THE MARKERS FOR IMMUNO-GENETIC SUSCEPTIBILITY IN CHILDHOOD DIABETES

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Abstract

Diabetes mellitus (DM) is a heterogenous sindrome characterized by a complex disturbance of the energetic metabolism, which affects the metabolismof both carbohydrates, lipids and proteins and also the other metabolisms. These disturbances result from an insulin secreting defect (the decrease of β cell mass / function), associated sometimes with a degree of peripheral insulin resistance. Prediction of type 1 DM, meaning the appreciation of the risk to develop the disease, raises a great theoretical and practical interest. This is based on the acceptance of the autoimmune pathogeny in most of the cases (DM type1A) and the understanding of the progressive, stadial evolution of the β-cell destructive process (1). The prediction strategies are using the genetic, immunologic and metabolic markers which define the risk of the patients to develop type 1 DM.

Key words: susceptibility, childhood, diabetes

Introduction

From a genetic point of view, diabetes is a complex, poligenic disease, involving numerous susceptibility genes and some protective genes, all with incomplete penetrance, reciprocally conditioning each other.

Actually, is unanimously accepted that the short prediagnosis period in type 1 DM is the top of a huge iceberg, just partially explored by the modern imunogenetic studies. These studies prefigure a stadial evolution of a variable duration (2) (months, years).

In this period of time the disease is ongoing through 6 evolutive phases:

- genetic susceptibility *(3),*
- precipitanting event (intervention of the trigger factors),
- overt immunologic abnormalities (autoantibodies: GAD, ICA),
- progressive loss of insulin release,
- overt diabetes,
- complete islet beta cell destruction.

The genetic markers used in association with the family history shows that the risk of type 1 DM is (4):
- 1/5.000 in cases without susceptibility alleles or family history
- 1/4 if two risk alleles exist and a positive family history.

A. Genetic markers:

In the last years numerous genes were studied (chromosomial regions); of these, two regions are mostly involved in the genetic susceptibility for type 1 DM:

- HLA region on short arm of chromosome 6 (6p21.3) noted IDDM1 and
- insulin gene region on the short arm of chromosome 11 (11p15), noted IDDM2. IDDM1 is responsible for almost 50% of the genetic susceptibility, while IDDM2 for 10-15% (5, 6, 7). Beside these two regions, genom –wide scan studies identified at least 18 chromosomial regions (noted IDDM3, IDDM4 etc.) associated with type 1 DM. For most of these regions, the susceptibility genes have not been precisely identified yet, the mechanism of their involvement in the pathogeny of the disease still remains to be clearified (8) (table 1).

The most known "diabetogenic genes" are those belonging to HLA system from the MHC region of the short arm of chromosome 6 (6p21.3) – with a major role in the immune response of the body (Fig. 1).

Presently is unanimously accepted that type 1 DM in the child is associated with:

- *DRB1*04-DQA1*0301-DQB1*0302* allele and
- *DRB1*03-DQA1*0501-DQB1*0201* and the decreased frequency should explain the low incidence of DM in some countries like Romania (9).

More than 90% of the diabetic patients with type 1 DM have predisposing alleles type DR3-DR4 – comparatively with 40-50% in the general population. The concomitant presence of DR3-DR4 in one patient increases the risk; actually this association is encountered in 30-50% of type 1 DM patients (compared to 1-6% in the general population).

Table I. Genome screensT1DM.

IDDM1	**6p21**	IDDM13	2q34-q35
IDDM2	**11p15**	IDDM15	6q21
IDDM3	15q26	IDDM17	10q25
IDDM4	11q13	IDDM18	5q31-q35 (IL2)
IDDM5	6q25-q27		1q42-qter
IDDM6	18q21		8q24
IDDM7	2q31	VDR, INFα	12q12-qter
IDDM8	6q27-qter		16p11-p16
IDDM9	3q21-q25		16q22-q24
IDDM10	10p11-q11		17q24-qter
IDDM11	14q24-q31	TGFβ1	19p13-q13
IDDM12	2q33 (CTLA4)		Xp13-p11

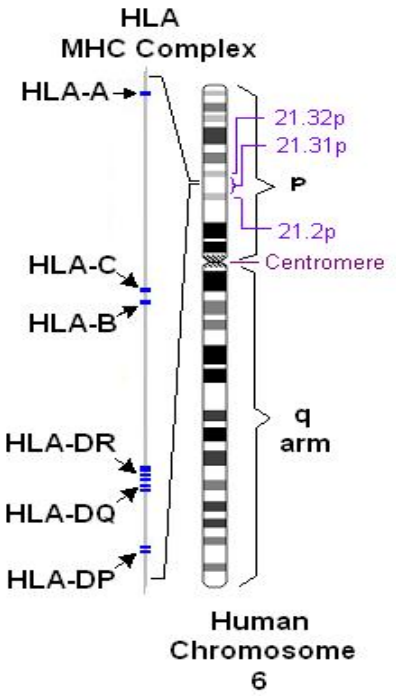

Fig. 1 - Schematic representation of HLA, projected on the short arm of chromosome 6 (Richard G Phelps and Andrew J Rees).

Numerous studies, confirm that the HLA DQ molecules have a primordial role in the predisposition to type 1 DM. DQA1*0301-DQB1*0302 is associated with an increased susceptibility for type 1DM in most of the populational group studied (10).

The study of the HLA-DP alleles didn`t offer any certain proof concerning their involvement in the predisposition for type 1 DM.

Some HLA alleles confer protection for the occurence of diabetes (11); we mean especially the following HLA molecules:

- DQ6 (DQB1*0602 si DQB1*0603),
- DQ7 (DQB1*0301/0304),
- DRB1*1401
- DQA1*0201

The protection confered is not absolute, however, less than 1% of type1 DM patients have these alleles. These protective alleles seem to have dominance upon the susceptibility alleles.

The second region proved to be associated with type1DM is the region for insulin gene on chromosome 11 - 11p15 (IDDM2). We talk about polimorphysms from a variable zone (VNTR – Variable Number of Tandem Repeats) situated in region 5' reported to the insulin gene promotor which influences the reglatory mechanism of insulin gene transcription.

At this level 3 classes of alleles may exist. Class I haplotype are associated with Type 1 DM while those from class III confer protection (12).

The other locuses prooved to be involved in the predisposition for type 1 DM, include (Fig. 2):

- The lymphoid-specific phosphatase (LYP) encoded by *PTPN22* is involved in preventing spontaneous T-

cell activation by dephosphorylating and inactivating T-cell receptor-associated Csk kinase *(13)*. An arginine-to-triptophan substitution at codon 620 of PTPN22 was considerently reported to be associated with type 1 DM as well as other autoimmune diseases, such as rheumatoid arthritis, systemic lupus erythematosis (SLE) and Grave's disease. Genoyping of PTPN22 revealed the following alleles:

- the homozygous genotype for the T allele and the heterozygous genotype C/T is associated with an increased risk for developing type 1 diabetes
- the C/C homozygous genotype is protective against type 1 diabetes.
- The presence of the heterozigous genotype C1858T in patients with type 1 DM, increases the risk to associate other autoimmune disturbances (14).
- gene CTLA - 4 (Citotoxic T Lymphocite antigen) on chromosome 2q33 – corresponding to IDDM12
- gene for α chain of the interleukine 2 receptor (IL2RA/CD25) on chromosome10p15.

Possibly implicated in the predisposition for type1 DM are some polymorphisms from gene ICAM -1 (Intercellular Cell Adhesion Molecule I) and the gene for Vitamine D Receptor (VDR - Vitamin D Receptor) (15).

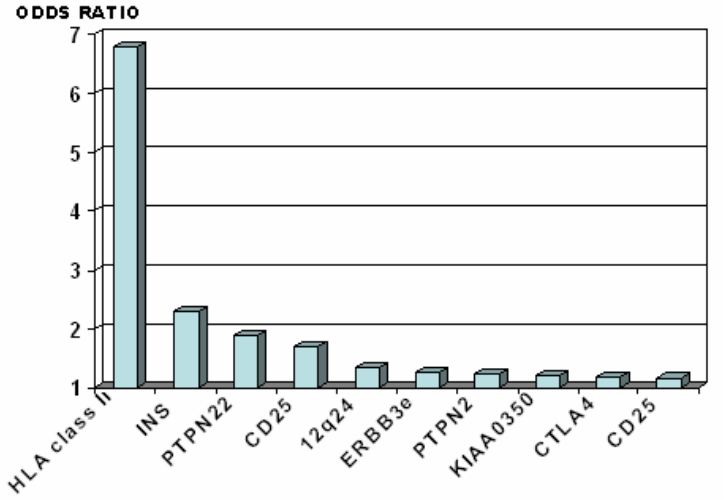

Fig. 2 - Summary of subset of confirmed loci from whole genome screens associated with type 1A diabetes (Modified from Todd et al. Nature Genetics, June 6, 2007).

B. The markers for autoimmunity

The autoimmune destructive process of the β cells is a chronic process, with variable duration and evolution velocity, individualised.

Within this period, the immunologic markers might be evidentiated, in the serum, including: islet cell antibodies (ICA), insulin autoantibodies (IAA), GAD65 antibodies etc.

Detection of antibodies in the serum has an important diagnostic significance, so, the high ICA is predictible for type1 DZ, before the occurence of the disease, fact that has been prooved in relatives of the diabetic patients. 8-10% of these, with an increased titre of these antibodies progress towards DM within one year.

The presence of markers in association, in the serum of some subjects, both in the general population and in some belonging to subgroups with increased risk for type 1 diabetes mellitus (type 1 DM), increases the probability for developing this disease(16).

- *ICA (islet cell antibodies)* was first described as being associated with type 1 DM. ICA titre is expressed as JDF conventional units (Juvenile Diabetes Foundation).
- ICA are present in serum in 70-80% of the diabetic patients even since onset (17). Allthough technically difficult to perform, they remain the most sensitive marker for the prediction of the risk to develop DM , titres above 20 U JDF showing a probability of 30-40% to develop the disease in the next 5 years.
- *IAA (Insulin Autoantibodies)* are present in serum since the onset (meaning before the initiation of insulin therapy) in 50-70% of the subjects, more frecquent in children than in adults. Their presence represents the proof of an ongoing β-cell destructive process and represents an important marker for the detection of the subjects at risk to develop type 1 DM (18).

- One of the most important autoantigenes that induces production of antibodies associated with DM is *GAD (Glutamic Acid Decarboxylase*) that is present in the β cells but also in CNS and the testicular tissue (19). The antibodies angainst the 65 kD peptide of GAD (GADA) are present in 70-80% of the patients type 1 DM and occur even since the prediagnostic period.
- Recently, a new family of β cell autoantigenes has been identified, the family of proteins PTP – Protein Tyrosine Phosphatase. The antibodies against a 40 kDa fragment of this protein also called *ICA512 or IA – 2*, occure in 60-70% of the subjects with type1 DM at the onset (20).
- There are also other citoplasmatic β cell antigenes responsible for the autoimmunity in DM. Of these, the most studied were*: ICA69, Carboxipeptidase H, Gangliozide GM2-1, Imogen 38, Glima 38, Peripherina, Hsp 60 (Heat Shock Protein 60) etc*.(21).

There are cases when healthy individuals are found with significant titres of diabetogenic antibodies that may persist years before the occurence of clinical DM or even without developing the disease (≈ 5% of the general population).

For the moment, prediction (expressed as the percentage probability of the risk to develop type1 DM) can`t offer an absolute precision (22). The association of the immunological tests and the genetic typing, more and more accesible, even in the newborn, because of the development of rapid, automatic and cheaper techniques, increases the accuracy of prediction comparatively to isolate evaluation of the humoral immunity.

In Romania there are just few studies concerning different aspects of the type 1 DM in children, but none of them prospective aiming for the evaluation of prediction and prevention in type 1 DM in the infantile population and the causal relationship *genetic predisposition – immune status – environment factors* (especially food).

There are still many questions to be answered, for instance if:

- the presence of the markers for cellular autoimmunity increase the risk for type 1 DM also in the general population,
- all subjects with autoimmune markers will develop type 1 DM,
- the detection of autoantibodies correlated with the reduction of first phase of the insulinic response increases the possibility of the disease to occure,
- the associations between antibodies increase the risk.

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