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Genetic polymorphism of inter-α-trypsin inhibitor (ITI) in the Basque Country (northern Spain)

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Summary. The genetic polymorphism of inter-α-trypsin inhibitor (ITI) was analyzed in 2 samples of 554 residents and 303 autochthonous healthy unrelated individuals from the Basque Country (northern Spain), by isoelectric focusing on miniaturized polyacrylamide gels followed by immunoblotting. The allele frequencies were ITI*1 = 0.586, ITI*2 = 0.402 and ITI*3 = 0.012 in residents and ITI*1 = 0.548, ITI*2 = 0.449 and ITI*3 = 0.003 in the autochthonous population. These allele frequencies were compared with those reported in other European populations.

Key words: ITI – Genetic polymorphism – Isoelectric focusing – Basque Country population – European distribution

Zusammenfassung. Der genetische Polymorphismus des Inter-α-Trypsin-Inhibitors (ITI) wurde in 2 Stichproben von 554 ansässigen und 303 autochthonen gesunden, nicht verwandten Individuen des Baskenlandes (Nordspanien) mittels isoelektrischer Fokussierung unter Verwendung von miniaturisierten Polyacrylamidgelen und anschließendem Immunoblotting untersucht. Die Allelfrequenzen betrugen für ITI*1 = 0,586, ITI*2 = 0,402, ITI*3 = 0,012 bei Ansässigen und ITI*1 = 0,548, ITI*2 = 0,449, ITI*3 = 0,003 für die autochthone Population. Diese Allelfrequenzen wurden mit jenen von anderen europäischen Populationen verglichen.

Schlüsselwörter: ITI – Genetischer Polymorphismus – Isoelectrische Fokussierung – Baskenland – Population – Europäische Verteilung

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Introduction

Human inter-α-trypsin inhibitor (ITI, now called ITIL according to the Human Gene Mapping 11) is a plasma glycoprotein of 220 KDa composed of 3 polypeptide chains, 2 different heavy chains and one light chain (Vogt et al. 1992). Heavy and light chains are covalently cross-linked by glucosaminoglycan chondroitin sulphate (Schreitmüller et al. 1987). At present, the structure of the ITI genes and the precise subunit composition of the polypeptide chain complexes remains to be established. It is synthesized in the liver and present in plasma at normal concentrations of 50 mg/ml (Yuasa et al. 1991). Genetic variations of ITI were first demonstrated by isoelectric focusing (IEF) in agarose gels followed by immunofixation or immunoblotting with specific ITI antisera (Vogt and Cleve 1990). Two common alleles (ITI*1, ITI*2) and one rare allele (ITI*3) determine 6 phenotypes in Europeans. Two additional rare alleles (ITI*4, ITI*5) were found in Asians and one rare allele (ITI*6) in African blacks (Vogt et al. 1992; Yuasa et al. 1991; Vogt and Cleve 1990; Vogt et al. 1991a; Luckenbach et al. 1991, 1992).

In this study, the ITI allele frequencies were determined in a resident and in an autochthonous population of the Basque Country (northern Spain) using isoelectric focusing in miniaturized gels.

Materials and methods

Plasma samples were obtained from 554 unrelated resident donors and from 303 unrelated autochthonous donors from the Basque Country. Only those individuals whose 8 surnames and birth places of their 4 grandparents were of Basque origin were considered as autochthonous.

Isoelectric focusing was carried out on miniaturized polyacrylamide gels (inter-electrode distance 55 mm) as previously de-

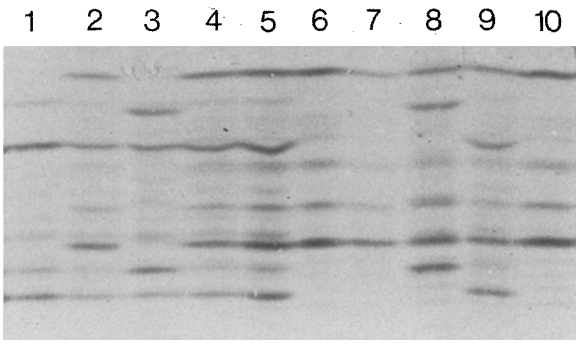

Fig. 1. Common ITI phenotypes demonstrated by isoelectric focusing of native samples followed by immunoblotting. Anode at the *top*. *Lane 1* = 2; *lane 2* = 2-1; *lane 3* = 3-2; *lane 4* = 2-1; *lane 5* = 2-1; *lane 6* = *1; lane 7* = 1; *lane 8* = 3-1; *lane 9* = 2-1; *lane 10* = 1

scribed (Alonso and Gascó 1987). Native samples were analysed (with a mixture of Ampholine pH 3.5–10 and Pharmalyte 4–6.5) according to Vogt et al. (1991b). The ITI band pattern was detected by a two-step enzyme immunoassay as previously described for the detection of GC protein (Alonso 1988) using the following antibodies: rabbit anti-human ITI followed by swine anti-rabbit immunoglobulins/HRP.

The diversity between observed and expected results has been tested using the χ^2-test for independence and genetic equilibrium according to the Hardy-Weinberg law.

Results and discussion

Figure 1 shows the isoelectric focusing band pattern in native samples of 5 different ITI phenotypes encountered in this population study. The resolution obtained using miniaturized gels could clearly distinguish allele products with similar isoelectric points (e.g. Fig. 1, lane 1 and lane 3) and is comparable to that obtained with conventional size gels (Vogt et al. 1991b). Furthermore, the method presented here is faster and more economical and has been successfully applied to the ITI typing from fresh sera and sera stored at −40°C for a period of 10 years. At the present time, the stability of this genetic marker in bloodstains is being studied. The discrimination probability of ITI in the resident population of the Basque Country is calculated to be 0.633 on the basis of the allele frequencies and the single exclusion chance is calculated to be 0.184, by means of the formula pq (1-pq).

The distribution of ITI phenotypes and the corresponding allele frequencies in a population sample of 554 unrelated resident individuals and 303 unrelated autochthonous individuals from the Basque Country are presented in Table 1. A total of 3 different alleles and 5 phenotypes was observed. The observed numbers are in agreement with the expectation according to the Hardy-Weinberg law. No significant differences were found between autochthonous and resident populations ($\chi^2 = 3.78$, 2 d.f.).

The ITI allele frequencies found in this study have been compared with those reported in other European populations (Table 2). The values of ITI allele frequencies are within the range of variation reported in other European populations. However, it is worth pointing out that the frequency of the ITI*3 allele in the Basque Country resident population is the highest found in

Table 1. ITI phenotypes and allele frequencies in the Basque Country (northern Spain)

Phenotypes	Population			Allele frequencies	
	Resident	Autochthonous		Resident	Autochthonous
1	200	97			
2–1	242	137	ITI*1 =	0.586	0.548
2	99	67	ITI*2 =	0.402	0.449
3–1	8	1	ITI*3 =	0.012	0.003
3–2	5	1			
Total	554	303			

$\chi^2 = 2.9922$; d.f. = 2; $0.30 > P > 0.20$ (resident population)
$\chi^2 = 1.9358$; d.f. = 2; $0.40 > P > 0.30$ (autochthonous population)
(The phenotypes with expected numbers below 5 were pooled for the calculation)

Table 2. Comparison between ITI allele frequencies found in the present study with those reported in other European populations

Population	Number	ITI*1	ITI*2	ITI*3	Reference
Germany	677	0.600	0.393	0.007	Luckenbach et al. 1991
	248	0.575	0.417	0.008	Vogt and Cleve 1990
Austria	124	0.577	0.423	–	Vogt and Cleve 1990
NW Portugal	78	0.552	0.448	–	Luckenbach et al. 1992
Basque Country Residents	554	0.586	0.402	0.012	This study
Basque Country Autochthonous	303	0.548	0.449	0.003	This study

Europe and the Basque Country autochthonous population has the ITI*2 and the lowest ITI*3 so far reported.

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