

**USE OF CARDIAC AUTOANTIBODIES AS A METHOD TO IDENTIFY INDIVIDUALS
AT INCREASED RISK OF DEVELOPING CARDIOVASCULAR EVENTS**

CROSS-REFERENCE TO RELATED APPLICATIONS

[001] This application claims priority to U.S. Provisional Application No. 63/552,279, filed February 12, 2024, the disclosure of which is hereby incorporated by reference in its entirety.

ELECTRONIC SEQUENCE LISTING

[002] This application contains a Sequence Listing in computer readable form entitled “01123-0017-00PCT”, created February 4, 2025, having a size of 7,768 bytes, which is incorporated by reference herein.

DESCRIPTION

[003] This invention was made with government support under Research Grant No. R01 DK125677 awarded by NIH/National Institute of Diabetes and Digestive and Kidney Diseases Diabetes. The government has certain rights in the invention. This invention was also made with support from Breakthrough T1D (previously JDRF), award number 2-SRA-2023-1426-M-B-Lipes.

FIELD

[004] This disclosure relates to the measurement of autoantibodies (AAb) to heart muscle proteins (“cardiac autoantibodies”, cardiac “AAb”, or “cAAb”), including AAb to myosin heavy chain 6 (MYH6), myosin heavy chain 7 (MYH7), S1-fragment of MYH6 (S1-MYH6), S2-fragment of MYH6 (S2-MYH6), and cardiac troponin I (cTnI or TnI), for assessing risk of developing future cardiovascular events in individuals with type 1 diabetes (T1D), and subjects treated with immune checkpoint inhibitors (ICI). The disclosure also describes that the presence of AAb to MYH6 not only predicts a high risk of developing cardiovascular events, but identifies a subset of individuals who develop cardiovascular events of autoimmune etiology, who may particularly benefit from immune-targeted therapies to prevent (“primary prevention”) or treat (“secondary prevention”) cardiovascular disease.

BACKGROUND

[005] Despite major advancements in diabetes management over past decades, individuals with type 1 diabetes (T1D) currently have a ~9-fold higher risk of premature cardiovascular disease (CVD) events, a 6-fold increased risk of death secondary to CVD and an 8-fold

increased risk of hospitalization for heart failure, compared to the general population. Remarkably, in a recent analysis of 10 million individuals followed in the UK National Health Service, T1D was more strongly linked to poor CVD outcomes than any other condition, including type 2 diabetes (T2D). However, less than half of the excess CVD risk in T1D is explained by known risk cardiovascular factors, and the underlying mechanisms are unknown.

[006] Inflammation plays a central role in the pathogenesis of atherosclerotic CVD in the general population. Targeting the inflammasome to IL-1B to IL-6 pathway of innate inflammation has been a major focus of recent successful clinical trials, in which a substantial proportion of participants had T2D. Innate inflammation is central to the pathophysiology of obesity, T2D, and their cardiovascular complications.^{8,9} Given the high prevalence of overweight and obesity in adults with T1D¹⁰ it has been assumed that inflammation in T1D is similar to that in T2D.¹¹ However, while shared inflammatory pathways contribute to the development of end-stage renal disease in T1D and T2D,¹² the importance of inflammation in the pathogenesis of CVD in T1D has been unclear.¹³

[007] T1D is an autoimmune disease postulated to arise when T cells of the adaptive immune system mistakenly attack and destroy the body's insulin-producing islet β -cells. The disease develops in genetically susceptible individuals, primarily from human leucocyte antigen (HLA) class II variants¹⁴. Although T1D is a T cell-mediated disorder,¹⁵ the presence of multiple (i.e., ≥ 2) islet AAbs is the strongest single predictor of future T1D. Following the unexpected discovery that the expression of the high T1D-risk HLA-DQ8 in humanized transgenic mice resulted in early mortality from chronic autoimmune myocarditis (inflammation of the heart muscle) in mice^{53,18}, it was shown that experimental myocardial infarction induces the development of T cell and autoantibody (AAb) responses against the heart-specific protein, α -myosin (encoded by *MYH6*), a key autoantigen¹⁸ with poor infarct healing¹⁶. Studying biosamples from recent-onset T1D participants during the landmark Diabetes Control and Complication Trial (DCCT),¹⁹ chronic hyperglycemia – that also causes myocardial injury and is the most powerful modifiable risk factor for CVD in T1D²⁰ – was shown to induce the expression of cardiac autoantibodies (AAbs). While these studies showed an association of cardiac AAbs with later CVD risk, CRP is a non-specific marker of inflammation, and the nature of the potential inflammatory state was unclear. Furthermore, previous studies involved a small subset of the DCCT cohort having very high or very low HbA1c levels with only 6 subsequent CVD events, and the studies were unable to evaluate whether cardiac AAbs are independent risk factors for CVD events.²¹

[008] Here, examining samples from ~900 post-DCCT participants in the Epidemiology of Diabetes Interventions and Complications (EDIC) follow-up study, MYH6 AAbs were identified in a subgroup of individuals who develop accelerated CVD complications of primary autoimmune etiology. This disease process is characterized by HLA-linked genetic susceptibility, markedly elevated levels of circulating cytokines – similar in magnitude to a severe acute infection or a systemic autoimmune disorder – and increased risk of accelerated CVD events driven primarily by IL-6 and GM-CSF, instead of traditional cardiovascular risk factors. These findings provide a rationale for using cardiac AAb screening to identify individuals with T1D at highest risk for future CVD events and as a tool to aid in the selection of agents targeting systemic inflammation, or the underlying autoimmune process itself, to prevent and treat cardiovascular disease, which is the leading cause of premature morbidity and mortality in T1D.

SUMMARY

[009] Disclosed herein are methods for identifying a patient at increased risk for developing cardiovascular disease events (CVD events) by analyzing blood (serum or plasma) for the presence of cardiac autoantibodies (AAbs).

[0010] This disclosure describes a method to identify individuals with T1D at greatly increased risk for the development of cardiovascular disease (CVD) events, such as acute myocardial infarction, entailing the detection of AAbs specific to full-length MYH6 alone or in combination with the presence of AAbs specific to the S1-fragment of MYH6; the S2-fragment of MYH6; full-length MYH7; and full-length cTnI. The presence of MYH6 AAbs is strongly associated with the HLA-DQ2 genotype and with extremely elevated levels of circulating proinflammatory cytokines. Further increased CVD risk was seen in subjects having AAbs specific to full-length MYH6 together with elevated levels of interleukin-6 (IL-6) and granulocyte-macrophage colony stimulating factor (GM-CSF). Positivity for such inflammatory cytokines would thus also identify high-risk individuals who may benefit the most from drugs/biologics that target the linked inflammatory mediators, and/or the use of treatments to block the autoimmune process itself. The disclosure also describes a new approach to CVD risk stratification combining the new diagnostic tool, i.e., screening for cardiac AAbs, particularly MYH6 AAbs, combined with HLA DQ typing and cytokine profiling, particularly measuring IL-6 and GM-GSF levels. Similar approaches could also be used to identify T1D patients with pre-existing CVD events who are at higher risk for recurrent CVD events/CVD death who could benefit from targeted anti-inflammatory/immunomodulatory therapies in “secondary” CVD prevention trials.

[0011] In one embodiment, the AAbs are specific for MYH6 (encoding α -myosin). MYH6 AAb positivity was discovered by the inventors to be strongly linked to the HLA-DQ2 genotype. In another embodiment, the presence of MYH6 AAbs was also associated with markedly elevated levels of numerous proinflammatory cytokines, particularly interleukin-6 (IL-6) (22.7 pg/mL versus 3.2 pg/mL in MYH6 autoantibody-negative subjects; $P<0.001$) and GM-CSF (59.2 pg/mL versus 13.8 pg/mL in MYH6 autoantibody-negative subjects; $P<0.001$). In another embodiment, the effect of MYH6 AAbs on increased CVD risk, was primarily mediated by IL-6, rather than traditional cardiovascular risk factors. These findings are the first to indicate that CVD may be of autoimmune etiology in T1D, and raise the potential of using immunological approaches to predict, prevent and treat this major cause of premature mortality. Provided herein, *inter alia*, are the following embodiments:

[0012] Embodiment 1: A method for identifying a subject with type 1 diabetes (T1D) at increased risk of developing a first cardiovascular disease event, wherein the method comprises:

- a. isolating blood, optionally a serum or plasma sample, from the subject;
- b. identifying the subject as having cardiac autoantibodies (AAbs) specific to full-length myosin heavy chain 6 (MYH6, SEQ ID NO: 1) in the blood; and
- c. predicting that the subject is at increased risk for developing a first cardiovascular disease event compared to a subject with T1D not having cardiac AAbs specific to full-length MYH6.

[0013] Embodiment 2: A method for identifying a subject with T1D at increased risk of developing a first cardiovascular disease event, wherein the method comprises:

- a. isolating blood, optionally a serum or plasma sample, from the subject;
- b. identifying the subject as having two or more cardiac AAbs, wherein the two or more cardiac AAbs are selected from those specific to:
 - i. full-length MYH6;
 - ii. the S1-fragment of MYH6;
 - iii. the S2-fragment of MYH6;
 - iv. full-length myosin heavy chain 7 (MYH7, SEQ ID NO: 2); and
 - v. full-length cardiac troponin I (cTnI, SEQ ID NO: 3); and
- c. predicting that the subject is at increased risk for developing a first cardiovascular disease event compared to a subject with T1D not having said cardiac AAbs.

[0014] Embodiment 3: The method of embodiment 1 or embodiment 2, wherein the first cardiovascular disease event is any one of:

- a. nonfatal myocardial infarction (MI);
- b. subclinical MI ("silent MI") detected on an electrocardiogram;
- c. angina confirmed by ischemic changes with exercise testing or by clinically significant obstruction on coronary angiography, or wherein the angina occurs at rest or does not respond to nitroglycerin treatment;
- d. coronary revascularization with angioplasty or coronary artery bypass;
- e. ischemic stroke;
- f. heart failure; or
- g. death secondary to cardiovascular disease.

[0015] Embodiment 4: A method for identifying a subject with T1D and a preexisting cardiovascular disease event at increased risk of having a recurrent cardiovascular disease event comprising:

- a. isolating blood, optionally a serum or plasma sample, from the subject;
- b. identifying the subject as having cardiac AAbs specific to full-length MYH6 in the blood; and
- c. predicting that the subject is at increased risk for developing a recurrent cardiovascular disease event compared to a subject with T1D not having AAbs specific to full-length MYH6.

[0016] Embodiment 5: A method for identifying a subject with T1D and a preexisting cardiovascular event at increased risk of having a recurrent cardiovascular event, wherein the method comprises:

- a. isolating blood, optionally a serum or plasma sample, from the subject;
- b. identifying the subject as having two or more cardiac AAbs, wherein the two or more cardiac AAbs are selected from those specific to:
 - i. full-length MYH6;
 - ii. the S1-fragment of MYH6;
 - iii. the S2-fragment of MYH6;
 - iv. full-length myosin heavy chain 7 (MYH7, SEQ ID NO: 2); or
 - v. full-length cardiac troponin I (cTnI, SEQ ID NO: 3)
- c. predicting that the subject is at increased risk for developing a recurrent cardiovascular disease event compared to a subject with T1D not having said cardiac AAbs.

[0017] Embodiment 6: The method of embodiment 4 or embodiment 5, wherein the preexisting cardiovascular disease event is any one of:

- a. nonfatal myocardial infarction (MI);
- b. subclinical MI ("silent MI") detected on an electrocardiogram;
- c. angina confirmed by ischemic changes with exercise testing or by clinically significant obstruction on coronary angiography, or wherein the angina occurs at rest or does not respond to nitroglycerin treatment;
- d. coronary revascularization with angioplasty or coronary artery bypass; or
- e. ischemic stroke.

[0018] Embodiment 7: The method of any one of embodiments 4-6 wherein the recurrent cardiovascular event is one or more or:

- a. nonfatal myocardial infarction (MI);
- b. coronary revascularization with angioplasty or coronary artery bypass;
- c. ischemic stroke;
- d. heart failure; or
- e. death secondary to cardiovascular disease.

[0019] Embodiment 8: The methods of any one of embodiments 1-7, wherein the increased risk for developing a first or recurrent cardiovascular disease event persists after adjustment for established cardiovascular risk factors selected from one or more of the following: age, sex, duration of diabetes, HbA1c levels, systolic blood pressure, diabetic kidney disease, retinopathy, cardiac autonomic neuropathy, pulse rate, smoking, triglycerides, and LDL cholesterol levels.

[0020] Embodiment 9: The method of any one of embodiments 1-8, further comprising:

- a. evaluating additional cardiac AAb-linked genetic and proinflammatory cytokine risk markers, wherein the additional markers comprise one or more of:
 - i. the presence of the human leucocyte antigen (HLA) genotype, DQ2 or DQ8;
 - ii. the presence of elevated blood levels of one or more proinflammatory cytokines, wherein the one or more proinflammatory cytokines are selected from: IL-6, GM-CSF, TNF- α , G-CSF, EGF, sCD40L, CCL2, IL-8, IL-12, CCL7, IFN- γ , and CCL22, and wherein the elevated levels of the one or more

proinflammatory cytokines are compared to levels in healthy control subjects or in subjects with T1D not having cardiac AAbs; and

iii. the presence of decreased IL-10 levels, wherein the decreased levels of IL-10 are compared to levels in healthy control subjects or subjects with T1D not having cardiac AAbs, and

b. predicting that the subject is at increased risk for developing a first or recurrent cardiovascular disease event compared to a subject having cardiac AAbs alone, i.e., without the one or more additional linked genetic and proinflammatory cytokine risk markers.

[0021] Embodiment 10: The method of embodiment 9, wherein the HLA-DQ2 genotype is DQ2.5 (DQA1*05:01/DQB1*02:01).

[0022] Embodiment 11: The method of embodiment 10, wherein a subject with a HLA-DQ2.5 homozygous genotype is at increased risk of developing a first or recurrent cardiovascular disease event than a subject with HLA-DQ2.5 heterozygous genotype.

[0023] Embodiment 12: The method of embodiment 9, wherein the HLA-DQ8 genotype is DQA1*03:01-DQB1*03:02.

[0024] Embodiment 13: The method of embodiment 12, wherein a subject with a HLA-DQA1*03:01-DQB1*03:02 homozygous genotype is at increased risk of developing a first or recurrent cardiovascular disease event than a subject with HLA-DQA1*03:01-DQB1*03:02 heterozygous genotype.

[0025] Embodiment 14: The method of any one of embodiments 9-13, wherein the additional markers comprise presence of elevated blood levels of IL-6 and GM-CSF, and wherein the elevated blood levels are 2-times or greater, 3-times or greater, 5-times or greater, 7-times or greater, or 10-times or greater, as compared to healthy control subjects or subjects with T1D not having cardiac AAbs.

[0026] Embodiment 15: The method of embodiments 9-14, wherein adding GM-CSF and IL-6 to a cardiovascular risk prediction model further improves the prediction of a first or recurrent cardiovascular disease event compared with a model containing full-length MYH6 AAbs alone.

[0027] Embodiment 16: The method of embodiments 9-15, wherein adding GM-CSF and IL-6 to a cardiovascular risk prediction model further improves the prediction of a first or recurrent cardiovascular event compared with a model containing two or more cardiac AAbs alone, wherein the two or more cardiac AAbs are selected from those that are specific to:

a. full-length MYH6;

- b. S1-fragment of MYH6;
- c. S2-fragment of MYH6;
- d. full-length MYH7; or
- e. full-length cTnI.

[0028] Embodiment 17: The method of any one of embodiments 1, 3, 4, 6-15, wherein identifying a subject as having AAbs specific to full-length MYH6 predicts the development of a cardiovascular disease event at a significantly higher rate and at a younger age than a subject not having AAbs specific to full-length MYH6.

[0029] Embodiment 18: The method of any one of embodiments 2, 3, 5-14, or 16, wherein identifying a subject as having 2 or more cardiac AAbs predicts the development of a first or recurrent cardiovascular disease event at a significantly higher rate and at a younger age than a subject with T1D not having said cardiac AAbs.

[0030] Embodiment 19: The method of any one of embodiments 1-18, wherein the subject has adult-onset T1D.

[0031] Embodiment 20: The method of embodiment 19, wherein the subject has adult-onset T1D and has a HLA-DQ2 genotype, such as a DQ2.5 genotype.

[0032] Embodiment 21: The method of any one of embodiments 1-18, wherein the subject has childhood-onset T1D.

[0033] Embodiment 22: The method of embodiment 21, wherein the subject has a HLA-DQ8 genotype, optionally wherein the DQ8 genotype is DQA1*03:01-DQB1*03:02.

[0034] Embodiment 23: A method for identifying a subject with cancer treated with at least one immune checkpoint inhibitor (ICI) who is at increased risk of developing myocarditis or who has suspected myocarditis or who is in need of treatment with an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor of T-cell activation, a B-cell antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6, wherein the method comprises:

- a. isolating blood, optionally a serum or plasma sample, from the subject;
- b. identifying the subject as having cardiac autoantibodies (AAbs) specific to full-length myosin heavy chain 6 (MYH6, SEQ ID NO: 1) in the blood; and
- c. predicting that the subject is at increased risk of developing myocarditis, or that the subject has suspected myocarditis, or that the subject is in need of treatment with an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor of T-cell activation, a B-cell

antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6, compared to a subject with cancer treated with at least one ICI not having cardiac AAbs specific to full-length MYH6.

[0035] Embodiment 24: A method for identifying a subject with cancer treated with an immune checkpoint inhibitor (ICI) who is at increased risk of developing myocarditis or who has suspected myocarditis or who or is in need of treatment with an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor of T-cell activation, a B-cell antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6, wherein the method comprises:

- a. isolating blood, optionally a serum or plasma sample, from the subject;
- b. identifying the subject as having two or more cardiac AAbs, wherein the two or more cardiac AAbs are selected from those specific to:
 - i. full-length MYH6;
 - ii. the S1-fragment of MYH6;
 - iii. the S2-fragment of MYH6;
 - iv. full-length myosin heavy chain 7 (MYH7, SEQ ID NO: 2); and
 - v. full-length cardiac troponin I (cTnI, SEQ ID NO: 3); and
- c. predicting that the subject is at increased risk for developing myocarditis or has suspected myocarditis or is in need of treatment with an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor of T-cell activation, a B-cell antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6, compared to a subject with cancer treated with at least one ICI not having said cardiac AAbs.

[0036] Embodiment 25: The method of any one of embodiments 23-24, wherein the at least one ICI comprises an anti-PD-1 antibody, an anti-CTLA4 antibody, an anti-PD-L1 antibody, a combination of an anti-PD-1 and anti-CTLA4 antibody, or a combination of an anti-PD-L1 antibody and an anti-CTLA4 antibody.

[0037] Embodiment 26: The method of any one of embodiments 23-25, further comprising:

- a. evaluating additional cardiac AAb-linked proinflammatory cytokine risk markers, wherein the additional markers comprise one or more of:

- i. the presence of elevated blood levels of one or more proinflammatory cytokines, wherein the one or more proinflammatory cytokines are selected from: IL-6, GM-CSF, TNF- α , G-CSF, EGF, sCD40L, CCL2, IL-8, IL-12, CCL7, IFN- γ , and CCL22, and wherein the elevated levels of the one or more proinflammatory cytokines are compared to levels in healthy control subjects or in subjects with cancer and treated with at least one ICI not having cardiac AAbs; and
 - ii. the presence of decreased IL-10 levels, wherein the decreased levels of IL-10 are compared to levels in healthy control subjects or in subjects with cancer and treated with at least one ICI not having cardiac AAbs, and
- b. predicting that the subject is at increased risk for developing myocarditis or has suspected myocarditis or is in need of treatment with an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor of T-cell activation, a B-cell antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6, compared to a subject having cardiac AAbs alone, i.e., without the one or more additional linked proinflammatory cytokine risk markers.

[0038] Embodiment 27: The method of embodiment 26, wherein the additional markers comprise presence of elevated blood levels of IL-6 and GM-CSF, wherein the elevated blood levels are 2-times or greater, 3-times or greater, 5-times or greater, 7-times or greater, or 10-times or greater, as compared to levels in healthy control subjects or subjects with cancer and treated with at least one ICI not having cardiac AAbs.

[0039] Embodiment 28: The method of any one of embodiments 26-27, wherein adding GM-CSF and IL-6 to a cardiovascular risk prediction model further improves the prediction of development of myocarditis compared with a model containing full-length MYH6 AAbs alone.

[0040] Embodiment 29: The method of any one of embodiments 26-28, wherein adding GM-CSF and IL-6 to a cardiovascular risk prediction model further improves the prediction of development of myocarditis compared with a model containing two or more cardiac AAbs alone, wherein the two or more cardiac AAbs are selected from those that are specific to:

- a. full-length MYH6;
- b. S1-fragment of MYH6;
- c. S2-fragment of MYH6;
- d. full-length MYH7; or
- e. full-length cTnI.

[0041] Embodiment 30: The method of any one of embodiments 1-29, wherein identifying cardiac AAbs comprises the use of a fluid-phase radioimmunoprecipitation assay.

[0042] Embodiment 31: The method of any one of embodiments 1-30, wherein having cardiac AAb is defined as having an AAb index above the threshold for positivity based on predetermined cutoff values at the 99th percentile of the levels in serum samples from healthy control subjects.

[0043] Embodiment 32: The method of any one of embodiments 30-31, where the cardiac AAb index is defined as $[\text{counts per million (CPM) in the unknown sample} - \text{CPM in the negative control standard}] / [\text{CPM in the positive standard} - \text{CPM in the negative standard}] \times 100$ in a fluid-phase radioimmunoprecipitation assay.

[0044] Embodiment 33: The method of any one of the embodiments 30-32, where the cutoff for having full-length MYH6 AAbs is about 0.701.

[0045] Embodiment 34: The method of any one of the embodiments 30-33, where the cutoff for having S1-fragment MYH6 AAbs is about 0.330.

[0046] Embodiment 35: The method of any one of the embodiments 30-34, where the cutoff for having S2-fragment MYH6 AAbs is about 0.763.

[0047] Embodiment 36: The method of any one of the embodiments 30-35, where the cutoff for having full-length MYH7 AAbs is about 0.799.

[0048] Embodiment 37: The method of any one of the embodiments 30-36, where the cutoff for having full-length cTnI AAbs is about 0.283.

[0049] Embodiment 38: The method of any one of embodiments 1-37, further comprising administering one or more treatment to a subject to treat the predicted increased risk for developing a first or recurrent cardiovascular disease event, or to treat the predicted increased risk for developing myocarditis and/or suspected myocarditis.

[0050] Embodiment 39: A method of selecting subjects to include in a clinical trial for the treatment or prevention of a cardiovascular disease event comprising:

- a. determining that the subject as having an increased risk of a first or recurrent cardiovascular disease event, using the method of any one of embodiments 1-37; and

- b. including the subjects in the clinical trial based on this increased risk.

[0051] Embodiment 40: A method of preventing or treating a cardiovascular disease event in a subject with T1D comprising:

- a. determining that the subject as having an increased risk of a first or recurrent cardiovascular disease event using the method of any one of embodiments 1-37, and
- b. administering one or more treatments for preventing or treating a cardiovascular disease event.

[0052] Embodiment 41: A method of preventing or treating a cardiovascular disease event in a subject with cancer treated with at least one ICI, comprising:

- a. determining that the subject has an increased risk of developing myocarditis or has suspected myocarditis using the method of any one of embodiments 23-40, and
- b. administering one or more treatments for preventing or treating myocarditis.

[0053] Embodiment 42: The method of any one of embodiments 38-41, wherein the one or more treatment comprises inhibiting the effect of one or more proinflammatory cytokines or inhibiting the autoimmune disease process itself.

[0054] Embodiment 43: The method of any one of embodiments 38-42, wherein the one or more treatment comprises administration of an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor of T-cell activation, a B-cell antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6.

[0055] Embodiment 44: The method of embodiment 43, wherein the IL-6 ligand inhibitor is sirukumab, olokizumab, clazakizumab, vobarilizumab, or ziltivekimab.

[0056] Embodiment 45: The method of embodiment 43, wherein the IL-6 receptor inhibitor is sarilumab or tocilizumab.

[0057] Embodiment 46: The method of embodiment 43, wherein the GM-CSF inhibitor is mavrilimumab, otilimab, namilumab, or lenzilumab.

[0058] Embodiment 47: The method of embodiment 43, wherein the JAK1/JAK2 inhibitor is baricitinib.

[0059] Embodiment 48: The method of embodiment 43, wherein the inhibitor of T-cell activation is an anti-CD3 antibody or a CTLA4-Ig fusion protein.

[0060] Embodiment 49: The method of embodiment 43, wherein the anti-CD3 antibody is teplizumab.

[0061] Embodiment 50: The method of embodiment 43, wherein the low-dose IL-2 is recombinant IL-2 (aldesleukin).

[0062] Embodiment 51: The method of embodiment 43, wherein the antigen is conjugated or delivered in nanoparticles or is a modified mRNA vaccine.

[0063] Embodiment 52: The method of embodiment 43, wherein the CTLA-Ig fusion protein is abatacept.

[0064] Embodiment 53: A method of preventing a first or recurrent cardiovascular disease event in a subject with T1D comprising:

- a. determining that the subject as having an increased risk of a first or recurrent cardiovascular disease event using the method of any one of embodiments 1-22 or 30-52, wherein the subject expresses the HLA DR3-DQ2 or HLA-DQ8 haplotype, and
- b. administering a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6, wherein the tolerogenic therapy restores immune tolerance to MYH6 and thereby prevents a first or recurrent cardiovascular disease event.

[0065] Embodiment 54: The method of embodiment 53, wherein the antigen is conjugated or delivered in nanoparticles or is a modified mRNA vaccine.

[0066] Embodiment 55: The method of embodiment 53 or 54, wherein the HLA-DQ2 genotype is DQ2.5 (DQA1*05:01/DQB1*02:01).

[0067] Embodiment 56: The method of embodiment 55, wherein a subject has a HLA-DQ2.5 homozygous genotype.

[0068] Embodiment 57: The method of embodiment 53 or 54, wherein the HLA-DQ8 genotype is DQA1*03:01-DQB1*03:02.

[0069] Embodiment 58: The method of embodiment 57, wherein a subject has a HLA-DQA1*03:01-DQB1*03:02 homozygous genotype.

[0070] Embodiment 59: The method of any one of embodiments 1-22 or 30-58, wherein the T1D is latent autoimmune diabetes of adults (LADA).

[0071] Additional objects and advantages will be set forth in part in the description which follows, and in part will be understood from the description, or may be learned by practice. The objects and advantages will be realized and attained by means of the elements and combinations particularly pointed out in the appended claims.

[0072] It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive of the claims.

[0073] The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate one (several) embodiment(s) and together with the description, serve to explain the principles described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0074] **Figures 1A-1C** show the risk of developing CVD events according to the number and type of cardiac autoantibody (AAb) present at baseline. (a) Kaplan-Meier curves showing the cumulative incidence of first CVD events according to the number of cardiac AAbs (No AAbs, 1 AAb, ≥ 2 AAbs) present at baseline, among AAbs to full-length (FL)-MYH6, S1-MYH6, S2-MYH6, FL-MYH7, and cTnI. (b) Kaplan-Meier curves showing the cumulative incidence of first CVD events according to the presence (MYH6 AAb+) or absence (MYH6 AAb-) of MYH6 AAbs at baseline. (c) Unadjusted (black) and adjusted (red) hazard ratios (HR) for the number (1 AAb versus ≥ 2 AAbs) and type (MYH6 AAb versus cTnI of cardiac AAB present at baseline for subsequent first CVD events. The adjusted models for 1 AAb, ≥ 2 AAbs, and MYH6 AAb+ included the following covariates: age, retinopathy at baseline, microalbuminuria/ESRD, DCCT/EDIC time-weighted mean HbA_{1c}, LDL, and systolic blood pressure. The cTnI AAb+ model included age, male sex, current triglycerides, cardiac autonomic neuropathy, and DCCT/EDIC time-weighted mean HbA_{1c} and systolic blood pressure. The hazard of developing a CVD event in the presence of only 1 AAb or ≥ 2 AAbs is compared to the hazard in the absence of cardiac AAbs. The hazard of having a CVD event in MYH6 AAb+ and cTnI AAb+ subjects is compared to the hazard in MYH6 AAb- and cTnI AAb- subjects, respectively. Data points at the center (circles) represent hazard ratios, and the length of the lines indicates the 95% confidence interval.

[0075] **Figures 2A-2D** show identification of IL-6 and GM-CSF as the primary mediators of excess CVD risk associated with ≥ 2 AAbs or MYH6 AAbs. (a) Baseline concentrations of circulating cytokines stratified by cardiac AAb number and type. Median (interquartile range) values of cytokine and chemokine concentrations (pg/mL) are presented in their natural scale, along with odds ratio (95% confidence intervals). Groups are compared with the no AAb group by logistic regression, considering one tertile change in cytokine/chemokine concentrations using log₂-transformed data. [†]P-values (red and blue stars indicate positive and negative associations, respectively) corrected for multiple testing using the Benjamini-Hochberg procedure (*P<0.05, **P<0.01, ***P<0.001, ****P<0.0001). (b) Heatmap of the median fold change relative to the No AAb group in concentrations of 37 cytokines in subjects with only 1 AAb, ≥ 2 AAbs, MYH6 AAbs, and cTnI AAbs. (c) Hierarchical cluster analysis (Ward's method) of the 12 core cytokines associated with MYH6 AAbs and ≥ 2

AAbs (dotted lines), revealing 4 clusters (C1-C4). The percentage of subsequent first CVD events in each cluster are as listed. (d) Forest plots of the effect of each of the 12 core cytokines, alone or combined, on the association between MYH6 AAbs (top panel) and ≥ 2 AAbs (bottom panel) and risk of incident CVD events. *Adjusted for age, retinopathy, and DCCT/EDIC time-weighted mean LDL. †Adjusted for retinopathy, and DCCT/EDIC time-weighted mean LDL and systolic blood pressure. IFN, interferon; TNF, tumor necrosis factor; G-CSF, granulocyte colony-stimulating factor; EGF, epidermal growth factor; sCD40L, soluble CD40 ligand; CCL, C-C motif chemokine ligand; GM-CSF, granulocyte macrophage colony-stimulating factor; IL, interleukin; IL-1RA, interleukin-1 receptor antagonist; TGF, transforming growth factor.

[0076] **Figures 3A-3C** show cytokine profiles in MYH6 AAb+ T1D participants overlap with rheumatoid arthritis (RA), and COVID-19, and are distinct from those found in patients with T2D. (a) Comparison of circulating cytokine concentrations in healthy controls (gray, $n = 32$), T1D no AAbs (purple, $n = 146$), T1D MYH6 AAb+ (red, $n = 41$), rheumatoid arthritis (blue, $n = 32$), COVID-19 (orange, $n = 32$), and T2D (green, $n = 50$). Group comparisons were made using analysis of variance with correction for multiple testing. Statistical significance: * $P < 0.05$ and **** $P < 0.0001$. (b) Principal component analysis (PCA) comparing cytokine profiles between T1D MYH6 AAb+ participants to patients with COVID-19, rheumatoid arthritis, or T2D. (c) PCA comparing cytokine profiles of T2D, healthy controls, and T1D no AAbs. The concentration of the 37 measured cytokines was normalized among healthy controls and various disease states using quantile normalization prior to PCA. AAb, autoantibody; ns, non-significant; T1D, type 1 diabetes; T2D, type 2 diabetes.

[0077] **Figures 4A-4D** show that among the T1D participants who subsequently developed first CVD events, MYH6 AAbs-positive participants are genetically and immunologically distinct from MYH6 AAb-negative participants. (a) Association between HLA-DQ genotype and MYH6 AAb status. DQ2.5 = DQA1*05:01-DQB1*02:01 and DQ8 = DQA1*03:01-DQB1*03:02. (b) Levels of MYH6 AAb levels according to DQ2 genotype status. Median MYH6 AAb levels for each group are represented by red bars, and the 99th percentile cutoff for MYH6 AAb positivity is shown as a dashed line. (c) Correlogram illustrating the pairwise relationships between cytokines among participants who developed first CVD events based on MYH6 AAb status. Spearman's rank correlations were used with correction for multiple testing. Significant correlations are denoted by circles, with the color representing the Spearman correlation coefficient. (d) Network analysis displaying the cytokine-to-cytokine

correlation profile. Nodes represent cytokines, and the color of edges between nodes indicates the Spearman correlation coefficient connecting them.

[0078] **Figure 5** shows the relationship between the number of cardiac AAbs (AAbs) at baseline and the risk of developing a subsequent first MACE. Kaplan-Meier curves showing the cumulative incidence of first CVD events according to the number of cardiac AAbs (No AAb, 1 AAb, ≥ 2 AAb) present at baseline. Cumulative incidence of a first of any CVD event within each group was estimated the Kaplan–Meier method and differences between groups were evaluated using the log-rank test.

[0079] **Figure 6** shows the association between the type of AAb, among subjects positive for only a single AAb type, and the risk of developing a subsequent first CVD event. Kaplan-Meier curves show the cumulative incidence of first CVD events according to the specific AAb type present at baseline among individuals who tested positive for only 1 AAb. The cumulative incidence of first CVD events within each group was estimated the Kaplan–Meier method and differences between groups were evaluated using the log-rank test.

[0080] **Figure 7** shows cytokine concentrations according to MYH6 AAb status at baseline in T1D DCCT/EDIC participants who developed subsequent first CVD events. Circulating cytokine concentrations in T1D MYH6 AAb+ (red, n = 15) and T1D MYH6 AAb– (blue, n = 63) were compared with the Student t test using log2-transformed data with subsequent adjustment of P values to account for multiple testing. AAb denotes autoantibody and T1D indicates type 1 diabetes.

[0081] **Figure 8** shows a hypothetical scheme of how MYH6 autoantibodies are linked to increased CVD risk in T1D. (1) Chronic hyperglycemia induces subclinical myocardial injury, stimulating the uptake of α -myosin (encoded by MYH6) by dendritic cells, which migrate into the heart-draining lymph node, where (2) α -myosin-reactive CD4+ T cells, that have escaped thymic negative selection, recognize α -myosin peptides preferentially presented by HLA-DQ2, leading to their differentiation into T helper cells (Th cells) that produce GM-CSF and IFN- γ , and signal B cells to produce MYH6 AAbs. The α -myosin-specific Th cells then migrate into (“attack”) the heart where (3) GM-CSF functions to polarize cardiac macrophages into a proinflammatory phenotype, resulting in the (4) Systemic production of proinflammatory cytokines. Elevated circulating GM-CSF stimulates bone marrow production of neutrophils and monocytes that home into inflamed atherosclerosis lesions, and also activates monocytes to produce IL-6, further augmenting IL-6 production. (5) Inflammation mediated by GM-CSF and IL-6 and orchestrated by CD4+ T cells (not MYH6 AAbs) leads to increased risk of CVD events.

[0082] **Figures 9A-9B** show the risk of developing CVD events according to the number of cardiac autoantibodies in the childhood-onset T1D CACTI cohort. (a) Kaplan-Meier curves showing the cumulative incidence of first CVD events according to the number of cardiac AAbs (No AAbs 1 AAb, ≥ 2 AAbs) present at baseline, among AAbs to full-length (FL)-MYH6, S1-MYH6, S2-MYH6, FL-MYH7, and cTnI. (b) Kaplan-Meier curves showing the cumulative incidence of first CVD events according to the presence (MYH6 AAb+) or absence (MYH6 AAb-) of MYH6 AAbs at baseline.

DESCRIPTION OF THE SEQUENCES

[0083] Table 1 provides a listing of certain sequences referenced herein.

Table 1: Description of the Sequences		
Description	Sequences	SEQ ID NO
myosin heavy chain 6 (MYH6)	MTDAQMADFGAAQYLRKSEKERLEAQTRPFDIRTECFVPDDKEEFVKAKIL SREGGKVIAETENGKTVTVKEDQVLQONPPKFDKIEDMAMLTFLHEPAVLFN LKERYAAWMIYTYSGLFCVTVPYKWLVPVYNAEVVAAAYRGKKRSEAPPHIFS ISDNAYQYMLTDRENQSILITGESGAGKTVNTKRVIQYFASIAAIGDRGKKD NANANKGTLEDQIIQANPALEAFGNAKTVRNDNSSRFGKFIRIHFGATGKLA SADIETYLLEKSRVIFQLKAERNYHIFYQILSNKKPELLDMLLVTNPNPYDYA FVSQGEVSVASIDDSEELMATDSAFDVLGFTSEEKAGVYKLTGAIMHYGNMK FKQKQREEQAEPDGTEDADKSAYLMGLNSADLLKGLCHPRVKVGNEYVTKGQ SVQQVYYSIGALAKAVYEKMFNWMVTRINATLETQKPRQYFIGVLDIAGFEI FDFNSFEQLCINFTEKLLQQFFNHMFVLEQEEYKKEGIEWTFIDFGMDLQA CIDLIEKPMGIMSILEEECMFPKATDMTFKAKLYDNHLGKSNNFQKPRNIKG KQEAHFSLIHYAGTVDYNILGWLEKNKDPLNETVVALYQKSSLKLMATLFSS YATADTGDGSKSGGKKKGSSFQTVSALHRENLNKLMTNLRTTHPHFVRCII PNERKAPGVMNDPLVMHQLRCNGVLEGIRICRKGFPNRILYGDFRQRYRILN PVAIPEGQFIDSRKGTEKLLSSLDIDHNQYKFGHTKVFFKAGLLGLEEMRD ERLSRIITRMQAQARGQLMRIEFKKIVERRDALLVIQWNIRAFMGVKNWPWM KLYFKIKPLLKSAETEKEMATMKEEFGRICKETLEKSEARRKELEKMKVSSLQ EKNDLQLQVQAEQDNLNDAEERCDQLIKNKIQLEAKVKEMNERLEDEEEMNA ELTAKKRKLEDECSELKKDIDDLETLAKVEKEKHATENKVKNLTEEMAGLD EIIAKLTKEKKALQEAHQALDDLQVEEDKVNSLSKSKVKLEQQVDDLEGLS EQEKKVRMDLERAKRKLEGLDLKTQESIMDLENDKLQLEEKLLKKKEFDINQQ NSKIEDEQVLALQLQKKLKENQARIEELEEELEAERTARAKVEKLRSDLSRE LEEISERLEEAGGATSVQIEMNKKREAEFQKMRRDLEEATLQHEATAAALRK KHADSVAELEGEQIDNLQRVKQKLEKEKSEFKLELDDVTSNMEQIIKAKANLE KVSRTLEDQANEYRVKLEEAQRSLNDFTTQRAKLQTENGELARQLEEKEALI SQLTRGKLSYTQOMEDLKRQLEEEGKAKNALAHALQSARHDCDLLREQYEEE TEAKAELQRVLSKANSEVAQWRTKYETDAIQRTTEELEEAKKKLAQRLQDAEE AVEAVNAKCSSLEKTKHRLQNEIEDLMVDVERSNAALDKKQRNFDKILA EWKQKYEESQSELESSQKEARSLSTELFKLKNAYEESLEHLETFKRENKNLQ EEISDLTEQLGEGGKNVHELEKVRKQLEVEKLELQSALEEAASLEHEEGKI LRAQLEFNQIKAEIERKLAEKDEEMEQAKRNHQRVDSLQTSLSDAETR SRNE VLRVKKKMEGDLNEMEIQLSHANRMAAEAQKQVKSLQSLKDTQIQLD DAVR	1

	ANDDLKENIAIVERNNLLQAELEELRAVVEQTERSRLAEQELIETSERVQ LLHSQNTSLINQKKKMESDLTQLQSEVEEAVQECRNAEEKAKKAITDAAMMA EELKKEQDTSAHLERMKKNMEQTIKDLQHRLDEAEQIALKGGKKQLQKLEAR VRELEGELEAEQKRNAESVKGMRKSERRIKELTYQTEEDKKNLLRLQDLVDK LQLKV KAYKRQAEAEAEQANTNLSKFRKVQHELDEAEERADIAESQVNKLRA KSRDIGAKQKMHDEE	
myosin heavy chain 7 (MYH7)	MGDSEMAVFGAAAPYLRKSEKERLEAQTRPFDLKKDVFPDDKQEFVKAKIV SREGGKVTAETEGKTVTVKEDQVMQONPPKFDKIEDMAMLTFLHEPAVLIN LKDRYGSWMIYTYSGLFCVTVPYKWLVPYTPPEVVAAYRGKKRSEAPPHIFS ISDNAYQYMLTDRENQSILITGESGAGKTVNTKRVIQYFAVIAAIGDRSKKD QSPGKGTLEDQIIQANPALEAFGNAKTVRNDNSSRFGKFIRIHFGATGKLAS ADIETYLLEKSRVIFQLKAERDYHIFYQILSNKKPELLDMLLITNNPYDYAF ISQGETTVASIDDAEELMATDNADFVLGFTSEEKNSMYKLTGAIMHFGNMKF KLKQREEQAEPDGTEEADKSAYLMGLNSADLLKGLCHPRVKVGNVEYVTKGQN VQQVIYATGALAKAVYERMFNWMVTRINATLETQPRQYFIGVLDIAGFEIF DFNSFEQLCINFTNEKLQQFFNHMFVLEQEEYKKEGIEWTFIDFGMDLQAC IDLIEKPMGIMSILEEECMFPKATDMTFKAKLFDNHLGKSANFQKPRNIKGGK PEAHFSLIHYAGIVDYNIIIGWLQKNKDPLNETVVGLYQKSSLKLLSTLFANY AGADAPIEKGGKAKKGGSSFQTVSALHRENLNKLMTNLRSTHPPHVRICIIPN ETKSPGVMDNPLVMHQLRCNGVLEGIRICRKGFPNRILYGDFRQRYRILNPA AIEGQFIDSRKGAEKLLSSLDIDHNQYKFGHTKVFFKAGLLGLLEEMRDER LSRIITRIQAQSRGVLARMEYKLLERRDSSLVIQWNIRAFMGVKNWPWMKL YFKIKPLLKSAEREKEMASMKEEFTRLKEALEKSEARRKELEEKMVSSLQEK NDLQLQVQAEQDNLADAEERCDQLIKNKIQLEAKVKEMNERLEDEEEMNAEL TAKKRKLEDECESELKRDIDDLLELTAKVEKEKHATENKVKNLTEEMAGLDEI IAKLTKEKKALQEAHQALDDLQAEEDKVNTLTAKVKLEQQVDDLEGSLEQ EKKVRMDLERAKRKLEGLKLTQESIMDLENDKQQLDERLKKKDFELNALNA RIEDEQALGSQKQKLKELQARIEELEELEEAERTARAKVEKLRSDDLRELE EISERLEEAGGATSVQIEMNKKREAEFQKMRRDLEEATLQHEATAAALRKKH ADSVAELGEQIDNLQRVKQKLEKEKSEFKLELDDVTSNMEQIIKAKANLEKM CRTLEDQMNEHRSKAEETQRSVNDLTSQRAKLQTENGELSRQLDEKEALISQ LTRGKLTYTQLEDLKRQLEEEVKAKNALAHALQSARHDCDLLREQYEEETE AKAELQRVLSKANSEVAQWRTKYETDAIQRTTEELEEAKKKLAQRLQEAEEAV EAVNAKCSSLEKTKHRLQNEIEDLMVDVERSNAALDKKQRNFDKILAEW KQKYEESQSELESSQKEARSLSTELFKLKNAYEESLEHLETFKRENKNLQEE ISDLTEQLGSSGKTIHELEKVRKQLEAEKMELOSALEAEASLEHEEGKILR AQLEFNQIKAEIERKLAEKDEEMEQAKRNHLRVVDSLQTS LDAETRSRNEAL RVKKKMEGDLNEMEIQLSHANRMAAEAQKQVKSLSLLKDTQIQLD DAVRAN DDLKENIAIVERNNLLQAELEELRAVVEQTERSRLAEQELIETSERVQLL HSQNTSLINQKKKMDADLSQLQTEVEEAVQECRNAEEKAKKAITDAAMMAEE LKKEQDTSAHLERMKKNMEQTIKDLQHRLDEAEQIALKGGKKQLQKLEARVR ELENELEAEQKRNAESVKGMRKSERRIKELTYQTEEDRKNLLRLQDLVDKLQ LKVKAYKRQAEAEAEQANTNLSKFRKVQHELDEAEERADIAESQVNKLRAKS RDIGTKGLNEE	2
cardiac troponin I (cTNI)	MADGSSDAAREPRPAPAPIRRRSSNYRAYATEPHAKKSKISASRKLQLKTL LLQIAKQELERAEERRGEKGRALSTRCPLELAGLGAELQDLCRQLHARV DKVDEERYDIEAKVTKNITEIADLTQKIFDLRGKFKRPTLRRVRISADAMMQ ALLGARAKESLDLRAHLKQVKKEDTEKENREVG DWRKNIDALSGMEGRKKKF ES	3

DETAILED DESCRIPTION OF THE EMBODIMENTS

I. Methods of predicting elevated risk of a first or recurrent cardiovascular disease event in a subject with T1D or treated with an immune checkpoint inhibitor

[0084] Described herein, in some embodiments, are methods of detecting AAbs that predict cardiac disease events in patients with T1D. The disclosure herein also relates to methods of detecting AAbs in cancer patients treated with at least one immune checkpoint inhibitor (ICI), that, for example, can aid in the early detection and diagnosis of cardiovascular disease events such as potentially-life threatening cardiac toxicities like myocarditis.

[0085] As used herein, “an autoantibody” or “AAb” is an antibody produced by an organism in response to a constituent of its own tissues. In other words, an AAb is an antibody expressed by a subject, wherein the antigenic target of the AAb is a protein normally expressed by the subject.

[0086] In some embodiments, an AAb disclosed herein may bind to a protein expressed by the heart, specifically cardiac muscle tissue, of the subject. Such antibodies may be termed “cardiac AAbs,” which may be abbreviated “cAAbs” herein. In some embodiments, the presence of the cardiac AAb may be linked to an increased risk of cardiovascular disease in the subject.

[0087] In some embodiments, provided is a method of identifying a subject with T1D at increased risk of developing a first cardiovascular disease event, wherein the method comprises (a) isolating blood, optionally a serum or plasma sample, from the subject; (b) identifying the subject as having cardiac AAbs specific to full-length myosin heavy chain 6 (MYH6, SEQ ID NO: 1) in the blood; and (c) predicting that the subject is at increased risk for developing a first cardiovascular disease event compared to a subject with T1D not having cardiac AAbs specific to full-length MYH6. As described herein, additional linked genetic and inflammatory markers may be used together with the presence of MYH6 AAbs to identify subjects at even greater risk of cardiovascular disease events.

[0088] In some embodiments, provided is a method for identifying a subject with T1D at increased risk of developing a first cardiovascular disease event, wherein the method comprises (a) isolating blood, optionally a serum or plasma sample, from the subject; (b) identifying the subject as having two or more cardiac AAbs, wherein the two or more cardiac AAbs are selected from those specific to full-length MYH6; the S1-fragment of MYH6; the S2-fragment of MYH6; full-length myosin heavy chain 7 (MYH7, SEQ ID NO: 2); or full-length cardiac troponin I (abbreviated herein as cTnI or TnI, SEQ ID NO: 3); and predicting

that the subject is at increased risk for developing a first cardiovascular disease event compared to a subject with T1D not having said cardiac AAbs. As described herein, additional linked genetic and inflammatory markers may be used together with the presence of said AAbs to identify subjects at even greater risk of cardiovascular disease events.

[0089] In some embodiments wherein the subject has T1D, the first cardiovascular disease event is any one of nonfatal myocardial infarction (MI); subclinical MI (“silent MI”) detected on an electrocardiogram; angina confirmed by ischemic changes with exercise testing or by clinically significant obstruction on coronary angiography, or wherein the angina occurs at rest or does not respond to nitroglycerin treatment; coronary revascularization with angioplasty or coronary artery bypass; ischemic stroke; heart failure; or death secondary to cardiovascular disease.

[0090] In some embodiments, provided is a method for identifying a subject with T1D and a preexisting cardiovascular disease event at increased risk of having a recurrent cardiovascular disease event comprising (a) isolating blood, optionally a serum or plasma sample, from the subject; (b) identifying the subject as having cardiac AAbs specific to full-length MYH6 in the blood; and (c) predicting that the subject is at increased risk for developing a recurrent cardiovascular disease event compared to a subject with T1D not having AAbs specific to full-length MYH6.

[0091] In some embodiments, provided is a method for identifying a subject with T1D and a preexisting cardiovascular disease event at increased risk of a recurrent cardiovascular disease event comprising (a) isolating blood, optionally a serum or plasma sample, from the subject; (b) identifying the subject as having two or more cardiac AAbs, wherein the two or more cardiac AAbs are selected from those that specific to full-length MYH6; the S1-fragment of MYH6; the S2-fragment of MYH6; full-length MYH7; or full-length cTnI; and predicting that the subject is at increased risk for developing a recurrent cardiovascular disease event compared to a subject with T1D not having said cardiac AAbs.

[0092] In some embodiments, the preexisting cardiovascular disease event comprises one or more of nonfatal myocardial infarction (MI); subclinical MI (“silent MI”) detected on an electrocardiogram; angina confirmed by ischemic changes with exercise testing or by clinically significant obstruction on coronary angiography, or wherein the angina occurs at rest or does not respond to nitroglycerin treatment; coronary revascularization with angioplasty or coronary artery bypass; or ischemic stroke.

[0093] In some embodiments, the recurrent cardiovascular disease event comprises one or more of nonfatal myocardial infarction (MI); coronary revascularization with angioplasty or

coronary artery bypass; ischemic stroke; heart failure; or death secondary to cardiovascular disease.

[0094] In some embodiments, identifying a subject as having AAbs specific to full-length MYH6 predicts the development of a cardiovascular disease event at a significantly higher rate and at a younger age than a subject not having AAbs specific to full-length MYH6. In some embodiments, identifying a subject as having 2 or more cardiac AAbs predicts the development of a first or recurrent cardiovascular disease event at a significantly higher rate and at a younger age than a subject with T1D not having said cardiac AAbs.

[0095] In some embodiments, the method of predicting elevated risk of a first or recurrent cardiovascular disease event further comprises administering one or more treatments to a subject to treat the predicted increased risk for developing a first or recurrent cardiovascular disease event.

[0096] In some embodiments, provided is a method of identifying a subject treated with at least one immune checkpoint inhibitor who is at higher risk of developing a cardiovascular disease event or who has a suspected cardiovascular disease event (e.g., a cardiac toxicity such as myocarditis) or who is in need of treatment with an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor of T-cell activation, a B-cell antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6, wherein the method comprises (a) isolating blood, optionally a serum or plasma sample, from the subject; (b) identifying the subject as having cardiac AAbs specific to full-length myosin heavy chain 6 (MYH6, SEQ ID NO: 1) in the blood; and (c) predicting that the subject is at increased risk for developing a cardiovascular disease event or has a suspected cardiovascular disease event (e.g., a cardiac toxicity such as myocarditis) or is in need of treatment with an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor of T-cell activation, a B-cell antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6, compared to a subject treated with at least one immune checkpoint inhibitor not having cardiac AAbs specific to full-length MYH6. In some cases, the subject has cancer. As also described herein, additional linked inflammatory markers may be used together with the presence of MYH6 AAbs to identify subjects at risk of developing a cardiovascular disease event or with a suspected cardiovascular disease event. In some embodiments, the cardiovascular disease event or suspected cardiovascular disease event is myocarditis.

[0097] In some embodiments, provided is a method for identifying a subject treated with at least one immune checkpoint inhibitor at increased risk of developing a cardiovascular disease event or suspected cardiovascular disease event (e.g., a cardiac toxicity such as myocarditis), or is in need of treatment with an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor of T-cell activation, a B-cell antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6, wherein the method comprises (a) isolating blood, optionally a serum or plasma sample, from the subject; (b) identifying the subject as having two or more cardiac AAbs, wherein the two or more cardiac AAbs are selected from those specific to full-length MYH6; the S1-fragment of MYH6; the S2-fragment of MYH6; full-length myosin heavy chain 7 (MYH7, SEQ ID NO: 2); or full-length cardiac troponin I (abbreviated herein as cTnI or TnI, SEQ ID NO: 3); and (c) predicting that the subject is at increased risk for developing a cardiovascular disease event or suspected cardiovascular disease event (e.g., a cardiac toxicity such as myocarditis) or is in need of treatment with an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor of T-cell activation, a B-cell antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6, compared to a subject treated with at least one immune checkpoint inhibitor not having said cardiac AAbs. In some cases, the subject has cancer. As described herein, additional linked inflammatory markers may be used together with the presence of said cardiac AAbs to identify subjects at risk of developing a cardiovascular disease event or with a suspected cardiovascular disease event. In some embodiments, the cardiovascular disease event or suspected cardiovascular disease event is myocarditis.

A. Type 1 diabetes

[0098] As used herein, “type 1 diabetes” or “T1D” refers to a condition wherein the body produces little or no insulin due to an attack of a subject’s own immune system against insulin-producing pancreatic beta cells. The expression of one or more antibodies against pancreatic beta cells is a marker of T1D and occurs many years before the clinical onset of diabetes.

[0099] Most commonly, diabetes mellitus is either type 1 or type 2. T1D (previously known as insulin-dependent diabetes or juvenile diabetes) is an autoimmune disease characterized by immune-mediated destruction of the insulin-producing beta cells of the pancreas. The

expression of one or more antibodies against pancreatic beta cells (“islet AAbs”) is a marker of T1D and occurs many years before and at the clinical onset of diabetes.

[00100] Generally, T1D presents with a constellation of symptoms (such as excessive thirst, frequent urination, and unexplained weight loss) and is diagnosed by blood glucose tests, glycosylated hemoglobin test, and a test for islet AAbs.

[00101] In one embodiment, T1D includes latent autoimmune diabetes of adults (LADA). This is sometimes also referred to as adult-onset T1D. Although T1D requires insulin at diagnosis to control blood sugar and prevent a dangerous condition called ketoacidosis, LADA has a slower course of onset and may initially be misdiagnosed as type 2 diabetes, especially if the person has risk factors such as obesity or family history. LADA can be treated with oral medications for some time, but eventually insulin is needed. Both T1D and LADA have similar symptoms, such as excessive thirst, urination, and blurred vision. The diagnosis of LADA can be made by the presence of AAbs against proteins expressed by pancreatic beta cells, such as glutamic acid decarboxylase (GADA). Most islet AAb tests for both T1D or LADA utilize a radioimmunoprecipitation format, the gold-standard assay format for islet AAb detection. Described herein is a radioimmunoprecipitation assay format to detect cardiac AAbs.

[00102] Recent interest has also focused on early stages of T1D, wherein the patient is not dependent on insulin but shows other markers of T1D particularly one or more AAbs related to beta cell autoimmunity. For example, recent research has identified subjects in the early stages of T1D who have ≥ 2 different types of islet AAbs and blood sugar abnormalities but do not yet require treatment with insulin, although the subject will progress over time to requiring insulin. Treatment of such patients before they require insulin with immunotherapies (such as the recently approved teplizumab (TZIELD®)) can substantially delay, and potentially even prevent, progression to overt diabetes⁵².

[00103] In some embodiments, the T1D is childhood-onset diabetes. Childhood-onset diabetes may also be referred to as “juvenile-onset” or “youth-onset” diabetes. Childhood-onset is defined as occurrence of disease prior to the age of 18 years. In other embodiments, the T1D is adult-onset T1D.

B. Cardiovascular disease events

[00104] As used herein, “a cardiovascular disease event” refers to any incident that may cause chest discomfort, shortness of breath, and/or damage to the heart muscle. In some embodiments, a first cardiovascular disease event is any one of nonfatal myocardial infarction (MI); subclinical MI (“silent MI”) detected on an electrocardiogram; angina

confirmed by ischemic changes with exercise testing or by clinically significant obstruction on coronary angiography, or wherein the angina occurs at rest or does not respond to nitroglycerin treatment; coronary revascularization with angioplasty or coronary artery bypass; ischemic stroke; heart failure; or death secondary to cardiovascular disease.

[00105] In some embodiments, the preexisting cardiovascular disease event comprises one or more of nonfatal myocardial infarction (MI); subclinical MI (“silent MI”) detected on an electrocardiogram; angina confirmed by ischemic changes with exercise testing or by clinically significant obstruction on coronary angiography, or wherein the angina occurs at rest or does not respond to nitroglycerin treatment; coronary revascularization with angioplasty or coronary artery bypass; or ischemic stroke. In some embodiments, the recurrent cardiovascular disease event comprises one or more of nonfatal myocardial infarction (MI); coronary revascularization with angioplasty or coronary artery bypass; ischemic stroke; heart failure; or death secondary to cardiovascular disease.

[00106] In some embodiments, the cardiovascular disease event is myocarditis or autoimmune myocarditis.

[00107] In some embodiments, the methods described herein identify subjects at high risk of having a first or recurrent cardiovascular disease event. In this way, a subject’s clinician more closely monitors or treats the subject to reduce this risk after a subject is identified to be at high risk of a future cardiovascular disease event.

[00108] In some embodiments, the subject has not had a prior cardiovascular disease event but has other traditional cardiovascular risk factors for developing a cardiovascular event. Such risk factors include hypertension or high lipid levels. In such settings, the presence of ≥ 2 cardiac AAbs, or MYH6 AAb alone, are a strong risk factors for developing for developing a cardiovascular event after taking into account (“adjusting for”) traditional cardiovascular risk factors.

[00109] In some embodiments, the increased risk for developing a first or recurrent cardiovascular disease event persists after adjustment for traditional cardiovascular risk factors selected from one or more of the following: age, sex, duration of diabetes, HbA1c levels, systolic blood pressure, diabetic kidney disease, retinopathy, cardiac autonomic neuropathy, pulse rate, smoking, triglycerides, and LDL cholesterol levels.

[00110] In some embodiments, a subject who does not have other risk factors for a developing a cardiovascular event (such as uncontrolled hypertension or high lipid levels) is identified as having risk of a cardiovascular event based on the presence of MYH6 AAbs.

[00111] In some embodiments, the present methods improve a clinician's ability to identify patients at high risk of a cardiovascular disease event using MYH6 AAbs.

[00112] In some embodiments, the present methods identify subjects at risk of a major adverse cardiovascular event (MACE). As used herein, a "major adverse cardiovascular event" or "MACE" is cardiovascular event that is likely to lead to disability or death. In some embodiments, a MACE is a nonfatal MI, stroke, or death secondary to cardiovascular disease. In some embodiments, the methods described herein identify subjects at high risk of a nonfatal MI, stroke, or death secondary to cardiovascular disease. In some embodiments, a subject who does not have other risk factors for a MACE (such as uncontrolled hypertension or high lipid levels) is identified as having risk of a MACE based on the subject having cardiac AAbs.

C. Cardiac AAbs

[00113] Myosin heavy chain 6 (also known as MYH6; α -myosin; myosin-6; ASD3; MYHC; MYHCA; or α -MyHC) is the cardiac muscle-specific alpha (α) isoform of cardiac myosin heavy chain. SEQ ID NO: 1 provides a representative sequence of MYH6 (Uniprot Reference: P13533). A full-length MYH6 cDNA clone is also commercially available and used in some experiments described herein (Origene catalogue number SC309209).

[00114] The S1-fragment of MYH6 (subfragment 1 or motor domain) can be obtained by proteolytic cleavage of myosin, or by PCR techniques as used in the results described herein, and corresponds to amino acids 1-865 of SEQ ID NO:1. The myosin S1 head contains the ATPase, the actin-binding site which is regulated by ATP binding, a long lever arm, which moves actin past myosin by a swinging motion. The S1 head domain is key to cardiac muscle contraction. The S2-fragment of MYH6 (subfragment 2) is a domain of myosin important for flexibility of myosin and the PCR-generated fragment used here corresponds to amino acids 822-1327 of SEQ ID NO:1.

[00115] Light meromyosin (LMM) of MYH6 mediates the dimerization of myosin heavy chains to form myosin and corresponds to amino acids 1237-1940 of SEQ ID NO: 1. The LMM is the tail or backbone portion of the molecule, which intertwines with the tails of other myosin molecules to form a thick filament.

[00116] Myosin heavy chain 7 (also known as MYH7; myosin-7; CMH1; MPD1; SPMD; SPM; CMD1S; MYHCB; CMYP7A; CMYP7B) is the beta (β) isoform of cardiac myosin heavy chain. SEQ ID NO: 2 provides a representative sequence of MYH7 (NCBI

Reference Sequence: NP_000248.2). The amino acid sequence of MYH7 is 93% identical to that of MYH6.

[00117] In some embodiments, the AAb is an antibody that binds to troponin, a protein involved in heart muscle contraction. In some embodiments, the troponin is cardiac troponin I (troponin I3, cardiac type; CMH7; RCM1; cTnI; CMD2A; TNNC1; CMD1FF). SEQ ID NO: 3 provides a representative sequence of cTnI (NCBI Reference Sequence: NP_000354.4).

[00118] AAbs to MYH6 can bind to any region of the protein. In some embodiments, AAbs only bind to the S1-fragment or the S2-fragment of MYH6. In some embodiments, the AAbs that bind to MYH6 bind to all or part of the S1- and/or S2-fragments of MYH6.

[00119] In some embodiments, AAbs are identified if the levels of AAbs are above the 99th percentile of index values obtained from healthy control subjects. In other words, the presence of AAbs may be defined as a levels of AAbs that is greater than the level of the same AAbs in 99% of control subjects.

[00120] In some embodiments, a subject having cardiac AAbs has AAbs specific to full-length MYH6 AAbs. In some embodiments, a patient having cardiac AAbs has AAbs specific to full-length MYH6, as well as AAbs to other antigens expressed in cardiac tissue.

[00121] In some embodiments, a subject having cardiac AAbs has two or more cardiac AAbs selected from those that are specific to full-length MYH6; the S1-fragment of MYH6; the S2-fragment of MYH6; full-length MYH7; or full-length cTnI. In some embodiments, a patient has AAbs specific to full-length MYH6, as well as at least one AAb specific to the S1-fragment of MYH6; the S2-fragment of MYH6; full-length MYH7; or full-length cTnI. In some embodiments, a patient does not have AAbs specific to full-length MYH6, and has two or more AAbs specific to S1-fragment of MYH6; the S2-fragment of MYH6; full-length MYH7; or full-length cTnI.

[00122] In some embodiments, identifying AAbs comprises measuring whether AAbs bind to an antigen comprising all or part of recombinantly-produced full-length MYH6. In some embodiments, identifying AAbs comprises measuring whether AAbs bind to recombinantly produced S1-fragment of MYH6. In some embodiments, identifying AAbs comprises measuring whether AAbs bind to recombinantly produced S2-fragment of MYH6. In some embodiments, identifying AAbs comprises measuring whether AAbs bind to recombinantly produced full-length MYH7. In some embodiments, identifying AAbs comprises measuring whether AAbs bind to recombinantly produced full-length cTnI.

[00123] The present assay is not limited to the method of measuring cardiac AAbs. In some embodiments, identifying cardiac AAbs comprises the use of a fluid-phase

radioimmunoprecipitation assay, as described herein. In some embodiments, having a cardiac AAb is defined as having an AAb index above the threshold for positivity based on predetermined cutoff values at the 99th percentile of the levels in serum samples from healthy control subjects. In some embodiments, the cardiac AAb index is defined as $[\text{counts per million (CPM) in the unknown sample} - \text{CPM in the negative control standard}] / [\text{CPM in the positive standard} - \text{CPM in the negative standard}] \times 100$ in a fluid-phase radioimmunoprecipitation assay. In some embodiments, the cutoff for having full-length MYH6 AAbs is about 0.701. In some embodiments, the cutoff for having S1-fragment MYH6 AAbs is about 0.330. In some embodiments, the cutoff for having S2-fragment MYH6 AAbs is about 0.763. In some embodiments, the cutoff for having full-length MYH7 AAbs is about 0.799. In some embodiments, the cutoff for having full-length cTnI AAbs is about 0.283.

D. Proinflammatory and genetic biomarkers

[00124] Proinflammatory and genetic biomarkers may be evaluated in the affected individual having cardiac AAbs. For example, the presence of AAbs to MYH6 and/or other cardiac AAbs may indicate that a subject is at risk for a first or recurrent cardiovascular disease event, but the presence in addition of one or more proinflammatory and genetic biomarkers can indicate that the subject is at even higher risk. In some embodiments, a subject is at a higher risk for a first or recurrent cardiovascular disease event when they have AAbs and a proinflammatory or genetic biomarker as compared to a subject identified with only AAbs.

[00125] In some embodiments, a method of identifying subjects at high risk for a first or recurrent cardiovascular disease events comprises evaluating the presence of AAbs and further comprises evaluating additional cardiac AAb-linked genetic and proinflammatory cytokine risk markers in the subject. In some embodiments, additional cardiac AAb-linked genetic and proinflammatory cytokine risk markers comprise the presence of the HLA genotype, DQ2 or DQ8; the presence of elevated levels of one or more proinflammatory cytokines, wherein the one or more proinflammatory cytokines are selected from IL-6, GM-CSF, TNF- α , G-CSF, EGF, sCD40L, CCL2, IL-8, IL-12, CCL7, IFN-gamma, and CCL22, and wherein the elevated levels of the one or more proinflammatory cytokine are compared to levels in subjects with T1D not having cardiac AAbs; and/or decreased IL-10 levels, wherein the decreased levels of IL-10 are compared to levels in subjects with T1D not having cardiac AAbs and without the additional linked genetic and proinflammatory cytokine risk markers. In some embodiments, a subject having AAbs and one or more genetic or proinflammatory

cytokine risk marker has a higher risk for a first or recurrent cardiovascular disease event than a subject identified with only AAbs.

1. Proinflammatory biomarkers

[00126] In some embodiments, an additional risk marker is one or more proinflammatory cytokines. As used herein, a “proinflammatory cytokine” is a signaling molecule that is secreted from a cell of the body and that promotes inflammation. In some embodiments, a proinflammatory cytokine is secreted from a type of immune cell called a CD4 T cell. In some embodiments, subjects with an elevated levels of proinflammatory cytokines together with the presence of AAbs are at increased risk of a cardiovascular disease event compared to subjects with AAbs.

[00127] In some embodiments, the one or more proinflammatory cytokines are selected from IL-6 (interleukin 6: Gene ID: 3569), GM-CSF (colony stimulating factor 2: Gene ID: 1437), TNF- α (tumor necrosis factor: Gene ID: 7124), G-CSF (colony stimulating factor 3: Gene ID: 1440), EGF (epidermal growth factor: Gene ID: 1950), sCD40L (soluble CD40 ligand: Gene ID: 959), CCL2 (C-C motif chemokine ligand 2: Gene ID: 6347), IL-8 (interleukin 8 or C-X-C motif chemokine ligand 8: Gene ID: 3576), IL-12 (interleukin 12: Gene ID: 3593), CCL7 (C-C motif chemokine ligand 7: Gene ID: 6354), IFN-gamma (interferon gamma: Gene ID: 3458), and CCL22 (C-C motif chemokine ligand 22: Gene ID: 6367).

[00128] In some embodiments, the one or more proinflammatory and genetic biomarker comprises elevated levels of one or more proinflammatory cytokines, wherein the one or more proinflammatory cytokines are selected from IL-6, GM-CSF, TNF- α , G-CSF, EGF, sCD40L, CCL2, IL-8, IL-12, CCL7, IFN-gamma, and CCL22, and wherein the elevated levels of the one or more proinflammatory cytokine are compared to levels in a subject with T1D not having cardiac AAbs. In some embodiments, the elevated levels of one or more proinflammatory cytokine are 2-times, 3-times or greater, 5-times or greater, 7-times or greater, or 10-times or greater, as compared to levels of the same cytokines in a subject with T1D not having cardiac AAbs and without the additional linked genetic and proinflammatory cytokine risk markers.

[00129] In some embodiments, the subject having elevated levels of one or more proinflammatory cytokine together with having cardiac AAbs specific to full-length MYH6 indicates an increased risk of a first or recurrent cardiovascular disease event in a subject with T1D as compared to AAbs to full-length MYH6 alone. In some embodiments, the subject

having elevated levels of one or more proinflammatory cytokine together with having two or more cardiac AAbs, wherein the AAbs are selected from those specific to full-length MYH6, the S1-fragment of MYH6, the S2-fragment of MYH6, full-length MYH7, or full-length cTnI, indicates an increased risk of a first or recurrent cardiovascular disease event in a subject with T1D as compared to having said AAbs alone.

[00130] In some embodiments, the elevated levels of IL-6 and GM-CSF are 2-times or greater, 3-times or greater, 5-times or greater, 7-times or greater, or 10-times or greater, as compared to healthy control subjects or subjects with T1D not having said cardiac AAbs.

[00131] In some embodiments, adding evaluating GM-CSF and IL-6 to a cardiovascular risk prediction model further improves the prediction of a first or recurrent cardiovascular disease event compared with a model containing full-length MYH6 AAbs alone. In some embodiments, adding evaluating GM-CSF and IL-6 to a cardiovascular risk prediction model further improves the prediction of a first or recurrent cardiovascular event compared with a model containing two or more cardiac AAbs alone, wherein the two or more cardiac AAbs are selected from those that are specific to full-length MYH6; S1-fragment of MYH6; S2-fragment of MYH6; full-length MYH7; or full-length cTnI.

[00132] The method of any one of claims 7-12, wherein the elevated levels of IL-6 and GM-CSF are 2-times or greater, 3-times or greater, 5-times or greater, 7-times or greater, or 10-times or greater than a control subject.

[00133] In some embodiments, a subject has cardiac AAbs and elevated GM-CSF levels. In some embodiments, a subject has cardiac AAbs and elevated levels of both IL-6 and GM-CSF. As described herein, subjects having cardiac AAbs and elevated levels of IL-6 and/or GM-CSF may be treated with agents that inhibit the effects of IL-6 and/or GM-CSF.

E. Genetic risk markers

[00134] In some embodiments, a subject having cardiac AAbs has a specific human leukocyte antigen (HLA) genotype. In some embodiments, a specific HLA genotype is associated with an increased risk of cardiovascular disease events in a subject having cardiac AAbs.

[00135] HLA-DQ2 and HLA-DQ8, are known to be more common in people with T1D. In contrast, the present findings identified that subjects with AAbs to MYH6 and the presence of a HLA-DQ2 allele have higher risk of a cardiovascular disease event. In some embodiments, the HLA-DQ2 allele is a genetic risk marker. In some embodiments, the HLA-DQ2 allele is DQ2.5 (DQA1*05:01/DQB1*02:01). In some embodiments, a subject with a

HLA-DQ2.5 homozygous genotype is at increased risk of developing a first or recurrent cardiovascular disease event than a subject with HLA-DQ2.5 heterozygous genotype.

[00136] In some embodiments, a subject having the HLA-DQ2 allele together with cardiac AAbs MYH6 indicates an increased risk of a first or recurrent cardiovascular disease event in a subject with T1D as compared to a subject having T1D and not having said cardiac AAbs. In some embodiments, the cardiac AAbs are MYH6. In some embodiments, the cardiac AAbs are two or more cardiac AAbs selected from those that are specific to full-length MYH6; S1-fragment of MYH6; S2-fragment of MYH6; full-length MYH7; or full-length cTnI.

[00137] In some embodiments, the subject has an HLA-DQ8 allele. In some such embodiments, the subject has childhood-onset T1D. In some embodiments, the HLA-DQ8 allele is (DQA1*03:01-DQB1*03:02). In some embodiments, a subject with a HLA-DQ8 homozygous genotype is at increased risk of developing a first or recurrent cardiovascular disease event than a subject with HLA-DQ8 heterozygous genotype.

[00138] In some embodiments, a subject having the HLA-DQ8 allele together with cardiac AAbs MYH6 indicates an increased risk of a first or recurrent cardiovascular disease event in a subject with T1D as compared to a subject having T1D and not having said cardiac AAbs. In some embodiments, the cardiac AAbs are MYH6. In some embodiments, the cardiac AAbs are two or more cardiac AAbs selected from those that are specific to full-length MYH6; S1-fragment of MYH6; S2-fragment of MYH6; full-length MYH7; or full-length cTnI. In some such embodiments, the subject has childhood-onset T1D.

II. Selection of subjects for clinical trials based on cardiac AAbs

[00139] In some embodiments, methods described herein of predicting risk of cardiovascular disease events can also be used to include or exclude subjects from a clinical trial. In some embodiments, a method of selecting subjects to include in a clinical trial for the treatment or prevention of a cardiovascular disease event comprises determining that the subject as having an increased risk of a first or recurrent cardiovascular disease event using any of the methods described herein and including the subjects in the clinical trial based on this increased risk. In some embodiments, a method of selecting subjects to include in a clinical trial for the treatment or prevention of a cardiovascular disease event comprises identifying the subject as having AAbs that specific to full-length MYH6 (SEQ ID NO: 1), plus or minus any of the above genetic or inflammatory biomarkers described herein, in the blood and including the subject from the clinical trial. In some embodiments, clinicians

choose to include subjects with T1D and having cardiac AAbs specific to full-length MYH6 due to their increased risk of a cardiovascular disease event. In some embodiments, subjects with T1D and having cardiac AAbs specific to full-length MYH6 are included in clinical trials of one or more treatments described herein. In some embodiments, clinicians may include the presence of cardiac antibodies specific to full-length MYH6 in inclusion or exclusion criteria for a clinical trial.

[00140] In some embodiments, a method of selecting subjects to include in a clinical trial for the treatment or prevention of a cardiovascular disease event comprises identifying the subject as having two or more cardiac AAbs, wherein the two or more cardiac AAbs are selected from those specific to full-length MYH6; the S1-fragment of MYH6; the S2-fragment of MYH6; full-length myosin heavy chain 7 (MYH7, SEQ ID NO: 2); or full-length cardiac troponin I (cTnI, SEQ ID NO: 3), plus or minus any of the above genetic or inflammatory biomarkers described herein, in the blood and including the subject from the clinical trial. In some embodiments, clinicians choose to include subjects with T1D and having two or more cardiac AAbs, wherein the two or more cardiac AAbs are selected from those specific to full-length MYH6; the S1-fragment of MYH6; the S2-fragment of MYH6; full-length MYH7; or full-length cTnI due to their increased risk of a cardiovascular disease event. In some embodiments, subjects with T1D and having two or more cardiac AAbs, wherein the two or more cardiac AAbs are selected from those specific to full-length MYH6; the S1-fragment of MYH6; the S2-fragment of MYH6; full-length MYH7; or full-length cTnI are included in clinical trials of one or more treatments described herein. In some embodiments, clinicians may include the presence of two or more cardiac AAbs, wherein the two or more cardiac AAbs are selected from those specific to full-length MYH6; the S1-fragment of MYH6; the S2-fragment of MYH6; full-length MYH7; or full-length cTnI, in inclusion or exclusion criteria for a clinical trial.

III. Treatment or prevention of cardiovascular disease events in subjects with cardiac AAbs

[00141] In some embodiments, subjects identified to have a risk of cardiovascular disease events using a method described herein are administered one or more treatments for treating or preventing a cardiovascular disease event.

[00142] In some embodiments, subjects having cardiac AAbs specific to full-length MYH6 will determine which subjects will be administered a treatment for treating or preventing a cardiovascular disease event. In some embodiments, a clinician selects the most

appropriate treatment for a subject based on whether the subject has cardiac AAbs specific to full-length MYH6, using a method described herein.

[00143] In some embodiments, subjects having cardiac AAbs specific to two or more cardiac AAbs selected from those specific to full-length MYH6; the S1-fragment of MYH6; the S2-fragment of MYH6; full-length MYH7; or full-length cTnI will determine which subjects will be administered a treatment for treating or preventing a cardiovascular disease event. In some embodiments, a clinician selects the most appropriate treatment for a subject based on whether the subject has two or more cardiac AAbs selected from those specific to full-length MYH6; the S1-fragment of MYH6; the S2-fragment of MYH6; full-length MYH7; or full-length cTnI, using a method described herein.

[00144] In some embodiments, subjects with one or more previous cardiovascular disease events are evaluated for whether they have cardiac AAbs.

A. Subjects with T1D

[00145] In some embodiments, a method of preventing or treating a cardiovascular disease event in a subject with T1D comprises determining that the subject as having an increased risk of a first or recurrent cardiovascular disease event using a method as described herein, and administering one or more treatments for preventing or treating a cardiovascular disease event.

[00146] In some embodiments, before treatment, a subject is also evaluated for one additional cardiac AAb-linked genetic and proinflammatory cytokine risk markers, as described herein. In some embodiments, the additional cardiac AAb-linked genetic and proinflammatory cytokine risk markers comprise the presence of the HLA genotype DQ2 or DQ8; the presence of elevated blood levels of one or more proinflammatory cytokines, wherein the one or more proinflammatory cytokines are selected from: IL-6, GM-CSF, TNF- α , G-CSF, EGF, sCD40L, CCL2, IL-8, IL-12, CCL7, IFN-gamma, and CCL22, and wherein the elevated levels of the one or more proinflammatory cytokines are compared to levels in subjects with T1D not having said cardiac AAbs; and the presence of decreased IL-10 levels, wherein the decreased levels of IL-10 are compared to levels in subjects with T1D not having said cardiac AAbs and without additional linked genetic and proinflammatory cytokine risk markers. In some embodiments, evaluating cardiac AAbs and additional cardiac AAb-linked genetic and proinflammatory cytokine risk markers will determine which subjects will be administered a treatment for treating or preventing a cardiovascular disease event.

[00147] In some embodiments, the subject with HLA genotype DQ2 (e.g., DQ2.5) was more than 18 years old at the onset of T1D. In some embodiments, the subject with HLA genotype DQ8 was less than 18 years old at the onset of T1D.

B. T1D Treatments

[00148] In some embodiments, appropriate treatments for subjects with T1D and cardiac AAbs include those to reduce an immune response. In some embodiments, the one or more treatments comprise inhibiting the effect of one or more proinflammatory cytokines or inhibiting the autoimmune disease process itself. In some embodiments, inhibiting the autoimmune process comprises inhibiting T-cell activation.

[00149] In some embodiments, a subject having cardiac AAbs is treated with an agent to inhibit the effects of IL-6 or GM-CSF. In some embodiments, a subject having cardiac AAbs and elevated levels of IL-6 and/or GM-CSF is treated with an agent to inhibit the effects of IL-6 or GM-CSF. In some embodiments, a patient is treated with an agent to inhibit the effects of IL-6 or GM-CSF if they have cardiac AAbs and also show elevated levels of IL-6 or GM-CSF. In some embodiments, subjects having cardiac AAbs and also show elevated levels of IL-6 or GM-CSF have a level of overall increased inflammation and are treated with an agent to reduce inflammation.

[00150] In some embodiments, the one or more treatments comprise administration of an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor of T-cell activation, B-cell antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6. In some embodiments, a subject with MYH6 and elevated levels of IL-6 is treated with an IL-6 inhibitor. In some embodiments, the IL-6 ligand inhibitor is sirukumab, olokizumab, clazakizumab, vobarilizumab, or ziltivekimab. In some embodiments, the IL-6 receptor inhibitor is sarilumab or tocilizumab.

[00151] In some embodiments, the GM-CSF inhibitor is mavrimumab, otilimab, namilumab, or lenzilumab. Mavrimumab targets the GM-CSF α receptor; whereas otilimab, namilumab, and lenzilumab bind directly to GM-CSF. In some embodiments, a subject having cardiac AAbs and elevated levels of GM-CSF is treated with a GM-CSF inhibitor.

[00152] In some embodiments, the JAK1/JAK2 inhibitor is baricitinib. In some embodiments, the inhibitor of T-cell activation is an anti-CD3 antibody or a CTLA4-Ig fusion protein. In some embodiments, the anti-CD3 antibody is teplizumab. In some embodiments, the CTLA4-Ig fusion protein is abatacept or the low-dose IL-2 is aldesleukin.

[00153] In some embodiments, treatment is a single-agent treatment. In some embodiments, treatment is with more than one agent. For all treatments described herein, treatments may be in addition to treatment for symptoms directly related to diabetes, such as insulin for glucose control.

[00154] In some embodiments, the treatment is a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6. As used herein, a “tolerogenic therapy” is one to induce tolerance and reduce autoimmunity to an antigen. Such types of tolerogenic therapies may also be termed “autoantigen therapies” and have been described^{16, 50}. In some embodiments, the treatment is a tolerogenic vaccine, wherein the vaccine is administered to reduce the immune response to an antigen comprised in the vaccine. In some embodiments, a treatment to inhibit the development of AAbs that bind to MYH6 comprises a tolerogenic therapy, wherein the antigen comprises all or part of MYH6. In some embodiments, the antigen is a recombinantly expressed protein. A tolerogenic therapy may be used with another treatment described herein or together with an agent for improving glucose control, such as insulin. In some embodiments, treatment of a subject cardiac AAbs with a tolerogenic therapy reduces or blocks the development of more of these AAbs.

[00155] In some embodiments, the antigen for a tolerogenic therapy is conjugated or delivered in nanoparticles or is a modified mRNA vaccine. A variety of means for antigen deliver to restore tolerance are described in⁵¹, which is incorporated herein in its entirety, and any of these delivery methods may be used.

[00156] In some embodiments, a method comprises identifying that a subject has cardiac AAb and also has subject also has the HLA DR3-DQ2 haplotype. In some embodiments, a method of preventing a first or recurrent cardiovascular disease event in a subject with T1D comprises determining that the subject as having an increased risk of a first or recurrent cardiovascular disease event using a method described herein, wherein the subject expresses the HLA DR3-DQ2 haplotype, or HLA-DQ8 haplotype, and administering a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6, wherein the tolerogenic therapy restores immune tolerance to MYH6 and thereby prevents a first or recurrent cardiovascular disease event. In some embodiments, the HLA-DQ2 genotype is DQ2.5 (DQA1*05:01/DQB1*02:01). In some embodiments, a subject has a HLA-DQ2.5 homozygous genotype. In some embodiments, the subject has a HLA-DQ8 haplotype.

C. Subjects undergoing cancer immunotherapy treatment

[00157] In some embodiments, methods herein comprise detecting cardiac AAbs in a subject treated with at least one immune checkpoint inhibitor (ICI). In some embodiments, the subject is a cancer patient. In some embodiments, the subject is a cancer patient prescribed at least one immune checkpoint inhibitor (ICI) therapy for treatment of the cancer. In some embodiments, the subject is at increased risk for developing or has developed an immune-related adverse event (Postow N Engl J Med, 378: 158; 2018) such as myocarditis (Johnson N Engl J Med 2016;375:1749-55). In some embodiments, the methods are performed to identify whether a subject treated with at least one immune checkpoint inhibitor is at higher risk of developing or has a suspected cardiovascular disease event. In some embodiments, the cardiovascular disease event is a cardiac toxicity such as myocarditis. In some embodiments the cardiovascular disease event is an atherosclerotic cardiovascular disease event. In some embodiments, the subject with cardiac AAbs is at increased risk of developing an atherosclerotic cardiovascular disease event. In some embodiments, the methods are performed to identify subjects in need of treatment with an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor of T-cell activation, a B-cell antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6.

[00158] Examples of immune checkpoint inhibitors (ICI) herein include but are not limited to antibodies and fusion proteins with antigen-binding domains that bind to inhibitory immune checkpoint molecules and combinations thereof (e.g., anti-PD-L1 antibodies, anti-CTLA4 antibodies, and anti-PD-1 antibodies or small molecule inhibitors of PD-L1, PD-1, or CTLA4). Further examples of immune checkpoint inhibitors include but are not limited to anti-PD-L2 antibodies, anti-PD-1 antibodies, anti-B7-H3 antibodies, anti-B7-H4 antibodies, anti-HVEM antibodies, anti-B- and T-lymphocyte attenuator (BTLA) antibodies, anti-Killer inhibitory receptor (KIR) antibodies, anti-GAL9 antibodies, anti-TIM-1 antibodies, anti-TIM3 antibodies, anti-TIM-4 antibodies, anti-A2AR antibodies, anti-CD39 antibodies, anti-CD73 antibodies, anti-LAG-3 antibodies, anti-phosphatidylserine antibodies, anti-CD27 antibodies, anti-CD30 antibodies, anti-TNF α antibodies, anti-CD33 antibodies, anti-Siglec-5 antibodies, anti-Siglec-7 antibodies, anti-Siglec-9 antibodies, anti-Siglec-11 antibodies, anti-TREM1 antibodies, anti-TREM2 antibodies, anti-TIGIT antibodies, anti-VISTA antibodies, anti-CD2 antibodies, and anti-CD5 antibodies. Additional examples of immune checkpoint inhibitors include but are not limited to small molecules that affect the activity of one or more of PD-L2, PD-1, B7-H3, B7-H4, HVEM, B- and T-lymphocyte attenuator (BTLA), Killer

inhibitory receptor (KIR), GAL9, TIM-1, TIM3, TIM-4, A2AR, CD39, CD73, LAG-3, phosphatidylserine, CD27, CD30, TNF α , CD33, Siglec-5, Siglec-7, Siglec-9, Siglec-11, TREM1, TREM2, TIGIT, VISTA, CD2, and CD5. Additional examples of immune checkpoint inhibitors include therapies that deliver such immune checkpoint inhibitors to the subject, such as certain cancer vaccines or cell therapies, which for example, may provide nucleic acids, vaccines or cells expressing one or more immune checkpoint inhibitor fusion protein or antibody. In some embodiments, the at least one immune checkpoint inhibitor is an anti-PD-1 antibody, an anti-CTLA4 antibody, or a combination of an anti-PD-1 antibody and an anti-CTLA4 antibody. In some embodiments, the immune checkpoint inhibitor is an anti-PD-L1 antibody.

[00159] In some embodiments, a subject treated with ICI has cardiac AAbs specific to two or more cardiac AAbs selected from those specific to full-length MYH6; the S1-fragment of MYH6; the S2-fragment of MYH6; full-length MYH7; or full-length cTnI. In some embodiments the subject is a cancer patient. Thus, in some embodiments, the presence of or a high relative level of such two or more cardiac Aabs compared to a reference population of subjects treated with ICI may be used to determine whether the subject should receive a treatment for treating or preventing a cardiovascular disease event such as myocarditis. In some such embodiments, a clinician may select a treatment for the subject based on whether the subject has two or more cardiac AAbs selected from those specific to full-length MYH6; the S1-fragment of MYH6; the S2-fragment of MYH6; full-length MYH7; or full-length cTnI, using results from a method described herein.

[00160] Myocarditis (also called ICI-related myocarditis in subjects receiving ICI) is one of the most serious adverse events that occurs as a result of immune checkpoint inhibition, and it can be fatal. (See e.g., Wang et al., *JAMA Oncol* 4(12):1721–1728, (2018), Axelrod et al., *Nature* 611(7937):818–826 (2022)). In some embodiments, the methods described herein are used to predict the risk of myocarditis or to aid in the diagnosis of (and serve as a biomarker of) suspected myocarditis in cancer patients treated with ICI. For example, there is a need in the art for noninvasive biomarkers for predicting and aiding in the diagnosis of severe adverse events. For example, myocarditis currently is often identified by costly or invasive procedures such as magnetic resonance imaging (MRI) or cardiac biopsy. In some embodiments, presence of cardiac AAbs specific to full-length MYH6 is an early biomarker of a cardiovascular disease event, such as a cardiac toxicity, such as myocarditis, or a high level of cardiac AAbs specific to full-length MYH6 compared to the level in a reference population of subjects treated with ICI identifies a subject at risk of developing or

who has a suspected cardiovascular disease event, such as a cardiac toxicity, such as myocarditis, or who should receive treatment typically given to subjects with suspected myocarditis or at risk of developing myocarditis. Such information, for example, may be used to determine whether a subject should be administered such a treatment. In some embodiments, a clinician may select an appropriate treatment for a subject based on whether the subject has cardiac AAbs specific to full-length MYH6 or a high relative level of such cardiac AAbs compared to a reference population of subjects treated with ICI, using the results from a method described herein.

[00161] Associations between ICI treatment and higher risk of cardiovascular disease events have also been found. (*See, e.g., Z.D. Drobni, et al., Circulation* 142: 2299-2311 (2020).) For example, Drobni et al. found a 3-fold higher risk for cardiovascular disease events in patients with a range of cancers treated with ICI compared to those not treated with ICI, noting that cardiovascular events were higher after initiation of ICI treatment in the subjects, potentially mediated by accelerated progression of atherosclerosis. Specifically, there was a more than 3-fold increase in the rate of atherosclerotic plaque progression after initiation of ICI therapy compared to cancer patients who did not receive ICI treatment. (*Id.*, at 2299 and 2305.)

[00162] In some embodiments, the treatment provided to subjects with cardiac AAbs, for example, to treat or prevent suspected myocarditis, comprises administration of an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor of T-cell activation, B-cell antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6. In some embodiments, a subject with MYH6 and elevated levels of IL-6 is treated with an IL-6 inhibitor. In some embodiments, the IL-6 ligand inhibitor is sirukumab, olokizumab, clazakizumab, vobarilizumab, or ziltivekimab. In some embodiments, the IL-6 receptor inhibitor is sarilumab or tocilizumab. In some embodiments, the GM-CSF inhibitor is mavrilimumab, otilimab, namilumab, or lenzilumab. Mavrilimumab targets the GM-CSF α receptor; whereas otilimab, namilumab, and lenzilumab bind directly to GM-CSF. In some embodiments, a subject having cardiac AAbs and elevated levels of GM-CSF is treated with a GM-CSF inhibitor. In some embodiments, the JAK1/JAK2 inhibitor is baricitinib. In some embodiments, the inhibitor of T-cell activation is an anti-CD3 antibody or a CTLA4-Ig fusion protein. In some embodiments, the anti-CD3 antibody is teplizumab. In some embodiments, the CTLA4-Ig fusion protein is abatacept or the low-dose IL-2 is aldesleukin.

[00163] In some embodiments a subject treated with ICI, such as a cancer patient, does not have T1D. In other cases, a subject treated with ICI, such as a cancer patient, also has T1D, or another ICI-related autoimmune disease.

[00164] In some embodiments, subjects with one or more previous or concurrent cardiovascular disease events are evaluated for whether they have cardiac AAbs.

[00165] In some embodiments, a method of preventing or treating a cardiovascular disease event (e.g. myocarditis) in a subject treated with ICI, such as a cancer patient, comprises determining that the subject has an increased risk of a first or recurrent cardiovascular disease event using a method as described herein and administering one or more treatments for preventing or treating a cardiovascular disease event such as myocarditis.

[00166] In some embodiments, before treatment, a subject is also evaluated for one additional cardiac AAb-linked genetic and proinflammatory cytokine risk markers, as described herein. In some embodiments, the additional cardiac AAb-linked proinflammatory cytokine risk markers comprise the presence of elevated blood levels of one or more proinflammatory cytokines, wherein the one or more proinflammatory cytokines are selected from: IL-6, GM-CSF, TNF- α , G-CSF, EGF, sCD40L, CCL2, IL-8, IL-12, CCL7, IFN-gamma, and CCL22, and wherein the elevated levels of the one or more proinflammatory cytokines are compared to levels in subjects treated with immune-checkpoint inhibitors not having cardiac AAbs; and the presence of decreased IL-10 levels, wherein the decreased levels of IL-10 are compared to levels in subjects treated with immune-checkpoint inhibitors not having cardiac AAbs and without proinflammatory cytokine risk markers. In some embodiments, evaluating cardiac AAbs and additional cardiac AAb-linked proinflammatory cytokine risk markers will determine which subjects will be administered a treatment for treating or preventing a cardiovascular disease event (e.g., myocarditis, e.g., ICI myocarditis).

IV. Prevention of the development of AAbs to myosin heavy chain

[00167] In some embodiments, treatment may block the development of AAbs to MYH6 in subjects who do not already have them. In some embodiments, blocking the development of AAbs to MYH6 or blocking upstream T-cell responses to MYH6/alpha-myosin that drive the production of MYH6 AAbs in subjects with recent onset T1D could block or reduce the subject's risk of cardiovascular disease events.

[00168] In some embodiments, a treatment inhibits the development of AAbs that bind to MYH6 in a subject who does not already have such AAbs.

[00169] In some embodiments, a method of preventing a cardiovascular disease event in a subject with recent-onset T1D (1) identifying a subject with recent-onset T1D, (2) identifying the absence of AAbs that bind to MYH6 (SEQ ID NO: 1) in the blood from the subject; and (3) administering a treatment to inhibit the development of MYH6 AAbs.

[00170] In some embodiments, a treatment to inhibit the development of MYH6 AAbs is a T-cell tolerogenic therapy. As such, a tolerogenic therapy as described herein can reduce autoimmunity to MYH6.

[00171] In some embodiments, a treatment to inhibit the development of AAbs that bind to MYH6 comprises a tolerogenic therapy specific for an antigen comprising all or part of MYH6. In some embodiments, a treatment to inhibit the development of AAbs that bind to MYH6 comprises a tolerogenic therapy, wherein the antigen comprises all or part of the LMM fragment of MYH6, S1-fragment, and/or S2-fragment. In some embodiments, a treatment to inhibit the development of AAbs that bind to MYH6 comprises a tolerogenic therapy, wherein the antigen comprises all or part of the LMM fragment of MYH6. In some embodiments, a treatment to inhibit the development of AAbs that bind to MYH6 comprises a tolerogenic therapy, wherein the antigen is a recombinantly expressed protein.

[00172] In some embodiments, a treatment to inhibit the development of AAbs that bind to MYH6 is administered to a subject wherein their HLA-DQ2 allele is DQ2.5. In some embodiments, the treatment to inhibit the development of AAbs that bind to MYH6 is selected based on the subject's HLA-DQ2 homozygous status.

[00173] In some embodiments, the elevated levels of one or more proinflammatory cytokines for determining subjects appropriate for treatment to inhibit the development of AAbs that bind to MYH6 are 2-times, 3-times or greater, 5-times or greater, 7-times or greater, or 10-times or greater, as compared to levels of the same cytokines in control subjects.

[00174] In some embodiments, the one or more proinflammatory cytokine for determining subjects appropriate for treatment to inhibit the development of AAbs that bind to MYH6 is IL-6 and/or GM-CSF. In some embodiments, a tolerogenic therapy is administered if the subject has elevated levels of IL-6 and/or GM-CSF.

Example 1. Risk of a first subsequent CVD event increases with increasing number of cardiac AAbs at baseline

[00175] A middle-aged cohort of 891 individuals with T1D who were free of CVD events at baseline were assessed. ²¹ One subject was excluded from further analysis compared to the data presented in the examples and figures of US Provisional Application No.

63/552,279, due to that subject having a different duration of follow-up after the initial study. Seventy-nine (79/891) (8.9%) subsequently developed the first of any CVD event (Table 2) and 37/891 (4.2%) developed MACE (Table 4), during a median 7.0 year follow-up.

Consistent with previous studies on this cohort, participants who developed CVD events were generally older and were more likely to be smokers, have a longer T1D duration, higher HbA1c levels and a greater prevalence of diabetic kidney disease, hypertension, cardiac autonomic neuropathy (CAN), retinopathy, and hyperlipidemia than those who remained free of CVD events.^{22,23} The prevalence of cardiac AAbs (to MYH6, MYH7, the S1-fragment of MYH6 (S1-MYH6), the S2-fragment of MYH6 (S2-MYH6), or cTnI)^{16,19} was markedly greater among subjects who had a first CVD event than among those who did not (34% vs. 15%, respectively, $P<0.0001$) (Table 2) and among those who developed a major adverse cardiovascular event (MACE) than among those who did not (41%, vs 15%, respectively, $P<0.0001$) (Table 4).

[00176] As shown in Fig. 1a, the cumulative incidence of a subsequent first CVD event increased with the number of cardiac AAbs present at baseline, with Kaplan–Meier estimates of 7% (52/746), 11% (11/102), and 36% (16/44) in participants with no AAbs, 1 AAb, and ≥ 2 AAbs, respectively (log-rank $P<0.0001$), over a maximum follow-up of 10 years. A similar pattern was observed for MACE, with estimates of 3%, 6%, and 20% respectively (Fig. 5). Of note, participants with ≥ 2 AAbs developed CVD events at a younger age than those without AAbs (mean age $\pm$ SD, 51 $\pm$ 5 vs 57 $\pm$ 6 years; $P=0.002$).

[00177] To assess the independent role of cardiac AAbs on CVD risk, Cox proportional-hazard models were performed that simultaneously controlled for risk factors associated with incident CVD events (Table 2) or MACE (Table 4) and the presence of cardiac AAbs (Tables 5-6). In an unadjusted model, positivity for a single AAb was not associated with risk of CVD events; however, the presence of ≥ 2 AAbs was associated with an extremely high risk of first CVD events (HR, 6.5; 95% CI, 3.7–11.5; $P<0.0001$) compared with no AAbs (Fig. 1c). This estimate was modestly reduced to 5.2 in a fully adjusted model including microalbuminuria/ESRD, retinopathy, and DCCT/EDIC time-updated mean systolic blood pressure, mean HbA1c, and mean LDL levels (95% CI, 2.9–9.4; $P<0.0001$) (Fig. 1c). Similarly, the presence of ≥ 2 AAbs was associated with a 7.7-fold HR for MACE (95% CI, 3.5–16.7; $P<0.0001$), which was reduced to 5.5 after adjustment for traditional cardiovascular risk factors (95% CI, 2.4–12.9; $P<0.0001$) (Table 6). Alternative models adjusting for other risk factors or potential confounders did not attenuate the effect of ≥ 2 AAbs on the risk of CVD (Tables 18 and 19) or MACE (Tables 20 and 21).

Example 2. AAbs to MYH6 are associated with the greatest risk of CVD events

[00178] In participants with only a single cardiac AAb, there was a striking difference in CVD risk according to which specific AAb type was present at baseline, with AAbs to MYH6 associated with the highest risk (log-rank $P=0.028$, Figure 6). In a competing risk regression to assess the independent role of each of the five cardiac AAb types on CVD risk, only MYH6 and cTnI AAbs remained in the model (Table 17).

[00179] As shown in Fig. 1b, the cumulative incidence of a first CVD event was 37% (15/41) in MYH6 AAb-positive subjects, compared to 8% (64/851) in MYH6 AAb-negative subjects (log-rank $P<0.0001$). Similar to participants with ≥ 2 AAbs, MYH6 AAb-positive participants were younger at the time of occurrence of a first CVD event compared to MYH6 AAb-negative participants (mean age, 53 ± 6 vs 57 ± 7 years, respectively $P=0.028$). In an unadjusted Cox regression model, MYH6 AAbs were associated with a markedly increased HR for first CVD events (HR, 5.9; 95% CI, 3.4–10.4; $P<0.0001$) (Fig. 1c). This HR was only slightly reduced to 5.3 (95% CI, 3.0–9.5; $P<0.0001$) (Fig. 1c) after adjusting for traditional cardiovascular risk factors associated with CVD and MYH6 AAbs (Tables 2 and 7), and was not further attenuated in other models (Table 22). Similar associations were found between MYH6 AAbs and incident MACE (Table 6, Table 21). In contrast, the risk associated with cTnI AAbs for composite CVD events was no longer significant after adjustment for traditional cardiovascular risk factors ($P=0.075$) (Fig. 1c, Tables 2 and 8). Of note, among participants with ≥ 2 AAbs, the majority (68%; 30/44) were positive for MYH6 AAbs (versus 27% for cTnI AAbs), suggesting that the effect of ≥ 2 AAbs on CVD risk was mostly due to MYH6 AAbs.

Example 3. MYH6 AAbs are HLA class II-linked

[00180] Since traditional cardiovascular risk factors did not explain the excess CVD risk associated with MYH6 AAbs, alternative mechanisms were evaluated. Among the susceptibility genes associated with autoimmune diseases, the strongest associations have been with HLA class II genes. Expression of the high T1D-risk molecule, HLA-DQ8 (*DQA1*03:01-DQB1*03:02*), in humanized transgenic mice was previously shown to result in the development of spontaneous autoimmune myocarditis with the production of AAbs against α - and β -myosin heavy chain (encoded by *Myh6* and *Myh7*).¹⁸

[00181] HLA-DQ typing was performed on 475 DCCT/EDIC participants, 146 with cardiac AAbs and 329 without cardiac AAbs. Unexpectedly, the presence of MYH6 AAbs was associated with another high T1D-risk genotype, HLA-DQ2, specifically *DQA1*05:01-DQB1*02:01*, that confers risk for a variety of autoimmune conditions in

addition to T1D.^{24,25} At least one DQ2 genotype was carried by 68% (28/41) of MYH6 AAb-positive subjects compared with 50% (218/434) of MYH6 AAb-negative subjects ($P=0.027$), with DQ2 homozygosity present in 27% (11/41) of MYH6 AAb-positive subjects versus 6% (26/434) in MYH6 AAb-negative subjects ($P=0.0001$) (Table 3). The presence of DQ2 in the absence of DQ8 was also strongly associated with MYH6 AAbs ($P=0.004$). Similar DQ2 associations were found with AAbs to MYH7, whose targeting appears to arise secondarily from epitope spreading starting from MYH6¹⁸ (Table 3). In contrast, no HLA-DQ associations were found with AAbs to cTnI (Table 3), S1- or S2-MYH6 (Table 9). These findings suggested an important role for CD4⁺ T cells in the pathogenesis of CVD events in MYH6 AAb-positive subjects.

Example 4. Correlation between the numbers and types of cardiac AAbs and the concentrations of circulating proinflammatory cytokines at baseline

[00182] To test whether the increased risk of CVD events was mediated by elevated circulating levels of the pro-atherogenic cytokine, IFN- γ ,²⁶⁻²⁸ that is abundantly produced by α -myosin-specific CD4⁺ T cells in the peripheral blood of T1D patients with multiple cardiac AAbs,^{16,18} an unbiased, multiplex assay was conducted to measure the concentrations of 42 cytokines/chemokines in the same baseline samples as was used for AAb measurements in 143 DCCT/EDIC participants with cardiac AAbs and 146 participants without cardiac AAbs. Owing to matching, the three AAb groups (0, 1 and ≥ 2 AAbs) were similar with respect to age, sex, and other cardiovascular risk factors, and showed similar proportions of incident CVD events compared to the larger cohort (Table 10, Figs. 1A-1C). Of the inflammatory mediators measured, five (IL-1 α , IL-3, IL-5, IL-9, and IL-13) had $>30\%$ measurements below the detection limit of the assay and were filtered out from subsequent analysis.

[00183] These studies revealed several notable findings (Fig. 2a). First, a stepwise increase in serum IFN- γ levels was seen with increasing cardiac AAb number (0, 1 and ≥ 2 AAbs; $P<0.001$ for trend) and in MYH6 AAb-positive subjects, confirming the original hypothesis.¹⁹ Second, progressive elevation in the levels of numerous other proinflammatory cytokines was seen with increasing cardiac AAb number, with particularly pronounced trends for TNF- α , G-CSF, EGF, sCD40L, and CCL22 ($P<0.001$ for trend). Conversely, the concentrations of the anti-inflammatory cytokine IL-10, a marker of T-regulatory cells that possesses multiple atheroprotective activities,²⁹⁻³¹ decreased across the AAb groups ($P=0.005$). Third, was the absolute magnitude of the proinflammatory cytokine elevations in the ≥ 2 AAb group, with many elevated at several-fold greater concentrations compared to the

T1D participants without AAbs. Fourth, although the cytokine profiles in the ≥ 2 and MYH6 AAb groups mostly overlapped, as was expected, the MYH6 AAb group showed particularly prominent (~ 7 -fold) elevations in IL-6, a pivotal mediator of CVD events in the general population,^{32,33} with median of levels of 22.7 pg/mL versus 3.2 pg/mL in subjects without AAbs ($P < 0.001$), and ~ 4 -fold elevated GM-CSF levels ($P < 0.001$, Fig. 2A). Conversely, the MYH6 AAb group showed reduced levels of IL-10, with median concentrations of 3.4 pg/mL versus 6.8 pg/mL in subjects with no AAbs ($P < 0.001$), suggesting inadequate negative feedback regulation to prevent MYH6-specific proinflammatory reactivity. Finally, Spearman's rank correlation analysis showed that while many of the elevated cytokines were strongly intercorrelated, the highest correlations were found between GM-CSF and IL-6, with $r_s = 0.85$ in the ≥ 2 AAb groups and $r_s = 0.73$ in the MYH6 AAb group. In contrast, IL-6 and GM-CSF were weakly correlated in participants with no AAbs ($r_s = 0.24$), 1 AAb ($r_s = 0.35$) or cTnI AAbs ($r_s = 0.37$). Of note, although many cytokines were elevated in the MYH6 and ≥ 2 AAb groups, they were skewed towards Th1 cytokines, with IL-4, IL-17, and IL-1 β levels similar to the no AAb group.

[00184] In contrast, the cTnI AAb group showed elevated levels of only a few cytokines, and lower levels of the proinflammatory cytokine, IL-15³⁴ (Fig. 2a), whereas participants with a single AAb showed similar proportions of elevated and reduced proinflammatory cytokines compared to the no AAb group. Intriguingly, considering the findings in Fig. 2a, along with the heatmap of all measurable cytokines shown in Fig. 2b, it appeared that the overall magnitude of the proinflammatory cytokine "load" at baseline across each of the AAb groups (no AAb, 1 AAb, ≥ 2 AAb; MYH6 AAb, and cTnI AAb) paralleled the risk of developing first CVD events (Figs. 1A-1C).

Example 5. Hierarchical cluster analysis of 12 core cytokines and first subsequent CVD events

[00185] These findings suggested that one or more of the cardiac AAb-linked circulating proinflammatory cytokines, many of which have been implicated in promoting atherosclerosis,³¹ contributed to the elevated risk of CVD events. As a first step, a hierarchical cluster analysis of the 12 "core" proinflammatory cytokines with significantly higher concentrations was performed, compared with the no AAb group, shared by the MYH6 AAb and ≥ 2 AAb groups (Figs. 2a, 2c). As shown in Fig. 2c, four clusters were identified, with the highest levels of proinflammatory cytokines in Cluster 1 (C1) and the lowest levels in Cluster 4 (C4). Strikingly, among the 13 CVD events occurring in the highest proinflammatory Cluster 1, 12 (92%) occurred in participants with ≥ 2 AAb. Similarly, among

the 15 CVD events occurring in Cluster 1 in the MYH6 AAb group, 14 (93%) occurred in participants positive for MYH6 AAbs. In both the MYH6 AAb and ≥ 2 AAb groups, the remaining CVD cases occurred in Cluster 2 (C2, Fig. 2c). Conversely, among the 146 control subjects without AAbs, the majority of CVD events occurred in Cluster 4 (C4), with a progressive decrease in the proportion of CVD events from Cluster 4 to Cluster 1.

Example 6. The effect of MYH6 AAbs on CVD risk is mediated primarily by IL-6 and GM-CSF

[00186] To identify which cytokine(s) shared by the MYH6 AAb and ≥ 2 AAb groups contributed the most to CVD risk, a series of analyses were performed adjusting for each of the 12 core cytokines added in one at a time, while controlling for traditional cardiovascular CVD risk factors. In this cytokine study (Fig. 2d), MYH6 AAb positivity was associated with an unadjusted 4.3-fold increased HR on subsequent first CVD events, compared to the absence of MYH6 AAbs (95% CI, 2.3–8.1; $P < 0.0001$). Similar to previous studies (Figs. 1A–1C), this estimate was modestly attenuated after adjustment for traditional cardiovascular risk factors (HR, 3.8; 95% CI, 2.0–7.3; $P < 0.0001$, Table 23). When further adjusted for each of the 12 core cytokines, most had minimal or modest effect on the model. However, the effect of MYH6 AAbs was markedly attenuated after adjustment for GM-CSF (HR, 2.7; 95% CI, 1.4–5.4; $P = 0.004$), with the greatest effect observed for IL-6 (HR, 2.3; 95% CI, 1.1–4.6; $P = 0.021$) (Fig. 2d). When both GM-CSF and IL-6 were included in the model, the risk of MYH6 AAbs for first CVD events became insignificant (HR, 1.5; 95% CI, 0.7–3.5; $P = 0.328$). This effect was specific to GM-CSF, with the other core cytokines showing minimal or no further attenuation when combined with IL-6 (Table 24). In contrast, the association between ≥ 2 AAbs and risk of CVD events (Fig. 2d) became non-significant after further adjustment for GM-CSF alone, with IL-6 having the second greatest attenuation on CVD risk.

Example 7. Cytokine profiles in MYH6 AAb-positive T1D subjects overlap with rheumatoid arthritis and COVID-19

[00187] The magnitude and breadth of the cytokine elevation in the MYH6 AAb-positive T1D participants – suggesting both innate and adaptive immune dysregulation – seemed to more closely resemble a systemic autoimmune disorder or acute infection than T2D, to which inflammation in T1D has been usually compared.¹³ To examine this possibility, serum cytokine levels were measured in three well-phenotyped cohorts: 1) rheumatoid arthritis (RA), an autoimmune disease characterized by high-grade systemic

inflammation and markedly elevated levels of IL-6 that is the target of inhibitors in clinical use for this condition^{35,36}; 2) severe acute coronavirus disease 2019 (COVID-19), that is also associated with cytokine dysregulation with IL-6^{37,38} and GM-CSF levels^{38,39} correlating with disease severity; and 3) T2D with obesity, that is associated with low-grade systemic inflammation.^{8,40} Since T1D participants without cardiac AAbs were previously used as a reference group (Fig. 2a), this analysis included healthy control subjects.⁴¹

[00188] The COVID-19 cohort⁴¹ was notable in having severe disease requiring hospitalization with 63% (20/32) requiring mechanical ventilation in the intensive care unit, and the remainder requiring supplemental oxygen (Table 11). All RA patients had moderate to severe disease activity, were seropositive for rheumatoid antibodies and were treated with methotrexate (Table 12). The T2D cohort was free of prior CVD events⁴² and is described in Table 13.

[00189] Remarkably, despite lacking a clinically apparent systemic inflammatory condition, the T1D MYH6 AAb-positive subjects showed similarly elevated levels of IL-6 to both RA and COVID-19, with GM-CSF levels similar to COVID-19 but slightly lower than those in RA (Fig. 3a). Numerous other proinflammatory cytokines, including CCL22, IFN- γ , IL-8, IL-12, sCD40L, G-CSF and MCP-3 and were at least as elevated in the T1D MYH6 AAb-positive subjects compared with either RA or COVID-19 (Fig. 3a, Table 14).

Interestingly, IL-6 and GM-CSF were among the most highly correlated cytokines in RA ($r_s = 0.79$) and COVID-19 ($r_s = 0.73$), similar to the MYH6 AAb-positive T1D subjects, above.

Despite these similarities, there were some distinct differences between the groups with elevated IL-1 β levels in RA and COVID-19, but not T1D subjects, who had IL-1 β levels similar to controls (Fig. 3a). It is interesting to note that T1D MYH6 AAb-positive and RA patients showed similar levels of CCL22 (Fig. 3a), which is regulated by GM-CSF.^{43,44} Remarkably, principal component analysis demonstrated almost complete overlap of cytokine profiles in T1D MYH6 AAb-positive participants with those found in RA, and partial overlap with COVID-19; however, there was no overlap with T2D (Fig. 3c).

Example 8. Different cytokine profiles in T1D and T2D

[00190] The T2D cohort showed relatively low levels of most cytokines except for IL-1 β (Fig. 3a) EGF, CCL2, and IL-18 (Table 14). Notably, IL-1 β and IL-18 were among the most highly correlated cytokines in T2D ($r_s = 0.65$) – consistent with the known role of the inflammasome and its key secreted products, IL-1 β and IL-18, in the pathogenesis of T2D⁹ – but were weakly correlated in RA ($r_s = 0.24$), COVID-19 ($r_s = 0.38$) and T1D MYH6 AAb-positives ($r_s = 0.32$). Conversely, IL-6 and GM-CSF were weakly correlated in T2D ($r_s =$

0.33). Despite the T1D-no AAb group being overweight with 38% being obese (BMI ≥ 30 kg/m²) (Table 10), their cytokine profiles were distinct from those found in T2D and mostly overlapped with healthy controls (Fig. 3c).

Example 9. Discovery of a potential autoimmune etiology (“endotype”) for CVD in T1D

[00191] These findings raised the possibility that a subgroup of individuals with T1D develop CVD of primary autoimmune etiology, defined by the presence of MYH6 AAbs. As previously shown, MYH6 AAb-positive patients had >5-fold increased risk of early CVD events compared to MYH6 AAb-negative subjects (Fig. 1b, Fig. 1c). MYH6 AAb positivity was also strongly associated with a particular DQ2 genotype, DQ2.5, *DQA1*05:01-DQB1*02:01* (Table 3).

[00192] When the analyses were restricted to the DCCT/EDIC participants who developed first CVD events, 87% of MYH6 AAb-positive subjects carried at least one DQ2 genotype, compared with 41% of MYH6 AAb-negative subjects ($P=0.002$) (Fig. 4a). The association was even more pronounced with DQ2 homozygosity, which was present in one third of MYH6 AAb-positive subjects versus none (0 of 64) of the MYH6 AAb-negative subjects ($P<0.001$) (Fig. 4a). Furthermore, either DQ2 or DQ8 was carried by 100% (15 of 15) of MYH6 AAb-positive subjects versus 75% (48 of 64) in subjects without MYH6 AAbs ($P=0.030$), (Fig. 4a). A striking HLA gene dosage effect was seen on MYH6 AAb levels (Fig. 4b), with at least one DQ2 genotype associated with a 1.8-fold higher MYH6 AAb levels (95% CI, 1.2–2.6; $P=0.002$), and DQ2 homozygosity associated with 9.0-fold higher MYH6 AAb levels (95% CI, 4.8–16.7; $P<0.0001$), compared with non-DQ2 participants.

[00193] In addition, significant differences in baseline levels of 16 of 37 cytokines were seen between participants with and without MYH6 AAbs who developed incident CVD events, with the most pronounced elevations observed for IL-6, IL-1RA, IL-8, IL-12 p40, and GM-CSF, and reduced levels of IL-10 (Fig. 7; Table 15). Furthermore, the strength of the pairwise relationships was compared between the differentially expressed cytokines, strongly correlated clusters of proinflammatory cytokines in the MYH6 AAb-positive group were found that were absent in the MYH6 AAb-negative group (Fig. 4c). Notably, correlations in the MYH6 AAb-positive group appeared to be coordinated around IL-6, particularly with IL-12 p40 ($r_s = 0.80$), IL-1RA ($r_s = 0.74$), IL-4 ($r_s = 0.83$), and GM-CSF ($r_s = 0.76$) (Fig. 4c). In addition, the MYH6 AAb-positive group showed negative pairwise correlations between IL-10 and numerous proinflammatory cytokines that were positively correlated in the MYH6 AAb-negative group (Fig. 4c). Network analyses further revealed a complex, closely

interconnected network of cytokines in the MYH6 AAb-positive group, in contrast to the sparse and scattered network in the MYH6 AAb-negative group (Fig. 4d).

[00194] Collectively, these findings provide the first evidence of the existence of a subgroup of T1D patients with accelerated CVD events of primary autoimmune etiology, defined by the presence of a specific cardiac AAb type, to MYH6, and characterized by HLA-linked genetic susceptibility and “high-grade” systemic cytokine production. Further, a CVD pathway was identified that is primarily driven by elevated IL-6 and GM-CSF, for which inhibitors are proven therapeutics in other autoimmune conditions (Fig. 8). Although current approaches to CVD reduction in T1D focus on the modification of traditional cardiovascular risk factors alone, these findings highlight individuals who might benefit more from targeted therapies⁴⁵ to combat inflammation or the underlying autoimmune process itself. These findings also point to the utility of cardiac AAb screening to identify individuals with T1D at highest risk for CVD events.

Example 10. Correlation between AAbs and lower left ventricular ejection fraction

[00195] In a separate study of T1D DCCT/EDIC participants with prior CVD events who underwent cardiac magnetic resonance (CMR) imaging exams (n=70), those with ≥ 2 cardiac AAbs showed a markedly lower left ventricular ejection fraction (mean $\pm$ SD, **51 $\pm$ 2%**) compared to those with only one or no AAbs, who had normal ejection fraction (**63 $\pm$ 1%**, $P<0.0001$) (Table 16). Left ventricular dysfunction is the strongest predictor of adverse outcome after acute myocardial infarction (MI) and is associated with a 3- to 4-fold increase in mortality risk in the general population. T1D is associated with a high case fatality following MI (Kerola AM, et al. *Diabetes Care* 45:1657-65 (2022)), raising the possibility that the presence of cardiac autoimmunity, as defined by ≥ 2 AAbs, contributes to this risk.

Example 11: Discussion

[00196] T1D has long been postulated to confer a specific risk for CVD;⁵⁴ however, the underlying biological pathways are unknown. In this prospective study of ~900 participants from the landmark T1D DCCT/EDIC study, a novel pathophysiological process was identified that may contribute to this excess risk. This pathway is defined by the presence of a specific cardiac AAb type, to MYH6, and is characterized by HLA-linked genetic susceptibility, systemic cytokine dysregulation, and markedly increased risk of early cardiovascular disease (CVD) events, driven primarily by elevated IL-6 and GM-CSF (Figure 8). To date, cardiovascular risk reduction in T1D has focused on traditional risk factor modification.⁵⁵ However, the findings of these Examples provide evidence of a subgroup of

Type I diabetes (T1D) patients with accelerated CVD complications of autoimmune etiology, who may particularly benefit from more targeted therapies^{45,56} to combat inflammation or the underlying autoimmune process itself.⁵⁰ The results also point to the potential utility of cardiac AAb screening to identify T1D patients at greatest risk for CVD events.

[00197] The dominant role of MYH6 AAbs in CVD prediction is notable in view of previous studies showing that CD4⁺ T cells specific for α -myosin drive autoimmune myocarditis in humans and mouse models.^{16,57,18,58} The major role of α -myosin as an autoantigen may relate to its absent expression in thymus that enables strongly self-reactive T cells specific for α -myosin to escape thymic negative selection (Figure 8),¹⁸ a mechanism recently implicated in other forms of myocarditis.^{16,57,59} The ability of MYH6 AAbs to identify high CVD-risk individuals also opens the possibility of future antigen-specific tolerogenic approaches to prevent CVD in T1D.

[00198] While autoimmune responses to atherosclerotic plaque antigens are commonly found in the general population,⁶⁰ these responses do not manifest as a classical autoimmune disease and are neither HLA-restricted nor associated with early breakdown of tolerance to self-antigens.⁶¹ Notably, the strongest risk factor for MYH6 AAb-positivity was the presence of diabetic retinopathy at DCCT baseline ~25 years prior to sampling, reinforcing the concept that persistent glycemic exposure early in the course of T1D⁶² may break cardiac self-tolerance (Figure 8).¹⁹

[00199] The data herein further show that MYH6 AAbs, and subsequent CVD events associated with MYH6 AAbs, are HLA-DQ2-linked (Figure 8). The striking HLA gene-dosage effect on both the prevalence and titers of MYH6 AAbs is reminiscent of the HLA effects on insulin AAb expression in new-onset T1D itself.⁶³ It has been suggested that the biologic basis for such effects is that homozygous individuals express more HLA molecules on the surface of antigen-presenting cells and thus may recruit CD4⁺ T cell responses—that are required for production of IgG AAbs—of greater magnitude.⁶⁴ Furthermore, MYH6 AAbs were associated with DQ2.5, which confers risk for many autoimmune disorders, including T1D.^{65,24} Indeed, GAD65 AAbs, that are also associated with DQ2.5, are considered markers of a general propensity to autoimmunity in T1D.⁶⁵ These findings thus suggest that CVD may be part of the spectrum of long-term autoimmune comorbidities in T1D.⁶⁶

[00200] Although T1D is generally considered a prototypical organ-specific autoimmune disease without systemic signatures,^{67,68} by stratifying subjects by cardiac AAb number and type, a subgroup was revealed exhibiting pathologically elevated levels of

proinflammatory cytokines—resembling systemic inflammatory illnesses. Furthermore, examining 37 measurable circulating cytokines as potential mediators of CVD risk associated with MYH6 AAbs, led to identification of IL-6 as the most important contributor to this risk.

[00201] IL-6 is a strong predictor of CVD events in the general population.^{33,69,70}

Recent clinical trials have identified the innate immune pathway leading from the NLRP3 inflammasome to IL-1 β to IL-6 as a central target for atheroprotection in T2D.^{6,7,71} However, neither IL-1 β nor IL-18 were elevated in MYH6 AAb-positive T1D subjects, suggesting that the process driving IL-6 production was inflammasome-independent. Furthermore, the IL-1 β to IL-6 pathway is generally associated with low-grade inflammation and modest IL-6 levels.^{35,8} However, MYH6 AAb-positive T1D participants exhibited extremely elevated IL-6 levels, ~10-fold higher than healthy controls and resembling RA and COVID-19, suggesting high-grade systemic inflammation.

[00202] The findings herein instead point to GM-CSF as the most likely upstream driver of IL-6 production.^{72,73,74,44} GM-CSF is notable not only for its role in mobilizing bone marrow cells to sites of inflammation (including atherosclerosis lesions (Figure 8),^{75,76} but as a CD4⁺ T cell-derived proinflammatory cytokine⁷⁷ that mediates numerous tissue-restricted autoimmune diseases,^{78,79} including myocarditis^{73,80} (Figure 8). In these settings, GM-CSF polarizes myeloid cells to a proinflammatory phenotype, driving production of numerous cytokines including IL-6, IL-8, IL-12, TNF- α , CCL22, CCL2, and CCL7,⁸¹ which were elevated in MYH6 AAb-positive T1D subjects. This function may explain the unexpectedly large number of elevated cytokines in MYH6 AAb-positive T1D subjects, their shared cytokine profiles with RA and COVID-19, and highly inter-correlated IL-6 and GM-CSF levels in all three groups. Finally, the high circulating levels of GM-CSF and IL-6 may explain how autoimmunity directed against a heart-restricted autoantigen, MYH6, can lead to accelerated atherosclerosis, a systemic disease process.

[00203] Another striking feature of MYH6 AAb-positive participants was their low levels of anti-inflammatory cytokine IL-10, suggesting uncontrolled proinflammatory responses. Although the basis for this finding is unclear, previous studies have shown that under steady state conditions, the cardiac microenvironment favors differentiation of α -myosin-specific CD4⁺ T cells into Treg cells,⁸² major sources of IL-10. Based on the results herein, it was postulated that the inflammatory milieu of cardiac autoimmunity shifts CD4⁺ T cell expansion towards a proinflammatory phenotype at the expense of Tregs,⁸³ with reduced

production of IL-10. It is interesting to note that elevated GM-CSF, IL-6, and reduced Tregs are immunological hallmarks of acute myocarditis.⁸⁰

[00204] T1D is recognized as a heterogeneous disease with distinct endotypes defined by different islet AAb types, HLA genotypes, and age-of-onset.⁸⁴ Our data indicate that CVD complications in T1D are also heterogeneous and we provide the first evidence for a distinct autoimmune subtype (“endotype”) defined by MYH6 AAbs, HLA-linked genetic susceptibility, and accelerated CVD events. The data herein further identify a cardiac AAb-linked pathogenic pathway for CVD primarily driven by GM-CSF and IL-6, for which inhibitors are proven therapeutics in other conditions.^{85,86}

[00205] Strengths of this study include availability of biosamples and clinical datasets from the DCCT/EDIC cohort, a large, extensively phenotyped T1D cohort with longitudinal follow-up for complications and rigorously adjudicated CVD outcomes over an extended period of time;⁴⁸ use of HLA genotyping, autoantibody and cytokine profiling to immunophenotype CVD risk; and validation of cytokine findings in multiple cohorts. In this study, cardiac AAb and cytokine measurements were performed at a single timepoint. However, the single participant positive for all 5 cardiac AAb types in a previous study on DCCT,¹⁹ remained positive for 4 cardiac AAbs in the current study ~25 years later (and subsequently developed a CVD event), suggesting that cardiac autoimmunity may be persistent. The number of MACE in this study was small and to enhance power in our cytokine studies, and a composite CVD endpoint was used as the primary outcome. Third, the lack of racial diversity may differ from other T1D populations.

[00206] In conclusion, the data herein identify a novel form of “autoimmune CVD” in T1D, distinct from the inflammatory pathways associated with CVD in T2D. The strong evidence presented here that CVD may be of autoimmune etiology raises the potential for using T1D-targeted^{45,56} immunological approaches to predict and prevent this leading cause of premature death and morbidity.

Example 12: The presence of multiple (≥ 2) cardiac autoantibodies, particularly MYH6 autoantibodies, is associated with increased incidence of first CVD events in the childhood-onset T1D CACTI cohort

[00207] A cohort of 652 childhood-onset T1D patients, 507 of whom with complete follow-up for CVD events, was analyzed from the Coronary Artery Calcification in Type 1 Diabetes (CACTI) study cohort.⁸⁷ As in Example 1 from the DCCT/EDIC cohort, it was found that ≥ 2 cardiac AAbs, particularly MYH6 AAbs, predict first CVD events in CACTI, after adjusting for traditional risk factors. Updated Kaplan-Meier curves (Figs. 9A-9B)

showed that positivity for ≥ 2 AAbs was associated with an increased incidence of first CVD events (Log-rank $P=0.004$ compared to no AAbs). A similar impact was observed for MYH6 AAbs (Log-rank $P<0.001$, compared to MYH6 AAb- negative participants), similar to our findings in the DCCT/EDIC cohort). Kaplan-Meier curves also showed a stepwise increase in the incidence of CVD events with increasing cardiac AAb number at baseline (Log-rank $P=0.003$). In multivariable models (Table 25) fully adjusted for age, sex, HbA1c, systolic blood pressure, LDL-cholesterol and urinary albumin/creatinine ratio, positivity for ≥ 2 AAbs is now associated with a 3.9-fold increased risk of CVD events ($p=0.002$), while MYH6 AAbs were associated with a 3.4-fold increased risk of CVD events ($p=0.002$).

[00208] Since established risk biomarkers hsTnT and NT-proBNP were measured and accessible in the shared CACTI database, the performance of cardiac AAbs was compared to these biomarkers (Table 26). Remarkably, in fully adjusted models, MYH6 AAbs performed similarly if not better than hsTnT and also better than NT-ProBNP for CVD prediction.

[00209] Intriguingly, when comparing the HLA-DQ genotype of subjects with MYH6 and MYH7 AAbs from the childhood-onset CACTI cohort, the levels of cardiac autoantibodies were higher in subjects with the DQ8 genotype (DQA1*03:01-DQB1*03:02), and highest in subjects with a homozygous DQ8 genotype (Table 27).

Example 13: A Distinctive circulating “hyperinflammatory” cytokine profile is also observed in MYH6 AAb+ CACTI participants who subsequently developed first CVD events

[00210] Cytokines were measured on 131 CACTI cohort samples using the Human Cytokine Proinflammatory-Focused 15-Plex Discovery Assay cytokine kit (HDF15; Millipore) by Eve Technologies (Calgary, Canada). The HDF15 panel included: GM-CSF, IL-6, IFN- γ , IL-1 β , IL-1RA, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12(p40), IL-12(p70), IL-13, TNF- α , and MCP-1.

[00211] The CACTI analyses comprised two studies: 1) a nested case:control study of 30 MYH6 AAb-positive “cases” with 60 control AAb-negative “control” subjects matched with cases for age, T1D duration, sex, smoking, and albumin/creatinine ratio, and 2) among the 56 subjects who had subsequent first CVD events, to determine whether the levels of circulating cytokines at baseline were significantly higher in MYH6 AAb-positive subjects compared to MYH6 AAb-negative subjects, as was found in the DCCT/EDIC cohort (Example 7).

[00212] The studies revealed significantly higher levels of 6 cytokines (IFN- γ IL-2, IL-4, IL-5, IL-10 and IL-13) in MYH6 AAb-positive compared to AAb-negative subjects.

Moreover, within the subgroup who developed first CVD events, dramatic differences were found in the baseline cytokine levels between MYH6 AAb-positive and MYH6 AAb-negative subjects (Table 28), with MYH6 AAb-positive subjects showing marked elevations in 87% (13 of 15) of cytokines, suggestive of a “hyperinflammatory state.”

[00213] Furthermore, when the 30 MYH6 AAb-positive subjects in the CACTI cohort were stratified according to incident CVD event status, the levels of IL-6, IFN- γ , and other cytokines were markedly higher in MYH6 AAb-positive subjects who developed CVD events compared to those who did not. These findings provide further evidence of the potential value of combining MYH6 AAbs with cytokine measurements to improve CVD risk stratification in patients with T1D.

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Materials and Methods

Study cohorts

[00214] The Diabetes Control and Complications Trial and Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) study cohort has been described in detail.^{46,47} In brief, the DCCT was a randomized clinical trial held between 1983 and 1993, that assigned 1,441 participants with T1D (age 13–39 years) to receive intensive glucose lowering therapy or conventional therapy, with the goal of evaluating whether intensive glycemic control resulted in lower risk of long-term diabetic complications. At the baseline DCCT visit, all participants were free of a history of clinical CVD, hypertension, and hypercholesterolemia. The DCCT consisted of two cohorts: a primary prevention cohort with 1–5 years' T1D duration (mean, 2.6 years), devoid of diabetic retinopathy at baseline (i.e., study entry); and a secondary intervention cohort with T1D for 1–15 years (mean, 8.7 years), with minimal to moderate non-proliferative diabetic retinopathy at DCCT baseline. After a mean follow-up of 6.5 years, the intensive and conventional groups achieved mean HbA1c levels of 7% and 9%, respectively. Subsequently, all subjects were encouraged to practice intensive therapy and invited to participate in the EDIC observational follow-up study, which started in 1994.⁴⁷ After the first 4 years of EDIC follow-up, the mean HbA1c levels in the two former treatment groups became equivalent (mean, 8.0%). During the DCCT, participants underwent yearly physical examinations, with HbA1c levels measured every quarter and fasting lipid levels, serum creatinine values, and albumin excretion assessed annually. Microalbuminuria was defined as urinary albumin excretion levels of ≥ 30 mg/24 h on two consecutive occasions, while macroalbuminuria was defined as ≥ 300 mg/24 h. Both conditions were considered present if end-stage renal/kidney disease (ESRD/ESKD) developed, defined as either kidney transplantation or dialysis. Electrocardiograms were conducted annually. In the EDIC study, methodologies from the DCCT were maintained with the exception of HbA1c measurements, which were reduced to once a year, and fasting lipid levels and kidney function assessments, which were measured every two years.

[00215] The current studies entail the analysis of a single serum sample from 891 EDIC participants with no prior CVD events during EDIC years 13–16 (2006–2009)²¹ with follow-up to EDIC year 24 (December 31, 2017).⁴⁸

Type 2 diabetes

[00216] Serum samples were obtained from 50 patients with T2D without clinical CVD defined by a negative cardiovascular history (i.e., normal resting electrocardiogram, absence of cardiac symptoms, and no prior hospitalization for CVD events) who were recruited between 2001 and 2006 as part of the Joslin Heart Study from at Joslin Diabetes Center.⁴² T2D samples were matched to the DCCT/EDIC cohort subjects for sex, smoking status, current HbA_{1c}, macroalbuminuria and ESRD.

Rheumatoid arthritis

[00217] Serum samples were obtained from 33 adult patients with rheumatoid arthritis (RA) from the Benaroya Research Institute (BRI) Diabetes and Immune-Mediated Disease Registry and Repository. Subjects were selected for being seropositive for rheumatoid factor (RF) or anti-citrullinated protein antibodies (ACPA), receiving ongoing methotrexate therapy, having moderate to severe disease activity (Routine Assessment of Patient Index Data 3 score) and for not receiving biologic disease-modifying therapies.

Acute COVID-19

[00218] Serum samples were obtained from 32 adults hospitalized during April and May 2020 with acute coronavirus disease 2019 (COVID-19) at Virginia Mason Medical Center, Seattle Washington, USA from the BRI Diabetes and Immune-Mediated Disease Registry and Repository. The cohort included 20 patients with severe disease requiring mechanical ventilation in the intensive care unit and 12 patients with moderate disease requiring supplemental oxygen, as described.⁴¹ All COVID-19 subjects were positive for SARS-CoV-2 by RT-PCR testing.

Healthy controls

[00219] Serum samples were obtained from 32 healthy control participants (BRI Diabetes and Immune-Mediated Disease Registry and Repository). Subjects were selected randomly but matched to T1D DCCT/EDIC participants by age (± 5 years). The control subjects were devoid of autoimmune disease or any family history of autoimmunity.

Childhood-Onset T1D, CACTI Cohort

[00220] Serum samples were obtained from 507 participants from the CACTI cohort. The CACTI study has been examining risk factors and incidence of CVD complications in

T1D since its inception in 2000.¹² It includes 652 participants with T1D (of whom 507 had complete follow-up for CVD events), 20–55 years of age and devoid of CAD at baseline (i.e., all subjects were asymptomatic for CAD with no history of CABG, coronary angioplasty, or unstable angina). All patients were <30 years of age at T1D diagnosis, predominantly in childhood (<18 years of age), treated with insulin within 1 year of diagnosis, and required to have a T1D duration of ≥10 years on enrollment. The CACTI cohort has been followed for 20 years for incident CAD outcomes that include MI, CABG, angioplasty, or CAD death as reported in the patient's medical record or death certificate.

[00221] CACTI study participants completed a baseline examination between 2000–2002 and returned for follow-up examinations 3, 6 and 12 years later. The entire cohort has undergone GWAS, with HLA alleles imputed using the TOPmed reference panel.

Cardiac autoantibody assays

[00222] AAb levels to five human myocardial antigens full-length MYH6; full-length MYH7; S1 fragment of MYH6 (S1-MYH6); S2-fragment of MYH6 (S2-MYH6); and cTnI] were measured using fluid-phase radioimmunoprecipitation assays as described.^{16,19,21} The cardiac AAb index was calculated as [(counts per minute [CPM] in the unknown sample – CPM in the negative control standard)] / [(CPM in the positive standard – CPM in the negative standard)] x 100. The cutoffs for having AAb were determined as the 99th percentile of the AAb index values obtained from healthy control subjects, and were as follows: 0.701 for FL-MYH6; 0.799 for FL-MYH7; 0.330 for S1-MYH6; 0.763 for S2-MYH6; and 0.283 for cTnI AAb.¹⁹ The cardiac AAb assays were highly reproducible, with the following intra- and inter- assay coefficients of variation (%): 7.4 and 10.1 for FL-MYH6; 5.7 and 10.3 for FL-MYH7; 6.0 and 11.1 for S1-MYH6; 5.0 and 7.8 for S2-MYH6; and 3.2 and 6.2 for cTnI AAb.

Cardiovascular disease outcomes

[00223] The composite cardiovascular disease (CVD) outcome was the time to the first of any of the following types of CVD events^{20,48,49}: nonfatal myocardial infarction (MI) or stroke, death judged to be secondary to CVD; subclinical (“silent”) MI detected on an annual electrocardiogram (ECG), angina confirmed by ischemic changes with exercise tolerance testing or by clinically significant obstruction on coronary angiography, coronary revascularization with angioplasty and/or coronary artery bypass, or heart failure defined as the presence of orthopnea, paroxysmal nocturnal dyspnea, or prominent limitation of physical activity due to heart disease with follow-up to EDIC year 24.⁴⁸ Major adverse cardiovascular events (MACE) was defined as nonfatal MI or stroke, or death secondary to CVD. The

occurrence of CVD events was ascertained during annual DCCT/EDIC study visits, with the assessment of medical history, electrocardiogram, and medical records to verify self-reported events, with all CVD events adjudicated by a review committee masked to DCCT treatment group and HbA1c levels.⁴⁸

Class II HLA genotyping

[00224] High-resolution HLA genotyping of DQA1 and DQB1 alleles at two-field (4-digit) resolution was performed as previously described¹⁹ on genomic DNA from 475 DCCT/EDIC participants, comprising 146 T1D subjects with cardiac AAbs and 329 T1D subjects without cardiac AAbs.

Serum cytokine measurements

[00225] Serum cytokines were measured in a nested case-control study using baseline serum samples from 146 DCCT/EDIC participants with cardiac AAbs matched with 146 participants without cardiac AAbs for age, sex, time-updated mean HbA_{1c}, and macroalbuminuria/ESRD. The multiplex cytokine analysis was carried out on the Luminex™ 200 system (Luminex, Austin, TX, USA) by Eve Technologies Corp. (Calgary, Alberta), using the Human Cytokine/Chemokine 42-Plex Discovery Assay® kit (MilliporeSigma, Burlington, Massachusetts, USA) on same baseline serum samples as were used for cardiac AAb measurements. The kit contained the following markers: sCD40L, EGF, Eotaxin, FGF-2, Flt-3 ligand, Fractalkine, G-CSF, GM-CSF, GRO α , IFN- α 2, IFN- γ , IL-1 α , IL-1 β , IL-1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p40), IL-12(p70), IL-13, IL-15, IL-17A, IP-10, MCP-1 (CCL2), MCP-3 (CCL7), MDC (CCL22), MIP-1 α , MIP-1 β , PDGF-AA, PDGF-AB/BB, RANTES, TGF α , TNF- α , TNF- β , and VEGF. To avoid batch effects, the same kit lot number (#3509890) was used for all cytokine assays, with the same standard curves applied. The same positive and negative control calibrator samples were run in all assays, and data were normalized before analysis to account for potential intra-assay effects. The company flagged four of 328 samples with analyte signals that extrapolated beyond the standard curve across the entire cytokine panel that was attributed to human anti-animal antibodies. These four samples were excluded from subsequent analysis.

Statistical analysis

[00226] The clinical characteristics were summarized using descriptive statistics, reported as mean $\pm$ SD or median (25th and 75th percentile) for continuous variables and frequencies (%) for categorical variables. Normality was assessed using the Shapiro-Wilk Test. Continuous and categorical variables were compared with the Wilcoxon rank-sum test and the Chi-square or Fisher's exact test, respectively. The follow-up period started from the

date of the first sampling (EDIC years 13–16) and ended at the occurrence of the first CVD event or EDIC year 24, whichever occurred first. The Kaplan–Meier method was used to estimate the cumulative incidence of a first any-CVD event or MACE, and group differences were compared using the log-rank test. Cox proportional hazards models were used to assess the risk (hazard ratio and 95% confidence interval) of developing any first CVD event or MACE associated with the number and type of cardiac AAbs at baseline as a function of fixed covariates (e.g., age, T1D duration, current smoking, current triglycerides) and time-dependent covariates (e.g., mean HbA_{1c}, mean systolic blood pressure, mean LDL). During the DCCT/EDIC study HbA_{1c} was measured annually in EDIC, and fasting lipids and renal function were measured in alternate years in EDIC (yearly in DCCT). Time-updated mean HbA_{1c} represents the total glycemic exposure during DCCT/EDIC with weights of 0.25 and 1 for quarterly DCCT and annual EDIC values, respectively, up to the measure immediately preceding the event or censoring for those without an event as described.^{22,46,47} The models were built on risk factors associated with both incident CVD events^{22,48} and the presence of cardiac AAbs.

[00227] Given the large number of potentially confounding variables in the DCCT/EDIC cohort,²² covariates were entered into the models and variables were deleted with $P > 0.08$. To identify the number of variables for adjustment, the final models were fit using age and other covariates that had the greatest attenuation on the effect of cardiac AAbs on the risk of developing any first CVD event (microalbuminuria/ESRD, retinopathy at DCCT baseline, DCCT/EDIC time-updated mean LDL, mean HbA_{1c}, and mean systolic blood pressure) or MACE (retinopathy at DCCT baseline, and DCCT/EDIC time-updated mean HbA_{1c} and mean systolic blood pressure). Alternative models were built using backward elimination or forward selection methods based on the association of traditional cardiovascular risk factors with cardiac AAbs or the CVD outcome. Some of the risk factors associated with the presence of cTnI AAbs (i.e., male sex and cardiac autonomic neuropathy) were different from those associated with MYH6 AAbs and ≥ 2 AAbs; therefore, they were included in the final model. To evaluate the accuracy of fitted models and examine potential problems related to overfitting and selection bias, the proportional hazards assumption was assessed in Cox regression models using the Schoenfeld residuals.

[00228] To assess the association between class II HLA alleles and specific AAb types, the proportions of different HLA genotypes in subjects who tested positive versus negative for a particular AAb type using either the Chi-square or Fisher's exact test, based on cell size, were compared with subsequent adjustment of P values to account for multiple

testing. To examine the relationship between the type or number of cardiac AAbs and cytokine concentrations, the distribution of cytokines was first assessed and transformed into their base 2 logarithms. The cytokine levels were compared among the cardiac AAb groups by logistic regression per one tertile change of their percent rank distribution. Orthogonal relationships among the cytokines associated with MYH6 AAbs and ≥ 2 AAbs were visualized using the R package *pheatmap* and examined with hierarchical cluster analysis using Ward's method. It was assessed whether presence of one or more proinflammatory cytokines shared between the MYH6 AAb and ≥ 2 AAb groups could contribute to the increased risk of first CVD events. Models were built for each of the AAb groups using the same approach as described earlier. The final model consisted of AAbs, along with three covariates that had the strongest impact on the relationship between MYH6 AAbs (age, retinopathy, and DCCT/EDIC time-weighted mean LDL) or ≥ 2 AAbs (retinopathy, and DCCT/EDIC time-weighted mean LDL and systolic blood pressure) and the risk of CVD events. A series of additional analyses were performed to adjust for each of the 12 core cytokines, one at a time, and for the combined effect of IL-6 and GM-CSF to account for collinearity, while controlling for traditional cardiovascular CVD risk factors.

[00229] Alternative models that included additional covariates, such as T1D duration, current pulse rate, and microalbuminuria/ESRD but these did not further attenuate the observed effect observed in the main models.

[00230] Cytokine concentrations in healthy controls and different disease states were compared by means of logistic regression using log₂-transformed data. PCA plots were constructed with ellipse probability for the 37 detectable cytokines in various disease states. First, a boxplot of each sample was plotted to see if their abundances are comparable. Then a quantile normalization of the cytokine data was performed so that they had the same distribution of values and were thus comparable to each other.

[00231] Correlation matrix analysis was performed using the R plotting packages ggplot2 and ggcorrplot, with pairwise interactions between the 12 core cytokines assessed by calculating Spearman's rank correlation coefficients. Correlation network analysis of quantile-normalized cytokine data was performed using the R packages corrr, ggraph, and igraph to visualize marker-to-marker interactions. The edges represent Spearman's rank correlation coefficient with an absolute value of >0.5 . Box plots, violin plots, forest plots, and Kaplan-Meier plots were generated in GraphPad Prism version 9.4.1 (GraphPad Software, San Diego, CA). *P* values were adjusted for multiple testing using the Benjamini-Hochberg

procedure to control the false discovery rate at 5%. All statistical analyses were performed using two-sided tests in SAS v9.4 (SAS Institute, Cary, NC) and R version 4.3.0.

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V. Summary of Tables

[00232] The following are Tables referenced throughout the application.

Table 2. Baseline characteristics of the DCCT/EDIC T1D participants stratified by the development of subsequent first CVD events				
Characteristic	Total (<i>n</i> = 891)	First CVD events		<i>P</i> value
		Yes (<i>n</i> = 79)	No (<i>n</i> = 812)	
Age, y	49±7	52±7	49±7	<0.001
T1D duration, y	28±5	29±5	28±5	0.023
Males	462 (52)	48 (61)	414 (51)	0.097
Current smoker	103 (12)	18 (23)	85 (10)	0.001
Current HbA _{1c} , %	7.9±1.2	8.3±1.4	7.8±1.2	0.001
BMI, kg/m ²	28±5	29±4	28±5	0.157
Hypertension	427 (48)	51 (65)	376 (46)	0.002
Cardiac autonomic neuropathy*	248 (29)	37 (49)	211 (27)	<0.0001
Microalbuminuria/ESKD	219 (25)	32 (41)	187 (23)	<0.001
Macroalbuminuria/ESKD	79 (9)	12 (15)	67 (8)	0.038
Retinopathy at DCCT baseline	448 (50)	51 (65)	397 (49)	0.008
Current pulse rate, bpm	70±12	73±13	70±11	0.035
Current LDL, mg/dL	97±29	102±33	97±29	0.150
Current triglycerides, mg/dL	78±53	103±70	76±51	<0.0001
Conventional treatment group	432 (48)	37 (47)	395 (49)	0.759
DCCT/EDIC time-updated mean†				
HbA _{1c} , %	7.9±0.9	8.3±1.0	7.9±0.9	0.004
Pulse rate, bpm	72±7	74±8	72±7	0.031
LDL, mg/dL	110±20	117±19	109±20	<0.001
Triglycerides, mg/dL	83±40	99±47	81±38	<0.0001
Systolic blood pressure, mm Hg	118±8	122±8	117±8	<0.0001
Cardiac AAb positivity	146 (16)	27 (34)	119 (15)	<0.0001
Values are presented as mean ± SD or <i>n</i> (%), unless otherwise specified. *Within the cardiac autonomic neuropathy group, the number of participants (<i>n</i>) who developed a CVD event is 75, while the number who did not develop a CVD event is 783. CVD events were defined as a composite of nonfatal myocardial infarction, nonfatal stroke, CVD death, silent MI, confirmed angina, revascularization, or heart failure.				

DQ genotype	Total (n = 475)	MYH6 AABs		P value*	MYH7 AABs		P value*	TnI AABs		P value*
		Positive (n = 41)	Negative (n = 434)		Positive (n = 34)	Negative (n = 441)		Positive (n = 24)	Negative (n = 451)	
DQA1*03:01-DQB1*03:02 (DQ8)	250 (53)	17 (41)	233 (54)	0.134	13 (38)	237 (54)	0.081	9 (38)	241 (53)	0.128
DQ8/8	32 (7)	3 (7)	29 (7)	0.749	1 (3)	31 (7)	0.718	3 (13)	29 (6)	0.214
DQA1*05:01-DQB1*02:01 (DQ2.5)	246 (52)	28 (68)	218 (50)	0.027 [†]	27 (79)	219 (50)	0.002 [†]	11 (46)	235 (52)	0.549
DQ2.5/2.5	37 (8)	11 (27)	26 (6)	0.0001 [†]	10 (29)	27 (6)	<0.001 [†]	2 (8)	35 (8)	0.710
DQ8 and not DQ2.5	137 (29)	8 (20)	129 (30)	0.168	4 (12)	133 (30)	0.023 [†]	5 (24)	132 (29)	0.374
DQ2.5 and not DQ8	105 (22)	17 (41)	88 (20)	0.004 [†]	15 (44)	90 (20)	0.002 [†]	6 (25)	99 (22)	0.726
DQ2.5/DQ8	125 (26)	9 (22)	116 (27)	0.507	9 (26)	116 (26)	0.983	4 (17)	121 (27)	0.346
Either DQ2.5 or DQ8	382 (80)	36 (88)	346 (80)	0.213	31 (91)	351 (80)	0.101	16 (67)	366 (81)	0.081
Values are presented as n (%). Data were analyzed by Chi-Square except when any expected value is <5, in which case Fisher's exact test was applied. *P value corrected for multiple testing using the Benjamini-Hochberg procedure. †P < 0.05 for positive association; †P < 0.05 for negative association. There were no associations between DQA1*02:01-DQB1*02:02 and cAAb types.										

Table 4. Baseline characteristics of the DCCT/EDIC T1D participants with no prior CVD events stratified by subsequent MACE

Characteristics	Total (<i>n</i> = 891)	First MACE		<i>P</i> value
		Yes (<i>n</i> = 37)	No (<i>n</i> = 854)	
Age, y	49±7	52±6	49±7	0.002
T1D duration, y	28±5	30±5	28±5	0.009
Males	462 (52)	23 (62)	439 (51)	0.200
Current smoker	103 (12)	8 (22)	95 (11)	0.051
Current HbA _{1c} , %	7.9±1.2	8.4±1.3	7.9±1.2	0.002
BMI, kg/m ²	28±5	29±5	28±5	0.273
Hypertension	427 (48)	22 (59)	405 (47)	0.151
Cardiac autonomic neuropathy*	248 (29)	18 (49)	230 (28)	0.007
Microalbuminuria/ESKD	219 (25)	16 (43)	203 (24)	0.007
Macroalbuminuria/ESKD	79 (9)	7 (19)	72 (8)	0.028
Retinopathy at DCCT baseline	448 (50)	27 (73)	421 (49)	0.005
Current pulse rate, bpm	70±12	72±11	70±12	0.215
Current LDL, mg/dL	97±29	100±30	97±29	0.357
Current triglycerides, mg/dL	78±53	113±81	76±51	0.002
Conventional treatment group	432 (48)	18 (49)	414 (48)	0.984
DCCT/EDIC time-updated mean				
HbA _{1c} , %	7.9±0.9	8.4±1.1	7.9±0.9	0.006
Pulse rate, bpm	72±7	74±8	72±7	0.173
LDL, mg/dL	110±20	115±19	109±20	0.117
Triglycerides, mg/dL	83±40	103±51	82±39	0.001
Systolic blood pressure, mm Hg	118±8	123±9	117±8	0.002
Cardiac autoantibody positivity	146 (16)	15 (41)	131 (15)	<0.0001

Values are mean ± SD or *n* (%). *Within the cardiac autonomic neuropathy group, the number of participants (*n*) for No first MACE is 821.

Table 5. Baseline characteristics of the DCCT/EDIC T1D participants stratified by cardiac autoantibody status			
Characteristics	Cardiac AAbs		P value
	Present (n = 146)	Absent (n = 745)	
Age, y	49±7	49±7	0.750
T1D duration, y	28±5	28±5	0.169
Males	83 (57)	379 (51)	0.186
Current smoker	16 (11)	87 (12)	0.804
Current HbA _{1c} , %	8.0±1.5	7.8±1.2	0.396
BMI, kg/m ²	28±4	28±5	0.440
Hypertension	71 (49)	356 (48)	0.852
Cardiac autonomic neuropathy*	48 (35)	200 (28)	0.073
Microalbuminuria/ESKD	50 (34)	169 (23)	0.003
Macroalbuminuria/ESKD	21 (14)	58 (8)	0.010
Retinopathy at DCCT baseline	77 (53)	371 (50)	0.516
Current pulse rate, bpm	72±12	70±11	0.138
Current LDL, mg/dL	99±32	97±29	0.560
Current triglycerides, mg/dL	84±62	77±52	0.467
Conventional treatment group	86 (59)	346 (46)	0.006
DCCT/EDIC time-updated mean			
HbA _{1c} , %	8.1±1.1	7.9±0.9	0.033
Pulse rate, bpm	74±8	72±7	0.045
LDL, mg/dL	111±21	109±20	0.190
Triglycerides, mg/dL	87±44	82±39	0.350
Systolic blood pressure, mm Hg	119±8	117±8	0.037
Incident CVD event	27 (18)	52 (7.0)	<0.0001
Values are presented as mean ± SD or n (%). *Within the cardiac autonomic neuropathy group, the number of participants (n) is 136 for those with cardiac autoantibodies (AAbs) and 722 for those without cardiac AAbs.			

Table 6. Risk of first MACE according to the number and type of cardiac autoantibodies present at baseline

Risk factor	MACE		Unadjusted HR (95% CI)	P value	Adjusted HR (95% CI)*	P value
	Yes (n = 37)	No (n = 854)				
cAAb number						
No cAAbs	22 (59)	723 (85)	1.0 [Reference]			
1 cAAb	6 (16)	96 (11)	2.0 (0.8–5.0)	0.124	1.9 (0.8–4.8)	0.149
≥2 cAAbs	9 (24)	35 (4)	7.7 (3.5–16.7)	<0.0001	5.5 (2.4–12.9)	<0.0001
cAAb type						
MYH6 AAbs	8 (22)	33 (4)	6.2 (2.8–13.6)	<0.0001	4.9 (2.2–11.1)	<0.001
TnI AAbs	5 (14)	19 (2)	6.5 (2.5–16.6)	<0.001	3.1 (1.1–9.0)	0.040

Values are presented as *n* (%) or HR with 95% CI. *The adjusted model for the number of cAAbs or MYH6 AAbs includes age, retinopathy at DCCT baseline, and DCCT/EDIC time-updated mean HbA1c and mean systolic blood pressure. The adjusted model for TnI AAbs includes age, cardiac autonomic neuropathy, and DCCT/EDIC time-updated mean HbA1c and mean systolic blood pressure. The hazard of having a first MACE in individuals with ≥2 cAAbs is relative to those without cAAbs. The hazard of having a first MACE in individuals positive for a specific type of cAAbs is relative to those with any other or no cAAbs.

Table 7. Baseline characteristics of the T1D participants stratified by MYH6 autoantibody status			
Characteristics	MYH6 AAb status		P value
	Positive (n = 41)	Negative (n = 850)	
Age, y	49±7	49±7	0.652
T1D duration, y	28±5	28±5	0.227
Males	26 (63)	436 (51)	0.129
Current smoker	5 (12)	98 (12)	0.896
Current HbA _{1c} , %	8.0±1.4	7.9±1.2	0.594
BMI, kg/m ²	28±4	28±5	0.998
Hypertension	19 (46)	408 (48)	0.836
Cardiac autonomic neuropathy*	8 (22)	240 (29)	0.366
Microalbuminuria/ESKD	13 (32)	206 (24)	0.278
Macroalbuminuria/ESKD	3 (7)	76 (9)	0.721
Retinopathy at DCCT baseline	28 (68)	420 (49)	0.018
Current pulse rate, bpm	72±12	70±11	0.407
Current LDL, mg/dL	104±30	97±29	0.071
Current triglycerides, mg/dL	82±47	78±54	0.356
Conventional treatment group	23 (56)	409 (48)	0.318
DCCT/EDIC time-updated mean			
HbA _{1c} , %	8.2±1.1	7.9±0.9	0.144
Pulse rate, bpm	74±7	72±7	0.176
LDL, mg/dL	115±16	109±20	0.044
Triglycerides, mg/dL	89±47	82±39	0.399
Systolic blood pressure, mm Hg	119±8	118±8	0.164
Incident CVD event	15 (37)	64 (8)	<0.0001
Values are presented as mean ± SD or n (%). *Within the cardiac autonomic neuropathy group, the number of MYH6 AAb-positive participants (n) is 36, while the number of MYH6 AAb-negative participants is 822. Any-CVD events were defined as a composite of nonfatal acute myocardial infarction (MI), stroke, CVD death, silent MI, confirmed angina, revascularization, or heart failure that occurred after sampling and EDIC year 24.			

Table 8. Baseline characteristics of the T1D participants stratified by TnI autoantibody status			
Characteristics	TnI AAb status		P value
	Positive (n = 24)	Negative (n = 867)	
Age, y	50±8	49±7	0.415
T1D duration, y	28±5	28±5	0.931
Males	18 (75)	444 (51)	0.021
Current smoker	1 (4)	102 (12)	0.346
Current HbA _{1c} , %	7.7±1.2	7.9±1.2	0.580
BMI, kg/m ²	28±4	28±5	0.804
Hypertension	14 (58)	413 (48)	0.301
Cardiac autonomic neuropathy*	10 (42)	238 (29)	0.162
Microalbuminuria/ESKD	8 (33)	211 (24)	0.313
Macroalbuminuria/ESKD	4 (17)	75 (9)	0.156
Retinopathy at DCCT baseline	14 (58)	434 (50)	0.424
Current pulse rate, bpm	69±12	70±11	0.540
Current LDL, mg/dL	98±25	97±30	0.857
Current triglycerides, mg/dL	95±64	78±53	0.259
Conventional treatment group	16 (67)	416 (48)	0.071
DCCT/EDIC time-updated mean			
HbA _{1c} , %	8.1±1.2	7.9±0.9	0.681
Pulse rate, bpm	71±8	72±7	0.336
LDL, mg/dL	114±16	109±20	0.223
Triglycerides, mg/dL	99±49	82±39	0.127
Systolic blood pressure, mm Hg	122±10	118±8	0.041
Incident CVD event	7 (29)	72 (8)	<0.001
Values are presented as mean ± SD or n (%). *Within the cardiac autonomic neuropathy group, the number of TnI AAb-negative participants (n) is 834.			

Table 9. Lack of significant association between HLA-DQ genotype and autoantibodies to S1-MYH6 or S2-MYH6

HLA type	S1-MYH6 AAb		P value	S2-MYH6 AAb		P value
	Positive (n = 54)	Negative (n = 421)		Positive (n = 26)	Negative (n = 419)	
DQA1*03:01-DQB1*03:02 (DQ8)	34 (63)	216 (51)	0.106	26 (46)	224 (53)	0.322
DQ8/8	3 (6)	29 (7)	1.000	4 (7)	28 (7)	0.781
DQA1*05:01-DQB1*02:01 (DQ2.5)	27 (50)	219 (52)	0.780	30 (54)	216 (52)	0.776
DQ2.5/2.5	6 (11)	31 (7)	0.333	4 (7)	33 (8)	1.000
DQ8 and not DQ2.5	18 (33)	119 (28)	0.439	12 (21)	125 (30)	0.192
DQ2.5 and not DQ8	9 (17)	96 (23)	0.306	16 (29)	89 (21)	0.214
DQ2.5/DQ8	16 (30)	109 (26)	0.557	14 (25)	111 (26)	0.812
Either DQ2.5 or DQ8	45 (83)	337 (80)	0.567	42 (75)	340 (81)	0.276

Values are presented as n (%). Data were analyzed using the Chi-Square test, except when any expected value was <5, in which case Fisher's exact test was applied.

Table 10. Baseline characteristics of the T1D participants in the cytokine substudy stratified by the number of cardiac autoantibodies

Characteristics	Number of cAAbs				P for trend*
	Total (n = 289)	No cAAbs (n = 146)	1 cAAb (n = 99)	≥2 cAAbs (n = 44)	
Age, y	49±7	48±7	50±7	48±7	0.702
T1D duration, y	28±5	27±5	28±5	29±5	0.103
Males	167 (58)	87 (60)	53 (54)	27 (61)	0.742
Current smoker	38 (13)	22 (15)	11 (11)	5 (11)	0.360
Current HbA _{1c} , %	7.9±1.3	7.8±1.1	8.1±2.0	7.7±1.3	0.638
BMI, kg/m ²	28±5	29±5	27±4	28±4	0.195
Overweight	125 (43)	59 (40)	47 (47)	19 (43)	0.429
Obesity	100 (35)	56 (38)	28 (28)	16 (36)	0.316
Hypertension	152 (53)	81 (55)	48 (48)	23 (52)	0.415
Cardiac autonomic neuropathy [†]	89 (32)	42 (30)	37 (40)	10 (25)	0.630

Microalbuminuria/ESKD	91 (31)	42 (29)	34 (34)	15 (34)	0.344
Macroalbuminuria/ESKD	43 (15)	22 (15)	15 (15)	6 (14)	0.874
Retinopathy at DCCT baseline	149 (52)	74 (51)	45 (45)	30 (68)	0.293
Current pulse rate, bpm	71±12	70±11	72±12	72±12	0.161
Current LDL, mg/dL	98±32	98±32	99±34	99±28	0.710
Current triglycerides	82±57	80±52	82±64	87±57	0.493
Conventional treatment group	158 (55)	73 (50)	61 (62)	24 (55)	0.196
DCCT/EDIC time-updated mean					
HbA _{1c} , %	8.1±1.0	8.0±1.0	8.2±1.0	8.1±1.2	0.184
Pulse rate, bpm	73±7	72±7	74 ± 8	74±6	0.075
LDL, mg/dL	110±22	109±23	109 ± 23	114±17	0.276
Triglycerides, mg/dL	86±43	85±42	82 ± 47	88±38	0.747
Systolic blood pressure, mmHg	119±8	118±8	119 ± 8	120±8	0.123

Values are presented as mean ± SD or *n* (%). [†]Within the cardiac autonomic neuropathy group, the total number of participants (*n*) is 275, with 142 for no cAABs, 93 for 1 cAAB, and 40 for ≥2 cAABs. Overweight was defined as BMI ≥25 and <30 kg/m²; obesity was defined as BMI ≥30 kg/m². **P* values for trend were calculated using the Jonckheere-Terpstra trend test and corrected for multiple comparisons with the post hoc Tukey-Kramer method.

Table 11. Clinical characteristics of the COVID-19 cohort	
Characteristics	(n = 32)
Age, y	61 (48, 65)
Males	19 (59)
Type 2 diabetes	19 (59)
COVID-19 severity*	
Moderate	12 (37.5)
Severe	20 (62.5)
Experimental medicines	
Hydroxychloroquine	7 (22)
Remdesivir	20 (63)
Tocilizumab	8 (25)
Convalescent plasma	15 (47)

Values are presented as median (interquartile range) or n (%).

*Moderate disease severity was defined as hospitalization requiring supplemental oxygen therapy, while severe disease was defined as requiring mechanical ventilation in the intensive care unit.

Table 12. Clinical characteristics of the rheumatoid arthritis cohort	
Characteristics	(n = 32)
Age, y	53 (46, 58)
Females	28 (88)
Disease duration, y	8±10
Rheumatoid factor positive	23 (72)
Anti-CCP2 positive	31 (97)
Rheumatoid factor or anti-CCP2-positive	32 (100)
CRP, mg/L	7.4±8.5
Erosive arthritis	10 (31)

Medications	
Methotrexate	32 (100)
Steroids	0
Biologics	0
Comorbidities	
Type 2 diabetes	2 (6)
Hashimoto's or Graves' disease	6 (19)

Values are presented as median (interquartile range) or *n* (%).

Table 13. Clinical characteristics of the type 2 diabetes cohort	
Characteristics	(<i>n</i> = 50)
Age, y	63±6
Diabetes duration, y	13±6
Males	26 (52)
BMI, kg/m ²	33±6
HbA1c, %	7.7±1.5
Current smoker	5 (10)
Hypertension	41 (82)
Treated hypercholesterolemia	43 (86)
Diabetic kidney disease	
Microalbuminuria	10 (20)
Macroalbuminuria	5 (10)
eGFR ≤30 mL/min/1.73m ²	7 (14)

Values are presented as mean ± SD or *n* (%).

Cytokine pg/mL	Healthy controls (n = 32)	Type 1 diabetes					COVID-19 (n = 32)	Type 2 diabetes (n = 50)
		Total (n = 289)	No cAABs (n = 146)	≥2 cAABs (n = 44)	MYH6 AABs (n = 41)	Rheumatoid arthritis (n = 33)		
IFN- γ	5 (2, 13)	11 (7, 25)	9 (6, 13)	22 (13, 54)	17 (12, 53)	24 (14, 64)	23 (11, 63)	5 (3, 10)
TNF- α	6 (4, 8)	9 (6, 12)	7 (5, 10)	17 (11, 25)	13 (7, 25)	30 (27, 42)	21 (16, 35)	7 (6, 12)
G-CSF	10 (6, 23)	21 (12, 29)	18 (12, 24)	39 (27, 85)	33 (23, 60)	23 (11, 40)	30 (23, 71)	7 (3, 18)
EGF	34 (17, 56)	37 (23, 60)	33 (23, 47)	57 (38, 83)	48 (26, 69)	86 (32, 159)	33 (25, 46)	187 (95, 284)
sCD40L	1090 (630, 1227)	1184 (707, 1717)	1012 (612, 1501)	1647 (1340, 2012)	1668 (1280, 1975)	1274 (962, 1863)	1160 (603, 1583)	1169 (957, 1345)
CCL22	337 (259, 456)	404 (300, 567)	368 (255, 538)	661 (475, 885)	604 (424, 770)	679 (488, 882)	350 (177, 447)	414 (336, 634)
IL-8	10 (5, 16)	10 (8, 20)	9 (8, 15)	16 (11, 30)	19 (10, 47)	11 (6, 22)	25 (21, 42)	5 (3, 8)
IL-12p70	2 (1, 5)	3 (1, 6)	3 (1, 5)	5 (3, 8)	6 (2, 13)	6 (4, 21)	2 (1, 5)	2 (1, 7)
IL-6	2 (1, 7)	4 (2, 11)	3 (2, 9)	9 (2, 28)	23 (3, 41)	26 (11, 44)	27 (16, 43)	3 (1, 8)
GM-CSF	14 (11, 25)	16 (8, 24)	14 (9, 20)	26 (12, 64)	59 (18, 75)	54 (37, 137)	57 (31, 105)	11 (8, 15)
CCL2	209 (135, 255)	227 (197, 296)	212 (192, 244)	236 (218, 305)	246 (230, 341)	280 (211, 363)	290 (188, 372)	294 (204, 390)
IL-10	3 (2, 4)	5 (2, 9)	7 (3, 13)	5 (3, 8)	3 (2, 5)	4 (2, 10)	10 (3, 20)	5 (1, 12)
CCL7	21 (13, 32)	26 (18, 42)	25 (19, 36)	31 (25, 49)	30 (22, 50)	31 (21, 58)	15 (9, 22)	20 (11, 34)
GRO- α	665 (535, 887)	910 (713, 1199)	918 (773, 1177)	759 (494, 1244)	1140 (568, 1390)	682 (529, 839)	717 (534, 1188)	792 (557, 1194)
CXCL10	79 (54, 116)	77 (52, 102)	82 (62, 101)	90 (38, 160)	105 (47, 148)	91 (61, 129)	577 (254, 926)	82 (68, 101)
IL-1RA	14 (10, 21)	21 (14, 35)	20 (15, 33)	30 (14, 41)	31 (16, 55)	46 (19, 147)	99 (45, 237)	1 (0, 1)
IL-7	2 (1, 3)	3 (2, 4)	3 (2, 4)	3 (2, 5)	3 (2, 5)	4 (2, 5)	4 (2, 6)	1 (0, 2)
PDGF-AA	496 (367, 618)	788 (627, 927)	794 (639, 920)	852 (646, 959)	789 (581, 927)	429 (360, 505)	387 (289, 526)	843 (652, 1407)
TGF- α	2 (1, 4)	4 (3, 6)	4 (3, 6)	5 (3, 7)	5 (2, 7)	4 (3, 8)	3 (2, 5)	2 (1, 3)
FGF2	62 (52, 86)	110 (73, 141)	109 (77, 132)	115 (81, 154)	110 (85, 154)	96 (70, 176)	66 (56, 81)	96 (85, 117)

Eotaxin	93 (69, 118)	118 (88, 145)	116 (92, 144)	120 (79, 142)	125 (82, 145)	96 (71, 160)	77 (42, 109)	133 (90, 186)
Flt-3L	8 (1, 27)	16 (6, 26)	15 (8, 25)	16 (11, 22)	17 (9, 24)	11 (3, 47)	3 (0, 12)	13 (2, 30)
Fractalkine	23 (16, 41)	70 (37, 110)	72 (45, 106)	76 (32, 109)	98 (31, 122)	55 (35, 190)	23 (15, 29)	71 (42, 145)
IFN- α 2	24 (13, 50)	48 (23, 70)	48 (24, 60)	48 (22, 68)	50 (21, 69)	73 (39, 233)	25 (14, 38)	27 (17, 51)
IL-12p40	25 (11, 51)	33 (16, 52)	34 (17, 49)	35 (25, 48)	38 (25, 57)	50 (42, 131)	18 (9, 28)	30 (13, 61)
PDGF-BB	2196 (1859, 2798)	3236 (2556, 4126)	3204 (2628, 3875)	3336 (2617, 4286)	3087 (1946, 4002)	2216 (1845, 3108)	2068 (1537, 2771)	3339 (2607, 4743)
IL-15	2 (1, 3)	3 (2, 6)	5 (2, 7)	4 (3, 5)	5 (3, 6)	6 (3, 18)	3 (2, 5)	2 (1, 3)
IL-17A	1 (1, 9)	3 (1, 7)	3 (1, 8)	4 (2, 10)	4 (2, 10)	2 (1, 5)	1 (0, 3)	3 (1, 6)
IL-1 β	3 (2, 7)	3 (1, 5)	3 (1, 6)	3 (2, 5)	4 (2, 6)	9 (5, 21)	16 (8, 30)	13 (7, 22)
IL-2	1 (1, 2)	2 (1, 3)	2 (1, 3)	2 (1, 4)	1 (1, 4)	3 (1, 7)	0 (0, 1)	1 (1, 3)
IL-4	19 (10, 53)	21 (9, 42)	23 (11, 34)	33 (10, 98)	33 (8, 139)	39 (17, 118)	18 (9, 27)	14 (7, 48)
MIP-1 α	4 (3, 5)	4 (3, 5)	4 (3, 5)	4 (3, 6)	4 (2, 7)	6 (4, 10)	4 (2, 6)	4 (3, 6)
MIP-1 β	36 (27, 46)	46 (32, 66)	48 (34, 67)	45 (38, 52)	44 (33, 54)	42 (26, 51)	43 (28, 60)	37 (30, 53)
RANTES	108 (78, 149)	366 (277, 473)	360 (279, 457)	386 (208, 523)	366 (183, 475)	136 (71, 178)	133 (89, 194)	203 (140, 288)
TNF- β	7 (1, 32)	24 (7, 51)	23 (7, 47)	29 (20, 40)	29 (16, 40)	23 (5, 47)	1 (1, 3)	0 (0, 2)
VEGF- α	51 (36, 71)	54 (40, 74)	51 (43, 64)	58 (40, 79)	55 (40, 85)	82 (32, 103)	55 (23, 132)	52 (29, 95)
IL-18	164 (98, 242)	190 (120, 384)	183 (99, 398)	223 (153, 385)	220 (149, 384)	138 (82, 384)	167 (88, 331)	281 (182, 793)

Values are presented as the median (interquartile range) of cytokine concentrations (pg/mL), shown on the natural scale.

Table 15. Baseline serum cytokine levels among T1D participants with subsequent first CVD events stratified by MYH6 autoantibody status				
Cytokine, pg/mL	MYH6 AAb status		OR (95% CI) *	P value [†]
	Positive (n = 15)	Negative (n = 63)		
IL-6	41.4 (30.3, 60.7)	9.2 (3.5, 26.6)	6.29 (2.11–18.75)	0.008
IL-1RA	99.0 (32.8, 764.7)	21.2 (10.6, 31.1)	4.76 (1.78–12.74)	0.008
IL-8	29.7 (19.5, 95.5)	11.9 (7.5, 22.1)	4.73 (1.78–12.54)	0.008
IL-12p40	66.5 (35.4, 416.4)	27.6 (14.0, 44.7)	4.73 (1.78–12.54)	0.008
GM-CSF	68.7 (59.2, 78.4)	31.1 (13.3, 71.0)	3.77 (1.53–9.25)	0.012
IL-4	143.1 (104.7, 214.1)	53.5 (18.3, 155.6)	3.09 (1.33–7.18)	0.019
CCL22	770.1 (651.3, 895.3)	484.1 (389.2, 670.9)	3.09 (1.33–7.18)	0.019
IL-10	3.2 (2.1, 5.6)	5.7 (2.7, 9.0)	0.39 (0.17–0.86)	0.022
sCD40L	1579.0 (1357.9, 1717.2)	1261.9 (623.5, 1694.7)	2.60 (1.17–5.77)	0.022
IL-12p70	9.0 (4.3, 72.1)	3.5 (1.4, 7.0)	2.60 (1.17–5.77)	0.022
CCL2	324.5 (235.8, 374.5)	253.2 (193.5, 315.5)	2.60 (1.17–5.77)	0.022
CCL7	54.9 (33.0, 112.9)	28.5 (13.0, 48.8)	2.60 (1.17–5.77)	0.022
FGF2	150.0 (110.2, 262.1)	115.4 (84.7, 155.1)	2.60 (1.17–5.77)	0.022
G-CSF	27.1 (22.0, 83.3)	16.0 (9.2, 29.8)	2.60 (1.17–5.77)	0.022
CXCL10	172.6 (122.4, 2022.1)	105.6 (68.5, 164.5)	2.22 (1.03–4.76)	0.042
IL-18	355.7 (159.3, 1298.9)	171.1 (108.7, 301.6)	2.22 (1.03–4.76)	0.042
IL-17A	11.5 (4.1, 137.5)	4.3 (1.7, 12.7)	1.67 (0.81–3.42)	0.166
PDGF-AA	685.6 (601.7, 831.0)	599.5 (470.6, 826.1)	1.67 (0.81–3.42)	0.166
IFN- α 2	50.1 (26.2, 73.2)	27.9 (17.5, 59.0)	1.67 (0.81–3.42)	0.166
GRO- α	428.1 (323.9, 1230.3)	588.2 (200.9, 896.9)	1.46 (0.72–2.96)	0.295
IFN- γ	19.5 (7.8, 85.3)	12.8 (6.9, 26.3)	1.46 (0.72–2.96)	0.295
IL-2	5.0 (3.6, 14.6)	3.6 (1.0, 11.3)	1.46 (0.72–2.96)	0.295
TGF- α	5.2 (3.9, 32.6)	4.8 (2.9, 7.2)	1.46 (0.72–2.96)	0.295

TNF- β	33.5 (17.8, 79.9)	32.6 (4.4, 100.4)	1.46 (0.72–2.96)	0.295
IL-1 β	4.3 (2.8, 45.4)	4.0 (1.4, 16.1)	1.32 (0.65–2.67)	0.438
TNF- α	15.8 (6.8, 25.9)	11.3 (7.4, 19.1)	1.28 (0.64–2.58)	0.483
IL-7	2.9 (2.0, 7.9)	3.2 (1.5, 5.2)	1.28 (0.64–2.58)	0.483
Fractalkine	113.3 (97.6, 751.6)	117.1 (43.8, 274.2)	1.28 (0.64–2.58)	0.483
VEGF- α	65.5 (39.8, 103.9)	53.6 (34.6, 81.1)	1.28 (0.64–2.58)	0.483
Eotaxin	130.5 (98.5, 138.3)	126.6 (108.0, 169.4)	0.78 (0.39–1.57)	0.483
PDGF-BB	2428.3 (1790.1, 3838.0)	2600.6 (1941.7, 3707.4)	0.78 (0.39–1.57)	0.483
MIP-1 β	41.2 (25.8, 68.1)	47.4 (30.2, 63.4)	0.78 (0.39–1.57)	0.483
Flt-3L	23.0 (13.6, 59.4)	17.5 (7.6, 33.0)	1.16 (0.58–2.34)	0.672
EGF	47.6 (36.0, 55.1)	47.9 (21.3, 81.5)	0.88 (0.44–1.76)	0.725
IL-15 [‡]	5.6 (2.9, 30.9)	6.0 (3.1, 11.4)	1.00 (0.50–1.99)	1.000
MIP-1 α [‡]	6.8 (2.4, 21.1)	6.5 (3.5, 12.9)	1.00 (0.50–1.99)	1.000
RANTES [‡]	318.7 (119.7, 368.4)	301.7 (210.0, 400.7)	1.00 (0.50–1.99)	1.000

Values are presented as the median (interquartile range) of cytokine/chemokine concentrations (pg/mL) on the natural scale. *Unadjusted OR and 95% CI are shown per one tertile change in cytokine/chemokine concentrations. Groups were compared by logistic regression using log2-transformed data. [‡]*P* values were corrected for multiple testing using the Benjamini-Hochberg procedure. [‡]Measures of association were not calculated because the predicted probabilities were indistinguishable. Significant negative association is shown in blue font.

Table 16. Among the T1D subjects with prior CVD events who underwent cardiac magnetic resonance (CMR) imaging exams, those with ≥ 2 cardiac AAbs (AAbs) showed markedly lower left ventricular ejection fraction compared to those with ≤ 1 AAb or no AAbs

CMR parameter	Fully adjusted*			Fully adjusted*		
	≥ 2 AAbs (n=17)	Only 1 or no AAb (n=53)	P value	≥ 2 AAbs (n=17)	No AAbs (n=44)	P value
Ejection fraction, %	51 $\pm$ 2	63 $\pm$ 1	<0.0001	52 $\pm$ 2	62 $\pm$ 1	0.001

Values are least square mean $\pm$ SE. *Full adjustment includes age at CMR exam, sex, body surface area, machine type, DCCT cohort (Primary prevention/Secondary intervention), smoking, alcohol use, macroalbuminuria/ESKD, and DCCT/EDIC time-weighted mean systolic blood pressure, HDL, LDL, and HbA_{1c}.

Table 17. Independent effects of specific cardiac autoantibody types on the risk for incident CVD events

AAb type	First CVD events		Unadjusted HR (95% CI)	P value	Adjusted HR (95% CI)*	P value
	Yes (n = 79)	No (n = 812)				
MYH6	15 (19)	26 (3)	5.9 (3.4–10.4)	<0.0001	7.1 (3.0–16.5)	<0.0001
S1-MYH6	11 (14)	43 (5)	2.7 (1.4–5.2)	0.002	1.5 (0.7–3.2)	0.294
MYH7	10 (13)	24 (3)	4.0 (2.1–7.8)	<0.0001	0.7 (0.3–1.9)	0.513
TnI	7 (9)	17 (2)	4.3 (2.0–9.4)	<0.001	2.5 (1.1–5.9)	0.036

Data are presented as n (%) or HR with 95% CI. *The adjusted model includes all four cardiac AAb types above along with traditional risk factors (age, current triglycerides, current smoking, and DCCT/EDIC time-updated mean HbA_{1c} and mean systolic blood pressure). The hazard of having a first CVD event in the presence of a specific cardiac AAb type is relative to having a first CVD event in the absence of that specific AAb type. S2-MYH6 AAbs are not shown since they were not associated with first CVD events in univariable analysis.

Table 18. Selection of potential covariates associated with ≥ 2 cardiac autoantibodies on the risk of subsequent first CVD events (for Model 2)

Risk factor	Unadjusted HR (95% CI)	P value	Adjusted HR (95% CI)*	P value
Current smoker	2.40 (1.42–4.07)	0.001	2.97 (1.61–5.49)	<0.001
Age	1.07 (1.03–1.11)	<0.0001	1.05 (1.01–1.09)	0.018
Current triglycerides	1.01 (1.00–1.01)	<0.0001	1.01 (1.00–1.01)	0.017
Mean systolic blood pressure	1.07 (1.04–1.10)	<0.0001	1.04 (1.01–1.08)	0.021
Mean HbA _{1c}	1.41 (1.14–1.74)	0.002	1.14 (0.84–1.54)	0.416
Cardiac autonomic neuropathy	2.49 (1.58–3.91)	<0.0001	1.43 (0.82–2.50)	0.213
Retinopathy at DCCT baseline	1.84 (1.16–2.92)	0.010	1.39 (0.67–2.87)	0.375
Microalbuminuria/ESKD	2.10 (1.34–3.29)	0.001	1.09 (0.60–1.98)	0.783
T1D duration	1.05 (1.00–1.09)	0.038	1.00 (0.93–1.08)	0.918
Mean pulse rate	1.04 (1.01–1.07)	0.016	1.00 (0.96–1.04)	0.945
Mean LDL	1.02 (1.01–1.03)	<0.001	1.01 (1.00–1.02)	0.219
≥ 2 cAAbs	6.53 (3.72–11.46)	<0.0001	6.46 (3.43–12.15)	<0.0001

*Adjusted for all the variables listed above. A covariate was retained in the final model if it was statistically significant at $P < 0.05$.

Table 19. Association between the number of cardiac autoantibodies or MYH6 autoantibodies and risk for subsequent first CVD events (Model 2)

Risk factor	Unadjusted HR (95% CI)	P value	Adjusted HR (95% CI)*	P value
No cAAbs	1.0 [Reference] [†]			
1 cAAb	1.6 (0.8–3.1)	0.159	1.4 (0.7–2.7)	0.326
≥ 2 cAAbs	6.5 (3.7–11.5)	<0.0001	6.5 (3.6–11.8)	<0.0001
MYH6 AAbs	5.9 (3.4–10.4)	<0.0001	6.8 (3.8–12.1)	<0.0001

Data are presented as n (%) or HR and 95% CI. *Model 2 for CVD events includes age, current triglycerides, current smoking, and DCCT/EDIC time-updated mean HbA_{1c} and mean systolic blood pressure. [†]The hazard of having a first CVD event in the presence of ≥ 2 cAAbs is relative to having a first CVD event in the absence of cAAbs. The hazard of having a first CVD event in the presence of MYH6 AAbs is relative to having a first CVD event in the absence of MYH6 AAbs.

Table 20. Selection of potential covariates associated with ≥ 2 cardiac autoantibodies on the risk for subsequent first MACE (for Model 2)				
Risk factor	Unadjusted HR (95% CI)	P value	Adjusted HR* (95% CI)	P value
Current triglycerides	1.01 (1.00–1.01)	<0.0001	1.01 (1.00–1.01)	0.063
Age	1.08 (1.03–1.14)	0.002	1.07 (1.01–1.14)	0.027
Mean HbA _{1c}	1.59 (1.18–2.15)	0.003	1.68 (1.09–2.58)	0.018
Mean systolic blood pressure	1.08 (1.04–1.13)	<0.0001	1.05 (1.00–1.10)	0.073
T1D duration	1.08 (1.02–1.15)	0.013	1.07 (0.96–1.19)	0.216
Retinopathy at DCCT baseline	2.69 (1.30–5.56)	0.008	1.13 (0.36–3.52)	0.839
Microalbuminuria/ESKD	2.32 (1.21–4.45)	0.011	0.86 (0.32–2.32)	0.765
Macroalbuminuria/ESKD	2.36 (1.03–5.36)	0.041	0.94 (0.26–3.37)	0.929
Cardiac autonomic neuropathy	2.38 (1.25–4.53)	0.008	0.91 (0.40–2.08)	0.831
≥ 2 cAAbs	7.69 (3.54–16.72)	<0.0001	6.61 (2.80–15.57)	<0.0001

*Adjusted for all the variables listed above.

Table 21. Association between cardiac autoantibody number or type and risk for subsequent first MACE (Model 2)				
Risk factor	Unadjusted HR (95% CI)	P value	Adjusted HR (95% CI)*	P value
No cAAbs	1.0 [Reference] [†]			
1 cAAb	2.0 (0.8–5.0)	0.123	1.7 (0.7–4.2)	0.263
≥ 2 cAAbs	7.7 (3.5–16.7)	<0.0001	6.4 (2.8–14.7)	<0.0001
MYH6 AAbs	6.2 (2.8–13.6)	<0.0001	6.3 (2.8–14.0)	<0.0001
TnI AAbs	6.5 (2.5–16.6)	<0.0001	3.3 (1.1–9.6)	0.029

Data are presented as *n* (%) or HR with 95% CI. *Model 2 for MACE includes age, current triglycerides, and DCCT/EDIC time-updated mean HbA_{1c} and mean systolic blood pressure; the latter was forced into the model. [†]The hazard of having a MACE in the presence of ≥ 2 cAAbs is relative to having a first MACE in the absence of cAAbs. The hazard of having a first MACE in the presence of MYH6 AAbs is relative to having a MACE in the absence of MYH6 AAbs.

Table 22. Selection of potential covariates associated with MYH6 autoantibodies on risk for subsequent first CVD events (for Model 2)

Risk factor	Unadjusted HR (95% CI)	P value	Adjusted HR (95% CI)*	P value
Current smoker	2.41 (1.42–4.07)	0.001	2.30 (1.28–4.13)	0.006
Age	1.07 (1.03–1.11)	<0.0001	1.05 (1.01–1.09)	0.007
Current triglycerides	1.01 (1.00–1.01)	<0.0001	1.01 (1.00–1.01)	0.001
Mean systolic blood pressure	1.07 (1.04–1.10)	<0.0001	1.04 (1.01–1.07)	0.022
Mean HbA _{1c}	1.41 (1.14–1.74)	0.002	1.15 (0.87–1.53)	0.321
Cardiac autonomic neuropathy	2.49 (1.58–3.91)	<0.0001	1.55 (0.92–2.61)	0.097
Retinopathy at DCCT baseline	1.84 (1.16–2.92)	0.010	1.29 (0.66–2.52)	0.455
Microalbuminuria/ESKD	2.10 (1.34–3.29)	0.001	1.23 (0.72–2.11)	0.455
T1D duration	1.05 (1.00–1.09)	0.038	1.02 (0.95–1.09)	0.617
Mean pulse rate	1.04 (1.01–1.07)	0.016	1.00 (0.96–1.04)	0.867
Mean LDL	1.02 (1.01–1.03)	<0.001	1.01 (1.00–1.02)	0.220
MYH6 AAbs	5.95 (3.39–10.45)	<0.0001	6.57 (3.52–12.31)	<0.0001

*Adjusted for all the variables listed above. A covariate was retained in the final model if it was statistically significant at $P < 0.05$.

Table 23. Baseline characteristics of the T1D participants in the cytokine substudy stratified by the development of incident CVD events

Characteristics	First CVD event		P value
	Yes (n = 40)	No (n = 249)	
Age, y	52±6	48±7	0.004
T1D duration, y	29±5	28±5	0.017
Males	26 (65)	141 (57)	0.320
Current smoker	7 (18)	31 (12)	0.380
Current HbA _{1c} , %	8.1±1.4	7.9±1.3	0.302

BMI, kg/m ²	29±4	28±5	0.031
Hypertension	26 (65)	126 (51)	0.091
Cardiac autonomic neuropathy*	17 (45)	72 (30)	0.079
Microalbuminuria/ESKD	19 (48)	72 (29)	0.019
Macroalbuminuria/ESKD	8 (20)	35 (14)	0.327
Retinopathy at DCCT baseline	29 (73)	120 (48)	0.004
Current pulse rate, bpm	76±12	70±11	0.005
Current LDL, mg/dL	105±31	97±32	0.078
Current triglycerides, mg/dL	109±73	77±52	0.003
Conventional treatment group	20 (50)	13 (55)	0.523
DCCT/EDIC time-updated mean			
HbA _{1c} , %	8.2±1.0	8.0±1.0	0.224
Pulse rate, bpm	76±7	73±7	0.015
LDL, mg/dL	119±19	109±22	0.006
Triglycerides, mg/dL	102±47	84±42	0.002
Systolic blood pressure, mm Hg	124±9	118±8	<0.001
Values are presented as mean ± SD or <i>n</i> (%). *Within the cardiac autonomic neuropathy group, the number of participants (<i>n</i>) with a first CVD event is 38, while the number without a first CVD event is 237.			

Table 24. Effect of each of the 12 core cytokines combined with IL-6 on the risk of CVD events associated with MYH6 autoantibodies		
MYH6 AAbs and risk	HR (95% CI)	<i>P</i> value
first CVD events		
Adjusted for traditional risk factors	3.81 (2.00–7.27)	<0.0001
IL-6	2.29 (1.13–4.64)	0.021
IL-6 + GM-CSF (Reference)	1.52 (0.66–3.53)	0.328

IL-6 + CCL22	2.06 (1.00–4.28)	0.052
IL-6 + IFN- γ	2.12 (1.00–4.49)	0.050
IL-6 + CCL7	2.12 (1.04–4.31)	0.039
IL-6 + G-CSF	2.16 (1.06–4.43)	0.035
IL-6 + CCL2	2.26 (1.11–4.59)	0.024
IL-6 + sCD40L	2.35 (1.17–4.72)	0.017
IL-6 + IL-8	2.41 (1.19–4.88)	0.014
IL-6 + TNF- α	2.98 (1.52–5.85)	0.002
IL-6 + IL-12p70	2.98 (1.51–5.91)	0.002
IL-6 + EGF	2.54 (1.28–5.05)	0.008

The MYH6 AAb model was initially adjusted for age, retinopathy at DCCT baseline, and time-updated mean LDL, and then further adjusted for IL-6 alone or in combinations with each of the 12 core cytokines.

Table 25: Risk of first CVD according to the number and type of cardiac autoantibodies present at baseline in the mostly childhood-onset T1D CACTI cohort

Risk factor			Unadjusted HR (95% CI)	P value	Fully adjusted [†] HR (95% CI)	P value
	Yes (n = 56)	No (n = 451)				
Autoantibody number						
No AAb	34 (61)	340 (75)	1.0 [Reference]		1.0 [Reference]	
Any AAb	22 (39)	111 (25)	2.1 (1.2–3.5)	0.010	2.1 (1.2–3.8)	0.015
1 AAb	13 (23)	75 (17)	1.7 (0.9–3.3)	0.102	1.7 (0.8–3.4)	0.160
≥2 AAbs	9 (16)	36 (8)	2.9 (1.3–6.0)	0.007	3.9 (1.7–8.9)	0.002
Autoantibody type						
MYH6	8 (14)	22 (5)	3.3 (1.6–7.1)	0.002	3.4 (1.6–7.4)	0.002
S1-MYH6	12 (21)	62 (14)	1.8 (0.9–3.4)	0.081	1.7 (0.9–3.4)	0.130
S2-MYH6	4 (7)	37 (8)	0.9 (0.3–2.5)	0.865	0.9 (0.3–2.9)	0.843
MYH7	6 (11)	14 (3)	3.1 (1.3–7.2)	0.010	3.4 (1.3–8.7)	0.012
cTnl	4 (7)	16 (4)	2.4 (0.9–6.7)	0.095	3.2 (1.1–6.7)	0.036

[†]Fully adjusted models include: age, sex, HbA1c, systolic blood pressure, LDL-cholesterol and urinary albumin/creatinine ratio

Table 26: Comparison of MYH6 AABs to established risk biomarkers for predicting CVD events in CACTI				
Biomarker	Adjusted for other biomarkers Hazard Ratio (95% CI)	P value	Adjusted for other biomarkers <u>and</u> <u>traditional risk factors</u> Hazard Ratio* (95% CI)	P value
MYH6 AABs	2.97 (1.33–6.64)	0.008	3.27 (1.44–7.41)	0.005
hsTnT	1.82 (1.37–2.42)	<0.0001	1.67 (1.15–2.43)	0.007
NT-ProBNP	1.14 (0.90–1.46)	0.273	1.11 (0.80–1.52)	0.542

*Traditional risk factors included: age, sex, HbA1c, systolic blood pressure, LDL, and urinary albumin/creatinine ratio. Hazard ratios for cTnT-Hs and NT-ProBNP are per one-quartile increase in the concentration of these markers

Table 27: Association between HLA-DQ genotype and cardiac autoantibody types in CACTI							
HLA-DQ genotype	Total (n = 496)	MYH6 AABs		P- value	MYH7 AABs		P- value
		Positive (n = 29)	Negative (n = 467)		Positive (n = 20)	Negative (n = 476)	
DQ2 (DQA1*05:01-DQB1*02:01)	253 (51)	15 (52)	238 (51)	0.937	7 (35)	246 (52)	0.144
DQ2/2	33 (7)	3 (10)	30 (6)	0.412	2 (10)	31 (7)	0.541
DQ2 and not DQ8	121 (24)	6 (21)	115 (25)	0.633	6 (30)	115 (24)	0.552
DQ8 (DQA1*03:01-DQB1*03:02)	326 (66)	23 (79)	303 (65)	0.113	14 (70)	312 (66)	0.682
DQ8/DQ8	45 (9)	5 (17)	40 (9)	0.115	7 (35)	38 (8)	<0.0001
DQ8 and not DQ2	194 (39)	14 (48)	180 (39)	0.298	13 (65)	181 (38)	0.015

Table 28: Cytokine profile among the CACTI participants who subsequently developed first CVD events			
	CACTI participants with subsequent first CVD events		
Median, pg/mL	MYH6 AAb- (n = 42)	MYH6 AAb+ (n = 8)	P value [†]
GM-CSF	4.2 (0.1, 15.0)	148.9 (8.2, 745.2)	0.009
IL-6	1.1 (0.3, 4.2)	18.7 (3.3, 47.2)	0.006
IFN- γ	1.2 (0.5, 6.8)	16.2 (12.8, 123.9)	<0.001
TNF- α	16.8 (12.8, 25.3)	65.2 (25.3, 203.2)	<0.001
IL-1 β	3.9 (1.8, 7.4)	32.4 (9.8, 100.3)	<0.001
IL-1RA	4.9 (2.9, 8.9)	19.1 (16.5, 199.5)	<0.001
IL-2	1.8 (1.2, 3.6)	18.9 (5.3, 48.4)	<0.001
IL-4	0.5 (0.3, 0.8)	7.3 (0.8, 39.5)	<0.001
IL-5	0.6 (0.3, 1.6)	14.4 (2.3, 23.0)	<0.001
IL-8	17.4 (9.5, 29.6)	31.3 (16.4, 80.9)	0.152
IL-10	3.0 (2.2, 6.1)	26.4 (8.4, 241.4)	<0.001
IL-12p40	96.2 (72.1, 163.7)	221.8 (81.9, 1030.0)	0.058
IL-12p70	5.4 (2.3, 10.6)	49.8 (7.4, 175.2)	0.003
IL-13	32.7 (18.4, 55.1)	208.3 (63.0, 976.2)	<0.001
CCL2	422.3 (324.2, 624.6)	529.9 (270.7, 739.1)	0.947

EQUIVALENTS

[00233] The foregoing written specification is considered to be sufficient to enable one skilled in the art to practice the embodiments. The foregoing description and Examples detail certain embodiments and describe the best mode contemplated by the inventors. It will be appreciated, however, that no matter how detailed the foregoing may appear in text, the embodiment may be practiced in many ways and should be construed in accordance with the appended claims and any equivalents thereof.

[00234] As used herein, the term about refers to a numeric value, including, for example, whole numbers, fractions, and percentages, whether or not explicitly indicated. The term about generally refers to a range of numerical values (e.g., +/-5-10% of the recited range) that one of ordinary skill in the art would consider equivalent to the recited value (e.g., having the same function or result). When terms such as at least and about precede a list of numerical values or ranges, the terms modify all of the values or ranges provided in the list. In some instances, the term about may include numerical values that are rounded to the nearest significant figure.

What is Claimed is:

1. A method for identifying a subject with type 1 diabetes (T1D) at increased risk of developing a first cardiovascular disease event, wherein the method comprises:
 - (a) isolating blood, optionally a serum or plasma sample, from the subject;
 - (b) identifying the subject as having cardiac autoantibodies (AAbs) specific to full-length myosin heavy chain 6 (MYH6, SEQ ID NO: 1) in the blood; and
 - (c) predicting that the subject is at increased risk for developing a first cardiovascular disease event compared to a subject with T1D not having cardiac AAbs specific to full-length MYH6.
2. A method for identifying a subject with T1D at increased risk of developing a first cardiovascular disease event, wherein the method comprises:
 - (a) isolating blood, optionally a serum or plasma sample, from the subject;
 - (b) identifying the subject as having two or more cardiac AAbs, wherein the two or more cardiac AAbs are selected from those specific to:
 - (i) full-length MYH6;
 - (ii) the S1-fragment of MYH6;
 - (iii) the S2-fragment of MYH6;
 - (iv) full-length myosin heavy chain 7 (MYH7, SEQ ID NO: 2); and
 - (v) full-length cardiac troponin I (cTnI, SEQ ID NO: 3); and
 - (c) predicting that the subject is at increased risk for developing a first cardiovascular disease event compared to a subject with T1D not having said cardiac AAbs.
3. The method of claim 1 or claim 2, wherein the first cardiovascular disease event is any one of:
 - (a) nonfatal myocardial infarction (MI);
 - (b) subclinical MI ("silent MI") detected on an electrocardiogram;
 - (c) angina confirmed by ischemic changes with exercise testing or by clinically significant obstruction on coronary angiography, or wherein the angina occurs at rest or does not respond to nitroglycerin treatment;
 - (d) coronary revascularization with angioplasty or coronary artery bypass;

- (e) ischemic stroke;
 - (f) heart failure; or
 - (g) death secondary to cardiovascular disease.
4. A method for identifying a subject with T1D and a preexisting cardiovascular disease event at increased risk of having a recurrent cardiovascular disease event comprising:
- (a) isolating blood, optionally a serum or plasma sample, from the subject;
 - (b) identifying the subject as having cardiac AAbs specific to full-length MYH6 in the blood; and
 - (c) predicting that the subject is at increased risk for developing a recurrent cardiovascular disease event compared to a subject with T1D not having AAbs specific to full-length MYH6.
5. A method for identifying a subject with T1D and a preexisting cardiovascular event at increased risk of having a recurrent cardiovascular event, wherein the method comprises:
- (a) isolating blood, optionally a serum or plasma sample, from the subject;
 - (b) identifying the subject as having two or more cardiac AAbs, wherein the two or more cardiac AAbs are selected from those specific to:
 - (i) full-length MYH6;
 - (ii) the S1-fragment of MYH6;
 - (iii) the S2-fragment of MYH6;
 - (iv) full-length myosin heavy chain 7 (MYH7, SEQ ID NO: 2); or
 - (v) full-length cardiac troponin I (cTnI, SEQ ID NO: 3)
 - (c) predicting that the subject is at increased risk for developing a recurrent cardiovascular disease event compared to a subject with T1D not having said cardiac AAbs.
6. The method of claim 4 or claim 5, wherein the preexisting cardiovascular disease event is any one of:
- (a) nonfatal myocardial infarction (MI);
 - (b) subclinical MI ("silent MI") detected on an electrocardiogram;

- (c) angina confirmed by ischemic changes with exercise testing or by clinically significant obstruction on coronary angiography, or wherein the angina occurs at rest or does not respond to nitroglycerin treatment;
 - (d) coronary revascularization with angioplasty or coronary artery bypass; or
 - (e) ischemic stroke.
7. The method of any one of claims 4-6 wherein the recurrent cardiovascular event is one or more or:
- (a) nonfatal myocardial infarction (MI);
 - (b) coronary revascularization with angioplasty or coronary artery bypass;
 - (c) ischemic stroke;
 - (d) heart failure; or
 - (e) death secondary to cardiovascular disease.
8. The methods of any one of claims 1-7, wherein the increased risk for developing a first or recurrent cardiovascular disease event persists after adjustment for established cardiovascular risk factors selected from one or more of the following: age, sex, duration of diabetes, HbA1c levels, systolic blood pressure, diabetic kidney disease, retinopathy, cardiac autonomic neuropathy, pulse rate, smoking, triglycerides, and LDL cholesterol levels.
9. The method of any one of claims 1-8, further comprising:
- (a) evaluating additional cardiac AAb-linked genetic and proinflammatory cytokine risk markers, wherein the additional markers comprise one or more of:
 - (i) the presence of the human leucocyte antigen (HLA) genotype, DQ2 or DQ8;
 - (ii) the presence of elevated blood levels of one or more proinflammatory cytokines, wherein the one or more proinflammatory cytokines are selected from: IL-6, GM-CSF, TNF- α , G-CSF, EGF, sCD40L, CCL2, IL-8, IL-12, CCL7, IFN- γ , and CCL22, and wherein the elevated levels of the one or more proinflammatory cytokines are compared to levels in healthy control subjects or in subjects with T1D not having cardiac AAbs; and

- (iii) the presence of decreased IL-10 levels, wherein the decreased levels of IL-10 are compared to levels in healthy control subjects or subjects with T1D not having cardiac AAbs, and
 - (b) predicting that the subject is at increased risk for developing a first or recurrent cardiovascular disease event compared to a subject having cardiac AAbs alone, i.e., without the one or more additional linked genetic and proinflammatory cytokine risk markers.
10. The method of claim 9, wherein the HLA-DQ2 genotype is DQ2.5 (DQA1*05:01/DQB1*02:01).
11. The method of claim 10, wherein a subject with a HLA-DQ2.5 homozygous genotype is at increased risk of developing a first or recurrent cardiovascular disease event than a subject with HLA-DQ2.5 heterozygous genotype.
12. The method of claim 9, wherein the HLA-DQ8 genotype is DQA1*03:01-DQB1*03:02.
13. The method of claim 12, wherein a subject with a HLA-DQA1*03:01-DQB1*03:02 homozygous genotype is at increased risk of developing a first or recurrent cardiovascular disease event than a subject with HLA-DQA1*03:01-DQB1*03:02 heterozygous genotype.
14. The method of any one of claims 9-13, wherein the additional markers comprise presence of elevated blood levels of IL-6 and GM-CSF, and wherein the elevated blood levels are 2-times or greater, 3-times or greater, 5-times or greater, 7-times or greater, or 10- times or greater, as compared to healthy control subjects or subjects with T1D not having cardiac AAbs.
15. The method of claims 9-14, wherein adding GM-CSF and IL-6 to a cardiovascular risk prediction model further improves the prediction of a first or recurrent cardiovascular disease event compared with a model containing full-length MYH6 AAbs alone.
16. The method of claims 9-15, wherein adding GM-CSF and IL-6 to a cardiovascular risk prediction model further improves the prediction of a first or recurrent cardiovascular event compared with a model containing two or more cardiac AAbs alone, wherein the two or more cardiac AAbs are selected from those that are specific to:
- (a) full-length MYH6;
 - (b) S1-fragment of MYH6;
 - (c) S2-fragment of MYH6;

- (d) full-length MYH7; or
- (e) full-length cTnI.

17. The method of any one of claims 1, 3, 4, 6-15, wherein identifying a subject as having AAbs specific to full-length MYH6 predicts the development of a cardiovascular disease event at a significantly higher rate and at a younger age than a subject not having AAbs specific to full-length MYH6.

18. The method of any one of claims 2, 3, 5-14, or 16, wherein identifying a subject as having 2 or more cardiac AAbs predicts the development of a first or recurrent cardiovascular disease event at a significantly higher rate and at a younger age than a subject with T1D not having said cardiac AAbs.

19. The method of any one of claims 1-18, wherein the subject has adult-onset T1D.

20. The method of claim 19, wherein the subject has adult-onset T1D and has a HLA-DQ2 genotype, such as a DQ2.5 genotype.

21. The method of any one of claims 1-18, wherein the subject has childhood-onset T1D.

22. The method of claim 21, wherein the subject has a HLA-DQ8 genotype, optionally wherein the DQ8 genotype is DQA1*03:01-DQB1*03:02.

23. A method for identifying a subject with cancer treated with at least one immune checkpoint inhibitor (ICI) who is at increased risk of developing myocarditis or who has suspected myocarditis or who is in need of treatment with an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor of T-cell activation, a B-cell antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6, wherein the method comprises:

- (a) isolating blood, optionally a serum or plasma sample, from the subject;
- (b) identifying the subject as having cardiac autoantibodies (AAbs) specific to full-length myosin heavy chain 6 (MYH6, SEQ ID NO: 1) in the blood; and
- (c) predicting that the subject is at increased risk of developing myocarditis, or that the subject has suspected myocarditis, or that the subject is in need of treatment with an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor

of T-cell activation, a B-cell antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6, compared to a subject with cancer treated with at least one ICI not having cardiac AAbs specific to full-length MYH6.

24. A method for identifying a subject with cancer treated with an immune checkpoint inhibitor (ICI) who is at increased risk of developing myocarditis or who has suspected myocarditis or who or is in need of treatment with an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor of T-cell activation, a B-cell antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6, wherein the method comprises:

- (a) isolating blood, optionally a serum or plasma sample, from the subject;
- (b) identifying the subject as having two or more cardiac AAbs, wherein the two or more cardiac AAbs are selected from those specific to:
 - (i) full-length MYH6;
 - (ii) the S1-fragment of MYH6;
 - (iii) the S2-fragment of MYH6;
 - (iv) full-length myosin heavy chain 7 (MYH7, SEQ ID NO: 2); and
 - (v) full-length cardiac troponin I (cTnI, SEQ ID NO: 3); and
- (c) predicting that the subject is at increased risk for developing myocarditis or has suspected myocarditis or is in need of treatment with an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor of T-cell activation, a B-cell antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6, compared to a subject with cancer treated with at least one ICI not having said cardiac AAbs.

25. The method of any one of claims 23-24, wherein the at least one ICI comprises an anti-PD-1 antibody, an anti-CTLA4 antibody, an anti-PD-L1 antibody, a combination of an anti-PD-1 and anti-CTLA4 antibody, or a combination of an anti-PD-L1 antibody and an anti-CTLA4 antibody.

26. The method of any one of claims 23-25, further comprising:

- (a) evaluating additional cardiac AAb-linked proinflammatory cytokine risk markers, wherein the additional markers comprise one or more of:
 - (i) the presence of elevated blood levels of one or more proinflammatory cytokines, wherein the one or more proinflammatory cytokines are selected from: IL-6, GM-CSF, TNF- α , G-CSF, EGF, sCD40L, CCL2, IL-8, IL-12, CCL7, IFN- γ , and CCL22, and wherein the elevated levels of the one or more proinflammatory cytokines are compared to levels in healthy control subjects or in subjects with cancer and treated with at least one ICI not having cardiac AAbs; and
 - (ii) the presence of decreased IL-10 levels, wherein the decreased levels of IL-10 are compared to levels in healthy control subjects or in subjects with cancer and treated with at least one ICI not having cardiac AAbs, and
 - (b) predicting that the subject is at increased risk for developing myocarditis or has suspected myocarditis or is in need of treatment with an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor of T-cell activation, a B-cell antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6, compared to a subject having cardiac AAbs alone, i.e., without the one or more additional linked proinflammatory cytokine risk markers.
27. The method of claim 26, wherein the additional markers comprise presence of elevated blood levels of IL-6 and GM-CSF, wherein the elevated blood levels are 2-times or greater, 3-times or greater, 5-times or greater, 7-times or greater, or 10- times or greater, as compared to levels in healthy control subjects or subjects with cancer and treated with at least one ICI not having cardiac AAbs.
28. The method of any one of claims 26-27, wherein adding GM-CSF and IL-6 to a cardiovascular risk prediction model further improves the prediction of development of myocarditis compared with a model containing full-length MYH6 AAbs alone.
29. The method of any one of claims 26-28, wherein adding GM-CSF and IL-6 to a cardiovascular risk prediction model further improves the prediction of development of

myocarditis compared with a model containing two or more cardiac AAbs alone, wherein the two or more cardiac AAbs are selected from those that are specific to:

- (a) full-length MYH6;
- (b) S1-fragment of MYH6;
- (c) S2-fragment of MYH6;
- (d) full-length MYH7; or
- (e) full-length cTnI.

30. The method of any one of claims 1-29, wherein identifying cardiac AAbs comprises the use of a fluid-phase radioimmunoprecipitation assay.

31. The method of any one of claims 1-30, wherein having cardiac AAb is defined as having an AAb index above the threshold for positivity based on predetermined cutoff values at the 99th percentile of the levels in serum samples from healthy control subjects.

32. The method of any one of claims 29-30, where the cardiac AAb index is defined as
$$\frac{[\text{counts per million (CPM) in the unknown sample} - \text{CPM in the negative control standard}]}{[\text{CPM in the positive standard} - \text{CPM in the negative standard}]} \times 100$$
 in a fluid- phase radioimmunoprecipitation assay.

33. The method of any one of the claims 30-32, where the cutoff for having full-length MYH6 AAbs is about 0.701.

34. The method of any one of the claims 30-33, where the cutoff for having S1-fragment MYH6 AAbs is about 0.330.

35. The method of any one of the claims 30-34, where the cutoff for having S2-fragment MYH6 AAbs is about 0.763.

36. The method of any one of the claims 30-35, where the cutoff for having full-length MYH7 AAbs is about 0.799.

37. The method of any one of the claims 30-36, where the cutoff for having full-length cTnI AAbs is about 0.283.

38. The method of any one of claims 1-37, further comprising administering one or more treatment to a subject to treat the predicted increased risk for developing a first or recurrent

cardiovascular disease event, or to treat the predicted increased risk for developing myocarditis and/or suspected myocarditis.

39. A method of selecting subjects to include in a clinical trial for the treatment or prevention of a cardiovascular disease event comprising:

- (a) determining that the subject as having an increased risk of a first or recurrent cardiovascular disease event, using the method of any one of claims 1-37; and
- (b) including the subjects in the clinical trial based on this increased risk.

40. A method of preventing or treating a cardiovascular disease event in a subject with T1D comprising:

- (a) determining that the subject as having an increased risk of a first or recurrent cardiovascular disease event using the method of any one of claims 1-37, and
- (b) administering one or more treatments for preventing or treating a cardiovascular disease event.

41. A method of preventing or treating a cardiovascular disease event in a subject with cancer treated with at least one ICI, comprising:

- (a) determining that the subject has an increased risk of developing myocarditis or has suspected myocarditis using the method of any one of claims 23-40, and
- (b) administering one or more treatments for preventing or treating myocarditis.

42. The method of any one of claims 38-41, wherein the one or more treatment comprises inhibiting the effect of one or more proinflammatory cytokines or inhibiting the autoimmune disease process itself.

43. The method of any one of claims 38-42, wherein the one or more treatment comprises administration of an IL-6 ligand inhibitor, IL-6 receptor inhibitor, GM-CSF inhibitor, JAK1/JAK2 inhibitor, inhibitor of T-cell activation, a B-cell antagonist, regulatory T cell (Treg) therapy, low-dose IL-2 therapy, or a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6.

44. The method of claim 43, wherein the IL-6 ligand inhibitor is sirukumab, olokizumab, clazakizumab, vobarilizumab, or ziltivekimab.

45. The method of claim 43, wherein the IL-6 receptor inhibitor is sarilumab or tocilizumab.

46. The method of claim 43, wherein the GM-CSF inhibitor is mavrilimumab, otilimab, namilumab, or lenzilumab.
47. The method of claim 43, wherein the JAK1/JAK2 inhibitor is baricitinib.
48. The method of claim 43, wherein the inhibitor of T-cell activation is an anti-CD3 antibody or a CTLA4-Ig fusion protein.
49. The method of claim 43, wherein the anti-CD3 antibody is teplizumab.
50. The method of claim 43, wherein the low-dose IL-2 is recombinant IL-2 (aldesleukin).
51. The method of claim 43, wherein the antigen is conjugated or delivered in nanoparticles or is a modified mRNA vaccine.
52. The method of claim 43, wherein the CTLA-Ig fusion protein is abatacept.
53. A method of preventing a first or recurrent cardiovascular disease event in a subject with T1D comprising:
- (a) determining that the subject as having an increased risk of a first or recurrent cardiovascular disease event using the method of any one of claims 1-22 or 30-52, wherein the subject expresses the HLA DR3-DQ2 or HLA-DQ8 haplotype, and
 - (b) administering a tolerogenic therapy specific for an antigen comprising all or part of full-length MYH6, wherein the tolerogenic therapy restores immune tolerance to MYH6 and thereby prevents a first or recurrent cardiovascular disease event.
54. The method of claim 53, wherein the antigen is conjugated or delivered in nanoparticles or is a modified mRNA vaccine.
55. The method of claim 53 or 54, wherein the HLA-DQ2 genotype is DQ2.5 (DQA1*05:01/DQB1*02:01).
56. The method of claim 55, wherein a subject has a HLA-DQ2.5 homozygous genotype.
57. The method of claim 53 or 54, wherein the HLA-DQ8 genotype is DQA1*03:01-DQB1*03:02.
58. The method of claim 57, wherein a subject has a HLA-DQA1*03:01-DQB1*03:02 homozygous genotype.

59. The method of any one of claims 1-22 or 30-58, wherein the T1D is latent autoimmune diabetes of adults (LADA).