The frequency of A91V in the perforin gene and the effect of tumor necrosis factor-α promoter polymorphism on acquired hemophagocytic lymphohistiocytosis

Perforin geninde A91V frekansı ve tümör nekrozis-α faktör promotor polimorfizminin edinsel hemofagositik lenfohistiositoza etkisi

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Abstract

Objective: Numerous acquired etiological factors, such as infections, malignancies, and collagen tissue disorders, are involved in the development of acquired hemophagocytic lymphohistiocytosis (AHLH). Not everyone with the same etiological factors developments AHLH, which suggests the role of additional genetic or environmental predisposing factors that remain to be identified.
Materials and Methods: Perforin gene A91V missense transition (C>T change at position 272 in exon 2 of the perforin gene) and TNF-α gene promoter-1031 T>C nucleotide substitution are 2 candidate genetic predisposing factors due to their potential to alter inflammatory responses. In the present study these changes were investigated in healthy controls and AHLH patients.
Results: A91V transition was observed in 7 of the 159 (4.4%) controls. Among the 44 AHLH patients, 5 (11.3%) were heterozygous and the difference in the frequency of A91V transition, although striking (odds ratio: 2.8), was not statistically significant (p=0.09). All A91V-positive patients had infection. TNF-α-1031 T>C polymorphism was examined in 164 healthy controls and 40 AHLH patients, and the CC risk-elevating genotype was noted in 7 (4.3%) of the controls and 1 (2.5%) of the AHLH patients. The frequency of C and T alleles was 22.5% (n=18) and 77.5% (n=62) among the AHLH patients, and 22% (n=72) and 78% (n=259) among the controls, respectively. There wasn't a statistically significant difference between the groups in terms of allele frequencies (p>0.05).
Conclusion: The present results indicate that compared to controls, A91V mutation was 2.8-fold more prevalent (according to the odds ratio) in the AHLH patients. A91V mutation is not uncommon in the general population and increases the risk of AHLH in patients with an underlying condition, especially those with an underlying infection. *(Turk J Hematol 2011; 28: 125-30)*

Key words: Acquired hemophagocytic lymphohistiocytosis, infection-related HLH, perforin, A91V mutation, TNF-α polymorphism

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Özet

Amaç: Edinsel hemofagositik lenfohistiosito zun (EHL) gelişmesinde enfeksiyonlar, habis hastalıklar, kollajen doku hastalıkları gibi çok çeşitli etmen rol oynamaktadır. Aynı tetikleyici faktörü bulunan hastaların tümünde EHL'un gelişmemesi EHL'ye yatkınlık yapan ek genetik ve çevresel faktörlerin varlığına işaret etmektedir.
Yöntem ve Gereçler: Perforin geninde A91V yanlış anlam değişikliği (perforin geninde ekzon 2, pozisyon 272'de C>T değişikliği) ve tumor nekrozis faktör (TNF)-α geninin promoter bölgesinde –1031T>C nükleotid değişikliği inflamatuvar yanıtı değiştirebilen ve bu nedenle EHL'ye yatkınlığa neden olabilen iki potansiyel adaydır. Çalışmamızda EHL'li hastalar ve kontrollerde bu değişiklikler incelenmiştir.
Bulgular: 159 sağlıklı Türk popülasyonunda A91V değişikliği 7 (%4.4) kişide saptanmıştır. 44 EHL olgusunun beşinde (%11.3) bu değişiklik saptanmış olup, fark dikkat çekici olmakla birlikte istatistiksel anlamlılık göstermemiştir (p=0.09); odds oranı 2.8 olarak hesaplanmıştır. A91V pozitif olan hastaların tümünde enfeksiyon altta yatan etiolojik nedendi. TNF-α -1031T>C polimorfizmi 164 sağlıklı birey ve 40 EHL'li hastada çalışıldı. Kontrollerin 7'sinde (%4.3) ve EHL bulunan hastaların 1'inde (%2.5) riski artıran CC genotipi saptandı. C ve T allel frekansları sırasıyla EHL'de 18 (%22.5) ve 62 (%77.5), kontrollerde 72 (%22) ve 259 (%78) olarak bulundu. Allel frekansları açısından gruplar arasında fark saptanmadı (p>0.05).
Sonuçlar: Çalışmamızın sonuçları edinsel HLH'li hastalarda sağlıklı kontrollara göre A91V sıklığının 2.8 kat odds oranına göre daha sık olduğunu, A91V'nin sağlıklı Türk populasyonunda nadir olmadığını ve özellikle enfeksiyonu olanlarda EHL'ye yatkınlık yapabileceğini göstermektedir.
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Anahtar kelimeler: Edinsel hemofagositik lenfohistiositoz, enfeksiyon ilişkili HLH, perforin, A91V mutasyonu, TNF-α, polimorfizmi

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Introduction

Hemophagocytosis is a mysterious abnormal cellular condition that accompanies several disorders. One such disorder is genetically transmitted familial (primary) hemophagocytic lymphohistiocytosis (HLH), which is characterized by high fever, hepatosplenomegaly, cytopenia, hyperferritinemia, hypertriglyceridemia and/or hypofibrinogenemia, a high level of the alpha chain of soluble interleukin-2 (sCD25), low natural killer (NK) cell activity, and hemophagocytosis in bone marrow, cerebrospinal fluid (CSF), or lymph nodes [1]. Mutations in 4 different genes, namely perforin on chromosome 10q21, munc 13-4 on 17q25, syntaxin 11 on 6q24, and syntaxin-binding protein 2 (STXBP2) on 19p13.2 are reported to be responsible for familial HLH [2-5].

Another hemophagocytosis-related disorder is acquired (secondary) HLH (AHLH), which is related to all of the above criteria of the Histiocyte Society, except for a genetic defect in 1 of the familial HLH-associated genes. AHLH is commonly associated with various etiologic agents, such as infectious agents-particularly viruses-malignancy, and autoimmune diseases such as juvenile rheumatoid arthritis [6-9]; however, why some patients with an underlying disorder develop AHLH and others don't in the presence of the same etiological factor is not known and requires further investigation.

Recently, 272C>T nucleotide changes in exon 2 of the perforin gene, which leads to alanine 91 valine (A91V) amino acid substitution, was described as a polymorphism because of its high incidence rate, which varies from 3% to 17% in healthy populations [10,11]. Subsequently, it was reported that A91V causes conformational changes and impairs the process by which perforin protein becomes the active form [12,13]. A91V is reported to be associated with mutations in the perforin and munc 13-4 genes [14]. In addition, it was suggested that homozygous A91V transition is a cause of infection-induced FHL, and it was also suggested that the transition is a predisposing factor in such disorders as autoimmune lymphoproliferative syndrome-a childhood acute lymphoblastic leukemia-and type 1 diabetes [15-19]. As such, it is very likely that A91V plays a role in the pathogenesis of AHLH by reducing the cytotoxicity of cytotoxic T lymphocytes and NK cells [11-13].

On the other hand, it was reported that a number of cytokines are elevated in HLH, such as tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and interleukin (IL)-6, IL-10, IL-12, IL-16, and IL-18. Hypercytokinemia results from uncontrolled activa-

tion of histiocytes and T cells [8]. Clinical and laboratory findings of HLH were reported to be due to organ infiltration by histiocytes and lymphocytes, as well as hypercytokinemia. Among these cytokines, TNF-α is a major cytokine that plays a critical role in the pathogenesis of inflammatory, malignant, and autoimmune disorders. The C allele of -1031 T>C polymorphism in the TNF-α gene promoter was reported to increase susceptibility to AHLH in a Korean population [20].

AHLH is relatively common in Turkey, but not much is known about its predisposing factors [6-9]. The present study aimed to investigate the frequency of the A91V mutation in healthy controls and to determine the roles of A91V and TNF-α-1031 T>C changes in the pathogenesis of AHLH.

Materials and Methods

Patients and controls

The study included 44 unrelated AHLH patients treated at 2 hospitals (42 from Hacettepe University and 2 from Kırıkkale University) between 2005 and 2009, and 164 healthy controls. The controls were blood bank donor samples that were used following receipt of written informed consent by the donors. Among the patients, 44 (32M/12) fulfilled the Histiocyte Society diagnostic criteria for AHLH. The patients ranged in age from 1 d to 16 years (median age: 2 years). The study protocol was approved by the Hacettepe University Ethics Committee (approval number: TBK 05/19-25) and written informed consent was obtained from all the participants.

Genotyping

Genomic DNA was isolated from EDTA-preserved peripheral venous blood cells using automatic isolation methods (MagnaPure Large Volume Nucleic Asit Isolation Kit, Roche). Individuals were genotyped for A91V transition using custom-designed probes for the LightCyclerTM instrument (Roche Applied Sciences, Mannheim, Germany), and for the TNF-α-1031.

T>C polymorphism using the 5' nuclease polymerase chain reaction (TIBMOLBIOL, Germany), as previously described [21]. Statistical analyses were performed using Fisher's exact and chi-square tests.

Results

The etiologic agents in the AHLH patients are shown in Table 1. In all, 3 patients had skin eruption, fever, and lymphocyte predominance in peripheral blood. These patients were accepted as having unknown viral infections. There was first-degree consanguinity between the parents of 15 patients and the parents of 3 other patients were from the same village, suggesting possible consanguinity; therefore, the families were further analyzed via haplotype analysis for HLH mutations, including perforin, munc 13-4, and syntaxin 11 genes, and none had haplotype homozygosity for these genes.

A91V status of the patients

Among the 159 healthy controls, 7 (4.4%) had A91V transition (Table 2). The controls that had the

Table 1. Etiologic agents and perforin A91V transition in the AHLH patients

Underlying Disease	Number of Patients	A91V mutation positive
EBV infection	6	2
Leukemia	5	
Autoimmune disease	4	
Combined *E. coli* and *Pseudomonas aeruginosa* sepsis	3	1
Pneumonia	3	1
Respiratory tract infection	3	
Sepsis (*Streptococcus viridians*)	1	
MAS	2	
CMV	2	
HHV-6	1	
Parvovirus B19	1	
Brucella	1	
Salmonella	1	
Unknown-possible viral infection	3	1
Metabolic disorder*	3	
Hepatitis of unknown origin	1	
Inflammatory bowel disease	1	
Langerhans cell histiocytosis	1	
Myelodysplastic syndrome	1	
Neonatal hemochromatosis	1	

EBV: Epstein-Barr virus; MAS: macrophage activation syndrome; HHV-6: human herpes virus 6; CMV: cytomegalovirus; JMML: juvenile myelomonocytic leukemia
*The underlying metabolic disorders were glycogen storage disease, Gaucher disease, and propionic acidemia

A91V mutation were confirmed to be free of any disease at the time the results were obtained. In all, 5 (11.3%) of the AHLH patients were heterozygous for the A91V transition (Table 2). The difference in the frequency of the transition between the AHLH and control groups was not statistically significant (Fisher's exact test, p=0.090), although it was strikingly higher in the AHLH group (odds ratio: 2.8). Among the 5 patients with A91V, 2 had infectious mononucleosis, 1 had *Escherichia coli* and *P. aeruginosa* sepsis, 1 had pneumonia, and 1 had a viral infection of unknown origin (Table 1).

TNFα promoter-1031 T>C polymorphism

TNF-α-1031 T>C polymorphism findings are given in Table 2. Among the 164 controls, 7 (4.3%) had homozygous risk of elevated CC and 58 (35.4%) had heterozygous TC genotypes. Among the 40 AHLH patients examined for the polymorphism, 1 (4%) had the CC genotype and 16 (40%) had the TC genotype. Among the 164 controls that were analyzed for TNF polymorphisms and the 40 patients whose DNA was available for analysis, there wasn't a statistically significant difference between allele status (p=0.78). The frequency of C and T alleles was 22.5% (n=18) and 77.5% (n=62) among the AHLH patients, and 22% (n=72) and 78% (n=259) among the controls, respectively. There wasn't a statistically significant difference between groups in terms of T and C allele frequencies (p>0.05).

Discussion

The present study obtained initial data on the frequency of A91V in Turkey (4.4%), indicating that it was quite common in the controls, as previously reported [10,15]. In the present study 5 (11.3%) AHLH patients with infection had A91V in the heterozygote state, suggesting that the transition may be a predisposing factor for infection-associated AHLH; the difference between the 2 groups was not statistically significant. Nonetheless, the difference between the AHLH patients and healthy controls in terms of A91V status was striking (odds ratio: 2.8); this problem might be overcome by increasing the number of participants in future studies. Among the 5 patients that had A91V, 2 had severe EBV infection, 1 had neonatal sepsis due to *E. coli* and *P. aeruginosa*, 1 had severe pneumonia, and 1 had a probable viral infection of unknown origin, which may indicate the additive effect of carrier state and infections in the development of HLH. Although the number of patients in the present study was small, the statistically non-significant but higher rate of A91V in the AHLH patients suggests that especially in the presence of infection as a triggering factor A91V transition may play a predisposing role in the emergence of full-blown AHLH. Additionally, the present results would have been more useful had perforin expression analysis been performed in all of the patients and controls.

TNF-α promoter-1031 T>C polymorphism, which is reported to have a significant affect on transcription, was not observed to increase the risk of AHLH in the present study. A study on patients with AHLH reported that this cytokine polymorphism and the TNFα-1031 C allele increased the risk of AHLH [19]. Discrepancies between the reported results may be due to differences in the genetic pools of the study populations included in these 2 studies. Additional research with different populations and larger patient cohorts will help in elucidating the contribution of this polymorphism to the development of AHLH.

In conclusion, the prevalence of A91V was relatively high in the present study. The rate of A91V transition was 4.4% among the controls and 11.3% among the AHLH patients; the difference was not statistically significant, but the incidence of A91V transition was approximately 3-fold higher in the patients according to the odds ratio. TNF-α polymorphisms did not contribute to AHLH according to the present findings. The presence of A91V might contribute to the development of AHLH, especially in

Table 2. Distribution of perforin gene A91V transition, and TNF-α promoter-1031 T>C polymorphism genotypes and alleles

Group	Perforin A91V		TNF-α-1031 T>C					
				Genotype			Allele	
	Total Studied (n)	A91V+ n (%)	Total Studied (n)	CC n (%)	TC n (%)	TT n (%)	C n (%)	T n (%)
AHLH	44	5 (11.3)	40	1 (2.5)	16 (40)	23 (57.5)	18 (22.5)	62 (77.5)
Control	159	7 (4.4)	164	7 (4.3)	58 (35.4)	99 (60.3)	72 (22)	256 (78)

the presence of severe concomitant infection; therefore, A91V transition should be screened in patients-particularly those with infection-induced AHLH-to ensure that patients are closely monitored. Additional research with larger AHLH patient groups is needed to more clearly delineate the role of A91V transition in AHLH.

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Electronic Database Information

Accession numbers and URLs for data presented in the study are as follows:

GenBank: http://www.ncbi.nih.gov/Genbank.

PRF1 human: [NM005041]

Online Mendelian Inheritance in Man (OMIM): http://www.ncbi.nlm.nih.gov/Omim.

Familial hemophagocytic lymphohistiocytosis numbers: OMIM #267700 and OMIM #603553.

Conflict of interest statement

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

References

1. Henter JI, Horne A, Aricó M, Egeler RM, Filipovich AH, Imashuku S, Ladisch S, McClain K, Webb D, Winiarski J, Janka G. HLH-2004: diagnostic and therapeutic guidelines for hemophagocytic lymphohistiocytosis. Pediatr Blood Cancer 2007;48:124-31. [CrossRef]
2. Stepp SE, Dufourcq-Lagelouse R, Le Deist F, Bhawan S, Certain S, Mathew PA, Henter JI, Bennett M, Fischer A, de Saint Basile G, Kumar V. Perforin gene defects in familial haemophagocytic lymphohistiocytosis. Science 1999;286:1957-9.
3. Feldmann J, Callebaut I, Raposo G, Certain S, Bacq D, Dumont C, Lambert N, Ouachée-Chardin M, Chedeville G, Tamary H, Minard-Colin V, Vilmer E, Blanche S, Le Deist F, Fischer A, de Saint Basile G. Munc13-4 is essential for cytolytic granules fusion and is mutated in a form of familial hemophagocytic lymphohistiocytosis (FHL3). Cell. 2003;115:461-73. [CrossRef]
4. zur Stadt U, Schmidt S, Kasper B, Beutel K, Diler AS, Henter JI, Kabisch H, Schneppenheim R, Nürnberg P, Janka G, Hennies HC. Linkage of familial hemophagocytic lymphohistiocytosis (FHL) type-4 to chromosome 6q24 and identification of mutations in syntaxin 11. Hum Mol Genet. 2005;14:827-34. [CrossRef]
5. Côte M, Ménager MM, Burgess A, Mahlaoui N, Picard C, Schaffner C, Al-Manjomi F, Al-Harbi M, Alangari A, Le Deist F, Gennery AR, Prince N, Cariou A, Nitschke P, Blank U, El-Ghazali G, Ménasché G, Latour S, Fischer A, de Saint Basile G. Munc 18-2 deficiency causes familial hemophagocytic lymphohistiocytosis type 5 and impairs cytotoxic granule exocytosis in patient NK cells. J Clin Invest 2009;119:3765-73. [CrossRef]
6. Gurgey A, Secmeer G, Tavil B, Ceyhan M, Kuskonmaz B, Cengiz B, Ozen H, Kara A, Cetin M, Gumruk F. Secondary hemophagocytic lymphohistiocytosis in Turkish children. Pediatr Infect Dis J. 2005;24:1116-7. [CrossRef]
7. Rouphael NG, Talati NJ, Vaughan C, Cunningham K, Moreira R, Gould C. Infection associated with haemophagocytic lymphohistiocytosis. Lancet 2007;7:814-22. [CrossRef]
8. Janka GE. Familial and acquired hemophagocytic lymphohistiocytosis. Eur J Pediatr 2007;166:95-109.
9. Oren H, Gulen H, Ucar C, Duman M, Irken G. Successful treatment of infection associated hemophagocytosis syndrome with intravenous immunoglobulin. Turk J Hematol 2003;20:95-9.
10. Zur Stadt U, Beutel K, Weber B, Kabisch H, Schneppenheim R, Janka G. A91V is a polymorphism in the Perforin gene not causative of FHLH phenotype. Blood 2004;104:1909-10. [CrossRef]
11. Molleran Lee S, Villanueva J, Sumegi J, Zhang K, Kogawa K, Davis J, Filipovich AH. Characterisation of diverse PRF1 mutations leading to decreased natural killer cell activity in North American families with haemophagocytic lymphohistiocytosis. J Med Genet 2004;41:137-44. [CrossRef]
12. Trambas C, Gallo F, Pende D, Marcenaro S, Moretta L, De Fusco C, Santoro A, Notarangelo L, Arico M, Griffiths GM. A single amino acid change, A91V, leads to conformational changes that can impair processing to the active form of perforin. Blood 2005;106:932-7. [CrossRef]
13. Risma K, Frayer R, Filipovich A, Sumegi J. Aberrant maturation of mutant Perforin underlies the clinical diversity of hemophagocytic lymphohistiocytosis. J Clin Invest 2006;116:182-92.
14. Zhang K, Johnson JA, Biroschak J, Villanueva J, Lee SM, Bleesing JJ, Risma KA, Wenstrup RJ, Filipovich AH. Familial haemophagocytic lymphohistiocytosis in patients who are heterozygous for the A91V perforin variation is often associated with other genetic defects. Intern J Immunogenet 2007;34:231-3. [CrossRef]
15. Mancebo E, Allende LM, Guzmán M, Paz-Artal E, Gil J, Urrea-Moreno R, Fernández-Cruz E, Gayà A, Calvo J, Arbós A, Durán MA, Canet R, Balanzat J, Udina MA, Vercher FJ. Familial hemophagocytic lymphohistiocytosis in an adult patient homozygous for A91V in the Perforin gene with tuberculosis. Haematologica 2006;91:1257-60.

16. Santoro A, Cannella S, Trizzino A, Lo Nigro L, Corsello G, Arico MA. A single amino acid change A91V in Perforin: a novel, frequent predisposing factor to childhood acute lymphoblastic leukemia? Haematologica 2005;90:697-8.
17. Mehta PA, Davies SM, Kumar A, Devidas M, Lee S, Zamzow T, Elliott J, Villanueva J, Pullen J, Zewge Y, Filipovich A; Children's Oncology Group. Perforin polymorphism A91V and susceptibility to B-precursor childhood acute lymphoblastic leukemia: a report from the Children's Oncology group. Leukemia 2006;20:1539-41. [CrossRef]
18. Clementi R, Chiocchetti A, Cappellano G, Cerutti E, Ferretti M, Orilieri E, Dianzani I, Ferrarini M, Bregni M, Danesino C, Bozzi V, Putti MC, Cerutti F, Cometa A, Locatelli F, Maccario R, Ramenghi U, Dianzani U. Variation of the Perforin gene patients with autoimmunity/lymphoproliferation and defective Fas function. Blood 2006;108:3079-84. [CrossRef]
19. Orilieri E, Cappellano G, Clementi R, Cometa A, Ferretti M, Cerutti E, Cadario F, Martinetti M, Larizza D, Calcaterra V, D'Annunzio G, Lorini R, Cerutti F, Bruno G, Chiocchetti A, Dianzani U. Variations of the Perforin gene in patients with type 1 diabetes. Diabetes 2008;57:1078-83. [CrossRef]
20. Chang YH, Lee DS, Jo HS, Cho SI, Yoon HJ, Shin S, Yoon JH, Kim HY, Hong YJ, Hong SI, Cho HI. Tumor necrosis factor alpha promoter polymorphism associated with increased susceptibility to secondary hemophagocytic lymphohistiocytosis in the Korean population. Cytokine 2006;36:45-50. [CrossRef]
21. di Giovine FS, Camp NJ, Cox A, et al. Detection and population analysis of IL-1 and TNF gene polymorphisms. In Cytokine Molecular Biology: A Practical Approach. Blackwill F, editor. Oxford: Oxford University Press. 2000;21-46.
22. Karapınar B, Yılmaz D, Aydınok Y, Türkoğlu E, Hekimgil M, Kavaklı K. Intense myelofibrosis in a child: unusual result of EBV-associated hemophagocytic lymphohistiocytosis. Turk J Hematol 2007; 24:32-5.