Available online at www.sciencedirect.com
ScienceDirect
Leukemia Research 32 (2008) 972–975
Leukemia Research
www.elsevier.com/locate/leukres

Clinical and molecular aspects of Turkish familial hemophagocytic lymphohistiocytosis patients with perforin mutations

Hamza Okur [a], Gunay Balta [a], Nurten Akarsu [a], Ahmet Oner [b], Turkan Patiroglu [c], Ali Bay [b], Tulin Sayli [d], Sule Unal [a], Aytemiz Gurgey [a,*]

[a] *Hacettepe University, Faculty of Medicine, Department of Pediatrics, Division of Pediatric Hematology, 06100-Sihhiye, Ankara, Turkey*
[b] *Yuzuncu Yil University, Faculty of Medicine, Division of Hematology, Van, Turkey*
[c] *Erciyes University, Division of Pediatric Hematology, Kayseri, Turkey*
[d] *SSK Ankara Children's Training Hospital, Ankara, Turkey*

Received 28 July 2007; received in revised form 25 November 2007; accepted 26 November 2007
Available online 10 January 2008

Abstract

The aim of this study was to elucidate the pathologic sequence changes and associated clinical phenotypes in 9 new patients showing homozygosity for perforin gene among a total of 37 (24%) Turkish FHL families studied by linkage analysis. These 9 unrelated patients (5M/4F) were coming from consanguineous families and their presentation ages of systemic symptoms were ranged from birth to 15 years. Direct sequencing of coding exons of the perforin gene led to the identification of five different homozygous alterations. The nonsense W374X mutation was identified in three patients while four different missense mutations namely G149S, V50M, A91V and novel A523D were detected in the rest six patients.

Keywords: FHL; Perforin gene; Mutations; Clinical presentations

1. Introduction

Hemophagocytic lymphohistiocytosis (HLH) may present on the basis of genetic and various acquired disorders. Familial hemophagocytic lymphohistiocytosis (FHL) is a rare, life threatening autosomal recessive disorder characterized by fever, cytopenias and hepatosplenomegaly, hyperferritinemia, hypertriglyceridemia and/or hypofibrinogenemia, a high level of α chain of the soluble interleukin 2 receptor (sCD25), hemophagocytosis in the bone marrow, cerebrospinal fluid or lymph nodes [1,2]. It is usually a fatal disorder during infancy or early childhood unless hematopoetic stem cell transplantation is performed [3]. The symptoms are caused by uncontrolled activation of T cells and macrophages and overproduction of inflammatory cytokines.

Recently, mutations of perforin (FHL-2), Munc 13-4 (FHL-3) and Syntaxin 11 (FHL-4) gene have been described in FHL [4–6]. In 1999, Dufourcq-Lagelouse et al. mapped a locus for FLH-2 to 10q21-q22 in a genetic linkage study [4]. In the same year, Stepp et al. described mutations in perforin gene located in this chromosomal region [7]. The perforin gene has three exons and its coding sequence is located in exon 2 and exon 3 [8]. Perforin plays an important role in the cytolytic process and is produced as a precursor form that is subsequently cleaved at the C terminus to produce an active mature form. Patients with perforin mutation have absent or low levels of perforin in natural killer (NK) cells [9]. Perforin deficiency usually causes a fatal disorder in patients with FHL-2 and more than 50 mutations have been described in the perforin coding region [10].

Familial and acquired forms of HLH are considerably common in Turkish population, probably due to frequent

* Corresponding author at: Hacettepe University, Faculty of Medicine, Department of Pediatrics, Division of Pediatric Hematology, Talatpasa Blvd., 06100-Sihhiye, Ankara, Turkey. Tel.: +90 312 305 11 72; fax: +90 312 305 42 08/310 57 00.
E-mail address: agurgey@hacettepe.edu.tr (A. Gurgey).

0145-2126/$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.leukres.2007.11.033

consanguineous marriages and high incidence of infections [11–13]. The aim of this study is to present the molecular defects and associated clinical phenotypes of nine new FHL patients showing homozygosity for the perforin gene.

2. Materials and methods

Thirty-seven unrelated children (21M/16F) were diagnosed as having primary HLH. Age at diagnosis ranged from 7 days to 16 years. Parental consanguinity was present in 33 and family history of the disease in 18 patients. All patients met the diagnostic criteria of HLH described previously [1]. Blood samples were obtained from the patients and relevant family members by written informed consent. The Ethics Committee of Hacettepe University approved the study (approval number: TBK 05/19-25).

DNA was extracted from peripheral blood using standard protocols. A total of four polymorphic DNA markers (D10S529, D10S676, D10S537 and D10S1685) tightly linked to the perforin gene were genotyped to test for homozygosity. Affected children demonstrating homozygosity were then subjected to sequence analysis (ABI 310 Applied Biosystems, Foster City California USA) by using primers: PRF2-F5′CCTTCCATGTGCCCTGATAA3′/R5′GCCAGGATTGCAGTTTCTTC3′; PRF3.1-F5′CATGTGACCTTGAGCAGTCC3′/R-5′ACACACTGGCATGGGTCTC3′; PRF3.2-F5′ACTCAGCCTGGGTAAACTCG3′/R-5′TGGACAAGCTTGGTCTAATGG3′.

3. Results

In the present study, homozygosity for perforin gene was observed in 9 families among the 37 primary HLH patients (24%) studied by linkage analysis. Direct DNA sequence analysis of the gene led to the identification of five different homozygous aberrations in these nine patients. Three patients (33%) carried the same nonsense W374X mutation in exon 3. Remaining six patients (67%) were found to have four different missense variations. Among these missense changes, G149S was detected in two, V50M in one, A91V in two patients. Parents of all families were heterozygous for the respective mutations. Linkage analysis in all respective families failed to identify homozygosity for Munc13-4 and Syntaxin 11 genes except one patient. In addition, a novel homozygous 1568 C to A substitution in exon 3 led to the replacement of alanin with aspartic acid at codon 523 (A523D) in one patient. The results of the linkage analysis of the respective family revealed that homozygosity was present only for the perforin gene but not for the Munc13-4 and Syntaxin 11 genes. One of the asymptomatic siblings was also homozygous for this substitution. The screening of the close relatives and at least 30 residents of the same village failed to identify this alteration in homozygous state while parents and three individuals were found to have this change in heterozygous state.

The clinical, laboratory and molecular characteristics of nine patients with perforin mutations are summarized in Table 1. The median age of the nine patients was 9.7 and 10 months at presentation (range: birth to 15 years) and at diagnosis (range: 7 days to 16 years), respectively. All patients had typical systemic findings for FHL including high fever, splenomegaly, bicytopenia or pancytopenia, hypertriglyceridemia. In addition, all cases also had hyperferritinemia except case 3 who had associated iron deficiency anemia without elevated ferritin levels. The patients with the nonsense W374X mutation had rather severe presenting symptoms. Presenting symptoms in cases with missense mutations, however, were variable in severity. For instance, the cases 6 and 7 carrying V50M and A91V mutations, respectively had a very late onset with initial presentation of central nervous system involvement in the absence of any finding of systemic symptoms. Therefore, they had been misdiagnosed as having neurological disorders called acute disseminated encephalomyelitis (ADEM) and lymphomatoid granulomatosis. They were diagnosed as HLH when systemic presenting symptoms have emerged 3 months and 1 year later (Table 1). Case 8 with homozygous A91V mutation was symptomatic for HLH at the age of 11 years and 9 months. Like two patients of this study, central nervous system involvement had led to the diagnosis of ADEM in his deceased sister. Case 9 with a novel A523D transition had family history of 5 deceased siblings. She was presented with fever, hepatosplenomegaly and pancytopenia, lymphadenopathy (Table 1).

4. Discussion

Because of the fact that FHL is quite common in Turkish population, identification of the molecular pathologies of the FHL patients has prime importance in this population. In the current study, 9 patients (24%) among the 37 Turkish FHL patients were assigned to FHL type 2 by linkage analysis. Genomic sequencing of the entire coding region of perforin gene led to the identification of five different pathologic changes in these patients. Among those, three patients (33%) were found to have W374X mutation. In a previously reported two studies, on the other hand, perforin mutations were identified in 30% and 44% of Turkish FHL patients [14,15]. Of those, 67% and 86% had W374X mutation. Overall these three studies indicated that perforin mutation incidence is relatively similar ranging from 24% to 44% and that W374X mutation is the most common type of perforin mutation in Turkish population. However, there is a remarkable difference between the frequencies of W374X mutation being 33% in the present and 67–86% in the previous studies [14,15]. This difference may be attributed to the fact that all of the patients diagnosed as FHL were analyzed in the present study; however, there may be a selection bias