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ALLELIC FREQUENCIES FOR ALA213/VAL213 POLYMORPHISM OF THE PI (ALPHA-1-ANTITRYPSIN) LOCUS IN A MOUNTAINOUS REGION OF MÉRIDA –VENEZUELA. ESTIMATION OF THE CAUCASOID CONTRIBUTION

FRECUENCIAS ALÉLICAS DEL POLIMORFISMO ALA213/VAL213 DEL LOCUS PI (ALFA-1-ANTITRIPSINA) EN UNA REGIÓN MONTAÑOSA DE MÉRIDA – VENEZUELA. ESTIMACIÓN DE LA CONTRIBUCIÓN CAUCÁSICA

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ABSTRACT

The Ala213/Val213 polymorphism of the PI locus was studied using PCR-RFLP in a sample of 33 genetically independent homozygous M1M1 individuals, with four grandparents, born in the Apartaderos geographic focus, a mountainous region of Mérida, Venezuela. The genotypic frequencies found were: Ala213/Ala213 homozygotes; 3% (one individual), Ala213/Val213 heterozygotes; 21% (7 individuals) and Val213/Val213 homozygotes; 76% (25 individuals). The allelic frequencies found were: 13.6% for the PI*M1(Ala213) allele and 86.4% for the PI*M1(Val213) allele. The reported frequencies for the PI*M1(Ala213) allele vary worldwide according to the ethnic group: 0.0% in Amerindians, 16.4 to 34.1% in Caucasoids and 54.0 to 73.1% in Negroids. The Caucasoid contribution to the sample studied was estimated at 40%.

Key words: Ala213/Val213, PI Locus, Alpha-1-antitrypsin, AAT, AAT Venezuela.

RESUMEN

Se estudió el polimorfismo Ala213/Val213 del locus PI, mediante PCR-RFLP, en una muestra de 33 individuos homocigotos M1M1, genéticamente independientes, con cuatro abuelos nacidos en el foco geográfico de Apartaderos, región montañosa de Mérida, Venezuela. Se encontraron las siguientes frecuencias genotípicas: 3% de homocigotos Ala213/Ala213 (un individuo); 21% de heterocigotos Ala213/Val213 (7 individuos) y 76% de homocigotos Val213/Val213 (25 individuos). Las frecuencias alélicas encontradas fueron: 13,6% para el alelo PI*M1(Ala213) y 86,4% para el alelo PI*M1(Val213). A nivel mundial las frecuencias reportadas para el alelo PI*M1(Ala213) varían de acuerdo al grupo étnico: 0,0 % en Amerindios, de 16,4% a 34, 1% en Caucasoideos y 54,0% a 73,1% en Negroideos. Se estimó un 40% de contribución Caucasoidea en la muestra estudiada.

Palabras clave: Ala213/Val213, Locus PI, Alfa-1-antitripsina, AAT, AAT Venezuela.

INTRODUCTION

The PI locus (14q32.1) (Lai *et al.* 1983; Turleau *et al.* 1984) codifies for alpha-1-antitrypsin (AAT), a serine protease inhibitor, the most abundant component in the serpins family (Guzdek *et al.* 1990; Crystal 1990). This small plasmatic sialoglycoprotein (394 aminoacids) (Long *et al.* 1984; Crystal 1991), is synthesized primarily in hepatocites (Perlmutter *et al.* 1985), and is capable of diffusing into the blood stream and many tissues. Its main physiological function is accomplished in lung alveoli: normal AAT inhibits neutrophil elastase (Meyer *et al.* 1975) and so, degradation of alveolar tissue elastin is avoided, without development of lung emphysema (Crystal 1991).

PI locus is one of the most polymorphic loci in the human genome. More than 100 alleles have been described (primarily by isoelectric focusing), many of which show polymorphic frequencies, including several

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normal alleles, as well as null, dysfunctional and deficient ones (Buist *et al.* 1989; Crystal 1989; Faber *et al.* 1994; Seixas *et al.* 2002; Gupta *et al.* 2005).

Normal most frequent allele is PI*M, with intermediate migration in isoelectric focusing gels, that has at least 9 subtypes: PI*M1(Val213 and Ala213), PI*M2, PI*M3 (Frants *et al.* 1978; Graham *et al* 1990), PI*M4 (Constans *et al.* 1980), PI*M5 Weidinger *et al.* 1985), PI*M6, PI*M7 and PI*M8 (Weidinger 1992).

PI locus polymorphism has been studied for many populations around the world. Nevertheless, frequencies for the Ala213/Val213 polymorphism have been reported only in a few populations (Cox *et al.* 1987; Nukiwa *et al.* 1987; Gaillard *et al.* 1994; Rocha *et al.* 1997; Seixas, 2001), none of which is in Latin-America. According to those reports, allelic frequencies of Ala213/Val213 differ broadly in different ethnic groups (Table 1).

In the present study we describe through PCR-RFLP, allelic frequencies for Ala213/Val213 polymorphism in a sample from the mountainous region of the State of Mérida-Venezuela and estimate the Caucasoid contribution to the studied population.

Table 1. Comparison of the estimated Caucasoid contribution.

Population	N	PI*M1(Ala213)	PI*M1(Val213)	Reference
Venezuela Páramo-Mérida	33	0,136	0,864	Present study
Venezuela Amerindian Yukpa-Irapa	9	0,000	1,000	Arias *et al*. (2001)
Japan	156	0,000	1,000	Nukiwa *et al*. (1987)
Spain Basque country	55	0,164	0,836	Seixas *et al*. (2001)
Portugal (North)	115	0,339	0,661	Rocha *et al*. (1997)
Portugal	182	0,341	0,659	Seixas *et al*. (2001)
Canada	38	0,34	0,66	Cox and Billingsley (1987)
United States (Caucasoid)	39	0,32	0,68	Nukiwa *et al*. (1987)
St. Thomas and Prince (Portuguese ex-colony of black population)	171	0,731	0,269	Seixas *et al*. (2001)
South Africa				
Blacks	65	0,54	0,46	Gaillard *et al*. (1994)
Whites	110	0,26	0,74	

MATERIALS AND METHODS

Sample

The studied sample included 33 (17♀ y 16♂) genetically independent individuals, each one with four grandparents born in the **geographic focus** (Arias 1994) of Apartaderos, a 2376 Km^2 circular area centred in the town of Apartaderos (8º48'30'' N; 70º51' W) (Figure 1). They were randomly chosen from a subgroup of 165 M1M1 homozygous, phenotyped in a previous study by isoelectric focusing of plasma samples (Fonseca-Pérez *et al.* 1996). This subgroup belonged to a total of 233 genetically independent elementary school students, with four grandparents born in the geographic focus of Apartaderos, randomly sampled within the *páramo* area (mountainous region higher than 3000 m above sea level with scant vegetation).

DNA extraction

Five ml of blood were collected from each of the 33 studied individuals, and the DNA present in each sample was extracted by saline precipitation method (Lahiri and Nurnberger 1991).

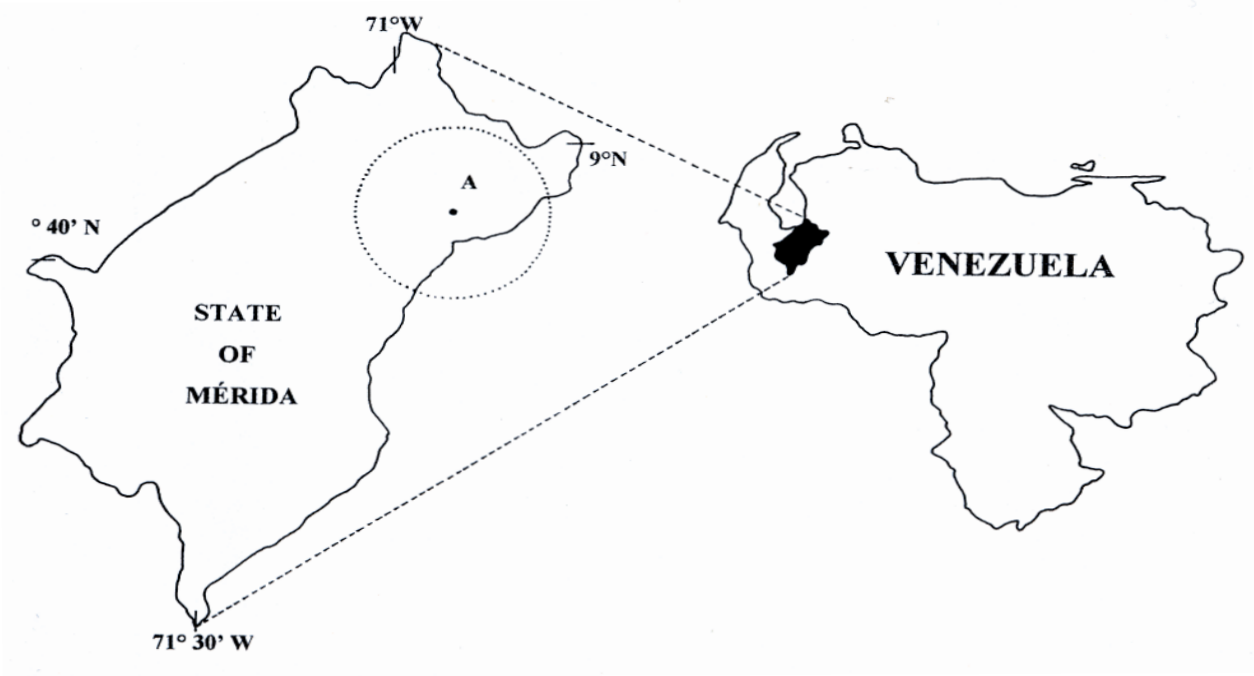

Figure 1. Geographic focus of Apartaderos, a 2376 Km^2 circular area centred in the town of Apartaderos (8º 48' 30'' N; 70º 51' W), Mérida state, Venezuela.

PCR-RFLP

Every DNA sample was amplified through PCR obtaining a 427 bp fragment from PI exon III using primers proposed by Nukiwa *et al.* (1987):

5' CACTCTTCCAAACCTTCACTCAC 3' and

5' TTCACCCTCCTCAGCCCTCT 3'

PCR conditions for amplification were as follows: one preliminary denaturation step at 94ºC for 6 min. followed by 32 PCR cycles. Strand denaturation was made at 94ºC for 1 min., annealing at 48ºC for 1 min. and primer extension at 72ºC for 1.5 min. A final extension at 72ºC for 7 min. was performed.

The 427 bp DNA fragments amplified were digested with *Bst* E II at 60ºC for 16 hours. The transition from C to T creates a restriction site for Bst E II in the PI*M1Val213 allele, which is absent in the PI*M1Ala213 allele, allowing differentiation of both alleles. Fragments obtained were then separated electrophoretically on 12% polyacrylamide:bisacrylamide (29:1) gels (12 cm long, 0.8 mm thick), run in TAE buffer (Tris/acetate 0.045M with EDTA 1mM, pH 8.1). DNA fragment sizes were compared to a molecular weight marker (pBR322 digested by *Msp* I) run on the same gel. Gels were silver stained (Lohmann *et al.* 1992).

Estimation of the Caucasoid contribution

The Caucasoid contribution to the studied sample was estimated with the Bernstein´s formula (1931), considering the allelic frequency of PI*M1Ala213 found in the sample, and the reported frequency for Caucasoid (Portuguese) population, as follows:

$$m_1 = \frac{P_{12} - P_2}{P_1 - P_2} = \frac{0.136 - 0.0}{0.34 - 0.0} = 0.40$$

where

m1 = contribution of population number 1, (Caucasoid) to the hybrid population (studied Venezuelan sample).

P_1 = allelic frequency (PI*M1Ala213) in population #1 (Caucasoid → Portuguese).

P_2 = allelic frequency (PI*M1Ala213) in population #2 (a Venezuelan Amerindian one).

P_{12} = allelic frequency (PI*M1Ala213) in the hybrid population (studied Venezuelan sample).

Considering the absence of PI*M1Ala213 in Asiatic populations (Table 1), and the Mongoliod origin of Amerindians, this allele frequency was assumed to be 0 for the Amerindian settled in Venezuela prior to the arrival of Spaniards (population 2). This assumption agrees with the absence of the allele in the Yukpa-Irapa, which is so far, the only Venezuelan Amerindian population phenotyped for this polymorphism (Arias *et al.* 2001).

The PI*M1Ala213 allele frequency used for caucasoid population, was the one reported for the Portuguese population since there are no reports for the Spaniartd population, except for the Basque, a very isolated group, whose genetic pool does neither represents the Iberian Peninsula, nor the Spanish region.

RESULTS

In the studied sample there were 3% (1 individual) Ala213/Ala213 homozygous, 21% (7 individuals) Ala213/Val213 heterozygous and 76% (25 individuals) Val213/Val213 homozygous.

Allelic frequencies found for the polymorphism Ala213/Val213, in the M1M1 homozygous were: 13,6% (9 alleles) for PI*M1Ala213 and 86.4% (57 alleles) for PI*M1Val213.

According to Bernstein (1931) formula, considering the estimated allelic frequency for the PI*M1Ala213 allele in the studied sample, and the frequency of this allele in a present Caucasoid population (Portuguese), there is a 40% Caucasoid contribution in this Venezuelan sample.

DISCUSSION

According to previous population studies (Table 1), the allelic frequency for PI*M1Ala213 fluctuates, with a clear ethnic dependence. The highest allelic frequencies correspond to Negroid populations (0.54 – 0.731), followed by Caucasoid ones (0.341 – 0.164). In populations of Asiatic origin, including an Amerindian one (Yukpa – Irapa, Venezuela), there is a complete absence of the PI*M1Ala213 allele.

The allelic frequency found for the studied sample (0.136), is higher than the previously reported for Asiatic populations, and lower than the reported for Caucasoid and Negroid ones (Table 1). This result was expected due to the hybrid origin of the "general" Venezuelan population.

Most Venezuelan populations are the product of the three mayor ethnic contributions: a major Amerindian contribution, followed by an important Caucasoid (Spaniard) one and a minor Negroid (African) contribution.

The studied sample is located in the Venezuelan Andes, in the Mérida state, considered the region with the highest Caucasoid contribution in the country (except for a particular Caucasoid isolate). This fact is evidenced when blood group frequencies are analyzed. Mérida's population has been registered with the country's highest frequency for negative Rh factor (12.02%) (González-Coira *et al.* 1998); while the national mean is 3% (Rodríguez-Larralde *et al.* 2001).

The above detailed conditions of the population of Mérida, makes the latter adequate for the estimation of the Caucasoid contribution through the frequency of the PI*M1(Ala213) allele. Assuming the absence of PI*M1Ala213 in Venezuelan Amerindians prior to the Spanish colonization, presence of this allele in the studied population nowadays must be the result of the contribution of one or more of the other ethnic groups that conform the present hybrid population. The very limited Negroid contribution (according to historical registries) to the studied geographical focus, enables us to assume the presence of PI*M1Ala213 in the hybrid population, as a Caucasoid contribution, and to calculate this contribution (40%) obviating the Negroid one (because of this consideration the estimated value is probably slightly overestimated).

Population from Mérida State has another peculiarity: it is one of the country's region with the lowest Negroid contribution. According to historical data (Osorio 1996), this contribution to colonial Mérida must have been scarce; in 1820, the rate of African slaves present in the towns within the studied geographical focus (Apartaderos), did not exceed 2.6% of the population in any case, and had a mean value of only 1.55% for the whole geographic focus.

The calculation of the Caucasoid contribution to the sample from the geographic focus of Apartaderos through the PI*M1(Ala213) allele frequency, is very similar (X^2 = 3.19, df. 2, p = 0.202) to the previously estimated one, through alleles PI*M2 and PI*S (Table 2) (Fonseca-Pérez *et al.* 1996) as could be expected.

Table 2. Comparison of the estimated Caucasoid contribution in previous study and the present study.

Allele used as ethnic marker	Caucasoid contribution	N (# of individuals)	Reference
PI*M1(Ala213)	40	33	Present study.
PI*S	41.8	233	Fonseca-Pérez *et al*. (1996).
PI*M2	42.2	233	Fonseca-Pérez *et al*. (1996).
	Mean value: 41.32		

The Caucasoid contribution to this geographic focus (Apartaderos is not necessarily the same as in others from the state of Mérida. The heterogeneity in the Colonial human settlements reflects an important difference in the genetic pool of the present populations. Comparative frequency studies for the haptoglobin alleles and the PI*S allele for the studied geographic focus and the geographic focus of Guaraque (also from Mérida state), show wide differences in respect to the Caucasoid contribution being the one for the Guaraque focus, twice that of Apartaderos focus (González-Coira *et al*. 2002).

The PI*M1(Ala213) may be considered an ethnic marker, particularly in populations with absent or scarce Negroid (or Caucasoid) contribution, as is the case with the population of the Mérida state.

CONCLUSIONS

The genotypic frequencies found in the sample from the mountainous region of Mérida State-Venezuela, for the Ala213/Val213 polymorphism of locus PI were: 3% of Ala213/Ala213 homozygous (one individual); 21% of Ala213/Val213 heterozygous (7 individuals) and 76% of Val213/Val213 homozygous (25 individuals). The allelic frequencies found were: 13.6% for the PI*M1(Ala213) allele and 86.4% for the PI*M1(Val213) allele. The Caucasoid contribution to the studied sample was estimated in 40%.

The PI*M1(Ala213) may be considered an ethnic marker for Mérida's population, and other populations with absent or scarce Negroid (or Caucasoid) contribution.

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REFERENCES

Arias S. 1994. Selective detection among the high risk populations, instead of mass screening, proposed for countries with various homogeneous origins and a stratified distribution of abnormal genes. In: New Horizons in Neonatal Screening (Farriaux, J.P. and Dhondt, J. –L., eds.). Amsterdam: Excerpta Med. Int. Congr. Ser. Nº 1041:97-100 pp.

Arias S., Paradisi I., Fonseca T., González N., Pérez E., Ramírez C. 2001. 32 sistemas fenotípicos polimórficos del ADN, definidores del aislado Yukpa Irapa (=Tucuco, Yuco, Maraca) de Perijá, Venezuela, para 14 cromosomas, en una muestra mínima. In: VIII Congreso Venezolano de Genética (Vargas, J., Moreno, N. & Martínez, J.A., eds.). Valencia: Av. Genét. VIII:341 pp.

Bernstein F. 1931. Die geographische Verteilung der Blutgruppen und ihre antropologische Bedeutung. In: Comitato Italiano per lo Studio dei Problemi della Populazione. Roma: Instituto Poligráfico dello Stato. 227-243 pp.

Buist A.S., Burrows B., Cohen A., Crystal R.G., Fallat R.G., Gadek J.E., Turino G.M. 1989. Guidelines for the approach to the patient with severe hereditary alpha-1-antitrypsin deficiency. Am. Rev. Respir. Dis. 140:1494-1497.

Constans J., Viau M., Gouaillard C. 1980. Pi^{M4}: An additional Pi^{M} subtype. Hum. Genet. 55:119-121 pp.

Cox D.W., Billingsley G.D., Mansfield T. 1987. DNA restriction site polymorphisms associated with the α1-antitrypsin gene. Am. J. Hum. Genet. 41:891-906 pp.

Crystal R.G. 1990. Alpha-1-antitrypsin deficiency, emphysema, and liver disease: genetic basis and strategies for therapy. J. Clin. Invest. 85:1343-1352 pp.

Crystal R.G. 1991. Alpha-1-antitrypsin deficiency: pathogenesis and treatment. Hospital Practice. 15:81-94 pp.

Faber J.P., Poller W., Weidinger S., Kirchgesser M., Schwaab R., Bidlingmaier F., Olek K. 1994. Identification and DNA sequence analysis of the 15 new alpha-1-antitrypsin variants, including two PI*Q0 alleles and one deficient PI*M allele. Am. J. Genet. 55:1113-1121 pp.

Fonseca-Pérez T., González-Coira M., Arias S. 1996. PI locus (alpha-1-antitrypsin) allelic frequencies in an Andean Venezuelan population. Gene Geogr. 10:65-74 pp.

Frants R.R., Noordhoek G.T., Eriksson A.W. 1978. Separator isoelectrofocusing for identification of alpha-1-antitrypsin (PiM) subtypes. Scand. J. Clin. Lab. Invest. 38:457-462 pp.

Gaillard M.C., Zwi S., Nogueira C.M., Ludewick H., Feldman C., Frankel A., Tsilimigras C., Kilroe-Smith T.A. 1994. Ethnic differences in the occurrence of M1(ala213) haplotype of alpha-1-antitrypsin in asthmatic and non-asthmatic black and white South Africans. Clin. Genet. 45:122-127 pp.

González-Coira M., Mora J.C., Sepúlveda S., Cuevas J., Pérez J., Román D., Velásquez N. 1998. Evolución temporal "aparente" de la frecuencia génica de los sistemas sanguíneos ABO y Rh en una población de Mérida, Venezuela. In: VII Congreso Venezolano de Genética. (Pineda, L., ed). Maracaibo: Av. Genét. VII:270 pp.

González-Coira M., Araque D., Fonseca-Pérez T. 2002. El locus PI (alfa-1-antitripsina) como estimador de mestizaje en dos poblaciones venezolanas. In: VII Congreso de la Asociación Latinoamericana de Antropología Biológica, Ciudad de México. 88 pp.

Gram A., Hayes K., Weidinger S., Newton C.R., Markham A.F., Kalsheker, N.A. 1990. Characterization of the alpha-1-antitrypsin M3 gene, a normal variant. Hum. Genet. 85:381-382 pp.

Gupta J., Bhadoria D.P., Lal M.K., Kukreti R., Chattopadhaya D., Gupta V.K., Dabur R., Yadav V., Chhillar A. K., Sharma G.L. 2005. Association of PIM3 allele of alpha-1-antitrypsin gene with chronic obstructive pulmonary disease. Clin. Biochem. 38(5):489-491 pp.

Guzdek A., Potempa J., Dubin A., Travis J. 1990. Comparative properties of human alpha-1-proteinase inhibitor glycosylation variants. FEBS. 72:125-127 pp.

Lahiri D.K., Nurnberger J.J. 1991. A rapid non-enzymatic method for preparation of HMW DNA from blood for RFLP studies. Nucl. Acid. Res. 19:5444 pp.

Lai E.C., Kao F.T., Law M.L., Woo S.L.C. 1983. Assignment of alpha-1-antitrypsin gene and a sequence-related gene to human chromosome 14 by molecular hybridization. Am. J. Hum. Genet. 35:385-392 pp.

Lohmann D., Hormsthenke B., Gillessen-Kaesbach G., Stefani F.H., Hoefler H. 1992. Detection of small RB1 deletions in retinoblastoma by multiplex PCR and high-resolution gel electrophoresis. Hum. Genet. 89:49-53 pp.

Long G.L., Chandra T., Woo S.L.C., Davie E.W., Kurachi K. 1984. Complete sequence of cDNA for human alpha-1-antitrypsin and the gene for S variant. Biochemistry. 23:4828-4837 pp.

López J.E. 1999. La emigración de la España peninsular a Venezuela, en los siglos XVI, XVII y XVIII. Caracas: Biblioteca de Autores y Temas Mirandinos en Coedición con el Consejo de Desarrollo Científico y Humanístico de la Universidad Central de Venezuela.

Meyer J.F., Bieth J., Metais P. 1975. On the inhibition of elastase by serum. Some distinguishing properties of alpha-1-antitrypsin and alpha-2-macroglobulin. Clinica Chimica Acta. 62:43-53 pp.

Nukiwa T., Brantly M., Ogushi F., Fells G., Satoh

K., Srier L., Courtney M., Crystal R.G. 1987. Characterization of the M1(Ala^{213}) type of alpha-1-antitrypsin, a newly recognized, common "normal" alpha-1-antitrypsin haplotype. Biochemistry. 26:5259-5267 pp.

Osorio E. F. 1996. Los Andes Venezolanos. Proceso social y estructura demográfica (1800 – 1873). Mérida, Venezuela: Universidad de Los Andes, Consejo de Publicaciones.

Perlmutter D.H., Code F.S., Kilbridge P., Rossaing I.H., Colten H.R. 1985. Expression of α_1-proteinase inhibitor gene in human monocytes and macrophages. Proc. Natl. Acad. Sci. USA. 82:795-799 pp.

Rocha J., Pinto D., Santos M.T., Amorim A., Amil-Dias J., Cardoso-Rodrigues, Aguiar A. 1997. Analysis of the allelic diversity of a (CA)n repeat polymorphism among α1-antitrypsin gene products from northern Portugal. Hum. Genet. 99:194-198 pp.

Seixas S., Mendoza C., Costa F., Rocha J. 2002. Alpha-1-antitrypsin null alleles: evidence for the recurrence of the L353fsX376 mutation and a novel G to A transition in position 1 of intron IC affecting normal mRNA splicing. Clin. Genet. 62:175-180 pp.

Turleau C., De Grouchy J., Chavin-Colin F., Dore F., Seher J., Dautzenberg M., Arthuis M., Jeanson C. 1984. Two patients with intersticial del(14q), one with features of Holt-Oram syndrome: exclusion mapping of PI(alpha-1-antitrypsin). Ann. Genet. 27:237-240 pp.

Weidinger S., Poller W., Faber J.-P., Schwarrzfischer F. 1985. Alpha-1-antitrypsin: evidence for a fifth PI M subtype and a new deficiency allele PI*Z(Augsburg). Hum.Genet. 71:27-29 pp.

Weidinger S. 1992. Reliable phenotyping of alpha-1-antitrypsin by hybrid isoelectric focusing in an ultranarrow immobilized pH gradient. Electrophoresis. 13:234-239 pp.