

# Comprehensive Analysis of Human Leukocyte Antigen System Class II DRB1 in children with Insulin Dependent Diabetes Mellitus in the North Azerbaijan and Iranian Azerbaijan

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**KEY WORDS:** Human Leukocyte Antigen system, DRB1 alleles

The Republic of Azerbaijan lies in south-western Asia, bordering with Armenia, Georgia, Iran, Russia and Turkey; it has no access to ocean but borders the Caspian Sea. Its land area is 86 600 km<sup>2</sup>; the average population density is 86 persons per square kilometer. The total population is slightly lower than eight million, with approximately 90% Azeri, and minorities of Dagestani, Russian, and other nationalities. The majority Azeri population belongs to the Caspian type of southern Europoid race. The Azeri live not only on the territory of Azerbaijan but also in Northern Iran, which is a result of division of the territory in 1828. The Azerbaijani language belongs to the Oguz group of the Turkish language family and is spoken not only by 95% population of Azerbaijan, but also by about one-fourth of the population of Iran. Diabetes mellitus is one of the diseases, the genetics of which has been most widely studied. In 40-50 % cases it is connected with Human Leukocyte Antigen system. The major genetic determinants of this disease are DQ and DR genes "DR3" and "DR4" haplotypes create high risk for diabetes. Insulin dependent diabetes mellitus risk is very high among the Iranian Azerbaijanis possessing HLA DR3-DQ2 haplotypes. In this ethnic group DRB1\*0301 (82/5% with 11.3%), DQA1\*0501 (82.5% with 6.3%), DQB1\*0201 (81.3% with 35%) alleles represent higher risk compared with healthy people. In Iranian Azerbaijani nationality the frequency of DRB1\*0401 is significantly higher (76.74% in comparison with 23.26%).

**AIM.** The research aimed to study the relationship between diabetes mellitus and HLA genes in two azerbaijani populations. In the populations the alleles of HLA polymorphic genes are observed in different versions.

## MATERIALS AND METHODS

HLA-DRB1 gene has been determined in children in North Azerbaijan (104 sick, 200 healthy). Saliva has been used in control groups and blood analysis in diabetic patients. The samples were genotyped for HLA alleles with high-resolution genotyping technology at the Children's Hospital Oakland Research Institute in California. HLA sequence data was generated using next-generation sequencing on the 454 GS FLX and GS Junior Systems (Roche, Basel, Switzerland) and analyzed using Conexio Genomics (Fremantle, Australia). HLA ASSIGNATF genotyping software to interpret the sequence files as HLA genotypes. Amplicons were generated from genomic DNA using DRB generic exon 2 454 fusion primers. The 454 fusion primers consist of a locus-specific primer on the 39 end, a 10-bp multiplex ID (MID) tag, and an "A" or "B" 454-specific primer sequence on the 59 end. The MID tag serves as a sample barcode recognized by the Conexio ASSIGNATF genotyping software. Amplicons were purified with AMPure beads (Becton Dickinson, Franklin Lakes, USA), quantified using the Quant-iTPicoGreen dsDNA reagent (Life Technologies, Foster City, USA), and mixed with capture beads after dilution. Individual DRB exon 2 amplicon molecules captured by these beads, and were amplified in an emulsion PCR and DNA-containing beads subsequently analyzed by pyrosequencing to obtain sequence readings originating from a single molecule. Sequencing and genotyping was done using the GS FLX and GS Junior Systems and Conexio ASSIGNATF software as previously described. The results have been calculated with Conexio ATF Assign™ and SCORE™ computer program. The statistic calculation have been analyzed using Pearson's Chi-squared and odds ratio has been computed.

## RESULTS AND THEIR DISCUSSION

While the DRB1 genotypes of the 209 control subjects conformed to expected HWE proportions, those of the 104 diabetes subjects deviated significantly from HWE (p-value < 1E-05). The primary contributor to this deviation was an excess of DRB1\*03:01 + DRB1\*04:02 heterozygotes (24 observed; 15 expected; p-value = 3.4E-03). DRB1\*04:05 homozygotes were also observed to be in excess (3 observed; 0.5 expected; p-value = 7.1E-03), as were DRB1\*09:01 + DRB1\*07:01 heterozygotes (3 observed; 0.25 expected; p-value = 6.0E-04). In addition, the absence of DRB1\*07:01 + DRB1\*03:01 heterozygotes was also significant (0 observed; 3.75 expected; p-value = 1.48E-02).

Genotyping of the DRB1 locus identified 38 alleles present in the population. Association analysis with the BIGDAWG revealed that 14 of these alleles were present in sufficient frequency to assess T1D association (Table 2). The remaining 24 alleles were binned for association analyses. Significant heterogeneity was observed between diabetes subjects and control subjects at the locus level (p-value < 2.22E-16). DRB1\*03:01 and DRB1\*04:02 showed the strongest positive association with disease (OR 5.06 and 4.47; p = 7.77E-13 and 2.27E-10, respectively). DRB1\*04:05 was also positively associated (OR 3.53; p = 1.90E-03). Six of the remaining 11 alleles showed negative disease association (protection), including alleles in the "DR2" group, DRB1\*15:01 and DRB1\*15:02.

Both European and Asian alleles exist in

North Azerbaijan population. DRB1\*03:01, DRB1\*04:02, DRB1\*04:05, DRB1\*09:01 alleles contribute a high risk for diabetes, but DRB1\*15:01 (Europe) and DRB1\*15:02 (Asia), DRB1\*11:01 alleles are protective in nature.

## CONCLUSIONS

DRB1 alleles vary widely in the studied populations. DR3 and DR4 haplotypes are associated with diabetes mellitus and they are found in broad intervals. Some alleles (such as, DRB1\*11:01) possess contradictory features in different populations. The complete study of DRB1, DRB3, DRB4, DRB5, DQA1, DQB1, DPA1, DPB1, A, B, C genes will provide possibility to enlarge upon the haplotypes.

Table 1. The frequencies of HLA alleles for 104 type 1 diabetes subjects versus 209 controls

DRB1 allele	Patient frequency (n)	Control frequency (n)	OR (95% CI)	p value	Significance
01:01	0.0385 (8)	0.0383 (16)	1.01 (0.37-2.54)	9.91E-01	NS
03:01	0.2885 (60)	0.0742 (31)	5.06 (3.08-8.4)	7.77E-13	*
04:02	0.25 (52)	0.0694 (29)	4.47 (2.67-7.57)	2.27E-10	*
04:04	0.0144 (3)	0.0311 (13)	0.46 (0.08-1.69)	2.13E-01	NS
04:05	0.0721 (15)	0.0215 (9)	3.53 (1.42-9.3)	1.90E-03	*
07:01	0.0625 (13)	0.0789 (33)	0.78 (0.37-1.56)	4.58E-01	NS
11:01	0.0144 (3)	0.0789 (33)	0.17 (0.03-0.56)	1.09E-03	*
11:04	0.0144 (3)	0.0789 (33)	0.17 (0.03-0.56)	1.09E-03	*
13:01	0.0096 (2)	0.0646 (27)	0.14 (0.02-0.57)	2.05E-03	*
13:02	0.0144 (3)	0.0383 (16)	0.37 (0.07-1.31)	1.01E-01	NS
14:01	0 (0)	0.0526 (22)	0 (0-0.35)	7.56E-04	*
15:01	0.0144 (3)	0.0694 (29)	0.2 (0.04-0.65)	3.28E-03	*
15:02	0.0048 (1)	0.0646 (27)	0.07 (0-0.43)	6.53E-04	*
16:01	0.0337 (7)	0.0335 (14)	1 (0.34-2.71)	9.92E-01	NS
Binned	0.1683 (35)	0.0335 (14)	0.78 (0.49-1.23)	2.63E-01	NS

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