Diabetologia (1997) 40: 95–99

Diabetologia

Rapid communications

IA-2-autoantibodies complement GAD_{65}-autoantibodies in new-onset IDDM patients and help predict impending diabetes in their siblings

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Summary IA-2 has been identified as an autoantigen that is recognized by immunoglobulins from insulin-dependent diabetic (IDDM) patients. Using a liquid phase radiobinding assay, we performed an IA-2-autoantibody (IA-2-Ab) assay in 474 IDDM patients and 482 non-diabetic control subjects aged 0–39 years. IA-2-Ab were detected in 58 % of the patients and 0.8 % of control subjects. Their prevalence in patients was lower than that of islet cell autoantibodies (ICA; 73 %) or glutamic acid decarboxylase (M_r 65 kDa)-autoantibodies (GAD_{65}-Ab; 82 %) but higher than that of insulin autoantibodies (IAA; 42 %). IA-2-Ab were more frequent in patients under age 20 years (70 %) than between 20 and 40 years (45 %; $p < 0.001$). In the whole IDDM group, 92 % of patients were positive for at least one of the three molecular assays, which is higher than the positivity for the ICA assay (73 %). Only 1 % was negative in the molecular assays and positive in the ICA assay. IA-2-Ab levels were positively correlated with ICA titres ($p < 0.001$) and HLA DQ A1*0301 – DQ B1*0302 ($p < 0.003$) by multivariate analysis. In a group of 481 non-diabetic siblings (age 0–39 years) of IDDM patients only 7 were IA-2-Ab positive (1.5 %). All seven were under age 20 years and positive for at least two other autoantibodies and for DQ A1*0301 – DQB1*0302. Four of these seven developed IDDM during the 6–70-month follow-up period. The positive predictive value of IA-2-Ab (57 %) was higher than that of ICA, GAD_{65}-Ab or IAA alone, or in combination (≤ 20 %) but these calculations are restricted by the relatively short observation period and the small number of cases. The only IA-2-Ab-negative case of pre-diabetes was also negative for IAA and GAD_{65}-Ab, while it was strongly positive for ICA. In conclusion, IA-2-Ab show a high diagnostic specificity for IDDM and are predictive markers of impending diabetes in siblings of patients. In combination with other molecular antibody assays they may replace ICA testing in future. Our data also indicate that other autoantibodies than IA-2-Ab, GAD_{65}-Ab and IAA contribute to ICA. [Diabetologia (1997) 40: 95–99]

Keywords Insulin-dependent diabetes mellitus, protein tyrosine phosphatases, autoantibodies, genetic susceptibility, diabetes prediction.

Received: 18 September 1996 and in revised form: 8 October 1996

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Abbreviations: IDDM, Insulin-dependent diabetes mellitus; IA-2-Ab, IA-2-autoantibodies; ICA, islet cell autoantibodies; GAD_{65}-Ab, autoantibodies against the 65 kDa isoform of glutamic acid decarboxylase; IAA, insulin autoantibodies; PTPase, protein tyrosine phosphatase; IA-2ic, intracellular domain of IA-2; CTLA-4, cytotoxic T-lymphocyte associated protein-4; 5′INS, 5′flanking region of the insulin gene; ROC, receiver-operating characteristics.

* See Acknowledgements

During the past few years, molecular cloning has identified a family of intracellular and transmembrane proteins with protein tyrosine phosphatase (PTPase)-activity which are believed to play a role in intracellular and intercellular signal transduction [1]. Two structurally related transmembrane PTPases, IA-2 (homologous to ICA512) and IA-2β, have recently been characterized as autoantigens in insulin-dependent diabetes mellitus (IDDM) [1]; they represent the precursor forms of the previously described 37/40 kDa tryptic fragments of islet cell membrane proteins which are recognized by circulating immunoglobulins from IDDM patients [1–3].

Antibodies to molecularly defined islet antigens such as IA-2 (IA-2-Ab) and the 65 kDa isoform of glutamic acid decarboxylase (GAD_{65}-Ab) appear to contribute most of the islet cell autoantibody (ICA) reactivity as measured by indirect immunofluorescence on cryosections of human blood group-O pancreata [4]. Like insulin autoantibodies (IAA), IA-2-Ab and GAD_{65}-Ab are preferably measured with liquid phase radiobinding assays using radioactively labelled human recombinant antigen (fragments) for substrate [2, 3].

The prevalence and levels of several immune markers of IDDM (ICA, GAD_{65}-Ab, IAA) vary according to age, gender, and the presence of genetic risk markers both at onset of IDDM and in siblings of diabetic patients [5]. In the present study we have examined whether this is also the case for IA-2-Ab, and, if so, whether detection of this marker adds predictive value to the currently available tests.

Subjects and methods

Subjects. A group of 474 Caucasian IDDM patients with diabetes onset before age 40 years were consecutively recruited nationwide by the Belgian Diabetes Registry [5]. Blood was sampled before the start or within 7 days of insulin treatment.

The 481 non-diabetic Caucasian siblings were also consecutively registered and sampled at their first visit since IDDM onset in the proband (median elapsed time since onset: 4 months; range 0–53 months). During follow up (median follow-up time: 29 months; range 6–70 months) five siblings developed IDDM. The 482 Caucasian control subjects were recruited in the provinces of Antwerp and Brabant from among blood donors aged 18–39 years, laboratory personnel aged 18–39 years and children or adolescents attending emergency departments for minor local surgery [5].

The diagnostic criteria and demographic characteristics of study subjects were as previously reported [5]. The study was approved by the ethical committees of the five participating universities. Informed consent was obtained from the study subjects.

Autoantibodies. IA-2-Ab were determined by a previously described liquid phase radiobinding assay using the ^{35}S-labelled intracellular domain of recombinant human IA-2 (IA-2ic) as tracer [2]. The latter was prepared by in vitro coupled transcription/translation of the corresponding cDNA [2] and purified by ultrafiltration through a Centricon 30 filter (Amicon, Brussels, Belgium) to over 95 % precipitability by trichloro-acetic acid, allowing expression of antibody levels as percentage tracer bound, independent of the use of an antibody-positive standard serum. Likewise IAA and GAD_{65}-Ab were determined by liquid-phase radiobinding assay [5] with the use of respectively HPLC-purified recombinant human ^{125}I-insulin and Centricon-purified recombinant human ^{35}S-GAD_{65} as tracer, hence also allowing expression of results as percentage tracer bound. The cut-off for IA-2-Ab positivity was determined to be greater than or equal to 0.5 % by Receiver-Operating Characteristics (ROC)-curve analysis [6] of the 474 IDDM patients and the 482 control subjects. The cut-off levels for GAD_{65}-Ab ($\geq$ 1.7 %) and for IAA ($\geq$ 0.7 %) were based on the analysis of results obtained in over 300 control subjects and over 300 IDDM patients, belonging to the populations used for IA-2-Ab cut-off determination. For IA-2-Ab and GAD_{65}-Ab the linearity range was up to at least 10 % tracer bound; samples with higher antibody levels were diluted until they yielded results within the linearity range and the final level was calculated by multiplication with the dilution factor. Strongly positive samples could thus display levels well above 100 % tracer binding. For all IAA-positive samples, the percentage tracer bound was less than 10 %, thus requiring no sample dilution for correctly determining the IAA-level. For an IA-2-Ab-positive control serum (mean level: 2.1 % tracer bound), both the intra- and inter-assay coefficients of variation amounted to 7 % (n = 10).

ICA were determined by indirect immunofluorescence and titres expressed as Juvenile Diabetes Foundation (JDF) units [5]. The cut-off for positivity was greater than 12 JDF units as determined by ROC-plot analysis [6]. The antibody prevalence in the control subjects was 0.8 % for IA-2-Ab, 2.6 % for GAD_{65}-Ab, 1.2 % for IAA and 1.3 % for ICA. Only one subject (0.2 %) was positive for more than one autoantibody (high levels of ICA, IA-2-Ab and GAD_{65}-Ab).

The IAA, ICA and GAD_{65}-Ab assays consistently performed well in consecutive external quality control programs (Immunology of Diabetes Workshops [IDW]- and European Nicotinamide Diabetes Intervention Trial [ENDIT]-serum exchange programs; University of Florida proficiency program). In the first IA-2-Ab proficiency program from the University of Florida, our method obtained a 100 % score for laboratory sensitivity, specificity, consistency and validity.

Genetic markers. DNA-polymorphisms at the HLA-DQ and CTLA-4 (cytotoxic T-lymphocyte-associated protein-4) gene loci and the 5′flanking region of the insulin gene (5′INS) were determined as before [5, 7].

Statistical analysis

Differences in prevalence were analysed by chi-square test with Yates' correction or Fisher's exact test whenever appropriate. Multivariate analysis was performed by logistic forward stepwise regression analysis after log-transformation of autoantibody levels in the IA-2-Ab-positive population. Differences were considered significant whenever $p < 0.05$ or, in case of k comparisons, whenever $p < 0.05/k$ (Bonferroni). If the overall p-value for comparing differences between groups was smaller than $0.05/k$, the p-values for significance between individual groups were calculated and again corrected for the number of comparisons made. All statistical tests were performed by "SPSS for Windows 6.1" (SPSS, Chicago, Ill., USA) and "Epi-Info 5" (USF, Stone Mountain, Ga., USA) statistical package for personal computers.

Table 1. Prevalence of autoantibodies or combinations of autoantibodies according to age at clinical onset of IDDM

Antibody marker	Prevalence			*p* value[a]
	0–9 years (n = 97)	10–19 years (n = 158)	20–39 years (n = 219)	
IA-2-Ab	69 (71)[c]	109 (69)[d]	98 (45)	< 0.001
IAA	69 (71)[de]	67 (42)[f]	61 (28)	< 0.001
GAD_{65}-Ab	67 (69)[eg]	139 (88)	185 (85)	< 0.001
ICA	77 (79)[d]	127 (80)[d]	140 (64)	< 0.001
IA-2-Ab or GAD_{65}-Ab	84 (87)	148 (94)	192 (88)	> 0.05
IA-2-Ab or GAD_{65}-Ab or IAA	87 (90)	151 (96)	196 (89)	> 0.05
IA-2-Ab or GAD_{65}-Ab or IAA or ICA	90 (93)	152 (96)	197 (90)	> 0.05
ICA-negative/IA-2-Ab-positive	11 (11)	9 (6)	13 (6)	> 0.05
ICA-negative/GAD_{65}-Ab-positive	7 (7)[d]	24 (15)	54 (25)	< 0.001
≥ 2 autoantibodies	87 (90)[c]	140 (90)[d]	159 (73)	< 0.001

Results are *n* (%); [a] overall chi-square test; to correct for multiple comparisons (Bonferroni method) tests were considered significant whenever $p < 0.05/10$ or $p < 0.005$, in which case, individual chi-square tests between age groups were performed which were also corrected for multiple comparisons and considered significant whenever $p < 0.05/3$ or $p < 0.017$. [e] $p < 0.001$ vs 10–19 years; [d] $p < 0.001$; [g] $p < 0.002$; [f] $p = 0.003$; [c] $p = 0.007$ vs 20–39 years

Results

IDDM patients. IA-2-Ab were detected in 276 of 474 patients (58 %), but only in 4 of 482 non-diabetic control subjects (0.8 %). They were overall less prevalent than GAD_{65}-Ab (391 of 474 or 82 %) and ICA (344 of 474 or 73 %) but more frequent than IAA (197 of 474 or 42 %). Unlike GAD_{65}-Ab but similar to IAA and ICA, IA-2-Ab were more frequent at clinical onset under age 20 years (70 vs 45 % between age 20 and 40 years; $p < 0.001$) (Table 1). Incidentally, the four IA-2-Ab-positive control subjects were between 6 and 20 years old. When positivity for at least one of the three molecular assays (IA-2-Ab, GAD_{65}-Ab or IAA) was considered, 434 of 474 patients (92 %) were scored as positive which is markedly higher than the positivity reached with the immunohistochemical reaction (ICA-positivity in 73 %) (Table 1). Among the 8 % of subjects who were negative for these three markers, only a minority (5 of 40 subjects) tested ICA-positive; the prevalence of ICA-positive patients who were negative for one of the three molecular assays, was highest in the age group younger than 10 years (3.1 vs 0.6 % in 10–19 years and 0.4 % in 20–39 years) (Table 1). Conversely, among the 27 % ICA-negative patients ($n = 130$) many were positive for IA-2-Ab ($n = 33$), GAD_{65}-Ab ($n = 85$) (Table 1) or both ($n = 93$; not shown). The prevalence of ICA-negative but GAD_{65}-Ab-positive patients was highest in the group aged 20–39 years.

Overall, 386 patients (81 %) were positive for at least two of the four types of autoantibodies tested as compared to only 1 of 482 control subjects (0.2 %) (see methods), with a significantly higher frequency in patients under age 20 years (227 of 255 or 89 % vs 159 of 219 or 73 % in the group aged 20–39 years; $p < 0.001$) (Table 1).

According to multivariate analysis in IA-2-Ab positive patients, IA-2-Ab levels were positively associated with ICA-titres ($p < 0.001$) and HLA DQA1*0301-DQB1*0302 ($p < 0.003$), but not with GAD_{65}-Ab levels, female gender, IAA, HLA DQ A1*0501-DQB1*0201 or other genetic risk markers (5′INS 1/1 genotype or CTLA-4 G-allele).

Siblings of IDDM patients. Among the 481 siblings tested, 7 (1.5 %) were positive for IA-2-Ab, 43 (9 %) for GAD_{65}-Ab, 27 (6 %) for ICA, 26 (5 %) for IAA and 20 (4 %) for at least two types of antibodies. The seven IA-2-Ab-positive siblings were all under age 20 years and positive for HLA DQA1*0301-DQB1*0302 and at least two other types of antibodies (Table 2).

Four of the IA-2-Ab-positive siblings belonged to the group of five siblings who developed IDDM within 6 to 36 months after initial blood sampling. The three other IA-2-Ab-positive siblings were young boys showing positivity for multiple IDDM markers and hence expected to develop diabetes within the next few years (Table 2). Five of the eight pre-diabetic or IA-2-Ab-positive siblings were sampled twice or more during diabetes-free follow-up (6–36 months) and tested consistently positive for multiple antibodies on these occasions. The pre-IDDM subject number 5 (Table 2) with borderline IA-2-Ab levels became IA-2-Ab negative at IDDM onset. The only pre-diabetic sibling who was IA-2-Ab-negative at first sampling was also negative for IAA and ICA but strongly ICA-positive, indicating that other autoantigens may contribute to ICA. From the number of marker-positive pre-diabetic siblings (Table 2) and the total number of marker-positive siblings, a positive predictive value can be calculated for subsequent development of IDDM; this value is higher for IA-2-Ab (57 %) than for ICA (15 %), GAD_{65}-Ab

Table 2. Clinical and biological data in prediabetic siblings of IDDM patients (n = 5) and in IA2-Ab-positive but non-diabetic siblings (n = 3)

Subject		Age (years)	Gender	Time to onset (months)	IA-2-Ab[a] (%)	IAA[a] (%)	GAD_{65}-Ab[a] (%)	ICA[a] (JDF units)	HLADQA1*-B1* 0301-0302
Pre-IDDM	#1	17	Male	27	**72.2**	0.5	**1.9**	**400**	present
	#2	12	Male	18	**0.6**	**1.3**	**226.3**	**50**	present
	#3	6	Male	36	0.0	0.4	0.5	**400**	present
	#4	12	Female	6	**25.8**	0.3	**508.9**	**200**	present
	#5	8	Male	6	**0.5**	**0.8**	**25.2**	0	present
IA-2-Ab positive	#1	5	Male	?	**0.7**	**0.8**	**279.2**	6	present
	#2	5	Male	?	**2.9**	**2.4**	**165.4**	**200**	present
	#3	4	Male	?	**0.9**	**7.2**	**12.9**	**100**	present

[a] Values above the threshold for positivity for immune markers are indicated in bold; five of the eight prediabetic or IA-2-Ab-positive siblings tested consistently positive for these markers during diabetes-free follow-up (6–36 months), the pre-IDDM subject #5 with borderline IA-2-Ab levels became IA-2-Ab negative at IDDM onset

(9 %), IAA (8 %) or for a combination of at least two of these four assays (20 %). However, these calculations are restricted by the small number of cases and the short duration of follow-up in the siblings (range 6–70 months).

Discussion

This study documents that, overall, the present IA-2-Ab assay yields a less sensitive IDDM marker than the assays for GAD_{65}-Ab and ICA. However, the diagnostic sensitivity of the various antibody tests we investigated tends to vary according to age at diabetes onset, being relatively higher in children for ICA and IA-2-Ab, and higher in adults for GAD_{65}-Ab. Moreover, the diagnostic sensitivity of a given marker in the prediabetic state may differ from that at clinical onset and is difficult to assess at present, especially with respect to IDDM-onset in (late) adulthood. On the other hand, positivity for IA-2-Ab represents a more specific marker than the other autoantibodies tested, in view of its lower frequency in non-diabetic control subjects (0.8 %), siblings of IDDM patients (1.5 %) and non-diabetic patients with other organ-specific autoimmune diseases (0.9 %) [8], and in view of its higher positive predictive value in siblings, especially when clinical onset was under age 20 years. Like GAD_{65}-Ab [9], IA-2-Ab occur in association with ICA and the HLA DQ A1*0301-DQB1*0302 risk haplotype in new-onset patients, in line with previous findings in first-degree relatives [10]. At variance with GAD_{65}-Ab [9], IA-2-Ab are more prevalent at clinical onset under age 20 years and their levels are not correlated with the presence of the HLA DQ A1*0501-DQB1*0201 risk haplotype or with the highest genetic risk as determined by the interaction of HLA DQ- and 5′INS-linked risk. Moreover, IA-2-Ab levels were not correlated with GAD_{65}-Ab- and IAA levels. Thus, IA-2-Ab usefully complement the latter two molecular radiobinding assays for the classification and prediction of diabetes. Regardless of age, 92 % of patients were positive for IAA, GAD_{65}-Ab or IA-2-Ab; only an additional 1 % were ICA-positive. Conversely, a considerable fraction of ICA-negative subjects (72 %, corresponding to 20 % of all patients) were positive for either IA-2-Ab or GAD_{65}-Ab.

In siblings, IA-2-Ab appeared to have a higher positive predictive value for impending IDDM than any other single antibody test or even than the combined positivity for at least two antibody types. A longer follow-up period on a larger group of siblings is required to determine more precisely the positive predictive value of the various combinations of autoantibodies tested in the present study. Nevertheless, our results are in agreement with the findings of Verge et al. [10] in a larger but partly selected group of first-degree relatives, where the positive predictive values of the different antibodies tended, however, to be higher probably because of the longer follow-up and the inclusion of a selected group of ICA-positive relatives. Only one of five pre-diabetic siblings in our study was IA-2-Ab-negative before clinical onset. Interestingly, this individual was negative for IAA and GAD_{65}-Ab, but was strongly ICA positive suggesting that other autoantibodies besides GAD_{65}-Ab, IA-2-Ab or IAA must also contribute to ICA. In this respect, the IA-2β PTP-ase may represent an additional relevant islet cell autoantigen [1].

In conclusion, IA-2-Ab are IDDM markers with a high diagnostic specificity, which complement GAD_{65}-Ab and help predict impending IDDM in siblings of patients. In combination with other molecular autoantibody assays such as IAA and GAD_{65}-Ab, IA-2-Ab may replace ICA testing for IDDM prediction in the future.

Acknowledgements. We are indebted to Dr. D. Eizirik (Department of Metabolism and Endocrinology, Vrije Universiteit Brussel, VUB) for critical reading of the manuscript and helpful discussion, to Dr. B. Van der Auwera (Department of Biochemistry, VUB) for the determination of genetic markers, to

Dr. D. Flamez (Department of Biochemistry, VUB) for amplifying the IA-2ic and GAD_{65} cDNA, to A. Demarré, V. Claessens, L. De Pree, S. Exterbille, C. Groven, A. Ivens, F. Lebleu, E. Quartier, G. Schoonjans and H. Thomas for excellent technical assistance, and to V. Bruylandt, N. Ringoot and L. Vermeir for expert secretarial work. Cristalline Humulin was a gift from Eli Lilly Co. Brussels, Belgium. Human GAD_{65} cDNA was a gift from Drs. Å. Lernmark and A. Falorni when at the Karolinska Institute, Stockholm, Sweden. The present work was supported by the Belgian Nationaal Fonds voor Geneeskundig Wetenschappelijk Onderzoek (FGWO grant 3-9007-91N and a fellowship for Fundamental Clinical Research to FKG), the Belgian National Lottery, the Flemish Ministry of Public Health and Environment and Johnson & Johnson.

The following members of the Belgian Diabetes Registry have also contributed to the recruitment of patients, siblings and control subjects or to the handling of samples for the present study: E. Balasse (Brussels), H. Becq (Wilrijk), J. Beirinckx (Izegem), L. Claeys (Zoersel), M. Coeckelberghs (Antwerp), J.-L. Coolens (Hasselt), W. Coucke (Roeselare), E. Couturier (Brussels), R. Craen (Gent), J.-C. Daubresse (Charleroi), I. De Leeuw (Antwerp), F. Defoer (Brussels), C. Delvigne (Antwerp), H. Dorchy (Brussels), M. Du Caju (Antwerp), F. Féry (Brussels), M. Gaham (Braine-l'Alleud), J. Gérard (Angleur), C. Gillet (Brussels), J. Guiot (Seraing), C. Herbaut (Mons), B. Keymeulen (Brussels), G. Krzentowski (Jumet), G. Lamberigts (Brugge), J.-P. Lauvaux (Brussels), M. Letiexhe (Liège), J. Monballyu (Ekeren), G. Moorkens (Antwerp), D. Nicolaij (Kortrijk), F. Nobels (Aalst), M.-C. Pelckmans (Lier), A. Purnode (Brussels), R. Rooman (Antwerp), R. Rottiers (Gent), J. Schutyser (Kortrijk), G. Somers (Brussels), L. Terriere (Hoboken), J. Teuwen (Kapellen), J. Tits (Genk), K. Van Acker (Bornem), P. Van Crombrugge (Aalst), L. Van Gaal (Antwerp), S. Van Imschoot (Brugge), P. Van Rooy (Antwerp), E. Vandenbussche (Herentals), S. Vanneste (Zoersel), C. Vercammen (Bonheiden), J. Vertommen (Antwerp). We dedicate this work to the memory of the late Prof. Dr. G. Somers.

References

1. Lu J, Li Q, Xie H et al. (1996) Identification of a second transmembrane tyrosine phosphatase, IA-2β, as an autoantigen in insulin-dependent diabetes: precursor of the 37-kDa tryptic fragment. Proc Natl Acad Sci USA 93: 2307–2311
2. Payton MA, Hawkes CJ, Christie MR (1995) Relationship of the 37,000- and 47,000 Mr tryptic fragments of islet antigens in insulin-dependent diabetes to the protein tyrosine phosphatase-like molecule IA-2 (ICA 512). J Clin Invest 96: 1506–1511
3. Bonifacio E, Lampasona V, Genovese S, Ferrari M, Bosi E (1995) Identification of protein tyrosine phosphatase-like IA_{-2} (islet cell antigen 512) as the insulin-dependent diabetes-related 37/40 k autoantigen and a target of islet-cell antibodies. J Immunol 155: 5419–5426
4. Myers MA, Rabin DU, Rowley MJ (1995) Pancreatic islet cell cytoplasmic antibody in diabetes is represented by antibodies to islet cell antigen 512 and glutamic acid decarboxylase. Diabetes 44: 1290–1295
5. Van der Auwera B, Schuit F, Lyaruu I et al. (1995) Genetic susceptibility for insulin-dependent diabetes mellitus in Caucasians revisited: the importance of diabetes registries in disclosing interactions between HLA-DQ- and insulin-gene-linked risk. J Clin Endocrinol Metab 80: 2567–2573
6. Zweig M, Campbell G (1993) Receiver-operating characteristics (ROC) plots: a fundamental tool in clinical medicine. Clin Chem 39: 561–571
7. Nisticò L, Buzzetti R, Pritchard LE et al. (1996) The CTLA-4 gene region of chromosome 2q33 is linked to, and associated with, type 1 diabetes. Hum Mol Genet 5: 1075–1080
8. Morgenthaler NG, Seissler J, Achenbach P et al. (1996) Antibodies to IA-2 in IDDM, Stiff man syndrome and autoimmune endocrine diseases: evidence of high specificity for IDDM. Diabetologia 39 [Suppl I]: A27 (Abstract)
9. Vandewalle C, Falorni A, Goubert P, Van der Auwera B, Gorus F, the Belgian Diabetes Registry (1995) GAD_{65}-Autoantibodies are associated with HLA-DQ- and 5′INS-linked genetic risk in adult new onset IDDM-patients. Autoimmunity 21: 31 (A119)
10. Verge CF, Gianani R, Kawasaki E et al. (1996) Prediction of type 1 diabetes in first-degree relatives using a combination of insulin, GAD, and ICA 512 bdc/IA-2 autoantibodies. Diabetes 45: 926–933