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Analysis Of Family Cases With Type I Diabetes Mellitus In A Group Of Pediatric Patients From Constanța County

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ABSTRACT. The aim of this study was to present the cases with family history of type 1 diabetes in a group of pediatric patients from the Clinic of Pediatrics, Constanta County Hospital, and to analyze the risk for relatives conferred by presence of particular DR genotypes. 8 out of 60 index cases had relatives affected with type 1 diabetes (13.33%). Of these, four had first-degree relatives affected (6.67%) and were presented here. Although high-risk genotypes were more frequent in the group of familial cases, no association was found between DR genotype and family history of type 1 diabetes when statistical significance was tested (p value 0.119).

Key words: type 1 diabetes, DR genotype, family history, genetic risk

Introduction

The natural progression of type 1 diabetes (T1D) suggests the presence of several stages: the presence of genetic susceptibility, development of autoimmunity without clinical signs and, finally, clinically manifest disease [1]. Genetic susceptibility, mainly encoded by major histocompatibility complex class II genes HLA-DRB1 and HLA-DQB1, modulates disease stages, producing a faster or slower progression of the autoimmune process, more or less important β cell destruction and therefore the variable onset age and severity of clinical manifestations and apparition of secondary cases in families. Identification of the associations between HLA alleles and diabetes is important both for assessing prognosis, although to a lesser extent, but especially for risk calculation in families of patients or in the general population [2].

Materials and Methods

The study group included 62 cases of T1D, represented by patients in the Clinic of Pediatrics, Constanta County Hospital during October 2008 - September 2009 (60 cases) and their first degree relatives who also had T1D (2 cases).

We determined HLA-DRB1 and DQB1 alleles by molecular methods, and haplotypes and genotypes resulting from their combination [3];

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than we performed a comparative study of clinical manifestations in different groups of patients defined by presence of HLA-DR genotypes recognized as with different risk for disease.

To examine associations between genetic factors and clinical manifestations we subdivided the 62 patients with T1D by categories of the DR genotypes, defined in order of risk that they confer [4]:

- DR3/DR4 heterozygous genotype (abbreviated 3/4) - high risk genotype;
- DR4/nonDR3 genotype (abbreviated 4/Y, including genotype homozygous DR4/DR4 and heterozygous genotype any DR4/Z, where 'Y' is any DR allele except DR3 and 'Z' is any DR allele other than DR3 and DR4) - this genotype confers moderate risk for disease;
- DR Genotype3/X (abbreviated 3/X, including genotype homozygous DR3/DR3 and heterozygous DR Genotype3/Z, where 'X' is any DR allele except DR4) - this genotype confers a low risk (but still higher than unit);
- Genotype nonDR3/nonDR4 (abbreviated Z/Z, heterozygous or homozygous genotype include any alleles, but without DR3 or DR4 alleles) - this genotype does not confer risk for type 1 diabetes, specifically in terms of relative risk (RR), it is either neutral ($RR \sim 1$) or confers protection ($RR < 1$).
- For analysis of family history of disease, patients were separated into two categories:
 - Family cases - patients who have relatives affected with T1D, regardless of their degree of kinship; and
 - Single cases - patients who don't have relatives affected with T1D.

Statistical methods. For all analyzes was considered significant a p value of 0.05. Testing statistical significance of differences observed between categories of variables was analyzed by chi square test (χ^2) using appropriate contingency tables. Chi square test was calculated using SSPS software version 17.0. Chi square test provided the p value for the comparisons made.

Results

Of the 60 patients enrolled in the study as probands (index cases), 8 had a history of affected relatives with T1D (13.33%). Of these, four had first-degree relatives affected (6.67%) and four other had relatives affected with higher degree of kinship (a patient had the paternal grandfather affected with T1D - second degree kinship and three others had affected relatives of 3rd or 4th degree). In Table no.I are presented the clinical and genetic data of the 10 patients.

Gender distribution was M/F = 6/4, genotype DR3/4 was present in 5 patients, same as genotype DR4/Y, age at onset was only in two cases before 5 years, blood glucose at the onset quite dispersed (220-600 mg/dl), all forms of onset were present, but only one case had severe DKA at onset. There are no genotypes DR3/X and DRZ/Z.

Because in multifactorial diseases (also called common diseases with genetic predisposition) risk increases/decreases exponentially with the degree of kinship, we discussed only the cases in which patients had first degree relatives affected. Two of these patients, B.C. and U.O. had siblings affected and P.D.A. and S.M. had the father with T1DM (see pedigrees).

Case 1 - B.C. (male) was enrolled in the study at the age of 18. The disease started at the age of 14 with symptoms that can be placed in mild DKA category. He has family history of diabetes type 1 - a sister I.M. enrolled in the study at 25 years, with onset at 17 years and mild symptoms.

Genotype of the proband B.C. is DR4/Y (*0402/*0302 & *1301/*0603) and his sister's I.M. is DR3/4 (*0301/*0201 & *0402/*0302). Parental genotypes are (see Figure no.1): father's genotype B.T. is DR4/Y (*0401/*0302 & *0402/*0302) and the mother's B.E. is DR3/X (*0301/*0201 & *1301/*0603). The proband has a healthy twin sister, from whom we did not obtain biological samples.

We observe that in this case that the two brothers don't have the same genotype: I.M. presents the high-risk genotype DR3/4, while B.C. has moderate-risk genotype DR4/Y; however B.C.

Table I - Summary of clinical data and DR genotypes in patients with family history of T1D

No.	Name (initials)	DR Genotype	Sex	Age	Age at onset		Blood glucose (mg/dl)	Mode of onset	Family history T1D
					years	interval			
1	B.C.	4/Y	M	18	14	>5	300	Mild DKA*	1 st degree
2	I.M. [§]	3/4	F	25	17	>5	350	Mild DKA	1 st degree
3	U.O.	3/4	M	17	8	>5	400	Mild DKA	1 st degree
4	U.I. [§]	3/4	M	24	13	>5	500	Moderate DKA	1 st degree
5	P.DA.	4/Y	F	9	4	0–5	400	Moderate DKA	1 st degree
6	S.M.	3/4	F	7	2	0–5	220	Mild DKA	1 st degree
9	S.G.	4/Y	M	11	9	>5	600	Moderate DKA	2 nd degree
8	L.M.	3/4	M	14	5	>5	600	Severe DKA	3 rd degree
7	H.E.	4/Y	F	17	5	>5	400	Mild DKA	3 rd degree
10	M.AA.	4/Y	M	11	9	>5	400	Moderate DKA	4 th degree

developed T1D at a younger age than I.M. (14 vs. 17 years).

Case 2 - U.O. (male) was enrolled in the study at the age of 17. The disease started at 8 years of age with symptoms that can be placed in mild DKA. He has family history of T1D – a brother U.I. enrolled in the study at 24 years, with onset at 17 years with moderate DKA.

Genotype of the proband U.O. is DR3/4 (*0301/*0201 & *0401/*0302), same as his brother's U.I. (*0301/*0201 & *0401/*0302). Parental genotypes are (Figure no. 2): father's U.C. genotype is DR3/4 (*0301/*0201 & *0401/*0302) and the mother U.Ca. has DR4/Y (*0101/*0501 & *0401/*0302).

We observe that the father of the two patients, U.C., also carries the high risk genotype and yet had not developed T1D. The brothers have haplotypes DRB1/DQB1 identical by descent where a large chromosomal region (in this case MHC) is inherited from parents in block. It is obvious that both brothers have inherited haplotype *0301/*0201 from the father and the *0401/*0302 from the mother. We presume that the *0401/*0302 haplotype from the mother carries other yet unidentified susceptibility genes that are absent in the father's.

Case 3 - P.DA. (female) - was enrolled in the study at age of 9. The disease started at age 4 ½ years with moderate DKA. She has family history of T1D - his father P.A. has the disease, but did not provided biological sample for testing.

Genotype of the proband P.DA. is DR4/Y (*0401/*0302 & *0407/*0302) and her mother's genotype M.A. (see Figure no. 3) is DR4/Y (*0401/*0302 & *1401/*0503). Father's genotype is unknown, but we know that his daughter inherited from him the haplotype *0407/*0302.

Particularities of this case are: (1) family with low socioeconomic status, parents didn't properly supervise patient's diet and treatment with insulin. P.DA. presented in the first year after onset several

Figure 1 - Pedigree of B.C. He has an elder sister affected with T1D, carrying a different DR genotype

Figure 2 - Pedigree of U.O. He has an elder brother affected, with a DR genotype identical by descent

Figure 3 - Pedigree of P.D.A. She has the father affected

bouts of hypoglycemia that led to hypoglycemic coma and is now diagnosed with secondary epilepsy; (2) father P.A. developed the disease at the age of 27 years (diagnosis confirmed by the presence of specific antibodies and the need for treatment with insulin) three years after his daughter P.D.A.

Case 4 - S.M. (female) - was enrolled in the study at age of 8. The disease started at 2 years and 8 months, with mild DKA symptoms. Father S.L. has type 1 diabetes from age 14, but was unavailable for biological sampling.

Genotype of the proband S.M. is DR3/4

Table II - Analysis of the association between DR genotype and family history

			Family history		
			Present	Absent	Total
DR Genotype	3/4	Number of cases	5	14	19
		% in DR Genotype	5.3%	94.7%	100.0%
		% in Family history	50.0%	26..9%	30.6%
		% din Total	8.06%	22.6%	30.6%
	4/Y	Number of cases	5	18	23
		% in DR Genotype	26.1%	73.9%	100.0%
		% in Family history	50.0%	34.6%	37.1%
		% din Total	8.06%	29.0%	37.1%
	3/X	Number of cases	0	17	17
		% in DR Genotype	0%	100.0%	100.0%
		% in Family history	0%	32.7%	27.4%
		% din Total	0%	27.4%	27.4%
	Z/Z	Number of cases	0	3	3
		% in DR Genotype	0%	100.0%	100.0%
		% in Family history	0%	5.7%	4.8%
		% din Total	0%	4.8%	4.8%
	Total	Number of cases	10	52	62
		% in DR Genotype	16.1%	83..9%	100.0%
		% in Family history	100.0%	100.0%	100.0%
		% din Total	16.1%	83.9%	100.0%
χ^2 5.839			p value = 0.119

Figure 4 - Pedigree of S.M. She has the father affected

(*0301/*0201 & *0401/*0302) and her mother's C.M. genotype is DR3/X (*0301/*0201 & *1101/*0301), as shown in Figure no.4. S.M. inherited from the mother haplotype *0301/*0201 and haplotype *0401/*0302 from the father.

Particularity of this case is the early onset; S.M. is actually the only patient with genotype DR3/4 and onset before the age of 5, enrolled in our study. Perhaps the haplotype inherited from the father, presents also other risk factors, outside DRB1 and DQB1 genes conferring susceptibility to T1D.

Analysis of DR genotype association with family history of T1D is shown in Table II. As mentioned, in the 10 patients with a family history were present only two categories of DR genotypes, namely DR3/4 and DR4/Y. Testing statistical significance of the association of DR genotype and family history for T1D produced a p-value > 0.05, which excludes the existence of an association between two variables. Moreover, with no cases in two of the eight cells of the contingency table, the results have to be interpreted with caution.

Discussion

Most patients with T1D did not have relatives affected by the disease. Analysis of family history for T1D showed the presence of affected relatives to 8

of index cases (13.3%), while 86.7% of index cases were single cases. In total we enrolled in the study 10 patients with a family history. This distribution is consistent with that observed in other studies in which over 85% of patients are unique cases [5]. In terms of risk for type 1 diabetes are important cases where there were first-degree relative are affected. 4 probands had first degree relatives with type 1 diabetes - in two cases probands had affected siblings who were also enrolled in the study and the two other cases had a parent affected, in both cases the father.

Although not validated by statistical testing, DR genotypes distribution in the categories of family history is consistent with that described in the literature [6], with high and moderate risk genotypes increased in the family cases: 50% of familial cases showed genotype DR3/4 vs. 26.9% in single cases and the remaining 50% had genotype DR4/Y vs. 34.6% of single cases.

Knowing DR genotype in patients with T1D is important for risk estimation in 1st degree relatives. As shown in the cases presented in the study, several situations may be found.

Risk in the offspring of diabetic parents is higher in children with diabetic father (6-8%) than those with diabetic mother (2-4%). Preferential transmission of T1D from diabetic father was described previously in numerous studies. This difference was attributed to several mechanisms, including preferential transmission of HLA risk haplotypes father, insulin gene imprinting or reduced fertility of women with T1D compared to affected men [7]. In our study, father of S.M. has diabetes, whose disease started at 14 years.

Another example is the risk in siblings of a diabetic patient. The overall risk of the brothers is about 6% [8]. However, depending on the identity with the proband, genetic risk for T1D varies quite widely: siblings who share with the proband 2, 1 or 0 haplotypes identical by descent have a risk for disease of 12.9%, 4.5% and 1.8%, respectively. This risk may increase to 20% in siblings who have haplotypes identical by descent with DR3/4 probands [9]. In our study we observed two cases where siblings developed the disease. In the first case, the proband B.C., developed the disease at the age of 14 years, 4 years after his sister I.M. who developed the disease

at 17 years. IM has genotype DR3/4 and B.C. shares with his sister a haplotype identical by descent with genotype 4/13; although I.M.'s genotype put her at higher risk for disease, she had the onset at an older age than her brother. In the second case, U.O. has a sibling with T1D, U.I. First U.O. developed T1D at 8 years, and after two years his elder brother developed the disease (17 years). The brothers have haplotypes identical by descent DR3/4, which have the greatest risk to cause disease in siblings.

Finally, there is the risk of patients' parents. Depending on the study, the risk is estimated at 1.9-3.8, regardless of the sex of the parent [10]. In our study we also met this situation, the proband P.DA. developed the disease at the age of 8, and her father presented with T1D three years later, at the age of 27.

Conclusions

1. Prevalence of family history on the study group was similar to that in other studies.
2. Four probands had 1st degree relatives affected with T1D.
3. Presentation of clinical cases highlighted and demonstrated the risk of secondary cases in families with type 1 diabetes patients.
4. Knowing the genotype and HLA-DRB1 and DQB1 haplotypes in patients allows correct estimation of risk for T1D in patients' relatives.

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