Significance of HLA-associated genetic factors for primary prevention in the development of type 1 Diabetes mellitus

U. N. Sherov*, *B. K.* Badrdinova, *Sh. M.* Akhmedova, *M. A.* Rizaeva, and *Z. A.* Zhurakulova
Bukhara State Medical Institute, Bukhara, Uzbekistan

Abstract. In recent years, diabetes mellitus remains one of the most pressing problems in clinical medicine. Along with, this scientific research illustrates this is due to its wide distribution, the severity of complications, and the lack of clear ideas about the etiology and pathogenetic mechanisms of the disease so far. Diabetes mellitus type 1 (DM type 1) is 20% of the total incidence of diabetes in the population. However, this form of the disease has a particularly high social significance, since it develops at a young age, and its numerous complications, especially vascular ones, affect patients in the most socially active period of their lives. In this regard, the urgent task of clinical medicine is the timely diagnosis and prevention of this disease, the successful solution of which depends primarily on the skillful use of modern diagnostic tools. In recent years, in connection with scientific and technological progress, it has become possible, on the basis of immunophenotyping, to determine the risk of a person's predisposition to many pathological conditions, including type 1 diabetes.

1 Introduction

Among the various etiological reasons for the formation of type 1 diabetes, hereditary factors are of significant importance, however, the methods used in medicine do not yet fully reveal the essence of this issue within the framework of classical genetics [1, 4, 6, 10, 14, 16, 23].

By its nature, type 1 diabetes, like many other diseases, refers to pathologies with a hereditary predisposition, or the so-called multifactorial diseases [2, 6, 11, 18, 21].

Most diseases with a complex type of inheritance are characterized by the action spectrum of many genes (polygenes) that contribute additively to this pathology [3, 5, 9, 13, 17, 19].

In this regard, it is relevant to use such genetic systems that allow for early diagnosis and prevention of type 1 diabetes by identifying a group of individuals with an increased risk of developing type 1 diabetes. In this regard, the study of the system of human leukocyte antigens (HLA - Human Leucocyte Antigens) turned out to be the most promising. This system is characterized by a high degree of polymorphism due to the allelic representation of the genes that form it. Due to the availability of antigen detection of this system, as well as the use of marker properties, researchers have acquired a new, high-quality tool in

* Corresponding author: parviz.feruza83@mail.ru

deciphering the clinic and pathogenesis of many diseases. There have been numerous studies devoted to the study of genetic factors associated with the HLA system of predisposition to type 1 diabetes [5, 7, 9, 12, 17, 20].

However, there are many contradictions and differences in this issue regarding the ethnic characteristics of the distribution of HLA antigens. The Uzbek population of Bukhara, which is characterized by its own characteristics in the representation of HLA antigens, is currently unexplored, it is also unexplored in relation to such a pathology as type 1 diabetes. The relevance of research in this area is obvious.

2 Purpose of work

To establish the significance of HLA-associated genetic factors in the development of type 1 diabetes mellitus in the Uzbek population as the basis for the primary prevention of this disease. Based on the goal in this study, we conducted a survey among patients suffering from type 1 diabetes mellitus. Among the examined patients were residents of the city of Bukhara, as well as residents of the Jondor and Karakul regions. Careful selection of patients belonging to people of Uzbek nationality was determined by the fact that when interviewing and collecting anamnestic data, the genealogical tree of two, if possible, three generations of the proband was taken into account. All patients who were examined in this work were registered in the regional endocrinological dispensary in Bukhara. A total of 80 patients with an accurately established diagnosis of type 1 diabetes were examined. The control group consisted of 100 practically healthy Uzbek donors living in the Bukhara region.

3 Characteristics of the examined patients

The study involved 80 patients with type 1 DM (39 women and 41 men) aged 3 to 45 years, the mean age was 23.5±1.2 years. The distribution of patients by sex and age is presented in Table 1.

From the presented data it can be seen that women and men of young age (18-35 years) predominated among the patients, in the children's age group - these are children aged 7 to 14 years. When analyzing the age of onset of the disease, it was found that 22 people fell ill at the age of 1.5 to 10 years; 23 people fell ill from 10 to 15 years; 15 people fell ill between the ages of 15 and 20; 21 people fell ill from 20 to 40 years old.

Table 1. Distribution of patients by sex and age.

sex	Children				Adults		Total
	0-3	4-6	7-14	15-17	18-35	36-45	
Men	-	1	9	2	20	9	41
		100	47,3	25	48,8	90	51,2
Women	1		10	6	21	1	39
	100	-	52,7	75	51,2	10	47,8
Total	1	1	19	8	41	10	80
	100	100	100	100	100	100	100

Note: The numerator is an absolute number, the denominator is a percentage.

In total, 60 people fell ill under the age of 20 years, which is 75% of the total number of examined persons, 20 people (25%) fell ill after the age of 20 years. These data are consistent with literature data on an earlier age of onset of type 1 DM.

The study of the statute of limitations in the examined persons revealed the following data: statute of limitations from several months to 1 year was established in 8 patients; from

1 to 5 years - in 23 people; from 5 years to 10 years - in 28 people; from 10 years to 20 years - 16 people; from 20 years and more - in 5 people.

The bulk of the patients, 49 people, had disease duration of 5 to 20 years. In 15 people, the onset of the disease is associated with stress; in 28 people, the development of type 1 diabetes was preceded by ARVI; 37 people did not associate the onset of the disease with anything. Among other diseases that patients most often associated with the occurrence of type 1diabetes were viral hepatitis, intestinal infections. Diseases preceding the development of type 1 diabetes are presented in Table 2.

Table 2. Diseases that preceded the development of IDSD.

Disease	Quantity sick	% of the total
1. Viral hepatitis	30	37,5%
2. flu, sore throat	23	28,7%
3. Frequent SARS, colds	25	31,3%
4. Intestinal infections	2	2,5%
5. Smallpox, measles, chicken pox	6	7,5%
6. Stress	15	18,8%

Among the examined patients, it was found that 70 patients (87.5%) suffer from severe DM. All of these patients had a severe, rapidly progressive course, in 10 patients (12.5%) moderate severity with a slowly progressive course was established. A special place in the clinic of diabetes is occupied by its specific vascular complications.

The most common complication observed in the patients examined by us is diabetic antipathy of the lower extremities and diabetic retinopathy. Of the patients examined by us, diabetic antipathy of the lower extremities was observed in 55 (68.8%) patients, diabetic retinopathy in 32 (40%) patients. The diagnosis was made on the basis of clinical data, patient complaints, and capillaroscopy data. Diabetic polyneuropathy was observed in 55 (68.8%) patients with type 1 diabetes. Diabetic neuropathy was observed in 25 patients; 20 (25%) patients with DM often had a history of coma.

One of the severe complications of type 1 diabetes in children is Mauriac's syndrome. In the studied group of patients, Mauriac's syndrome was observed in 9 (11.3%) patients, 37 (46.3%) patients had a complication in the form of periodontitis. 20 (25%) patients had a burdened heredity in terms of DM in ascending and lateral lines. Diabetic nephropathy was observed in 18 (22.5%) patients. Thus, all these complications indicate the predominance of patients with severe type 1 diabetes. In addition to complications of the underlying disease, all patients also had concomitant symptoms in the form of various diseases, which are presented in Table 3.

Table 3. Accompanying disease.

Disease	Number of patients	% of total
1. Chronic hepatitis	43	53,7
2. IHD	3	3,8
3. Anemia	12	15,0
4. Chron. Pyelonephritis	7	8,8
5. Chron. Cholecystitis	7	8,8
6. Diffuse goiter	2	2,9
7. Chron. Tonsillitis	13	16,3

Treatment of patients was carried out differentiated, taking into account the severity of diabetes, damage to organs and systems, complications and concomitant diseases. Therapy included the N9 diet, insulin preparations. In addition, patients received vitamins A, B, C, ascorutin, anabolic steroids.

4 Typing of HLA antigens

The HLA phenotype was determined in a standard microlymphocytotoxic test (Terasaki P.I., 1964) using a panel of HLA antisera from the St. Petersburg Research Institute of Hematology and Blood Transfusion. When conducting the microlymphocytotoxic test, the recommendations of the NIH (National Institute of Health, USA) were taken into account, according to which, when digging lymphocytes into the wells of the Terasaki plate, the "shooting" technique was used, which largely ensured the purity of the "readable" wells. This method is based on the combination of the corresponding antibodies with the lymphocyte antigens of the subject in the presence of rabbit complement, which is a mixture of sera of 10-14 non-immunized rabbits. If there are corresponding HLA antigens on the surface of lymphocytes, then a lymphocytotoxic reaction occurs, as a result of which the permeability of the membrane of mononuclear cells is disturbed, and they are intensely stained with a dye, while living cells remain unstained.

Isolation of lymphocytes

For serological typing of HLA class I antigens, a purified suspension of lymphocytes isolated by the method of Boyum A. (1968) is used.

Blood was taken from the cubital vein in the amount of 2 ml. with heparin at a dose of 15 units / ml of blood. This volume was then mixed with 5 ml. Media 199 and layered on a mixture of ficoll-verografin with a density of 1.077 - 1.078 g/sm. After centrifugation at 400 G for 20 minutes, the dull lymphocytic cloud formed at the interface between the two media was aspirated with a Pasteur pipette and washed twice in 199 medium at 200 G for 10–15 minutes. The supernatant was removed, the cells were resuspended to a concentration of 3-4 million in 1 ml. medium 199. Cells were counted in a Goryaev chamber.

B. Microlymphocytotoxicity test.

A standard microlymphocytotoxic test is used to detect class I HLA antigens (loci A, B, C). The resulting suspension of lymphocytes was introduced into the wells of the Terasaki plate using a Hamilton-type syringe with a dispenser. In advance, specific HLA anti-sera were placed in each well under a layer of vaseline oil in a volume of 1 μl. Incubated for 0.5 hour at 22° C.

After that, whole rabbit complement, previously tested for activity and cytotoxicity, was added to each well with a Hamilton syringe in a volume of 1 μl. The second incubation lasted for 1 hour at 37° C, after which 1 μl was added. 5% eosin solution. After 2-3 minutes, 1 μl was added. formalin solution with pH=7.0 - 7.2. Microplates were left in the refrigerator at 4°C. The reaction results were read the next day using an inverted microscope.

Accounting for the results of staining with eosin.

When reading the results of the cytotoxic reaction, the percentage of dead and living cells visible in the field of view in the well is estimated.

Living lymphocytes are intact cells of a spherical shape and highly refracting light, as a result of which they look like small yellowish discs that shine brightly against a red eosin background.

Dead lymphocytes - cells of a flatter shape, larger than bright living cells, dull, dark gray, do not stand out against the background of the environment. Fragments of dark-colored material outline the contours of the cells and give the cell surface a spotty appearance. Typically, the percentage of dead cells in each well is measured above the background level determined in the negative control.

1. If the absolute level of cell death in the negative control is about 50%, then the evaluation by degrees of results is stopped and only positive reactions are taken into account.
2. Check the positive control, giving one hundred percent cell death.
3. Look at well №3 after a few seconds and evaluate the percentage of dead cells. Contribute as a result a copy of the map of the corresponding panel.
4. Evaluate the remaining wells sequentially, recording the results in the appropriate column of the map.

In each well, it is necessary to carefully and skillfully assess the cell death caused by antibodies to HLA antigens.

The intensity of the cytotoxic reaction is evaluated according to the following scale:

- 81-100% cell death is a strongly pronounced positive reaction;
- 61-80% cell death is a pronounced positive reaction;
- 31 -60% cell death is a weak positive reaction;
- 11-30% cell death is a doubtfully positive reaction;
- 0 -10% cell death is a negative reaction.

5 Interpretation of typing results

The accuracy of cell typing depends on the quality and specificity of the HLA-antisera of the typing panel, the experience of the researcher, and the correctness of the technology for setting up the complement-dependent cytotoxicity reaction. To interpret the results of typing, the following points should be taken into account:

1. Disregard results if only mild cytotoxicity is detected in the positive control or if cell death is greater than 70% in the negative control.
2. Any result with spontaneous cell death greater than 40% should be interpreted with caution.
3. Always consider alternative explanations for obvious definitions, looking for refutation of results in unusual or unexpected positive or negative reactions.
4. By reaction with known cells, the belonging of the studied to this phenotype is established. Re-examine any well in which typing is not consistent with this definition.
5. Antigen cleavages are determined if a common antigen is found and the serum corresponding to the antigen cleavage phenotype gives a clear ambiguous reaction.
6. Mentally compare the known linkage disequilibrium in the population with the most probable haplotypes, which may consist of the found antigens.
7. Verify that the identified HLA type matches previous findings for the individual or family members.

Record the results, noting any weak, odd, or otherwise questionable findings, and recommend further investigation, such as:

1. Repeat typing with fresh blood and type in panels with a wider range of specificities.
2. It is confirmed by family studies that the “blank” antigen at any locus is a consequence of homozygosity.
3. Blood is drawn again and, with the consent of an experienced or more specialized laboratory, any sera are sent for analysis in the case when the “blank” antigen cannot be due to homozygosity, as well as in cases of triple antigens of the same locus and other interesting or doubtful cases.

6 Immunogenetic methods of data processing

The frequency of occurrence of the antigen.

To calculate this indicator, a proportion is drawn up, which takes into account the total number of patients, taken as 100%, and the number of occurrence of this antigen in the studied sample.

For example: 50 - 100%
12 - X

$X = \frac{12 \cdot 100}{50} = 24\%$, where

50 - number of examined
12 - the number of occurrences of a given antigen
X - antigen frequency
The frequency of occurrence of the HLA gene.
Calculated using the Bershtein formula:

$X = 1 - \sqrt{1 - AG \cdot \left(\frac{12}{100}\right)}$, where AG- antigen frequency

The significance of frequencies.

To determine the significance of different frequencies of HLA antigens in the compared samples, the criterion is used x^2, presenting the primary material in the form of a four-field table:

sick	a	b
healthy	c	d

N=a+b+c+d

where a - the number of patients with the presence of this antigen;
b - the number of patients who do not have this antigen;
c - the number of healthy people with the presence of this antigen;
d - the number of healthy people with the absence of this antigen.

Such tables are compiled for all determined HLA specificities. The value x^2 calculated by the formula:

$$x^2 = \frac{[(a \cdot b - d \cdot c) - 1/2 \cdot N] \cdot N}{(a + c) \cdot (b + d) \cdot (a + b) \cdot (c + d)}$$

x^2 is also calculated using a special computer statistical program.

Values x^2, exceeding 3.84 (which corresponds to P<0.05) is considered as an indicator of a significant difference between the frequencies in the compared groups. To confirm the detected associations of HLA antigens and to establish the validity of the associations, it is necessary to calculate the corrected value of P, denoted Ps, which is determined by the Bonferoni formula:

Ps = P x N, where N - the number of detected antigens.

Relative risk criterion (RR).

To determine the strength of association of HLA antigens, the relative risk criterion RR (Relative risk) is calculated:

$RR_{\_} = \frac{a \cdot d}{c \cdot b}$ Values a, b, s, d taken from a four-field table for a given antigen

Criteria for etiologic fraction (EF)

Indicates the primacy or predominance of one or another HLA antigen in the etiopathogenesis of the disease. This criterion is calculated for those antigens that are more common than in the control.

$$EF = \frac{RR - 1}{RR} \cdot \frac{a}{a + b}$$

Preventive Fraction Criterion (PF)

Shows the degree of protection against diseases with a given HLA phenotype. This criterion is calculated for those antigens that are significantly less common than in the control.

$$PF = \frac{1 - RR}{RR \cdot \left(1 - \frac{a}{a + b}\right) + \left(\frac{a}{a + b}\right)}$$

When conducting immunogenic studies and when studying populations and when analyzing immunogenic parameters in the studied pathologies, it is also necessary to study haplotype associations, that is, haplotypes (a haplotype is a set of alleles on one chromosome). To do this, it is necessary to calculate the number of occurrence of different haplotypes from the data of the identified HLA phenotypes.

For example: a four-field haplotype table is compiled.

10	15	25	- HLA-A1 frequency
9	16	25	- the difference between the number of examined and the frequency of occurren A1
19	31	50	- number of examined HLA-B8 frequency

a - HLA haplotype occurs 10 times

b - the difference between the frequency of occurrence of the HLA-AI antigen and the frequency of occurrence of the A1-B8 haplotype (15)

c - difference between the frequency of occurrence of the HLA-B8 antigen and the frequency of occurrence of the A1-B haplotype 8 (9)

d - calculated according to the compiled table (16)

N - number of ice-covered persons (50)

According to the table, x2 and other indicators (P, Ps, RR, EF, PF) are calculated. If the haplotype “passes” along x2 (which means x2 > 3.84), then this indicates a significant value of linkage imbalance (gamete association) - D.

To calculate linkage disequilibrium, you need to know the data a, b, c, d, N from the four-field haplotype table:

$$D = \sqrt{\frac{d}{N}} - \sqrt{\frac{(b + d) \cdot (c + d)}{N^2}}$$

7 Conclusion

1. The distribution of HLA antigens in the healthy Uzbek population of Bukhara has its own characteristics. Genetic markers of the Uzbek population of Bukhara are HLA antigens: A28, B7.

2. It has been established that genetic factors associated with the HLA system are important in the susceptibility of individuals to type 1 diabetes. An increased risk of developing type 1 diabetes is determined by the following HLA antigens: A2, B12, B16, B22, Cw1, Cw2. Genetic markers of type 1 diabetes in this population are genes: HLA-B16, Cw2.
3. A significant contribution to the realization of predisposition to type 1 diabetes is made by the following HLA haplotypes: A3/B18, A1/B16, A2/B22, A9/B16, A2/B40.
4. HLA antigens A25 and B7 act as protectors; their presence in the phenotype of individuals reduces the risk of developing type 1 diabetes.
5. For various clinical signs of type 1 diabetes, there are HLA markers associated with predisposition. The age of onset of the disease, prescription, hereditary burden are characterized by certain combinations of HLA phenotypes, which can act as criteria confirming the diagnosis.
6. Various complications in patients with type 1 diabetes are associated with various HLA antigens (diabetic polyneuropathy is associated with antigens A2, B22; retinopathy is associated with antigens A2, A25; Mauriac's syndrome is associated with antigens A1, B8; nephropathy is associated with antigens A2, B7; diseases oral cavity associated with antigens A3, B22, B35). According to these markers, it is possible to carry out early diagnosis, prognosis of the threat of development and, in combination with other methods, differential diagnosis of the disease.

References

1. B. K. Badridinova, “Risk factors for the development of diabetic nephropathy in patients with type 1 diabetes”, in *Bulletin of the Doctor* (2020)
2. J. J. Bakhronov, S. J. Teshaev, M. S. Shodieva, International Journal of Pharmaceutical Research **13(1)**, 683-686 (2020)
3. S. S. Davlatov, B. Z. Khamdamov, Sh. J. Teshaev, Journal of Natural Remedies **22(1(2))**, 147-156 (2021)
4. S. Davlatov, Sh. Teshayev, X. Fayziev, N. Khamidova, International Journal of Pharmaceutical Research **13**, 970-976 (2020)
5. Sh. A. Djuraeva, B. K. Badridinova, British Medical Journal **2(1)** (2022)
6. F. Nurutdinova, Z. Tuksanova, Y. Rasulova, E3S Web of Conferences **474**, 01002 (2024)
7. O. E. Idiev, S. Z. Teshaev, Journal of Pharmaceutical Negative Results **13** (2022)
8. B. Z. Khamdamov, R. M. Akhmedov, A. B. Khamdamov, International Journal of Pharmaceutical Research **11(3)**, 1193-1196 (2019)
9. S. S. Davlatov, B. Z. Khamdamov, Sh. J. Teshaev, Journal of Natural Remedies **22(1(2))**, 147-156 (2021)
10. B. Z. Khamdamov, U. B. Oltiev, A. B. Khamdamov, Science Asia **48**, 61-67 (2022)
11. B. Z. Khamdamov, A. Sh. Rakhimov, I. B. Khamdamov, Eur. Chem. Bull. **12(8)**, 8342-8351 (2023)
12. O. M. Kurbanov, M. S. Sharapova, A. N. Zulfikorov, I. S. Muhammadiev, International Journal of Current Research and Review **12(24)**, 135-139 (2020)
13. K. F. Rakhmatillaevna, European Journal of Molecular and Clinical Medicine **7(3)**, 1518-1523 (2020)

14. K. F. Rakhmatillaevna, E. G. Torakulovich, European Journal of Molecular and Clinical Medicine **7(3)**, 2468-2472 (2020)
15. B. B. Safoyev, O. M. Kurbanov, M. S. Sharopova, International Journal of Pharmaceutical Research **13(1)**, 694-701 (2020)
16. S. J. Teshaev, R. R. Baymuradov, N. K. Khamidova, D. A. Khasanova, International Journal of Pharmaceutical Research **12(3)** (2020)
17. S. Zh. Teshaev, S. V. Yanchenko, A. V. Malyshev, L. M. Petrosyan, S. Sh. Ramazonova, Oftalmologiya **20(4)**, 772-779 (2023)
18. Sh. Zh. Teshaev, S. V. Yanchenko, A. V. Malyshev, R. R. Boboeva, G. B. Juraeva, Oftalmologiya **20(4)**, 780-786 (2023)
19. S. J. Teshayev, D. K. Khudoyberdiyev, S. S. Davlatov, International Journal of Pharmaceutical Research **13(1)**, 679-682 (2021)
20. S. Zh. Teshaev, S. V. Yanchenko, A. V. Malyshev, L. M. Petrosyan, S. Sh. Ramazonova, Oftalmologiya **20(4)**, 772-779 (2023)
21. Sh. Zh. Teshaev, S. V. Yanchenko, A. V. Malyshev, R. R. Boboeva, G. B. Juraeva, Oftalmologiya **20(4)**, 780-786 (2023)