CRR
JOURNAL
OF CARDIORESPIRATORY RESEARCH

ISSN 2181-0974
DOI 10.26739/2181-0974
Impact Factor SJIF 2022: 5.937

Journal of CARDIORESPIRATORY RESEARCH

Volume 6, Issue 3
2025

МИНИСТЕРСТВО ЗДРАВООХРАНЕНИЯ
РЕСПУБЛИКИ УЗБЕКИСТАН

Журнал кардиореспираторных исследований

JOURNAL OF CARDIORESPIRATORY RESEARCH

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Учредитель:

Самаркандский государственный медицинский университет

Tadqiqot.uz

Ежеквартальный научно-практический журнал

ISSN: 2181-0974
DOI: 10.26739/2181-0974

№ 3
2025

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№3 (2025) DOI http://dx.doi.org/10.26739/2181-0974-2025-3

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№3 (2025) DOI http://dx.doi.org/10.26739/2181-0974-2025-3

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ISSN: 2181-0974
www.tadqiqot.uz
JOURNAL OF CARDIORESPIRATORY RESEARCH
ЖУРНАЛ КАРДИОРЕСПИРАТОРНЫХ ИССЛЕДОВАНИЙ

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РАННЯЯ ДИАГНОСТИКА И ПРОГНОЗИРОВАНИЕ СЕМЕЙНОЙ БРОНХИАЛЬНОЙ АСТМЫ ПУТЕМ ОПРЕДЕЛЕНИЯ ГЕНЕТИЧЕСКИХ МАРКЕРОВ

For citation: Okboev T. A. EARLY DIAGNOSIS AND PREDICTION OF FAMILY BRONCHIAL ASTHMA BY IDENTIFYING GENETIC MARKERS. Journal of cardiorespiratory research. 2025, vol. 6, issue 3.

http://dx.doi.org/10.26739/2181-0974/2025/6/3/1

АННОТАЦИЯ

Целью исследования было определение частоты генотипа 4b/4a и аллелей гена eNOS3 среди больных бронхиальной астмой узбекской национальности и членов их семей для ранней диагностики и прогнозирования заболевания. С этой целью было проведено исследование 173 человек из 49 семей с наследственной предрасположенностью к семейной бронхиальной астме. У лиц, включенных в семейное исследование, методом полимеразной цепной реакции (молекулярно-генетическая диагностика) была изучена идентификация гена eNOS3, т. е. выявление 27 пар нуклеотидов, повторяющихся 4/5 раз в интроне 4 (4b, 4a) гена eNOS3. Выявленная ассоциация аллельного варианта 4a/4a гена eNOS3 с развитием семейной бронхиальной астмы в узбекской популяции свидетельствует о необходимости нового подхода к пониманию генетических механизмов патогенеза заболевания, и на этой основе особое внимание следует уделять оптимизации методов ранней диагностики и прогнозирования бронхиальной астмы.

Ключевые слова: Семейная бронхиальная астма, ген eNOS3, ранняя диагностика и прогнозирование.

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EARLY DIAGNOSIS AND PREDICTION OF FAMILY BRONCHIAL ASTHMA BY IDENTIFYING GENETIC MARKERS

ANNOTATION

The aim of the study was to determine the frequency of the 4b/4a genotype and alleles of the eNOS3 gene among patients with bronchial asthma of Uzbek nationality and their family members for early diagnosis and prognosis of the disease. For this purpose, a study was conducted on 173 people from 49 families with a hereditary predisposition to familial bronchial asthma. In individuals included in the family study, the identification of the eNOS3 gene was studied by polymerase chain reaction (molecular genetic diagnostics), i.e., the detection of 27 nucleotide pairs repeated 4/5 times in intron 4 (4b, 4a) of the eNOS3 gene. The identified association of the 4a/4a allelic variant of the eNOS3 gene with the development of familial bronchial asthma in the Uzbek population indicates the need for a new approach to understanding the genetic mechanisms of the pathogenesis of the disease, and on this basis, special attention should be paid to optimizing the methods of early diagnosis and prognosis of bronchial asthma.

Key words: familial bronchial asthma, eNOS3 gene, early diagnosis and prognosis.

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GENETIK MARKERLARNI ANIQLASH ORQALI OILAVIY BRONXIAL ASTMANI ERTA TASHXISLASH VA BASHORATLASH

ANNOTATSIYA

Tadqiqot maqsadi o‘zbek millatiga mansub bo’lgan bronxial astma bilan xastalangan bemorlar va ularning oila a’zolari o‘rtasida eNOS3 genining 4b/4a genotip va allellari chastotasini aniqlash orqali kasallikni erta tashxislash va bashoratlashdan iborat bo’lgan. Buning uchun oilaviy

bronxial astma kasalligi irsiy moyil bo'lgan 49 nafar oiladagi 173 nafar shaxslarda tadqiqot olib borilgan. Oilada tadqiqotga kiritilgan shaxslarda eNOS3 geni identifikatsiyasi, ya'ni eNOS3 geni 4 (4b, 4a) intronida 4/5 marta takrorlangan 27 nukleotidlar juftligini aniqlash polimeraza zanjir reaksiyasi (molekulyar-genetik tashxis) usuli orqali o'rganilgan. Bunda o'zbek populyatsiyasida oilaviy bronxial astma rivojlanishida eNOS3 genining 4a/4a allel variantining aniqlangan assotsiatsiyasi kasallik patogenezining genetik mexanizmlarini tushunishga yangicha yondashish va shu asosda bronxial astmani erta diagnostika usullari va bashoratlashni optimallashtirish kerakligini aloxida e'tibor qaratish lozimligi aniqlangan.

Kalit so'zlar: Oilaviy bronxial astma, eNOS3 geni, erta tashxislash va bashoratlash

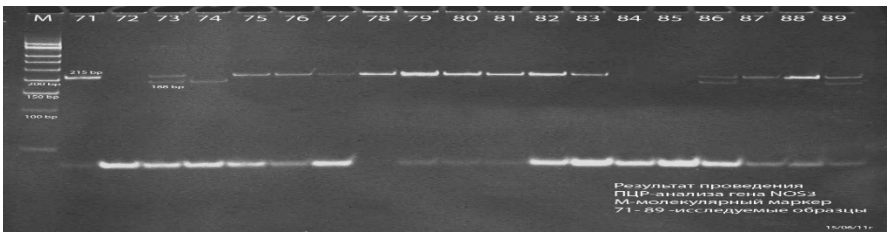
Relevance. Bronchial asthma(BA), considered as one of the diseases belonging to the group of multifactorial diseases, still remains one of the pressing problems of modern pulmonology and allergology. Today, scientific achievements in the study of the human genome and advanced results of molecular biology make it possible to identify specific tasks for the development of diagnostic methods using new technologies. The late 20th century saw many in-depth studies using polymerase chain reaction (PCR) in candidate gene studies and positional cloning. As a result of these studies, candidate areas of structural changes contributing to the formation of bronchial asthma were studied. In particular, a wide range of polymorphic variants of the promoter and coding regions of bronchial asthma genes has been studied. Currently, family studies and relationship analysis have identified many chromosomal regions associated with susceptibility to the development of bronchial asthma. In addition to the genes that determine susceptibility to bronchial asthma, special attention is paid to studying the role of the endothelial nitric oxide synthase gene (eNOS3), which is one of the candidate genes for the metabolism of inflammatory mediators, in the development of this disease. Information about the genetic polymorphism of candidate genes in different populations and their impact on the development of the disease allows us to better understand the features of the pathogenesis of bronchial asthma and develop effective approaches to early diagnosis, prognosis and targeted primary prevention.

The purpose of the study is to determine the frequency of genotypes and alleles 4b/4a of the gene eNOS3 among patients with familial bronchial asthma and their family members in the Uzbek population.

Materials and methods of research. The study was conducted in 49 Uzbek families with a hereditary predisposition to bronchial asthma. The study included 173 people from 49 families. Of these, 116 people in the family were diagnosed with bronchial asthma, 57 people are healthy relatives in the family. Family members are 73 (42.19%) men and 100 (57.81%) women aged 4 to 78 years. Their average age is 33.55 [4; 78] years.

All patients with familial asthma underwent a comprehensive clinical, functional and laboratory examination. The examination of patients was carried out in accordance with the diagnostic criteria of the WHO international classification (based on the revised ICD-10) and the global strategy for the treatment and prevention of asthma (GINA, 2022).

The identification of the eNOS3 gene in 173 individuals included in a family study was studied, that is, the identification of 27 nucleotide pairs repeated 4/5 times in intron 4 (4b, 4a) of the eNOS3 gene by PCR (molecular genetic diagnostics). DNA extraction, amplification, and electrophoresis were performed from lymphocytes in the patient's peripheral deposits. For amplification, oligoprimers of the structure F: 5'-AGGCCTATGGTAGTGCCTT-3' R: 5'-TCCTTTAGTGCTGTGGTCAC-3' were used. One of the results obtained is presented in the figure.



Picture1. Electrophorogram of the result of amplification

The control group was made up of 45 practically healthy people aged 17-62 without signs of respiratory pathology. 24 (52.38%) of them are men and 21 (47.61%) are women, their average age is 28.64 [17; 62] is equal to.

The results of the statistical processing of the received data were performed using the Microsoft Excel program on the Pentum-IV computer. Arithmetic mean (M), error of arithmetic mean value (m) was calculated for the normal distribution of the sample. Reliability differences between means were assessed by Student's t-test. In order to determine the relationship between the tested parameters, a correlation analysis was performed by calculating the Spearman (R) rank correlation coefficient. The statistical significance of the difference between indicators was calculated at r<0.05 [Rebrova O.Yu. 2002]. Statistical processing of molecular genetic testing data was performed using an online calculator (https://medstatistic.ru/index.php).

Research result. Based on the obtained results, when the frequency of genotypes and alleles of the eNOS3 gene was evaluated in 49 individuals of the family, the distribution was as follows in 173 individuals of the family: 4b/4b genotype - 152 (87.9%), 4b/4a genotype - 17 (9.8%)), 4a/4a genotype - 4 (2.3%), 4b allele - 321 (92.8%), 4a allele - 25 (7.2%). 4b/4b genotype - 38 (84.5%), 4b/4a genotype - 6 (13.3%), 4a/4a genotype - 1 (2.2%) in 45 practically healthy individuals examined as a control group. , 4b allele was recorded in 82 (91.1%), 4a allele - 8 (8.9%) [Table 1].

Table1

Frequency of occurrence of eNOS3 gene in family members

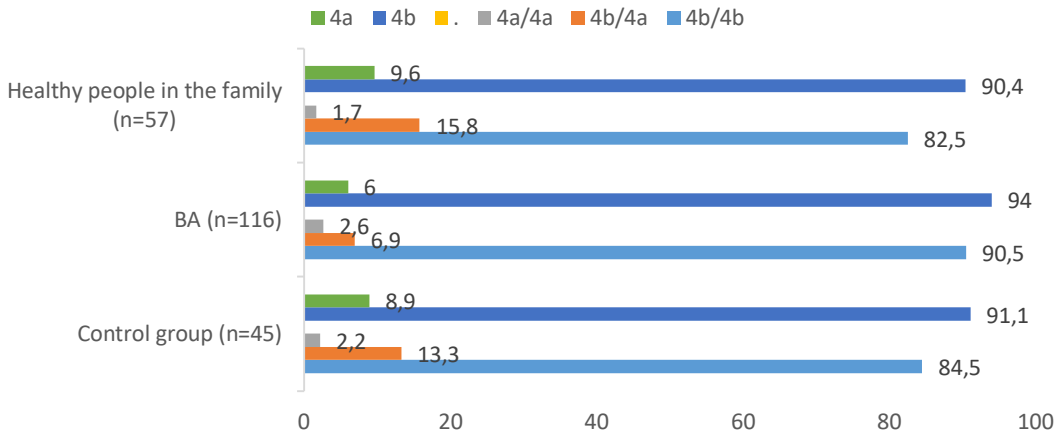
Genotype and allele	Individuals in families (173)	Controls (45)	χ2	P	OR[95%CI]
4b/4b	152(87,9%)	38(84,5%)	0.46	p=0.793	1.33[0.53-3.37]
4b/4a	17(9,8%)	6(13,3%)			0.71[0.26- 1.91]
4a/4a	4 (2,3%)	1 (2,2%)			1.04[0.11- 9.55]
4b	321 (92,8%)	82 (91,1%)	0.28	p=0.596	1.25[0.54-2.88]
4a	25 (7,2%)	8 (8,9%)			0.79[0.34-1.81]

Note: the reliability of the results is obtained relative to those in the control group (p<0,05)

The eNOS3 gene polymorphism 4b/4a genotype and alleles found in the subjects included in the study were divided into groups with BA and those without BA. It was noted that 105 (90.5%) carriers of the homozygous genotype consisting of 4b/4b alleles in patients with familial BA compared to 38 (84.5%) of the control group (OR=1.76, χ2=1.70, p=0.429) . 47 (82.5%) were not identified in the family. It was found that carriers of the heterozygous genotype consisting of 4b/4a alleles in the family were 8 (6.9%) in patients with familial BA, twice

less than in 9 (15.8%) patients without BA. In the control group, it was 6 (13.3%). Patients with familial BA carrying the homozygous genotype of the 4a/4a allele in the family were 3(2.6%), compared to the control group 1 (2.2%) significantly more often (OR=1.17, $\chi 2$=1.70, p= 0.429). 1 (1.7%) of undiagnosed BAs in the family.

In patients with familial BA, the occurrence of eNOS3 gene 4b allele carriers 218 (94.0%) was significantly higher compared to the control group 82 (91.1%) (OR=1.52, $\chi 2$=0.83, p=0.363). 103 (90.4%) individuals without BA were identified in the family. It was noted that eNOS3 gene 4a allele carriers 11 (9.6%) were significantly more frequent in individuals without BA in the family compared to the control group 8 (8.9%) (OR=1.09, $\chi 2$=0.03, p=0.853). 4a allele carriers of eNOS3 gene accounted for 14 (6.0%) of patients with familial BA [Figure 2].



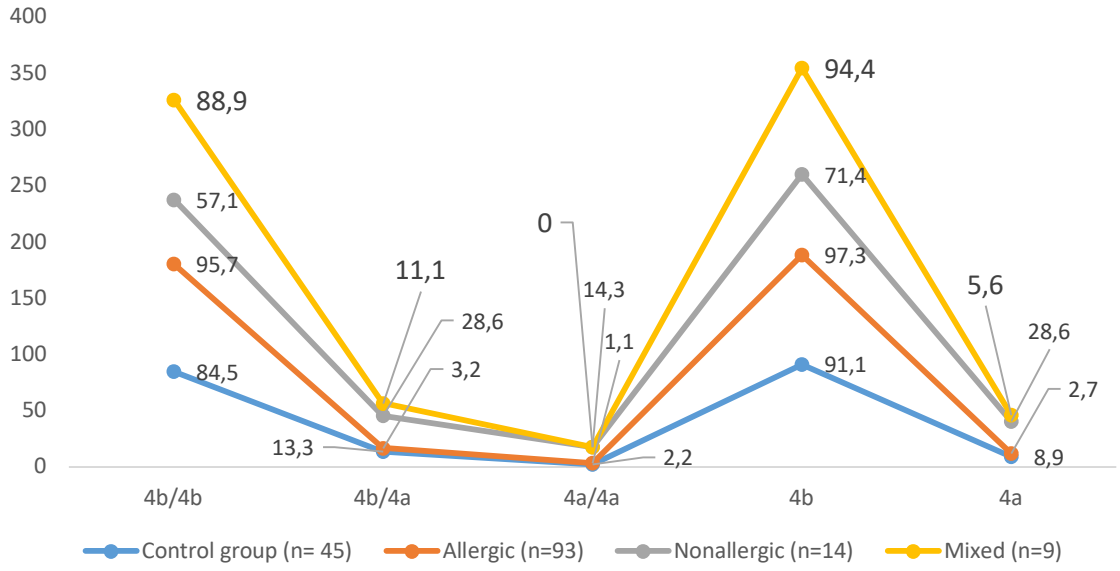
Note: the reliability of the results is obtained relative to those in the control group (p<0,05)

Figure 2. Meeting of eNOS3 gene genotypes and alleles among family members

Thus, it was found that there were many cases of familial BA in individuals carrying the eNOS3 gene 4b/4b genotype and 4b allele in the family. The disease was significantly higher in the family carrying the 4a/4a allele homozygous genotype. This shows that eNOS3 gene 4b/4b, 4a/4a genotypes and carriers of the 4b allele are more likely to develop the disease in the Uzbek population.

The analysis of genotypes and alleles of the eNOS3 gene was carried out in patients with familial BA according to pathogenetic types of the disease. It was noted that carriers of the eNOS3 gene homozygous genotype consisting of 4b/4b alleles in the family had a significantly higher incidence of familial BA allergic type 89 (95.7%) compared to the control group 38 (84.5%) (OR=4.10, $\chi 2$=5.24 , p=0.023). It was noted that carriers of the eNOS3 gene heterozygous genotype consisting of 4b/4a alleles in the family had twice the incidence of non-allergic type of familial BA 4(28.6%) compared to the control group 6(13.3%) (OR=2.17, $\chi 2$=5.08 , p=0.025). Also, carriers of the homozygous genotype of eNOS3 gene 4a/4a alleles in the family had familial BA six times more often than non-allergic type 2 (14.3%) compared to control group 6 (13.3%) (OR=7.33, $\chi 2$=5.54, p =0.063). It was also noted that carriers of the homozygous genotype of the eNOS3 gene 4b/4b alleles in the family had a mixed type of familial BA in 8 (88.9%) compared to the control group 38 (84.5%) (OR=1.47, $\chi 2$= 0.25, p=0.885).

In patients with familial BA, it was noted that 4b allele carriers of the eNOS3 gene were reliably more frequent in the allergic type of familial BA 181 (97.3%) than in the control group 82 (91.1%) (OR=3.53, $\chi 2$=5.20, r< 0.05). It was noted that eNOS3 gene 4a allele carriers in patients with familial BA had a significantly higher frequency of 8 (28.6%) in the non-allergic type of familial BA compared to 8 (8.9%) in the control group (OR=4.10, $\chi 2$=7.06, r <0.01). It was also noted that in patients with familial BA, 4b allele carriers of the eNOS3 gene were significantly more frequent in the mixed type of familial BA 17 (94.4%) than in the control group 82 (91.1%) (OR=1.66, $\chi 2$=0.25, r =0.641). [Figure 3].



Note: the reliability of the results is obtained relative to those in the control group (p<0,01, p<0,05)

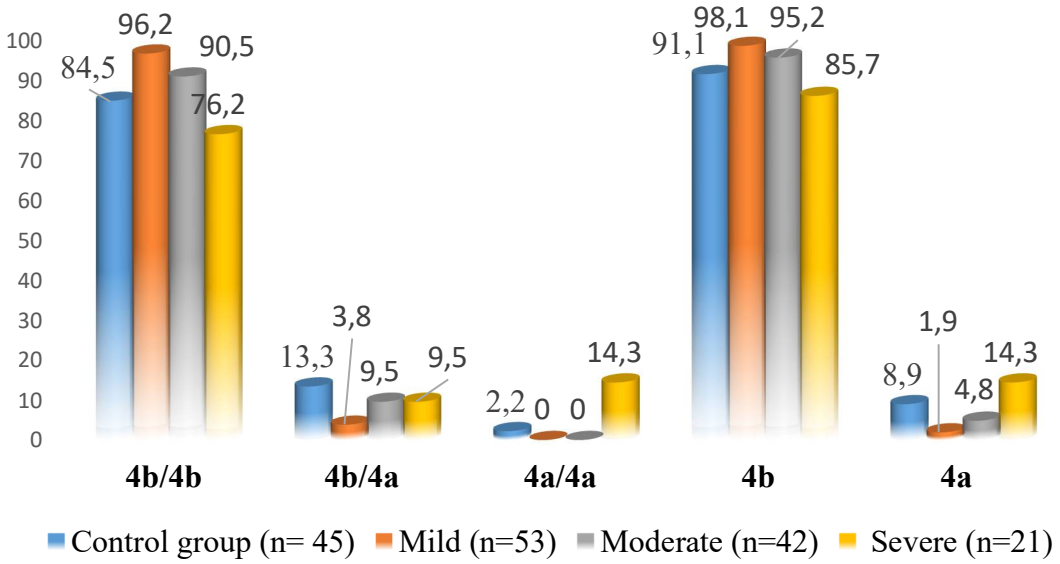
Figure 3. Comparison of eNOS3 gene genotype and alleles according to pathogenetic types of the disease

Thus, in the Uzbek population, it was found that carriers of the 4b/4b homozygous genotype and 4b allele of the eNOS3 gene with the allergic type of familial BA, and the carriers of the 4a/4a homozygous genotype and the 4a allele with the nonallergic type of familial BA were found to be more ill.

An analysis of the distribution of genotypes and alleles of the eNOS3 gene was performed according to the severity of the disease in patients with familial BA. It was noted that eNOS3 gene 4b/4b allele homozygous genotype carriers in the family had a mild disease in 51 (96.2%) compared to 38 (84.5%) control group (OR=4.70, $\chi2$=4.27, p=0.118). In patients with familial BA, eNOS3 gene 4b allele carriers were significantly more frequent in mild familial BA 104 (98.1%) compared to control group 82 (91.1%) (OR=5.07, $\chi2$=4.93, r< 0.05).

It was noted that carriers of homozygous genotype of eNOS3 gene 4b/4b alleles in the family were more likely to have moderate disease 38 (90.5%) compared to 38 (84.5%) control group (OR=1.75, $\chi2$= 1.30, p=0.523). In patients with familial BA, carriers of the 4b allele of the eNOS3 gene were found to be more frequent in moderate familial BA in 80 (95.2%) compared to 82 (91.1%) controls (OR=1.95, $\chi2$= 1.15, p=0.284).

It was noted that carriers of the homozygous genotype of eNOS3 gene 4a/4alleles in the family had more severe disease in 3 (14.3%) compared to control group 1 (2.2%) (OR=7.33, $\chi2$=3.73, p= 0.155). In patients with familial BA, eNOS3 gene 4a allele carriers were more frequent in severe familial BA in 8 (14.3%) compared to 8 (8.9%) controls (OR=2.28, $\chi2$=2.43, p =0.120) [Figure 4].



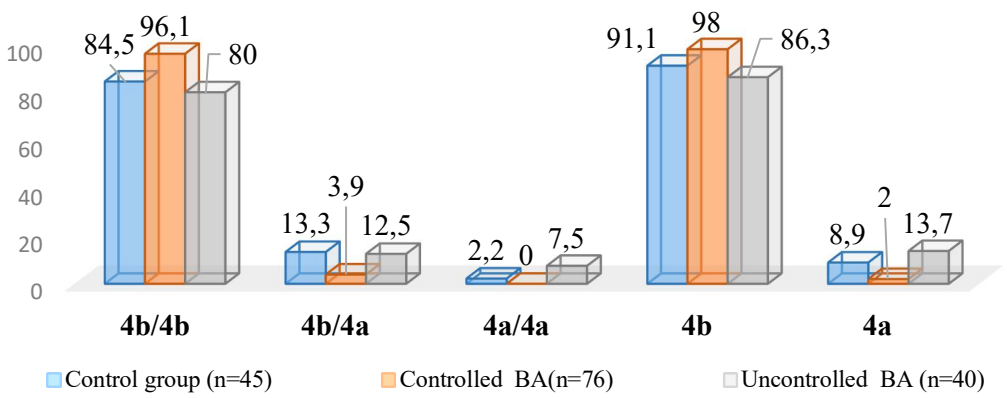
Note: the reliability of the results is obtained relative to those in the control group (p<0,05)

Figure 4. Correlation of eNOS3 gene genotype and alleles in disease severity

Thus, in the Uzbek population, it was found that carriers of the 4b/4b homozygous genotype and 4b allele of the eNOS3 gene are more likely to have a mild degree of familial BA. It was found that carriers of 4a/4a homozygous genotype and 4a allele were more likely to suffer from severe familial BA.

An analysis of the distribution of genotypes and alleles of the eNOS3 gene according to the level of disease control in patients with familial BA was performed. In patients with controlled familial BA, 73(96.1%) homozygous genotype carriers of 4b/4b alleles were reliably increased compared to 38(84.5%) of the control group (OR=4.48, $\chi2$=5.45, p=0.066). It was noted that 149 (98.0%) of eNOS3 gene 4b allele carriers in patients with controlled familial BA compared with 82 (91.1%) of the control group were reliably observed (OR=4.85, $\chi2$=6.23, r<0.05).

Among patients with uncontrolled familial BA, 3(7.5%) homozygous genotype carriers of 4a/4a alleles were significantly increased compared to control group 1(2.2%) (OR=3.57, $\chi2$=1.32, p=0.518). It was noted that 11 (13.7%) carriers of the eNOS3 gene 4a allele in patients with uncontrolled familial BA compared with 8 (8.9%) of the control group (OR=1.63, $\chi2$=1.008, r=316) [Picture 5].

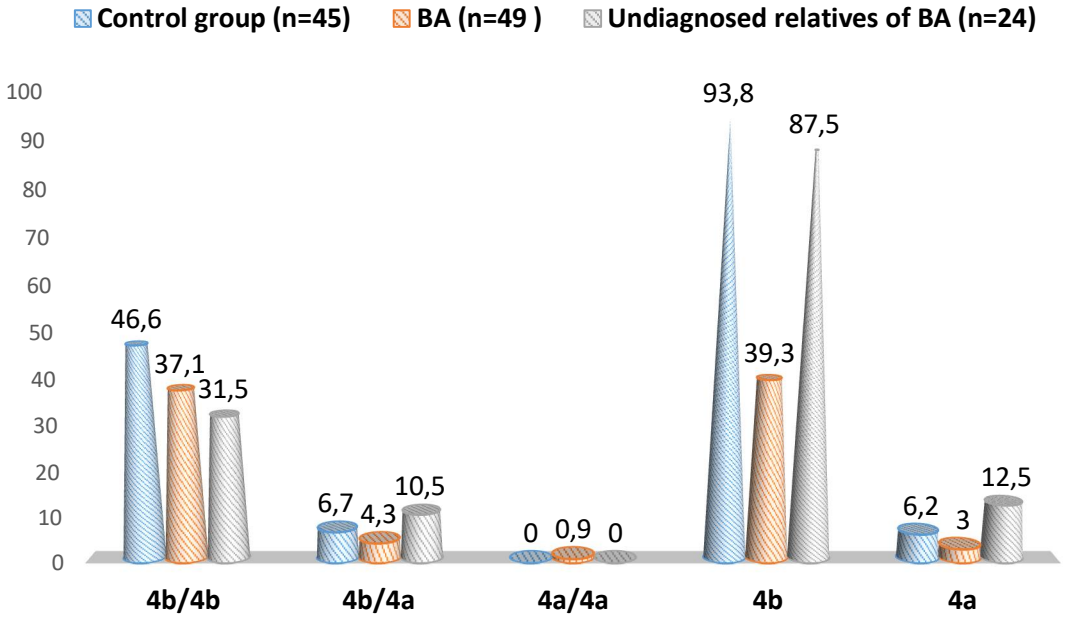


Note: the reliability of the results is obtained relative to those in the control group (p<0,05)

Figure 5. Occurrence of genotype and allele of the eNOS3 gene for disease control.

Thus, in the Uzbek population, it was found that the 4b/4b homozygous genotype and 4b allele carriers of the eNOS3 gene were more prevalent in patients with disease control, and the 4a/4a homozygous genotype and 4a allele carriers in patients who did not achieve disease control.

An analysis of the distribution of genotypes and alleles of the eNOS3 gene by gender of patients with familial BA was performed. It was noted that 6 (10.5%) carriers of the heterozygous genotype consisting of 4b/4a alleles in men without BA in the family were more frequent compared to 3 (6.7%) of the control group (OR=2.33, $\chi 2$=1.23, r=268). In men without family history of BA, 4a allele carriers 6(12.5%;) were more frequent compared to control group 3(6.2%;) (OR=2.14, $\chi 2$=1.10, r=294) [Figure 6].

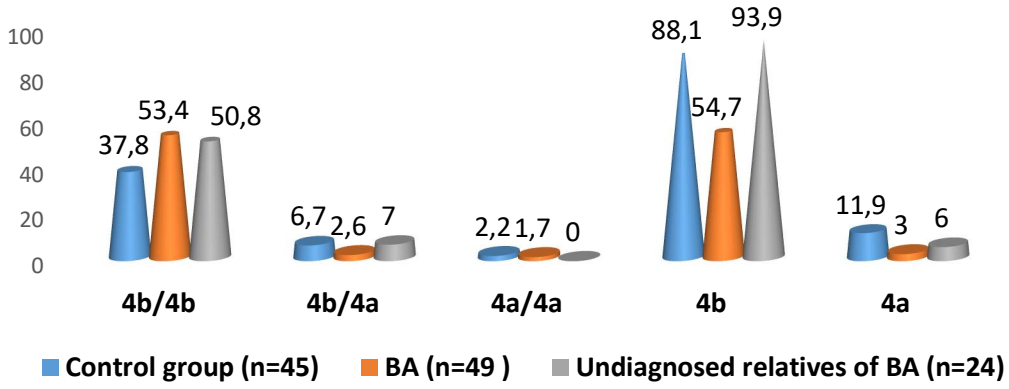


Note: the reliability of the results is obtained relative to those in the control group (p<0,05)

Figure 6. Genotype and alleles of the eNOS3 gene in males in the family

An analysis of the distribution of genotypes and alleles of the eNOS3 gene by gender of patients with familial BA was performed. Homozygous genotype of the eNOS3 gene consisting of 4b/4b alleles and 4b allele carriers in the family had BA (62/53.4%; and 127/54.7%; respectively), undetected BA (29/50.8%; and 62 /93.9%;) in women compared to the control group (17/37.8%; and 37/88.1%; respectively) was noted to be more common.

It was noted that 62 (53.4%) women with BA with homozygous genotype of 4b/4b alleles in the family compared to 17 (37.8%) women in the control group (OR=2.92, $\chi 2$=2.64, r= 267). It was noted that 29 (50.8%) women without family history of BA were more frequent compared to 17 (37.8%) of the control group (OR=1.71, $\chi 2$=1.69, r=430). It was noted that 62 (93.9%) women carrying the 4b allele of the eNOS3 gene in the family were more frequent compared to 37 (88.1%) of the control group (OR=2.09, $\chi 2$=1.19, r=285) [Picture7].



Note: the reliability of the results is obtained relative to those in the control group (p<0,05)

Figure 7. The occurrence of genotypes and alleles of the eNOS3 gene in women in the family

Thus, in the Uzbek population, in patients with familial BA, it was noted that eNOS3 gene 4b/4b homozygous genotype carriers were more common in women, and 4b/4a heterozygous genotype carriers were more common in men without family BA. This shows that eNOS3 gene is inherited with this genotype in patients with familial BA in the Uzbek population.

Summary. Thus, the determined association of the 4a/4a allele variant of the eNOS3 gene in the development of familial BA in the Uzbek population allows a new approach to understanding the genetic mechanisms of the pathogenesis of the disease and, on this basis, to optimize the methods of early diagnosis and prediction of BA.

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