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Frequency of Genetic Polymorphism of the Gene Encoding 16kDa Clara Cell Secretory Protein (CC16) in Shiraz, Iran

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Abstract

The Clara cell protein (CC16) is a small and readily diffusible protein of 16 kDa secreted by bronchiolar Clara cells in the distal airspaces. Mutation detection methods identified an adenine to guanine substitution in the CC16 gene at position 38 (A38G) downstream from the transcription initiation site within the non-coding region of exon 1. In the present study, the genetic polymorphism of CC16 was detected by PCR-based method in 175 normal individuals from Shiraz population, Fars province (south of Iran). Initially the subjects were divided into two sex groups. Considering that there was no statistically significant differences between males and females ($\chi^2 = 5.52$; df = 2; $P<0.05$) the sex groups were pooled. The frequencies of 38A and 38G alleles were 0.24 and 0.76 percent, respectively. The study population was at Hardy-Weinberg equilibrium ($\chi^2 = 2.61$; df = 1; $P<0.05$). The present results indicated that this polymorphism might have a geographic distribution.

Keywords: *CC16, Polymorphism, Shiraz, Iran*

Introduction

The Clara Cell is one of the most heterogeneous and multi-functional cell types of the mammalian lung, showing a great interspecies variability in abundance and spatial distribution. One of the major proteins secreted by Clara cells is the 16kDa Clara Cell protein (CC16) (1). Even though the exact in vivo function of the CC16 remains to be clarified, there is growing evidence that this protein plays a protective role against pulmonary inflammatory response(2, 3). Interspecies homologus of the CC16 are reffered in the literature by different names which allude to their origin [human protein 1, urine protein-1, uteroglobin (UG), Clara cell secretory protein (CCSP)]. The human CC16 first identified in urine of patients with renal failure and purified later from lung lavage (3). The amino acid sequence of CC16 presents a high similarity with CC16 from other mammalian species (monkey, rodents), indicating a high level of phylogenic conservation and suggesting a physiological importance (4).

The gene encoding CC16 is localized to chromosome 11q13, a region occupied by several genes involved in the regulation of allergy and inflammation (5). CC16 is a potent natural immuno-suppressor and anti-inflammatory agent. In vitro, CC16 inhibits both monocytes and polymorphonuclear neutrophils chemotaxic and phagocytosis (2). Mice deficient in CC16 expression exhibit a higher susceptibility to oxidant lung injury and an excessive inflammatory response (6, 7).

Mutation detection methods identified an adenine to guanine substitution in the CC16 at position 38 (A38G) downstream from the transcription site within the non-coding region of exon 1. It is suggested that the 38G allele is more likely to be the wild type; and the 38A

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allele is the mutant type (8). This polymorphism was associated with an increased risk of asthma in populations of Australian (8) and Iranian (9). However, studies on populations of Japanese and British adults (5) and North American children (10) did not replicate these associations.

The distribution of serum proteins, blood groups, and red cell enzymes in Iranian populations has been studied by different investigators (11-13). Very recently we had reported the frequencies of some genetic polymorphisms in Shiraz population using DNA analysis (14-16). In order to get more insight into the genetic structure of Iranian populations the present study was done.

Materials and Methods

A total of 175 healthy individuals (91 males, 84 females) were studied (mean age 28.5 ±3.1 years, range from 18 to 48 years). The studied group was unrelated Iranian Muslims from Shiraz, Fars Province (South of Iran).

Blood samples were obtained from subjects. Immediately after collection, whole blood was stored at-20^{o} C until use. Genomic DNA for PCR was isolated from whole blood using the thawed blood samples by standard procedure (17). The PCR method for determining the CC16 polymorphism was the same as that reported previously (8). The primers for amplifying the CC16 gene segment corresponding to exon 1 were 5'CAGT ATCTTATGTAGAGCCC3' and 5'CCTGAGAGTTCCTAAGTCCAGG3'.

The PCR was performed in 50µl reaction buffer containing 200 µM dNTPs, 1.5 µM $MgCl_2$, 1µM primers, about 1 µg DNA and 2 units of thermo-stable *Tag* DNA polymerase using a programmable thermo-cycler (Progene, Techne, England). After 5 min of pretreatment at 94^{o}C, 25 cycles of 1 min denaturation at 94 ^{o}C, 1 min annealing at 58^{o}C and 1 min extension at 72 ^{o}C were performed. Amplification products were digested with *Sau96I*. Restriction digestion of amplified DNA samples showed three altered digested patterns identifying heterozygous and homozygous subjects for both 38A and 38G alleles. Genetic polymorphism was defined as AA (absence of restriction site on both alleles), GG presence of restriction site on both alleles) or AG (heterozygous).

For evaluating the genetic polymorphism the digested DNA samples were analyzed by gel electrophoresis (2.0% agarose). To test for contamination, negative controls (tubes containing the PCR mixture, without the DNA template) were incubated in every run.

Allelic frequency was calculated using counting method. The Chi-square test was applied. The p-value less than 0.05 was considered statistically significant.

Results

Initially the subjects were divided into two sex groups (Table 1). There was no statistically significant differences between males and females (χ^2 = 5.52; df = 2; P<0.05). Because the gene encoding CC16 was located to human chromosome 11q13 (5), the genetic polymorphism of CC16 inherited as an autosomal trait. Therefore the sex groups were pooled. The allelic frequency of 38A and 38G was 0.24 and 0.76 percent, respectively. The study population was at Hardy-Weinberg equilibrium (χ^2 = 2.61; df = 1; P<0.05).

Table 1: Frequencies of CC16 genotypes in Shiraz population

Genotype	Male	Female	Total
AA	9	5	14
AG	35	21	56
GG	47	58	105
Total	91	84	175

Discussion

This polymorphism did not show a significant difference between Australian (8), British (5), and Japanese (5) populations. Table 2 shows

the allelic frequencies of CC16 in the other three populations. However, the frequency of 38A in Shiraz population was significantly lower than that of reported from Australian, British, and Japanese populations. However, the present study showed that there is a similarity between Iranian and Turkish populations (18). It should be noted that Iranian and Turkish populations showed several similarity and showed several differences for polymorphic markers (11, 14-16). Because there is no data about the frequency of 38A allele in other Asian and European populations, it is very difficult to interpret the present data. However, it may be suggested that the 38A allele has a geographical distribution.

Table 2: Distribution of the 38A and 38G alleles of CC16 in the other populations

Population	38A	38G	Number of samples	Reference
Australian	33	67	266	8
British	38	62	150	5
Japanese	39	61	100	5
Turkey	26	74	55	18
Iranian	24	76	175	Present study

Note: The allelic frequencies were expressed as percent.

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References

1. Bernard A, Dumont X, Roles H, Lauwerys R, Dierynck I et al (1993). The molecular mass and concentrations of protein 1 or Clara cell protein in biological fluids: a reappraisal. *Clin Chim Acta*, 223: 189-91.
2. Milele L, Cordella-Miele E, Mukherjee AB (1987). Uteroglobin: Structure, molecular biology and new perspective on its function as a phospholipase A2 inhibitor. *Endocrine Rev*, 8: 474-90.
3. Singh G, Singh J, Katayal SL, Brown WE, Kramps JA, Paradis IL, Dauber JH, Macpherson Ta, Squeglia N (1988). Identification, cellular localization, isolation and charatrization of human Clara cell-specific 10 KDa protein. *J Histochem Cytochem*, 36:73-80.
4. Hashimoto S, Nakagawa K, Sueishi K (1996). Monkey Clara cell 10 KD1 protein (CC10): a characterization of the amino acid sequence with an evolutional comparison with humans, rabbits, rat, and mice. *Am J Respir Cell Mol Biol*, 15:361-66.
5. Gao PS, Mao XQ, Kawai M, Enomoto T, Sadaki S et al (1998). Negative association between asthma and variants of CC16 (CC10) on chromosome 11q13 in British and Japanese populations. *Hum Genet*, 03: 57-9.
6. Johston CJ, Mango GW, Finkelstein JN, Stripp BR (1997). Altered pulmonary response to hyperoxia in Clara cell secreatory pritein deficient mice. *Am J Respir Cell Mol Biol,* 17:147-55.
7. Mango GW, Johanston CJ, Reynolds SD, Finkelstein JN, Plopper CG, Stripp BR (1998). Clara cell secretory protein deficiency increases oxidant stress response in conducting airways. *Am J Physiol,* 275: L348-L356.
8. Liang IA, Goldblatt J, Eber E, Hayden CM, Rye PJ, et al (1998). A polymorphism of the CC16 gene is associated with an increased risk of asthma. *J Med Genet*, 35: 463-67.
9. Saadat M, Saboori Z, Emad A. Saadat I (2004). Combination of CC16, GSTM1 and GSTT1 genetic polymorphism is associated with asthma. *J Allergy Clin Immunol*, 113: 996-98.
10. Bakhu M, Graves PE, Erikson RP (1998). Lack of association between a polymor-

phism of the CC16 gene and increased susceptibility to asthma (abstract). *Am J Pespir Cri Care Med,* 157: A771.

11. Amirshahi P, Sunderland E, Farhud DD, Tavakoli SM, Daneshmand P, Papiha SS (1992). Population genetics of the peoples of Iran. I. Genetic polymorphisms of blood groups, serum proteins and red cell enzymes. *Inter J Anthrop*, 7: 1-10.
12. Saadat M, Amirshahi P, and Farhud DD (1997). ABO and Rh blood groups distribution in the populations of Larestan and Lamerd, Fars province, Iran. *Iranian J Publ Health,* 25: 21-26.
13. Saadat M, Amirshahi P, and Farhud DD (1999). [Genetic drift in populations of Larestan Fars provience, Iran]. *Iranian J Publ Health*, 28: 39-46. (In Persian).
14. Kamkar M, Saadat M, Saadat I, Haghihi G (2003). Report of VNTR with 13 repeats linked to phenylalanine hydroxylase locus in unaffected members of two PKU families. *Iranian Biomed J,* 7: 89-90.
15. Saadat M, Farhud DD, Saadat I (2001). Frequency of glutathione S-transferase M1 (GSTM1) and GSTT1 null genotypes in Fars population (south of Iran). Iranian *J Publ Health*, 30: 83-6.
16. Saadat M, Kamkar M, Mohabbatkar H, Saadat I (2003). High frequency of IVS10nt546 linked to VNTR8 in Iranian PKU patients from Fars Province. *Iranian Biomed J,* 7: 145.
17. Newton CR (1995). Mutational analysis: Known mutations. In: *PCR2*. *A practical approach*. Eds, McPherson MJ, Hames D and Taylor GR. IRL-Press, Oxford. PP: 219-22.
18. Kalyoncu AF, Karakaya G, Yilmaz E, Balci B, Karaduman A, Yasavul U (2003). Analgesic intolerance with or without bronchial asthma: is there a marker? *J Invest Allergol Clin Immunol*, 13: 162-69.