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Heterogeneity of Diabetes: β-Cells, Phenotypes, and Precision Medicine: Proceedings of an International Symposium of the Canadian Institutes of Health Research's Institute of Nutrition, Metabolism and Diabetes and the U.S. National Institutes of Health's National Institute of Diabetes and Digestive and Kidney Diseases

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One hundred years have passed since the discovery of insulin—an achievement that transformed diabetes from a fatal illness into a manageable chronic condition. The decades since that momentous achievement have brought ever more rapid innovation and advancement in diabetes research and clinical care. To celebrate the important work of the past century and help to chart a course for its continuation into the next, the Canadian Institutes of Health Research's Institute of Nutrition, Metabolism and Diabetes and the U.S. National Institutes of Health's National Institute of Diabetes and Digestive and Kidney Diseases recently held a joint international symposium, bringing together a cohort of researchers with diverse interests and backgrounds from both countries and beyond to discuss their collective quest to better understand the heterogeneity of diabetes and thus gain insights to inform new directions in diabetes treatment and prevention. This article summarizes the proceedings of that symposium, which spanned cutting-edge research into various aspects of islet biology, the heterogeneity of diabetic phenotypes, and the current state of and future prospects for precision medicine in diabetes.

It has been 100 years since the discovery of insulin—without question one of the most impactful medical achievements of the 20th century. Before Frederick G. Banting and his colleagues made this momentous discovery, diabetes was fatal, claiming the lives of people who developed it within a few months to a few years. However, the

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isolation and extraction of insulin, and its subsequent commercialization, transformed diabetes into the manageable chronic condition it is today, made even more so as therapeutic, technological, and clinical research advances in diabetes continued to improve diabetes management.

Indeed, although the discovery of insulin changed the diabetes landscape forever, one could reasonably argue that the modern era of diabetes management only really began in the final quarter of the last century. For example, who could have imagined in 1976—more than 50 years after the advent of insulin—that the glycated hemoglobin (HbA_{1c}) test, first performed at that time on five hospitalized patients with diabetes (1), would become a gold standard test for assessing diabetes, determining therapeutic indications, and even diagnosing diabetes? The initial report describing the HbA_{1c} test stated that "hemoglobin A_{1c} concentration appears to reflect the mean blood sugar concentration best over previous weeks to months" and that "the periodic monitoring of hemoglobin A1c levels provides a useful way of documenting the degree of control of glucose metabolism in diabetic patients" (1). Since the publication of that landmark article, the translational advances in terms of both clinical application of HbA_{1c} measurement and resulting improvements in diabetes medical management have been impressive and have transpired at a much quicker pace.

So much has occurred that it is hard to believe that it was only 1993—less than 30 years ago—when the results of the Diabetes Control and Complications Trial were published (2) and the world fully understood both the crucial importance of achieving near-normal glycemic control and the substantial value of the HbA_{1c} test in monitoring that effort. Less than 20 years ago, in 2002, we learned from the Diabetes Prevention Program research study (3) that the onset of type 2 diabetes can be delayed or avoided through lifestyle modification or pharmacotherapy. And, less than 3 years ago, yet another landmark study was published, this one reporting that a course of the anti-CD3 antibody teplizumab could delay progression to clinical type 1 diabetes in high-risk individuals (4). These were all major research achievements that further altered the course of diabetes management.

Other important breakthroughs also occurred in rapid succession. New drug classes—specifically sodium–glucose cotransporter 2 inhibitors and glucagon-like peptide 1 receptor agonists—have come to market, offering cardiovascular and renoprotective benefits beyond their glycemic effects as assessed by HbA_{1c} (5). There have also been tremendous advances in bariatric surgery, and the American Diabetes Association's (ADA's) *Standards of Medical Care in Diabetes* now includes guidance on its role in the treatment of appropriate patients with type 2 diabetes (6). Technology has also contributed significantly to the improved management of diabetes, with impressive advances made in continuous glucose monitoring (7) and the advent of commercially available hybrid closed-loop insulin delivery systems (8). There have also been expansions in the knowledge base supporting diabetes care, education, and support, emphasizing that there is no "one-size-fits-all" diet for individuals with diabetes (9), recognizing that patients should be at the center of the clinical decision-making cycle for diabetes management (10), and underscoring the need to mitigate inequities in the distribution of social determinants of health and the provision of medical care (11).

All of these developments have informed the currently recommended strategies for managing diabetes, as reflected in guidelines from numerous professional organizations, including the ADA's *Standards of Medical Care in Diabetes*. Collectively, these advancements have led us to today's more individualized approach to treatment. Using type 2 diabetes as an example, we are now encouraged to base the selection of pharmacological therapies on the presence or absence of comorbidities such as atherosclerotic cardiovascular disease and chronic kidney disease independent of a patient's HbA_{1c}—a change based on clinical research evidence to date. In addition, providers are also encouraged to factor in the relative importance of each patient's weight status, hypoglycemia risk, and financial constraints (12).

However, these more individualized recommendations, although invaluable, are generally not considered to be "precision medicine," which can be defined as "an emerging approach for disease prevention and treatment that takes into account people's individual variations in genes, environment, and lifestyle" (13). Essentially, precision medicine is the process of applying biological science to match the most appropriate therapy to the most appropriate person at the most appropriate time. As we enter the second century of diabetes care since the discovery of insulin, precision medicine truly represents the next frontier for diabetes, and in the coming years, diabetes research will be increasingly focused on furthering this approach.

In 2013, Franks et al. (14) reported that the future of research on how best to stratify diabetes medicine will require a full understanding of the interaction of all nongenetic elements to which people may be exposed (nutrition, physical activity, sleep, stress, etc.) with the quantifiable elements of our physiome (e.g., genome, proteome, and metabolome). Only in this way will precision therapies become a routine part of medical management for all people with diabetes, as they are now only for those with rare monogenic forms of the disease for which physiomic factors have been more fully elucidated.

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See accompanying articles, pp. 1 and 23.

To celebrate the completion of the first century of diabetes innovation and usher in a new era in which precision medicine is certain to flourish, the Canadian Institutes of Health Research's Institute of Nutrition, Metabolism and Diabetes (CIHR-INMD) and the U.S. National Institutes of Health's National Institute of Diabetes and Digestive and Kidney Diseases (NIH-NIDDK) recently held a joint symposium. Titled "Heterogeneity of Diabetes: β-Cells, Phenotypes, and Precision Medicine," this first-ever collaboration between the premier diabetes research institutes of Canada and the U.S. gathered researchers from both countries and beyond virtually to discuss the challenges and opportunities in their quest to better understand the heterogeneity of diabetes and thus gain insights that could chart new directions in treatment and prevention.

This article summarizes the proceedings of that symposium.

SESSION 1: ISLET BIOLOGY IN HEALTH AND DIABETES

The symposium opened with an in-depth discussion of the heterogeneity of diabetes at its most basic level, particularly focusing on the islet microenvironment. Discussions centered on islet cell interactions, heterogeneity in β-cell function, pericytes, and exocrine pancreas inflammation. Specifically, 14 researchers presented cutting-edge work elucidating both intercellular diversity within the islets and key intracellular functional differences among subpopulations of β-cells, as well as the role of islet vasculature in the pathogenesis of diabetes. They described emerging bioengineering and biomimetic strategies to advance disease modeling for preclinical testing of potential new treatments and optimization of cell transplantation procedures. Additionally, they discussed various stressors that contribute to β-cell death and dysfunction and the ways in which these stress pathways could become therapeutic targets to influence diabetes development and progression.

Part 1: Heterogeneity of the Islet Microenvironment

The first topic within this session focused on the heterogeneity of the islet microenvironment, including the diversity of β-cells in terms of phenotype and function. Presenters also explained how interactions involving other cell populations within and outside of the islets may affect β-cells' ability to respond physiologically to metabolic changes and challenges and the role these diverse interactions may play in the pathogenesis and maintenance of a diabetic state.

Presentation 1: Heterogeneity of Endocrine Islet Cells (Mark O. Huising)

Multiple endocrine cell types constitute the pancreatic islet, supported by a constellation of ancillary cell types. Attention is most often and understandably directed at pancreatic β-cells, as the only source of endogenous insulin and the only islet cells that cause endocrine disorders upon dysfunction. However, we are beginning to appreciate that pancreatic α- and δ-cells, which release glucagon and somatostatin, respectively, each play an important role in overall pancreas function. Discussion centered on these cell types and the roles they may play in activating multiple signaling cascades and in modulating β-cell activity to meet the body's insulin needs (15,16). A better understanding of the full islet landscape and interactions between the cell types is necessary to grasp the heterogeneity of diabetes at a cellular level.

To that end, Dr. Huising, of the University of California, Davis, in the U.S., presented on the physiological role of δ-cells as local integrators in the regulation of insulin secretion in response to glucose. Data demonstrate that δ-cells restrain insulin and glucagon secretion during hyperglycemia and remain active during normoglycemia, increasing the β-cell glucose threshold. In the absence of δ-cell function, this threshold is reduced, with an acute and lasting drop in the glucose set point (16). Endogenous Ucn-3 (urocortin-3) produced by β-cells plays an important role in this feedback regulation, promoting somatostatin secretion by δ-cells, which, in turn, inhibits insulin release (Fig. 1) (17,18).

In support of the importance of δ-cells, recent research by Dr. Huising's laboratory, in collaboration with Vincent Poitout from the University of Montreal, showed that the free fatty acid receptor GPR120 stimulates insulin and glucagon release, which is mediated at least in part by the suppression of δ-cell activity. This finding was confirmed by the observation that specific knockout of GPR120 in δ-cells prevents GPR120 agonists from increasing insulin release (19).

Presentation 2: Heterogeneity of β-Cell Functionality (Richard K.P. Benninger)

Not only is the islet landscape heterogeneous in terms of different types of cells (i.e., α-, β-, and δ-cells), but there is also heterogenity within the β-cell population itself (20–23). The presentation by Dr. Benninger, of the University of Colorado, Denver, in the U.S., discussed the heterogeneous functionality of β-cells, which encompasses insulin secretion, metabolic activity, and calcium ion (Ca^{2+}) dynamics.

Understanding how β-cells with differing functionality contribute to overall islet function is an area of active research. Dr. Benninger and his research team have identified multiple subpopulations of β-cells based on signatures of Ca^{2+} dynamics (24). He described their investigations, in animal and human tissue studies, of the role of one such subpopulation, termed "first-responder" cells, in islet function, using two-photon laser ablation; he also described investigations of the functional properties of these cells using pyridine nucleotide autofluorescence, glibenclamide stimulation, and network analysis (25). Their analyses showed that first-responder cells are distinct from previously identified functional subpopulations and display characteristics of high membrane excitability and slightly lower-than-average coupling to their neighbors. They also observed a hierarchy of the first-phase response time, through which cells that were next earliest to respond often took over the role of the first-responder cells upon ablation.

This distinctive first-responder β-cell is functionally important to a phase of insulin release that is crucial for glucose homeostasis and disrupted early in diabetes progression. The researchers speculate that recovering the functional capacity of subpopulations of β-cells may restore the dynamics of insulin release needed for effective glycemic control.

This work underscores the importance of expanding research in preclinical models to studies on human tissue, as initial data from Dr. Benninger's team indicate that heterogeneity in human islets may differ from what has been described in animal models. Differences between β-cell populations in human and experimental animals may determine different

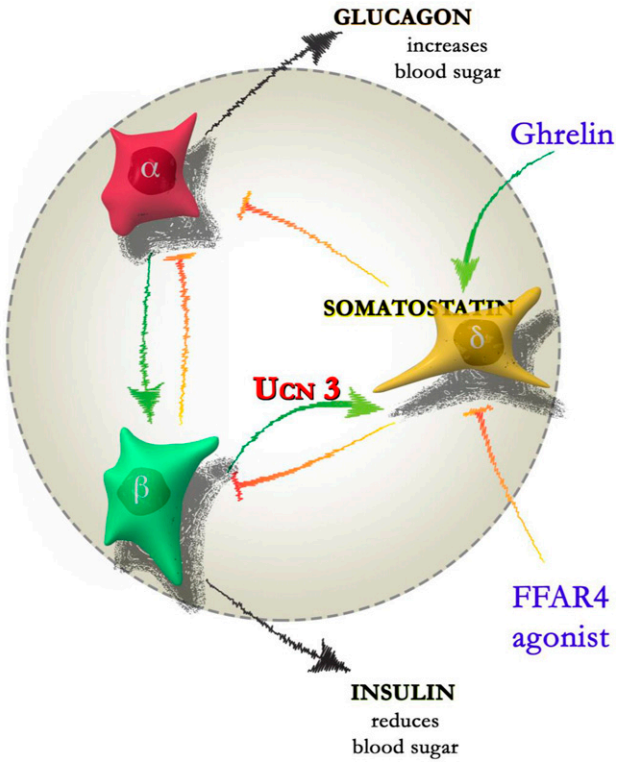


Figure 1—δ-Cells inhibit β- and α-cells via the inhibitory actions of somatostatin. Controlling the activity of δ-cells are a multitude of nutrients, neurotransmitters, and paracrine and endocrine factors. Examples shown here are not comprehensive.

pathogenic mechanisms and eventual therapeutic approaches (20,21,26,27). Also, it is important to study how responsiveness by other islet cell types, including δ- and α-cells, and the resulting paracrine cross talk, as described by Dr. Huising, may affect glucose regulation.

Presentation 3: Role of Dysfunctional Islet Pericytes (Joana Almaça)

In addition to inter- and intracellular diversity, islets are also equipped with a complex network of blood vessels that enable efficient exchanges among cells (28). In her presentation, Dr. Almaça, of the University of Miami in the U.S., explained that these blood vessels are not just passive conduits, but rather play a dynamic role in controlling the hormonal output of the islets. Islet blood vessels are made of endothelial cells covered by pericytes, which regulate capillary diameter and local blood flow (Fig. 2) (29). High glucose levels inhibit pericyte contractile activity by dilating islet capillaries and increasing blood flow in vivo, a physiological response known as "functional hyperemia" (29).

Dr. Almaça referred to studies of type 1 (J. Almaça, unpublished observations) and type 2 diabetes (27,29),in which microvascular alterations were seen early and during the evolution of the disease process, concomitant with evident impaired pericyte responses to high glucose that may also be heterogeneous. She proposed that dysfunctional pericytes may lead to uncontrolled islet blood flow that, together with increased intraislet amyloid deposition and fibrosis, can exacerbate islet dysfunction and loss of glucose homeostasis. In her model, vascular dysfunction may be a common pathogenic mechanism within the heterogeneous spectrum of the disease. Deeper research and mechanistic elucidation in this field by Dr. Almaça and other researchers may have significant translational impact with therapeutic implications. One important question will be to determine just how heterogeneous the pericyte functional response may be throughout the pathophysiological process.

Presentation 4: Contribution of Exocrine Pancreas Inflammation (Rebecca L. Hull-Meichle)

Islets are embedded within the exocrine pancreas and exist in close proximity to both acini and pancreatic ducts (30). However, the impact of these exocrine cell types on islet function and survival is poorly understood (31). The presentation by Dr. Hull-Meichle, of the University of Washington in the U.S., reviewed data from a retrospective analysis of archived autopsy pancreas tissue collected from subjects with cystic fibrosis–related diabetes (CFRD), cystic fibrosis (CF) without diabetes, and control subjects with no CF (32). The histologic analysis identified islet interleukin-1β (IL-1β) immunoreactivity and increased glucagon as prominent abnormalities in islet morphology of CF tissues with or without diabetes that were not present in tissue of control subjects with no CF. Importantly, they observed islet IL-1β immunoreactivity in pediatric subjects (10 years of age—the currently recommended age to begin screening CF patients for CFRD). This finding suggests that islet inflammation could begin very early in patients with CF, consistent with clinical studies showing reduced β-cell function in very young subjects with CF (33). It was also shown that the increased presence of islet amyloid deposition was only seen in CFRD and therefore is unlikely to mediate islet inflammation, as it is thought to do in type 2 diabetes (32). Finally, the investigators found a surprising decrease in intraislet macrophages, at least in adults with CF (with or without diabetes) (33), whereas others have found evidence of increased T cells in the islets of subjects with CF (34).

Given that IL-1β is known to contribute to impaired islet function and viability, these findings may help to explain the decreased insulin secretory function and abnormal glucose tolerance that occur starting in early childhood in patients with CF. Islet dysfunction may therefore be accompanied or perhaps even preceded by an inflammatory process within islets. Further research is needed to better understand the mechanisms underlying islet inflammation, especially in the context of cross talk between the exocrine and the endocrine pancreas that could eventually lead to preventive interventions (e.g., the use of anti-inflammatory drugs).

Presentation 5: Lessons from Human Islet Profiling Studies (Patrick MacDonald)

Dr. MacDonald, of the University of Alberta in Canada, focused his presentation on studies of α-cell functional heterogeneity and how the α-cell state is

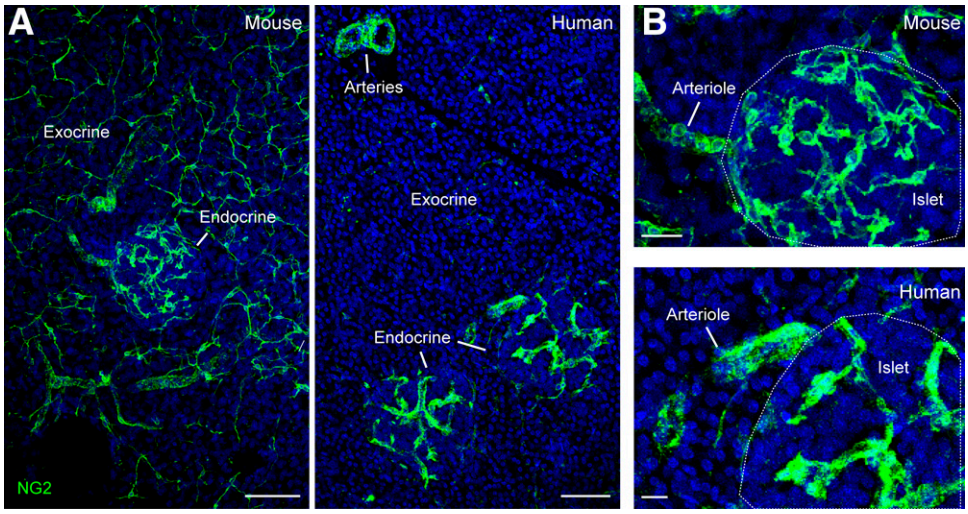


Figure 2—Pancreatic islets are full of pericytes. *A*: Z-projection of confocal images of mouse and human pancreatic tissue sections immunostained with an antibody against the mural marker NG2 (neuron-glial antigen 2; green). *B*: Zoomed images of regions containing islets in image *A*. Pericyte density in mouse and human islets is higher than in surrounding acinar tissue. This difference is even more striking in the human pancreas. These findings suggest that pancreatic islets in mice and humans are equipped with a mechanism that allows the control of their blood flow independently of the surrounding exocrine tissue. Scale bars are 50 mm in image *A* and 10 mm in image *B*.

linked to the dysfunction observed in type 2 diabetes. It has been previously shown that, in concert with reduced insulin secretion from pancreatic islet β-cells in type 2 diabetes, disrupted glucagon secretion from islet α-cells contributes to hyperglycemia and impaired hypoglycemia counter-regulation (35). Through an electrophysiological fingerprinting strategy and single-cell transcriptomics (36), researchers in Dr. MacDonald's laboratory have been able to assess human islet cell phenotypes, showing that α-cells enriched for markers of mitochondrial function and transcription factors such as *NEUROD1*, *ISL1*, *NKX2–2*, and *FEV* that define pancreatic endocrine lineage exhibit an impaired electrophysiological phenotype and dysregulated exocytosis (37). This work demonstrates that the function of all α-cells is not equally affected by the disease and that a subset of α-cells defined by their maturation state may be key drivers of impaired glucagon responses in type 2 diabetes.

Part 2: Islet Engineering to Further Precision Medicine

The second part of the Islet Biology session focused on bioengineering and biomimetic strategies for disease modeling, mechanistic elucidation, preclinical testing, and optimization of cell replacement therapies (primary islet cells or those derived from human pluripotent stem cells [hPSCs] that are able to evade allo- and/or autoimmune rejection).

Human islet transplantation has been a successful cell-based therapy for type 1 diabetes; it can normalize blood glucose and provide insulin independence for patients, and it may prevent severe hypoglycemia (38,39). However, because of the limited availability of cadaveric donor islets, the need for systemic immunosuppression, long-term graft function/viability issues, and problems associated with the transplant site, there remain hurdles to overcome to realize the promise of this option as a broad-reaching therapy for type 1 diabetes (40,41). This session discussed strategies for not only engineering islet endocrine cells from stem cells as an unlimited cell source for transplantation, but also applying bioengineering principles to generate islet organoids that can evade immune destruction; engineer islet vasculature, a critical component of the islet niche; produce biomaterials that can enhance islet graft survival and modulate local immune responses; and create novel microfluidic platforms that could enhance our understanding of human islet–immune interactions in type 1 diabetes. Methodologies for vascularizing genetically tractable hPSC islets will facilitate studies of genotype-phenotype relationships and precision medicine approaches to diabetes.

Presentation 1: Engineering Endocrine Islet Cells (Francis Lynn)

The first presentation in this topic area was from Dr. Lynn, of the University of British Columbia in Canada, whose laboratory is working toward engineering a stem cell–based cell source for cell replacement therapy for type 1 diabetes. Dr. Lynn explained that differentiation protocols for generating β-cells from hPSCs have improved greatly in the past decade; laboratories can now produce insulin-producing stem cell–derived β-cells (SCβ-cells) with high efficiency, and, when transplanted, these SCβ-cells can reliably reverse diabetes in mouse models of diabetes (42,43). However, protocols for generating SCβ-cells remain unable to generate populations of pure endocrine cells similar to those found in human islets. Furthermore, glucose-stimulated calcium influxes are blunted in SCβ-cells (44) and protocols to direct the functional maturation of SCβ-cells—a process that appears to be coupled through metabolism and calcium-regulated expression—remain elusive (45). Dr. Lynn described the considerable heterogeneity in SCβ-cells that are generated in vitro, noting that unexpected cell types are contained in glucose-responsive SCβ-cell clusters (45,46). Moreover, Dr. Lynn explained, after FACS (fluorescence-activated cell sorting) purification, reaggregation, and

transplantation, cells initially identified as *INS-GFP*–expressing cells can adopt other endocrine cell fates, suggesting some post-transplantation phenotypic plasticity (45). Questions remaining for Dr. Lynn and his colleagues to attempt to answer include whether functional maturation of SCβ-cells is necessary for unencapsulated transplantation, whether stem cell–derived islet cells are appropriately heterogeneous, and whether this heterogeneity is important for achieving durable cell therapy.

Presentation 2: Engineering the Islet Vasculature (Juan Melero-Martin)

Although islets comprise only 1–2% of the pancreatic mass, they receive 5–10% of the pancreatic blood flow, and almost all β-cells are in contact with a blood vessel. The islet vasculature provides many essential functions; it not only provides oxygen and nutrients, removes tissue waste, regulates hemostasis, and mediates inflammation, but also provides important endocrine functions by facilitating the sensing of blood glucose, regulating hormone secretion, and mediating insulin secretion (47,48). Given the essential role of the islet vasculature, one strategy to improve both the engraftment and efficacy of a transplanted cellular therapeutic product is to generate the accompanying vasculature and transplant it along with SCβ-cells.

Starting with human induced PSCs (i.e., PSCs that can be generated directly from a somatic cell), Dr. Melero-Martin, of Harvard Medical School in the U.S., and his collaborators have developed robust differentiation protocols that allow for the efficient production of endothelial and perivascular cells (Fig. 3). Combining these cells with a scaffold (collagen/fibrin hydrogel) creates a functional vascular network that can be transplanted with the SCβ-cells (49–51). This research has broad potential applications. It could reliably provide an unlimited number of vascular cells for engineering the islet vasculature and could also optimize in vitro preclinical and disease modeling microphysiological systems. The latter include those being developed within the NIDDK's Human Islet Research Network (HIRN) and its Consortium on Human Islets Biomimetics, as described later in the program by Dr. Maike Sander, of the University of California, San Diego, in the U.S. (see Part 2, Presentation 5 below). This work also complements the research presented earlier in the program by Dr. Almaça on vascular flow regulation by pericytes; their potential incorporation into the engineering modalities described by Dr. Melero-Martin could yield better understanding of tissue-specific, vascular, and endocrine cellular cross talk and its impact on designing novel cell therapies.

Presentation 3: Engineering Islet Organoids (Eiji Yoshihara)

Generating "immune evasive" functional pancreatic islets for transplantation holds great promise in obviating the need for life-long immunosuppression or encapsulation (placement of an immune-protective device) to prevent autoimmune attack in people with type 1 diabetes. Dr. Yoshihara, of the David Geffen School of Medicine in the U.S., and his colleagues developed a novel three-dimensional (3D) culture system that generates hPSC-derived islet-like organoids (HILOs) and forced the overexpression of PD-L1 (programmed death-ligand 1), an immune checkpoint protein, in HILOs (Fig. 4) (52). When these organoids were transplanted into immune-competent humanized mice, a significant reduction in allograft rejection was observed (52). When HILOs underwent a multi-pulse stimulation of interferon γ, they were found to not only express PD-L1, but also develop de novo cytokine tolerance via a newly identified transcriptional memory system (52). This research furthers our understanding of transplant immune tolerance and provides insight into the mechanisms of both graft and autoimmune rejection and future immune-evasive and tolerizing therapeutic interventions.

Presentation 4: Engineering Bioactive Biomaterials (Cherie Stabler)

The success of clinical islet transplantation is hindered by the location of the implant site, which is prone to mechanical stresses, inflammatory responses, and exposure to high drug and toxin loads, as well as strong inflammatory and immunological responses to the transplant in spite of systemic immunosuppression. Dr. Stabler, of the University of Florida in the U.S., presented data showing how her laboratory uses principles of engineering and material science to overcome some of the key

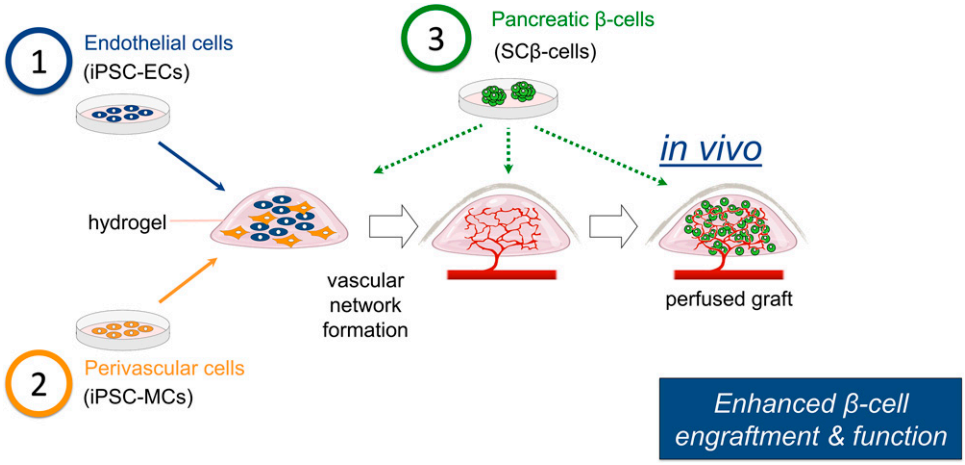


Figure 3—Schematic depicting a PSC-based strategy to bioengineer human vascular networks that support the engraftment and function of SCβ-cells. iPSC-ECs, induced PSC endothelial cells; iPSC-MCs, induced PSC perivascular cells.

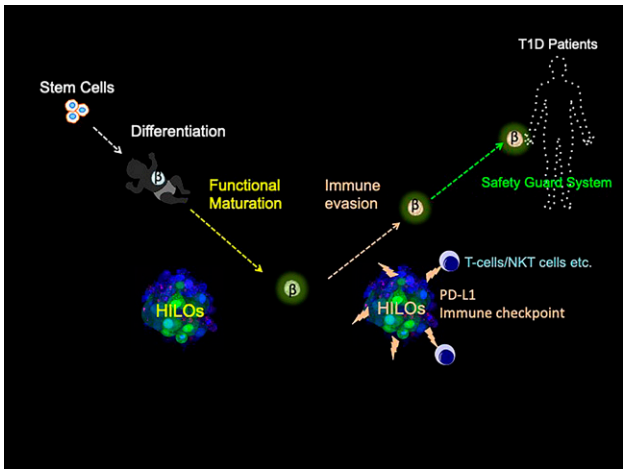


Figure 4—The generation of immune functional HILOs may provide an alternative approach for treating type 1 diabetes without the requirement for systemic immunosuppression. NKT, natural killer T; T1D, type 1 diabetes.

obstacles in cellular replacement therapy for type 1 diabetes. Dr. Stabler highlighted three primary strategies: the development of scaffolds to house islets at alternative transplant sites (53), the fabrication of encapsulation protocols for the immuno-camouflage of the transplant (54), and the production of bioactive biomaterials for the local delivery of oxygen and immunomodulatory drugs and/or cells (55) (Fig. 5). She described how 3D scaffolds can serve to create a more favorable islet engraftment site by ensuring optimal distribution of the transplanted cells, creating a desirable niche for the islets, and promoting vascularization. This strategy, combined with novel encapsulation methods, may substantially decrease the need for systemic immunosuppression by preventing host recognition of surface antigens. Finally, she emphasized how localization of supportive agents to the site of the transplant can serve to enhance efficacy while minimizing the side effects commonly observed with systemic delivery (56).

Presentation 5: Engineering Islets on a Chip (Maike Sander)

A major limitation for identifying mechanisms of human diabetes is the absence of an in vitro model in which human β-cells can be studied under in vivo–mimicking conditions. To fully understand the pathophysiology of diabetes, it is crucial to develop a human model, whereby the interactions of all cells involved in the disease process (e.g., β-cells, vascular endothelial cells, stromal cells, and innate and adaptive immune cells) can be studied in the context of the normal islet architecture, including vasculature and islet matrix. Dr. Sander, of the University of California, San Diego, and her HIRN collaborators have developed a microfluidic-based platform in which primary human islets are supported by a network of perfused human microvessels (57). This 3D vascularized islet microorgan platform allows for physiologic, microvessel-mediated delivery of nutrients, disease-relevant stimuli, or immune cells to the islets.

Using this model, they have demonstrated extravasation of activated immune cells from the microvessels into the islets, providing proof of principle that immune cell–β-cell interactions can be studied in this platform. Toward the goal of establishing a fully autologous and genetically tractable islet model (Fig. 6), they have established a similar model with hPSC-derived islet-like clusters. To analyze β-cell function in real time, they generated an hPSC line with GCaMP6f (a genetically encoded Ca^{2+} indicator), in which green fluorescent protein fluorescence serves as a reporter for Ca^{2+} influx and the triggering pathway of insulin secretion. Analysis of real-time Ca^{2+} responses revealed responsiveness of vascularized SCβ-cells to high glucose, the glucagon-like peptide 1 analog exendin-4, and potassium chloride.

Part 3: β-Cell Stress and Death in Diabetes

The Islet Biology session drew to a close with a review of our understanding of the stressors in both type 1 and type 2 diabetes that contribute to β-cell death and dysfunction. In recent years, it has become recognized that most individuals with type 1 diabetes have residual β-cells that are likely dysfunctional and are subject to multiple stresses, including inflammatory cytokines and endoplasmic reticulum (ER) stress that contribute to their dysfunction and demise (58). Evidence for ER stress early in the course of type 1 diabetes has raised the possibility that stress pathways in β-cells could be targeted therapeutically to prevent or delay disease (59,60). In addition, the β-cell stress response may help shape anti-islet autoimmunity in type 1 diabetes; in this way, β-cells are not passive players in disease pathogenesis. Recent evidence suggests that a subpopulation of residual β-cells in type 1 diabetes may also be subject to senescence, a cellular process that includes loss of proliferation and resistance to apoptosis (61). Pathways of cell stress and senescence may contribute to β-cell dysfunction in both type 1 and type 2 diabetes. Understanding the triggers of β-cell stress in diabetes, and how cell stress pathways may lead to β-cell dysfunction and death, has potential to lead to new therapies that target cell stress to preserve β-cell function.

Presentation 1: Exploring the Balance Between β-Cell Health and Stress (Carmella Evans-Molina)

Dr. Evans-Molina, of Indiana University School of Medicine in the U.S., presented on her group's study of the balance between β-cell health and stress in type 1 diabetes, both in the laboratory and in clinical trials. Their work has enabled new insight into disease pathogenesis and led to the identification of biomarkers of type 1 diabetes of value for stratifying patients in clinical trials. Dr. Evans-Molina explained that the premise that β-cell stress is an early event in type 1 diabetes and that the β-cell response to stress contributes to autoimmunity (and, thus, β-cells play a role in their own demise) is gaining increasing attention. Understanding the pathways that lead to β-cell stress in type 1 diabetes may suggest therapeutic targets that could be coupled with traditional immunomodulatory therapies to slow or prevent disease, while also improving biomarkers for tracking disease progression.

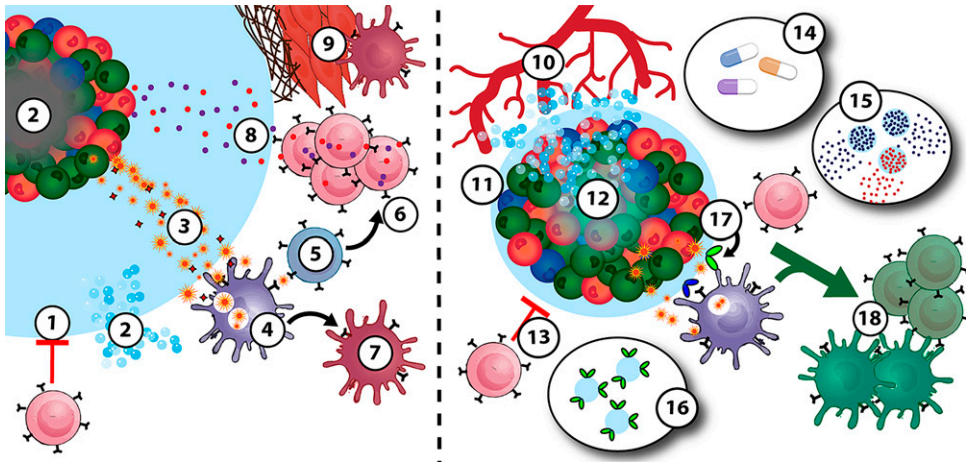


Figure 5—While islet encapsulation blocks direct interactions between immune cells and the islet (*1*), the large biomaterial barrier creates insufficient nutrient delivery and central necrosis (*2*). Shedding of antigen and stress signals (*3*) also leads to activation of innate and adaptive immune cells (*4* and *5*), resulting in antigen-specific T-cell expansion/activation (*6*) and broad macrophage recruitment (*7*). Although unable to directly attack the encapsulated graft, immune cells impart damage by secreting reactive oxygen species and cytokines, which diffuse through the hydrogel (*8*), and by recruiting fibroblasts and macrophages to create a fibrotic capsule (*9*). Recent approaches can address these challenges by improving islet vascularization (*10*) and decreasing the capsule size (*11*), which improves nutrient delivery and supports islet viability (*12*). With direct islet–immune interactions still blocked (*13*), the modulation of indirectly activated immune cells is feasible through moderate systemic immunosuppressants (*14*), localized soluble drug delivery (*15*), and/or the use of immunomodulatory materials near (*16*) or attached to the encapsulating material (*17*). These approaches can not only stop immune activation, but also convert immune cells toward a tolerogenic/regulatory phenotype (*18*).

Subgroup and responder analyses from recent clinical trials, including the teplizumab (62) and oral insulin (63) trials, indicate that stratifying patients by β-cell function upon entry into immunotherapy trials has the potential to improve trial design and possibly outcomes (64). This approach involves staging of disease progression in at-risk individuals into metabolic "checkpoints" (65,66). The goal of metabolic staging for clinical trial is to identify a window of opportunity for optimal therapeutic intervention with immune therapy and/or β-cell protective agents, aided by the application of new biomarkers of β-cell function such as the proinsulin–to–C-peptide ratio (67,68). Such approaches could complement standard measures such as meal-stimulated C-peptide response, as well as potentially newer, experimental measures of β-cell stress and function in diabetes such as exosomal microRNAs (69). Dr. Evans-Molina described recent and ongoing work to identify differentially expressed islet and exosomal microRNAs in an in vitro model of type 1 diabetes. A future area of research is to correlate these metabolic checkpoints with the molecular events occurring during disease pathogenesis (e.g., β-cell stress, senescence, neoantigen production, and inflammation) to design β-cell–targeted interventions.

Presentation 2: Targeting the β-Cell Unfolded Protein Response (Feyza Engin)

When ER is stressed in cells, the unfolded protein response (UPR) is triggered as an adaptation that leads to attenuation of ER stress or to β-cell apoptosis; however, the molecular mechanisms by which the UPR regulates pancreatic β-cell death or

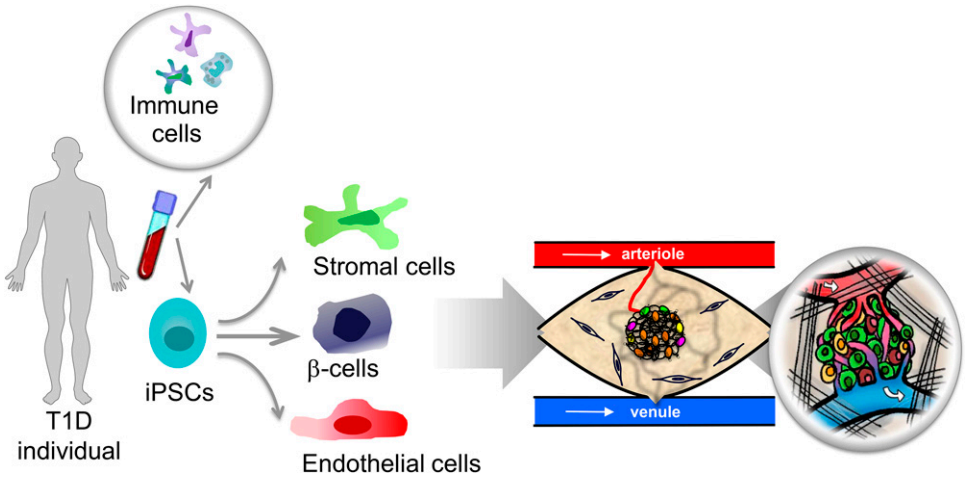


Figure 6—A fully autologous system to model diabetes, as shown here, could serve as a platform to study immune cell–β-cell interactions and to test therapeutics. iPSC, induced PSC; T1D, type 1 diabetes.

survival, or β-cell-immune cell dialogue during type 1 diabetes progression, still remain largely unknown. Dr. Engin, of the University of Wisconsin-Madison in the U.S., presented her laboratory's work showing that the chemical chaperone and ER stress reliever TUDCA (tauroursodeoxycholic acid) prevents autoimmune diabetes when administered to prediabetic nonobese diabetic (NOD) mice, pointing to the importance of β-cell stress in the pathogenesis of type 1 diabetes (70). Her group generated NOD mice with β-cell–specific deletion of IRE1α (inositol-requiring enzyme 1α), an ER transmembrane protein and key UPR sensor, and found that ablation of β-cell IRE1α prior to insulitis prevented diabetes in these mice (71). This effect was attributed to a transient β-cell dedifferentiation that occurred in these animals, associated with a downregulation of expression of key autoantigens, including insulin, and an attenuation of the anti-islet immune response. These findings add another layer to our understanding of how β-cell stress and dysfunction may play a role in promoting autoimmunity in type 1 diabetes and suggest new therapeutic targets in the β-cell stress pathway to slow or prevent disease.

Presentation 3: Elucidating β-Cell Senescence in Diabetes (Peter Thompson)

An alternative pathway to the UPR that β-cells can enter when subjected to stress is the DNA damage response (DDR), which can lead to cell senescence, a state characterized by growth arrest, apoptosis resistance, and secretion of a proinflammatory secretome called SASP (senescent-associated secretory phenotype). In his presentation on this topic, Dr. Thompson, of the University of Manitoba in Canada, described his work in identifying β-cell senescence as a characteristic state of a subpopulation of β-cells in type 1 diabetes in both NOD mice and humans (61). Senescent β-cells also accumulate during the pathogenesis of type 2 diabetes in mouse models and humans (72). Dr. Thompson's team has found that DDR, SASP, and apoptosis resistance distinguish β-cell senescence from normal aging and the UPR. The development of senescent β-cells is progressive, accumulating as the disease progresses, and heterogeneous, with differences in the proportion of senescent β-cells among islets and individuals. Importantly, senescent β-cells are potentially targetable (73), and indeed antisenescence drugs were found to reduce the incidence of diabetes in NOD mice (61). Much more work needs to be done to understand senescence in both type 1 and type 2 diabetes, including an understanding of the specific triggers, how senescence relates to other states of β-cell dysfunction in diabetes, and how senescent β-cells may be best targeted in disease.

Presentation 4: Focusing on β-Cell Stress Pathways (Anath Shalev)

Rounding out the final portion of this session, Dr. Shalev, of the University of Alabama at Birmingham in the U.S., presented her research pointing to thioredoxin-interacting protein (TXNIP) as a central mediator of stress responses in β-cells and suggesting that it is a promising therapeutic target in both type 1 and type 2 diabetes. TXNIP expression is increased in β-cells by stressors such as cytokines, ER stress, and hyperglycemia. Genetic deletion of TXNIP relieves diabetes in mouse models, including streptozotocin (STZ)-induced diabetes and genetic obesity (74). Dr. Shalev's team further showed that the calcium channel blocker and antihypertensive drug verapamil, which decreases TXNIP expression, normalizes glycemia in STZ-induced diabetic mice (75). In a small clinical trial, her group demonstrated that verapamil treatment preserved β-cell function in new-onset type 1 diabetes, reducing the increase in insulin needs by 43% and the number of hypoglycemic episodes by 82% (76). Because verapamil is not specific for TXNIP, this group then screened a small molecule library for TXNIP inhibitors and identified and optimized an orally available molecule that can attenuate diabetes in STZ-treated mice or in mice with genetic obesity, therefore suggesting therapeutic promise for human diabetes (77).

SESSION 2: HETEROGENEITY OF DIABETIC PHENOTYPES BEFORE AND AFTER DIAGNOSIS: IMPACT ON MANAGEMENT AND TREATMENT

Recent advances in experimental research and human medicine have added complexity to our once simplistic understanding of diabetes phenotypes. Continuous enrichment of both clinical data at the population level and molecular and genetic data at the cellular level has revealed the existence of many more distinct presentations of the disease than were once recognized. These different presentations can be categorized into more refined types, and this was the focus of the symposium's second session. Scientists have embraced the ever-richer, ever-growing body of data drawn from the environment, genetics, medical and surgical treatment responses, and individual human differences to further our understanding of and develop treatment strategies for managing heterogenic diabetes.

Part 1: Diversity of Phenotypes and Pathophysiological Endotypes

Traditionally, most people with diabetes have been grouped into one of two major categories: type 1 diabetes, which reflects a condition of autoimmunity and results in pancreas destruction and absence of endogenous insulin, or type 2 diabetes, characterized by the lack of an adequate response to insulin given the insulin needs (i.e., increased insulin resistance).

We also recognize monogenic forms of diabetes, in addition to individuals reported as having latent autoimmune diabetes in adults (LADA). More recently, an additional classification, type 3c diabetes, has been described that is associated with disease or deficiency of the exocrine pancreas. However, these classifications do not capture the significant heterogeneity exhibited within each class or the rare or atypical other forms of the disease (Fig. 7) (78,79). In all cases, correct identification of underlying cause(s) is crucial for effective disease management.

Presentation 1: Type 1 Diabetes: Multiple Factors Affect Its Course (Maria J. Redondo)

Dr. Redondo, of Baylor College of Medicine in the U.S., enumerated several factors that contribute to heterogeneity in type 1 diabetes, including age at clinical diagnosis, islet autoantibody characteristics, and genetics, and explained how these factors can affect diabetes progression and phenotype. For example, children diagnosed with type 1 diabetes before the age of 7 years have shown a unique histopathology (80), immunological differences (81), and

more rapid loss of β-cell function (82). People who develop type 1 diabetes at an older age present with nonautoimmune factors, including obesity and genes typically associated with type 2 diabetes (e.g., transcription factor 7-like 2 single-nucleotide polymorphisms [83]). Older individuals are also more likely to have a high BMI (84), and there is a correlation between the presence of human leukocyte antigen isoforms, autoantibody appearance, and age of onset (85). Indeed, individuals who develop type 1 diabetes at older ages more closely resemble the type 2 diabetes phenotype.

Dr. Redondo advocated for further development of type 1 diabetes endotypes (pathophysiologically distinct subtypes of the disease) based on genetics, autoantibody presence, and response versus nonresponse to immunotherapies. Describing these endotypes will allow treatment of all clinically significant mechanisms in a patient-specific manner.

There is also a need to develop prognostic models for the trajectory, complications, and associated conditions of type 1 diabetes.

Presentation 2: Type 2 Diabetes: Phenotypes Differ Between Youth and Adults (Kristen Nadeau)

Age also has a significant effect on the clinical presentation of type 2 diabetes. Youth and adolescents with type 2 diabetes exhibit reduced endothelial function and abnormal mitochondria in insulin-resistant muscle, similar to those with type 1 diabetes. However, youth with type 2 diabetes differ from those with type 1 diabetes in that they exhibit a more severe metabolic phenotype, with increases in liver and visceral fat, triglycerides, and alanine aminotransferase levels and decreases in adiponectin and HDL cholesterol (86,87).

The phenotype of youth and adolescents with type 2 diabetes also differs markedly from that of their adult counterparts in both mechanisms underlying pathophysiology and response to therapy. Dr. Nadeau, of the University of Colorado–Anschutz in the U.S., presented data from the TODAY study showing that youth and adolescents with type 2 diabetes, when compared with adults with type 2 diabetes, exhibit increased insulin resistance, more rapid β-cell failure, and more rapid onset of complications, including hypertension, renal hyperfiltration, proteinuria, and cardiac hypertrophy (88–90). Youth also have shown an altered response to therapy, with 52% not responding to metformin therapy compared with 12% of adults (91). In addition, they are more likely to be female, have a higher BMI, belong to a minority racial or ethnic group, and have a strong family history of diabetes compared with adults with type 2 diabetes (91). Within this youth population, females are more likely to respond to rosiglitazone, whereas males respond better to lifestyle interventions (91). Conversely, youth may respond better to bariatric surgery than to traditional medical therapy (92). Further understanding the differences in phenotype between adults and youth with type 2 diabetes is necessary to identify the best treatments for these youth.

Presentation 3: Type 3c Diabetes: Under-Recognized Forms of Pancreatogenic Diabetes (Melena Bellin)

Type 3c diabetes refers to forms of diabetes resulting from primary pathologies of the exocrine pancreas, including acute or chronic pancreatitis, cystic fibrosis, and pancreatic cancer (93). The presentation by Dr. Bellin, of the University of Minnesota in the U.S., focused on post-pancreatitis diabetes (Fig. 8), which is often misdiagnosed or mislabeled as type 2 diabetes or occasionally as type 1 diabetes, making its prevalence difficult to estimate. Recent epidemiological studies suggest that type 3c diabetes is at least as prevalent as type 1 diabetes in adults (94,95). Approximately one in three adults with chronic pancreatitis and one in five adults with past acute pancreatitis is diagnosed with type 3c diabetes, although this

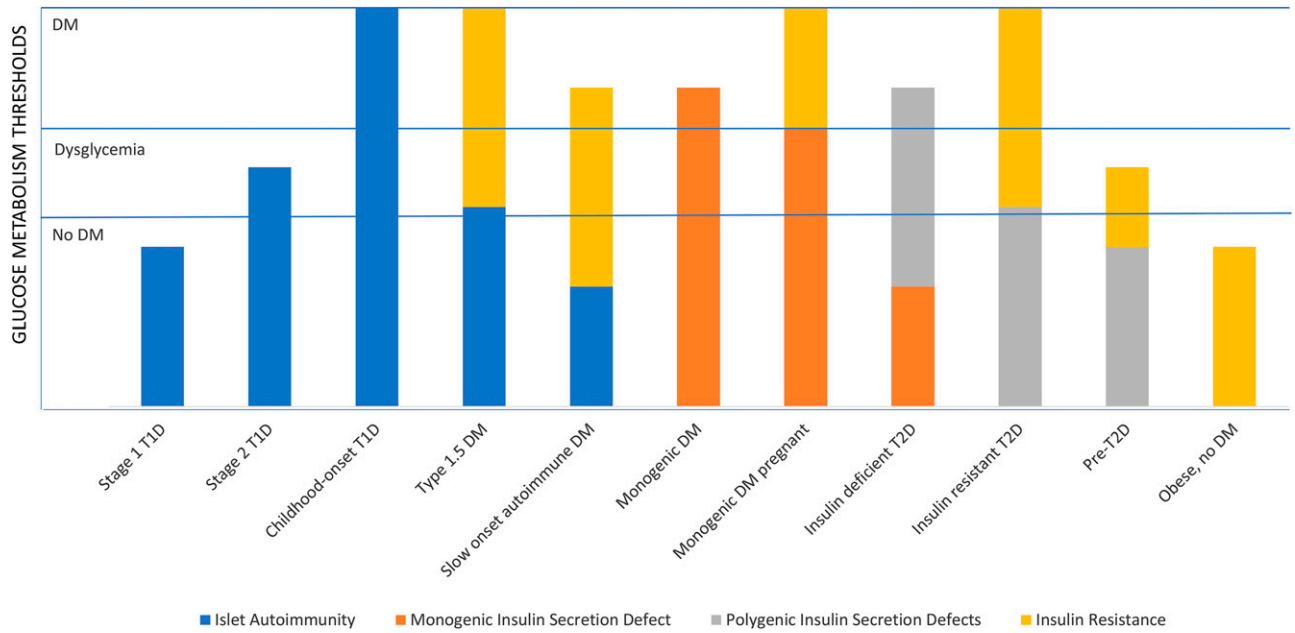


Figure 7—Model of diabetes etiopathogenesis based on the Palette Disease Model (78) and Threshold Hypothesis (79) that explain heterogeneity within diabetes types and overlap between diabetes types. Individuals (represented by bars) may have several diabetogenic mechanisms (represented by different colors) that, in combination, may cause glucose to reach a threshold. DM, diabetes mellitus; T1D, type 1 diabetes; T2D, type 2 diabetes.

incidence may be higher, as pancreatitis often goes undiagnosed.

Type 3c diabetes shares partial clinical presentation with both type 1 and type 2 diabetes, and insulin deficiency and insulin resistance are likely primary pathogenic mechanisms. Type 3c diabetes features reduced β-cell mass and insulin deficiency similar to type 1 diabetes, but also exhibits insulin resistance, which is characteristic of type 2 diabetes (96). Type 3c diabetes also shares common risk factors with type 2 diabetes, including obesity, family history, and genetic risk score (97). However, type 3c diabetes is more likely to require insulin therapy and may be associated with poorer glycemic control and an increased risk for pancreatic cancer (98).

Making an accurate diagnosis and determining whether the prevalent phenotype is insulin deficiency or insulin resistance is crucial for guiding treatment and prognosis. Therefore, treatment of post-pancreatitis diabetes should be undertaken in a multidisciplinary setting, including management of exocrine insufficiency, if present (96).

Presentation 4: Atypical Forms of Diabetes: Rare and Important to Accurately Diagnose (Miriam S. Udler)

The identification of monogenic and polygenic variants underlying the pathogenesis of rare and atypical diabetes has aided understanding of the disease spectrum. Monogenic variants account for ∼1–4% of all cases in young adults, most belonging to the MODY (maturity-onset diabetes of the young) family (99). Individuals within MODY have a classical presentation of young onset with a family history of diabetes but lack metabolic syndrome and are negative for autoantibodies, thereby distinguishing them from people with either type 1 or type 2 diabetes (100).

Although the presence of a specific monogenic variant informs therapeutic management, the additional presence of more common genetic variants in individuals within this group results in heterogeneity in presentation (Table 1) (101,102). For example, individuals with a combination of specific monogenic variants and more common variants generally have a younger age of diagnosis (103). Similarly, examination of the genetic contribution of monogenic variants to the development of type 2 diabetes has revealed extensive heterogeneity in underlying glycemic dysfunction (104).

Dr. Udler, of Harvard Medical School in the U.S., presented a model in which monogenic and polygenic variation represents a continuum of phenotypic variation. Using a "genetics first" approach, almost two-thirds of individuals with a MODY variant did not show classical features of diabetes (105). Machine learning and cluster analysis of type 2 diabetes–related variants and clinical presentation identified five type 2 diabetes genetic clusters that suggest unique underlying mechanisms in pathophysiology (106). In a research initiative funded by the NIDDK, the Rare and Atypical Diabetes Network (RADIANT), involving 14 centers across the U.S., now aims to further stratify individuals by identifying and characterizing rare and atypical forms of diabetes through whole-genome sequencing combined with phenotypic data (107).

Based on the presentations in the first part of this session, current classifications appear to be inadequate in accurately reflecting the heterogeneity in diabetes. Rather, presentation of diabetes represents more of a continuum, in which individual cases are affected to differing degrees by genetics, age, sex, phenotype, and environment.

Part 2: Determinants of Pathophysiological and Clinical Phenotypes and the Tools and Strategies Available for Their Characterization

A wide spectrum of tools and strategies can be used to define and understand the different classifications of diabetes, from carrying out deep characterizations of individuals presenting with prediabetes or a certain form of diabetes to using various rich data sets to identify and characterize different types of diabetes among subpopulations. These methods hold promise in helping us understand the complexity of diabetes

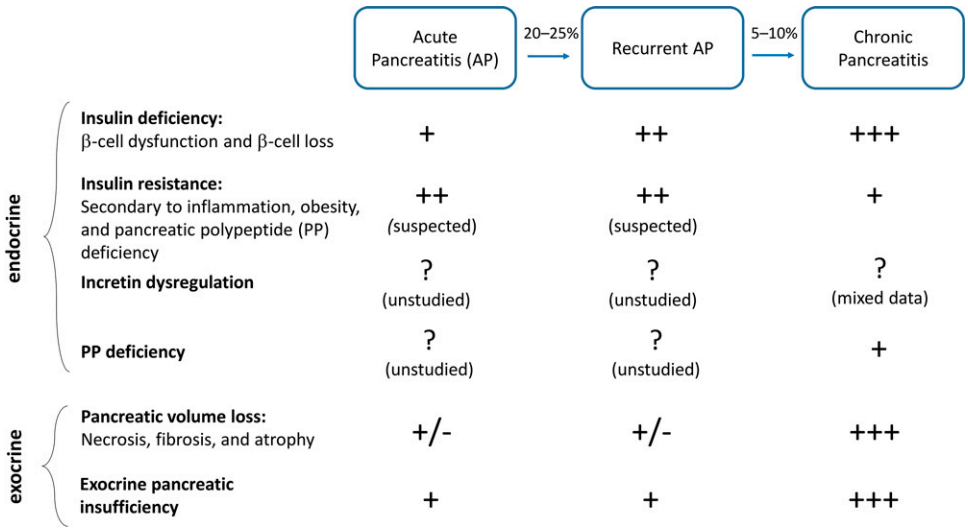


Figure 8—Potential endocrine and exocrine contributors to the development of post-pancreatitis diabetes in the setting of acute pancreatitis, recurrent acute pancreatitis, or chronic pancreatitis. Research is ongoing to better define the mechanisms of diabetes in these populations. Not displayed on this figure is β-cell autoimmunity, which has been reported in case series of patients with acute or chronic pancreatitis. Acute and chronic pancreatitis represent a spectrum of disease; the percentages above the arrows indicate the approximate proportion of patients whose disease progresses for each diagnosis.

Table 1—Clinical implications of some common and important causes of monogenic diabetes

Gene	Inheritance/phenotypes	Importance of genetic diagnosis
GCK	AD: *GCK*-MODY (common) AR: *GCK*-NDM (very rare)	*No treatment needed* for most patients (except possibly during pregnancy)
HNF1A	AD: *HNF1A*-MODY (common)	Excellent glycemic control usually possible with low-dose oral sulfonylureas
HNF4A	AD: *HNF4A*-MODY (uncommon)	Often responsive to low-dose oral sulfonylureas
HNF1B	AD: *HNF1B*-MODY (uncommon)	Optimal treatment for diabetes not well established; genetic diagnosis will inform monitoring and management of other features
ABCC8	AD/AR: *ABCC8*-NDM (common) *ABCC8*-MODY (rare)	Usually responds to high-dose oral sulfonylureas; genetic diagnosis facilitates monitoring/ intervention for neurodevelopmental problems
KCNJ11	AD: *KCNJ11*-NDM (common) *KCNJ11*-MODY (rare)	Usually responds to high-dose oral sulfonylureas; genetic diagnosis facilitates monitoring/ intervention for neurodevelopmental problems
6q24 (imprinted locus)	Most common cause of transient NDM	Diabetes recurring later in life is often responsive to noninsulin therapies
INS	AD/AR: *INS*-NDM (common) AD: *INS*-MODY (rare)	Early intensive insulin treatment; future treatments may feasibly target molecular mechanism(s)

Adapted from Riddle et al. (101). AD, autosomal dominant; AR, autosomal recessive; NDM, neonatal diabetes mellitus.

phenotypes and ultimately improve treatment and prevention interventions.

Presentation 1: Selecting Diabetes Treatments Based on Individual-Level Clinical Features (John Dennis)

Dr. Dennis, of the University of Exeter in the U.K., highlighted the wide-ranging clinical presentations that reflect the heterogeneity of type 2 diabetes, in addition to the variety of treatment strategies that address different aspects of diabetes-related defects. Current recommended treatment guidelines rely largely on broad, population-level data and average treatment effects observed in clinical trials (108,109). However, an approach to personalizing diabetes care and thereby refining treatment efficacy was proposed that could be achieved through a strategy based on easily measured clinical and biomarker characteristics such as sex, BMI, and kidney function metrics (110,111). Such a strategy can inform a "rational precision" approach to matching individuals to the most effective drug for their needs using individualized prediction models to determine the most favorable risk/benefit profile (112).

Dr. Dennis presented two such models (Fig. 9) (112). Once validated, he explained, such predictive models could be deployed worldwide at low cost to provide clinicians with individualized information when selecting from among the various available glucose-lowering therapies for a particular patient.

Presentation 2: Insights From the Diabetes Remission Effects of Bariatric Surgery (Satya Dash)

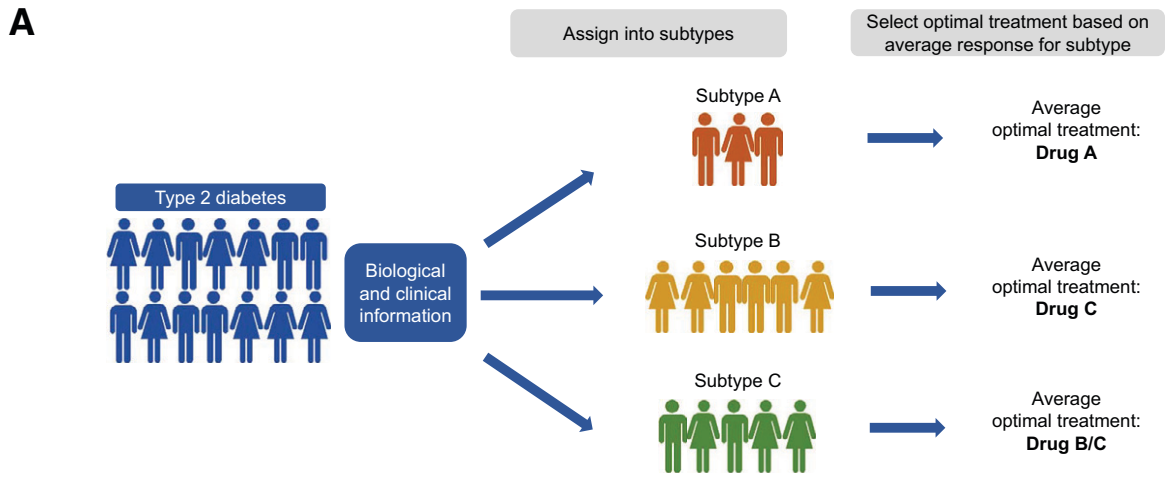
The global obesity pandemic is a major driver of the increasing prevalence of type 2 diabetes. Dr. Dash, of the University of Toronto in Canada, discussed the efficacy of diabetes remission after bariatric surgery in the individuals with obesity and diabetes.

Bariatric surgery is the most effective treatment for type 2 diabetes in people with obesity (113,114). The most commonly performed procedures in North America are the sleeve gastrectomy (a restrictive procedure) and Roux-en-Y gastric bypass (RYGB; a restrictive and malabsorption-generating procedure) (115). Both procedures have very good safety profiles, although the sleeve gastrectomy likely has fewer adverse effects (115). The available evidence suggests that the early glycemic benefits of bariatric surgery are largely mediated by reduced caloric intake and weight loss (116,117). Bariatric surgery can achieve type 2 diabetes remission in up to 60% of patients after 1 year, with longer-term remission of ~25% over 5–10 years (113,114). RYGB is likely the more effective procedure for inducing weight loss and achieving diabetes remission (118,119).

Individual factors, including older patient age, longer duration of diabetes, higher HbA_{1c}, greater number of diabetes medications, and preoperative insulin use are all negatively correlated with the likelihood of remission, likely because they are associated with greater β-cell dysfunction. These factors have been incorporated into clinical predictive tools, which generally perform well (120–122). However, there is substantial heterogeneity in diabetes remission response to surgery, suggesting that additional factors likely play a role. A more detailed understanding of the mechanisms underlying this heterogeneity will yield important insights into the pathophysiology and reversibility of type 2 diabetes and inform the optimal timing and choice of bariatric surgical procedures for people with obesity and type 2 diabetes.

Presentation 3: Role of Multi-Omics Profiling (Wenyu Zhou)

Dr. Zhou, of Tempus Laboratories in the U.S., presented on the power of longitudinal multi-omics profiling to assess inter- and intraindividual variability and how they progress over time, which can correlate with the development of diabetes (123). Longitudinal multi-omics profiling can provide an unbiased way to track the progression of variations in molecular and clinical characteristics.

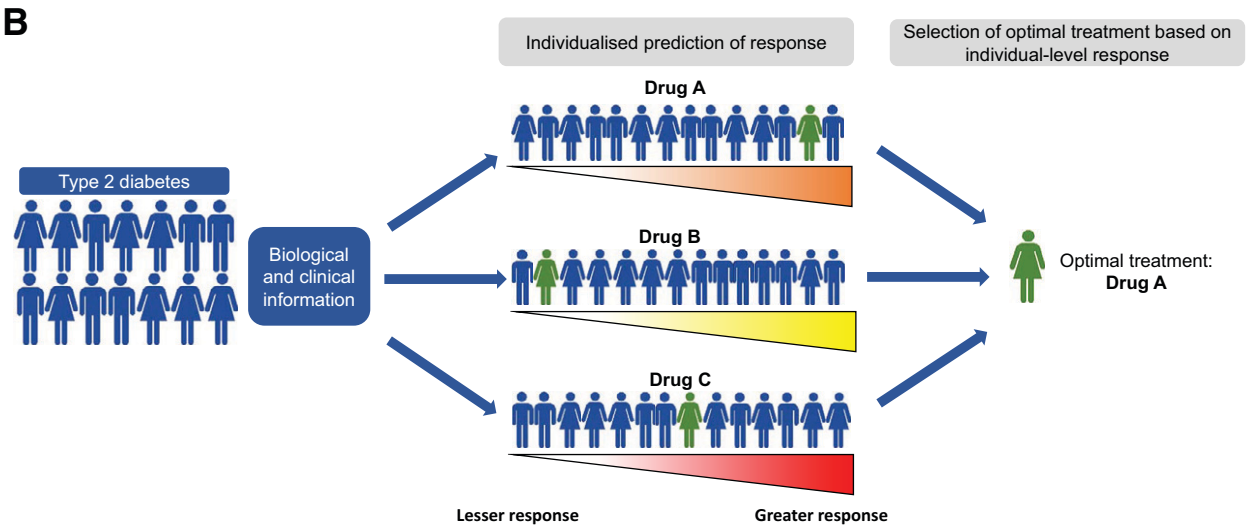


Advantages

- Simple to communicate.
- Can assess risk of multiple outcomes based on subgroup assignment.
- Classification could take place at one time point only e.g. around diagnosis (important if non-routine testing is required).
- May enhance understanding of the pathophysiological basis for type 2 diabetes

Disadvantages

- People within a subtype may be very different but are assumed to have the same outcome.
- Cannot be assumed to represent true pathophysiological subtypes - highly dependent on features used to classify them.
- Subtypes are not discrete, but overlap in phenotypic characteristics.
- Subtypes not stable – unless defined solely by genetics, a person can shift from one subgroup to another over time.



Advantages

- Optimal prediction of outcome, as predictions based on precise individual level characteristics.[39]
- Predictions specific to a person's characteristics at the point in time an optimal treatment strategy is being considered.

Disadvantages

- Complexity – specific models required for different outcomes e.g. risk of complications.
- Challenging to weigh up models for different outcomes and communicate these.
- Input data for prediction required at different time points.

Figure 9—Individualized prediction compared with classification into subtypes: advantages and disadvantages of two strategies to apply a precision medicine approach in type 2 diabetes. *A*: Classification into subtypes. In this approach, people with type 2 diabetes are subclassified into specific subtypes of type 2 diabetes based on clinical, genetic, phenotypic, and/or biomarker traits with the assumption that these subgroupings may enable more defined stratification for treatment responses and other outcomes. *B*: Individualized prediction. In this approach, markers from biological and clinical information are used as continuous traits to better predict a person's individual treatment responses to each drug option, thereby guiding the selection of the optimal treatment for that person. Approach *A* will propose treatment based on response for the particular subtype identified, whereas approach *B* recognizes differences in treatment response at an individual level. Reprinted with permission from Dennis (112).

However, further development of this strategy is warranted before it can be effectively implemented in routine clinical diagnosis and care.

Dr. Zhou described a study in which she and her colleagues followed healthy individuals and individuals with prediabetes for ~4 years with intensive molecular profiling, including transcriptomes, metabolomes, cytokines, proteomes, and the microbiome (124). This effort revealed distinct healthy profiles that nonetheless displayed diverse patterns of intra- and interpersonal variability. Responses to environmental triggers such as respiratory viral infection and immunization were also examined and found to be associated with extensive host and microbial changes with diverse molecular processes. For example, subjects with insulin resistance were found to respond differently from those who were insulin sensitive in global co-associations among thousands of profiled molecules and host–microbe interactions (125). Intra- and interpersonal variabilities were also evident during the aging process, with individuals showing patterns of molecular trajectories that may reveal underlying aging mechanisms (126).

Presentation 4: Precision Diabetes Analysis Using Advanced Technologies (Michael Snyder)

Precision health care relies on the ability to assess disease risk at an individual level, which can facilitate early detection of preclinical conditions and enable initiation of preventive strategies. Toward that end, Dr. Snyder, of Stanford University in the U.S., discussed the utility of precision diabetes analysis using advanced technologies. Recent technological advances, including the development of various -omics measurements and hundreds of wearable biosensors, enable in-depth and comprehensive molecular and physiological profiling.

Such work has led to numerous discoveries in multiple disease states and has enabled more precise phenotyping of prediabetes and type 2 diabetes (127,128). This work has identified heterogeneity of both insulin resistance and diabetes onset, with some individuals gradually progressing to type 2 diabetes, whereas others appear to experience a more sudden triggering event. Continuous glucose monitoring, in particular, has enabled the identification of different patterns of glycemic responses, or "glucotypes," that illustrate the heterogeneity within traditional diagnostic categories of glucose dysregulation (129).

These combined strategies can identify relevant molecular pathways that are associated with standard clinical measurements. The resulting "personalized, longitudinal big data" can be monitored prospectively to identify enriched signatures that can lead to personalized risk stratification for various subtypes of diabetes. Prediction models can then be developed to more specifically define and respond to individual patients' unique health risks.

Presentation 5: Leveraging Administrative Health Care Data and Electronic Health Records (Gillian Booth)

Moving from enriched data from individuals to aggregated data from many individuals, Dr. Booth, of the University of Toronto, described the integration of population-based data from administrative health care data sets and electronic health records (EHRs) as part of a multidimensional phenotyping approach. The recent rapid growth of health-related data collection has revolutionized our ability to characterize heterogeneity across patients and populations with respect to diabetes susceptibility, treatment responses, and outcomes (130–133). When combined with deep, rich data from individuals, these macro-level data could lead to the provision of more targeted and effective health care for each patient.

Large administrative health care data sets and EHRs offer a practical and cost-effective means of studying large populations over time, which can enable the disentangling of multiple associations, address confounding factors, and identify populations who are at particularly high risk for disease complications or who may be protected from adverse outcomes. For example, population-based studies using data from administrative sources such as insurance claims and patient registries were able to quantify the excess risks of cardiovascular disease and mortality conferred by diabetes (134–136). Such research approaches have been aided by advances in computational analysis, including artificial intelligence (AI) and machine learning (137), as well as the explosion in available data capturing human behavior and interactions from mobile apps, wearable devices, social media, e-commerce activity, and the natural and built environments in which people live.

Combinations of administrative, EHR, laboratory, and environmental data can provide valuable insights into the heterogeneity in diabetes risk factors and outcomes. Work by Dr. Booth and her colleagues has drawn on neighborhood-level built environment data, provincial health records, and other administrative data sources to explore the relationship between neighborhood walkability and incidence of diabetes and obesity (138,139). They have also used administrative health records and environmental data to study the influence of outdoor air temperature on the risk of gestational diabetes (140).

Macro-data available through administrative, EHR, and community sources can provide an efficient means of understanding disease heterogeneity and can also be leveraged to test novel associations and form new hypotheses. However, along with these opportunities come significant challenges that must be overcome, including a lack of uniformity in the reliability, accuracy, and completeness of data from different sources. Standardization is needed in approaches to big data–based research, and the replicability of findings can only be ensured through open, uniform methods of sharing data sources, algorithms, and codes.

Overall, precision medicine requires cross collaboration among multiple disciplines within the fields of biology, epidemiology, computer science, social science, and others. The use of administrative and EHR data can aid in the translation of research into clinical practice by enabling the study of diabetes heterogeneity on a much larger scale.

SESSION 3: PRECISION MEDICINE IN DIABETES

The symposium's final session centered on the impact and potential of AI and precision medicine in diabetes. The topic followed from a 2020 ADA–European Association for the Study of Diabetes consensus report describing the foundation for precision diabetes medicine and outlining future steps needed to realize its potential (141). Precision medicine holds the promise to provide the right treatment for the right patient at the right time, with the expectation

of better health at a lower cost for patients with complex disorders (142). It incorporates the concepts of precision diagnosis—including classifying diabetes into subtypes through assessment of genomics, metabolomics, epidemiology, ancestry, geography, clinical features—and diagnostic testing. It also includes precision therapeutics, designed to prevent or treat diabetes through an understanding of each patient's unique biology, and precision prognostics, through which a patient's risk of complications and response to treatment can be accurately predicted (141).

Presentation 1: Applying AI to an Integrated Clinic Network (Atul Butte)

The development of precision medicine requires assessment of large amounts of data from diverse sources, a challenge for which AI has been applied with increasing frequency. Dr. Butte, of the University of California, San Francisco, in the U.S., described how AI has been applied to publicly available molecular databases to find new autoantigens for type 1 diabetes (143) and new therapeutics for type 2 diabetes (144). He also explained how the wide variety of type 2 diabetes treatment approaches can be used to program AI to drive a new era of evidence-based medicine (145).

Dr. Butte described an initiative of University of California Health (UC Health) and United Healthcare to form an accountable care organization and clinically integrated network incorporating data from the five major academic health centers in the UC Health system to assess the care provided to 7 million patients in the past 9 years (145). Through this initiative, demographic and geographical data are being combined with diagnostic and treatment data to improve quality of care, reduce costs, and centralize the management of patients in primary care clinics. For example, the initiative screened more than 1 million patient records to identify ~41,000 patients with type 2 diabetes whose data can be accessed through one EHR dashboard to characterize features of diabetes and responses to treatment.

Using this rich resource, researchers have quantified social determinants of health by incorporating data on patients' socioeconomic status, residential neighborhoods, race, sex, age, and ethnicity into an area deprivation index (ADI) and then examining the association of the ADI with adverse health outcomes. Patients with a higher ADI score were found to have higher HbA_{1c} levels independent of age, sex, race, and ethnicity. In another initiative, an analysis of treatment outcomes within the network revealed that the initial therapy for patients with type 2 diabetes was modified multiple times in the course of their disease management. The analysis revealed statistically different treatment utilization patterns not only between health systems but also among individual providers within health systems (145). Population-wide analysis of these treatment modifications led to the development of an algorithm to predict patients' responses to treatment, enabling more efficient prescribing of first-line medications.

Dr. Butte noted that data in this network are de-identified to allow researchers to pose queries through a secure process that protects both patients' privacy and data integrity (146). Applying AI methods to the digital database enables the provision of clinical decision-support feedback to providers to guide their patient care. The system has broad applications in the management of multiple diseases and conditions.

Presentation 2: Looking Toward a Future of Disease-Modifying Therapies (Jose Florez)

Dr. Florez, of Harvard Medical School, then addressed the future of precision medicine and how it will transform diabetes care. He stressed that a revolution in our understanding of diabetes pathophysiology should be coupled with elucidation of its heterogeneity, interrogation of differential outcomes depending on molecular phenotype, and adoption of preventive and therapeutic strategies that are based on the specific metabolic processes at play in each individual. He noted that we stand on the verge of a paradigm shift in diabetes care, where we recognize that hyperglycemia, which drives the diagnosis of diabetes, is only the end result of multiple metabolic derangements at work in a diseased individual (147). Therapeutics must now pivot from controlling the symptom of hyperglycemia to addressing its root causes. Only by introducing true disease-modifying therapies can we hope to reverse the pathophysiological process and effect a cure.

In this quest to elucidate the various pathogenic mechanisms that conspire to cause hyperglycemia in each person with diabetes, we can now leverage high-throughput technologies that capture entire axes of biology. The integration of big data across the genome, epigenome, transcriptome, proteome, metabolome, microbiome, and exposome may yield mechanistic insights about the onset of diabetes, its progression, and the incidence of related complications. However, these big-data explorations must be reproducible, interpretable, and actionable (78). As recognizable patterns emerge, we should be increasingly able to refine subtypes, test them for differential drug responses, and examine the occurrence of complications within them. Where rigorous evidence-based approaches demonstrate discernible impacts on outcomes, methods that can perform the necessary measurements at scale and in a cost-effective manner must be developed and disseminated to the point of care. Algorithms and decision-support tools should be designed and deployed in health care systems. Ensuring that these advances are applicable to all racial and ethnic groups and can be implemented in resource-limited settings remains an ethical imperative.

Factors that could or will affect our ability to diagnose diabetes include genomic data, including the results of clinical testing and "entertainment genomics" from publicly available commercial sources, and wearable technologies, including activity and vital signs monitors and continuous glucose monitoring systems. Biomarkers discovered through the study of metabolomics, proteomics, and microbiomics, when combined with EHR data and imaging studies, will inform the clustering of patients into subtypes for further analysis of patterns of diabetes and responses to treatment (148). These cluster assignments may change over time based on the pattern of disease and responses to treatment, as a consequence of somatic mutations and alterations in lifestyle factors and the environment.

Moving forward, large-scale cooperative programs will be needed to achieve the scale necessary to meet these goals. One example already in existence is the Common Metabolic Diseases Knowledge Portal (www.cmdkp.org), a cooperative effort of academic groups and industry partners to provide an open-access

resource for integrating and analyzing genetic and genomic data, allowing insights into complex metabolic diseases and traits. It combines data from 277 data sets from 50 countries, provides graphic representations of data, and has revealed novel insights of associations across multiple traits.

Challenges to the management of diabetes will continue to include the pressure to control health care expenditures, the constraint of time for clinical encounters, the difficulty of interpreting complex clinical data to allow binary treatment decisions, the obesity epidemic, health disparities, and limited resources in low- and middle-income countries. Clinical translation of advances in the understanding of disease mechanisms will have to be interpretable, sustainable, reproducible, and affordable to be adopted.

Dr. Florez concluded by forecasting exactly what will be required to advance precision medicine in diabetes going forward. These elements include robust and high-throughput methods of data collection from appropriately scaled sources, rigorous analytics by multidisciplinary teams, open data sharing, expert panels to distill findings into actionable interventions, educators to disseminate the information, and outcomes studies to evaluate the public health impact of resulting innovations. A process involving all of these components toward achieving the goal of precision medicine has been presented (Fig. 10) (141).

In the panel discussions that followed each Session 3 presentation, Drs. Butte and Florez agreed that, for precision medicine to continue to advance, there is a need to first show the clinical relevance of precision medicine applications in small cohorts of patients and then apply approaches found to be successful to larger groups. They also agreed that environmental factors make tremendous contributions to the development (and therefore the prevention) of diabetes and that lifestyle changes can override the effects of genetic risk in reversing diabetes.

CONCLUSION

As we end our celebration of the 100th anniversary of the discovery of insulin, we are thankful for the many recent technological, therapeutic, and research advances that have contributed to enhanced quality of life for people with diabetes and increased their chances of living free of devastating complications. We now know we can prevent both type 1 and type 2 diabetes, and we are at an exciting moment with regard to cell replacement therapy. Clearly, the first century of diabetes innovation has positioned us well as a scientific community to usher in a new era in which precision medicine will flourish. As important as the discovery of insulin was to diabetes management 100 years ago, so, too, will be precision medicine, as it leads us to further landmark achievements in the next century.

This symposium strengthened our belief in a future in which the promise of personalized medicine will be fully realized. Discussion throughout this symposium emphasized that next steps will require more precise diagnostic classifications of diabetes, more detailed identification of subtypes, and more elucidation of the heterogeneity of treatment patterns. However, symposium participants also clearly recognized that, regardless of technological advances, individual patient preferences will still need to be considered, and patients should and will remain at the center of diabetes care. The familiar adage that "medicine is an art, not a science" is particularly relevant to precision medicine, as the ultimate success and utility of this approach will depend on both providers' understanding and patients' acceptance and interaction. We believe that the further development and expansion of precision medicine is the new

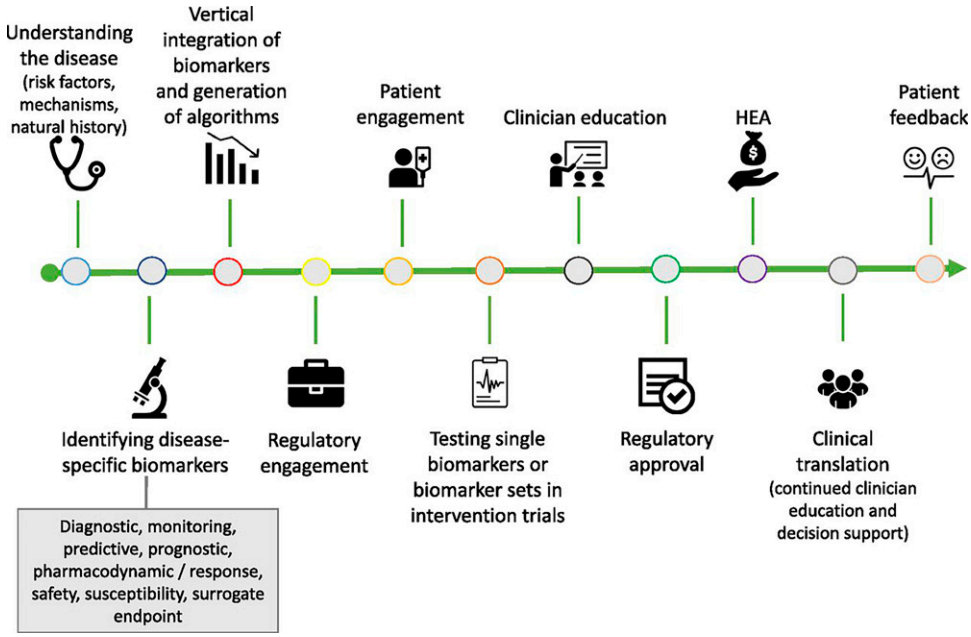


Figure 10—The path to precision diabetes medicine. HEA, health economic assessment. Adapted from Fitipaldi H, McCarthy MI, Florez JC, Franks PW. A global overview of precision medicine in type 2 diabetes. Diabetes 2018;67:1911–1922. Reprinted with permission from Chung et al. (141).

frontier for this next century of diabetes innovation.

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