49 A > G). One hundred twenty-six patients with HA, including 35 inhibitor-positive and 91 inhibitor-negative control patients, were included in the study and were subdivided into those with severe HA (n=60 cases; 20 with and 40 without inhibitors) and those with mild/moderate HA (n=66; 15 with and 51 without inhibitors). According to standard International Society on Thrombosis and Hemostasis definitions, patients were considered to express relevant inhibitors when they were documented on two separate occasions within a 1- to 4-week period and had a level of $\geq$0.6 Bethesda units (BU) per mL using the Nijmegen modification of the Bethesda assay.[22] High-response inhibitors represent patients with an inhibitor titer $\geq$5 BU/mL at any time point, and low-response inhibitors were patients who persistently presented an inhibitor titer <5 BU/mL despite repeated challenge with FVIII replacement therapy.[23] Clinical data included relevant patient information such as age, gender, ethnicity, age at first exposure to FVIII (recombinant or other blood products), and number of exposure days (ED).

FVIII level and inhibitor detection

Blood was collected in tubes containing 3.18% trisodium citrate at a 9:1 volumetric ratio. FVIII activity (FVIII:C) was determined using an aPTT-based one-stage clotting assay with an aPTT reagent sensitive to coagulation factor deficiency (^{STA}-C.K. PREST 5, ^{STA}compact Max; Diagnostica Stago, Asnières sur seine, France) and FVIII-deficient plasma (STA®-Deficient VIII, ^{STA}compact Max; Diagnostica Stago) performed with the Stago (^{STA}compact Max) fully automated blood coagulation

analyzer which was calibrated and controlled according to the manufacturer's instructions. The aPTT mixing study was performed to differentiate between a coagulation factor deficiency and the presence of inhibitors. Time-dependent inhibitors were assessed by measuring the aPTT of a mixture composed of one part patient plasma and one part normal plasma after incubation for 2 hours at 37°C. Time-independent inhibitors were determined by measuring the aPTT of an immediate mixture of patient and normal plasma that were incubated separately. The Nijmegen modification of the Bethesda assay, which is applied for more specific antibody detection in the lower range (cut-off point: 0.6 BU/mL) by virtue of the dilution of patient plasma with buffered normal pooled plasma, was performed to detect antibodies against FVIII. Normal pooled plasma was used as a negative control.[24]

DNA extraction and genetic analysis

High molecular weight genomic DNA was extracted from peripheral blood leucocytes anti-coagulated in K_2EDTA using a salting-out procedure.[25] The biallelic polymorphism CTLA-4-318 C > T (rs5742909) was analyzed using restriction fragment length polymorphism–PCR with the forward primer 5′-AAATGAATTGGACTG GATGGT-3′ and the reverse primer 5′-TTACGAGAAAGGAAGCCGTG-3′ as described in previous publications.[9,26] Amplification was performed in a final volume of 25 µl with 0.1 to 0.4 µg of genomic DNA, 0.4 µM of each primer (SinaClon Company, Tehran, Iran), 0.2 mM of each dNTP (Gen Fanavaran Co., Tehran, Iran), 1.5 U Super Taq DNA polymerase (Gen Fanavaran Co.), 0.8× PCR buffer, 2 µl dimethyl sulfoxide ≥99.9% (Sigma-Aldrich, St Louis, MO, USA), and 1.4 mM $MgCl_2$ (Gen Fanavaran Co.). The PCR program was set to an initial denaturation of 94°C for 5 minutes, followed by 28 cycles of 1 minute denaturation at 94°C, 1 minute of annealing at 60°C, 1 minute of elongation at 72°C, and a final 10-minute extension at 72°C. The 247-bp PCR product (in 7 µl) was digested with 10 U *Mse* I restriction enzyme (Thermo Fisher Scientific Inc., Rockford, IL, USA) and 1× digestion buffer R at 65°C for 3 hours in a total volume of 25 µl. The digested PCR products were resolved on a 4% agarose gel. After *Mse* I digestion, the T allele produces two fragments (131 bp and 116 bp) while the C allele produces the 247 bp undigested fragment. CTLA-4-318 C > T was also analyzed by direct sequencing of the amplified PCR product.

The dimorphism in the CTLA-4 + 49 A > G allele (rs 231775) was determined by PCR amplification of genomic DNA using the in-house designed forward primer 5′-GTGTAATACATATCTGGGATCAA AGC-3′ and reverse primer 5′-CCC AG GTAGGAG AAACACCTC-3′. The amplification mixture and PCR conditions were identical to those used to identify the previous polymorphism. CTLA-4 + 49 A > G was then detected by direct sequencing of the 300-bp PCR product. Amplified fragments were sequenced commercially using an ABI 3130 XL sequencer (Applied Biosystems, Foster City, CA, USA). The FinchTV sequence analysis software package (Geospiza Inc., Seattle, WA, USA) was used for sequence reading and analysis.

Statistical analysis

Statistical package IBM SPSS version 23 (IBM Corp., Armonk, NY, USA) was used to analyze the data. Continuous data are presented as medians, means ± SD, and ranges. The Chi-squared test was used to compare differences in the following categorical data: genotypes, alleles, and phenotype frequencies between inhibitor-positive and inhibitor-negative patients. All

P-values were two-sided with one degree of freedom, and $P < 0.05$ was regarded as statistically significant. Risk associations with a particular allele were reported as odds ratios (ORs) with 95% confidence intervals (CIs).

Results

Patient characteristics

A cohort of 126 male patients with HA from 88 unrelated families were enrolled in this study. The median age of all patients with inhibitors was 14 years (range, 5–30 years), while the median age in patients without inhibitors was 19 years (range, 5–51 years). Disease severity was determined by measuring the FVIII clotting activity. Of the 126 patients, 60 were diagnosed with severe HA (47.6%), 48 with moderate HA (38.1%), and 18 with mild HA (14.3%). Thirty-five patients were inhibitor-positive, of whom 80% (28/35) had a high titer of inhibitors (≥5 BU/mL) and 20% (7/35) had a low titer (<5 BU/mL). The frequency of inhibitors in patients with severe, moderate, and mild HA was 33.3% (20/60), 27% (13/48), and 11.1% (2/18), respectively. Patients exhibited a mean antibody titer of 67.27 BU/mL (range, 3–825 BU/mL).

The characteristics of patients with respect to inhibitors and the severity of HA are listed in Table 1. Among patients with severe HA, the mean age of inhibitor-positive patients was 15 ± 8 years (range, 5–30 years) and the mean age of inhibitor-negative controls was 22 ± 8 years (range, 5–38 years). Among patients with severe HA, 30% and 27.5% with and without inhibitors, respectively, received recombinant FVIII or other blood products before 6 months of age. The mean number of EDs among inhibitor-positive patients was 58 ± 27 SD (range, 9–120), while that in inhibitor-negative patients was 188 ± 82 (range, 35–450) (Table 2). The mean ages of inhibitor-positive and inhibitor-negative patients with mild/moderate HA were 16 ± 7 years (range, 6–28 years) and 18 ± 11 years (range, 5–51 years), respectively. Among patients with and without inhibitors, 13.3% and 15.7%, respectively, received FVIII before 6 months of age, while the mean ED was 68 ± 42 (range, 10–140) and 84 ± 42 (range, 30–200), respectively (Table 2).

Overall, our patients had no history of surgery. Further, apart from four patients with moderate HA who developed inhibitors after major gastrointestinal bleeds that required intensive treatment, the remaining patients suffered from joint and mucocutaneous bleeds or received prophylaxis therapy.

CTLA-4 genotype distribution

CTLA-4-318 C/T and CTLA-4 + 49 A/G polymorphisms were analyzed in 60 patients with severe HA (20 with and 40 without

Table 1. Characteristics of the study group with respect to inhibitor status

	Distribution by severity of hemophilia A and inhibitor status, N (%)				
Type of Hemophilia A	Mean FVIII level %	HR	LR	No Inhibitors	Total *N*
Severe (<1%)	0.8	18 (30)	2 (3.3)	40 (66.7)	60
Moderate (1%–5%)	2.1	8 (16.6)	5 (10.4)	35 (73)	48
Mild (6%–30%)	13.6	2 (11.1)	0 (0)	16 (88.9)	18
Total *N* (%)	–	28 (22.2)	7 (5.6)	91 (72.2)	126

HR: high responder, LR: low responder

Table 2. Characteristics of the study group with respect To age, age at first exposure to FVIII, and exposure days

	Mild/Moderate (n = 66)		Severe (n = 60)	
	Inhibitor-positive (n = 15)	Inhibitor-negative (n = 51)	Inhibitor-positive (n = 20)	Inhibitor-negative (n = 40)
Age (years)				
Mean (±SD)	16 (±7)	18 (±11)	15 (±8)	22 (±8)
Range	6–28	5–51	5–30	5–38
Age at first exposure to FVIII *N* (%):				
<6 months	2 (13.3)	8 (15.7)	6 (30)	11 (27.5)
≥6 months	13 (86.7)	43 (84.3)	14 (70)	29 (72.5)
Mean months (±SD)	29 (±33)	50 (±62)	18 (±15)	35 (±47)
Exposure days *N* (%):				
<150	15 (100)	45 (88.2)	20 (100)	12 (30)
≥150	0 (0)	6 (11.8)	0 (0)	28 (70)
Mean exposure days (±SD)	68 (±42)	84 (±42)	58 (±27)	188 (±82)
Range	10–140	30–200	9–120	35–450

SD, standard deviation.

inhibitors) and 66 patients with mild/moderate HA (15 with and 51 without inhibitors). No significant association was detected between a C/T substitution at position –318 in the promoter region and inhibitor development among both groups. Among patients with severe HA, the T allele (homozygous TT or heterozygous CT) was found in 50% of inhibitor-negative patients and in 35% of inhibitor-positive patients (Table 3). Thirteen (65%) patients with inhibitors were homozygous for the C allele (CC), three (15%) were homozygous for the T allele (TT), and four (20%) were heterozygous (CT), compared with 20 (50%), 10 (25%), and 10 (25%), respectively, of the control inhibitor-negative patients (Table 4). Total allele frequencies were 37.5% for the T allele and 62.5% for the C allele in the inhibitor-negative group compared with 25% and 75%, respectively, in the inhibitor-positive group. Based on our findings, there was no significant difference in the frequency of the T-positive phenotype in inhibitor-negative patients (50%) compared with inhibitor-positive patients (35%) (Table 4). Similarly, no significant correlation between CTLA-4-318 C/T SNP and inhibitor development was observed among patients with mild/moderate HA (Table 4).

Analysis of CTLA-4 + 49 A/G revealed that this polymorphism appeared at a significantly higher frequency in inhibitor-positive patients among both groups (severe and mild/moderate HA). A significantly higher inhibitor risk association was observed for the G allele. The frequency of the G allele was 47.5% in inhibitor-positive patients with severe HA while the frequency of the A allele was 52.5%, compared with 22.5% and 77.5%, respectively, in inhibitor-negative patients, corresponding to an OR of 3.1 (95% CI = 1.383–7.024, $P = 0.005$ (Table 5). This significant association was persistent after considering the combination of genotypes, i.e., GG and AG, with a dominant effect revealing an OR of 3.5 (95% CI = 1.112–11.017, $P = 0.028$) (Table 3). Five (25%) inhibitor-

Table 3. Relationship between CTLA-4 and inhibitor development based on division into CC, AA, and combined (CT, TT) and (AG, GG) genotypes

	Mild/Moderate (n = 66)				Severe (n = 60)			
	Inhibitor-positive (n = 15)		Inhibitor-negative (n = 51)		Inhibitor-positive (n = 20)		Inhibitor-negative (n = 40)	
CTLA-4 polymorphisms	*N*	%	*N*	%	*N*	%	*N*	%
–318 C/T								
Genotype frequencies[a,b]								
CC	10	66.7	37	72.6	13	65	20	50
CT and TT	5	33.3	14	27.4	7	35	20	50
+49 A/G								
Genotype frequencies[c,d]								
AA	5	33.3	32	62.7	6	30	24	60
AG and GG	10	66.7	19	37.3	14	70	16	40

[a]Difference between inhibitor-positive and -negative in mild/moderate group: OR = 1.3 (95% CI = 0.383–4.554).
[b]Difference between inhibitor-positive and -negative in severe group: OR = 0.5 (95% CI = 0.178–1.631).
[c]Difference between inhibitor-positive and -negative in mild/moderate group: OR = 3.4 (95% CI = 1–11.345, $P = 0.044$).
[d]Difference between inhibitor-positive and -negative in severe group: OR = 3.5 (95% CI = 1.112–11.017, $P = 0.028$).

Table 4. Genotype, allele, and phenotype frequencies of CTLA-4-318 C/T in Kurdish hemophilia A patients with and without inhibitors

	Mild/Moderate (n = 66)				Severe (n = 60)			
	Inhibitor-positive (n = 15)		Inhibitor-negative (n = 51)		Inhibitor-positive (n = 20)		Inhibitor-negative (n = 40)	
	N	%	*N*	%	*N*	%	*N*	%
–318 C/T								
Genotype frequencies[a,b]								
CC	10	66.6	37	72.6	13	65	20	50
TT	3	20	10	19.6	3	15	10	25
CT	2	13.4	4	7.8	4	20	10	25
Allele frequencies[c,d]								
C	22	73.3	78	76.5	30	75	50	62.5
T	8	26.7	24	23.5	10	25	30	37.5
Phenotype frequencies[e,f]								
C-positive	12	80	41	80.4	17	85	30	75
T-positive	5	33.3	14	27.5	7	35	20	50

[a]Difference between inhibitor-positive and -negative in mild/moderate group.
[b]Difference between inhibitor-positive and -negative in severe group.
[c]Difference between inhibitor-positive and -negative in mild/moderate group: OR = 1.2 (95% CI = 0.466–2.994).
[d]Difference between inhibitor-positive and -negative in severe group: OR = 0.5 (95% CI = 0.238–1.296).
[e]Difference between inhibitor-positive and -negative in mild/moderate group: OR = 1.2 (95% CI = 0.365–4.079).
[f]Difference between inhibitor-positive and -negative in severe group: OR = 0.6 (95% CI = 0.217–1.759).

Table 5. Genotype, allele, and phenotype frequencies of CTLA-4 + 49 A/G in Kurdish hemophilia A patients with and without inhibitors

	Mild/Moderate (n=66)				Severe (n=60)			
	Inhibitor-positive (n=15)		Inhibitor-negative (n=51)		Inhibitor-positive (n=20)		Inhibitor-negative (n=40)	
	N	%	*N*	%	*N*	%	*N*	%
+49 A/G								
Genotype frequencies[a,b]								
AA	5	33.3	32	62.7	6	30	24	60
GG	7	46.7	6	11.8	5	25	2	5
AG	3	20	13	25.5	9	45	14	35
Allele frequencies[c,d]								
A	13	43.3	77	75.5	21	52.5	62	77.5
G	17	56.7	25	24.5	19	47.5	18	22.5
Phenotype frequencies[e,f]								
A-positive	8	53.3	45	88.2	15	75	38	95
G-positive	10	66.7	19	37.3	14	70	16	40

[a]Difference between inhibitor-positive and -negative in mild/moderate group: $P = 0.011$.
[b]Difference between inhibitor-positive and -negative in severe group: $P = 0.026$.
[c]Difference between inhibitor-positive and -negative in mild/moderate group: OR = 4 (95% CI = 1.719–9.437, $P = 0.001$).
[d]Difference between inhibitor-positive and -negative in severe group: OR = 3.1 (95% CI = 1.383–7.024, $P = 0.005$).
[e]Difference between inhibitor-positive and -negative in mild/moderate group: OR = 3 (95% CI = 1.012–8.659, $P = 0.043$).
[f]Difference between inhibitor-positive and -negative in severe group: OR = 2.2 (95% CI = 0.871–5.639).

positive patients were homozygous for the G allele (GG), nine (45%) were heterozygous (AG), and six (30%) were homozygous for the A allele (AA) compared with two (5%), 14 (35%), and 24 (60%), respectively, of inhibitor-negative patients ($P = 0.026$) (Table 5). Although not significant, the frequency of the G-positive phenotype was higher in patients with inhibitors (70%) than in those without inhibitors (40%), with an OR of 2.2 (95% CI = 0.871–5.639) (Table 5). A similar trend in the correlation between CTLA-4 + 49 A/G and inhibitor development was observed among patients with mild/moderate HA as shown in Table 3 and Table 5.

Discussion

The ability to anticipate which HA patients have the potential to develop inhibitors and to recognize factors that lead to inhibitor formation would allow the application of appropriate therapies to avoid the inhibitor response. In the current study, we focused on patients with severe and mild/moderate HA, each presenting with and without inhibitors to assess the pertinence of two *CTLA-4* polymorphisms in the risk of developing FVIII inhibitors.

CTLA-4 is a surface molecule expressed on activated T cells that plays a crucial role as a negative regulator of T cell activation.[15,16,27] The CTLA-4-318 C/T SNP in the promoter region –318 bp from the ATG start codon is associated with increased promoter activity, increased protein expression, a negative effect on the immune response, and hence a lower risk of inhibitor formation.[28,29] In the present study, we found no significant protective correlation between inhibitor formation

and the –318 T allele in patients with either severe or mild/moderate HA, similar to the findings reported in an Indian study,[30] a Chinese cohort,[31] and a Brazilian study,[32] but in contrast to MIBS and Argentinean cohorts[9,12] that identified a substantial protective correlation between CTLA-4-318 C/T and inhibitor formation. It is noteworthy that most previous reports included only patients with severe HA. The current study further investigated the correlation between CTLA-4 and inhibitor development in a new cohort of patients with mild/moderate HA and, to our knowledge, represents the first such study on this group of patients.

Previous *in vitro* studies showed that CTLA-4 + 49 A > G at position +49 in coding sequence 1 produces a missense variant that results in a threonine to alanine exchange (p.Thr17Ala) in the leader peptide. This causes incomplete glycosylation in the endoplasmic reticulum, eventually leading to a decreased surface/total ratio of the protein that might affect its function.[28,33,34] The inhibitory effect of the CTLA-4 protein on activated T cells is less potent in subjects carrying the G allele than in those carrying the A allele.[20,33] Moreover, this A > G SNP has been linked to susceptibility to a number of antibody-mediated autoimmune diseases.[18,20,21,35,36] Additionally, an increased frequency of the CTLA-4 + 49 G allele was observed in patients with acquired HA compared with healthy controls.[37] In this series, a significantly higher inhibitor risk was observed for patients carrying the +49 G allele in patients with both severe and mild/moderate HA, which is consistent with the Argentinian cohort study.[12] A similar but non-significant trend was obtained in a study of North European patients with HA, showing an OR of 2.2 (95% CI = 0.6–7.8) for the presence of the G allele among patients with severe HA and inhibitors.[9] However, this association was not established in other populations, including a Brazilian study,[32] a Chinese cohort,[31] a group of Indian patients with HA,[30] and a series of Italian patients.[38] Pavlova *et al*. also reported the lack of a significant difference with regard to analysis of the +49 A > G SNP between patients with severe HA presenting with and without inhibitors.[11]

Currently endorsed treatments for HA patients presenting with inhibitors are substandard, so these patients have worse outcomes than those without inhibitors.[39] Therefore, a major focus of HA research has been to determine risk factors for inhibitor development and optimal management strategies to decrease inhibitor risk. Because of the low level of resources and economic issues in our developing country, the prevalence of mutations established as major determinants of inhibitor development in HA patients were not analyzed in the current cohort. Nevertheless, our study is important because no published report has yet defined the unmodifiable risk factors associated with inhibitor formation in the Iraqi Kurdish population. Moreover, secondary genetic factors inducing inhibitor development in HA patients (e.g., immune gene polymorphisms) from diverse regions and ethnicities show significant differences worldwide. These divergent findings were clarified in massive international studies, such as the Hemophilia Inhibitor Genetics study[40] and other regional studies.[9,10]

Conclusion

This study highlighted the association between CTLA-4 + 49 A > G and inhibitor formation in HA patients, justifying the need for an analysis of modifying risk factors for inhibitor development. Improved knowledge about such risk factors would allow researchers to develop regionally relevant inhibitor risk scores that consider all non-modifiable factors to calculate the genetic predisposition of each patient to develop inhibitors.

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