Published in final edited form as:

Diabetes Metab Res Rev. 2017 November ; 33(8): . doi:10.1002/dmrr.2922.

Autoantibodies Against PFDN2 Are Associated With An Increased Risk of Type 2 Diabetes: A Case-Control Study

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Abstract

Background: The adaptive immune system is involved in type 2 diabetes mellitus (T2DM), indicating the presence of unidentified autoantibodies that might be useful biomarkers for emerging immunomodulatory therapy. A prior microarray study with a small number of participants suggested the association of novel autoantibodies with T2DM in Southwest American Indians (SAIs). We therefore sought to determine whether antibodies against 14 target proteins are associated with T2DM in a large case-control study.

Methods: Participants were adults (age 20–59 years) of SAI heritage. Plasma antibodies against 14 possible target proteins were measured in 476 cases with type 2 diabetes of less than 5-years duration and compared with 424 controls with normal glucose regulation.

Results: Higher levels of antibodies against prefoldin subunit 2 (PFDN2) were associated with type 2 diabetes ($p = 0.0001$; Bonferroni-corrected threshold for multiple tests = 0.0036 ($\alpha = 0.05$)). The association between anti-PFDN2 antibodies and type 2 diabetes remained in multivariable logistic regression (OR 1.27; 95% confidence interval 1.09–1.49; per one SD difference in anti-PFDN2 antibody). The odds of T2DM were increased in the highest anti-PFDN2 antibody quintile by 66% compared with the lowest quintile. Differences in anti-PFDN2 antibodies were most prominent among cases with earlier onset of disease (i.e. age 20–39 years) compared with controls.

Conclusions: Anti-PFDN2 antibodies are associated with T2DM and might be a useful biomarker. These findings indicate that autoimmunity may play a role in T2DM in SAIs, especially among adults presenting with young onset of disease.

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Author contributions

D.C.C. and P.P. contributed to the research design, data analysis, and writing of the manuscript. R.L.H., W.C.K., C.B., and J.K. contributed to research design, obtaining the clinical data, data analysis, and writing of the manuscript. D.C.C. had access to all the data and is the guarantor of this work.

Conflicts of Interest

The authors having nothing to disclose.

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Keywords

autoantibody; immunity; type 2 diabetes; epidemiology

Introduction

In contrast to type 1 diabetes (T1DM) which is well-recognized as an autoimmune disease resulting from immune-mediated pancreatic beta-cell destruction and associated with clinically useful autoantibodies [1,2], type 2 diabetes (T2DM) has been traditionally regarded as a metabolic disease with a defect in insulin action preceding or occurring concurrently with pancreatic beta-cell failure [3]. However, the immune system is increasingly recognized as a pathogenic component of T2DM and obesity, which is a strong risk factor for T2DM [4–6]. Diminished obesity-associated insulin action is characterized by chronic inflammation involving infiltration of macrophages and both T and B cells into adipose tissue [7]. A subgroup of human subjects with phenotypic T2DM have pancreatic islet-specific T-cell responses and most individuals in this subgroup lacked the presence of autoantibodies associated with T1DM [8]. In a mouse model, B cells appears to play an instrumental role in worsening insulin action via modulation of T cells and production of pathogenic IgG antibodies, indicating a role for adaptive immunity in the pathophysiology of T2DM [4]. Humans with obesity and T2DM have higher levels of antibody secretion and polyclonal B cell activity [9]. Elevated polyclonal B cell activity seen in T2DM and obesity may increase likelihood of developing autoantibodies by overwhelming immune checkpoints against autoimmunity, as has been proposed in the pathogenesis of lupus autoantibodies [10]. Autoantibodies have been detected in subgroups of subjects with T2DM who were at increased risk for hypertension or cardiovascular complications (G-protein coupled receptors [11]), who had maculopathy and macroalbuminuria (rho-kinases [12]), and Charcot neuroarthropathy (type 2 collagen [13]). In addition, IL-6 autoantibodies have been detected in sera from 2.5% of Danish subjects with T2DM [14].

There is evidence of a role for the innate and adaptive immune systems in the development of T2DM specifically in Southwest American Indians (SAIs), a group in which T2DM and obesity are highly prevalent, but with low prevalence of GAD65 antibody and other known islet cell antibodies [15–17]. Markers of macrophage activation were associated with insulin action [18], and elevated leukocyte count predicted worsening insulin action and the development of T2DM [19]. Serum concentrations of gamma globulin, a nonspecific measure of the humoral immune system, were also positively associated with development of T2DM in SAIs [20]. T cell receptor complementarity determining region 3 length is shorter in SAI subjects with T2DM and associated with increased risk of diabetes [21]. Many autoimmune diseases show an association with certain HLA haplotypes, usually involving the major histocompatibility complex class II which encodes for genes that are important for immune response regulation [22]. A single-nucleotide polymorphism (SNP) that tags an HLA haplotype (*HLA-DRB1*16:02*) protective for T2DM and associated with increased insulin secretion was identified in this SAI population [15]. These findings from animal models and both SAI and non-SAI populations may be interpreted as supporting a potential role for autoimmunity and suggests the presence of unidentified autoantibodies. As

new therapies targeting the immune system emerge for treatment of T2DM [23], new biomarkers reflecting autoimmunity (e.g. autoantibodies) may be useful.

In this SAI population, we previously screened 9480 target proteins in a microarray in 18 individuals [24]. Based on specified statistical criteria or possible role in underlying biology of type diabetes, we then tested 70 of these proteins in a second confirmatory study in a moderately larger group (n=90) identifying 14 as promising biomarkers [24]. However, in both initial exploratory studies, to maximize the potential difference between groups, we selected individuals with T2DM and without the protective SNP that tags the *HLA-DRB1*16:02* haplotype for comparison with those with normal glucose regulation (NGR) and the presence of the protective haplotype.[24]. Thus, prior studies were limited by small sample sizes and by the fact that it could not be ascertained whether the identified autoantibodies were associated with T2DM, the protective SNP, or a combination. To further evaluate these autoantibodies, we tested the hypothesis that autoantibodies identified in our prior studies were associated with T2DM, independent of the protective SNP. We therefore conducted a frequency matched case-control study involving 476 cases with T2DM and 424 controls with NGR.

Materials and Methods

Subjects

Subjects participated in a longitudinal study of the etiology of T2DM among SAIs between 1965 and 2007, described in detail previously [25]. This study was approved by the Institutional Review Board of the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK). All subjects provided written informed consent before participation. Individuals in this population aged five years and over were invited for a research examination approximately every two years, which included a 75-gram OGTT. Plasma glucose concentrations for the OGTT were measured by enzymatic methods. For the present study, cases and controls were selected from those who were age 20 to 59 years at the time of examination, at least 6/8 SAI heritage, and had date of examination after January 1, 1990, to minimize storage effects. Cases of T2DM were determined by OGTT according to the 2003 American Diabetes Association criteria (i.e. fasting plasma glucose ≥ 126 mg/dl or 2h-plasma glucose ≥ 200 mg/dl) [26]. Cases were those with T2DM diagnosed on the date of exam or, if diagnosed before the exam, having a duration of T2DM less than 5 years. If a case had more than one exam within the 5-year period after the onset of T2DM, the earliest examination was chosen to minimize possible influence of diabetes duration. Controls had NGR (fasting plasma glucose < 100 mg/dl and 2h-plasma glucose < 140 mg/dl) on the date of the exam and were never known to be diagnosed with T2DM. Of the initial 12,647 subjects in the entire longitudinal study, 3715 subjects had an examination meeting the date, age, and SAI heritage criteria. Including only those with NGR (for controls) and limiting the T2DM duration (for cases), 739 case and 1343 control subjects were eligible. We sought to frequency match cases and controls by sex and age-decade strata (20–29 years, 30–39 years, 40–49 years, and 50–59 years) and chose a random sample from each stratum. About 125 samples were chosen from each age group, except for the highest age group (50–59 years) which had fewer cases and controls so all available cases and controls from this group were

chosen. For this study, we measured antibodies in 476 cases and 424 controls; this sample size achieved at least 90% power as described below.

Measurement of Antibodies

The reactivity of plasma samples to 14 target antigens (Table 2) was measured using the Luminex system, which is similar to ELISA and were assessed blinded to the case or control status. Eleven of the target antigens (peroxisome proliferator-activated receptor gamma transcript variant 2 (PPARG2), ubiquitin-conjugating enzyme E2M (UBE2M), transaldolase (TAL), hypothetical gene supported by BC001801 (LOC284912), TEA domain family member 4 transcript variant 3 (TEAD4), regulator of G-protein signaling 17 (RGS17), prefoldin subunit 2 (PFDN2), mitochondrial ribosomal protein S7 nuclear gene encoding mitochondrial protein (MRPS7), oxysterol binding protein-like 11 (OSBPL11), cystathionine-beta-synthase (CBS), mitogen-activated protein kinase kinase 3 transcript variant B (MAP2K3-B)) were selected since they were significantly associated with subjects with T2DM who lacked the protective HLA haplotype based on our prior study [24], and the remaining three target proteins (LXR-alpha, interleukin 1 beta (IL1B), and IAPP) were marginally associated in the pilot study but were known to be biologically relevant to obesity and T2DM based on the literature [27–29]. Test plasma was previously stored at -70°C . All 14 antigens were produced in an baculovirus-insect cell expression system [30,31] and captured on anti-glutathione S-transferase (anti-GST) conjugated MagPlex beads. Negative and positive controls were multiplexed and assayed together with captured antigens and all experimental samples. Negative controls consisted of bovine serum albumin (BSA) bound directly to beads and GST captured on anti-GST conjugated beads. Positive controls included anti-human IgG and human IgG. Beads were incubated with samples at a 1:200 dilution of plasma for one hour, followed by incubation with biotinylated anti-human IgG for one hour. After removal of excess biotinylated anti-human IgG, streptavidin conjugated to fluorescent protein R-Phycoerythrin (Streptavidin-RPE) was added and incubated for 30 minutes. After washing to remove unbound Streptavidin-RPE, the beads were analyzed with Luminex 200 analyzer to assess antibody signal, median fluorescence intensity (MFI), against the target antigens. Samples were assayed in triplicate for each analyte with CV ranging between 2.7% to 3.3%.

Additional Covariates and Fixed Factors

BMI (kg/m^2) was calculated by measured height and weight with the subject wearing light clothing and no shoes. SAI heritage was determined by self-report and classified into those with full heritage (8/8 SAI heritage) and those with mixed heritage (6/8 or 7/8 SAI heritage) and included as a covariate since SAI heritage was previously associated with greater risk of T2DM [32]. SNP genotyping data were obtained to identify those with the *HLA-DRB1*16:02* haplotype as previously described [15]. The A allele at rs9268852 which tags *HLA-DRB1*16:02* was previously associated with decreased risk of T2DM [15]; subjects were therefore classified as having the protective haplotype if they had a least one copy of the A allele at this genomic locus.

Statistical Analysis

All statistical analyses were performed using SAS software, version 9.3 (SAS Institute, Cary, NC, USA). Based on results from our prior study [24] and using a one-sided unpaired t-test using the lowest mean difference of the LOG_{10} values between cases and controls (0.079, liver X receptor alpha (LXR-alpha)) and the highest SD of the LOG_{10} values (0.42, islet amyloid polypeptide (IAPP)) and an alpha of 0.001 (0.05 divided by 14 target proteins and rounded down to 0.001), 90% power is achieved by a sample size of 756 with an allocation ratio of 1:1, and sample size of 849 with an allocation ratio of 1:2. A one-sided p-value was chosen to confirm the directionality of the relationships found in the prior two studies since we had deliberately chosen proteins with antibody levels more elevated among cases than controls.

Antibody MFI had a skewed distribution and LOG_{10} transformation was applied. To assess for association between antibody level and T2DM status independent of any differences in sample storage time or potential non-specific reactivity from the antibody assay, we adjusted MFI by storage time and background MFI (BSA bound directly to beads and GST captured on anti-GST conjugated beads) by linear regression analysis and then used the remaining unexplained variance (i.e. residuals) for subsequent analysis. For comparison of subject characteristics between cases and controls, two-sided p values were calculated using unpaired t, Wilcoxon rank-sum, and chi-squared tests where appropriate. An alpha level of 0.05 was chosen for these analyses. For comparison of antibodies between cases and controls, a one-tailed unpaired t-test was used since proteins with antibody levels that were more elevated among cases than controls were deliberately chosen based on our prior studies [24] and confirmation of this relationship was sought in the present study. To account for multiple testing, the Bonferonni-corrected threshold of $p < 0.0036$ was used for testing of the 14 antibodies.

Using unconditional logistic regression, we calculated ORs with 95% CIs for the associations between antibodies and T2DM. For these logistic regression models, we standardized continuous variables by using normalizing inverse Gaussian transformation of the ranks. The ORs were adjusted for age (continuous) and sex (model 1), age, sex, and BMI (continuous) (model 2), age, sex, BMI, and full SAI heritage (dichotomous) (model 3), and age, sex, BMI, full SAI heritage, and protective SNP that tags *HLA-DRB1*16:02* (dichotomous) (model 4).

To evaluate potential association between the protective SNP that tags *HLA-DRB1*16:02* and antibody response, linear models using PROC GLM were created. Antibodies which were significant in analysis without covariates were evaluated in multivariable linear models that adjusted for age, sex, BMI, SAI heritage, and T2DM status.

Results

Characteristics

Most cases had antibody assays from blood samples taken at the time of diagnosis of T2DM (82%) and the remaining cases (18%) had diabetes duration of less than 5 years (Table 1). By the frequency matched sampling, the proportion of cases who were men were

comparable in cases (50%) and controls (49%) ($p = 0.61$). There were fewer controls in the oldest age-decade (50 to 59 years) compared with cases due to availability of eligible controls (Table 1). Storage time of blood samples were comparable between cases (median 16 years, IQR 12 to 20 years) and controls (median 17 years, IQR 13 to 21 years) ($p = 0.13$). Consistent with *a priori* knowledge, BMI was greater among cases (median 38 kg/m², IQR 33 to 43 kg/m²) compared with controls (median 33 kg/m², IQR 28, 37 kg/m²) ($p < 0.001$) and the proportion of those with full SAI heritage was also greater among cases (80%) than controls (72%) ($p = 0.007$). Genetic data regarding the protective SNP that tags *HLA-DRB1*16:02* were available for most cases (90%) and controls (88%). Consistent with prior reports from this population [15], the proportion of controls with the SNP protective for T2DM (SNP rs9268852) was greater among controls (17%) than cases (11%) ($p = 0.004$). None of the antibodies were correlated with fasting or 2-hour glucose concentrations or BMI (all $p > 0.05$). None of the antibodies differed by sex except for OSBPL11 (men vs. women, mean of LOG₁₀ MFI values 0.020 vs. -0.019, $p = 0.007$). Since immunosenescence is associated with increased production of autoantibodies [33], we evaluated the association between age and autoantibodies and found that 12 of the 14 antibodies (PPARG2, UBE2M, LOC284912, TEAD4, PFDN2, MRPS7, OSBPL11, CBS, MAP2K3-B, LXR-alpha, IL1B, and IAPP) were weakly directly correlated with age (r ranging from 0.06 to 0.22, $p < 0.05$).

Antibodies and Type 2 Diabetes

Anti-PFDN2 antibody (mean of LOG₁₀ MFI values) was greater among cases than controls ($p = 0.0001$) which was significant at the multiple-testing corrected (Bonferroni) threshold of $p < 0.0036$ (Table 2). Anti-PPARG2 and anti-TEAD4 antibody MFI were nominally greater ($p = 0.008$ and $p = 0.041$, respectively) in cases than controls, but this was not significant at the multiple-testing corrected threshold.

Logistic regression models were created to control for risk factors identified *a priori*. In these models, continuous variables (age, BMI, antibodies) were rank standardized to a normal distribution and ORs were expressed per one SD change in these variables. Anti-PFDN2 antibody were associated with increased prevalence of T2DM in univariate analysis (OR 1.33, 95% CI 1.15–1.54, $p = 0.0001$) and remained associated with increased prevalence of T2DM in different multivariate logistic regression models (Table 3). In the full model adjusting for age, sex, SAI heritage, BMI, and the protective SNP that tags *HLA-DRB1*16:02*, anti-PFDN2 antibody (OR 1.27, 95% CI 1.09–1.49, $p = 0.003$) remained independently associated with increased prevalence for T2DM, (Table 3). In this full model, the SNP that tags *HLA-DRB1*16:02* (OR 0.61, 95% CI 0.40–0.93, $p = 0.02$) remained protective for T2DM, consistent with previous reports in this population [15]. The odds of T2DM were increased in the highest anti-PFDN2 antibody quintile by 66% (OR 1.66, CI 1.01–2.73, $p = 0.046$), compared with those in the lowest quintile also adjusting for age, sex, SAI heritage, BMI, and the protective SNP. There were no significant interactions between anti-PFDN2 antibody and age (continuous), age group (by decade), BMI (continuous), or sex. Results were similar in sensitivity analyses that evaluated these models in subgroups without the protective haplotype and restricting cases to those with T2DM diagnosed at the time of the exam. The statistical association between anti-PFDN2 antibody and T2DM appeared to be driven largely by differences in the younger age groups (Figure 1). In the age

groups 20–29 years and 30–39 years, those with T2DM had significantly greater anti-PFDN2 antibody level than controls ($p = 0.002$ and $p = 0.009$, respectively), even after adjusting for age group, T2DM status, sex, SAI heritage, BMI, and HLA haplotype.

Taking as the cutoff, two standard deviations above the overall mean of the controls, 10.5% of individuals age 20–29 years were classified as positive, compared with 3.2% in the same age group ($p = 0.03$). Using a similar classification for the age group 30–39 years, 6.5% of cases and 3.2% of controls were positive, though not significantly different. In the older age groups (40–49 and 50–59 years), the proportions of those who were positive were between 6.0% to 8.0% and proportions were not significantly different between cases and controls.

Antibody and HLA haplotype

We evaluated whether the presence of the SNP protective for T2DM that tags *HLA-DRB1*16:02* was associated with antibody levels using linear models. In all subjects including those with and without T2DM, the presence of the protective SNP was associated with a 39% lower anti-PPARG2 antibody level compared to those without the protective haplotype ($b = -0.21$; $p = 0.027$). Other antibodies were not associated with the SNP. The relationship between the protective SNP ($b = -0.22$; $p = 0.023$) and lower anti-PPARG2 antibody remained after adjusting for age, sex, SAI heritage, BMI, and T2DM. However, PPARG2 was not associated with increased prevalence of T2DM in an unadjusted logistic regression model (OR 1.12, CI 0.98 to 1.29, $p = 0.11$); similar results were observed with other models adjusting for other factors such as age, sex, SAI heritage, BMI, and the protective SNP (data not shown)

Discussion

We hypothesized that there were unidentified circulating autoantibodies in T2DM. PFDN2 and other protein targets were previously identified via a microarray experiment that was confirmed in a second sample via a microsphere-based experiment [24]. However, these experiments had a small sample and could not differentiate an association with T2DM from an association with the protective SNP that tags *HLA-DRB1*16:02* [15]. The current study involving a large sample in a well-defined population with detailed information on important risk factors, demonstrated that T2DM is associated with elevated anti-PFDN2 antibody levels. This finding was independent of the SNP which tags *HLA-DRB1*16:02* previously T2DM [15].

The differences in anti-PFDN2 antibody levels among cases and controls were most prominent in adults with earlier onset (20–29 years and 30–39 years) for T2DM, indicating that the adaptive immune response may be playing a more important role in those with early onset T2DM in this population of SAIs. This observation in T2DM mirrors observations in diseases undisputed as autoimmune (e.g. anti-cyclic citrullinated peptide antibodies in rheumatoid arthritis and anti-muscarinic 3 receptor antibodies in Sjögren's syndrome) [34] in which autoantibodies differ by the age of disease onset.

To our knowledge, PFDN2 has not been previously associated with T2DM. PFDN2 is one of the subunits of prefoldin, a molecular chaperone which facilitates proper protein folding

through binding to newly unfolded proteins or through preventing protein aggregation [35]. The mechanism by which these autoantibodies form is unknown. Depending on the immune model, these autoantibodies may reflect failure of self-tolerance or the continued presence of danger signals from chronic inflammation and tissue damage (e.g. from chronic adipose tissue inflammation or from pancreatic beta-cell injury from lipo- or glucotoxicity) causing an autoimmune activation in T2DM [36]. PFDN2 is located intracellularly which raises the question how this protein becomes exposed to the immune system. There is increasing evidence from other clearly autoimmune diseases (e.g. lupus) that outflow of contents from apoptotic cells associated with pro-inflammatory signals or absent anti-inflammatory ones contributes to presentation of normally silent intracellular autoantigens [37]. Evidence from rodents and humans indicates adipocyte apoptosis as a key event leading to infiltration of macrophages into adipose tissue and insulin resistance [38]. It is therefore possible that anti-PFDN2 antibodies are a consequence of adipocyte apoptosis. Mechanism aside, since a variety of immunotherapies for T2DM have been explored or are under investigation, additional biomarkers, particularly ones reflecting autoimmune activation, might be useful to guide decisions about who may benefit from immunotherapy and for monitoring treatment response [23].

Using two standard deviations above the mean of the controls as a cutoff, the proportion of those who were positive for anti-PFDN2 antibody was fairly low (e.g. 10.5% in the youngest age group with T2DM), though, possibly clinically useful nevertheless. If a goal of precision medicine is to match patients with the most appropriate treatment, then even low prevalence biomarkers may be useful in identifying those who might potentially benefit from targeted prevention efforts or treatment using either currently available diabetes medications or emerging immunotherapies. Like GAD65 antibody in T1DM which is a continuous variable and whose prevalence varies with the cutoff selected [39], the prevalence of anti-PFDN2 antibody may also be higher (or lower) depending on the choice of cutoff.

Since other autoantibodies (e.g. IA-2 in T1DM) are known to decline rapidly with increasing duration of disease [40], we restricted cases to subjects with known T2DM duration of less than 5 years with most of our cases having antibodies measured at the time of diagnosis. However, it is also possible that presence of these antibodies, similar to GAD65 antibodies in T1DM, persist despite longer disease duration [40]. It is also possible that these autoantibodies may increase with diabetes duration as more epitopes are exposed by worsening adipose tissue inflammation and declining beta cell function.

Lower anti-PPARG2 antibody was also found to be associated with the SNP protective for T2DM that tags *HLA-DRB1*16:02* indicating that the HLA haplotype may be influencing propensity to produce this antibody. PPARG2 is highly expressed in adipose tissue [41] and it is possible the antibodies may develop from apoptotic adipocytes. However, anti-PPARG2 antibody was not significantly associated with T2DM and the importance of anti-PPARG2 antibody is unclear.

Several limitations should be acknowledged. First this study was performed in a population with a high risk of T2DM. Although the pathophysiology of the development of diabetes in SAIs mirrors those of other populations, the autoantibody associations described may be

unique to this population. We also did not measure T1DM associated antibodies, although levels of these antibodies were not different between cases and controls in our prior study of these protein targets [24] and that these antibodies are uncommon in the SAI population [15–17]. Furthermore, the clinical phenotype in the SAI population is most consistent with type 2 diabetes. Whereas individuals with T1DM or latent autoimmune diabetes in adults are often lean, affected individuals in this SAI population have greatly increased adiposity and reduced insulin action, and typically go for years without an absolute need for insulin treatment [42].

Due to the cross-sectional design of this study, it is not known if antibodies developed before or after the onset of T2DM. In addition, it is also unclear if anti-PFDN2 antibodies are elevated alongside inflammatory biomarkers such as C-reactive protein which are also associated with increased risk of T2DM [43], since such markers were not available in the current study.

It is also unknown whether the same results would have been obtained using other methods such as a radioligand-binding assay, western blot, and ELISA. This study involved a microsphere-based assay that has the advantages of using small volumes to target multiple autoantibodies simultaneously in the same sample. Compared with western blot which cannot detect conformational epitopes since the target protein is denatured and ELISA which may introduce distortions of the 3-dimensional protein structure following protein binding to the plastic well, this bead-based approach is thought to have less distortion effects on target proteins [44] and has been used successfully to detect autoantibodies in autoimmune diseases such as celiac disease and anti-phospholipid disease [45].

In summary, anti-PFDN2 antibodies were increased in subjects with recently diagnosed T2DM, a finding that further supports the role of autoimmunity in T2DM. This association was driven by higher antibodies in those with younger onset T2DM indicating a greater role for adaptive immunity in earlier onset disease. In order for investigational therapies for T2DM targeting the immune system to reach the bedside, new autoimmunity biomarkers, such as anti-PFDN2 antibody, may be useful as indicators of disease.

Acknowledgments

The research was supported by the Division of Intramural Research of NIDDK, NIH. We thank the participants and the staff of the Phoenix Epidemiology and Clinical Research Branch, NIDDK, NIH.

Abbreviations:

BMI	body mass index
BSA	bovine serum albumin
GST	glutathione S-transferase
HLA	human leukocyte antigen
MFI	median fluorescence intensity

NGR	normal glucose regulation
OGTT	oral glucose tolerance test
SAI	Southwest American Indian
SNP	single-nucleotide polymorphism
T1DM	type 1 diabetes mellitus
T2DM	type 2 diabetes mellitus

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Figure 1. Anti-PFDN2 antibodies. Adjusted for age group, sex, Southwest American Indian heritage, BMI, SNP rs9268852, and type 2 diabetes. NGR, normal glucose regulation.

Table 1.

Subject characteristics

	Type 2 diabetes (cases)	NGR (controls)	p-value
No. of individuals	476	424	
Diabetes duration, n (%)			
At time of diagnosis	391 (82)	-	
0 to 2 years	53 (11)	-	
2 to 5 years	32 (7)	-	
Fasting glucose (mg/dl), median (IQR)	139 (115, 200)	90 (85, 94)	< 0.001
2h-glucose (mg/dl), median (IQR)	263 (225, 355)	104 (88, 119)	< 0.001
Male sex, n (%)	213 (50)	231 (49)	0.61
Age, years, median (IQR)	39 (30, 49)	37 (28, 45)	< 0.001
Age group, both sexes, n (%)			< 0.001
20–29	124 (26)	125 (29)	
30–39	124 (26)	124 (29)	
40–49	123 (26)	129 (29)	
50–59	105 (22)	50 (12)	
Age group, male only, n (%)			0.13
20–29	63 (27)	63 (30)	
30–39	62 (27)	63 (30)	
40–49	61 (26)	63 (30)	
50–59	45 (19)	24 (11)	
Age group, female only, n (%)			0.01
20–29	61 (25)	62 (29)	
30–39	62 (25)	61 (29)	
40–49	62 (25)	62 (29)	
50–59	60 (24)	26 (12)	
Full SAI heritage, n (%)	380 (80)	306 (72)	0.007
Storage time (year), median (IQR)	16 (12, 20)	17 (13, 21)	0.13
BMI (kg/m ²), median (IQR) ^a	38 (33, 43)	33 (28, 37)	< 0.001
SNP rs9268852, n (%)			0.004
Protective haplotype ^b	53 (11)	74 (17)	
Non-protective haplotype	377 (79)	301 (71)	
Missing	46 (10)	49 (12)	

Abbreviations: BMI, body mass index; IQR, interquartile range; NGR, normal glucose regulation; No., number; SAI, Southwest American Indian; SNP, single-nucleotide polymorphism

^a BMI data missing for 8 cases and 4 controls

^b Homozygous or heterozygous for protective allele

Table 2.

Autoantibodies against 14 target proteins among cases and controls.

Antibody target protein	Database ID	Cases, mean ^a	Controls, mean ^a	Case to control ratio ^b	p value ^c
PPARG2	NM_015869.2	0.0174	-0.0196	1.09	0.008
UBE2M	BC058924.1	0.0039	-0.0044	1.02	0.29
TAL	BC018847.1	-0.00003	0.00003	1.00	0.50
LOC284912	BC001801.1	0.0065	-0.0073	1.03	0.18
TEAD4	NM_201443.1	0.0108	-0.0121	1.05	0.041
RGS17	NM_012419.3	0.0083	-0.0093	1.04	0.18
PFDN2	NM_012394.2	0.0358	-0.0402	1.19	0.0001 ^d
MRPS7	NM_015971.2	0.0036	-0.0040	1.02	0.27
OSBPL11	NM_022776.3	0.0009	-0.0010	1.00	0.45
CBS	NM_000071.1	0.0058	-0.0065	1.03	0.14
MAP2K3-B	NM_145109.1	0.0056	-0.0063	1.03	0.20
LXR-alpha	BC041172.1	0.0098	-0.0110	1.05	0.064
IL1B	NM_000576.1	-0.0857	0.0096	0.96	0.84
IAPP	NM_000415.1	0.0075	-0.0085	1.04	0.15

^aMean of log10 values of residuals of median fluorescence intensity after adjustment for storage time and signal background^bRatio of unlogged means^cUnpaired t-test, one-tailed^dSignificant after correcting for multiple testing at Bonferonni corrected ($\alpha = 0.05$) threshold 0.0036

Table 3.

Logistic regression models for type 2 diabetes in relation to anti-PFDN2 antibodies

Model adjustments	OR	(95% CI)	p value
Unadjusted model			
Anti-PFDN2 antibody	1.33	(1.15, 1.54)	0.0001
Adjusted model 1:			
Anti-PFDN2 antibody	1.30	(1.12, 1.50)	0.0006
Adjusted model 2:			
Anti-PFDN2 antibody	1.30	(1.12, 1.50)	0.002
SAI heritage	1.33	(0.95, 1.87)	0.10
Adjusted model 3:			
Anti-PFDN2 antibody	1.27	(1.09, 1.49)	0.003
SAI heritage	1.11	(0.77, 1.60)	0.58
BMI	2.31	(1.93, 2.76)	<0.0001
Adjusted model 4:			
Anti-PFDN2 antibody	1.27	(1.09, 1.49)	0.003
SAI heritage	1.08	(0.75, 1.57)	0.68
BMI	2.31	(1.93, 2.76)	<0.0001
Protective SNP haplotype (rs9268852)	0.61	(0.40, 0.93)	0.02

Abbreviations: BMI, body mass index; SAI, Southwest American Indian; SNP, single-nucleotide polymorphism

Models 1–4 are also adjusted for age and sex

Anti-PFDN2 antibody and BMI were standardized to a normal distribution with SD = 1; ORs are given for a one SD difference in antibody level

n = 796 with complete data for all variables