EFFECT OF HLA-DR GENOTYPES ON THE CLINICAL MANIFESTATIONS AT THE ONSET OF TYPE 1 DIABETES IN A GROUP OF PATIENTS FROM CONSTANTA COUNTY

Irina Durbala [1], *Cristina Maria Mihai* [2], *Doina Catrinoiu* [3], *Pietro Maffei* [4]

[1] Cell and Molecular Biology Department
[2] Clinic of Pediatrics
[3] Clinic of Diabetes, Nutrition and Metabolic Diseases Department
Faculty of Medicine, "Ovidius" University Constanta, Romania
[4] Department of Medical and Surgical Sciences, University School of Medicine, Padua, Italy

Abstract

Type 1 diabetes mellitus (T1DM) is a metabolic disease with multifactorial etiology characterized by β-cell autoimmune processes. The strongest genetic determinants were proved to be class II genes of the major histocompatibility complex, mainly HLA-DR and -DQ genes. The aim of the study was to investigate HLA-DR genotype in association with chronological age at the disease onset. Additionally, the genotype frequencies in relation to the mode of onset and glucose blood concentration at the time of the presentation were analyzed. We typed 62 children with T1DM for alleles of HLA-DR locus and data about the onset of the disease were collected. The statistical analysis of clinical data in correlation with the DR genotype showed an association between DR genotype and the age at onset (p=0.031), but failed to show a correlation between the DR genotype and the mode of onset, or blood glucose at onset (p=0.78 and 0.57, respectively). In conclusion, in our group the DR genotype had effects only on the age of onset and not on the mode of onset or blood glucose at onset.

key words*: Type 1 diabetes, HLA-DR genes, clinical manifestations at the onset.*

Background

Though the complex association and linkage of HLA class II antigens with type 1 diabetes are not yet fully understood, HLA-DRB1 and -DQB1 alleles provide the strongest genetic contribution to the disease [1], accounting for approximately 50% of the genetic risk in type 1 diabetes [2]. In Caucasians, DR4 and DR3 and their linked DQ specificities DQB1*03:02 and DQB1*02 provide disease susceptibility, particularly in heterozygous combination [3]. An age dependent heterogeneity was observed in Caucasians with T1DM, with the high risk genotypes found more frequently in children while the late onset is associated with a higher frequency of low-risk genotypes [4, 5]. It can be assumed in such patients that strong predisposing immunological factors linked to the DR3 and DR4 haplotypes trigger the disease early in life, explaining the

predominance of DR3/4 patients in the childhood-onset group [6].

Material and methods

Subjects. The group of patients consisted of 60 index cases aged 2 to 18 registered at the Pediatrics Department of the Clinical Emergency Hospital Constanta during the year of 2009 and two cases detected by family history, siblings of two probands, also with T1DM, aged 25 and 24, respectively. From the 60 index cases 52 had no relatives with T1DM, while eight cases presented positive family history for T1DM – four had first degree relatives affected and four had distant relatives affected.

Methods. Clinical data were recorded in a standardized protocol. This included age at diabetes onset, the presence or absence of acute symptoms before diagnosis (polydipsia, polyuria, unintentional loss of weight, and ketosis or ketoacidosis), and family history of diabetes. We also collected data on blood glucose at first admittance. We separated patients in two age groups: onset before 5 years (0-5, early onset) and onset after 5 years of age [5-…). In respect with the mode of onset we divided the patients in three categories: mild, moderate and severe ketoacidosis (KA). Mild KA category included patients with polydipsia, polyuria, loss of weight ± ketotic halitosis and anorexia. Moderate KA patients had also nausea and/or vomiting and tachypnea. Severe KA was diagnosed in patients with added neurological signs (disorientation to stupor or coma). Values of pH and serum bicarbonate concentration at onset were recorded only for those patients with the first admission in our hospital after 2001; this accounted for less than half of the patients (26 patients – 41.9%). The information about pH and/or serum bicarbonate, when available, was used to separate patients into the three KA categories listed above. The cut-off values were: for mild KA pH > 7.30 and/or bicarbonate concentration >15 mEq/L, for moderate KA pH >7.2 and/or bicarbonate >10 mEq/L and for severe KA pH >7.1 and/or bicarbonate >5 mEq/L.

HLA typing was performed, as previously mentioned [7], for all 62 patients. Total genomic DNA was extracted from EDTA-anticoagulated venous blood with the QIAmp DNA Blood Mini Kit. HLA genotyping was performed with PCR-based sequence-specific oligonucleotide probe assays, the RELI SSO HLA typing systems from Invitrogen, and the ambiguities were solved with two sequence-specific primers systems, Domino HLA SSP System from Protrans and AllSet Gold SSP System from Invitrogen. Four digits HLA-DR alleles were detected, but for the purpose of this study we separated the patients in four groups based on the presence or absence of the high risk alleles of the DR3 or DR4 groups [8]. The HLA conferred susceptibility was graded into the following categories: DR3/4 (high-risk), DR4/Y (moderate risk, Y signifies any non-DR3 allele), DR3/X (low risk, X signifies any non DR-4 allele) DR Z/Z (no risk, Z signifies any non-DR3 and non DR-4 allele). A summary of these data is presented in Table 1.

Statistical analysis. For all analyses, a two-tailed P value of 0.05 was considered significant. All statistical analyses were performed using the Statistical Package for Social Science (SPSS version 17.0). Frequencies were calculated by count, and

comparisons were made in a contingency table using the Pearson's chi-square test for independence.

Results and discussions

One or two high risk alleles were found in roughly 95% of our patients (Table 1). Only three out of 62 patients (4.9%) carried no high risk allele. This is consistent with findings in other studies that analyzed the DR genes in children with T1DM [6]. However, when the studies included adult onset T1DM patients a greater DR heterogeneity was observed, with a greater proportion of patients carrying the no risk genotype. [6, 9] The frequency of the high risk genotype was 30.6%, slightly lower when compared to 36-38% in other studies [6, 10], but still higher than in other Romanian T1DM groups (20.75%) [11].

Table 1. Clinical and genetic aspects of the 62 patients with T1DM.

DR Genotype	3/4 (n = 19)	4/Y (n = 23)	3/X (n = 17)	Z/Z (n = 3)
Percent	30.6	37.1	27.4	4.9
Age at onset (years)	9.2 ± 4.1	6.1 ± 4.2	8.4 ± 3.8	6 ± 4.3
Family history (%)	9.7	6.5	-	-
Mode of onset				
Mild KA* (%)	14.5	16.1	12.9	3.2
Moderate KA (%)	12.9	16.1	8.1	1.6
Severe KA (%)	3.2	6.4	4.9	-
Blood glucose (mg/dl)	475 ± 163	462 ± 115	507 ± 128	370 ± 121

* category includes 6 patients with ketosis without KA

The DR genotype was found to be associated with the age of onset, when the patients were separated into two categories – onset before and after age of 5, respectively (p=0.031, Table 2), but no association was found when patients where separated in 5-year groups (data not shown).

We observe a very low frequency of the DR 3/4 genotype in the 0-5yrs onset group. This is much lower than expected, even when compared with other Romanian groups (17.4%); the international studies report as much as 50% of children with onset on T1DM before age of 5 carrying the high risk genotype [10, 11]. Same distribution, also not so striking is also found for the moderate risk DR 4/Y genotype, where a higher proportion of patients had the onset after the age of 5 (73.9% compared with 26.1%); however this is a group that is genetically more complex, been known that there is a great variation in the susceptibility conferred by the alleles in DR 4 group, depending also on the DQ alleles harbored [12]. No significant difference is observed in the age distributions of patients with the low risk DR 3/X and no risk DR Z/Z genotypes.

The association of DR genotypes with the mode of onset could not be demonstrated (p=0.786, Table 3). The frequencies were similar between groups and the null hypothesis could not be rejected leading to the conclusion that DR genotype has no influence on the mode of onset. Similarly, no association of the DR genotype with the levels of the blood glucose at onset was found; when

the mean values of the blood glucose at onset in the three DR genotypes including the majority of patients (DR 3/4, DR 4/Y and DR 3/X) were compared in one-way Anova test, the association could not be demonstrated (p=0.579, Table 4).

Table 2. Analysis of the association between DR Genotype and Age at Onset

			Age at Onset		
			(0-5)	[5-...)	Total
DR Genotype	3/4	Count	1	18	19
		% within DR Genotype	5.3%	94.7%	100.0%
		% within Age at Onset	6.3%	39.1%	30.6%
		% of Total	1.6%	29.0%	30.6%
	4/Y	Count	6	17	23
		% within DR Genotype	26.1%	73.9%	100.0%
		% within Age at Onset	37.5%	37.0%	37.1%
		% of Total	9.7%	27.4%	37.1%
	3/X	Count	7	10	17
		% within DR Genotype	41.2%	58.8%	100.0%
		% within Age at Onset	43.8%	21.7%	27.4%
		% of Total	11.3%	16.1%	27.4%
	Z/Z	Count	2	1	3
		% within DR Genotype	66.7%	33.3%	100.0%
		% within Age at Onset	12.5%	2.2%	4.8%
		% of Total	3.2%	1.6%	4.8%
	Total	Count	16	46	62
		% within DR Genotype	25.8%	74.2%	100.0%
		% within Age at Onset	100.0%	100.0%	100.0%
		% of Total	25.8%	74.2%	100.0%
Pearson Chi-Square **8.902**			*P* value **.031**		

Table 3. Analysis of the association between DR Genotype and Mode of Onset

			Mode of onset			
			Severe KA	Moderate KA	Mild KA	Total
DR Genotype	3/4	Count	2	8	9	19
		% within DR Genotype	10.5%	42.1%	47.4%	100.0%
		% within Mode of Onset	22.2%	33.3%	31.0%	30.6%
		% of Total	3.2%	12.9%	14.5%	30.6%
	4/Y	Count	3	10	10	23
		% within DR Genotype	13.0%	43.5%	43.5%	100.0%

		% within Mode of Onset	33.3%	41.7%	34.5%	37.1%
		% of Total	4.9%	16.1%	16.1%	37.1%
	3/X	Count	4	5	8	17
		% within DR Genotype	23.5%	29.4%	47.1%	100.0%
		% within Mode of Onset	44.5%	20.8%	27.6%	27.4%
		% of Total	6.4%	8.1%	12.9%	27.4%
	Z/Z	Count	0	1	2	3
		% within DR Genotype	0.0%	33.3%	66.7%	100.0%
		% within Mode of Onset	0.0%	4.2%	6.9%	4.9%
		% of Total	0.0%	1.6%	3.2%	4.9%
	Total	Count	9	24	29	62
		% within DR Genotype	14.5%	38.7%	46.8%	100.0%
		% within Mode of Onset	100.0%	100.0%	100.0%	100.0%
		% of Total	8%	38.7%	46.8%	100.0%
Pearson Chi-Square **1.725**			*P* value **.786**			

Table 4. ANOVA analysis of DR Genotype (3/4, 4/Y, 3/X) and Mean Blood Glucose at Onset

Descriptive Statistics

DR Genotype		N	Minimum	Maximum	Mean	Std. Deviation
3/4	Blood Glucose at Onset	19	220	900	475.79	163.752
4/Y	Blood Glucose at Onset	23	300	700	462.35	115.866
3/X	Blood Glucose at Onset	17	350	800	507.65	128.186
Z/Z	Blood Glucose at Onset	3	260	500	370.00	121.244

ANOVA	Sum of Squares	df	Mean Square	F	*P* value
Between Groups	20493.403	2	10246.702	.551	.579

When we ignored the DR genotype effect, and, instead, compared the mode of onset between the age groups, we found an association (p=0.040, Table 5). The different distribution of the severe KA cases in the age groups accounted for this finding – the severe KA onset was better represented in the early onset group (31.3% compared with 8.7% in the late onset group), while the moderate KA had a closer distribution with a slight predominance in the late onset group (45.7% vs 18.8%) and the mild KA onset was equally distributed between age groups (50% vs 45.7%). This can be explained by the delayed recognition of the symptoms in the early age group; younger children usually go undiagnosed for longer periods of time.

Table 5. Analysis of the association between Mode of Onset and Age at Onset

			Age at Onset		
			(0-5)	[5-...)	Total
Mode of Onset	Severe KA	Count	5	4	9
		% within Mode of Onset	55.6%	44.4%	100.0%
		% within Age at Onset	31.3%	8.7%	14.5%
		% of Total	8.1%	6.5%	14.5%
	Moderate KA	Count	3	21	24
		% within Mode of Onset	12.5%	87.5%	100.0%
		% within Age at Onset	18.8%	45.7%	38.7%
		% of Total	4.8%	33.9%	38.7%
	Mild KA	Count	8	21	29
		% within Mode of Onset	27.6%	72.4%	100.0%
		% within Age at Onset	50.0%	45.7%	46.8%
		% of Total	12.9%	33.9%	46.8%
	Total	Count	16	46	62
		% within Mode of Onset	25.8%	74.2%	100.0%
		% within Age at Onset	100.0%	100.0%	100.0%
		% of Total	25.8%	74.2%	100.0%
Pearson Chi-Square **6.427**			*P* value **.040**		

Conclusions

In our study, we found a correlation of the DR genotype with the age at onset of T1DM. However, we found an unusual distribution of the DR genotypes in the age groups with very low frequency of the high risk genotype DR 3/4 in the early onset group. The mode of onset and the blood glucose levels at onset were not influenced by the DR genotype. We also found an association between the age at onset and the mode of onset, probably due to the delayed recognition of the T1DM symptoms in the very young children.

REFERENCES

1. Sheehy MJ, Scharf SJ, Rowe JR, Neme de Gimenez MH, Meske LM, Erlich HA, Nepom BS. A diabetes-susceptible HLA haplotype is best defined by a combination of HLA-DR and -DQ alleles. *J Clin Invest* 83: 830-5. 1989.

2. Lermmark Å 1999 Type I diabetes. *Clin Chem* 45: 1331-1338. 1999.

3. Aly TA, Ide A, Jahromi MM, Baker JM, Fernando MS, Babu SR, et al. Extreme genetic risk for type 1A diabetes. *PNAS* 103(38): 14074-9. 2006.

4. Valdes AM, Thomson G, Erlich HA, Noble JA. Association between type 1 diabetes age of onset and HLA among sibling pairs. *Diabetes* 48: 1658-61. 1999.

5. Steenkiste A, Valdes AM, Feleo M, Hoffman D, Concannon P, Noble J, et al. 14th International HLA and Immunogenetics Workshop: report on the HLA component of type 1 diabetes. *Tissue Antigens* 69(suppl.1): 214-25. 2007.

6. Caillat-Zucman S, Garchon H J, Timsit J, Assan R, Boitard C, Djilali-Saiah I, et al. Age-dependent HLA genetic heterogeneity of type 1 insulin-dependent diabetes mellitus. *J Clin Invest*. 90(6): 2242-50. 1992.

7. Durbala I. Assessment of the risk for type 1 diabetes mellitus conferred by HLA class II genes. *Annals of the RSCB* 14(2): 123-9. 2009.

8. *Mrena S, Virtanen S, Laippala P, Kulmala P, Hannila M-L, Åkerblom HK, Knip M, the Childhood Diabetes in Finland Study Group*. Models for predicting type 1 diabetes in siblings of affected children. ***Diabetes* Care** 29: 662-*667,* 2006.

9. Erlich H, Valdes AM, Noble J, Carlson JA, Varney M, Concannon P, et al. HLA DR-DQ haplotypes and genotypes and type 1 diabetes risk. *Diabetes* 57(4): 1084-92. 2008.

10. Cucca F, Guja C. Genetic factors in the etiology of autoimmune type 1 diabetes. In: Cheta D, editor. *Genetics of diabetes: the truth unveiled.* The Publishing House of the Romanian Academy and S. Karger AG, Bucharest. 46-9. 2008.

11. Guja C. Particularitati genetice la DZ tip 1 la populatia din Romania. In: Guja C. *Factori genetici implicati in etiopatogenia diabetului zaharat tip 1*. The Publishing House of the Romanian Academy, Bucharest. 223-31. 2006.

12. Pugliese A, Eisenbarth GS. Human type 1 diabetes mellitus: genetic susceptibility and resistance. In: Eisenbarth GS, Lafferty KJ, editors. *Type 1 diabetes: molecular, cellular, and clinical immunology.* Oxford University Press, Inc. New York 134-45. 1996.

Correspondence Data:
Durbală Irina
Cell and Molecular Biology Department, Faculty of Medicine, "Ovidius" University Constanţa
Aleea Universităţii nr.1
900437 Constanţa, Romania
Tel: (+40) 723-016601 fax: (+40) 241-605001
e-mail: irina_durbala@yahoo.com