Severe Course of Community-Acquired Pneumonia in an Adult Patient Who Is Heterozygous for Q481P in the Perforin Gene: Are Carriers of the Mutation Free of Risk?

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Abstract

Most cases of autosomal recessive hemophagocytic lymphohistiocytosis (HLH) are associated with over 50 mutations in the perforin gene. Some of these mutations have no clear functional association. Only homozygous patients display a full-blown syndrome, whereas no severe disease has been described in heterozygous carriers of these mutations despite the presence of functional and phenotypic alterations in cytotoxic cells. We study the family of a child who died from HLH at 6 months of age due to a Q481P mutation in the perforin gene. The study is particularly interesting because the patient's heterozygous father experienced severe community-acquired pneumonia that could be attributed to deficient in vitro NK cell activity despite normal perforin expression. This case report suggests that impaired NK cell activity in a heterozygote can result in poorer initial control of infections with severe clinical expression.

Key words: Hereditary hemophagocytic lymphohistiocytosis. NK cells. Perforin. Q481P.

Resumen

La mayoría de los casos de linfohistiocitosis hemofagocítica autosómica recesiva (HLH) se asocian con algo más de 50 mutaciones en el gen de la perforina, algunas de ellas sin clara asociación funcional. Sólo los individuos homocigotos sufren un síndrome completo mientras que no se han descrito enfermedades graves en los heterocigotos, a pesar de mostrar alteraciones funcionales y fenotípicas de las células citotóxicas. Presentamos un estudio familiar a partir de una niña que murió a los 6 meses de edad por HLH secundaria a la mutación Q481P en el gen de la perforina. El padre heterocigoto para la mutación sufrió una neumonía extrahospitalaria grave que podría ser atribuida a la función deficiente de las células NK demostrada in vitro, a pesar de la expresión normal de perforina. Este caso clínico sugiere que una actividad deficiente de las células NK en un sujeto portador puede provocar un peor control temprano de las infecciones con una expresión clínica agresiva.

Palabras clave: Linfohistiocitosis hemofagocítica hereditaria. Células NK. Perforina. Q481P.

Introduction

Familial hemophagocytic lymphohistiocytosis (FHL) is the primary form of hemophagocytic lymphohistiocytosis (HLH). It is an autosomal recessive disease with an estimated incidence of 1 case/50,000 births [1-5]. This life-threatening condition is characterized by uncontrolled cellular activation with overproduction of inflammatory cytokines [6,7] and impaired function of natural killer (NK) cells and cytotoxic T cells [8].

The primary form of FHL is genetically heterogeneous [9] and involves several independent loci [6]. Perforin 1 (*PRF1*) was the first gene identified as causing FHL. Over 50 mutations have been identified. Most are located at exons 2 and 3 [10,11], and are the cause of the disease in approximately 20% to 40% of cases [12]. Only patients who are homozygous for the mutations develop the disease. Heterozygous patients may display disturbances in the phenotype and/or function of NK and cytotoxic T cells [13].

Perforin is a critical effector of cytotoxicity, since it is a soluble pore-forming cytolytic protein [14]. Impairment in its activity renders the immune system unable to kill infected target cells and leads to the clinical manifestations that are characteristic of FHL [12].

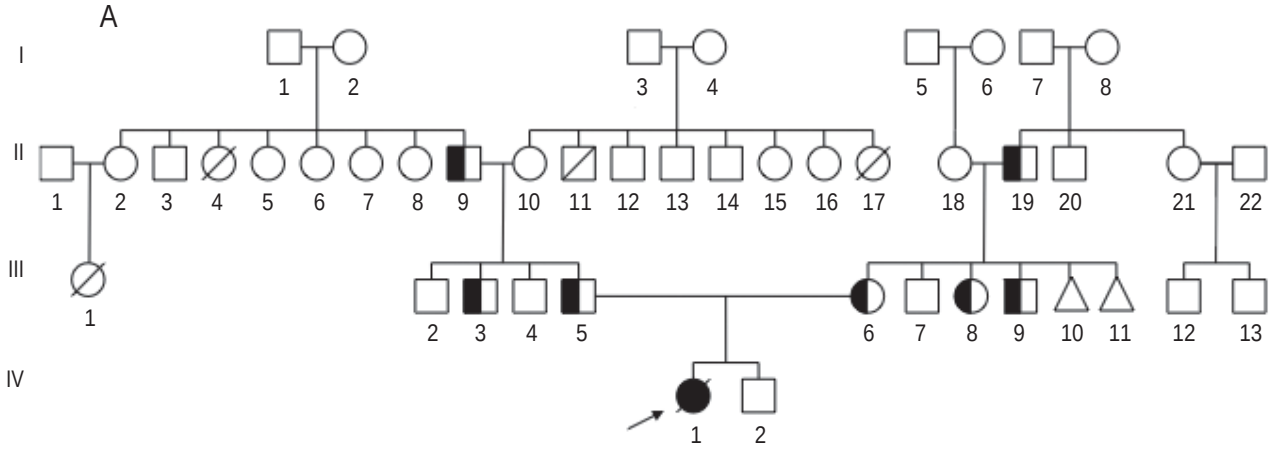
We report the case of a 6-month-old girl diagnosed with HLH associated with a homozygous mutation in *PRF1*. We also present the case of her father, who, to our knowledge, is the first heterozygous carrier of a mutation in the *PRF1* gene to experience a severe infectious episode.

Case Description

Our patient was the first child of nonrelated healthy parents. Her maternal grandmother had 2 miscarriages and 1 premature birth. Her paternal grandmother's sister and grandfather's sister and second-degree cousin died within the first year of life from an undefined disease (Figure 1).

Pregnancy and delivery were normal, and the patient had always been healthy. She was admitted to the intensive care unit at 6 months of age with a 3-day history of

A



B

Family members	Q481P	Perforin expression (%) CD8⁺ Cells	NK Cells
II 9	Heterozygous	18	89
II 10	No mutation	45	94
II 18	No mutation	46	89
II 19	Heterozygous	25	91
II 21	No mutation	30	91
III 2	No mutation	34	93
III 3	Heterozygous	32	88
III 4	No mutation	53	90
III 5	Heterozygous	23	83
III 6	Heterozygous	2	88
III 7	No mutation	3	85
III 8	Heterozygous	3	90
III 9	Heterozygous	14	86
IV 1	Homozygous	ND	ND
Normal values		8-35	82-93

Figure 1. A, Family pedigree and detection of the Q481P mutation. The patient was homozygous (full black) for the Q481P mutation at the perforin locus. The same mutation was identified as heterozygous (half-black) in her parents and some of the family members studied. B, Presence of the Q481P mutation in the perforin gene and expression of perforin in $CD3^+CD8^+$ and NK cells in the family members analyzed. Normal values were established by analysis of samples from 40 blood donors. ND indicates not determined.

fever and hepatosplenomegaly. Laboratory tests (Table) showed thrombocytopenia, anemia, hypofibrinogenemia, and progressive hepatic failure with severe alteration of coagulation. Infectious agents such as Epstein-Barr virus (EBV), cytomegalovirus, hepatitis B virus, and human immunodeficiency virus were ruled out as the primary cause of the illness. Culture of cerebrospinal fluid, blood, and urine gave negative results.

Culture of bone marrow aspirate was normal except for an increase in the macrophagic reticular cell count and a slight increase in the rate of erythropoiesis accompanied by dyserythropoiesis. A lymph node biopsy was consistent with hemophagocytosis, and revealed signs of cellular activation ($Ki67^+$) with an increased $CD3^+$ T cell count and depletion of B cells. The diagnosis of HLH was based on these findings.

Treatment with etoposide and dexamethasone was initiated. Daily transfusions of platelets and fresh plasma were also necessary. The patient received antimicrobial therapy with ceftriaxone, erythromycin, fluconazole, and ganciclovir. One week later, she died of multiple organ failure (Table) and disseminated infection by *Aspergillus fumigatus*.

Histopathology samples taken at autopsy confirmed the diagnosis of HLH, revealing infiltrating activated macrophages with hemophagocytosis in lymph nodes, spleen, bone marrow, and liver. Perforin expression and NK cell cytotoxicity could not be studied, since viable cells were not available because of rapid progression of the disease. The mutation analysis was performed in a DNA sample extracted from frozen liver tissue. It disclosed a homozygotic polymorphism (H300H) not associated with HLH and a homozygotic mutation of *PRF1* (A>C) at nucleotide 1442 changing the glutamine at position 481 to proline (Q481P). This change could have rendered the perforin labile or inactive [8], with gross protein misfolding and absence of lytic function. Such a mutation has been associated with early onset of the disease [11].

The patient's father was 36 years old and had always been healthy. Two years after the patient died, he had community-acquired pneumonia caused by *Streptococcus pneumoniae* with a rapid and severe course that required admission to hospital. He presented secondary bacteremia with a poor response to the antibiotics he was sensitive to in the antibiogram, and a systemic inflammatory response. Laboratory tests revealed leukopenia

Table. Hematologic and Biochemical Parameters During Admission

Variable	Admission	Day 3	Day 7	Reference Range
Hematocrit, %	21.6	28.4	26.8	33-35
Erythrocytes, $\times 10^6$ µL	2.83	3.3	3.1	4.1-5.1
White-cell count, $\times 10^3$ µL	10	3.4	3.8	6-17
Differential count, %				
Neutrophils	15	15.6	57.0	18-30
Lymphocytes	60	69.2	28.0	50-70
Monocytes	20	14.3	1.0	0-13
Platelet count, $\times 10^3$ µL	20	25	29	150-450
Mean corpuscular volume, fL	78.3	84.8	86	70-85
Prothrombin time, sec	21.10	00	00	10.1-15.4
Partial thromboplastin time, sec	69		00	25.3-48.3
Prothrombin activity, %	45	35	<10	90-100
Fibrinogen, mg/dL		65	ND	200-400
Hemoglobin, g/dL	7.4	10.2	9.5	12-15
Glucose, mg/dL	57	80	379	65-110
Conjugated bilirubin, mg/dL	3.9		3.3	0.01-0.25
Sodium, nEq/L	131	140	139	137-145
Potassium, nEq/L	5.4	2.6	4.4	3.5-5
Chloride, nEq/L	105	104	106	80-120
AST, nEq/L	235	404	866	2-85
ALT, U/L	167	128	149	2-58
GGT	275	244	248	7-32
Lactate dehydrogenase, U/L	843	2004	2728	460-1380
Alkaline phosphatase, U/L	225	171	188	58-522
Total bilirubin, mg/dL	3.3	9.6	4.2	0.1-1
Triglycerides, mg/dL		251	380	30-200
Ferritin, ng/dL			>1000	10-291

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gammaglutamyl transferase; ND, not determined.

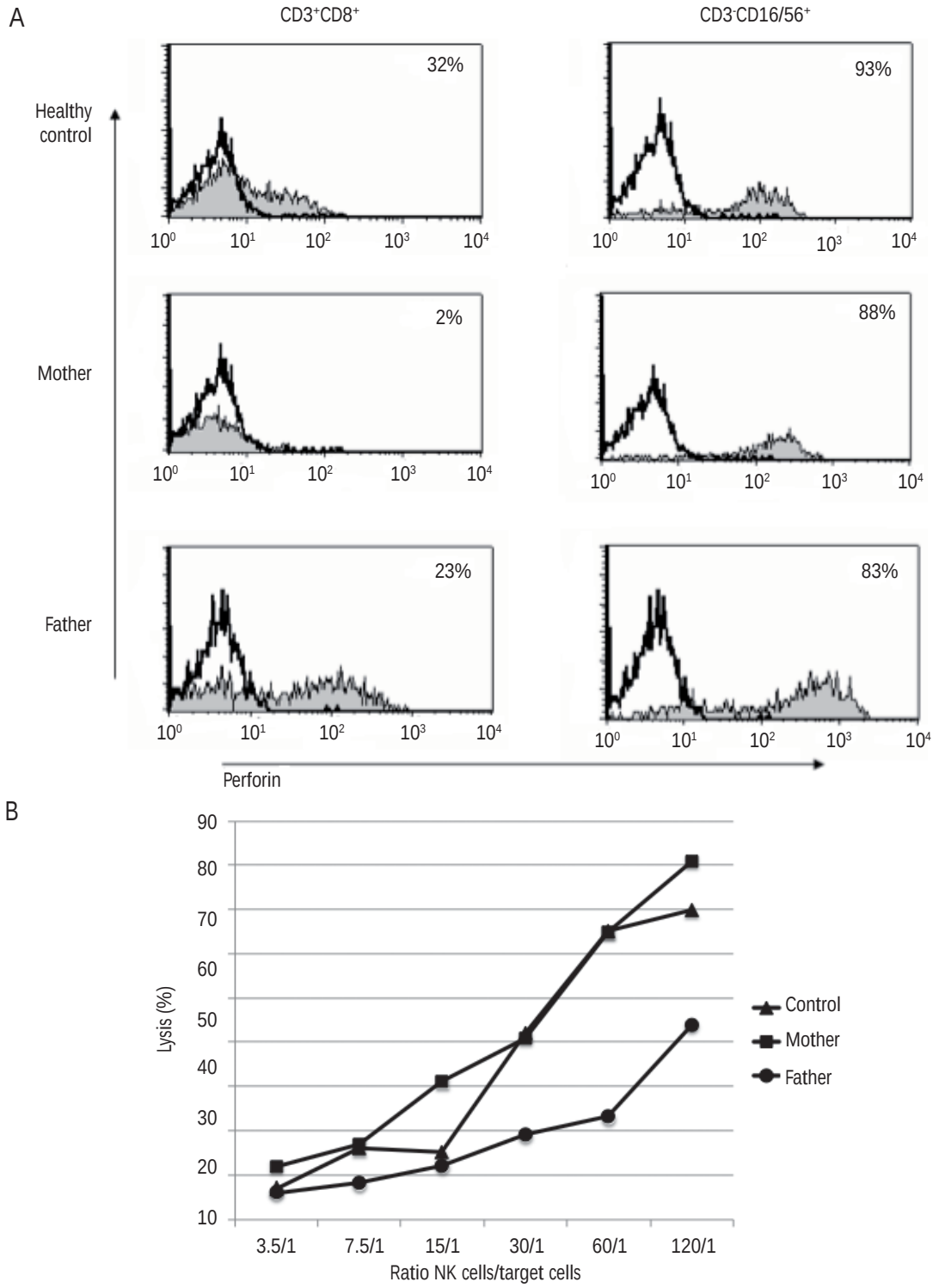


Figure 2. Perforin expression (A) and NK cytotoxicity assays (B) in the parents (heterozygotes). Grey histograms (A) represent perforin expression in the $CD3^+CD8^+$ T cells and $CD3^-CD16/56^+$ NK cells of parents and the percentages of gated cells in each case are indicated in each quadrant. White histograms represent isotype control staining. The mother expressed decreased levels of perforin on $CD3^+CD8^+$ T cells and normal levels on $CD3^-CD16/56^+$ NK cells, whereas perforin expression was normal in the father on both cell subsets. Perforin staining in a healthy individual is shown in the upper histograms as a control. Cytotoxicity activity of NK cells from the parents (B) was assessed at different NK cell/target K562 cell ratios (x-axis). Results are expressed as the percentage of specific lysis (y-axis) as measured by release of sodium chromate Cr 51. The father, but not the mother, proved to have a defective NK cytotoxic capacity in vitro. NK, indicates natural killer.

with a left deviation and a slight alteration of transaminases. Infection by EBV was ruled out, and bone marrow biopsy revealed a normal cell profile. The clinical course reached its peak on the fourth day of intensive antimicrobial therapy and gradually remitted thereafter. He was discharged 12 days after admission. The patient's mother is healthy.

Once the Q481P mutation was detected in the patient, a family study was proposed. All the members studied gave their informed consent for the analyses, as well as for reporting of the results. Screening for the A>C mutation at position 1442 using the ABI prism 3700 Sequence Detection System (PE Biosystems, Foster City, California, USA) was performed on those family members who agreed to it. The primers used were forward 5'-GAGGTGACCTTCATCCAAGCA-3' and reverse 5'-GAACCCTTCAGTCCAAGCATAC-3'. The mutational analysis in *PRF1* showed that the progenitors were carriers of the 1442 A>C mutation. The remaining family members who are heterozygous for the mutation are shown in Figure 1.

Intracellular expression of perforin on peripheral blood NK and cytotoxic T ($CD3^+CD8^+$) cells was also studied using flow cytometry after staining with specific monoclonal antibodies for cell surface molecules (CD3, CD8, CD16/56) together with cell permeabilization and intracellular staining of perforin [2]. Normal expression of perforin on NK cells was observed in all family members, but decreased expression on $CD3^+CD8^+$ was detected in 3 members, 2 of whom were heterozygous and 1 of whom had no mutation (Figure 1).

NK cell cytotoxicity was evaluated in both parents using a classic cytotoxicity assay (K562 cells labeled with sodium chromate Cr 51 [1]). The mother expressed low levels of perforin on $CD3^+CD8^+$ T cells and had normal NK cell activity, whereas the father displayed an impaired NK cell function with normal levels of perforin (Figure 2).

Despite the risk involved, the parents wanted to have more children, and preimplantation genetic testing and in vitro fertilization were performed at a referral center. The fertilization was successful and a healthy child with no mutation was born.

Discussion

Considering the variety of underlying conditions that can lead to HLH, a differential diagnosis was necessary. No signs of albinism were detected and no EBV or other viral or bacterial infection could be proved. Therefore, Griscelli syndrome, Chediak-Higashi syndrome, and the acquired form of HLH were ruled out. Furthermore, the lack of a history of rheumatic or autoimmune disease ruled out macrophage activation syndrome as a possible cause. A presumptive diagnosis of FHL was made, and this was subsequently confirmed by identification of the Q481P mutation in *PRF1*.

We think that the presence of the heterozygous mutation in the father and the small functional deficit that it causes played an important part in the severe symptoms he experienced. To date, variable alteration of perforin expression and in vitro NK cell function has only been reported in carriers of *PRF1* mutations [13], although no associated clinical abnormality has been described. In the case we report, the severe episode of community-acquired pneumonia could be attributed to deficient NK activity. Moreover, there was no proof of any other alteration of the immune system, since the values for leukocyte counts, perforin expression, and activation markers were normal (not shown). The in vitro dysfunction of NK cells could be at least partially responsible for a poor initial response to the infectious challenge. Normally, such a deficiency is subclinical, but in some conditions, or with an associated genetic background, it could lead to clinical manifestations. NK cells are part of innate immunity and their function is to provide a rapid initial response while the adaptive immune response is adjusting to the antigen. Heterozygosity could alter maturation of perforin and lead to the residual NK function [10]. It could also generate a borderline scenario in terms of cytotoxic competence. This is a hypothetical explanation, and the severe pneumonia could be a casual finding, although, to our knowledge, no severe infectious diseases have been described in relatives of patients with HLH.

The mother and 2 of the mother's brothers presented impaired expression of perforin on $CD3^+CD8^+$ cells, with normal expression on NK cells and preserved NK cytotoxicity, although there have been no clinical manifestations to date. Curiously, one brother with altered expression of perforin in $CD3^+CD8^+$ displayed no mutations. This alteration may not be associated with the mutation, and another genetic defect might be responsible for altered expression of perforin in the family. Indeed, a number of missense mutations in *PRF1* have been described and associated with diverse instability in perforin, which undergoes many posttransductional modifications [8].

In summary, carriers of a mutation in *PRF1* have been considered to be free from severe disease; however, our results support the hypothesis that *PRF1* mutations may have important clinical consequences for heterozygotes.

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References

1. 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 Gen. 2004;41:137-44.
2. Clementi R. Emmi L, Maccario R, Liotta F, Moretta L, Danesino C, Aricó M. Adult onset and atypical presentation of hemophagocytic lymphohistiocytosis in siblings carrying PRF1 mutations. Blood. 2002;100:2266-7.
3. Ménasché G. Feldman J, Fischer A, de Saint Basile G. Primary hemophagocytic syndromes point to a direct link between lymphocyte cytotoxicity and homeostasis. Immunol Rev. 2005;203:165-79.
4. Ueda I, Kurokawa Y, Koike K, Ito S, Sakata A, Matsumora T, Fukushima T, Morimoto A, Ishii E, Imashuku S. Late-onset cases of familial hemophagocytic lymphohistiocytosis with missense perforin gene mutations. Am J Hematol. 2007;82:427-32.

5. Mancebo E, Allende LM, Guzman M, Paz-Artal E, Gil J, Urrea-Moreno R, Fernández-Cruz E, Gayá 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 infection. Haematologica. 2006;91:1257-60.
6. Janka GE, Schneider EM. Modern management of children with haemophagocytic lymphohistiocytosis. Br J Haematol. 2004;124:4-14.
7. Schneider EM, Lorenz I, Müller-Rosenberger M, Steinbach G, Kron M, Janka-Schaub GF. Hemophagocytic lymphohistiocytosis is associated with deficiencies of cellular cytolysis but normal expression of transcripts relevant to killer-cell-induced apoptosis. Blood. 2002;100:2891-8.
8. Voskoboinik I, Thia M-C, Trapani JA. A functional analysis of the putative polymorphisms A91V and N252S and 22 missense perforin mutations associated with familial hemophagocytic lymphohistiocytosis. Blood. 2005;105:4700-6.
9. Filipovich AH. Hemophagocytic lymphohistiocytosis and related disorders. Curr Opin Allergy Clin Immunol. 2006;6:410-5.
10. Risma KA, Frayer RW, Filipovich AH, Sumegi J. Aberrant maturation of mutant perforin underlies the clinical diversity of hemophagocytic lymphohistiocytosis. J Clin Invest. 2006;116:182-92.
11. Schneider EM, Lorenz I, Walther P, Janka-Schaub GE. Natural killer deficiency: a minor or major factor in the manifestation of hemophagocytic lymphohistiocytosis? J Pediatr Hematol Oncol. 2003;25:680-3.
12. Russell JH, Ley TJ. Lymphocyte-mediated cytotoxicity. Annu Rev Immunol. 2002;20:323-70.
13. Sullivan K, Delaat C.A, Douglas S.D, Filipovich AH. Defective natural killer cell function in patients with hemophagocytic lymphohistiocytosis and in first degree relatives. Pediatr Res. 1998;44:465-8.
14. Voskoboinik I, Trapani JA. Addressing the mysteries of perforin function. Immunol Cell Biol. 2006;84:66-71.

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