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Genetic Polymorphism of Protease Inhibitor (PI) in Korean Population

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The genetic polymorphism of Protease inhibitor (PI) in Korean population was investigated by using isoelectric focusing (IEF) in an ultra-narrow pH range, 4.2~4.9, and immunoblotting. Three common alleles (*PI* * *M1*, *PI* * *2*, *PI* * *M3*) were observed and the frequencies for the alleles were *PI* * *M1*=0.7843, *PI* * *M2*=0.1613, *PI* * *M3*=0.0323. In addition to the three common alleles, rare alleles (*PI* * *S*, *PI* * *Z*, *PI* * *N*) were detected at low-level frequency. Two unknown variants, which were not reported on previous studies in Korean population, were also found.

KEY WORDS: Protease Inhibitor, Polymorphism, Isoelectric Focusing, Korean Population

Human protease inhibitor (PI) or α_1-antitrypsin is normally present in serum at concentration of 200~220 mg/100 ml. PI is the major inhibitor of proteolytic enzymes and its serum concentration is increased in response to inflammation or estrogens.

It is synthesized in liver and has a molecular weight of 50 kDa~55 kDa consisting of a single polypeptide chain of 394 amino acids (Carrell *et al.*, 1981). Its gene has been assigned to human chromosome 14q (Cox *et al.*, 1982).

The PI phenotypes were classically determined by acid starch gel electrophoresis (Fagerhol and Braend, 1965). The PI phenotyping was extended by appliction of polyacrylamide gel isoelectric focusing (IEF) (Allen *et al.*, 1974). Using the IEF, more than 60 variants have been reported at the PI locus (Klasen and Rigutti, 1982; Thymann, 1986). The most common PI type is M and some variants like Z and S are correlated with an PI deficiency. An association of PI deficiency and chronic obstructive pulmonary disease was observed first in 1963 by Laurell and Eriksson. Low concentration of PI may be also associated with progressive liver disease in infants (Eriksson, 1965; Sharp *et al.*, 1969). The product of the Z allele, when present in the homozygous state, gives concentration equal to 15% of that of normal M allele (Laurell and Eriksson, 1963), and the S allele gives concentration equal to 60% of that of the normal M allele (Fagerhol, 1969).

The polymorphism of PI in Korean population has been previously studied by Oh (1986) using the IEF (pH 3.5~5) and Coomassie blue staining. However, there are no data of the study by using the IEF in an ultra-narrow pH range and immunoblotting. Therefore, we have studied the distribution of phenotypes and allele frequencies of PI in Korean population by using the method of IEF in the ultra-narrow pH range (pH 4.2~4.9) and immunoblotting. This method is highly resolvable enough to detect minute difference of electrophoretic band patterns.

Materials and Methods

Blood samples were collected from unrelated healthy individuals living in Seoul, Taejeon, and Kongju, Korea. The serum was prepared by

centrifugation the coagulated blood samples at 2,000 g for 10 min at 4°C. All samples were stored at -20°C until use.

For the PI typing, thin layered polycrylamide gel isoelectric focusing (pH 4.2~4.9) was performed by the method of Eap *et al.* (1988), and Kim (1989) with slight modification. 1M H_3PO_4 and 1N NaOH were used for the anode and cathode electrode solution, respectively. The samples were applied on gel suface with Whatman No. 3 paper (4 × 6 mm) at a distance of 1 cm from the cathode end of the gel. The sample applicators were removed after running at 1,000 V for 45 min and then a main running was continued at 1,400 V for 3 hrs. All experiments were conducted at 8°C.

The transfer of PI onto a nitrocellulose filter (Sigma, 0.45 μm) was carried out according to the method of Yuasa *et al.* (1985) and Kim (1989) with slight modifications. After completion of passive blotting, the filter was soaked in 1% BSA-PBS solution (pH 7.2) to block the remaining protein binding sites on nitrocellulose filter. Then the filter was incubated for 1hr with goat anti-PI antibodies as the 1st antibody (Sigma) diluted 1:500, and was incubated for 1hr with peroxidase conjugated anti-goat IgG antibodies as the 2nd antibody (Sigma) diluted 1:1000.

For the colar reaction, the filter was soaked in the mixture solution consisting of 25 ml of 0.05 M Tris-HCl buffer (pH 7.2), 5 mg of 3-3′-diaminobenzidine tetrahydrochloride (Sigma) and 10 μl of 30% H_2O_2.

Results and Discussion

Fig. 1 shows the immunospecific PI banding pattern obtained from IEF in pH range 4.2~4.9 followed by immunoblotting with polyclonal monospecific anti-human PI. A total of 12 different PI phenotypes containing two unknown variants were observed. *Unknown* I and *Unknown* II are located more cathodal than the Z band, as illustrated in Fig. 1.

The phenotypes distribution and allele frequencies of PI in Korean population is given in Table 1. In addition to the common *PI*M1*, *PI*M2*, and *PI*M3* alleles, *PI*N*, *PI*S*, *PI*Z*, and *Null* alleles were observed at low frequencies, but other rare alleles (*PI*4*, *PI*5*, and *PI*C*) which were identified in Cheju population (Oh, 1986) were not observed. The frequencies for the three common alleles were *PI*M1*=0.7843, *PI*M2*=0.1613, and *PI*M3*=0.0323. The distribution was in good agreement with Hardy-Weinberg equilibrium (χ^2=10.40, df=7, 0.10 < P < 0.20).

The allele frequencies of PI in various populations are shown in Table 2. There are some differences among the various ethinic groups. The differences may be partially due to the different

Fig. 1. PI phenotypes in Korean population represented by immunoblotting after isoelectric focusing (pH4.2~4.9). From left to right each lane showed: M1M2, Unknown I, M1, M1, M1, M1, Null, M1M2, M2M3, M3, M1M3, M1M2, M1, M1M2, M2, M1, M2M3, M1N, M1S, M1N, M1Z, Unknown II.

Table 1. PI phenotypes and allele frequencies in Korean Population.

Phenotypes	Observed No.	Expected No.	Allele Frequencies
M1M1	315	310.57	(Common alleles)
M1M2	126	125.49	*PI* * *M1*=0.7843±0.0131
M1M3	14	25.15	*PI* * *M2*=0.1613±0.0117
M2M2	12	14.68	*PI* * *M3*=0.0323±0.0056
M2M3	10	5.16	
M3M3	4	1.72	(Rare alleles)
M1N	6	4.66	*PI* * *N*=0.0060±0.0025
M1S	1	0.79	*PI* * *S*=0.0010±0.0010
M1Z	1	0.79	*PI* * *Z*=0.0010±0.0010
Null	5	5.01	*PI* * *Nu*=0.0101±0.0032
Unkown	2	1.98	*PI* * *Un*=0.0040±0.0020
Total	496		

χ^2=10.40, df=7, 0.10 < P < 0.20

Table 2. PI allele frequencies in the various populations.

Populations	No.	Allele frequencies							References
		PI * *M1*	*PI* * *M2*	*PI* * *M3*	*PI* * *M4*	*PI* * *S*	*PI* * *Z*	Variants	
Caucasoid									
Switzerland	1148	0.7121	0.1381	0.0979	-	0.0383	0.0113	0.0026	Bär & Kratzer, 1988
Netherlands	708	0.7210	0.1228	0.1124	-	0.0297	0.0049	0.0092	Klasen *et al.*, 1977
South Gemany	752	0.6894	0.1649	0.0904	0.0179	0.0173	0.0127	0.0074	Weidinger *et al.*, 1982
Sweden	1062	0.6940	0.1384	0.1139	-	0.0245	0.0231	0.0062	Hjalmarsson, 1988
US White	284	0.6808	0.1577	0.1013	0.0025	0.0485	0.0025	0.0067	DeCroo *et al.*, 1991
Mongoloid									
West Japan	1000	0.7065	0.2390	0.0480	0.0015	-	-	0.0050	Yuasa *et al.*, 1984
Korea*	496	0.7843	0.1613	0.0323	-	0.0010	0.0010	0.0201	The present study
Korea	984	0.7713	.01900	0.0208	0.0092	0.0041	0.0020	0.0026	Oh, 1986
Negroid									
US Black	235	0.9258	0.0367	0.0303	-	0.0050	-	0.0020	DeCroo *et al.*, 1991
Nigeria	114	0.9605	0.0176	0.0219	-	-	-	-	DeCroo *et al.*, 1991
Mali	102	0.9314	0.0245	0.0392	-	-	0.0049	-	Frants & Eriksson, 1978

*The total of Seoul (108), Taejeon (208), Kongju (180)

IEF methods previously published paper. As indicated in the Table, the most common allele is *PI* * *M1*. The Negroid populations have much higher *PI* * *M1* frequency ranging from 0.9258 to 0.9605. Mongoloid populations present intermediate value ranging from 0.7065 to 0. 7843. Caucasoid populatons show somewhat lower *PI* * *M1* frequency ranging from 0.6808 to 0.7210. The *PI* * *M1* frequency (0.7713~0.7843) in Korean population is higher than that of Japanese (0.7065). In this study, we could not detect *PI* * *M4*, which was found in Cheju (Oh, 1986) and Japanese (Yuasa *et al.*, 1984) population. *PI* * *Z* and *PI* * *S* alleles were present with polymorphic frequency in Caucasoid, but they were present with nonpolymorphic in Mongoloid. These 2 alleles have not observed in the black gene pool (Pascali *et al.*, 1986;

Vandeville *et al.*, 1974) and their sporadic occurrences in some native black and US black population were due to white admixture (DeCroo *et al.*, 1991). Two unknown variants which are identified in this study, were not reported at previous studies in Korean population. Therefore, further studies of Korean population are required to find out their exact distribution pattern.

References

Allen, R.C., R.A. Haeley, and R.C. Talamo, 1974. A new method for the determination of alpha-1-antitrypsin phenotypes using isoelectric focusing on polyacrylamide gel slabs. *Am. J. Clin. Path.* **62:** 732-739.

Bär, W. and A. Kratzer, 1988. Polymorphism of alpha-1-antitrypsin (Pi) in the Swiss population determined by isoelectric focusing with an immobilized pH gradient. *Hum. Hered.* **38:** 106-110.

Carrell, R.W., J.O. Jdppsson, L. Vaughan, S.O. Brennan, M.C. Owen, and D.R. Boswell, 1981. Human alpha-1-antitrypsin: Carbohydrate attachment and sequence homology. *FEBS Lett.* **2:** 461-467.

Cox, D.W., V.D. Markovic,and I.E. Teshima, 1982. Genes for immunoglobulin heavy chains and for α_1-antitrypsin are localized to specific regions of chromosome 14q. *Nature* **297:** 428-430.

DeCroo, S., M.I. Kamboh, and R.E. Ferrell, 1991. Population genetics of alpha-1-antitrypsin polymorphism in US whites, US blacks and African blacks. *Hum. Hered.* **41:** 215-221.

Eap, C.B., C. Cuendet, and P. Baumann, 1988. Orosomucoid phenotyping by use of immobilizied pH gradients with 8M urea and immunoblotting. *Hum. Genet.* **80:** 183-185.

Eriksson, S., 1965. Studies in alpha-1-antitrypsin deficiency. *Acta. Med. Scand.* **432:** 5-85.

Fagerhol, M.K. and M. Braend, 1965. Serum prealbumin: polymorphism in man. *Science* **149:** 986-987.

Fagerhol, M.K., 1969. Quantitative studies on the inherited variants of serum alpha-1-antitrypsin. Scand. *J. Clin. Lab. Invest.* **23:** 97-103.

Frants, R.R. and A.W. Eriksson, 1978. Reliable calssification of six PiM subtypes by separator isoelectric focusing. *Hum. Hered.* **28:** 201-209.

Hjalmarsson, K., 1988. Distribution of alpha-1-antitrypsin phenotypes in Sweden. *Hum. Hered.* **38:** 27-30.

Kim, H.S., 1989. Studies on the polymorphism of serum proteins in Korean population. Thesis for the degree of Ph. D. at Seoul Natl. Univ., Seoul.

Klasen, E.C., C. Franken, W.S. Volkers, and L.F. Bernini, 1977. Population genetics of α_1-antitrypsin in the Netherlands. *Hum. Genet.* **37:** 303-313.

Klasen, E.C. and A. Rigutti, 1982. Isoelectric focusing of α_1-antitrypsin (PI) using restricted pH-range carrier ampholytes in combination with a crosslinked gel and a separator. *Electrophoresis* **3:** 168-171.

Laurell, C.B., and S. Eriksson, 1963. The electrophoretic alpha-globulin pattern of serum alpha-1-antitrypsin deficiency. *Scand. J. Clin. Lab. Invest.* **15:** 132-140.

Oh, M.Y., 1986. Studies on the polymorphism of serum proteins in Cheju population of Korea. Thesis for the degree of Ph. D. at Seoul Natl. Univ., Seoul.

Pascali, V.L., D. Ranalleta, and G. Spedini, 1986. Antitrypsin and Gc polymorphisms in some populations of Congo: An unusual, highly frequent mutant, PI * S, in Black and Babenga. *Ann. Hum. Biol.* **13:** 267-271.

Sharp, H.L., R.A. Bridges, and W. Krivit, 1969. Cirrhosis associated with alpha-1-antitrpsin deficiency: A previously unrecognized inherited disorder. *J. Lab. Clin. Med.* **73:** 934-939.

Thymann, M., 1986. Distribution of alpha-1-antitrypsin (Pi) phenotypes in Denmark determined by separator isoelectric focusing in agarose gel. *Hum. Hered.* **36:** 19-23.

Vandeville, D., J.P. Martin, and C. Ropartz, 1974. α_1-antitrypsin polymorphism of a Bantu population: Description of a new allele PiL*. *Humangenetik* **21:** 33-38.

Weidinger, S., M. Cleve, and W. Patutschnick, 1982. Alpha$_1$-antitrypsin: evidence for a fifth PiM subtype and a new deficiency allele PI*$Z_{Augsburg}$. *Hum. Genet.* **71:** 27-229.

Yuasa, I., K. Suenaga, Y. Gotoh, K. Ito, N. Yokoyama, and K. Okada, 1984. Pi (α_1-antitrypsin) polymorphism in the Japanese: confirmation of PI * M4 and description of new Pi variants. *Hum. Genet.* **67:** 209-212.

Yuasa, I., T. Taira, K. Suenaga, K. Ito, and K. Okada, 1985. Determination of alpha2-HS-glycoprotein phenotypes by isoelectric focusing and immunoblotting: Polymorphic occurrence of AHSG * 5 in Okinawa. *Hum. Genet.* **70:** 32-34.

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한국인 집단에서 Protease Inhibitor(PI)의 유전적 다형

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한국인 집단에서 Protease inhibitor(PI)의 유전적 다형 현상을 등전점 전기영동(pH4.2~4.9)과 면역 블로팅 방법을 이용하여 연구하였다. 그 결과 3개의 흔한 대립 유전자(*PI*M1*, *PI*M2* 및 *PI*M3*)들과 드문 대립 유전자(*PI*N*, *PI*S* 및 *PI*Z*)들이 관찰되었다. 3개의 흔한 대립 유전자들의 빈도는 *PI*M1*=0.7843, *PI*M2*=0.1613, *PI*M3*=0.0323으로 나타났다. 또한 본 연구에서 한국인을 대상으로 한 지금까지의 연구에서는 알려지지 않았던 2개의 변이형을 발견하였다.