

## PI ( $\alpha$ -1-ANTITRYPSIN) SUBTYPES IN NW PORTUGAL

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### INTRODUCTION

The study of  $\alpha$ -1-antitrypsin (PI) locus at protein level by isoelectric focusing has revealed a high degree of genetic variation both within and between most human populations.

Among European populations, Iberia (including the Basque Country) shows a peculiar distribution, with the highest PI\*S frequencies reported so far.

In this work we present formal and population genetics data on PI subtypes in NW Portugal.

### MATERIAL AND METHODS

Blood samples were obtained by venipuncture and EDTA plasmas were stored at  $-20^{\circ}\text{C}$ .

PI subtyping was done by hybrid isoelectric focusing (HIEF) at pH ranges 4.3-4.8 and 4.52-4.72 in polyacrylamide gels (T=5%; C=3%) at  $8^{\circ}\text{C}$ . Immobilized pH gradients were generated according to LKB Application Note 324. The dried gels were rehydrated for 2 hours with 20% (w/v) sucrose and 2% (v/v) carrier ampholytes pH 4.2-4.9 (Pharmacia). Electric settings were: 4000V, 4mA, 12W for 6 hours and 5000V, 25mA, 8W for 8 hours for the pH ranges 4.3-4.8 and 4.52-4.72, respectively. Anolyte was glutamic acid 10mM and catholyte was NaOH 10mM. Samples were directly applied using Whatman nr.1 filter paper (0.5x0.5cm). Staining was done with Coomassie Brilliant Blue.

### RESULTS AND DISCUSSION

Fig.1 presents PI phenotypes as revealed by HIEF with the 4.3-4.8 pH range. Using this interval PI M3 and PI M4 gene products were not discriminated. A rare variant (V) with intermediate mobility between the so-called m4 and m6 zones was detected, showing a decreased band intensity when compared to both M and S.

Fig.2 shows the separation achieved in the m8 zone with the pH range 4.52 - 4.72. PI Z1 is a gene product with a pI higher than PI Z and similar band intensity. These properties are coincident with PI Zaug gene product characterized by FABER et al.(1990) suggesting that the Z substitution ( $\text{Glu}^{342} \rightarrow \text{Lys}^{342}$ ) occurred in a M subtype different from M1.

#### Population Genetics

Tab.1 shows the distribution of PI subtypes in a random sample of 241 individuals from NW Portugal.

The high frequency of PI\*S of our sample (in association with a reduction of PI\*M1 frequency) is consistent with other reports on Spanish populations (Alonso et al., 1991); however, the

Portuguese sample is characterized by increased PI\*M2 and decreased PI\*M3 frequencies.

#### Formal Genetics

Segregation analysis was performed in a sample with 132 families with 301 offspring. No mother-child exclusions were found. Due to the small numbers per mating type inherent to the high degree of polymorphism of this system, the segregation of each gene product against all others was studied sequentially. With this analysis, a significant deficiency of M1 offspring from M1- x M1- mating type was found (Table 2).

TABLE 1. PI phenotype distribution in NW Portugal

<u>Phenotype</u>	<u>observed</u>	<u>expected</u>	
M1	86	79.13	PI*M1= 0.573 ± 0.023
M1M2	54	58.55	PI*M2= 0.212 ± 0.019
M1M3	17	21.28	PI*M3= 0.079 ± 0.012
M1M4	5	2.76	PI*M4= 0.010 ± 0.005
M1S	28	30.93	PI*S = 0.112 ± 0.014
M2	14	10.83	PI*V = 0.004 ± 0.003
M2M3	5	8.07	PI*Z = 0.008 ± 0.004
M2S	12	11.44	PI*Z1= 0.002 ± 0.002
M2V	1	0.41	
M2Z	2	0.82	chi-square = 5.68
M3	5	1.50	2 df
M3S	6	4.26	.10 > P > .05
S	3	3.02	
SZ	1	0.43	
SZ1	1	0.11	
VZ	1	0.02	
<u>others</u>	<u>0</u>	<u>7.44</u>	
total	241		

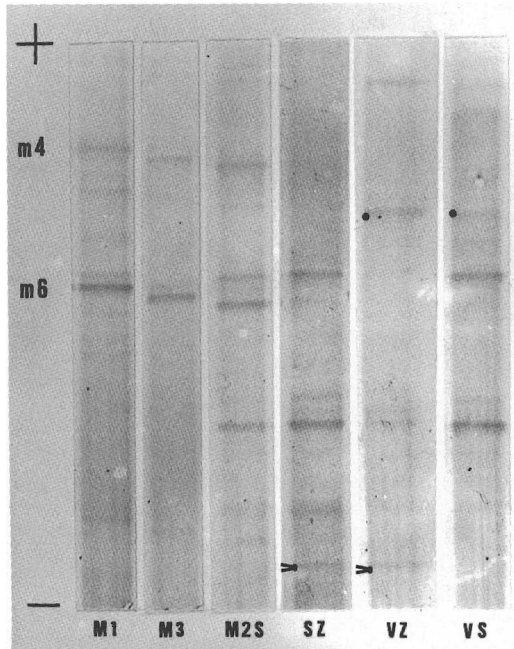
TABLE 2. Segregation analysis of M1- x M1- mating type

<u>nr. of</u>		<u>offspring phenotypes</u>			<u>chi<sup>2</sup></u>	<u>df</u>	<u>P</u>
<u>families</u>	<u>offspring</u>	<u>M1</u>	<u>M1-</u>	<u>-</u>			
24	50	5	31	14	6.12	2	.050-.025
		(12.50)	(25.00)	(12.50)			

"-" stands for any allele except M1

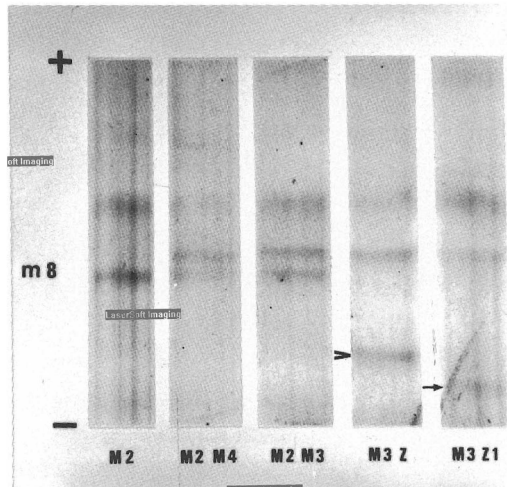
**Figure 1.**

PI subtypes obtained by HIEF in a 4.3-4.8 pH gradient. The main bands corresponding to PI V and PI Z are marked with · and > respectively.



**Figure 2.**

PI subtypes obtained by HIEF in a 4.52-4.72 pH gradient. The main bands corresponding to PI Z and PI Z1 are marked with > and -> respectively.



#### REFERENCES

- ALONSO A, VISEDO G, SANCHO M, FERNÁNDEZ-PIQUERAS J (1990) Isoelectric focusing in miniaturized gels; application to GC, PI, Tf, and ORM subtyping in Central Spain. in POLESKY HF, MAYR WR (eds) *Advances in Forensic Haemogenetics* 3: 255-259.
- FABER J-P, WEIDINGER S, OLEK K (1990) Sequence data of the rare deficient alpha-1-antitrypsin variant PI Zaugsburg. *Am J Hum Genet* 46: 1158-1162.