alpha-Antitrypsin (A1AT) is a serine protease inhibitor required for the prevention of proteolytic tissue damage, principally in the lung, by neutrophil elastase released by inflammatory cells [1]. While severe A1AT deficiency is the major factor leading to emphysema and related pulmonary diseases, it is also associated with neonatal hepatitis and cirrhosis [1, 2]. A1AT deficiency is an autosomal codominant disorder with a prevalence of about 1:3000 in Caucasians [3]. The A1AT gene has 7 exons spanning ~12 kb. The most common gene defect resulting in A1AT deficiency is that of a protease inhibitor (PI)-system Z mutation Glu342 to Lys, which is a single base substitution [4].

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BRL. After an initial denaturation at 94 °C for 5 min, a 35-cycle PCR program was carried out on a Perkin-Elmer (Norwalk, CT) thermal cycler: 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min; final extension at 72 °C for 10 min. After PCR, the restriction digestion mixture was prepared: 10 µL of PCR product, 1 U of Taq I restriction endonuclease (Gibco BRL) [12], and buffer to a final volume of 15 µL. Restriction digestion was completed after 2 h of incubation at 65 °C.

The digested PCR products were analyzed by 3% agarose electrophoresis at constant voltage of 200 V for 1 h. Control samples of known genotype were electrophoresed in each gel along with a size calibration mixture. For fast screening, 0.2 µg of DNA from five individuals were added to a PCR mixture for amplification and subsequent Taq I restriction digestion. When an abnormal result appeared, the individual DNA specimens were analyzed separately to identify the DNA sample carrying the mutant allele. To validate this protocol, all the DNA specimens in this study were analyzed individually and in groups of five. Identical results were obtained.

We obtained DNA specimens from 2005 unrelated Chinese subjects free of primary lung and liver diseases. Two individuals were found to be heterozygous for Z, i.e., MZ, and another two heterozygous for S, i.e., MS (Fig. 1). No SS, SZ, or ZZ were found. There were therefore two Z and two S alleles from a total of 4010 alleles, giving a frequency of 0.05% for both the Z and S mutations and 0.1% for the MZ and MS genotypes in the Chinese population. Combined with simultaneous phenotyping observation that showed absence of other mutations, our study suggests that 99.8% of the Chinese are of the MM genotype.

Such a dominance of the MM genotype in Chinese is unexpected, although people of Asian origin are known to have fewer Z mutations than Caucasians. While northern Europeans have a higher prevalence of the Z genotypes than southern Europeans, the Z and S mutations range from 1% to 4% and from 5% to 10% respectively in most Caucasian populations [7, 13]. In the Chinese, even the MZ and MS heterozygotes are less prevalent, at 0.5%, similar to the SS or ZZ homozygotes, at 0.25% and 0.3% respectively, of the British population [13]. To ascertain the association between the Z mutation and A1AT phenotypes in the Chinese population, we are analyzing the genotypes and phenotypes of normal Chinese subjects and of patients with emphysema. Meanwhile, we have shown our protocol of a double PCR with 5 DNA templates to be rapid, reliable, and economical for screening a large number of specimens directly for the Z and S mutations. There is, however, a limitation in this approach of batch analysis. If a single DNA sample in the batch of five samples failed to amplify by PCR, it would not be recognized. If this sample happened to be from a patient with a mutation, it would have been missed. In our individual analysis of 2005 samples, we did not have any sample that failed to amplify, showing the PCR protocol to be robust.

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References