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Identification of polymorphic variants of modifier gene B2AR (ADRB2) in patients with cystic fibrosis

ENILDA RRAPAJ[1], ETHEM RUKA[2] AND GRIGOR ZORAQI[3]

[1]Faculty of Natural Sciences, Tirana;

[2]Department of Molecular Biology, Faculty of Natural Sciences, Tirana;

[3]Center of Molecular Diagnosis and Genetic Research, University Hospital of Obstetrics and Gynecology "Queen Geraldine", Tirana.

*Corresponding author e-mail: rukaethem@gmail.com

Abstract

Mutations of CFTR gene are identified as the molecular basis of cystic fibrosis (CF) disease, but there are also other "modifier" genes, that influence the pathogenesis of the patients with CF. One such modifier genes is β2-adrenoreceptor gene (ADRB2). The aim of this study is the identification of polymorphic variants of this gene, in the locus Gln27Glu, in Albanian CF patients, in order to elucidate possible relations with clinical signs of the patients. We analyzed 47 patients with CF, which have the same genotype for CFTR gene, delF508 homozygous. DNA was extracted using standard methods from 5 ml of blood samples with EDTA. To identify genetic variants of this gene we used AS-PCR method and gel electrophoresis. Calculation of allele frequencies was done according to formulas in genetics of population.The method used for this study (AS - PCR) was very effective, with high sensitivity and low cost. We found out that in Gln27Glu locus, the frequency of Gln27 and Glu27 alleles were 0,62 and 0,38 respectivily.After genotyping ADRB2 gene in locus 27 for each patient, we defined groups of patients with the same genotype. In future, we will use subgroups of CF patients with the same genotypes for ADBR2 gene, in order to analyze the relationship between genotypes Gln27Glu and clinical parameters of CF disease. Identification of polymorphic variants of B2AR modifier gene in CF patients, could be useful in predicting clinical status of the patients and can help to perform differentiated treatments.

Keywords:B2AR gene, AS-PCR, allele frequency, Gln27Glu, CF-cystic fibrosis

1. Introduction

Cystic fibrosis is a common autosomal recessive disorder that primarily affects the epithelial cells in intestine, respiratory system, pancreas, gall bladder and sweat glands [9]. CFTR gene encodes for a chloride channel in the apical membrane of epithelial cells. Absence or abnormal function of this protein leads to the recessively inherited disease, cystic fibrosis (CF). Of the 1500 or so mutations which have been identified in CFTR, by far the most common is a deletion of phenylalanine at position 508 (ΔF508), accounting for approximately 70% of CF chromosomes in Albanian patients[8,14].

Despite CFTR itself, there are other factors which affect phenotype. Based on the understanding of the sequence events in cystic fibrosis, modifiers might include non-CFTR ion channels or genes involved in host defense, inflammation, epithelial repair, mucus production and airway responsiveness [3]. ADRB2 are important regulators of cAMP in the airway. Recent *in vitro* data demonstrated that ion transport via cAMP-dependent CFTR can be activated by β2 agonists [11,1,12].ADRB2 have been found in a complex with CFTR and ezrin/radixin/moesin-binding phosphoprotein 50 (EBP50), and ADRB2 and CFTR have been co-localized at the apical membrane [7,11]. An increase in β2-AR activity would be predicted to result in an increase in CFTR activity via effects on cAMP. Such findings provide a theoretical rationale to consider genetic variants of the β2-AR as gene modifiers in CF[11].

Buscher et al. reported worse function and decline in individuals who carried a Gly/Gly or Arg/Gly genotype at codon 16 or a Glu/Glu or Gln/Glu genotype at codon 27. In addition, Gly16 was significantly less common in the CF population than in several groups of healthy controls, possibly implying a survival disadvantage. Busher et al.found an increase in the frequency of the Arg16 allele compared with controls in a young population (average age 13 years old), whereas Hart et al. found

no differences in allelic frequencies for either codon 16 and 27 compared with controls[1,2].Considering this data,we propose this studywith the aim to investigate the role of different beta-2 AR polymorphisms in Albanian CF patients in order to find out any influence of the β2-AR as a modifier gene in cystic fibrosis disease.

First of all, we propose to genotype Gln27Glu variants of ADRB2 gene, in a sample of Albanian CF patients (mean age about 10 years old) identified previously by genetic screening of Cystic Fibrosis as ΔF508/ΔF508 genotype. In this paper we show preliminary results on genotyping ADBR2 gene, Gln27Glu variants, on CF Albanian patients.

2. Materials and methods

2.1 CF patients group.

Our study group was constituted of 47 CF patients under treatment in the Center of Cystic Fibrosis, at Pediatric Hospital "SelaudinBekteshi", in Tirana and were identified previously as ΔF508/ΔF508 genotypes, by the Center of Molecular Diagnosis and Genetic Research, at University Hospital of Obstetrics and Gynecology "Mbreteresha Geraldine", in Tirana. DNA samples from CF patients were conserved at - 20^0C and were used for the analysis of Gln27Glu polymorphisms of ADRB2 gene. The samples of CF patients were taken from a population with an average age of 7 years old.

2.2 DNA extraction from blood.

Genomic DNA was extracted from 5 ml blood using EDTA as anticoagulant by standard methods[5].

2.3 Genotyping of ADBR2 polymorphism: AS-PCR (Allele Specific - Polymerase Chain Reaction).

The polymorphisms of ADRB2 gene was assigned by an allele-specific approach: a modification of PCR, that depends on the synthesis of a PCR oligonucleotide primer that precisely matches with one of the alleles but mismatches with the other. Performing two PCR reactions on every DNA sample, using two different primer pairs,gives us the possibility to determine directly the genotypes of the individuals according to the results of PCR [13].

The primer pairs used for detection of two polymorphisms at nucleic acid 79 (aminoacid 27, ADBR2-27), Gln27 and Glu27, respectively, are 5'-GGA CCA CGA CGT CAC GCA AC -3' and 5'-GGA CCA CGA CGT CAC GCA AG-3'. The reverse primer is the same 5'- TGA TGA AGT AGT TGG TGA CC-3'. The size of the PCR product is 192 bp long for β2AR-27. Additional PCR primer pair, 5'-GAA CTG CCA CTT CAG CTG TCT-3' and 5'-CAG CTG CAT TTG GAA GTG CTC-3', were used to amplify a 320-bp DNA fragment of CYP1A1 gene, which serve as internal positive control of PCR reactions. PCR reactions were carried out in a volume of 50µl. A modification of PCR program was done as follow: an initial denaturation step at 94 ºC for 10 minutes, following by 40 cycles of 94 ºC for 60s, 57 ºC for 60s and 72 ºC for 60s, with a final extension step of 7 min at 72 ºC.

2.4 Identification of Gln27Glu genotypes by gel electrophoresis of AS-PCR products

The PCR products were run on 2% agrose gel electrophoresis in TBE buffer and visualized with ethidiumbromide staining and ultraviolet illumination. All genotype assignments were made without knowledge of any clinical variables. Genotype determination was done according to PCR products. We identify a heterozygote genotype when two PCR products were present for each sample, and a homozygote genotype when only one PCR product was present.

We calculate the allele frequencies of ADBR2 polymorphism at locus 27 of 47 CF patients after we have determined the genotypes by AS-PCR. Allele frequencies were calculated according to the standard formula used in population genetics.

2.5 Testing of Hardy-Weinberg equilibrium.

We tested the deviations from Hardy-Weinberg equilibrium of the ADRB2 genotype frequencies of Glu27Gln locus in our sample using Chi-Square test.

3. Results and discussions

In Fig.1, are represented the results of gel electrophoresis of AS-PCR reactions of 10 DNA samples, showing amplified fragments in both PCR reactions.

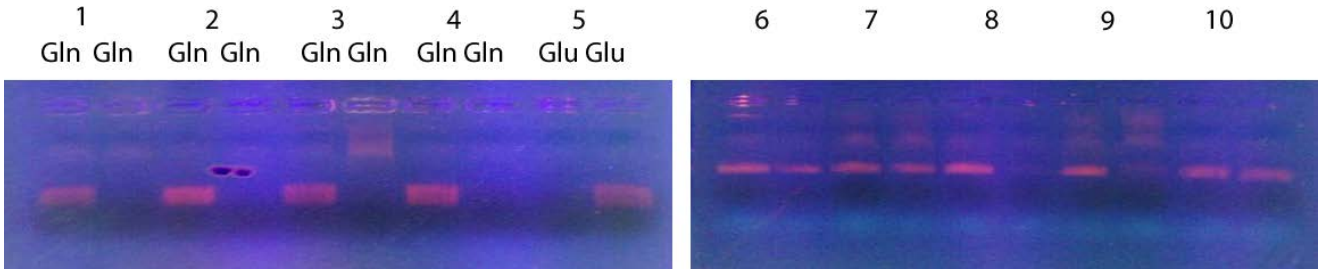

Figure 1:Gel electrophoresis of genotypes Gln27/Glu27 for ADRB2 gene.

Sample 1-4, 8, 9 are homozygotes for Gln/Gln genotype, and sample 5 is a homozygote for Glu/Glu genotype. Other samples are heterozygotes Gln/Glu.

From 47 CF patients analyzed, we found 19 genotypes of Gln/Gln, 20 genotypes of Gln/Glu and 8 genotypes of Glu/Glu. The frequency of genotype Gln/Gln,Gln/Glu and Glu/Glu was 0.4, 0.43 and 0.17 respectively(Fig.2).

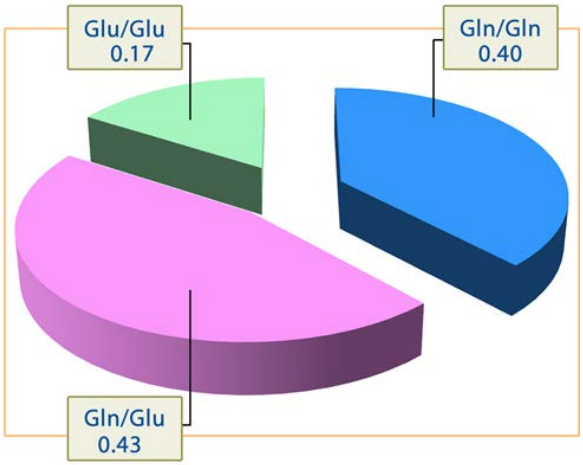

Figure 2.The pie chart showing the frequency of genotypes Gln27Glu found in 47 CF patient.

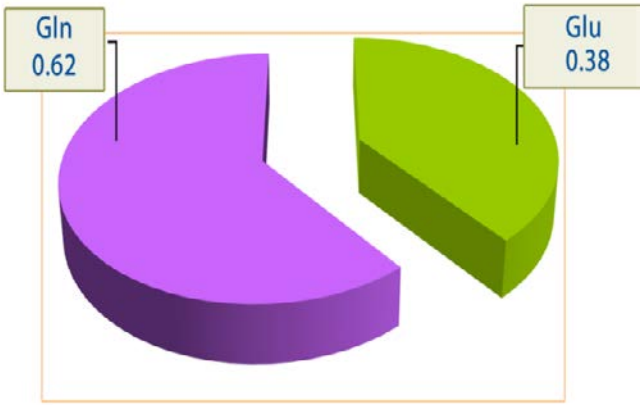

Figure 3.The graph showing the allele frequency found in 47 CF patients.

This data shows thatGln/Glugenotype have the highest frequency (0.43), while Glu/Glu genotype has the lowest frequency (0.17). Using standard formulas of population genetics we found out that the frequency of Gln27 and Glu27allele was 0.62, 0.38respectively(Fig. 3).

We performed Chi-square test for the analysis of Hardy-Weinberg equilibrium of the sample for Gln27Glu locus and found out that our samples were in genetic equilibrium. Our data are partially different from those reported from other researchers[10,6].

ADRB2 gene polymorphisms as a modifier gene, has attracted the attention of many researchers, who remain interested to discover the correlation between different genotypic variants with the variables used as CF´s severity markerson one hand and response to bronchodilators treatment on the other hand[4].The use of bronchodilators, and inhaled corticosteroidshave been suggested for the management of airway inflammation in CF, but the data for the correlation between bronchodilatation and ADRB2 polymporphysm are very limited[1,10].However, in CF, the effectiveness of these drugs is controversial and little is known about the response to broncodilatators and the association of CF´s severity with the different polymorphisms in ADRB2 gene. In this context, our future objective is to verify the possible roleof the Arg16Gly and Gln27Glu polymorphisms of ADRB2 gene in the severity and in bronchodilator response in Albanian CF patients.

4. Conclussions

We have used a simple allele-specific PCR assay to determine the alleles of locus 27 of the ADRB2 gene, responsible for genetic variants of β_2-AR receptors. We genotyped the ADRB2 gene variants, Gln27Glu, in a sample of 47 Albanian patients having the same CFTR genotype, ΔF508/ΔF508 homozygote.

The frequency of Gln27 and Glu27 alleles of ADRB2 gene resulted 0.62 and 0.38, respectively. We divided our sample of CF patients in two subgroups,

one group having the genotype Gln27Gln and the other group of patients having the genotypes Gln27Glu and Glu27Glu. We will increase the number of the CF sample up to 70 patients, and the final subgroups for the locus 27 will be compared with clinical parameters observed in CF patients under treatment.

5. References

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