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Bakhtadze, Ekaterine; Borg, Henrik; Stenstrom, G; Fernlund, Per; Arnqvist, H; Ekbom-Schnell, A; Bolinder, J; Eriksson, J; Gudbjornsdottir, S; Nystrom, L; Groop, Leif; Sundkvist, Göran

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HLA-DQB1 genotypes, islet antibodies and beta-cell function in the classification of recent onset diabetes among young adults in the nationwide Diabetes Incidence Study in Sweden

E. Bakhtadze[1], H. Borg[1], G. Stenström[2], P. Fernlund[3], H. J. Arnqvist[4], A. Ekbom-Schnell[5], J. Bolinder[6], J. W. Eriksson[7], S. Gudbjörnsdottir[8], L. Nyström[7], L.C. Groop[1], G. Sundkvist[1]

[1]Department of Clinical Sciences Malmö, Division of Endocrinology and Diabetes, Lund University, Malmö University Hospital, Malmö, [2]Department of Medicine, Kungsbacka Hospital, Göteborg University, Göteborg, [3]Clinical Chemistry, Department of Laboratory Medicine Malmö, Lund University, Malmö University Hospital, Malmö, [4]Department of Medicine and Care, Linköping University, Linköping, [5]Department of Medicine, Uppsala Academic Hospital, Uppsala, [6]Department of Medicine, Karolinska University Hospital Huddinge, Stockholm, [7]Department of Medicine, Umeå University Hospital, Umeå, [8]Department of Medicine, Sahlgrenska University Hospital, Göteborg, Sweden

Corresponding author: Professor Göran Sundkvist, Department of Endocrinology, Malmö University Hospital, SE-205 02 Malmö, Sweden; Tel: (+46 40) 331000, Fax: (+46 40) 33 62 01; e-mail: goran.sundkvist@med.lu.se

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Abbreviations: DISS, Diabetes Incidence Study in Sweden; HLA-DQB1, human leukocyte antigen-DQB1 locus; ICA, islet cell antibodies; JDF, Juvenile Diabetes Foundation; DASP, Diabetes Autoantibody Standardization Program; GADA, glutamic acid decarboxylase antibodies; IA-2A, IA-2-protein tyrosine phosphatase-like protein antibodies; F-P-C-peptide, fasting plasma C-peptide; BMI, body mass index; CPM, counts per minute.

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Abstract

Aims/hypothesis: WHO considers an etiological classification of diabetes to be essential. To evaluate whether HLA-DQB1 genotypes facilitates the classification of diabetes as compared with islet antibodies, young adult diabetic patients were investigated.

Methods: Blood samples were available at diagnosis from 1872 (90%) of the 2077 young adult patients (15-34 years old) during a five-year period in the nationwide Diabetes Incidence Study in Sweden (DISS). Islet antibodies were measured at diagnosis in 1869, fasting-plasma-C-peptide (F-P-C-peptide) after diagnosis in 1522, while HLA-DQB1genotypes were determined in 1743 patients.

Results: Islet antibodies were found among 83% clinically considered to have type 1 diabetes, 23% with type 2 and 45% with unclassifiable diabetes. F-P-C-peptide concentrations after diagnosis were markedly lower in patients with than in those without islet antibodies (median 0.24 nmol/l vs. median 0.69 nmol/l; $p<0.0001$). Irrespective of clinical classification, patients with islet antibodies showed increased frequencies at least one of risk HLA-DQB1 genotypes compared with patients without. Antibody negative patients with risk HLA-DQB1 genotypes had significantly lower fasting P-C-peptide concentrations than those without risk genotypes (0.51 nmol/l vs. 0.74 nmol/l; $p=0.0003$).

Conclusions/interpretation: Assessment of islet antibodies is necessary for an etiological classification of diabetic patients. HLA-DQB1 genotyping does not improve the classification in patients with islet antibodies. However, in patients without islet antibodies, HLA-DQB1 genotyping together with C-peptide measurement may be of value in the differentiation between idiopathic type 1 diabetes versus type 2 diabetes.

Introduction

Type 1 and type 2 diabetes have different aetiology and clinical courses. However, if only a clinical assessment is used at diagnosis, it is difficult to distinguish the two main types of diabetes from each other [1-4]. Among patients with the type 2 diabetes phenotype, depending on age at onset, 8-30% have islet antibodies indicating that the correct diagnosis would be autoimmune type 1 diabetes [5-7]. Besides autoimmune markers, certain HLA genotypes confer increased risk of type 1 diabetes [8-10]. Compared with islet antibodies and fasting plasma C-peptide (F-P-C-peptide) concentration as measure of beta-cell function, the value of type 1 diabetes associated HLA genotypes in the classification of diabetes among young adults is not established.

Using a 5-year cohort of incident diabetic patients in the Diabetes Incidence Study in Sweden (DISS), we genotyped key genes in the HLA-DQB1 locus and measured islet antibodies (in 1869) and F-P-C-peptide (in 1522) newly diagnosed diabetic patients 15-34 years of age. The aim was to evaluate the usefulness of HLA-DQB1 genotypes in the classification of diabetes as compared with islet autoantibodies and F-P-C-peptide among young adults.

Subjects, material and methods

Subjects

During a five-year period (Jan 1, 1998 to December 31, 2002), 2077 young adult (15-34 years of age) diabetic patients were reported to the Diabetes Incidence Study in Sweden (DISS). Type of diabetes, age, gender, height, body weight, symptoms at diagnosis, duration of symptoms, date of diagnosis, family history of diabetes, and blood glucose values at diagnosis were recorded on a special form by the reporting physician. The patients were invited to donate a blood sample for determination of islet antibodies (ICA, GADA, IA-2A) and HLA-DQB1 genotypes at diagnosis, and a blood sample for determination of F-P-C-peptide after

diagnosis (median=4 month, Q_1=3 month, Q_3=6 month). Blood samples were available from 1872 (90%) of the 2077 patients. Islet antibodies were measured in 1869, F-P-C-peptide in 1522, while HLA-DQB1genotypes were determined in 1743 patients. Fig 1 gives a flow chart of the different assessments conducted in the patients.

By use of a computer-based patient administrative register as a second source, the level of ascertainment in DISS during 1983-1987 was estimated at 78% in women and at 79% in men in the two southernmost counties, covering 9.2% of the population at risk. For clinical type 1 diabetes the level was 86% [11]. A similar study in the county of Västerbotten in the Northern Sweden, covering 2.9% of the population at risk, found no trend in the level of ascertainment during 1986-1997. The level of ascertainment for clinical type 1 diabetes was 91%. Further, the median level of ascertainment for clinical type 1 diabetes at six diabetic clinics continuously using the software DIABASE (Kungälv, Sweden) was 82% for their patients. Therefore, the ascertainment rate in DISS during the years seems to be constant. Level of ascertainment was assessed using the two-sample capture-recapture method [11]. The Ethics Committee at the Karolinska Institute, Stockholm, approved the study conducted after the patients had given informed consent.

HLA-DQB1 genotyping

Using a primer pair with biotinylated 3´ primers, the 158 bp second exon of HLA-DQB1 gene was amplified via the polymerase chain reaction (PCR). The amplification product was bound to streptavadin coated microtitration plates and denatured with NaOH. After washing, bound DNA was assessed using two different hybridization mixtures with lanthanide (III) chelate labelled DNA probes specific for the HLA-DQB1 alleles. One mixture contained europium (Eu)-labelled internal reporter probe for DQB1 *0602 and *0603 alleles (*0602-*0603), samarium (Sm)-labelled for *0603 and *0604 (*0603-*0604) alleles, and terbium (Tb)-labelled consensus sequence specific probe (Tb-DQB1 control) as control of PCR

amplification. The other mixture contained Tb-, Sm-, and Eu-labelled probes specific for DQB1*0201, *0301, *0302 alleles, respectively. To measure probe hybridization, microtitration plates were evaluated by time-resolved fluorescence (Delfia Research Fluorometer, Wallac OY, Turku, Finland). Different emission wavelengths and delay times were used to distinguish the signals of each lanthanide label [12]. 216 subjects without diabetes from the county of Skaraborg, Sweden were used as controls for HLA-DQB1 genotyping. In control subjects, HLA-DQB1 locus was amplified by PCR, followed by dot-blotting onto nitrocellulose filters, and hybridization using the radioactively end labelled sequence-specific oligonucleotide probes, then autoradiography was done [13].

Islet cell antibodies (ICA)

ICA were determined by a prolonged two-colour immune-fluorescence assay [14]. The detection limit for ICA was 4 JDF units for the first pancreas used in samples tested until April 1999 and 5 JDF units for the second pancreas used in samples tested from April 1999 and onwards. In the last ICA Proficiency Test (13^{th}) our ICA assay performed with 100% sensitivity and 100% specificity (ICA is not included in the Diabetes Autoantibody Standardization Program [DASP]).

GAD 65- glutamic acid decarboxylase antibodies (GADA)

GADA were measured by a radioligand binding assay, based on human ^{35}S-labeled recombinant GAD 65 [15]. The results are presented as an index: GADA index=100 * (u-n)/(p-n), where u is CPM (mean activity of all four measurements for a sample) of the unknown sample, n is CPM of the negative control, and p is CPM of the positive control. A GADA index >4.6 were considered as positive (97.5 percentile of 165 non-diabetic controls aged 7-34 years). In the first DASP (in 2000) our GADA assay showed a sensitivity of 80% and a specificity of 96%, respectively, in the second (in 2002), a sensitivity of 88% and a

specificity of 87%, respectively, and in the third DASP (in 2003), a sensitivity of 82% and a specificity of 93%, respectively.

IA-2-protein tyrosine phosphates-like protein antibodies (IA-2A)

IA-2A were measured by a similar assay based on human ^{35}S-labeled recombinant IA-2 [16]. An IA-2A index >1.0 was considered as positive (97.5 percentile of 165 non-diabetic controls aged 7-34 years). In the first DASP (in 2000), our IA-2A assay showed a sensitivity of 58% and a specificity of 100%, respectively, in the second (in 2002), a sensitivity of 62% and a specificity of 100%, respectively, and in the third DASP (in 2003), a sensitivity of 64% and a specificity of 100%, respectively.

Plasma C-peptide

Plasma C-peptide was determined by radioimmunoassay. The detection limit was 0.10 nmol/l and the normal range for fasting plasma C-peptide 0.25-0.75 nmol/l. A F-P-C-peptide concentration 0.1-0.25 nmol/l was considered as low and <0.10 nmol/l as immeasurable (beta-cell failure) [17].

Clinical classification

At diagnosis, based on clinical judgment by the reporting physician, the patient was classified as having type 1, type 2, or unclassifiable diabetes.

Statistical analysis

Comparisons of genotype frequencies between diabetic patients and control subjects were tested by two-tailed Fisher's exact test or chi-square (χ^2) test with Bonferroni adjustment (0.05/19 [number of genotypes in the study]) of *p*-values for multiple comparisons ($p<0.0026$ [0.05/19] was considered significant). Odds ratios (OR) and 95% confidence intervals (CI) were calculated by use of the formula *a*d/b*c*, where *a* is the number of diabetic patients with one of the genotypes; *b* is the number of control subjects with the corresponding genotype; *c* is the number of patients without this genotype; and *d* is the number of control subjects

without the corresponding genotype. Differences in continuous variables between groups were assessed by non-parametric Wilcoxon Signed-Rank test. Regression analysis was used for multiple comparisons. All statistical tests were performed by SPSS version 11.0 or JMP version 5 for MAC OS X. In univariate analysis $p<0.05$ was considered significant whereas in multivariate analysis $p<0.0026$ was considered significant. Continuous data are presented as median and 25^{th} and 75^{th} percentiles (Q1 and Q3) and dichotomous data as absolute values and percentage.

Results

Of the 2077 incident diabetic patients, 2018 were classified by the reporting physicians: 1395 (69%) were given a diagnosis of clinical type 1 diabetes, 366 (18%) of clinical type 2 diabetes and 257 (13%) could not be classified (unclassifiable diabetes). There was a clear male preponderance among clinical type 1 diabetic patients (male/female ratio= 1.9) (Table 1). Patients with clinical type 1 diabetes were younger (24 vs. 30 and 28 years, respectively, $p<0.0001$) and had lower BMI (22 vs. 32 and 25, respectively, $p<0.0001$) than patients with clinical type 2 or unclassifiable diabetes. A family history of diabetes was more frequent in clinical type 2 (53%) than in clinical type 1 (22%; $p<0.0001$) and unclassifiable (38%; $p=0.004$) diabetes (Table 1).

Among all patients, 1250 (67%) were positive for islet antibodies (Ab+). Islet antibodies were found in 83% with clinical type 1, in 23% with clinical type 2, and in 45% with unclassifiable diabetes. The prevalence of islet antibodies was significantly higher in clinical type 1 diabetic patients compared with unclassifiable patients (83% vs. 45%; $p<0.0001$), who, in turn, showed a higher prevalence of islet antibodies than patients with clinical type 2 diabetes (45% vs. 23%; $p<0.0001$). Irrespective of the clinical classification, almost half of Ab+ individuals had 3 different antibodies. Among patients positive for ICA or IA-2A, median ICA and IA-2A titre, respectively, was significantly higher in patients with clinical

type 1 diabetes (58 [Q_1=26, Q_3=130] JDF units vs. 45 [Q_1=17, Q_3=87; p=0.008] JDF units, and 95 [Q_1=36, Q_3=115] index values vs. 51 [Q_1=7, Q_3=114] index values; p=0.01, respectively) compared with those with unclassifiable diabetes. Similar differences were not seen for GADA.

HLA-DQB1 genotypes

Table 2 shows that, irrespective of their clinical classification, patients with islet antibodies had a significantly higher prevalence of risk HLA-DQB1 genotypes. However, among clinical type 1 diabetic patients without islet antibodies some protective and neutral HLA-DQB1 genotypes were significantly more frequent than in Ab+.

Patients with young age at onset of diabetes (≤25 year) had a twofold increased risk of having HLA-DQB1 *02/*0302 genotype (OR=1.6; 95% CI 1.3-2.1; p<0.0001) than those with older age at onset (>25 year); however, this effect disappeared in multivariate analysis when islet antibodies were included. In this analysis age at onset was included as a dependent categorical and HLA-DQB1 genotypes and islet antibodies as an independent categorical variables.

Islet antibodies in relation to HLA-DQB1 genotypes, age at onset and gender

Patients with the HLA-DQB1 *02/*0302 genotype had a threefold increased risk of having GADA (OR=3.4; 95% CI 2.4-4.7; p<0.0001) than patients with protective/neutral genotypes. In contrast, patients with HLA-DQB1 *0302/X and *0302/*0604, respectively, had a tree- and fivefold, respectively, increased risk of having IA-2A (OR=3.2; 95% CI 2.3-4.4; p<0.0001) and (OR=5.6; 95% CI 3.1-10.2; p<0.0001), respectively, than patients with protective/neutral genotypes.

As compared with Ab-, Ab+ was significantly associated with young age at onset of diabetes (≤25 year): 654 (52%) vs. 180 (29%), p<0.0001. Age at onset and gender (independent categorical variables) in relation to different islet antibody combinations

(dependent categorical variable) included in a multinomial logistic regression analysis showed that young age at onset (≤25 year) was independently and significantly (p<0.0001) associated with islet antibodies, particularly with IA-2A, alone (OR=7.2; 95% CI 3.2-16.4; p<0.0001) or in combination with ICA (OR=7.0; 95% CI 4.1-12.0; p<0.0001). In addition, male gender was independently associated with IA-2A, alone (OR=5.7; 95% CI 1.7-18.9; p=0.005) or in combination with ICA (OR=4.0; 95% CI 2.0-8.0; p<0.0001), whereas female gender was independently associated with GADA in combination with ICA (OR=1.7; 95% CI 1.3-2.4, p=0.001). Amongst patients positive for GADA, median GADA concentration was significantly higher in women than in men (82 [Q_1=27, Q_3=112] index values vs. 39 [Q_1=16, Q_3=91] index values; p<0.0001).

F-P-C-peptide in relation to islet antibodies and HLA-DQB1 genotypes

Table 1 shows that the median F-P-C-peptide concentration after diagnosis was significantly (p<0.0001) lower among clinical type 1 diabetic patients (0.24 nmol/l) compared both with clinical type 2 (0.75 nmol/l) and unclassifiable (0.48 nmol/l) diabetic patients. Fig. 2 shows that Ab+ patients had a significantly lower median F-P-C-peptide concentration than Ab- patients (0.24 nmol/l vs. 0.70 nmol/l; p<0.0001), irrespective of the presence of risk HLA-DQB1 or not. On the other hand, Ab- patients with risk HLA-DQB1 genotypes had a significantly lower median F-P-C-peptide concentration than patients with protective/neutral genotypes (0.51 nmol/l vs. 0.74 nmol/l; p=0.0003). After stratification for islet antibody presence, multiple regression analysis was performed in each group (Ab+ and Ab-). This analysis included F-P-C-peptide (dependent continuous variable), F-P-C-peptide follow-up time (≤ 6 month and > 6 month, respectively) and HLA-DQB1 genotypes (independent categorical variables). The analysis showed that among Ab- patients, risk HLA-DQB1 genotypes were significantly (p=0.001) associated with decreased F-P-C-peptide concentration, irrespective of when the follow- up sample was taken. In contrast, among Ab+

patients, a long F-P-C-peptide follow-up time (> 6 month) was significantly (p<0.0001) associated with decreased F-P-C-peptide concentration, irrespective of risk HLA-DQB1 genotypes.

Categorization of the F-P-C-peptide concentrations into low (≤0.25nmol/l) and normal (> 0.25nmol/l), respectively, showed that low F-P-C-peptide (≤ 0.25nmol/l) concentrations was significantly associated with islet antibodies (p<0.0001) but not with HLA-DQB1 genotypes in multinomial logistic regression analysis. In the regression analysis, nominal F-P-C-peptide was included as a dependent categorical variable and islet antibodies, HLA-DQB1 genotypes, follow-up time, age at onset, gender, BMI and family history of diabetes as an independent categorical variable.

Ketonuria, acidosis, diabetic symptoms in relation to islet HLA-DQB1 genotypes, islet antibodies, F-P-C-peptide, age at onset and gender

At diagnosis 1138 (60%) of diabetic patients had ketonuria, 149 (10%) acidosis and 1835 (93%) had diabetic symptoms; 1401 (75%) had a short duration of diabetic symptoms (<3 month). Multinomial regression analysis showed that presence of ketonuria was significantly (p<0.0001) associated with islet antibodies: particularly ICA combined with IA-2A (OR=4.0; 95% CI 1.9-8.1) or 3 antibodies (OR=3.2; 95% CI 2.3-4.6) or male gender (OR=1.8; 95% CI 1.4-2.3), but not with risk HLA-DQB1 genotypes. Prevalence of diabetic symptoms was significantly (p<0.0001) associated with 3 antibodies (OR=4.3; 95% CI 2.0-9.1) and male gender (OR=2.5; 95% CI 1.5-4.0). Moreover, short length of symptomatic period (<3 month) was significantly (p<0.0001) associated with 3 antibodies (OR=2.4; 95% CI 1.6-3.6). In the regression analysis, presence of ketonuria, the prevalence of diabetic symptoms and length of symptomatic period were separately included as a dependent categorical variable, whereas islet antibodies, HLA-DQB1 genotypes, F-P-C-peptide, follow-up time, gender, age at onset, BMI and family history of diabetes were included as an independent categorical variable.

Discussion

This study of 2077 young adults (15-34 year olds) with recently diagnosed diabetes, in whom islet antibodies were measured in 1869, showed that 1250 of 1869 (67%) had autoimmune type 1 diabetes if islet antibodies were used as an objective diagnosis of autoimmune type 1 diabetes. Among those classified as having type 1 diabetes, 83% had objective type 1 diabetes, however, also seen among 23% classified as with type 2 and 45% classified as with unclassifiable diabetes. If immeasurable or low F-P-C-peptide after diagnosis was included as a further objective criteria for clinical type 1 diabetes, only another 70 patients without islet antibodies were added to the clinical type 1 diabetes group. Hence, including patients with low or immeasurable F-P-C-peptide, the proportion of type 1 diabetes would increase with only 4% leading to 71% with objective type 1 diabetes among all incident young adults between 15 and 34 years of age developing diabetes. Accordingly, islet antibodies, not F-P-C-peptide, are most important in the classification of diabetes among young adults. Nevertheless, our study showed, that non-autoimmune diabetes (presumably mostly type 2 diabetes) is not rare among young adult diabetic patients, found in about every third patient. Hence, the differential diagnosis between clinical type 1 and clinical type 2 diabetes is a major issue among 15-34 years old incident diabetic patients.

Based on islet antibodies at diagnosis, we have previously reported, that around 25% of clinical type 2 and 50% of unclassifiable young adult diabetic patients should be considered as having type 1 diabetes [1, 18]. Noteworthy, the current DISS study conducted in 1998-2002, gives similar frequencies of islet antibodies among clinical type 2 and unclassifiable diabetic patients, as shown in the previous DISS studies of 1987-1988, and 1992-1993. This underlines that, among 15-34 year olds, to achieve a reliable classification, an objective classification based on islet antibodies is necessary.

Our study emphasizes that, although risk HLA-DQB1 genotypes are closely associated with islet antibodies, risk HLA-DQB1 genotypes *per se* do not contribute to the classification of type 1 diabetes. Indeed, the only suggestion that risk HLA-DQB1 genotypes may contribute to an etiological classification was among patients without islet antibodies. In line with the Belgian Diabetes Registry [3], we found that, among Ab- those with risk HLA-DQB1 genotypes had lower median F-P-C-peptide concentration than those with protective/neutral genotypes, irrespective of F-P-C-peptide follow-up time. However, patients with islet antibodies and samples taken 6 month after diagnosis had low F-P-C-peptide concentrations, irrespective of risk HLA-DQB1 genotypes, again underlining that in the presence of islet antibodies, genetic risk assessment based on HLA is not important in the classification. It was also observed previously, that in the presence of islet antibodies genetic risk or protection does not matter in the prediction of diabetes development [19-21]. Our observation that low F-P-C-peptide was associated with risk HLA-genotypes in Ab- indicates, that risk HLA-DQB1 genotypes *per se* may be associated with impaired beta-cell function. This corresponds to the previous findings regarding risk genotype presence in Ab- patients that conferred an increased risk of insulin requirement at a later follow-up [22]. However, antibodies not detected by current assays may be present in patients without islet antibodies, with risk HLA-DQB1 genotypes and low F-P-C-peptide. Another option is a later development of islet antibodies. Previous studies have shown that up to 10% of young adult onset diabetic patients without islet antibodies convert to positivity after the diagnosis of diabetes [23-26]. The disappearance of previous islet antibodies has also to be considered.

Interesting associations between risk HLA-DQB1 genotypes, age at onset of diabetes and islet antibodies were detected. It has been reported that patients with young age at onset of diabetes (≤25 year) have increased frequencies of HLA-DQB1 *02/0302 genotypes [27-30]**,** however; according to our study significant associations between HLA-DQB1 genotypes and

young age at onset was due to islet antibodies. In agreement with previous observations [31-33], GADA were associated with HLA-DQB1 *02/*0302, whereas IA-2A were associated with HLA-DQB1 *0302/X and *0302/*0604, respectively. Indeed, IA-2A concentration was highest among patients with the HLA-DQB1 *0302/X genotype. IA-2A are known to be associated with a rapid onset of type 1 diabetes as well as with young age at onset [34-36]. Hence, our study infers that the association between IA-2A and rapid onset may be HLA-DQB1 locus dependent. Our study also confirmed that IA-2A were associated with male gender, whereas GADA presence and high levels were associated with female gender [37, 38]. However, logistic regression analysis showed that the associations between gender and age at onset to the types of islet antibodies were not related to risk HLA-DQB1 genotypes, as indeed previously shown [39]. Thus, the well known increased incidence of type 1 diabetes among young adult men [40-43], as also demonstrated in our study, does not seem to be HLA-DQB1 related, but being an effect of gender in itself. As emphasized by our finding that ketonuria and diabetic symptoms were clearly associated with male gender.

A clear finding in our study was that, up to 6 months after diagnosis normal F-P-C-peptide does not exclude type 1 diabetes. This finding gives further support for the concept, that islet antibodies are the method of choice as tools in the classification of diabetes. The preserved beta-cell function in most patients with islet antibodies, demonstrates, that the process of beta-cell destruction is not always fast among young adult diabetic patients. Indeed, it fits with the previous observation that it may take 12 years before severe beta-cell failure develops in adult patients with islet antibodies [44].

It has been reported, that high concentrations of islet antibodies are associated with low F-P-C-peptide values [45], but no such association was shown in our study. Neither a correlation was found between the number of islet antibodies and F-P-C-peptide, as previously reported [46, 47]. Most likely this reflects, that beta-cell failure was not yet

frequent in our study patients. Prospective follow-ups may show that high concentrations and/or high number of islet antibodies are associated with beta-cell failure in the future among our patients.

The major strength of this study is that we recruited a large material of incident population based and representative diabetic patients aged 15-34 years age from a whole country. Indeed, since 1983 DISS have included > 9000 patients 15-34 years old at diagnosis of diabetes. In this 5- year study of 1998-2002, blood samples were taken in most incident cases and we could relate complete data regarding HLA-DQB1 genotypes, islet antibodies and F-P-C-peptide in 1455 newly diagnosed young adult diabetic patients (Fig 2). It can be argued that we did not access HLA-DQA1 genotypes in our patients. No doubt than extended genotyping is helpful for relative risk estimation. The sensitivity increases when new genotypes conferring risk are included. However, the linkage disequilibrium between alpha and beta chain alleles is very strong. Additional information obtained by typing for HLA-DQA1 would be of limited importance for our study [12]. Further, our association study of HLA-DQB1 loci may be considered as a cost-effective way to identify HLA contribution in the classification of diabetes in 15-34 year olds.

In conclusion, this study shows that 1) irrespective of clinical classification, 67% of patients with newly diagnosed diabetes at the age of 15-34 years have autoimmune type 1 diabetes 2) islet antibodies strongly contribute to the etiological classification of diabetes, 3) islet antibodies are more closely associated with beta-cell impairment than risk HLA-DQB1 genotypes, 3) risk HLA-DQB1 genotypes are associated with islet antibodies and do not contribute to the classification of diabetes in Ab+ 4) risk HLA-DQB1 genotypes are, however, associated with low F-P-C-peptide concentrations in the absence of islet antibodies presumably identifying non-autoimmune type 1 diabetes, and 5) absence of islet antibodies and high F-P-C-peptide concentrations predict a type 2 diabetes phenotype. Taken together

the data re-emphasize the need to measure islet antibodies for the diagnosis of autoimmune diabetes in young adults in clinical practise whereas HLA-DQB1 genotyping may be of interest in patients without islet antibodies.

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Conflict of interest

L.C Groop has been a consultant and served on advisory boards for Aventis-Sanofi, Bristol-Myers Squibb, Kowa, and Roche.

References

1. Landin-Olsson M, Karlsson FA, Lernmark Å, Sundkvist G, and the Diabetes Incidence Study in Sweden Group (1992) Islet cell and thyrogastric antibodies in 633 consecutive15-34 yr-old patients in the Diabetes Incidence Study in Sweden. Diabetes 41: 1022-1027

2. Tuomi T, Carlsson A, Li H, et al. (1999) Clinical and genetic characteristics of type 2 diabetes with and without GAD antibodies. Diabetes 48: 150-157

3. Weets I, Siraux V, Daubresse JC, et al. (2002) Relation between disease phenotype and HLA-DQ genotype in diabetic patients diagnosed in early adulthood. J Clin Endocrinol Metab 87: 2597-2605

4. Arnqvist HJ, Littorin B, Nyström L, et al. (1993) Difficulties in classifying diabetes at presentation in the young adult. Diabet Med 10: 606-613

5. Wroblewski M, Gottsäter A, Lindgärde F, Fernlund P, Sundkvist G (1998) Gender, autoantibodies, and obesity in newly diagnosed diabetic patients aged 40-75 years. Diabetes Care 21: 250-255

6. Turner R, Stratton I, Horton V, et al. (1997) UKPDS 25: autoantibodies to islet-cell cytoplasm and glutamic acid decarboxylase for prediction of insulin requirement in type 2 diabetes. Lancet 350: 1288-1293

7. Littorin B, Sundkvist G, Hagopian W, et al. (1999) Islet cell and glutamic acid decarboxylase antibodies present at diagnosis of diabetes predict the need for insulin treatment. A cohort study in young adults whose disease was initially labeled as type 2 or unclassifiable diabetes. Diabetes Care 22: 409-412

8. Nerup J, Platz P, Anderson OO, et al. (1974) HLA antigens and diabetes mellitus. Lancet ii: 864-866

9. Todd JA, Bell JI, McDevitt HO (1987) HLA-DQ beta gene contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature 329: 599-604

10. Thorsby E, Ronningen KS (1993) Particular HLA-DQ molecules play a dominant role in determining susceptibility or resistance to type 1 (insulin-dependent) diabetes mellitus. Diabetologia 36: 371-377

11. Littorin B, Sundkvist G, Scherstén B, et al. (1996) Patient administrative system as a tool to validate the ascertainment in the diabetes incidence study in Sweden (DISS). Diabetes Res Clin Pract 33: 129-133

12. Ilonen J, Reijonen H, Herva E, et al. (1996) Rapid HLA-DQB1 genotyping for four alleles in the assessment of risk for IDDM in the Finnish population. The Childhood Diabetes in Finland (DiMe) Study Group. Diabetes Care 19: 795-800

13. Stenström G, Berger B, Borg H, Fernlund P, Dorman JS, Sundkvist G (2002) HLA-DQ genotypes in classical type 1 diabetes and in latent autoimmune diabetes of the adult. Am J Epidem 156: 787-796

14. Landin-Olsson M, Sundkvist G, Lernmark Å (1987) Prolonged incubation in the two-colour immunofluorescence test increases the prevalence and titres of islet cell antibodies in Type 1 (insulin-dependent) diabetes mellitus. Diabetologia 30: 327-332

15. Borg H, Fernlund P, Sundkvist G (1997) Measurement of antibodies against glutamic acid decarboxylase (GADA)-two new 1^{125}-assays compared with S^{35}-GAD 65-ligand binding assay. Clin Chem 43: 779-785

16. Borg H, Fernlund P, Sundkvist G (1997) Protein tyrosine phosphatase IA2-antibodies plus glutamic acid decarboxylase 65 antibodies (GADA) indicates autoimmunity as frequently as islet cell antibodies assay in children with recently diagnosed diabetes mellitus. Clin Chem 43: 2358-2363

17. Gottsäter A, Landin-Olsson M, Fernlund P, Gullberg B, Lernmark Å, Sundkvist G (1992) Pancreatic beta-cell function evaluated by intravenous glucose and glucagon stimulation. A comparison between insulin and C-peptide to measure insulin secretion. Scand J Lab Invest 52: 631-639

18. Törn C, Landin-Olsson M, Östman J, et al. (2000) Glutamic acid decarboxylase antibodies (GADA) is the most important factor for prediction of insulin therapy within 3 years in young adult diabetic patients not classified as Type 1 diabetes on clinical grounds. Diab Metab Res Review 16: 442-447

19. Greenbaum CJ, Schatz DA, Cuthbertson D, Zeidler A, Eisenbarth GS, Krischer JP (2000) Islet cell antibody-positive relatives with human leukocyte antigen DQA1*0102, DQB1*0602: identification by the Diabetes Prevention Trial-type 1. J Clin Endocrinol Metab 85: 1255-1260

20. LaGasse JM, Brantley MS, Leech NJ, et al. (2002) Successful prospective prediction of type 1 diabetes in schoolchildren through multiple defined autoantibodies: an 8-year follow-up of the Washington State Diabetes Prediction Study. Diabetes Care 25: 505-511

21. Redondo MJ, Babu S, Zeidler A, et al. (2006) Specific HLA DQ Influence on Expression of Anti-Islet Autoantibodies and Progression to Type 1 Diabetes. J Clin Endocrinol Metab doi: 10.1210/jc.2005-1695

22. Pietropaolo M, Becker DJ, LaPorte RE, et al. (2002) Progression to insulin-requiring diabetes in seronegative prediabetic subjects: the role of two HLA-DQ high-risk haplotypes. Diabetologia 45: 66-76

23. Sundkvist G, Hagopian WA, Landin-Olsson M, et al. (1994) Islet cell antibodies (ICA), but not glutamic acid decarboxylase antibodies (GAD65-Ab), are decreased by plasmapheresis in patients with newly diagnosed insulin-dependent diabetes mellitus. J Clin End Metab 78: 1159-1165

24. Landin-Olsson M, Arnqvist HJ, Blohmé G, et al. (1999) Appearance of islet cell autoantibodies after clinical diagnosis of diabetes mellitus. Autoimmunity 29: 57-63

25. Decochez K, Tits J, Coolens JL, et al. (2000) High frequency of persisting or increasing islet-specific autoantibody levels after diagnosis of type 1 diabetes presenting before 40 years of age. The Belgian Diabetes Registry. Diabetes Care 23: 838-844

26. Borg H, Arnqvist HJ, Bjork E, et al. (2003) Evaluation of the new ADA and WHO criteria for classification of diabetes mellitus in young adult people (15-34 yrs) in the Diabetes Incidence Study in Sweden (DISS). Diabetologia 46: 173-181

27. Caillat-Zucman S, Garchon H-J, Timsit J, et al. (1992) Age-dependent HLA genetic heterogeneity of Type 1 insulin-dependent diabetes mellitus. J Clin Invest 90: 2242-2250

28. Tait BD, Harrison LC, Drummond BP, Stewart V, Varney MD, Honeyman MC (1995) HLA antigens and age at diagnosis of insulin-dependent diabetes mellitus. Hum Immunol 42: 116-122

29. Valdes AM, Thomson G, Erlich HA, Noble JA (1999) Association between type 1 diabetes age of onset and HLA among sibling pairs. Diabetes 48: 1658-1661

30. Emery LM, Babu S, Bugawan TL, et al. (2005) Newborn HLA-DR,DQ genotype screening: age- and ethnicity-specific type 1 diabetes risk estimates. Pediatr Diabetes 6: 136-144

31. Hagopian WA, Sanjeevi CB, Kockum I, et al. (1995) Glutamate decarboxylase-, insulin- and islet cell-antibodies and HLA typing to detect diabetes in a general population-based study of Swedish children. J Clin Invest 95: 1505-1511

32. Graham J, Hagopian WA, Kockum I, et al. (2002) Genetic effects on age-dependent onset and islet cell autoantibody markers in type 1 diabetes. Diabetes 51: 1346-1355

33. Genovese S, Bonfanti R, Bazzigaluppi E, et al. (1996) Association of IA-2 autoantibodies with HLA DR4 phenotypes in IDDM. Diabetologia 39: 1223-1226

34. Savola K, Bonifacio E, Sabbah E, et al. (1998) IA-2 antibodies--a sensitive marker of IDDM with clinical onset in childhood and adolescence. Childhood Diabetes in Finland Study Group. Diabetologia 41: 424-429

35. Bottazzo GF, Bosi E, Cull CA, et al. (2005) IA-2 antibody prevalence and risk assessment of early insulin requirement in subjects presenting with type 2 diabetes (UKPDS 71). Diabetologia 48: 703-708

36. Gorus FK, Goubert P, Semakula C, et al. (1997) IA-2-autoantibodies complement GAD_{65}-autoantibodies in new-onset IDDM patients and help predict impending diabetes in their siblings. Diabetologia 40: 95-99

37. Verge CF, Howard nJ, Rowley MJ, et al. (1994) Anti-glutamate decarboxylase and other antibodies at the onset of childhood IDDM: a population-based study. Diabetologia 37: 1113-1120

38. Sabbah E, Kulmala P, Veijola R, et al. (1996) Glutamic acid decarboxylase antibodies in relation to other autoantibodies and genetic risk markers in children with newly diagnosed insulin-dependent diabetes. J Clin Endocrinol Metab 81: 2455-2459

39. Weets I, Van Autreve J, Van der Auwera BJ, et al. (2001) Male-to-female excess in diabetes diagnosed in early adulthood is not specific for the immune-mediated form nor is it HLA-DQ restricted: possible relation to increased body mass index. Diabetologia 44: 40-47

40. Blohmé G, Nyström L, Arnqvist HJ, et al. (1992) Male predominance of Type 1 (insulin-dependent) diabetes mellitus in young adults: results from a 5-year prospective nationwide study of the 15-34 year age group in Sweden. Diabetologia 35: 56-62

41. Vandewalle CL, Coeckelberghs MI, Leeuw IH, et al. (1997) Epidemiology, clinical aspects, and biology of IDDM patients under age 40 years. Diabetes Care 20: 1556-1561

42. Gale EA, Gillespie KM (2001) Diabetes and gender. Diabetologia 44: 3-15

43. Kyvik KO, Nystrom L, Gorus F, et al. (2004) The epidemiology of Type 1 diabetes mellitus is not the same in young adults as in children. Diabetologia 47: 377-384

44. Borg H, Gottsater A, Fernlund P, Sundkvist G (2002) A 12-year prospective study of the relationship between islet antibodies and beta-cell function at and after the diagnosis in patients with adult-onset diabetes. Diabetes 51: 1754-1762

45. Borg H, Gottsater A, Landin-Olsson M, Fernlund P, Sundkvist G (2001) High levels of antigen-specific islet antibodies predict future beta-cell failure in patients with onset of diabetes in adult age. J Clin Endocrinol Metab 86: 3032-3038

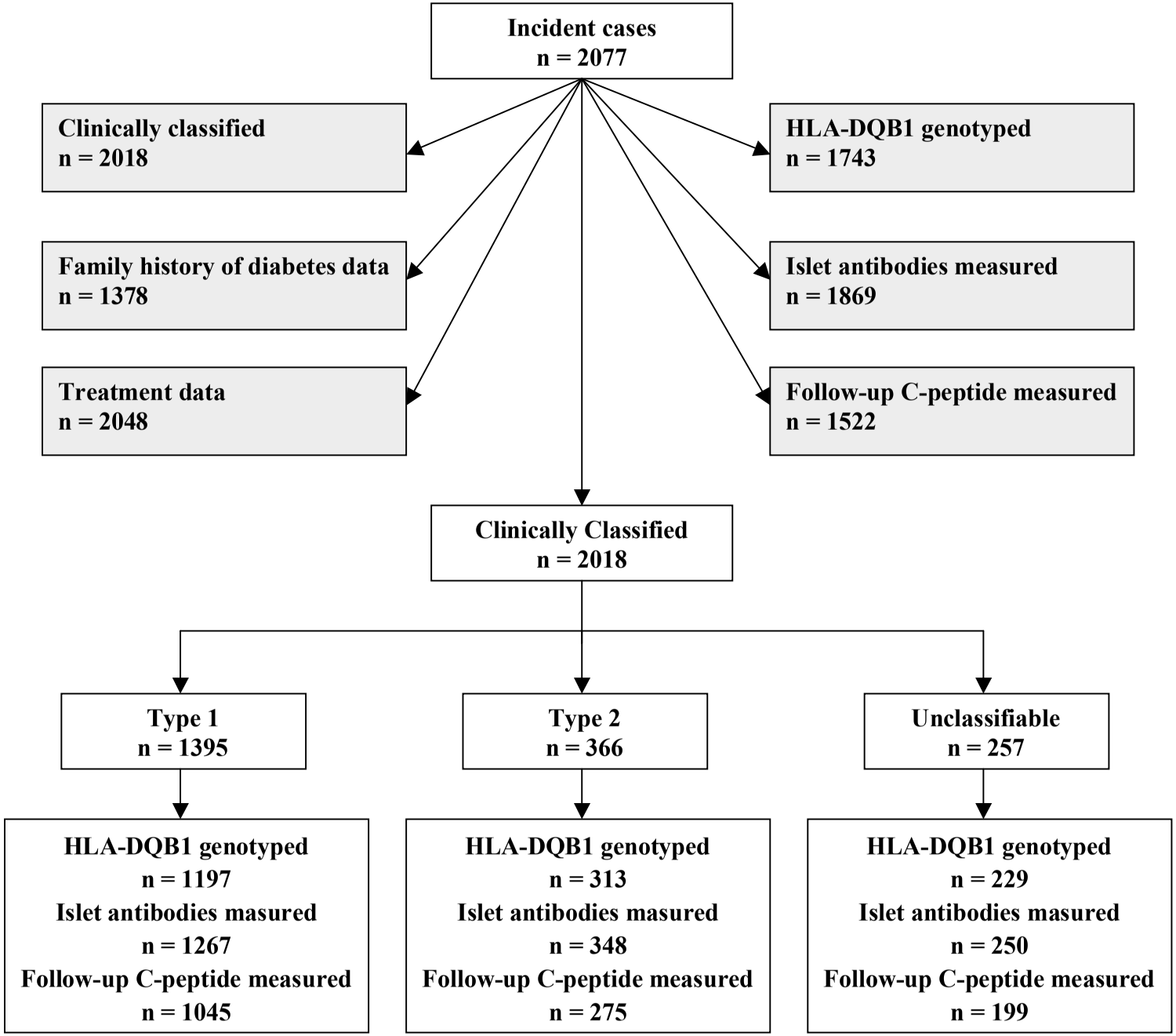
46. Schölin A, Törn C, Nyström L, et al. (2004) Normal weight promotes remission and low number of islet antibodies prolong the duration of remission in type 1 diabetes. Diabet Med 21: 447-455

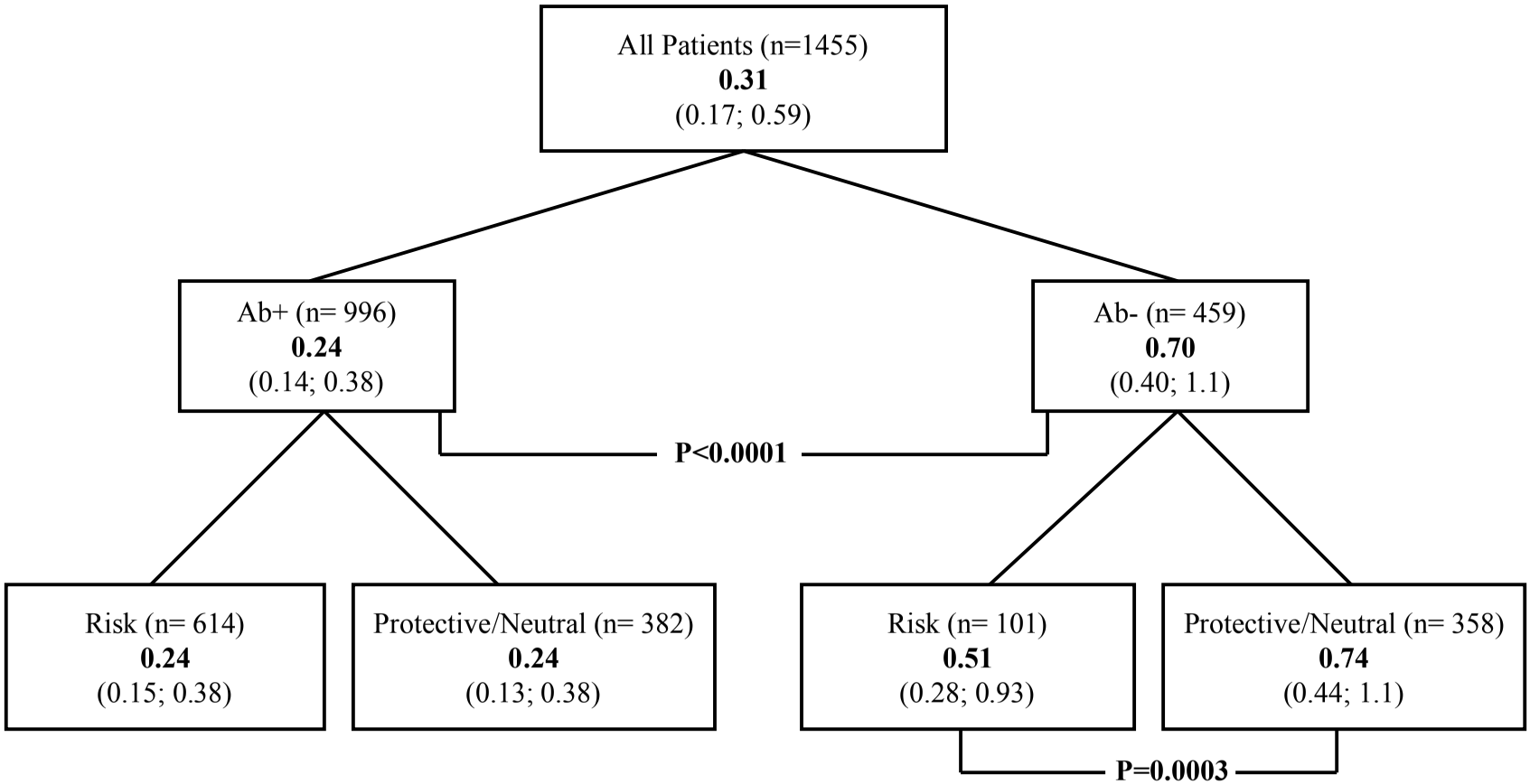
47. Schölin A, Björklund L, Borg H, et al. (2004) Islet antibodies and remaining ß-cell function eight years after diagnosis of autoimmune diabetes in young adults. A prospective follow-up of the nation-wide Diabetes Incidence Study in Sweden (DISS). J Intern Med 255: 384-391

Legends to figures

Fig. 1. Flow chart of DISS patients recruited during the 5-year study period (1998-2002). Upper part of figure shows 2077 incident cases of young adult (15-34 years of age) diabetic patients in the study with all available collected clinical and biological data. Lower part of the figure shows the 2018 clinically classified diabetic patients with their available data regarding HLA-DQB1 genotypes, islet antibodies and follow-up F-P-C-peptide.

Fig. 2. F-P-C-peptide (fasting plasma C-peptide) measured at follow-up in relation to islet antibodies and HLA-DQB1 genotypes. Altogether 1455 patients were tested whom had complete data regarding follow up F-P-C-peptide, HLA-DQB1 genotypes and islet antibodies. Figure shows median F-P-C-peptide concentrations (nmol/l), and 25th percentile (Q1), and 75th percentile (Q3). Ab+ indicates patients with and Ab- patients without islet antibodies. Median F-P-C-peptide concentration was significantly (p<0.0001) lower in Ab+ vs. Ab-, irrespective of risk HLA-DQB1 genotypes. However, Ab- with risk HLA-DQB1 genotypes had significantly (p=0003) lower median F-P-C-peptide than Ab- with protective/neutral genotypes.


Incident cases
n = 2077
Clinically classified
n = 2018
HLA-DQB1 genotyped
n = 1743
Family history of diabetes data
n = 1378
Islet antibodies measured
n = 1869
Treatment data
n = 2048
Follow-up C-peptide measured
n = 1522
Clinically Classified
n = 2018
Type 1
n = 1395
Type 2
n = 366
Unclassifiable
n = 257
HLA-DQB1 genotyped
n = 1197
Islet antibodies masured
n = 1267
Follow-up C-peptide measured
n = 1045
HLA-DQB1 genotyped
n = 313
Islet antibodies masured
n = 348
Follow-up C-peptide measured
n = 275
HLA-DQB1 genotyped
n = 229
Islet antibodies masured
n = 250
Follow-up C-peptide measured
n = 199


All Patients (n=1455)
0.31
(0.17; 0.59)
Ab+ (n= 996)
0.24
(0.14; 0.38)
Ab- (n= 459)
0.70
(0.40; 1.1)
P<0.0001
Risk (n= 614)
0.24
(0.15; 0.38)
Protective/Neutral (n= 382)
0.24
(0.13; 0.38)
Risk (n= 101)
0.51
(0.28; 0.93)
Protective/Neutral (n= 358)
0.74
(0.44; 1.1)
P=0.0003