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(54) **METHODS FOR DIAGNOSIS OF TYPE 1 DIABETES**

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(58) **Field of Classification Search**

None

See application file for complete search history.

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(57) **ABSTRACT**

Type 1 diabetes (T1D) patients make antibodies to self-proteins that are potential biomarkers for early detection and risk prediction. We have identified seventeen antigens as biomarkers for early diagnosis and risk prediction of T1D, including the antigens MLH1, MTIF3, PPIL2, NUP50, TOX4, FIGN, C9orf142, ZNF280D, HES1, QRFPR, CTRC, SNX6, SYTL4, ELA2A, IGRP, PAX6, and HMGN3.

6 Claims, 1 Drawing Sheet

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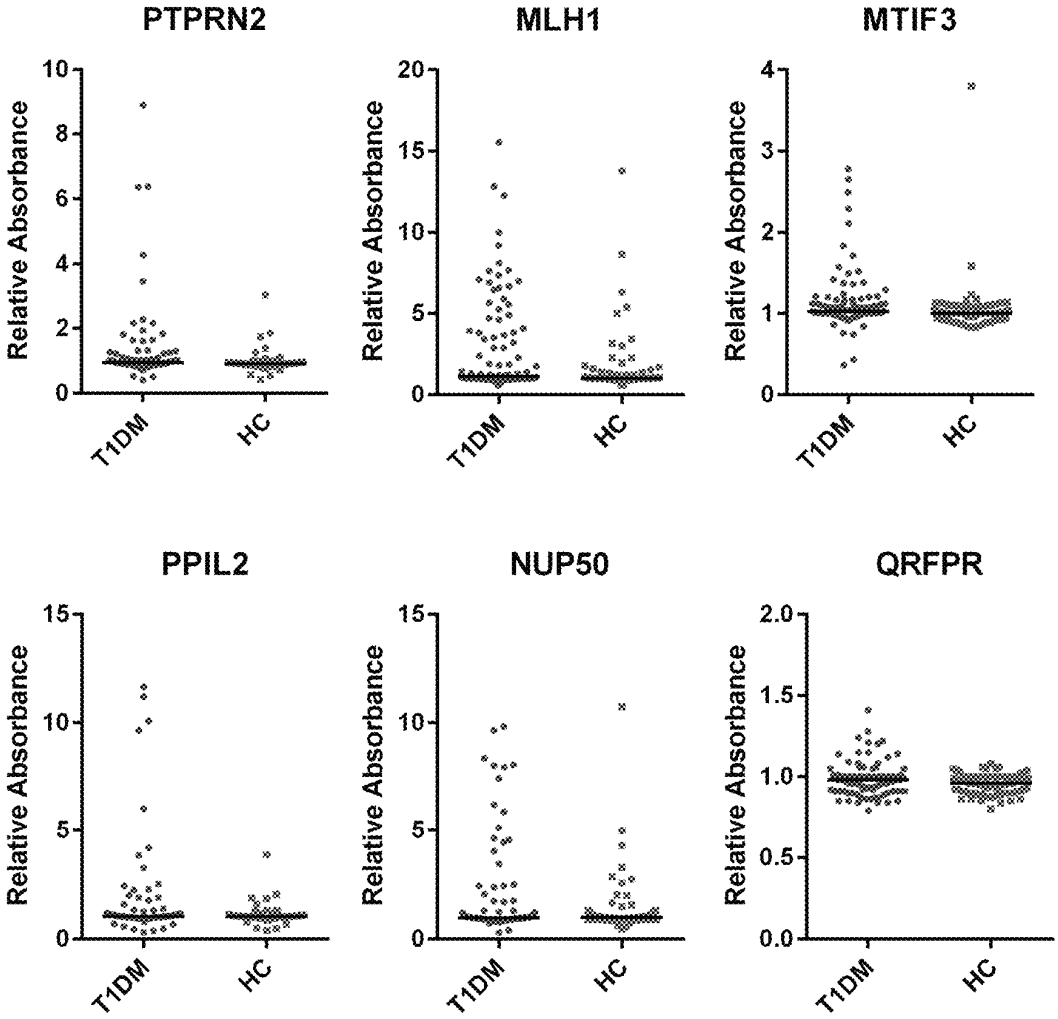
PTPRN2
MLH1
MTIF3
PPIL2
NUP50
QRFPR
Relative Absorbance
T1DM
HC

METHODS FOR DIAGNOSIS OF TYPE 1 DIABETES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 14/990,504, filed Jan. 7, 2016, which claims priority to U.S. Provisional Patent Application No. 62/100,775, filed Jan. 7, 2015, each of which is incorporated herein by reference as if set forth in its entirety.

FIELD OF THE INVENTION

This disclosure relates to biomarkers for the prediction of type 1 diabetes (T1D) onset and for diagnosing T1D.

BACKGROUND OF THE INVENTION

T1D is one of the most common juvenile autoimmune diseases. It is characterized by progressive autoimmune destruction of pancreatic beta cells. The incidence of T1D is increasing worldwide. T1D patients are dependent on life-long exogenous insulin but this is not a cure and in the long term there are serious co-morbidities. This leads to both personal and societal burdens in terms of financial and quality of life indicators. At the time of T1D diagnosis, it is thought that potentially 70%-90% of pancreatic beta cells have been destroyed. Therefore early diagnostic and prognostic markers of T1D prior to symptomatic disease onset will be of great value in identifying individuals that could benefit from intervention protocols while significant beta cell function still exists.

Prevention of T1D will only be possible if individuals with high risk for progression to T1D can be identified. The incidence of T1D in general population is around 22/100,000 in the US. The majority of T1D cases are diagnosed in non-relatives with 85% of new T1D cases occurring in individuals with no known family history. Thus, biomarkers are needed to improve our prediction models and enable the selection of subjects with, for example, high 5-year risk of disease onset. Such markers could be deployed immediately to identify high-risk subjects for intervention trials. Additionally in differentiating type 1 diabetes from other forms of diabetes mellitus autoantibodies are helpful and yet there are still some individuals with T1D that are negative for the current known autoantibodies, thus the discovery of additional autoantibodies aids in the differential diagnosis of T1D.

SUMMARY OF THE INVENTION

Identifying markers that present prior to the development of our currently used autoantibodies (AAbs) could improve the risk prediction models. Thus, the embodiments disclosed herein relate to the identification of AAb biomarkers in T1D so as to increase the sensitivity of detection in T1D patients and improve the T1D risk prediction model.

All references disclosed throughout are hereby incorporated herein in their entirety.

In one embodiment, we used a novel protein microarray technology termed "Nucleic Acid Programmable Protein Array" (NAPPA) (see, e.g., EP 1360490B1). This innovative protein microarray format avoids the need to express and purify the proteins by substituting the printing of full length cDNAs on the arrays. Proteins corresponding to the cDNAs are produced in situ as needed at the time of the assay by in vitro transcription and translation (IVTT)-coupled cell lysates. The cDNAs are configured to append a common epitope tag to all of the proteins on their C-termini so that they can be captured by a high-affinity capture reagent that is immobilized along with the cDNA.

In another embodiment, a method of diagnosing type 1 diabetes onset includes the step of contacting an antibody-containing fluid sample from a subject with one or more of antigens selected from the group consisting of MLH1, MTIF3, PPIL2, NUP50, TOX4, FIGN, C9orf142, ZNF280D, HES1, QRFPR, CTRC, SNX6, SYTL4, ELA2A, IGRP, PAX6, and HMGN3, wherein detection of antibodies with a suitable detection agent to one or more of these antigens indicates a diagnosis of type 1 diabetes onset in comparison to a healthy control sample.

In a further embodiment, a method of screening for a risk factor associated with type 1 diabetes onset includes the step of contacting an antibody-containing fluid sample from a subject with one or more antigens selected from the group consisting of MLH1, MTIF3, PPIL2, NUP50, TOX4, FIGN, C9orf142, ZNF280D, HES1, QRFPR, CTRC, SNX6, SYTL4, ELA2A, IGRP, PAX6, and HMGN3, wherein detection of antibodies with a suitable detection agent to one or more of these antigens indicates an elevated risk of type 1 diabetes onset in comparison to a healthy control sample.

These and other aspects of the embodiments disclosed herein will be apparent upon reference to the following disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. **1** depicts jitter plots of representative autoantigens from screening and knowledge based approaches (T1D stands for new-onset T1D patients; HC stands for healthy controls).

DETAILED DESCRIPTION OF THE INVENTION

The embodiments disclosed herein relate to 17 antigens that have been identified as biomarkers for early detection and risk prediction of T1D. These antigens are: MLH1, MTIF3, PPIL2, NUP50, TOX4, FIGN, C9orf142, ZNF280D, HES1, QRFPR, CTRC, SNX6, SYTL4, ELA2A, IGRP, PAX6, and HMGN3.

In general, two approaches were used to discover the disclosed antigens: a screen based approach and a knowledge based approach. To profile the serological antibody response, 40 T1D patients and 40 age/gender matched healthy controls were screened against 10,000 human proteins across 5 NAPPA array sets.

40 antigens were chosen for enzyme-linked immunosorbent assay (ELISA) verification on the same sample set. 19 antigens verified by ELISA were processed to the validation stage with 60 T1D patients and 60 healthy controls. In the knowledge based approach, 126 pancreas enriched genes were selected from literature mining and bioinformatics analysis and measured for their sero-reactivity among 46 T1D patients and 46 healthy controls. 15 antigens were chosen for validation in 50 T1D patients and 50 healthy controls.

Kits for assessing the presence of antigens for type 1 diabetes are also contemplated. An exemplary kit includes an antigen selected from MLH1, MTIF3, PPIL2, NUP50, TOX4, FIGN, C9orf142, ZNF280D, HES1, QRFPR, CTRC, SNX6, SYTL4, ELA2A, IGRP, PAX6, and HMGN3 to test

serological antibodies, as well as a suitable detection agent (e.g., a labeled secondary antibody).

EXAMPLE

Sera from T1D patients contain AAbs to human self-proteins. Thus, the sero-reactivity to 10,000 human proteins with sera from T1D patients and measured bound IgG. We scaled down the candidate number for validation in an independent sample set. In the knowledge based approach, we performed ELISA on 126 pancreas enriched genes and validate the candidates in an independent sample set. Taken together, 17 potential autoantigens were identified with sensitivities ranging from 10-27% at 95% specificity (Table 1).

Rapid antigenic protein in situ display (Rapid) ELISA was performed to confirm the sensitivities of autoantibodies biomakers. 96-well ELISA plates (Corning, ME) were coated with 10 ng/mL anti-glutathione S-transferase (GST) antibody (GE Healthcare, PA) in coating buffer (0.5 M carbonate bicarbonate buffer, pH 9.6) overnight at 4° C. On the next day, coated plates were washed 3 times with PBST and blocked with 5% milk-PBST (0.2% Tween) for 1.5 hrs at room temperature (RT).

Meanwhile, 40 ng/μL plasmids encoding candidate autoantigens were expressed in the human Hela cell-lysate based expression system at 30° C. for 1.5 hrs. After expression, candidate autoantigens were diluted in milk-PBST and captured in ELISA plates at 500 rpm for 1 h at RT. Plates were washed 5 times with PBST and incubated with diluted serum samples at 500 rpm for 1 h at RT. Then plates were washed again and incubated with HRP labeled anti-human secondary antibody (Jackson ImmunoResearch Laboratories, PA) for 1 h.

Finally, the plates were washed and incubated by 1-Step Ultra TMB—ELISA Substrate (Thermo scientific, IL) for detection and sulfuric acid to stop the reaction. OD450 was measured by Envision Multilabel Reader (Perkin Elmer, MA). Expression of candidate autoantigens was confirmed by mouse monoclonal anti-GST primary antibody and HRP labeled anti-mouse secondary antibody detection on the same plate. Relative absorbance was obtained by using the raw ELISA data dividing by the medium signal of each sample across all the antigens tested on the same day. The sensitivities for each antigen were determined at 95% specificity in comparison to a healthy control sample.

Prior work indicated that there are four known AAb biomarkers identified in T1D. The 5-year risk for T1D is 20-25% for subjects with one AAb, 50-60% for subjects with two AAbs, near 70% for subjects with three AAbs and 80% for those with four AAbs. Additional AAb biomarkers will help to improve the risk prediction in the general population. Thus, for example, the presence of autoantibodies to the antigen proteins as disclosed herein could be tested by immunoassays. The presence of one or more autoantibodies disclosed herein could be used as prediction of T1D onset.

The embodiments and example described above are not intended to be limiting.

TABLE 1

Discovery and Validation Statistics for 17 T1D biomarkers

	Discovery		Validation		All	
Antigen	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity	Specificity
MLH1	0.15	0.95	0.33	0.95	0.27	0.95
MTIF3	0.15	0.95	0.20	0.95	0.25	0.95
QRFPR	0.13	0.95	0.06	0.95	0.20	0.95
PPIL2	0.20	0.95	0.18	0.95	0.19	0.95
NUP50	0.15	0.95	0.17	0.95	0.16	0.95
CTRC	0.17	0.95	0.08	0.95	0.15	0.95
SNX6	0.13	0.95	0.04	0.95	0.15	0.95
TOX4	0.20	0.95	0.12	0.95	0.13	0.95
FIGN	0.13	0.95	0.12	0.95	0.13	0.95
SYTL4	0.20	0.95	0.02	0.95	0.13	0.95
ELA2A	0.11	0.95	0.04	0.95	0.13	0.95
C9orf142	0.18	0.95	0.05	0.95	0.11	0.95
ZNF280D	0.13	0.95	0.08	0.95	0.11	0.95
HES1	0.10	0.95	0.15	0.95	0.11	0.95
IGRP	0.17	0.95	0.02	0.95	0.11	0.95
PAX6	0.15	0.95	0.08	0.95	0.11	0.95
HMGN3	0.28	0.95	0.08	0.95	0.10	0.95

What is claimed is:

1. A method comprising:

contacting an antibody-containing fluid sample from a subject with proteins in a nucleic acid programmable protein array (NAPPA), wherein the NAPPA comprises proteins mutL homolog 1 (MLH1), mitochondrial translational initiation factor 3 (MTIF3), peptidylprolyl isomerase (cyclophilin)-like 2 (PPIL2), nucleoporin 50 (NUP50), detecting antibody binding to the NAPPA proteins with a suitable detection agent.

2. A method comprising:

contacting an antibody-containing fluid sample from a subject with proteins linked to a rapid antigenic protein in situ display enzyme linked immunosorbent assay (rapid ELISA) plate, wherein the proteins comprise mutL homolog 1 (MLH1), mitochondrial translational initiation factor 3 (MTIF3), peptidylprolyl isomerase (cyclophilin)-like 2 (PPIL2), and nucleoporin 50 (NUP50);

detecting antibody binding to the proteins with a suitable detection agent.

3. The method of claim **1**, wherein the proteins further comprise tox high mobility group box family member 4 (TOX4), fidgetin (FIGN), paralog of XCRCC4 and XLF (PAXX), zinc finger protein 280D (ZNF280D), Hes family BHLH transcription factor 1 (HES1), pyroglutamylated RF-

amide peptide receptor (QRFPR), chymotrypsin C (CTRC), sorting nexin 6 (SNX6), synaptotagmin like 4 (SYTL4), elastase 2A (ELA2A), glucose-6-phosphatase-related protein (IGRP), paired box 6 (PAX6), and high mobility group nucleosomal binding domain 3 (HMGN3).

4. The method of claim **1**, wherein the detection agent comprises a label.

5. The method of claim **2**, wherein the proteins further comprise tox high mobility group box family member 4 (TOX4), fidgetin (FIGN), paralog of XCRCC4 and XLF (C9orf142), zinc finger protein 280D (ZNF280D), Hes family BHLH transcription factor 1 (HES1), pyroglutamylated RF-amide peptide receptor (QRFPR), chymotrypsin C (CTRC), sorting nexin 6 (SNX6), synaptotagmin like 4 (SYTL4), elastase 2A (ELA2A), glucose-6-phosphatase-related protein (IGRP), paired box 6 (PAX6), and high mobility group nucleosomal binding domain 3 (HMGN3).

6. The method of claim **2**, wherein the detection agent comprises a label.

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