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Proinsulin levels and the proinsulin:C-peptide ratio complement autoantibody measurement for predicting type 1 diabetes

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Abstract *Aims/hypothesis:* We investigated whether random proinsulin levels and proinsulin:C-peptide ratio (PI:C) complement immune and genetic markers for identifying relatives at high risk of type 1 diabetes. *Materials and methods:* During an initial sampling, random glycaemia, proinsulin, PI:C and *HLA DQ* genotype were determined in 561 non-diabetic first-degree relatives who had been positive for islet autoantibodies on one or more occasions and in 561 age- and sex-matched persistently antibody-negative relatives. *Results:* During follow-up (median 62 months), 46 relatives with antibodies at entry developed type 1 diabetes. At baseline, antibody-positive relatives (n=338) had higher PI:C values (p<0.001) than antibody-negative subjects with (n=223) or subjects without (n=561) later seroconversion. Proinsulin and PI:C were graded according to risk of diabetes as expressed by positivity for (multiple) antibodies or IA-2 antibodies, especially in persons carrying the high-risk *HLA DQ2/DQ8* genotype and in prediabetic relatives. In the presence of multiple or IA-2 antibodies, a PI:C ratio exceeding percentile 66 of all antibody-negative relatives at entry (n=784) conferred a 5-year diabetes risk of 50% and 68%, respectively (p<0.001 vs 13% for same antibody status with PI:C<percentile 66). Cox regression analysis confirmed random PI:C as an independent predictor of the risk of diabetes (p≤0.001). *Conclusions/interpretation:* Random proinsulin and PI:C represent dynamic markers of the state of beta cell function that complement immune markers in identifying relatives who are at homogeneously high risk of contracting type 1 diabetes and are therefore eligible for secondary prevention trials.

Keywords C-peptide · *HLA DQ* · IA-2 antibodies · Islet antibodies · Prediction · Proinsulin · Type 1 diabetes

Abbreviations GADA: antibodies to M_r 65,000 glutamic acid decarboxylase · IA-2A: antibodies to insulinoma-associated protein-2 · IAA: insulin autoantibodies · ICA: islet cell cytoplasmic antibodies · P66: 66th percentile · PI:C: proinsulin:C-peptide ratio · TRFIA: time-resolved fluorescence immunoassay

Introduction

To identify persons at risk of diabetes among first-degree relatives of type 1 diabetic patients we currently use detection of islet cell cytoplasmic antibodies (ICA), insulin autoantibodies (IAA), antibodies to the M_r 65,000 isoform of glutamic acid decarboxylase (GADA) and/or antibodies to the intracellular domain of insulinoma-associated protein-2 (IA-2A) [1–6]. Relatives thus identified can then be enrolled in prevention trials aimed at preserving the residual beta cell mass by stopping or delaying the pathogenic process [7–9]. However, the rate of beta cell destruction among antibody-positive relatives varies considerably and some do not develop hyperglycaemia for many years [1–9]. In first-degree relatives of type 1 diabetic patients the presence of multiple autoantibodies or of IA-2A—even on one occasion—is associated with a high 5-year risk of diabetes, which may reach 80% in carriers of *HLA DQA1*0301-DQB1*0302* (*DQ8*)/*DQA1*0501-DQB1*0201* (*DQ2*) [5, 10, 11]. However, the predictive value of these marker combinations goes at the expense of diagnostic sensitivity, as they detect only about 30% of prediabetic relatives [10, 11]. There is therefore a need for additional biological markers capable of identifying more prediabetic relatives.

Measuring early hormonal markers may further improve risk assessment in relatives [12–21]. Loss of (first-phase)

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insulin response—during an intravenous glucose tolerance test—is considered highly predictive of impending diabetes in antibody-positive first-degree relatives [15–19] and has been used as an inclusion criterion in some diabetes prevention trials [9, 19]. However, this test is not optimally reproducible and is difficult to carry out on a large scale in non-diabetic risk groups comprising children, students and active young adults [20].

Elevated fasting proinsulin levels and proinsulin:C-peptide (PI:C) ratios have been described in recent-onset type 1 diabetic patients [21] and in prediabetic antibody-positive siblings with low first-phase insulin responses [12]. We investigated whether measurement of proinsulin and the PI:C ratio in random blood samples could improve the predictive value of immune and genetic markers in identifying persons at risk of developing type 1 diabetes. The long-term goal is to facilitate identification of those first-degree relatives of type 1 diabetic patients with a homogeneously high 5-year risk of diabetes, with a view to including them in secondary prevention trials designed to lead to conclusions within that time-frame.

Subjects and methods

Subjects and data collection Siblings (n=1,899), offspring (n=2,806) and parents (n=792) of type 1 diabetic patients were consecutively recruited by the Belgian Diabetes Registry between 1 January 1989 and 27 November 2002 according to previously defined criteria [10, 11, 22]. This group of first-degree relatives (n=5,497), aged between 0 and 39 years, was followed for a median (interquartile period) of 52 (43–59) months. The relatives were not preselected on the basis, for example, of ICA-positivity or known prediabetic state. Their probands are considered representative of the whole Belgian population of type 1 diabetic patients [23]. After obtaining written informed consent from each subject or the subject's parents, we asked them to complete a short questionnaire with demographic, familial and personal information. Blood was sampled randomly at inclusion and (as a rule) at yearly intervals during follow-up.

Relatives who developed diabetes during follow-up were identified through repeated contacts with Belgian endocrinologists and paediatricians, self-reporting through yearly questionnaires and through a link with the Belgian Diabetes Registry patient data base, where newly diagnosed diabetic patients who are younger than 40 years and reside in Belgium are registered. The study was conducted in accordance with the guidelines in The Declaration of Helsinki as revised in 2000 (http://www.wma.net/e/policy/b3.htm) and approved by the ethics committees of the Belgian Diabetes Registry and the participating university hospitals.

Serum, plasma and whole-blood samples were divided in aliquots and stored at −80°C until analysed for glucose, HbA_1c and diabetes-associated autoantibodies. Autoantibody-positive relatives were genotyped for *HLA DQ*. Random proinsulin, C-peptide and PI:C ratio were determined in the initial sample of relatives who developed autoantibodies on at least one occasion during the observation period (561 out of the 5,497 relatives) and in the initial sample of 561 individually age- and sex-matched relatives who remained antibody-negative throughout follow-up. The median (interquartile range) follow-up time of these 1122 relatives was 62 (50–69) months. Of the 338 relatives who were antibody-positive at first sampling, 194 (57%) were confirmed as antibody-positive at ≥1 later time-point. To assess whether elevated PI:C values persisted in these 338 antibody-positive relatives, PI:C was determined in the follow-up sample after 1 year when available (n=284). For prediabetic relatives all available samples up to clinical diagnosis were analysed.

Analytical methods ICA were determined by indirect immunofluorescence, IA-2A, GADA and IAA by liquid-phase radiobinding assays, and *HLA DQ* polymorphisms by allele-specific oligonucleotide genotyping as previously described [24–26]. Plasma glucose was measured on NaF plasma as before [10]. HbA_1c levels were determined by immunoassay (Roche, Basel, Switzerland) on a Cobas Integra 400 analyzer (Roche). Proinsulin and C-peptide were determined in plasma collected in K-EDTA monovettes (Sarstedt, Darmstadt, Germany) supplemented with aprotinin (Trasylol; Bayer, Brussels, Belgium; final concentration 600 kallikrein inactivator units/ml). Plasma proinsulin was measured by ELISA [27]. The lower and upper level of quantification amounted to 0.9 and 300 pmol/l, respectively. Up to a 500-fold excess of C-peptide did not interfere in the proinsulin assay. Because of high cross-reactivity with conversion intermediates [74% for split (32–33) proinsulin, 65% for des(31,32) proinsulin, 78% for split(65–66) proinsulin and 99% for des(64,65) proinsulin], the assay was considered to measure total proinsulin immunoreactive material [27]. It comprises two separate overnight incubations allowing the monoclonal antibodies used in the assay to compete with IAA (if present in the plasma tested) for binding to proinsulin. In these conditions IAA may only cause an underestimation of total proinsulin concentration at very high IAA levels (>4% tracer binding; P. De Pauw, personal communication). Such high IAA values were observed in less than 5% of the prediabetic and non-diabetic relatives at first sampling in the present study. The proinsulin ELISA can thus be considered to measure the sum of free and IAA-bound proinsulin. Total plasma C-peptide levels were determined by a two-step time-resolved fluorescence immunoassay (TRFIA) using a commercial kit (AutoDelfia-C-peptide kit B081-101; Perkin-Elmer, Wallac Oy, Turku, Finland) with in-house modifications. Fetal bovine serum (10% [v/v], Hyclone FBS; Perbio Science, Erembodegem, Belgium) was added to the incubation buffer to avoid heterophile antibody binding [28] and an additional washing step, preceding the incubation with the labelled antibody, was carried out to eliminate interference by K-EDTA from the samples. The assay measures the total C-peptide level (i.e. true C-peptide and proinsulin including its split- and des-forms) with a lower level of quantification at 30 pmol/l

Table 1 Metabolic and hormonal characteristics at study entry in relatives classified according to their baseline antibody status

Characteristic	Ab-positive[a]	Ab-negative[b]	*p*
n	338	784	
Age, years	14 (8–23)	13 (6–24)	0.263
Male/female, *n/n* (ratio)	168/170 (0.99)	378/406 (0.93)	0.647
Siblings/offspring/parents (*n/n/n*)	156/149/33	293/411/80	0.019
Glucose (mmol/l)	4.8 (4.4–5.3)	4.8 (4.4–5.1)	0.169
Proinsulin (pmol/l)	12.6 (7.1–25.4)	11.3 (6.8–20.9)	0.071
C-peptide (pmol/l)	698 (438–1025)	702 (451–1070)	0.475
Proinsulin:C-peptide ratio (%)	2.0 (1.3–2.9)	1.7 (1.2–2.4)	<0.001

Data are *n*; *n/n* (ratio); *n/n/n* or medians (interquartile range). Antibody-negative relatives who seroconverted to antibody positivity in follow-up samples (n=223) and those who remained persistently antibody-negative (n=561) did not differ in the parameters tested (not shown). The threshold for significance between Ab-positive and Ab-negative relatives was: p<0.007 (Bonferroni adjustment)

Ab antibody

[a]Positive for ICA, GADA, IA-2A, and/or IAA

[b]Negative for ICA, GADA, IA-2A and IAA

and was calibrated towards the international reference reagent for C-peptide (IRR C-peptide, code 84/510; NIBSC, South Mimms, UK). The level of true C-peptide was calculated by subtracting the proinsulin level measured by ELISA from the total C-peptide level measured by TRFIA and was used for further statistical analysis and for the exact calculation of the PI:C ratio using the formula: PI:C ratio (%)=100×proinsulin (pmol/l; ELISA)/[total C-peptide (pmol/l; TRFIA)−proinsulin (pmol/l; ELISA)].

Interassay coefficients of variation determined on human control sera amounted to 3% (n=40) for C-peptide and 8% (n=84) for proinsulin at the level of 896 pmol/l (2.7 μg/l) and 28 pmol/l (252 ng/l), respectively.

Statistical analysis Statistical differences between groups were assessed by means of the Mann–Whitney *U*-test or by Kruskall–Wallis analysis for continuous variables and by the chi-square test, using Yates' correction or Fisher's exact test for categorical variables. Mc Nemar's test was used to compare sensitivities of different variables in the same cohort. In prediabetic subjects Spearman's rank correlation was used to investigate the relation between PI:C ratio and time to diabetes. Differences in diabetes-free survival were investigated by Kaplan–Meier analysis and log-rank test [10, 11]. Cox proportional hazards model, performed by forward stepwise method, was used to investigate the independent contributions of risk factors identified by univariate analysis (whenever p<0.1), with 95% confidence intervals on hazard ratios [10, 11]. All statistical tests were performed two-tailed by SPSS for Windows 11.0 (SPSS, Chicago, IL, USA) or by Epi Info Version 6 (USD, Stone Mountain, GA, USA) and considered significant whenever p< 0.05 or, in case of k comparisons, whenever p<0.05/k (Bonferroni adjustment) [10, 11].

Results

Changes in subject status During the study period, 50 of 5497 relatives (0.9%) developed type 1 diabetes with a median (interquartile range) time to clinical onset of 33 (11–50) months. Of these, 46 (i.e. 92%) were initially antibody-positive at entry (13% progression to diabetes within 5 years; Kaplan–Meier analysis; not shown). The four others were antibody-negative at baseline but became antibody-positive at a later time (0.5% progression to diabetes within 5 years; Kaplan–Meier analysis; not shown). None of the persistently antibody-negative relatives developed diabetes during follow-up.

Hormonal characteristics according to antibody status at first sampling Antibody-positive relatives had similar plasma glucose, C-peptide and proinsulin levels to antibody-negative relatives (Table 1), regardless of whether or not the latter seroconverted to antibody-positivity at a later time (not shown). However, antibody-positive relatives had higher

Table 2 Metabolic and hormonal characteristics at initial random sampling of relatives according to antibody status and development of type 1 diabetes during follow-up

Characteristic	Ab-positive[a]		Ab-negative[b]	Overall *p*
	Prediabetic	Non-diabetic		
n	46	292	784	
Glucose (mmol/l)	5.0 (4.4–5.7)	4.8 (4.4–5.2)	4.8 (4.4–5.1)	0.078
Proinsulin (pmol/l)	17.7 (11.4–38.8)	11.7 (6.7–23.4)[c]	11.3 (6.8–20.9)[d]	0.001
C-peptide (pmol/l)	642 (410–1038)	703 (441–1026)	702 (451–1070)	0.490
Proinsulin:C-peptide ratio (%)	3.1 (2.3–5.9)	1.9 (1.3–2.7)[d]	1.7 (1.2–2.4)[d]	<0.001

Data are *n* or medians (interquartile range). Threshold for overall significance:p<0.012 (Bonferroni adjustment). In case of significant overall p value, the threshold for significance between individual groups is p<0.017 (Bonferroni adjustment)

Ab antibody

[a]Positive for ICA, GADA, IA-2A, and/or IAA

[b]Negative for ICA, GADA, IA-2A and IAA

[c]p=0.002 compared with initially antibody-positive prediabetic relatives

[d]p<0.001 compared with initially antibody-positive prediabetic relatives

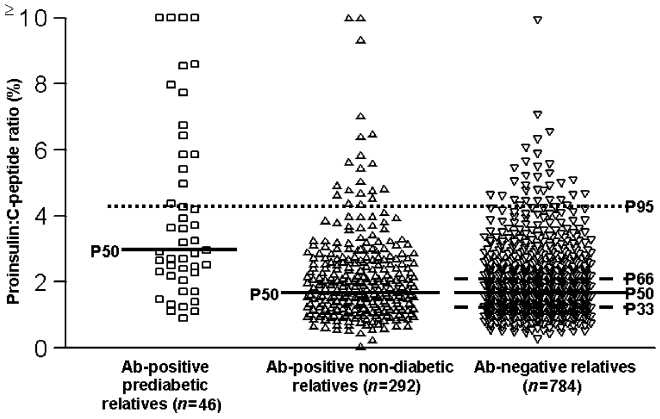

Fig. 1 Proinsulin:C-peptide (PI/C) ratio (%) at initial sampling in antibody-positive prediabetic relatives (n=46), antibody-positive non-diabetic relatives (n=292) and antibody-negative relatives (n=784). The *dotted line* at 4.1% indicates the cut-off value (percentile 95 in antibody-negative relatives) for increased PI:C ratio. *Solid lines*, median values (percentile 50 [P50]) of each group; *interrupted lines*, percentiles 33 (P33) and 66 (P66) of the control group

PI:C ratios (p<0.001) than antibody-negative relatives (Table 1). The PI:C ratios of the latter were indistinguishable from values observed in 22 healthy control subjects from the general population (E. Vandemeulebroucke, personal communication). Whenever available (1,072 of 1, 122 initial samples) HbA_1c levels were within the normal range (<6%; not shown).

PI:C ratio increased significantly according to the number of antibodies present (median [interquartile range] for n=113 with ≥2 antibodies: 2.28 [1.50–3.25]%; p<0.001 vs 1.84 [1.19–2.69]% for n=225 with 1 antibody); a similar association of increased PI:C ratio with multiple antibody-positivity was found, when only molecular antibodies were considered (not shown). PI:C was also significantly higher in IA-2A-positive relatives (n=68; 2.45 [1.60–3.94]%) than in relatives with other antibody specificities (IAA, GADA or ICA) (n=270; 1.88 [1.25–2.71]%; p=0.001). When IA-2A-positive and IA-2A-negative relatives were studied separately, PI:C ratio no longer increased with antibody number, but was only elevated (after stratification according to *HLA DQ* status) in IA-2A-positive *DQ2/DQ8* heterozygotes (n=20; 3.7 [2.3–6.3]; overall p<0.001) (not shown).

Hormonal characteristics of prediabetic relatives at first sampling The subgroup of 46 prediabetic antibody-positive relatives had higher random proinsulin levels and PI:C ratios than non-diabetic antibody-positive relatives (p≤0.002) or than antibody-negative (p<0.001) relatives. However, the three groups did not differ in glycaemia (Table 2) or in glucose:C-peptide ratio (not shown). Initial PI:C values (Fig. 1) and proinsulin levels (not shown) of prediabetic subjects and non-diabetic relatives with or without autoantibodies showed a considerable overlap. Taking the 95th percentile of PI:C in antibody-negative relatives (n=784) as cut-off (≥4.1%) (Fig. 1) an elevated ratio occurred in 37% of antibody-positive prediabetic relatives vs 7% of nondiabetic antibody-positive relatives and 5% of antibody-negative relatives (p<0.001 vs prediabetic relatives) (Fig. 1).

Predictive value of high PI:C in antibody-positive relatives In 338 antibody-positive relatives at first sampling a PI:C ratio above the 66th percentile of antibody-negative relatives (P66; PI:C=0.021; Fig. 1) was associated with a 24% 5-year risk of diabetes (36 diabetes events; p<0.0001 vs 4% in relatives with a PI:C ratio≤P66) (Fig. 2(a)). In subjects with positivity for ≥2 antibodies, progression to diabetes was significantly higher when the PI:C ratio was greater than P66 (50%; p=0.0003 vs 13% for PI:C ratio ≤P66) (Fig. 2(b)). The screening sensitivity [95% CI] for combined presence of high PI:C ratio and multiple antibodies was 76% [62–90%] (29 of 38 multiple antibody-positive diabetes cases predicted).

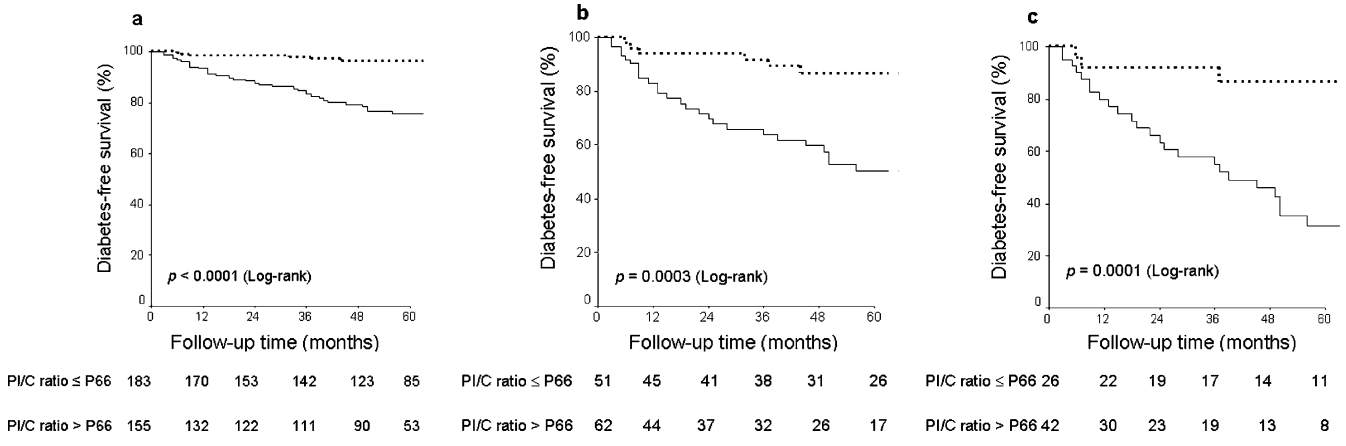

Fig. 2 Diabetes-free survival (%) in all antibody-positive first-degree relatives at initial sampling (n=338) (**a**), in multiple antibody-positive relatives (≥2 antibodies among IAA, ICA, GADA and IA-2A; n=113) (**b**), and in IA-2A-positive relatives (n=68) (**c**), stratified according to PI:C ratio (≤P66, *dotted line* vs >P66, *unbroken line*). Five-year diabetes-free survival for PI:C ratio ≤P66: 96% (CI 93–99) (**a**), 87% (CI 76–97) (**b**), 87% (CI 72–100) (**c**); for PI:C ratio >P66: 76% (CI 68–83) (**a**), 50% (CI 36–64) (**b**), 32% (CI 15–48) (**c**). Numbers of subjects still under follow-up in each category (at 12-month intervals) are indicated beneath the time scale

Table 3 Cox regression analysis in 338 initially antibody-positive first-degree relatives

Variable	Univariate analysis	Multivariate analysis (forward stepwise method)	
	p	*p*	Hazard ratio for diabetes (95% CI)[a]
IAA	0.716	NT	NT
ICA	<0.001	NS	NS
GADA	<0.001	NS	NS
IA-2A	<0.001	<0.001	7.2 (2.8–18.8)
Number of autoantibodies	<0.001	0.030	1.5 (1.0–2.2)
HLA DQ2/DQ8 genotype	<0.001	NS	NS
HLA DQ8 haplotype	0.002	NS	NS
HLA DQ2 haplotype	0.052	0.021	2.1 (1.1–4.0)
Proinsulin	0.005	NS	NS
C-peptide	0.332	NT	NT
Proinsulin: C-peptide ratio	<0.001	<0.001	1.2 (1.1–1.3)
Age	0.106	NT	NT
Sex	0.577	NT	NT
Sibling	0.002	NS	NS

Calculations were done with Cox regression models. All variables were considered at initial sampling
NT not tested
[a]Data are hazard ratios (95% CI)

In IA-2A-positive relatives, the presence of a high PI:C ratio was associated with 68% progression to diabetes within 5 years (p=0.0001 vs 13% progression for PI:C ratio ≤P66) (Fig. 2c). The screening sensitivity [95% CI] of this marker combination was 87% [75–99%] (26 of the 30 IA-2A-positive prediabetes cases were predicted).

Cox regression analysis The above analysis was confirmed by multivariate forward stepwise Cox regression analysis in the 338 antibody-positive relatives (Table 3). IA-2A was selected first as predictor of diabetes risk (p<0.001). The PI:C ratio was selected in the next step (p<0.001), followed by the presence of the *HLA DQ2* risk haplotype and the number of autoantibodies (p=0.021 and p=0.030, respectively). The other parameters (including the high-risk genotype *HLA DQ2/DQ8*) were statistically not significant and therefore dropped from the final model (Table 3).

Changes of PI:C over time When baseline PI:C ratios of prediabetic relatives were plotted against time to clinical onset (Fig. 3a), an inverse correlation was found (Spearman r_s=−0.44, p=0.002). Fig. 3b shows that the individual PI:C ratios tended to increase closer to diagnosis. A high PI:C ratio >P66 persisted 1 year later in 16 of 25 IA-2A-positive relatives (64%; 10 of 14 [71%] in prediabetic subjects) and in 55 of 88 (63%) antibody-positive relatives lacking IA-2A.

Discussion

This study indicates that elevated levels of proinsulin immunoreactive material and an elevated PI:C ratio precede onset of hyperglycaemia in randomly sampled non-diabetic first-degree relatives of type 1 diabetic patients. Antibody-negative relatives had partly overlapping but significantly lower proinsulin and PI:C values. The increase in ratio was more prominent in prediabetic subjects sampled within 40 months prior to disease onset than in prediabetic subjects sampled 40–110 months before. This possibly reflects an increased beta cell demand in those who are at risk of rapidly developing the disease. This is in line with Røder and co-workers, who reported that first-degree relatives who had diminished first-phase insulin release and were studied 1–28 months prior to disease onset exhibited a higher PI:C ratio than those with normal first-phase insulin response and no disease onset within this period [12].

To date we have found no difference in proinsulin and PI:C between antibody-negative relatives and a limited number of control subjects from the general population. This is at variance with a number of previous studies describing raised fasting proinsulin levels [14, 29, 30] and PI:insulin ratios [14], independent of antibodies and *HLA DQ* status, in subjects at familial risk of type 1 diabetes. However, no such elevated values were observed in cord blood from newborn siblings of diabetic children [31]. These findings were interpreted as evidence of beta cell

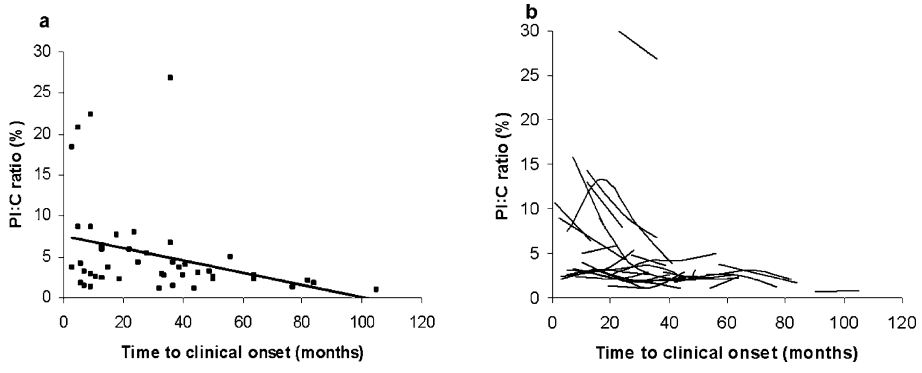

Fig. 3 PI:C ratio (%) according to time before clinical onset of type 1 diabetes. **a** At entry in 46 antibody-positive prediabetic first-degree relatives (non-parametric Spearman correlation coefficient:r_s=−0.44, p=0.002). **b** Evolution in all antibody-positive prediabetic relatives with ≥2 samples before diagnosis (n=32)

dysfunction occurring after birth but not leading to type 1 diabetes. The discrepancy with the present observation may relate to the use of random vs fasting values, different hormone and antibody assays, and low numbers both in older studies and our control group. However, it does not invalidate our observation of a(n) (further) increase in proinsulin and PI:C closer to diagnosis, which was also noted by others [14, 32, 33]. In particular, the use of random hormone levels may lead to more overlapping results between groups tested, but remains the only option to screen large groups of active non-diabetic relatives nationwide. Moreover, glucose stimulation was not found to vastly increase PI:C in healthy subjects [34].

It is conceivable that the elevated proinsulin levels and PI:C ratios measured at normal random glycaemia levels during the prediabetic period indicate an adaptation of the beta cell mass to maintain metabolic control in the face of increasing demands. In vitro studies on human beta cells isolated from normal donors have shown that a sustained activation of the cells by hyperglycaemia results in higher rates of proinsulin release and a higher PI:insulin ratio in the medium [35, 36]. A similar beta cell activation increasing glucose sensitivity and proinsulin release may occur when a reduced islet mass has to secure glucose homeostasis. It is so far unknown whether such an activated state of the beta cells can accelerate their functional failure and/or shorten their life span. Alternatively, an elevated ratio in the medium may also result from cytokine influences on beta cells shown by in vitro experiments [37]; if so, this could then reflect an altered phenotype of a subpopulation of cells with reduced insulin-producing activities [37, 38], whereby the other beta cells maintain metabolic control. Finally, increased proinsulin and PI:C may relate to insulin resistance, identified as a risk factor for progression to diabetes [39].

At present there is no evidence that differences in proinsulin and PI:C ratio are caused by differences in hormonal clearance or by variations in autoantibody levels against insulin and/or proinsulin [12]. IAA have been detected in prediabetes and are known to contribute to a circulating pool of antibody-bound proinsulin, thereby increasing its half-life and its plasma concentration [40]. In our study this also remains a possible, but very unlikely explanation, since antibody-positive prediabetic and non-diabetic relatives did not differ in terms of prevalence or levels of IAA at first sampling and hormonal differences persisted between prediabetic and non-diabetic relatives after exclusion of IAA-positive subjects (not shown). Cox regression analysis also supported the notion that the PI:C ratio and IAA were independent of each other in diabetes prediction. Finally, proinsulin-specific autoantibodies are relatively infrequent [41].

Measurement of an increased PI:C ratio is in itself not a marker for progression to diabetes within 5 years, but serves this function when combined with a positive antibody assay in the sample. Antibody-positivity in randomly sampled first-degree relatives predicted 13% risk for diabetes within 5 years versus 0.5% for antibody-negative samples. The hormonal changes were also more marked in the presence of ≥2 (molecular) antibodies or IA-2A, which indicates their significance as markers for 5-year risk of diabetes. Both in multiple antibody-positive and in IA-2A-positive relatives, a high PI:C ratio (>P66 of antibody-negative non-diabetic relatives) identified a subpopulation at higher risk of rapid progression (50 and 68% 5-year risk respectively), whereas both marker combinations have a similar screening sensitivity. Moreover, in IA-2A-positive relatives an elevated PI:C ratio (>P66) improved disease prediction to the same extent as did the presence of the *HLA DQ2/DQ8* genotype (5-year risk of 68 and 76%, respectively). However, the PI:C ratio had a significantly higher screening sensitivity (87% [95% CI 75–99%] versus 47% [95% CI 29–65%] for presence of the *DQ2/DQ8* genotype) ($p<0.001$; not shown).

Cox regression analysis in antibody-positive relatives confirmed the independent diabetes predictor capacity of the random PI:C ratio as a complement to IA-2A. In contrast with *HLA DQ* genotyping, which is more useful for screening programmes during the early preclinical phase [3, 34, 42–44], hormonal markers may be better suited to select high-risk antibody-positive subjects for prevention trials studying the efficiency of a given intervention within a 5-year time frame [12, 13, 15]. The two screening strategies may provide a different time-window for testing preventive interventions during the prediabetic period. Unlike genotyping, the random PI:C ratio also provides a dynamic parameter, whose increase over time indicates impending clinical onset in prediabetic relatives and is more amenable to large-scale monitoring than fasting or glucose-stimulated hormone measurements (e.g. intravenous glucose tolerance test) [12–19]. However, follow-up data indicate that in subjects with a high PI:C ratio this finding is not always confirmed in subsequent samples; hence this marker may not consistently identify high-risk subjects, possibly due to its ‘random’ nature. Parallel implementation of more elaborate dynamic tests (e.g. glucose clamps) may further clarify the changes in beta cell function during this evolution [45].

In conclusion, measurement of PI:C ratio in first-degree relatives of type 1 diabetic patients complements current diabetes risk assessment based on antibody assays. This marker for the functional state of beta cells provides a dynamic parameter for identifying relatives at risk of rapid progression to diabetes. An elevated PI:C ratio in IA-2A-positive relatives identifies a subpopulation with a 68% risk of developing the disease within 5 years.

Duality of interest
None declared

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