Rapid Communication

Functional impact of A91V mutation of the PRF1 perforin gene

Natalia Martínez-Pomar,⇑ Nallibe Lanio, Neus Romo, Miguel Lopez-Botet, Núria Matamoros

Department of Immunology, University Hospital of Son Espases, Palma de Mallorca, Spain
Immunology Unit, University Pompeu Fabra, Barcelona, Spain
IMIM (Hospital del Mar Research Institute), Spain

Abstract

Perforin (PRF1) gene mutations have been associated with Familial Hemophagocytic Lymphohistiocytosis type 2 (FHL2). Substitution p.A91V (c.272C>T) in exon 2 was first described as a neutral polymorphism. Nonetheless, recent clinical evidence and functional assays, suggest a potential pathogenic role for p.A91V, especially in compound heterozygous individuals. Moreover, p.A91V homozygosity has been linked to various pathological states including FHL and lymphocytic leukaemias.

In the present report we evaluated the impact of this mutation in a compound heterozygous A91V/G149S 31 year-old asymptomatic female. Functional assays revealed low perforin expression levels, as well as an impaired NK cell-mediated cytotoxicity, partially reconstituted after incubation with IL-2. These results support that p.A91V mutation, associated to another mutated PRF1 allele, may potentially predispose seemingly healthy carriers to suffer a milder FHL2 clinical phenotype, including later onset of the disease. Thus, clinical monitoring of p.A91V carrier individuals bearing another mutation in PRF1 is warranted.

1. Introduction

FHL is a heterogeneous disorder characterized by a hyperinflammatory syndrome with fever, hepatosplenomegaly, cytopenia and, less commonly, central nervous system involvement. FHL is a lethal autosomal recessive defect [1] that typically occurs in infancy or early childhood [2]. To date, described genetic defects associated with this pathology disrupt mechanisms involved in triggered or activation-induced cell death. Perforin-encoding PRF1 was the first gene linked to FHL2 [3] being responsible for 15–30% of cases [4]. Perforin is a pore-forming cytolytic protein synthesized and stored in secretory granules of cytotoxic T lymphocytes (CTLs) and natural killer cells (NK). Its function is essential for killing virus-infected or transformed cells through the granule exocytosis pathway [5]. The exact contribution of the various PRF1 mutations reported in the literature is not yet clear. The pathophysiological potential of the common variant c.C271T (p.A91V), found in up to 20% of healthy individuals [6,7] remains to be fully elucidated [8–10]. Initial studies considered A91V a benign polymorphism [11,12] but recent reports have proposed it as a disease-modifying gene [13–15]. Functional studies associated A91V to decreased cytotoxicity activity [16–18] but most clinical observations implying a pathogenic role for A91V have been limited by the small sample size. In the current study we describe a healthy 31 year-old female compound heterozygous for A91V/G149S who displayed diminished perforin expression levels and impaired NK and CD8+ T cell-mediated cytotoxicity.

2. Material and methods

2.1. Subjects

We studied a woman native from Morocco whose daughter developed FHL. The study protocol was conducted according to the ethical guidelines of the 1975 Declaration of Helsinki and approved by our hospital research ethics committee. Informed consent was obtained from all subjects.

2.2. PRF1 mutation analysis

Genomic DNA was extracted from peripheral blood (QiAmp DNA Blood mini Kit) in accordance with the manufacturer’s Instructions. Mutation analysis of PRF1 gene was performed by direct DNA sequencing using specific primers. In all samples the PRF1 gene encoding exon 2 and 3 was amplified using primers as follows: for exon 2, forward (F2) 5′-CCTTCCATGTGCCTGATAATC-3′; reverse (R2) 5′-GATGTTCTGGCTCCGAGTCTG-3′; for exon 3, forward (F3) 5′-GATGTTCTGGCTCCGAGTCTG-3′; reverse (R3) 5′-CCAGCGCCGGCTGAGTCCTG-3′.
3' and reverse (R2) 5'-GAAGCAGCCTCCAAGTTTGATTG-3'; and for exon 3, forward (F3) 5'-CATGCTAGTCTGACCACAATA-3' and reverse (R3) 5'-CTAATGGGAATACGAAGACAGCC-3'. PCR analyses were carried out using standard reaction mixes, and PCR products were purified (QiAquick PCR purification Kit) and sequenced directly on an automated sequencer (ABI-3100; Applied Biosystems) adding the following pair of internal primers to improve sequence analysis: reverse (R2int) 5'-GCCCTCCTGTAGGGCATTTTC-3' and forward (F2int) 5'-AGTGGACACACAAAGGTTCCTG-3'. Sequence data were analyzed with Chromas software and compared with the reported gene structure (gene number 190339, NCBI).

2.3. Flow cytometry analysis

Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation on Ficoll-Paque PLUS (GE Healthcare Bio-Sciences AB, Uppsala, Sweden). An intracellular staining was performed using the Cytofix/Cytoperm kit (Becton Dickinson, CA) as described by the manufacturer. Briefly, cells were incubated with anti-CD3-PerCP and anti-CD56-PE (BD Pharmingen, CA). After washing, cells were permeabilized and incubated with the anti-Perforin-FITC mAb (BD Pharmingen, CA). Samples were analyzed by flow cytometry in a FACSscan (Becton Dickinson, CA); data were processed with Cell Quest Pro (Becton Dickinson, CA) and FlowJo (Tree Star, Inc., OR) softwares. Appropriate isotype-matched control mAbs were used to assess nonspecific binding.

The surface expression of a panel of activating NK cell-associated receptors (i.e. NKG2A, NKG2D, NKG2C, NKp46, NKp30 and CD16) was analysed by multicolour flow cytometry as previously described [19].

2.4. Cytotoxicity assays

The cytolytic activity of PBMCs, freshly isolated or incubated 48 h with hrIL-2 (1000 U/ml), was analyzed against the human K562 erythroleukemia cell line in a 4-h 51Cr-release assay (effector/target ratios ranged from 100/1 to 12.5/1). All assays were set up in triplicate and specific lysis was calculated as previously described [19].

3. Results and discussion

FHL2 frequently occurs in children under 2 years of age but atypical forms with delayed onset have been reported [13]. In the present report we studied a woman whose daughter (G149S homozygous) presented severe FHL in early infancy (2 month old). Both parents were carriers and furthermore, the mother presented the A91V variant in trans (Fig. 1). Perforin expression is mainly confined to NK cells, as well as CD8+, CD56+, TcRαβ+ and some activated CD4+ T cells [20–22]. An analysis of NK and T cells from the asymptomatic A91V/G149S compound heterozygous revealed decreased perforin expression levels as compared to a healthy control (Fig. 2); as expected, perforin was undetectable in cells from the (G149S/G149S) patient. The proportions of CD56dim and CD56bright NK cell subsets and the expression of NK cell-associated receptors (i.e. NKG2A, NKG2D, NKG2C, NKp46, NKp30, NKp44 and CD16) were comparable in both individuals (data not shown).

Cytotoxicity mediated by PBMC derived from the compound heterozygous (A91V/G149S) individual was tested against the NK-sensitive K562 cell line, in parallel to samples from the homozygous (G149S/G149S) FHL patient and a healthy control. As shown in Fig. 3A, compound heterozygous cells displayed an impaired cytotoxic function against K562 cells, comparable to perforin-deficient G149S/G149S cells. When the samples were tested after in vitro IL-2 stimulation, cytotoxicity was enhanced in A91V/G149S cells and, to a lesser extent, in G149S/G149S cells, remaining in every case lower than that of control cells (Fig. 3B).
Analysis of predicted structural and functional effects of the A91V variant was tested using Polyphen (Polyorphism Phenotyping) ([http://genetics.bwh.harvard.edu/pph/](http://genetics.bwh.harvard.edu/pph/)) and the Sorting Intolerant From Tolerant ([http://blocks.fhcrc.org/sift/SIFT.html](http://blocks.fhcrc.org/sift/SIFT.html)) programs, predicting a possibly damaging (PSIC score difference: 1.791) and affect protein function (score 0.01), respectively.

The high frequency of the A91V mutation in healthy populations and the low numbers of FHL patients harboring this mutation has rendered its putative pathogenic role a controversial issue. Yet, its association to an impaired in vitro perforin function has been reported [23,24]. Moreover, a recent retrospective review [25] of genetic and immunological observations in patients developing late FHL revealed that around 7% presented the A91V genotype. Moreover, Chia et al. identified a subgroup of individuals from non-consanguineous families with two mutated PRF1 alleles, in which onset of FHL was markedly delayed or presented an hematological malignancy. Some of these patients were found to be A91V homozygous or compound heterozygous with a second PRF1 mutated allele. Our data are consistent with the observations of Chia et al. [23], who demonstrated that cytotoxic function of mutant A91V cells was completely rescued by culturing at 30 °C.

In summary, our results further support that this frequent polymorphism associated to another PRF1 allele mutation may predispose seemingly healthy carriers to suffer a milder clinical phenotype, including later onset of the disease. Thus, a close clinical follow-up of p.A91V carrier individuals bearing another PRF1 mutation is recommended. Moreover, the high incidence of p.A91V in the general population warrants an extended molecular analysis of this mutation in carrier relatives of FHL2 patients.

Acknowledgments

This work was supported by Grant Plan Nacional I+D (SAF2010-22153-C03-01) from University Pompeu Fabra.

References


