International Journal of
Biological Engineering and Agriculture

ISSN: 2833-5376
Volume 2 | No 9 | Sep-2023

The Use of Real Time Multiplex Pcr Analysis to Identify the Most Common Mutations of the Cyp21a2 Gene Associated with Cah

[1]Nazirova Mehribon Bakhromovna, [2]Abdurakhimov Abror Akramovich , [3]Khodzhaeva Svetlana Atakhanovna, [4]Dalimova Dilbar Akbarovna

[1]Junior researcher, Institute of Biochemistry and Biophysics at the National University of the Republic of Uzbekistan. mehryunya@mail.ru
[2]Phd, senior researcher, head of the department of genomics, Center for Advanced Technologies
[3]Assistant, Samarkand State Medical University
[4]Deputy Director for Science, Center for Advanced Technologies

Abstract: The use of molecular genetic research methods has become increasingly popular in recent years. This is due to the fact that their use allows for a personalized approach to diagnosis, choice of treatment tactics, disease prevention and differential diagnosis. The use of multiplex PCR analysis of the CYP21A2 gene, associated with CAH, allows us to reach a completely new level in the diagnosis of this disease.
Keywords: CYP21A2 gene, real-time PCR analysis, congenital adrenal hyperplasia.

INTRODUCTION

In the literature, congenital adrenal hyperplasia is often described as adrenogenital syndrome and is the autosomal recessive hereditary disease. It is characterized by the damage of one of the transport proteins enzymes, which leads to defects in the biosynthesis of the main steroid hormones in the adrenal cortex [1,5]. Thus, the level of cortisol in the patient's body decreases, which leads to the increase in adrenocorticotropic hormone (ACTH) and, as a consequence, the development of the adrenal hyperplasia and the accumulation of metabolites preceding the defective stage of steroidogenesis [4,2].

Currently, there are several manifestations of the disease, they vary depending on the affected gene. Thus, there are classical (salt-wasting and virile) forms and non-classical forms of the disease [3,4,6]. The classical form is characterized by almost complete absence of cortisol production, which leads to severe manifestations of the disease, up to a fatal outcome. The non-classical form has mild course and is often diagnosed at puberty. Diagnosis of classical forms of the disease is carried out in the first weeks of life of the newborn children, which is often vital. The non-classical form of CAH has hidden signs, and therefore its diagnosis is very difficult. The complexity of diagnosis is also associated with the variety of manifestations and signs of the disease, as well as with the difficulty of differential diagnosis of diseases such as PCOS and PSD in children. In 90-95% of cases, the disease is associated with the enzymatic defects of 21-hydroxylase [3,7]. The 21-hydroxylase enzyme is

encoded by the CYP21A2 gene. The decrease in the activity of 21-hydroxylase or its complete loss is the main cause of mutational damage to the CYP21A2 gene, which regulates the production of steroid hormones. The use of molecular genetic research methods using real-time PCR diagnostics allows not only early diagnosis, but also differential diagnosis from PCOS and PSD. But recently, along with the advantages of the real-time PCR method, its disadvantages are increasingly being noted, such as a large consumption of reagents, a large amount of time spent on setting up each individual mutation, as well as the cost of operating laboratory equipment. All this has led to the need to search for the new methods that include all the advantages of real-time PCR analysis and exclude its disadvantages. Thus, the method of real time multiplex PCR analysis was improved and developed. The essence of the method is to determine the optimal conditions, the design of allele-specific primers and fluorescent probes, as well as temperature conditions that will allow real-time PCR analysis to determine several mutations at once. This method increases the productivity of the analysis and reduces the cost of its formulation by more than 4 times.

Materials and methods. The material for the study was venous blood taken from CAH patients. The material was collected on the basis of two clinics. The "Genmedical" LLC clinic and multidisciplinary clinic at SamSMU. The total of 60 women and children participated in the study. Genomic DNA isolated from the patient's blood was used to carry out the reaction. The next step was to conduct a locus-specific PCR analysis to determine the CYP21A2 gene, on the PCR amplifier with the participation of specifically selected primers. Qualitative and quantitative evaluation of the PCR product was carried out and its further dilution according to the requirements required for further real time multiplex PCR analysis. Real time multiplex PCR analysis was carried out with the participation of developed specific primers and fluorescent probes necessary for the detection of several types of genetic markers at the same time in one multiplex reaction.

The results of the study. Based on the results of our work, we identified the 2 most common mutations of the CYP21A2 gene associated with the CAH in the Uzbek region. All these mutations are associated with the impaired production of the 21-hydroxylase enzyme. Mutation of the C89T occurs in 1.6% of the examined people. This mutation is associated with the replacement of the cytosine residue with thymine and leads to a loss of enzymatic activity of 21-gd by 20-50%. The second mutation we studied was the C1994T mutation, it was found in 8.3%, and is the most common mutation causing CAH. With this mutation, the enzymatic activity of 21-GD is completely terminated, since the mutation in the CYP21A2 gene is a replacement of cytosine with thymine, which in turn causes the formation of the stop-codon. To amplify the mutations described above, specific oligonucleotide primers and fluorescent probes were specially designed. During each reaction, the pair of primers and the pair of probes were used to detect each mutation.

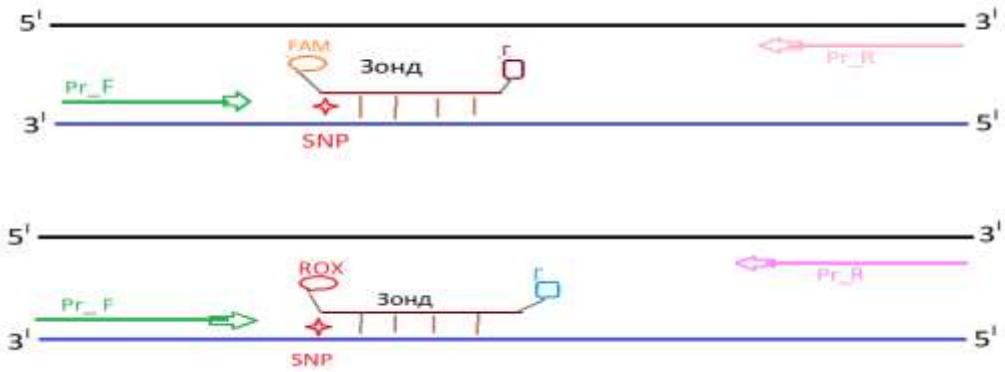
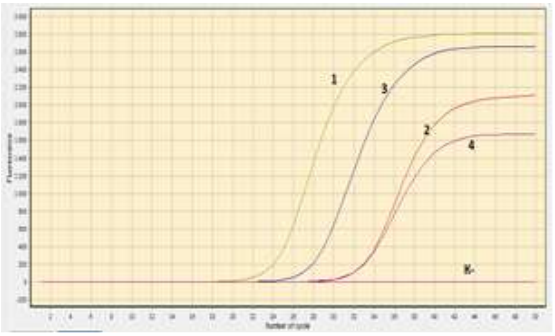
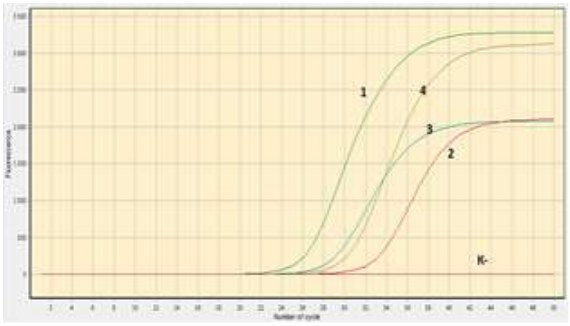


Fig. 1. The design of primers and probes for real-time multiplex PCR.

Thus, based on the results of the real time multiplex PCR analysis, 4 curves can be seen on the screen in real time. The absence of mutations characterizes the early increase in the fluorescence

of the dye located on the wild-type allele and a later glow of the dyes located on the mutant-type alleles. Heterozygous mutations on the graph will have a one-time glow of dyes. The presence of a homozygous mutation is indicated by the early glow of dyes located on the mutant allele and the later glow of dyes located on the wild-type allele.





a) this graph shows the detection of twob) this graph shows the detection of two mutations having normal homozygousmutations, one of which has a normal genotypes, where 1- C89T mutation representshomozygous genotype, and the second one - the wild type of allele (C-allele), 2- C89Tmutant heterozygous genotype, where 1- C89T mutation represents the mutant type of allele (T-mutation represents the wild type of allele (C-allele) 3- T999A mutation is represented by theallele), 2- C89T mutation represents the mutant wild type of allele (T-allele), 4- T999Atype of allele (T-allele) 3- T999A mutation is mutation is represented by a mutant type ofrepresented by the wild type of allele (T-allele), allele (A-allele), K- negative control (H_2O). 4- T999A mutation is represented by a mutant Where: 1,3 (wt, mut) C89T mutations. type of allele (A-allele), K- negative control (H_2O).

Conclusion. The data obtained in the course of the study prove that the developed method of real time multiplex PCR analysis has advantages over the real time PCR analysis carried out the detection of only one mutation. The developed method proved that it is reliable, fast, accurate and can be used as a diagnosis of CAH in molecular genetic laboratories.

References:

1. Баранник А.П., Колтунова А.А.,Озолиня Л.А., Лаврова Н.В., Шилов И.А., и др. Новая система ДНК-диагностики мутаций в гене CYP21 человека, ассоциированных с врожденной гиперплазией коры надпочечников. Биоорганическая химия. М.№3. 2010. С354-365.
2. Болезни органов эндокринной системы: Руководство для врачей ; под ред. И.И. Дедова. - М: Медицина 2000; 382-396.
3. Гапаров К.З. Генетические аспекты врожденной гиперплазии коры надпочечников на территории РСО-Алания. // Материалы XII всероссийской Бурденовской студенческой научной конференции. Молодежный инновационный вестник ТомV,№1.2016. .С107-111.
4. Далимова Д.А., Давлетчурин Д.Х., Хайдарова Ф.А., Нурматова С.Б., Адилов Б.Р. Первичные данные о полиморфизме Q318X цитохрома P450c241 – фермента стероид 21-гидроксилазы. Сборник тезисов докладов Республиканской научно-практической конференции молодых ученых, посвященный 70-летию Академии наук Республики Узбекистан; Ташкент. 2013. С.38.
5. Dracopoulou-Vabouli M., Maniati-Christidi M., and Dacou-Voutetakis C., “The spectrum of molecular defects of the CYP21 gene in the Hellenic population: variable concordance between genotype and phenotype in the different forms of congenital

adrenal hyperplasia," The Journal of Clinical Endocrinology and Metabolism, vol. 86, no. 6, pp. 2845–2848, 2001

6. Fanta M, Cibula D, Vrbikova J. Prevalence of nonclassic adrenal hyperplasia (NCAH) in hyperandrogenic women. Gynecol Endocrinol. 2008.
7. Krone N., Dhir V., ivison H.E., Arlt W.//Clin.Endocrinol (Oxf.) 2007.V.66.P.162-172.