

Screening for Type 1 diabetes genetic risk in newborns of continental Italy. Primary prevention (Prevefin Italy) – Preliminary data

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Introduction

Type 1 diabetes mellitus (T1DM) is one of the most serious and frequent chronic diseases in children with incidence rates increasing in many parts of the world (1-3).

The young age of the patients, the need for long-term insulin therapy and the high prevalence of complications account for the great social impact of T1DM. For these reasons, the discovery of new strategies to prevent T1DM would affect public health deeply (4).

It is proposed that T1DM is precipitated by environmental factors, operating in a genetically susceptible host to initiate a destructive islet β -cell specific autoimmune process (5). In fact T1DM is caused by autoimmune destruction of the β -cells of the pancreas. Numerous T1DM susceptibility loci have been identified on the entire genome, but the greatest genetic contribution is provided by the polymorphism of the HLA class II genes (DRB1, DQA1 and DQB1) (6).

In the prodromic phase of the islet autoimmune process there are increased blood levels of autoantibodies against islet cell antigens, such as insulin (IAA), glutamic acid decarboxylase (GADA) and protein tyrosine-phosphatase (IA-2A) (5). These autoantibodies do not play a direct role in the destruction of β -cells, but they can be considered as a marker of the autoimmune response against pancreatic islets. Recent

studies show a 20% prevalence of anti- β -cell autoantibodies in high-risk subjects at 24 months of age (7).

The autoimmune process against islet cells causes a gradual impairment of β -cell function, which can occur at different times in different subjects. The final outcome is the decline of insulin secretion, which can be demonstrated by metabolic tests (8).

The reasons why only some genetic susceptible individuals proceed to diabetes have not been completely explained. Several theories have been proposed to explain the way utilized by some factors in activating autoimmunity against β -cells (5). A great number of environmental factors have been investigated as the possible triggers of autoimmune response against β -cells: 1) viral infections (e.g. Coxsackievirus and CMV); 2) early infant diet (e.g. breastfeeding versus early introduction of cow milk components); 3) toxins (n-nitroso derivatives) and 4) vitamin D deficiency.

Early intake of cow milk proteins – above all β -casein – has been reported as a risk factor for T1DM in high genetic risk subjects. Although cow milk β -casein molecular structure is different from human β -casein, its sequence is homologue to some pancreas β -cell autoantigens. Furthermore, a cell-mediated immune response against β -casein has been demonstrated in most patients with recent onset T1DM (9, 10).

Vitamin D deficiency seems to play a crucial role in the development of β -cell autoimmunity, as vitamin D acts as an immunosuppressive agent, by reducing

lymphocyte proliferation and cytokine production (11) and promotes the maturation of the intestinal mucosa. The supplementation of vitamin D early in life is associated with a lower prevalence of T1DM in humans (12).

Since only approximately 10% of newly diagnosed T1DM patients have a first degree relative affected by the same disease, population-wide genetic screening is needed in order to identify all high-risk subjects and to acquire relevant information on T1DM pathogenesis. Moreover, population-wide genetic screening for T1DM and the follow up of high genetic risk subjects have been implemented, including DAISY (13) and PANDA (14) in USA, DIPP (15) in Finland, and DIABFIN (16) in Italy.

Aims of the study

The general aim of this phase II study is to validate the efficacy of two primary prevention strategies (vitamin D supplementation and β -casein-free diet) in preventing the autoimmune aggression against pancreatic β -cells and, ultimately, the onset of T1DM. This should provide useful information to design clinical prevention trials for T1DM (phase III studies).

The specific aim of the Prevefin study is to reduce the prevalence of β -cell autoantibodies (GADA, IA-2A and IAA) in newborns identified as to be at high genetic risk for developing T1DM. A large cohort of newborns has been enrolled; and high genetic risk infants are strictly monitored for the appearance of β -cell autoantibodies.

Subjects and methods

Study population

After informed consent, according to the Helsinki rules, we enrolled Caucasian newborns from 11 centers of 4 regions in continental Italy: 1) Liguria (S. Martino H and Gaslini Institute, Genoa), 2) Umbria (Foligno H, Spoleto H), 3) Marche (Ancona H, Ancona University, Jesi H, Macerata H, Ascoli Piceno

H) 4) Lazio (La Sapienza University, Pertini H). Newborns with at least one parent of non-Caucasian origins were excluded from the study.

The study was approved by the Ethical Committees of G. Gaslini Institute, Umbria Region, Marche Region and La Sapienza University. Written informed consent was obtained from parents of all participating infants.

Methods

Before hospital discharge, a study recruiter meets the parents of the newborns whose cord blood has been stored, and asks for written informed consent for genetic screening.

- *Genetic screening.* After genomic DNA extraction and PCR amplification, HLA DRB1 and DQB1 second-exon sequences are typed in order to define genetic risk for T1DM, as previously described (16, 17).

Infants with the HLA genotype DRB1*03, DRB1*04, DQB1*0302, in the absence of the protective allele DRB1*0403 are considered at high risk and eligible for the study (16, 17). All other infants, at low or moderate genetic risk, are not further investigated. Parents of high genetic risk children are recalled before their infant is 1 month old, and a second informed consent for randomizing the child to the treatment is then collected. At this time, we collect clinical history and a capillary blood sample is taken in order to analyze β -cell autoantibodies. Clinical history includes family history, with special attention to autoimmune diseases, pregnancy history (infections, diet, drugs and vaccinations) and data on the newborns, including neonatal weight, diet and vitamin supplementation during the first month. All newborns are then randomly assigned to one of the two groups of treatment: group I, Vitamin D supplementation (500 units a day) plus diet without β -casein for the first 12 months of life; group II, vitamin D supplementation and free diet. The general pediatrician is contacted as soon as possible in order to explain the project. In the first group a seroprotein hydrolysate formula (Similac RA, Abbott) is introduced when exclusive breastfeeding cannot be continued. At the moment of weaning, β -casein free diet is continued

by using products without cow milk; an example of diet is provided to the family and pediatrician, and calorie and calcium intake is monitored by means of a diet history interview.

Every 3–6 months – and until the 5th year of age – a clinical and auxological examination is performed and a peripheral venous blood sample is taken for β -cell autoantibody (GADA, IA-2A and IAA) and coeliac disease autoantibody (TGAA) determinations. Positivity for any of the autoantibody investigated is confirmed when it is found in two consecutive samples. In this case, a glucagon metabolic test is performed in order to evaluate the insulin secretion profile (18) (Fig. 1).

GADA, IA-2A and IAA are determined in radio-binding assays (RBAs). For infants born in Liguria and Lazio, islet-related autoantibody assays are tested in the autoimmunity laboratory of the Milan University (19), while samples collected in infants from Umbria and Marche are tested in the immunogenetics laboratory of the Perugia University (20).

- *Efficacy* (evaluated on the basis of O'Brian–Fleming criteria)

The expected population is 120 high genetic risk newborns, 60 for each group, in order to evaluate the efficacy of primary prevention in reducing the prevalence of autoantibodies. Previous studies have demonstrated that 20% of high risk subjects develop autoantibodies before 18 months of age (7). According to these data, 24 of the 120 newborns are expected to develop β -cell antibodies in the first 18 months of life.

Our population will be compared with previous Italian study (DIABFIN) (16).

Results

The recruitment started in February 2001. Up to March 2004, cord blood samples from 9409 Caucasian newborns have been collected and screened for T1DM associated HLA class II markers. Seventy-three newborns (0.77%) were found to be at “high gene-

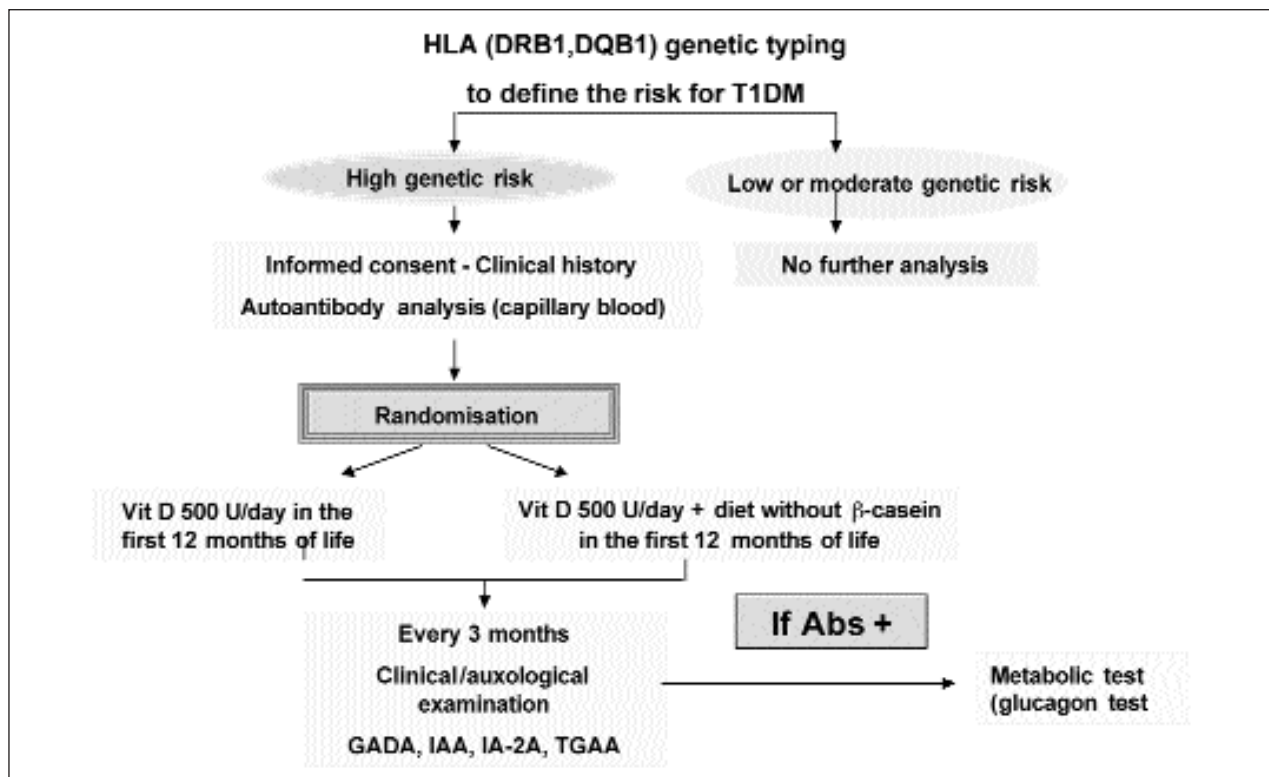


Figure 1. DNA collection (cord blood) in caucasian population

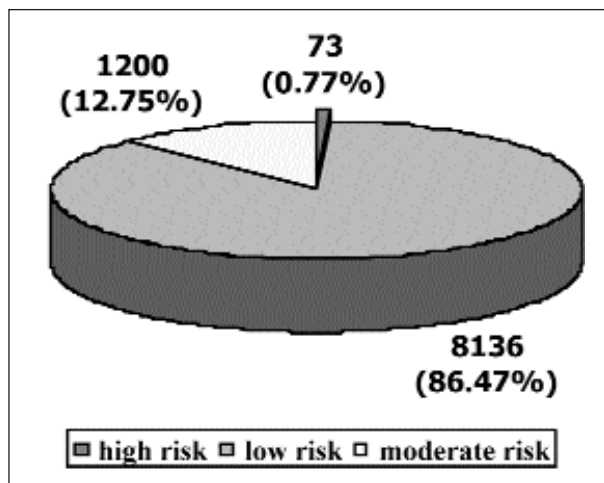


Figure 2. HLA analysis

tic risk” for developing T1DM (Fig. 2). Out of them, 42 babies are now in long- term follow up, each randomized into one of the two groups of primary prevention. The average follow up is 6.8 months (range 0.7-20.1 months).

Sixteen children dropped out of the study (10 from group I, 6 from group II). The causes of drop-out were mainly referred to the low tolerability of hydrolysate formula and to anxiety about the sampling of venous blood.

Three females developed GADA (at 6, 9 and 18 months of age respectively); in one case GADA were only transient (i.e., negative in the following samples) and in the other two cases, they have not been confirmed yet. In one male, IAA had been detected at 6 months of age, but positivity was not confirmed in the sample collected after 3 months. Among the 4 islet-related autoantibody positive children, three belong to the group I of treatment, and one belongs to the group II (Table 1).

None of the children has developed Type 1 diabetes or any other autoimmune disorder so far.

Table 1. Follow-up of subjects with autoantibodies

Subject	1 m	3 m	6 m	9 m	12 m	15 m	18 m
F AN Vit D	neg	neg	neg	GADA ++			
F AN Vit D + diet	neg	neg	neg	neg	neg	neg	GADA ±
F GE Vit D + diet	neg	neg	GADA ±	GADA ±	neg	neg	
M GE Vit D + diet	neg	neg	IAA +	neg	drop out		

Discussion

During the first three years of the Prevefin Italy study, genetic susceptibility to T1DM was assessed in a large cohort of newborns. The prevalence of subjects with the high-risk genotype (DRB1*03/DRB1*04, DQB1*0302, in the absence of the protective allele DRB1*0403) is lower than we expected: 0.77% versus 1.5-2% reported in literature for non-Italian population (13).

This implies that the recruitment of newborns needs to be prolonged for several months, in order to obtain the expected number of randomized high risk subjects. The prevalence of children at high genetic risk for developing T1DM is similar to the one observed in the DIABFIN study, a previous research performed on newborns in continental Italy (16).

Until now, no side effect was recorded as far as vitamin D supplementation is concerned, but casein hydrolysate diet caused compliance problems in some families that stopped the study after a few weeks/months from the beginning.

Further efforts need to be made in order to prevent drop-outs, mainly directed to implement connections with family pediatricians and to reduce the frequency of blood sampling. For this reason, we decided to continue the autoantibody follow-up every six months instead of the three months we had stated at the beginning.

The first preliminary data show a low rate of either transient or persistent autoantibody positivity but such data are not conclusive because the mean follow up is 6.8 months.

Primary prevention is easily accepted by Italian families and general pediatricians, but there is some resistance about modifying the infant’s diet by using a hydrolysate formula, mainly because the hydrolysate milk appears to be sometimes less palatable than the

regular infant formula. Moreover, we were able to obtain a greater compliance in PREVEFIN study than we did in DIABFIN study, where the genetic identification of at risk individuals was not followed by the purpose of any strategy of primary prevention.

In conclusion, these preliminary data show that a population-based screening and primary prevention for T1DM in the Italian population is feasible. For the majority of the Italian families, the assessment of the genetic risk for T1DM in their child is not associated with elevated anxiety and, when present, it is dissipated over the time anyways.

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