Abstract

Type 1 diabetes (T1DM) is a chronic autoimmune disease characterized by the T cell mediated destruction of the pancreatic beta cells. Its pathogenesis involves multiple genes and environmental factors. The major diabetes genes reported so far are the HLA class II (*IDDM1*) and the insulin gene (*IDDM2*) loci. In order to assess the role of genetic and environmental factors in T1DM for the Romanian population (with one of the lowest reported incidence of T1DM in Europe), we typed HLA/*IDDM1* and *INS/IDDM2* in 423 nuclear families. The study group comprised 1,515 individuals with 439 T1D patients (206 male/224 female) and 1,076 unaffected first degree relatives. Genotyping was done by PCR-SSOP (*IDDM1*) and Taqman® 5' nuclease assay (*IDDM2*). We defined as HLA(+) the subjects positive for DR3-DQ2 or DQ8 and as HLA(-) those DR3-DQ2(-) and DQ8(-) negative. We defined as *INS*(+) the AA homozygotes for the -23*Hph*I SNP of the insulin gene and as *INS*(-) carriers of the AT or TT genotypes.

We found 90% HLA(+) patients (395 of 439) versus 10% HLA(-) (44 patients). *INS*(+) patients represented 84.3% (370 of 439) while 15.7% were *INS*(-). In the unaffected group there were 73% HLA(+) subjects (786 of 1076) vs. 27% HLA(-) and 70% *INS*(+) subjects (753 of 1076) vs. 30% *INS*(-). We found 74 HLA(-)/*INS*(-) unaffected first degree relatives (6.87%) vs. only 7 (1.59%) of 439 diabetic patients. Overall 537 non-diabetic subjects (49.90%) were HLA(+)/*INS*(+) while 50 (4.64%) carried the most diabetogenic genotype: HLA DR3-DQ2/DQ8 plus -23*Hph*I A/A.

In conclusion, there are extremely rare (around 1.5%) cases of T1DM without apparent HLA and *INS* genetic susceptibility. Meanwhile, almost 50% of the unaffected first degree relatives are genetically susceptible (HLA+ and *INS*+) without developing the disease, almost 5% of them carrying the most diabetogenic known genotype. So, our data indicate that the genetic susceptibility is necessary for T1DM occurrence but it is not sufficient, suggesting the conjugate intervention of different environmental factors and other genes that remain to be elucidated.

Effect of HLA (*IDDM1*) and insulin gene (IDDM2) in type 1 diabetes patients and their first-degree relatives in Romania.

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INTRODUCTION

Type 1 diabetes mellitus (T1DM) is a common chronic autoimmune disease that appears in genetically predisposed individuals.

The maximum reported concordance rate for T1DM in monozygotic twins of just 30-50% indicates the importance of environmental factors and suggests that genetic factors are not sufficient for T1DM pathogenesis.

In a previous report we showed that **only 0.47%** of a group of T1DM patients from Romania were **"Genetic negative"** i.e. without diabetogenic alleles at three of the main genetic susceptibility loci for Romanians: ***IDDM1***, ***IDDM2*** and ***ICAM1***.

This strongly suggests that **genetic susceptibility is NECESSARY** for T1DM occurrence.

AIM

Our aim was to **determine** the prevalence of susceptibility ***IDDM1*** **(HLA class II)** and ***IDDM2*** **(Insulin gene INS-VNTR)** genes in T1DM patients and in their first degree relatives.

This allowed us **to evaluate indirectly** the role of genetic and **environmental factors** in T1DM **in Romania**, country with one of the lowest incidences of the disease in Europe.

MATERIALS AND METHODS (1)

- **423 Romanian T1DM families** from all over Romanian territory.
  - 407 simplex families
  - 7 multiplex families
  - 9 families with a parent/sibling pair affected
- **1515 individuals** (706 M/809 F)
- **1076 unaffected individuals**
- **439 type 1 diabetic patients** (208 M/231 F) - 430 siblings and 9 parents
  - Onset of disease between 9 months and 43 years.
  - The median age at the onset of disease was 12.4 years.
  - Most patients (> 70%) had diabetes onset before the age of 14 years ("juvenile" type 1 diabetes)

MATERIALS AND METHODS (2)

- Genotyping was done by **PCR-SSOP (*IDDM1*)** and **Taqman® 5' nuclease assay (*IDDM2*)** typing for the -23*Hph*I A/T SNP, an accurate indicator of the *INS*-VNTR alleles.

- We define as HLA(+) the subjects positive for DR3-DQ2 or DQ8 (diabetogenic HLA's) and as HLA(-) those DR3-DQ2 and DQ8 negative.

- We define as INS(+) the AA homozygotes for the -23*Hph*I SNP of the insulin gene and as INS(-) carriers of the AT or TT genotypes.

RESULTS (1)

***IDDM1* and *IDDM2* status in 439 T1DM subjects**

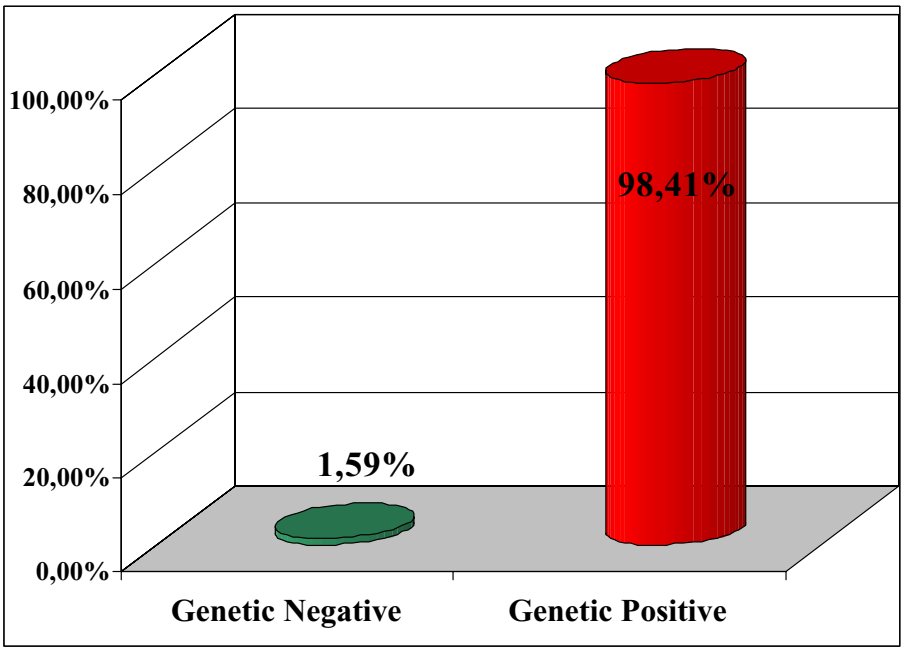
	Absent	**Present**
***IDDM1* (HLA+)**	**44 – 10.03%**	**395 – 89.97%**
***IDDM2* (INS+)**	**69 – 15.71%**	**370 – 84.3%**
"Genetic suceptibility" HLA(+) or INS(+)	**7 – 1.59%**	**432 – 98.41%**

RESULTS (2)

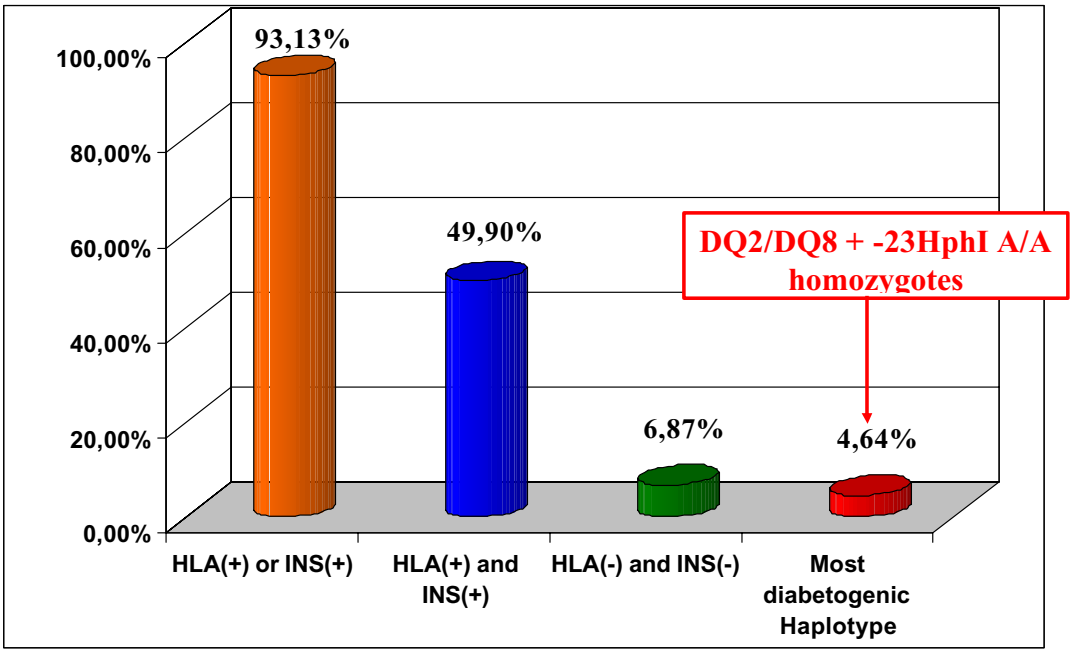
***IDDM* and *IDDM2* status in 1076 unaffected first degree relatives**

	Absent	Present
IDDM1 **(HLA+)**	290 – 26.95%	786 – 73.05%
IDDM2 **(INS+)**	323 – 30.02%	753 – 69.98%
HLA(+) and INS(+)	539 – 50.10%	537 – 49.90%
DR3-DQ2/DQ8 and -23Hph A/A	1026 – 95.36%	50 – 4.64%

Prevalence of "***genetic susceptibility***" in T1DM subjects



Prevalence of "***genetic susceptibility***" in unaffected first degree relatives



CONCLUSION

- There are **extremely rare** (around 1.5%) the cases of **T1DM without** apparent **genetic susceptibility**.
- **Almost 50%** of the **unaffected** first degree relatives are **genetically susceptible** (HLA+ and INS+) without developing yet the disease, **almost 5%** of them carrying the **most diabetogenic** known **genotype**
- Our data show that **the presence of diabetogenic alleles does not "guarantee" T1DM occurrence**.
- In conclusion, our data sustain that the **genetic susceptibility**, though necessary, **is NOT SUFFICIENT for T1DM** occurrence suggesting the **conjugate interventions** of different **environmental factors** (and other genes) that remain to be elucidated.