Polymorphisms of the *ELANE* gene promoter region in end-stage chronic kidney disease patients

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OBJECTIVES

End-stage renal disease (ESRD) is a growing public health problem with an increasing worldwide prevalence. Inflammation is a common feature in ESRD patients under hemodialysis (HD) [1,2]; however, the mechanisms/factors triggering the inflammatory process are still poorly clarified. The HD procedure induces neutrophil activation and elastase release, which might be important in the inflammatory process and in the development of oxidative stress, two factors that increase the anemia and the risk of cardiovascular diseases [1-3]. Neutrophil elastase, a protease able to degrade several extracellular matrix proteins, is encoded by *ELANE* gene. Polymorphisms of *ELANE* gene have been associated with the development of several pathologies [4–6]. Indeed, the presence of mutations and single nucleotide polymorphisms (SNPs) in the codifying region and in the six repetitive tandem motifs of the promoter appear to influence the level of elastase expression, promoting proteolytic disturbances [5].

This study aimed to identify polymorphisms of the *ELANE* promoter region and assess their impact in the plasma levels of elastase, clinical and hematological data, iron metabolism, dialysis efficiency, inflammatory and nutritional markers.

METHODS

We performed a cross-sectional study with 123 ESRD patients (69 males and 54 females, mean [± SD] age: 65.3 [±13.9] years) on regular hemodialysis. The promoter region of the *ELANE* gene was screened in all patients by PCR-direct sequencing with forward (5'-GGAAGGACCAGAGAAGTGC-3') and reverse (5'-CTGCCAAACCTAGACCTGAG-3') primers, which amplify a 397bp DNA fragment. Plasma elastase levels were quantified by ELISA. We also evaluate the association of each polymorphism with clinical and hematological data, inflammatory and iron metabolism markers, nutritional status and dialysis adequacy, according to the methods previously described [7]. Data were analyzed using the SPSS 23.0 for Windows (SPSS, Inc., Chicago, IL), student t-test, Mann-Whitney test, and one-way ANOVA test. The association between categorical variables was analyzed using the chi-squared test or Fisher's exact test. $p<0.05$ was accepted as statistically significant.

RESULTS

In 6 out of 123 ERSD patients we found PCR products with two patterns of DNA fragments: 502bp/397bp and 449bp/397bp (Figure 1A). Sequencing of these fragments revealed two duplications in heterozygosity: an extra block composed by the 4th and 5th repetitions of the promoter region between the 5th and 6th repetitions, and an extra 52bp between the 4th and 5th repetitions, respectively (the latter previously described) [4]. In the remaining 117 patients, we identified two SNPs already described: c.-741G>A (Figure 1-B.1, 1-B.2, 1-B.3) and c.-903T>G (Figure 1-C.1, 1-C.2). Moreover, we found a new SNP, the c.-801G>A (Figure 1-D.1, 1-D.2). Table 1 shows the prevalence and allele frequencies for each polymorphism identified.

Heterozygosity for the c.-903T>G polymorphism did not influence the circulating levels of elastase [TT: 30.7 (21.2-41.1) ng/mL; TG: 28.7 (19.3-38.9) ng/mL; $p = 0.673$)], neither none of the other evaluated variables, as well as for the three genotypes associated with the polymorphism c.-741G>A [GG: 32.3 (23.7-40.2) ng/mL; GA: 27.9 (18.3-44.1) ng/mL; AA: 18.9 (17.2-20.4) ng/mL; $p = 0.441$)]. Moreover, we found that patients with the c.-741G>A allele, but not the GG genotype, had a dominant effect on leucocyte counts and neutrophil counts, in neutrophil/lymphocyte ratio as well as levels on oxLDL and serum albumin.

Figure 1

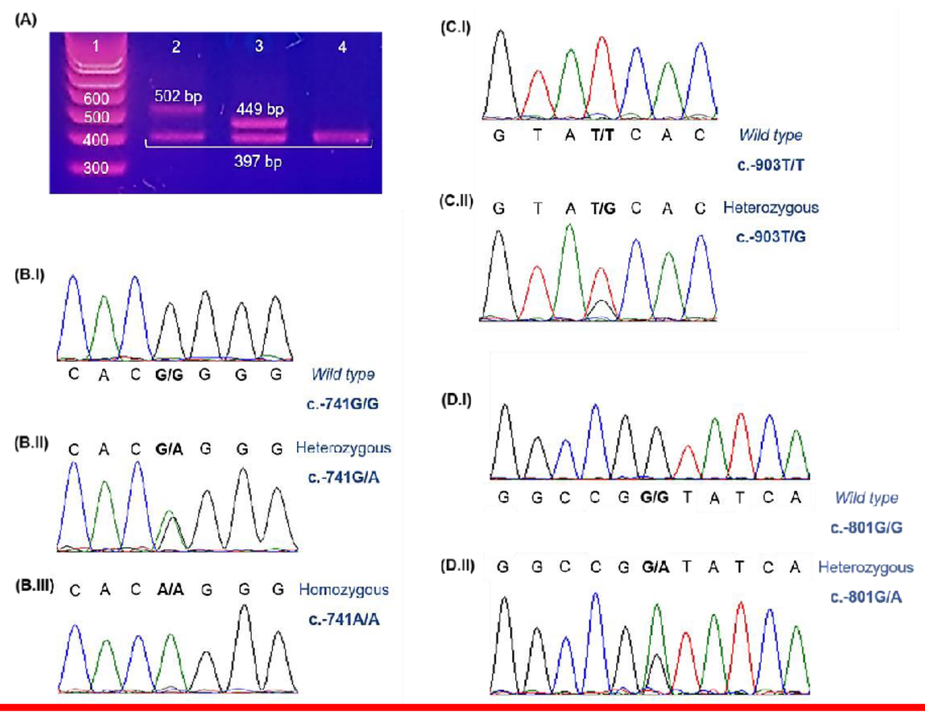

Table 1

	Polymorphism	Genotype	Number of cases		Allelic frequencies	
			N	%	Allele	%
Previously described	c.-903T>G	TT	111	90.2	T	95.1
		TG	12	9.8		
		GG	0	0.0	G	4.9
	c.-741G>A	GG	84	68.3	G	82.9
		GA	36	29.3		
		AA	3	2.4	A	17.1
	Extra 52 bp	*Wild type*	119	96.7	*Wild type*	98.4
		Hetero	4	3.3		
		Homo	0	0.0	Extra 52 bp	1.6
New	c.-801G>A	GG	122	99.2	G	99.6
		GA	1	0.8		
		AA	0	0.0	A	0.4
	Extra block	*Wild type*	121	98.4	*Wild type*	99.2
		Hetero	2	1.6		
		Homo	0	0.0	Exra block	0.8

CONCLUSIONS

ESRD patients under dialysis present high elastase plasma levels, which has been associated with the rise in neutrophils, common in inflammatory processes and also associated with the hemodialysis procedure. The c.-741G>A polymorphism in the *ELANE* promoter seems to be associated with higher neutrophil counts, and therefore, with an enhanced inflammatory process that is usually related with a poor outcome. Further studies with a larger population are required to confirm the influence of the GG genotype for c.-741G>A polymorphism in the inflammatory process and to assess the impact of the new mutation and the extra blocks.

References

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