



## Type 1 Diabetes and Prediabetic State in a Monozygotic Triplet

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**Abstract.** Type 1 diabetes mellitus (IDDM) results from a chronic process of autoimmune destruction of  $\beta$  cells of the Langerhans islets. The presence of autoantibodies (ICA, GADA, anti-IA2, IAA) in serum precedes the clinical onset of the disease. Genetic predisposition for IDDM is connected with HLA, CTLA-4 and insulin gene region.

The aim of the study was the genetic and immunological analysis of a triplet. One of them developed Type 1 diabetes mellitus. We analysed HLA class II, CTLA-4 and insulin gene polymorphisms in the whole family. Besides, we investigated immunological status of three brothers.

All patients present identical genotype for VNTR loci: D1S80, D17S5 and Apo B, as well as for HLA-DRB1, – DQA1, – DQB1, CTLA-4 gene and all studied insulin gene polymorphisms. That proves their monozygosity. The triplet presents strong genetic predisposition for IDDM. The two patients without overt diabetes have increased levels of ICA, GADA, IA2 and IAA.

**Key words:** Triplet, Type 1 diabetes, Prediabetes, Monozygosity, Genetic predisposition, HLA, CTLA-4, Insulin gene polymorphisms, Autoantibodies

## INTRODUCTION

Type 1 (insulin dependent) diabetes mellitus results from a chronic process of autoimmune destruction of  $\beta$  cells of the Langerhans islets. The first sign of  $\beta$ -cell destruction is the appearance of autoantibodies (ICA-islet cell antibodies, GADA-glutamic acid decarboxylase antibodies, anti-IA2 (tyrosine phosphatase) [12], IAA (autoantibodies to insulin) [13, 14]. The presence of these autoantibodies in serum may precede the clinical onset of the disease over several years [4]. Type 1 diabetes has a strong genetic component [2, 18]. Genetic predisposition for the disease is mainly connected with HLA [1], CTLA-4 and insulin gene [11].

Family studies show that relative risk of the disease development is related to the affinity with diabetic subject [3]. It rises from 0,2 % for general population to 30-50 % for monozygotic siblings [10, 15, 17].

Here we present an immunological and genetic analysis of three brothers (a triplet) aged 15 years old. One of them developed Type 1 diabetes mellitus (patient 1) three years ago. Two others present immunological markers of *prediabetes* stage.

## MATERIALS AND METHODS

HLA class II was performed using sequence-specific oligonucleotide (SSO) hybridisation after PCR amplification of the HLA-DRB1, -DQB1 and -DQA1 genes according to the XII International Histocompatibility Workshop protocols.

CTLA-4 exon 1 position 49 A/G dimorphism was analysed using allele-specific dot-blot hybridisation following PCR amplification.

Insulin gene region polymorphisms: -4217 PstI, -2221 MspI, -23 HphI, +805 Dra III, and +3580 MspI were analysed using the PCR-RFLP (restriction fragment length polymorphism).

ICA was analysed with an indirect immunofluorescence assay and the results were expressed in Juvenile Diabetes Foundation units (JDF-u) by a standard curve based on the international JDF-u reference sera sample.

Anti-GAD and anti-IA2/ICA512 antibodies were analysed using radioimmunoprecipitation assay with <sup>35</sup>S-methionine-labelled recombinant human protein GAD 65 and IA2 respectively. Normal levels of measured antibodies were: < 5,2 AU for GADA and < 8,1 AU for anti-IA2.

IAA antibodies were determined in a competitive binding radioligand assay using monoiodinated insulin as antigen and polyethylene glycol (PEG) as precipitating agent. Normal level of IAA was below 6,8%.

## RESULTS

The three brothers present identical genotype for VNTR loci: D1S80, D17S5 and Apo B, as well as for HLA-DRB1, -DQA1, -DQB1, CTLA-4 gene and all five studied insulin gene polymorphisms. In our opinion these results definitely prove the monozygosity of our patients.

The results of genetic typing of HLA alleles predisposing to Type 1 diabetes are shown in table 1. The analysis of genetic susceptibility linked both to insulin and CTLA-4 gene is presented in table 2.

The two patients without overt diabetes have increased levels of ICA, GADA, IA2 and IAA from the beginning of observation (Fig. 1 and 2).

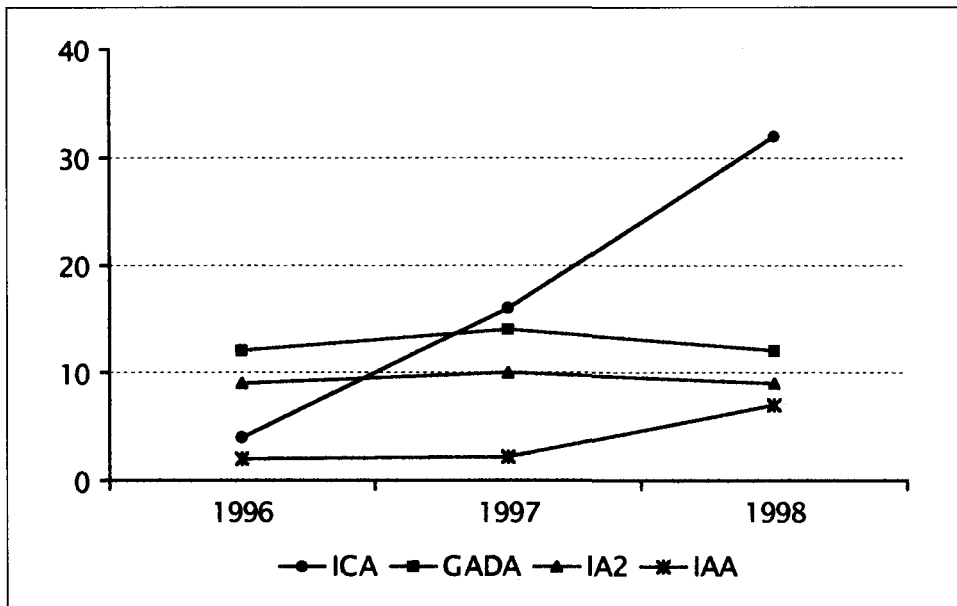
In case of these two boys without overt diabetes we performed periodically intravenous and oral glucose tolerance test (IVGTT and OGTT). Glycemic curve, FPIR (first phase insulin secretion) and C peptide levels present no abnormalities.

**Table 1 - Analysis of HLA genes of the family**

	DRB 1		DQA 1		DQB 1	
Patient 1 - IDDM	16	0401	0102	0301	0502	0302
Patient 2	16	0401	0102	0301	0502	0302
Patient 3	16	0401	0102	0301	0502	0302
Mother	0401	0401	0301	0301	0302	0302
Father	16	0401	0102	0301	0502	0301

**Table 2 - Analysis of genetic susceptibility linked to insulin gene region and CTLA-4 gene**

	-2221 MspI	-23 HphI	+805 DraIII	CTLA-4
Patient 1 - IDDM	pp	pa	pa	GG
Patient 2	pp	pa	pa	GG
Patient 3	pp	pa	pa	GG
Mother	pa	pa	pa	GA
Father	pa	pa	pa	GA



**Fig. 1 - Relative levels of autoantibodies in Patient 2. Norm = 1.**

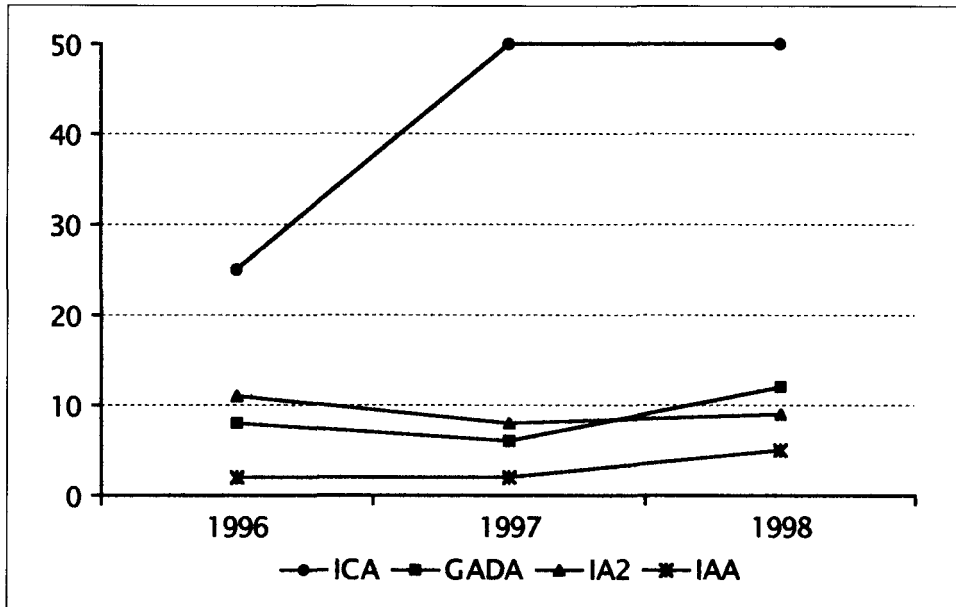


Fig. 2 - Relative levels of autoantibodies in Patient 3. Norm = 1.

## DISCUSSION

All three patients shared remarkably similar risk factor profiles for diabetes. The results indicate exactly the same genetic predisposition. The analysis of HLA class II alleles distribution shows DRB1\*0401-DQA1\*0301-DQB1\*0302 haplotype. Our studies revealed that this haplotype is strongly associated with IDDM in Polish population [6]. All three patients present GG polymorphism in CTLA-4 gene [5] – the most frequent genotype among Polish diabetics.

They present *pp-pa-pa* genotype in -2221 MspI, -23 HphI and +805 DraIII insulin gene polymorphism respectively. In our studies of Polish population *pp-pp-aa* constellation of these polymorphisms was strongly correlated with IDDM [9]. The similarity of three brothers concerns also immunological markers of Type 1 diabetes. All of them have definitely increased levels of autoantibodies. Interestingly, only one of monozygotic brothers developed overt diabetes. Two others are staying in a silent phase of the disease – *prediabetes* [19]. However, the increasing levels of autoantibodies prove that the immunological process of  $\beta$  cell destruction is still proceeding [17] *Prediabetic* brothers present normal levels of C-peptide, FBG (fasting blood glucose) and FPIR, which indicate normal residual and postprandial insulin secretion. These data suggest that two brothers without clinical disease symptoms have smaller degree of  $\beta$  cell destruction.

Our analysis did not reveal a clear difference between one diabetic and two *prediabetic* brothers. These results support the notion that genetic and immunological factors are not the unique elements playing a role in the pathogenesis of IDDM.

This is the first report about triplet suffering from Type 1 diabetes in Poland. Further studies of monozygotic siblings will shed light on essential risk factors for Type 1 diabetes.

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