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SHORT REPORT

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Sequence analysis of the granulysin and granzyme B genes in familial hemophagocytic lymphohistiocytosis

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Abstract Familial hemophagocytic lymphohistiocytosis (FHL) is an autosomal recessive disorder of immune regulation. Mutations in the gene encoding perforin were previously identified in a subset of FHL patients. The present analysis of two novel candidate genes, granzyme B and granulysin, by direct sequencing in a total of 16 FHL families, disclosed several sequence variations. However, none of these sequence variations were associated with the manifestations of FHL. These data do not support the notion that granulysin and granzyme B are candidate genes for FHL.

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Introduction

Familial hemophagocytic lymphohistiocytosis (FHL; OMIM 267700) is a rare autosomal recessive disorder characterised by fever, hepatosplenomegaly, pancytopenia and a multi-visceral accumulation of T-lymphocytes and macrophages (Henter et al. 1998). Natural killer (NK) cell activity is low or absent in these children, and recent investigations have revealed mutations in the gene encoding perforin in 20–40% of FHL patients (Stepp et al. 1999; Göransdotter Ericson et al. 2001). Hence, the majority of FHL cases have an unknown cause. Granzyme B and granulysin are known to co-localise with perforin in the lytic granules of cytotoxic T and NK cells. Granzyme B is critical for the induction of apoptotic death of target cells and as such represents a likely candidate gene in FHL. We initially identified granulysin as an antibacterial peptide (Andersson et al. 1995), but recent studies suggest that granulysin may also induce apoptosis in tumour cells (Kaspar et al. 2001). Moreover, a previously characterised "perforin-enhancing protein", which is able to restore activity to purified, non-lytic perforin, was suggested to be the rat homologue of human granulysin (Winkler et al. 1997). Thus, granulysin may also be considered a reasonable candidate gene in FHL. In the present study, we therefore investigated these two genes in 16 well-defined FHL families.

Patients and method

Patients

Fourteen families, previously found negative for perforin mutations (Göransdotter Ericson et al. 2001), were analysed for mutations in the granulysin gene, and ten of these and two additional families were assessed for granzyme B mutations. The patients (n=16) had either a family history of FHL (n=10), or fulfilled the Histiocyte Society criteria for hemophagocytic lymphohistiocytosis and had, in addition, either undergone bone marrow transplantation (BMT) (n=2) or died prior to BMT (n=4).

Table 1 Polymorphisms identified in FHL children and their parents in the granulysin and granzyme B genes

Codon	Sequence alteration	Effect on protein	Allele frequency in normal controls (%/%)	Conserved amino acid in mouse
Granulysin				
119	ACC→ATC	Ile→Thr	45/55	No[a]
Granzyme B				
55[b]	CAA→CGA	Gln→Arg	74/26	No
80[c]	AAG→AAA	Lys→Lys	Not analysed	–
94[d, e]	GCC→CCC	Ala→Pro	Not analysed	No
107[c]	AAT→AAC	Asn→Asn	Not analysed	–
247[e]	TAC→CAC	Tyr→His	Not analysed	No

[a]No murine homologue identified. Corresponding amino acid in the porcine homologue, NK-lysin, is Ser
[b]Both variants reported in NCBI, accession number AAA75490 (Gln) and CAA01810 (Arg)
[c]No change of amino acid
[d]Both variants reported in NCBI, accession number CAA01810 (Ala) and XP_032600 (Pro)
[e]These sequence variations were found in homozygous state in healthy parents

Mutation analysis

The amplification and cycle sequencing were performed as described elsewhere (Göransdotter Ericson et al. 2001). The obtained sequences were compared with the published sequences for granulysin (NCBI accession number NM_012483 and XM_002560) and granzyme B (CAA01810, XP_032600 and AAA55490), respectively. Primers for amplification and cycle sequencing of the coding region of the granulysin and granzyme B genes are available on request.

Gene symbols used in this article follow the recommendations of the HUGO Gene Nomenclature Committee (Povey et al. 2001).

Results and discussion

All translated exons of the granulysin and granzyme B gene were amplified and sequenced. Analysis of the granulysin gene disclosed a sequence variation resulting in the transition of isoleucine to threonine at codon 119 (Table 1). However, this sequence variation was found in both heterozygote and homozygote forms in patients and parents, as well as in a large proportion of the normal controls sequenced (Table 1), thus indicating that there is no association with the manifestations of FHL. We found no other sequence variations in the granulysin gene. Several sequence variations were identified in the granzyme B gene: a transition of glycine→arginine at codon 55, alanine→proline at codon 95 and tyrosine→histidine at codon 247. None of the sequence variations segregated with the manifestations of FHL. The current strategy did not allow us to exclude mutations in the non-coding regions of granulysin and granzyme B. However, immunoblotting for granulysin and granzyme B in spleen tissue obtained post mortem from one patient revealed expression of both proteins, thus arguing against such pathogenic mutations, at least in this patient (unpublished observations).

To conclude, our data do not provide support for granulysin or granzyme B as candidate genes for FHL. However, further studies are warranted to determine whether defective expression of these lytic granule constituents may play a role in the pathogenesis of FHL despite the absence of mutations in the coding region.

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