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Short Report

Two polymorphisms in the epithelial cell-derived neutrophil-activating peptide (ENA-78) gene

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Abstract. Increased expression of epithelial cell-derived neutrophil-activating peptide (ENA-78) has been reported in several immune and inflammatory conditions suggesting its role in inflammatory response. We have identified two single nucleotide polymorphisms in the promoter and exon 2 of the ENA-78 gene by scanning the full length gene using DHPLC DNA fragment analysis and DNA sequencing.
The polymorphism at position +398 (A/G from the first ATG codon) in exon 2 results in a synonymous substitution not resulting in an amino acid change. The promoter polymorphism was found at position −156 (C/G from the first ATG codon). An assay was designed for the detection of the polymorphisms using SNapshot ddNTP primer extension, followed by capillary electrophoresis (ABI 3100).
Allele and genotype frequencies for the promoter −156 polymorphism are presented for 107 healthy Spanish and 54 UK Caucasians. Frequencies for the exon 2 polymorphism are also presented for 63 UK Caucasians.

Keywords: ENA-78, polymorphism, biallelic

1. Introduction

Epithelial cell-derived neutrophil-activating peptide ENA-78 belongs to the CXC subfamily of chemokines and is expressed by epithelial cells after stimulation with pro-inflammatory cytokines such as IL-1B and TNFA inducing polymorphonuclear neutrophil (PMN) adhesiveness [1]. Zimmerman et al. have shown that ENA-78 is also released by stimulated endothelial cells in human lung and other tissues and can act in concert with IL-8 to induce neutrophil pro-adhesive activity [2]. ENA-78 biological activity appears to be similar to IL-8, however its synthesis and release by endothelial cells differs from IL-8, possibly due to different transcription regulation pathways.2 Higher levels of ENA-78 have been detected in chronic pancreatitis compared to normal and also it is raised in severe acute pancreatitis suggests involvement of this chemokine in the initiation and perpetuation of disease [3,4]. It has also been observed as being an important chemokine expressed in a number of inflammatory diseases such as Crohn's disease, ulcerative colitis and Rheumatoid arthriris [5,6]. In diabetic patients an increased neutrophil activation is observed which is evidenced by augmented basal level of metabolic processes in unstimulated neutrophil, enhanced concentration of neu-

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Table 1
Primers used for PCR amplification of the ENA-78 gene

Fragment	Gene position	Primer Sequence Nucleotide position*	
Fragment 1	Promoter, Exon1		
	Intron1, Exon 2	F 5′-ACT CCC TTC TAG CTG GAG CC-3′	34–53
		R 3′-CAC TTC CAC CTT GGA GCA CT-3′	758–739
Fragment 2	Exon2, Intron2	F 5′-CGT TGC GTT TGT TTA CAG AC-3′	660–679
	Exon3, Intron3	R 5′-ATC AAA GTG ACA AGT ACC CGT-3′	971–951
Fragment 3	Intron3, Exon4	F 5′-ACG GGT ACT TGT CAC TTT GAT-3′	951–971
	3′UTR	R 5′-CAG AGG TAC TAT GCT AAA CAC TTC-3′	1563–1540
Fragment 4	3′UTR	F 5′-GAA GTG TTT AGC ATA GTA CCT CTG-3′	1540–1563
		R 5′-TCT ATA TAA ACT AGC AGT CAG GA-3′	2173–2151

*GeneBank accession number: HSU12709.

trophil elastase and increased activity of neutrophil alkaline phosphatase (ALP) [7]. Diabetic patients with microvascular complications have a higher neutrophil activity than patients without complications [8].

The ENA-78 gene has been mapped to chromosome 4q13-q21 in the same region as other CXC subfamily genes and consists of 4 exons and three introns, resembling the organisation of the IL-8 gene [9].

In order to identify polymorphisms in the ENA-78 gene, the full sequence of the gene was scanned using DHPLC (wave^{TM} analysis) and two novel polymorphisms have been detected.

For mutation detection the full length sequence of the gene was examined as four separate segments and amplified using the primers shown in Table 1. PCR fragments were scanned and analysed in the DHPLC WAVE^{TM} system at two or three different temperatures according to the length and melting temperature predicted for each fragment. PCR products containing a mutation were visualised as a characteristic pattern.

Individual samples carrying different DHPLC peak pattern on each fragment were subjected to DNA sequencing.

Two single nucleotide polymorphisms (SNPs) one in the promoter (−156 C/G from the first ATG codon) and another in exon 2 (+398 A/G from the first ATG codon) of the ENA-78 gene were identified. Analysis using Expasy software (www.expasy.ch/tools/#translate) suggested that the SNP in exon2 does not result in an amino acid change. Presence of these two polymorphisms has also been reported in single nucleotide polymorphism database (http://www.ncbi.nlm.nih.gov/SNP/) confirming our results.

Assays have been designed for detection of the promoter polymorphism (−156 C/G) and exon 2 polymorphism (+398 A/G) using the ABI PRISM SNapshot ddNTP primer extension Kit. The G allele for the promoter polymorphism and G allele for exon 2 polymorphism has been assigned EMBL accession numbers AJ315731 and AJ315732 respectively.

The genomic DNA was amplified using following primers:

Promoter polymorphism:

Forward 5′-ACT CCC TTC TAG CTG GAG CC-3′

Reverse 5′-GTG CCT TCT GCA CTC CTT TT -3′

Exon 2 polymorphism:

Forward 5′-CGT TGC GTT TGT TTA CAG AC-3′

Reverse 5′-ATC AAA GTG ACA AGT ACC CGT-3′

For the promoter polymorphism a total of 100 ng genomic DNA was amplified in a 10 uL final volume PCR reaction containing 1x NH_4 buffer (Bioline), 1.5 mM $MgCl_2$, 0.2 mM dNTPs (Bioline), 5 pmol of each primer and 1 U Taq polymerase(Bioline) and 1mM Betaine (Sigma). The same conditions were used for exon the 2 polymorphism. Thermal cycling for the promoter polymorphism were as follows: 95°C for 2 min followed by 40 cycles each of 95°C for 45s, 59°C for 45s and 72°C for 45s and a final extension of 72°C for 2 min. A PCR product of 246 bp was visualised on a 3% agarose gel stained with ethidium bromide.

For the exon 2 polymorphism PCR was carried out by 95°C for 2 min followed by 40 cycles each of 95°C for 45s, 49°C for 45s and 72°C for 45s and a final extension of 72°C for 2 min. A PCR product of 312 bp was visualised on a 3% agarose gel.

The probes used for the single nucleotide extension in the primer extension kit were:

Promoter polymorphism:

5′-CAG ACA ATG GGA ACT GGT-3′

Exon 2 polymorphism:

5′-ATC ATT TTG GGA TGA ACT CC-3′

After extension and purification, the product was electrophoresed on an 3100 ABI analyser and the results were analysed with Genescan software.

Genotype and allele frequencies for the −156 polymorphism are summarised in Table 2 for 107 Northern Spanish and 54 UK healthy Caucasoids. Frequencies for the +398 polymorphism are also given for a group of 63 healthy UK Caucasoids.

Table 2
Allele and Genotype frequencies of ENA-78 exon 2 promoter and exon 2 polymorphism

Genotype	UK	Spanish
−156 G/C	N = 54	N = 107
G/G	34(63%)	79(74%)
G/C	18(34%)	25(23%)
C/C	2(3%)	3(3%)
Allele		
G	86(80%)	183(86%)
C	22(20%)	31(14%)
Exon 2 A/G	N = 63	
G/G	20(32%)	
G/A	38(60%)	
A/A	5(7%)	
Allele		
G	78(62%)	
A	48(38%)	

Both polymorphisms were found to conform to Hardy-Weinberg equilibrium, using Chi-square analysis (Statistical package Stata v6).

Altough the polymorphism in exon 2 is a synonymous single nucleotide change (not changing conserved amino acids), −156 G/C polymorphism which is located in the promoter region can influence gene expression level. These polymorphisms may be useful in the study of the genetics of diabetes and its microvascular complications which chemokines and neutrophil activations play an important role. *In vitro* studies for functional relevance of the −156 G/C polymorphism on the expression of the gene is needed.

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