α-1 Antitrypsin phenotypes by isoelectric focusing in a metropolitan southern Chinese population

S S Lee, J W M Lawton, K H Ko, K M Lam, C K Lin

Abstract

***Aims/Background*—α-1 Antitrypsin (α1AT) is an abundant protease inhibitor in human plasma. Its phenotypic variability has been reported to be associated with pulmonary emphysema and chronic liver diseases. However, α1AT deficiency is an uncommon condition in the Chinese population. The aim of this study was to describe the phenotypic distribution of α1AT in a southern Chinese population.**

***Methods*—A total of 1085 healthy blood donors underwent α1AT phenotyping by isoelectric focusing.**

***Results*—Two thirds (66.1%) were homozygous for either M1 or M2, whereas 32.6% were heterozygous for two different M phenotypes. The frequency of allelic variants was only 0.007, and deficiency variants were absent. Compared with earlier studies on southern Chinese populations, this study found a lower frequency of M2, and a higher number of allelic variants, including E, L, N, P, and S. This phenomenon can be attributed to population migration and mixing.**

***Conclusions*—An understanding of the α1AT pattern is important for evaluating the predisposition of the population to selected clinical diseases.**

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Keywords: α-1 antitrypsin; isoelectric focusing; phenotypes

α-1 Antitrypsin (α1AT) is the most abundant protease inhibitor in human plasma. Encoded by the gene locus PI on chromosomal segment 14q32.1, this 52 kDa proteinase can exist as a wide range of variants. The variability of α1AT has attracted the attention of clinical scientists, biochemists, and anthropologists. For example, deficiency mutants have long been shown to be associated with specific clinical entities such as chronic obstructive airway diseases, cirrhosis, and primary liver cancer.[1] More recent studies reported a relation between variant alleles and antineutrophil cytoplasmic antibody (ANCA) associated diseases.[2 3] In addition, the delineation of α1AT variant patterns has contributed to the understanding of population movement and mixing.[4 5] Therefore, the determination of the phenotypic distribution of α1AT is important in analysing the predisposition of a population to disease conditions. This is, understandably, a dynamic situation that can evolve with population changes over time.

We set out to establish the distribution of α1AT phenotypes in healthy adults in Hong Kong, a dynamic southern Chinese society with a high degree of human mobility. The results are discussed in association with reported results in mainland China and other Asian communities.

Materials and methods

SUBJECTS

Blood samples of healthy Chinese adults were obtained from unselected consecutive blood donors at the Hong Kong Red Cross Blood Transfusion Service. These samples were aliquoted and kept at −70°C before assays.

ISOELECTRIC FOCUSING

Isoelectric focusing was used for investigating the α1AT patterns, which were evaluated on stained gels, and confirmed by immunoblotting. Briefly, all serum specimens were treated with cysteine to reduce and block the active thiol group in α1AT. Thin layer acrylamide gels were prepared on GelBond PAG film (Amersham Pharmacia, UK) using the "SDS and native PAGE, IEF kit" (Amersham Pharmacia). A 5 ml aliquot of 30% (wt/vol) acrylamide was mixed with 0.8% bisacrylamide (Sigma, USA), 10.8 ml 25% (vol/vol) glycerol, 2.25 ml pharmalyte, pH 4.2–4.9 (Amersham Pharmacia), and 0.3 ml of 20 mg/ml ammonium persulphate (Sigma) in 11 ml distilled water, which was de-gassed before 20 µl TEMED (Sigma) was added. The mixture was poured on to the gel setting kit, and isopropanol was carefully overlayed on top of the gel solution to prevent oxidation. Polymerisation was completed after one hour, after which the GelBond PAG film supported gel was transferred to a Multiphor II IEF apparatus (Amersham Pharmacia), and cooled to 10°C by an external cryostat MultiTemp II (Amersham Pharmacia). The electrode strips were saturated with 0.04M DL-glutamic acid at the anode, and 0.1M NaOH at the cathode. The gel was prerun for 30 minutes with a constant power supply EPS3500 (Amersham Pharmacia) at 3000 V, 150 mA, 5 W.

The IEF sample application strip (Amersham Pharmacia) was used for the dispensing of cysteine treated serum samples. The gel was run at 3000 V, 150 mA, 15 W for 2.5 hours. The gel was fixed for 30 minutes in fixing solution (10% trichloracetic acid, 5% sulphosalicylic acid), followed by immersion for 30 minutes in destaining solution I (35% methanol, 10% acetic acid). The gel was then stained with 0.2% (wt/vol) filtered Coomassie blue R250 (Sigma) in destaining solution I. This was followed by immersion in destaining solution I until the gel background became clear. The gel was placed in destaining solution II (16% methanol, 7% acetic acid, 9% glycerol) for one hour, and then layered with a cellophane sheet (Pharmacia) and air dried.

Department of Pathology, The University of Hong Kong, Hong Kong Special Administrative Region, China
S S Lee
J W M Lawton
K H Ko
K M Lam

Hong Kong Red Cross Blood Transfusion Service, Hong Kong Special Administrative Region
C K Lin

Correspondence to:
Dr Lee, Department of Microbiology, The University of Hong Kong, Pokfulam Road, Hong Kong
microssl@hkucc.hku.hk

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Table 1 Frequency of α1 antitrypsin phenotypes in healthy Chinese adults

Type/subtypes	*Variants*	*Number*	*Frequency (f)*
Homozygous M	M1 M1	649	0.598
n = 717, *f* = 0.661	M2 M2	68	0.063
	M3 M3	0	0.000
	M4 M4	0	0.000
Heterozygous M	M1 M2	312	0.288
n = 353, *f* = 0.326	M1 M3	11	0.010
	M2 M3	30	0.028
Other heterozygous	M1 S	3	0.003
n = 15, *f* = 0.015	M2 S	1	0.001
	M1 E	4	0.004
	M1P	4	0.004
	M1L	2	0.002
	M2 N	1	0.001
Total		1085	1.000

Table 2 Frequency of α1 antitrypsin alleles in healthy Chinese adults

Allele	*Number*	*Frequency*
M1	1634	0.753
M2	480	0.221
M3	41	0.019
M4	0	0.000
S	4	0.002
Z	0	0.000
E	4	0.002
P	4	0.002
L	2	0.001
N	1	0.000
F	0	0.000
Total	2170	1.000

For confirmation of the patterns, serum proteins were transferred from the gel to a nitrocellulose membrane (Schleicher and Scheull, Germany). The gel and membrane were soaked in phosphate buffered saline (PBS), followed by the transferal of the membrane to another tank. The membrane was treated with blocking buffer (PBS with 1% bovine serum albumin, 0.05% Tween 20) for 30 minutes at room temperature, and then allowed to react with rabbit antihuman α1AT (Dako, Glostrup, Denmark; 200 µl/ml diluted 1/400 in blocking buffer) for one hour at room temperature. After washing four times with PBS in 0.05% NP40, the membrane was incubated overnight at 4°C with horseradish peroxidase labelled goat antirabbit IgG (Dako; 200µl/ml, diluted 1/1000 in blocking buffer). Washing was repeated and the bands were developed with 0.06% 4-chloro-1-naphthol, 0.02% H_2O_2 in PBS for 10 minutes. The reaction was stopped by rinsing with running tap water, after which the membrane was blotted dry and the α1AT patterns read.

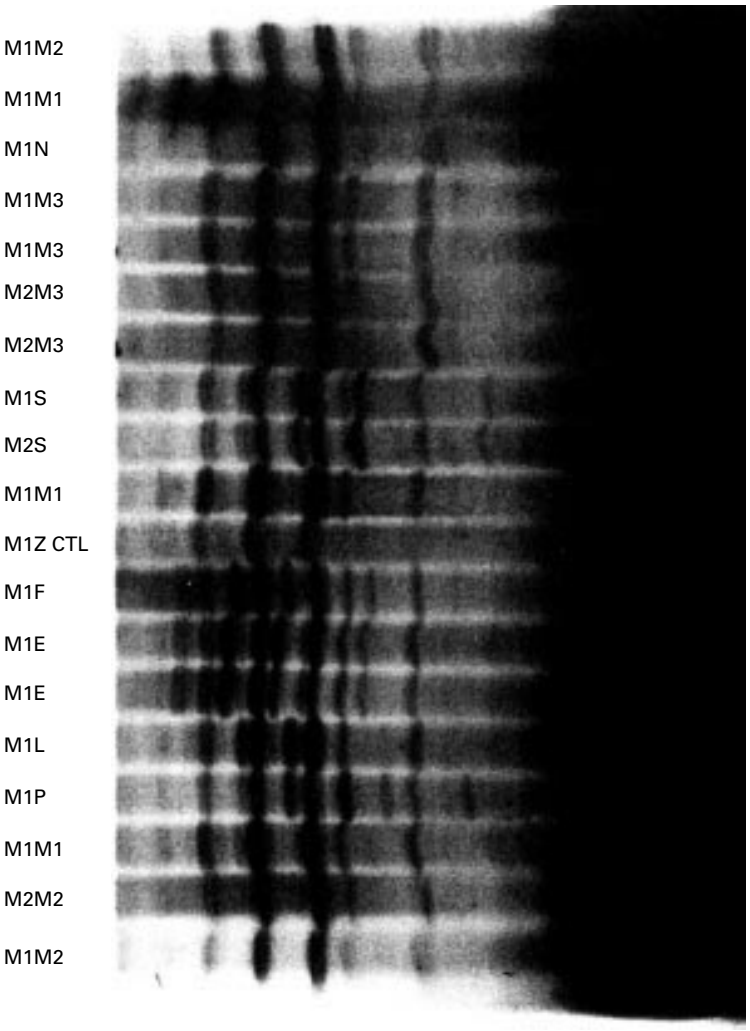

Figure 1 The isoelectric focusing patterns of selected subjects (staining with Coomassie blue). CTL, control.

Results

We tested 1085 Chinese adults for α1AT using isoelectric focusing. The male to female ratio was 1.5 : 1, with a mean age of 30.8 years (SD, 8.6; range, 14 to 59). Three categories of phenotypes were found: (1) homozygous for one of the M alleles, (2) heterozygous for two different M alleles, and (3) heterozygous for M and a variant allele (table 1). Overall, 717 (66.1%) were homozygous for the M phenotype—either M1 homozygotes (59.8%) or M2 homozygotes (6.3%). There were no M3 or M4 homozygotes. Heterozygous M phenotypes accounted for 32.6% of the total. The rest were heterozygous for M and a variant allele (1.5%) and none was homozygous for any variant.

The allelic frequencies of M1, M2, M3, and M4 were 0.753, 0.221, 0.019, and 0.000, respectively (table 2). Five types of allelic variants were identified—E, L, N, P, and S, accounting for only 0.7% of all alleles. Figure 1 shows the isoelectric focusing patterns of selected subjects.

Discussion

Our study offers a unique opportunity to examine the α1AT patterns in a group of healthy southern Chinese adults in Hong Kong. It is an international city and, in the presence of extensive human movement, it was envisaged that the pattern might differ from patterns reported in the literature. Analysis of two aspects can be made, namely: the distribution of normal M variants and the detection of other allelic variants.

In our study, the most common M allele was M1, followed by M2 and then M3, whereas M4 was absent. A previous study on Chinese people suggested that M2 was more common in the south than in the north.[6] They reported a frequency of 0.27 in Haikou, a southern city, compared with 0.16 in Beijing, the capital city

in the north. The allelic frequency of 0.22 for M2 in our study lies midway between the two extremes, reflecting the possibility of extensive population mixing in Hong Kong. The absence of M4 in Hong Kong is not entirely surprising, because the frequency even in Beijing was very low, at only 0.0018.

α1AT deficiency variants are uncommon in Chinese and black populations. The allelic frequency of Z could be over 0.2 in Scandinavian populations, somewhat lower in other Europeans, but zero in China and Japan.[7] An extremely rare form of frameshift mutation designated $null_{Hong\ Kong}$ has been described in a family of Chinese descent.[8] On the other hand, S is prevalent in Spanish and Portuguese populations but is uncommon in Chinese. We report an allelic frequency of 0.0018 for S but zero for Z. This is similar to the frequencies 0.0005 for both S and Z in another recent study on a southern Chinese population, using multiplex polymerase chain reaction for genotyping.[9] This observation contrasts with the invariably negative finding reported in earlier studies,[10] and could again reflect the phenomenon of population mixing between Chinese and other ethnic groups. Another variant S_{iiyama} has been described in Japan. Its relevance in the Chinese population is not known.

F and I are non-deficiency variants commonly described in white populations. In our study, these are absent. Instead, we detected two anodal variants (E, L) and two cathodal variants (N, P). E (notably E_{tokyo}), L ($L_{beijing}$), and P (P_{weishi}) have been described in other Chinese series,[6 10] although the frequency was very low. E_{tokyo} was the more common variant in Chinese, with an allelic frequency of 0.0052, compared with that of 0.0018 for E in our study. The N variant is reported for the first time in a Chinese population, at a frequency of 0.00046. It is not associated with protease deficiency. Its relevance with regard to population movement in Chinese is not known.

In summary, our study confirms the rarity of α1AT deficiency in ethnic Chinese. However, the allelic pattern described in our current study demonstrates some interesting features. First, the differentiation between north and south could become blurred with time when there is much population movement and mixing. Second, there is a higher prevalence of variants in Hong Kong, although the overall frequency has remained low. Finally, understanding the α1AT pattern is important for the evaluation of, not only genetic deficiency diseases, but also genetic predisposition to other clinical entities, such as vasculitides. In assessing the importance of these variant alleles, the study is limited by its application of isoelectric focusing alone as the method for delineating the α1AT phenotypes. Microheterogeneity needs to be evaluated by additional studies using crossed immunoelectrophoresis and the application of molecular biology techniques.

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