Type 1 Diabetes: Clinical and Experimental

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Type 1 diabetes (T1D) is one of the most common chronic diseases in children and adolescents; it is due to an autoimmune β -cell destruction. Because of the increasing incidence of T1D worldwide, particularly in preschool children, improved knowledge on pathogenetic mechanisms as well as new perspectives in diagnostic and therapeutic options are urgently needed. In this respect, relevant progress have recently been made by the growing development of technologies. In fact, technological advances have been offering patients and physicians new means in daily diabetes management and care with the aim of improving quality of life and possibly preventing acute and long-term complications. In particular, new types of insulin and less invasive and more comfortable devices for blood glucose measurement are available and they should offer new hope for achieving better metabolic control and reducing the psychological burden of the disease. Furthermore, recent technical advances have offered new perspectives for determination of both genetic predisposition and identification of novel biomarkers linked to the risk of diabetic complications, in particular the possibility to perform large genome-wide analyses contributed to find new genes involved in the genetic clustering of T1D. On the other hand, advances in proteomic analyses have improved our knowledge on early markers of diabetic micro- and macrovascular complications. The identification of the high-risk population and novel biomarkers will possibly improve our strategies for prevention and treatment of T1D in children.

Mechanism of the Year

Exhaled methyl nitrate as a non-invasive marker of hyperglycemia in type 1 diabetes

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Background: Recent technical advances may allow to detect several hundred volatile organic compounds (VOCs) in human exhaled air, many of which reflect unidentified endogenous pathways. In a previous study, Galassetti et al. estimated plasma glucose levels in healthy adults during a standard oral glucose tolerance test via exhaled VOC analysis. As a result of the metabolic characteristics of hyperglycemia in the diabetic (low insulin and increased free fatty acids and ketones), it was hypothesized that different exhaled VOC profiles may be present in children with T1D during spontaneous hyperglycemia. A strong correlation was demonstrated between exhaled methyl nitrate and specifically with the acute, spontaneous hyperglycemia of T1D children.

Methods: 18 experiments were conducted among 10 T1D children. Plasma glucose and exhaled gases were monitored during either constant euglycemia ($n = 5$) or initial hyperglycemia with gradual correction ($n = 13$); all subjects received i.v. insulin and glucose as needed. Gas analysis was performed on 1.9-liter breath samples via gas chromatography using electron capture, flame ionization, and mass selective detection.

Results: Among the approximately 100 measured exhaled gases, the kinetic profile of exhaled methyl nitrate, commonly present in room air in the range of 5–10 parts per trillion, was most strongly statistically correlated with that of plasma glucose ($p = 0.003$ – 0.001).

Conclusion(s): Indeed, the kinetic profiles of the two variables paralleled each other in 16 of 18 experiments, including repeat subjects who at different times displayed either euglycemia or hyperglycemia.

Since the 1980s, patients with diabetes have adopted portable meters in order to adjust insulin treatment and carbohydrate amount according to blood glucose levels. Soon after, the DCCT [1] and later

studies (EDIC) [2] provided clear evidence in adults and adolescents with T1D that better metabolic control is associated with fewer and delayed microvascular complications. The frequency of SMBG was also demonstrated to be associated with improved HbA1c [3]. Since then, technology is moving on in order to generate more accurate and less invasive devices able to minimize the burden of diabetes monitoring [4, 5].

Based on recent advances in volatile organic compound (VOC) analytical technology [6, 7], Novak et al. investigated whether exhaled gas profiles reflect glycemic homeostasis in children with T1D. Blood glucose monitoring was combined with simultaneous measurements of more than 100 gases detected in the exhaled breath of 18 children with T1D. It was shown that methyl nitrate (CH_3ONO_2), an isomeric protonated methyl-nitrate form, derived from the oxidative stress-dependent reaction of superoxide with nitric oxide (NO) showed the greatest correlation with glucose among the VOCs. In fact, methyl nitrate had a kinetic profile closely paralleling that of plasma glucose in 16–18 experiments, during which plasma glucose was either constantly euglycemic or initially hyperglycemic (160–410 mg/dl) with gradual correction. These preliminary data open new interesting perspectives for new devices to detect easily, non-invasively and as painlessly as possible blood glucose levels in patients with T1D. Furthermore, as methyl nitrate is an oxidative stress-derived compound, exhaled gas could be a reliable marker of the alterations in inflammatory and oxidative markers, now considered as the main determinants of diabetic vascular complications. Therefore, confirmation and improvement of these results and technology could offer an important means both in monitoring glycemic control as well as in determining the actual pathogenic potential of a given glycemic state in terms of onset/progression of diabetic complications.

New Paradigms

Multiple variants in vascular endothelial growth factor are risk factors for time to severe retinopathy in type 1 diabetes: the DCCT/EDIC Genetics Study

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Background: In this study, the authors sought to determine if any common variants in the gene for vascular endothelial growth factor (VEGFA) are associated with long-term renal and retinal complications in T1D.

Methods: A group of 1,369 Caucasian subjects with T1D from the Diabetes Control and Complications Trial (DCCT)/Epidemiology of Diabetes Interventions and Complications (EDIC) Study had an average of 17 retinal photographs and 10 renal measures over 15 years. The DCCT/EDIC performed an assessment of 18 single nucleotide polymorphisms (SNPs) in VEGFA that represent all linkage disequilibrium bins (pairwise $r^2 \geq 0.64$) and tested them for association with time to development of severe retinopathy, a three or more step progression of retinopathy, clinically significant macular edema, persistent microalbuminuria, and severe nephropathy.

Results: In a global multi-SNP test, a highly significant association of VEGFA SNPs with severe retinopathy ($p = 6.8 \times 10^{-5}$) was demonstrated while non-significant results were documented for the four other outcomes. In addition, in a survival analysis controlling for covariate risk factors, a significant association between 8 SNPs and severe retinopathy has been demonstrated ($p < 0.05$). The most significant single SNP association was rs3025021 (hazard ratio 1.37 [95% CI 1.13–1.66], $p = 0.0017$). Family-based analyses of severe retinopathy provide evidence of excess transmission of C at rs699947 ($p = 0.029$), T at rs3025021 ($p = 0.013$), and the C-T haplotype from both SNPs ($p = 0.035$). Multi-SNP regression analysis including 15 SNPs, and allowing for pairwise interactions, independently selected 6 significant SNPs ($p < 0.05$).

Conclusion(s): These data demonstrate that multiple VEGFA variants are associated with the development of severe retinopathy in T1D.

Data from longitudinal studies have clearly demonstrated the critical role of genetic predisposition on the development of chronic complications in patients with T1D (DCCT, EDIC) [8, 9]. In fact, although the most important risk factor is glycemic exposure, the development of diabetes complications has been shown also in patients with good glycemic control; in addition, a familial clustering of severe diabetic retinopathy and nephropathy has been demonstrated.

A number of susceptibility gene loci have been investigated; those involved in the synthesis of proteins, such as vascular endothelial growth factor (VEGF), have given relevant results. In particular VEGF, a mitogen mediating several effects associated to diabetes complications [10, 11], has been shown to be hyperexpressed in plasma and vitreous fluid of patients with vascular complications [12, 13]. Consequently, genetic-related up-regulation could represent an important mechanism involved in the pathogenesis of diabetic retinopathy. This study has examined longitudinal data for retinal and renal complications in 1,369 Caucasian type 1 diabetic patients from the DCCT/Epidemiology of Diabetes Interventions and Complications (EDIC) study, and the association between VEGFA SNPs and time to early signs of three retinal and two renal outcomes in the cohort. Single SNP multivariate and univariate Cox proportional hazards analyses detected nominally significant associations between severe retinopathy and more than one SNP in VEGFA. In addition, by performing a family-based analysis for four SNPs that were genotyped in available first-degree relatives, excess transmission was observed for alleles C of rs699947 and T of rs3025021, confirming the significant association of these SNPs observed in multi-SNP regression analyses. Therefore, the current study provides robust evidence that more than one SNP in VEGFA is independently associated with the risk of developing severe diabetic retinopathy in patients with T1D. This suggests that allelic heterogeneity is operating through one or more pathological mechanisms. A better understanding of the genetic risk to diabetic angiopathy will allow to develop a new and individualized therapeutic target related to the interindividual susceptibility to diabetes complications.

New Hope

Insulin intervention in slowly progressive insulin-dependent (type 1) diabetes mellitus

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Background: In this study, Maruyama et al. have evaluated whether insulin therapy rather than sulfonylurea treatment is effective to reverse or preserve β -cell function among patients with slowly progressive insulin-dependent (type 1) diabetes (SPIDDM) or latent autoimmune diabetes in adults (LADA).

Methods: In a multicenter, randomized, non-blinded clinical study, 4,089 non-insulin-dependent diabetic patients were screened for glutamic acid decarboxylase autoantibodies (GADAb). 60 GADAb-positive non-insulin requiring diabetic patients with ≤ 5 -year duration of diabetes were assigned to either the sulfonylurea group ($n = 30$) or insulin group ($n = 30$). Serum C-peptide responses to annual OGTTs were followed up for a mean of 57 months. An insulin-dependent state defined by the sum of serum C-peptide values during OGTT (sigma C-peptide) < 4 ng/ml (1.32 nmol/l) was established as the primary endpoint of the study.

Results: It was shown that the progression rate to an insulin-dependent state in the insulin group (3/30, 10%) was lower than that in the sulfonylurea group (13/30, 43%; $p = 0.003$, log-rank). Furthermore, as documented by longitudinal analysis, sigma C-peptide values were better preserved in the insulin

group than in the sulfonylurea group. In addition, as shown in a multiple regression analysis, insulin treatment, a preserved C-peptide response, and a low GADAb titer at entry resulted in independent factors in preventing progression to an insulin-dependent state. Subgroup analysis suggested that insulin intervention was highly effective for SPIDDM patients with high GADAb titers (≥ 10 U/ml; 180 WHO U/ml) and preserved β -cell function (sigma C-peptide ≥ 10 ng/ml [3.31 nmol/l]) at entry. No severe hypoglycemic episodes were reported during the study.

Conclusion(s): These results demonstrate that insulin intervention, adopted in order to preserve β -cell function, is effective and safe for patients with SPIDDM/LADA.

T1D occurs in genetically predisposed persons as a consequence of the immune-mediated destruction of insulin-secreting islet β cells. It is well known that the onset of clinically overt diabetes, which may occur from younger to elderly, represents the endpoint of an insidious, relatively long and progressive decline in the function of β cells which damages or destroys the majority of β cells [14]. This study has explored the issue of whether early intervention and insulin administration, given before the onset of diabetes, could alter the course of the disease in high-risk subjects, and in particular in patients with slowly progressive insulin-dependent diabetes mellitus (SPIDDM/LADA) [15]. SPIDDM is a particular subtype of type 1 diabetes which is characterized by persistently positive islet cell antibody, late age of onset, non-insulin-dependent diabetes, and slowly progressive β -cell failure. Therefore, the relative long duration gap between established autoimmunity-dependent β -cell destruction and the insurgence of the insulin-dependence state may represent an optimal opportunity to establish interventions to preserve β -cell failure. This multicenter randomized clinical trial was conducted in 61 patients with persistently high islet cell autoantibodies and criteria for SPIDDM; compared to sulfonylurea (SU) treatment, insulin intervention was effective and safe for treating gradual β -cell failure especially in patients with SPIDDM whose β -cell function was preserved (Σ CPR ≥ 10 ng/ml) and who have a high titer of GADAb (≥ 10 U/ml; 180 WHO U/ml) at the start of insulin therapy. In fact, the progression to an insulin-dependent state was documented in 43% of patients assigned to SU treatment compared to the 10% in the insulin group. Furthermore, the proportion of participants who progressed to a complete insulin-dependent state was 11 and 2% per annum in the SU and insulin groups, respectively. The cumulative probability of progression to an insulin-dependent state significantly differed between the groups ($p = 0.003$, log-rank test).

Although these results contrast with those of a previous study (Diabetes Prevention Trial-1: DPT-1) [16], which showed no effect of insulin administration on delaying or preventing T1D in relatives of type 1 patients at high risk for diabetes, this could be explained by several differences related to the study populations. In addition, the comparison is not with placebo but with sulfonylurea which could have a detrimental effect on β -cell reserve. In fact, several etiopathogenetic characteristics between the two forms of T1D may determine a different T-cell response to β -cell antigens with a possible less aggressive β -cell damage in SPIDDM than in typical acute-onset type 1 diabetes in DPT-1 probands or different genetic backgrounds of the patients. Therefore, the outcome of insulin intervention should differ in various populations according to HLA and/or other genetic factors. All these elements raise new questions to be solved which should open new perspectives in intervention strategies aimed to prevent or delay the onset of overt diabetes in high-risk populations.

Induction of robust diabetes resistance and prevention of recurrent type 1 diabetes following islet transplantation by gene therapy

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Background: In a previous study, Tian et al. have shown that the development of T1D can be prevented in non-obese diabetic (NOD) mice by reconstitution with autologous hemopoietic stem cells retrovirally transduced with viruses encoding MHC class II I-A β -chain molecules associated with protection from the disease.

Methods: This study examined whether a blockade of the programmed death-1 (PD-1)–programmed death ligand-1 (PD-L1) pathway (a major pathway known to control diabetes occurrence) could precipitate

T1D in young NOD mice following reconstitution with autologous bone marrow retrovirally transduced with viruses encoding protective MHC class II I-A β -chain molecules. In addition, Tian et al. have examined whether the expression of protective MHC class II alleles in hemopoietic cells could be used to prevent the recurrence of diabetes in mice with pre-existing disease following islet transplantation.

Results: Protection from the occurrence of T1D diabetes in young NOD mice by the expression of protective MHC class II I-A β -chain molecules in bone marrow-derived hemopoietic cells was resistant to induction by PD-1-PD-L1 blockade. In addition, reconstitution of NOD mice with preexisting T1D autologous hemopoietic stem cells transduced with viruses encoding protective MHC class II I-A β -chains determined the successful transplantation of syngeneic islets, resulting in the long-term reversal of T1D. Reversal of diabetes was resistant to the induction by PD-1-PDL-1 blockade and depletion of CD25+ T cells.

Conclusion(s): These data suggest that expression of protective MHC class II alleles in bone marrow-derived cells establishes robust self-tolerance to islet autoantigens and is sufficient to prevent the recurrence of autoimmune diabetes following islet transplantation.

T1D is a chronic autoimmune disease characterized by a permissive immune system that fails to impose tolerance to arrays of self antigens. Although, islet transplantation has progressed [17], the preexisting unbalanced immune self-tolerance represents one of the most important factors minimizing the results. In fact, because the production of a large amount of T cells against the β -cell antigens cannot be reversed, diabetes recurrence cannot be prevented. In this view, recent studies have pointed out an important role of negative immunoregulatory signals delivered by the programmed death 1 (PD-1)–PD-1 ligand (PD-L) pathway in regulating T-cell activation and tolerance [18]. In fact, it has been shown that the PDL pathway plays an essential role on dendritic cells (DCs), both directly during DC–T-cell interactions and indirectly through signaling into the DC. Furthermore, expression of MHC class II β -chains has been shown to prevent diabetes in NOD mice, when its expression is amplified [19]. In the present study, Tian et al. showed that the protective MHC class II I-A β -chain on hemopoietic cells following retroviral transduction of bone marrow is sufficient to prevent the destruction of transplanted syngeneic islets; furthermore, the recurrence of diabetes in NOD mice with preexisting T1D is prevented despite the presence of peri-insulitis within the islet transplants. The potential therapeutic opportunity offered by this study represents an important element in the determination of a novel therapeutic approach in T1D.

New Concerns

Altered neuroendocrine sleep architecture in patients with type 1 diabetes

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Background: The modulatory influence of nocturnal sleep on neuroendocrine secretory activity is increasingly recognized as a factor critical to health. Disturbances of sleep may arise from and contribute to the disease process in chronically ill patients suffering from T1D.

Methods: Neuroendocrine sleep architecture has been assessed under well-controlled non-hypoglycemic conditions in 14 T1D patients and 14 healthy controls matched for age, gender, and BMI, using standard polysomnography and repetitive blood sampling.

Results: As expected, plasma glucose ($p = 0.02$) and serum insulin ($p < 0.001$) levels were constantly higher in T1D patients than in healthy subjects throughout the night. Beside these characteristic alterations of glucose metabolism, T1D patients displayed higher blood levels of growth hormone ($p = 0.001$) and epinephrine ($p = 0.02$) during the entire night, and higher levels of ACTH ($p = 0.01$) as well as a tendency towards higher cortisol levels ($p = 0.072$) during the first half of the night, compared with healthy controls. Patients spent slightly less time in slow-wave sleep ($p = 0.09$) during the first half of the night (where this sleep stage predominates), and overall exhibited an increased proportion of

stage 2 sleep ($p = 0.01$). In addition, assessment of mood and symptoms after sleep showed that subjective sleep was less restorative in T1D patients than in healthy subjects.

Conclusion(s): These results indicate distinct alterations in the neuroendocrine sleep architecture of patients with T1D which add to the generally adverse impact of the disease on the patients' health.

The neuroendocrine pattern is a relevant factor of physiological changes in patients with T1D [20, 21]. Biological rhythms characteristically result in a complex regulation of periodically secreted hormones. In particular, nocturnal sleep is characterized by an initial increase of growth hormone (GH) secretion associated with a rise in prolactin and leptin synthesis, and a suppression of hypothalamo-pituitary-adrenal (HPA) secretory activity. Thereafter, GH, TSH and prolactin secretion progressively decrease while HPA secretory activity increases. In this study, Jauch-Chara et al. aimed to characterize neuroendocrine sleep architecture in patients with T1D compared to healthy controls. While preventing hypoglycemia with endovenous glucose infusion, whenever necessary, a relevant alteration in the hormonal pattern during sleep was shown. In fact, whereas GH and epinephrine levels were increased during the whole night in patients with T1D, HPA hormone concentrations were elevated essentially during the first half of the night. This characteristic hormonal pattern was associated with the tendency toward more shallow sleep in patients with a decreased amount of slow-wave sleep during the first half of the night and an overall increased proportion of stage 2 sleep during the night. In addition, an increased numbness and fatigue point to a weakened restorative effect of sleep were shown in patients with T1D. Whereas the night-time hormonal pattern has been evaluated in previous studies, relevant confounding factors, such as hypoglycemia, have minimized the correct interpretation of the results [21–23]. In the present study, changes in hormonal secretion during night-time sleep were shown during non-hypoglycemic events and they were characteristically associated to a less restorative sleep in patients with T1D than in healthy subjects. These findings represent a novel characteristic of patients with diabetes. Because of the association between impaired habitual sleeping patterns and all-cause mortality risk [24], these data might reveal a novel adverse impact of the disease in patients with T1D. Further longitudinal studies are now needed in order to fully clarify the impact of diabetes on sleep architectures and its adverse effects.

Concepts Revised

Impaired short-term blood pressure regulation and autonomic dysbalance in children with type 1 diabetes mellitus

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Background: Reduction in baroreceptor sensitivity (BRS) has been associated with hypertension in the normal population and with increased cardiovascular morbidity and mortality in patients with diabetes mellitus; in this study, Dalla Pozza et al. aimed to measure BRS in children with T1D.

Methods: 208 children (150 patients with T1D [mean age 13.9 ± 2.8 years, 70 boys, mean HbA1c $7.8 \pm 1.4\%$ and 58 healthy controls [mean age 14.1 ± 3.1 years, 32 boys]) were studied. BRS and heart rate variability (HRV) were analyzed from a short-time ECG and BP recording using the sequence method (BRS) and the frequency domain method (HRV).

Results: Impaired BRS was documented in 111 of 150 patients (74%) and 5 of 58 controls (8.6%). Mean BRS differed significantly between patients and controls (18.4 ± 7.2 vs. 25.8 ± 8.2 ms/mm, $p < 0.001$). BRS correlated inversely with systolic BP ($r = -0.23$, $p = 0.009$) and was related to diabetes duration ($r = -0.194$, $p = 0.027$). Analysis of HRV showed greater sympathetic and less parasympathetic influence in patients than in controls (low-frequency/high-frequency ratio 1.3 ± 0.8 vs. 0.9 ± 0.6 , $p < 0.05$); the low-frequency/high-frequency ratio was inversely correlated with BRS ($r = -0.28$, $p = 0.001$).

Conclusion(s): Reduced BRS is documented in children with diabetes and appears to be associated with the duration of disease. Furthermore, the degree of BRS impairment is related to the degree of autonomic dysbalance.

It is well known that morbidity and mortality are increased in patients with T1D mainly because of cardiovascular complications. In fact, adult patients with diabetes show a two- to tenfold risk of developing atherosclerotic lesions compared to the normal population [25]. Although several factors have been demonstrated to play an important role in the pathogenesis of diabetes-related cardiovascular disease (CVD), impaired blood pressure (BP) has been recognized as a major cofactor [26]. In fact, in well-controlled type 1 diabetic children, an independent association between systolic BP and increased intima-media thickness (IMT) of the common carotid artery (CCA) – universally accepted as an early manifestation of atherosclerosis [26] – has recently been confirmed. In addition, baroreceptor sensitivity (BRS) has been postulated to be involved in the development of systemic hypertension. In fact, the baroreflex itself refers to the mechanism of interaction between BP receptors and the autonomic nervous system; it is characterized by a complex regulatory circuit which results in intricate interaction of excitatory and inhibitory neurons together with local mediators (resulting in a direct modulation of vagal and sympathetic activities). The efficacy of this circuit can be calculated as the regression line of changes in BP against heart rate and is given as BRS, which has been showed to be a good predictor of hypertension. In addition, assessment of sympathovagal balance may be detected by the low-frequency (LF)/high-frequency (HF) band ratio defined by a power spectral analysis of heart rate variability (HRV).

In this cross-sectional study, by testing both BRS and HRV in a population of 150 children and adolescents with T1D, a precocious imbalance of autonomic nervous system was demonstrated. Children with diabetes showed an impairment of the beat-to-beat BP control and a reduction of BRS compared to healthy controls. Furthermore, the function of the baroreflex circuit was inversely related to systolic BP, suggesting a possible role of altered short-term BP control in the development of hypertension. Autonomic function studied by HRV analysis was impaired and correlated to vagal dysfunction. A thorough characterization of early abnormalities involved into the complex mechanisms of blood pressure regulation represents a main issue for clinicians in order to detect as early as possible children and adolescents at increased risk for hypertension and to establish preventive programmes. Longitudinal studies are now needed to assess whether these parameters may be used for clinical assessment cardiovascular risk in patients with childhood-onset diabetes.

Important for Clinical Practice

Validation of the WHO-5 Well-Being Index in adolescents with type 1 diabetes

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Background: Clinical guidelines suggest a periodical assessment of the psychological status of adolescents with diabetes, as part of ongoing care. The World Health Organization-Five Well-Being Index (WHO-5) is a short self-report instrument that appears suitable for this purpose. Firstly, in this study, de Wit et al. have aimed to assess the reliability and validity of the WHO-5 in adolescents with T1D.

Methods: 91 adolescents with T1D (aged 13–17 years) from four pediatric clinics completed the WHO-5, along with other psychological measures: the Center for Epidemiologic Studies Depression Scale (CES-D), the Diabetes Family Conflict Scale (DFCS), and the mental health and self-esteem subscales of the Child Health Questionnaire (CHQ-CF87). Confirmatory factor analysis (CFA) and exploratory factor analysis were conducted. Readability, homogeneity, and item-total and inter-item correlations were determined. Concurrent validity was examined by calculating correlation coefficients among all measures. Sensitivity and specificity of the WHO-5 were tested against those for the CES-D using receiver operating characteristic (ROC) curves.

Results: CFA confirmed the one-factor structure; Cronbach's α of this 5-item scale was 0.82. The WHO-5 showed a moderate to strong correlation with the CES-D ($r = -0.67$), with the mental health ($r = 0.60$) and self-esteem ($r = 0.43$) subscales of the CHQ-CF87, and with the DFCS ($r = -0.34$), confirming concurrent validity. ROC curve analysis confirmed the WHO-5 cutoff point of <50 for identification of mild to severe depressive affect (sensitivity 89% and specificity 86%).

Conclusion(s): The WHO-5 is a brief, patient-friendly measure of positive well-being with good psychometric properties that appears suitable for routine use in adolescents with T1D.

T1D represents the most common chronic disease in children and adolescents resulting from autoimmune β -cell destruction [27]. Due to the chronic absolute dependence on insulin treatment and to the high rate of acute and chronic complications occurring even at relatively young ages, diabetes places a particularly psychological heavy burden on the affected subjects [28]. In fact, several studies have clearly demonstrated a two- to threefold increased prevalence of depression in adolescents with diabetes compared to healthy subjects [29, 30]. Furthermore, less favorable quality of life (QoL) also appears to be related with youths' perceptions that diabetes is upsetting, difficult to manage, and stressful and is related to poor glycemic control; therefore, when QoL is assessed in diabetic youngsters, they tend not to rate themselves very differently from their healthy peers [29, 30]. Thus, knowledge on QoL in young diabetic patients as well as its determinants is crucial; in addition, monitoring of health-related QoL of adolescents is increasingly advocated in clinical practice. Anyway, although psychological status of adolescents with diabetes has been recommended to be assessed periodically as part of ongoing care, no consensus on which measure to use for this purpose was obtained. In this study, de Wit et al. attempt to validate, in a total of 91 adolescents with T1D, the World Health Organization-Five Well-Being Index (WHO-5), a short, positively worded instrument designed to assess the level of emotional well-being over a 14-day period. It was shown that the WHO-5 could represent a reliable and valid instrument suitable for routine use in adolescents. It requires only several minutes to perform and is patient-friendly. This result represents an important issue in daily diabetes care and could help to detect and address emotional problems in adolescents with diabetes as part of routine clinical care.

Clinical Trials, New Treatments

Omega-3 polyunsaturated fatty acid intake and islet autoimmunity in children at increased risk for type 1 diabetes

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Background: Retrospective studies have postulated a strong association between cod liver oil supplements in infancy and the decreased risk of T1D. In this study, Norris et al. have examined whether the intake of ω -3 and ω -6 fatty acids is associated with the development of islet autoimmunity in children.

Methods: For this purpose, a longitudinal, observational study, the Diabetes Autoimmunity Study in the Young (DAISY), was performed in Denver, Colorado, between January 1994 and November 2006; 1,770 children at increased risk for T1D (defined as either possession of a high diabetes risk HLA genotype or having a sibling or parent with T1D) were enrolled. The mean age at follow-up was 6.2 years. Islet autoimmunity was assessed in association with the reported dietary intake of polyunsaturated fatty acids starting at age 1 year. A case-cohort study ($n = 244$) was also conducted in which risk of islet autoimmunity by polyunsaturated fatty acid content of erythrocyte membranes (as a percentage of total lipids) was examined. Risk of islet autoimmunity was defined as being positive for insulin, glutamic acid decarboxylase, or insulinoma-associated antigen-2 autoantibodies on two consecutive visits and still autoantibody positive or having diabetes at last follow-up visit.

Results: 58 children developed islet autoimmunity. Adjusting for HLA genotype, family history of T1D, caloric intake, and ω -6 fatty acid intake, ω -3 fatty acid intake was inversely associated with risk of islet autoimmunity (hazard ratio [HR] 0.45; 95% confidence interval [CI] 0.21–0.96; $p = 0.04$). The association was strengthened when the definition of the outcome was limited to those positive for two or more autoantibodies (HR 0.23; 95% CI 0.09–0.58; $p = 0.002$). In the case-cohort study, ω -3 fatty acid content of erythrocyte membranes was also inversely associated with islet autoimmunity risk (HR 0.63; 95% CI 0.41–0.96; $p = 0.03$).

Conclusion(s): These results demonstrate that the dietary intake of ω -3 fatty acids is associated with reduced risk of islet autoimmunity in children at increased genetic risk for T1D.

T1D is a chronic autoimmune disease with a subclinical prodromic period characterized by selective loss of pancreatic insulin-producing β cells in genetically predisposed subjects [27]. As shown in epidemiological studies, less than 10% of those with increased genetic susceptibility progress to clinical disease, suggesting a strong environmental modification of the prediabetic process. Among the several exogenous triggers, dietary factors are thought to be an important causal issue implicated in the initiation and progression of β -cell destruction. In addition to their role as triggers, environmental factors are also likely to affect the outcome of the process and the rate of progression to clinical disease [14, 31]. Among the several candidate dietary factors, discussed as factors involved in islet autoimmunity and T1D, short duration of breastfeeding, uptake of cow's milk proteins during the first months of life, early introduction of cereals, as well as vitamin D intake seem to be implicated [14, 31]. The aim of this longitudinal observational study was to study, in a large high-risk population, whether the intake of polyunsaturated fatty acids (PUFA) during infancy might have a protective role on the risk of diabetes. By examining the reported ω -3 and ω -6 fatty acid dietary intake, Norris et al. were able to demonstrate an increased rate of islet autoantibodies (IA) appearance in oil-consuming children at increased genetic risk of T1D. Furthermore, the association was further confirmed by the higher proportion of ω -3 fatty acids detected in the erythrocyte membranes in subjects at decreased risk of IA. They postulate that adequate ingestion of ω -3 fatty acids throughout childhood may decrease the risk of IA by positively increasing production of a class of anti-inflammatory eicosanoids, known as resolvins and protectins [32, 33]. These molecules may exert favorable anti-inflammatory effects (including anti-inflammatory lipid molecules), suppress inflammatory cytokines (e.g., interleukin [IL]-1 β , tumor necrosis factor- α , IL-12), induce a reduction of Th1 responses, and suppress antigen-presenting cell maturation – all relevant for the prevention of T1D [32, 33]. The protective role of PUFAs intake, demonstrated in this study, represents an important finding and may justify large clinical trials aimed at confirming its potential role in blocking early islet inflammatory events and thus preventing the development of early IA in infants with a high genetic risk for this disease. Therefore, dietary supplementation with ω -3 fatty acids could possibly be of some help in the prevention of T1D.

Treatment with CD20-specific antibody prevents and reverses autoimmune diabetes in mice

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Background: The precise roles of B cells in promoting the development of T1D remain undefined.

Methods: To determine the efficacy and potential mechanism of therapeutic B-cell depletion, the authors generated a transgenic NOD mouse expressing human CD20 (hCD20) on B cells.

Results: A single cycle of treatment with an antibody specific for hCD20 temporarily depleted B cells and significantly delayed and/or reduced the onset of diabetes. Furthermore, disease established to the point of clinical hyperglycemia could be reversed in over one-third of diabetic mice. Why B-cell depletion is therapeutic for a variety of autoimmune diseases is unclear, although effects on antibodies, cytokines, and antigen presentation to T cells are thought to be important. In B-cell-depleted NOD mice, Hu et al. have identified a postulated novel mechanism by which B-cell depletion may lead to long-term remission through expansion of Tregs and regulatory B cells.

Conclusion(s): This study demonstrates that B-cell depletion in mice can prevent or delay diabetes, reverse diabetes after frank hyperglycemia, and lead to the development of cells that suppress disease. Therefore, these results demonstrate clinical efficacy, even in established disease, and identify mechanisms for therapeutic action that will guide design and evaluation of parallel studies in patients.

Improved understanding of the pathogenesis of T1D has completely changed our view of this disease in the past 25 years – from an acute, fulminant disease, to a chronic, autoimmune process. The increasing and not yet completely defined autoimmune-dependent destruction of insulin-secreting β cells has clearly demonstrated that, despite the primary role of autoreactive T cells, autoreactive B cells are required for the instauration and progression of autoimmune loss of insulin secretion [34, 35]. In fact, via their production of circulating autoantibodies and/or their role as antigen-presenting cells for autoreactive T cells, autoreactive B cells have shown to play a determinant role in autoimmune diseases, showing a possible new therapeutic opportunity in prevention of diabetes [34, 35]. By developing a bacteria artificial chromosome-transgenic NOD mouse that expresses hCD20 on B cells (which allows B-cell depletion with anti-hCD20), Hu et al. were able to demonstrate a new potential therapeutic target for preventing autoimmune diabetes in mice. They showed that a temporary depletion of B cells, achieved by an antibody specific for hCD20, significantly prevents T1D and blocks the disease progression; in addition, diabetes was reversed in over one-third of mice with established disease. Furthermore, as a direct consequence of B-cell depletion, they reported a significant expansion of two populations of T cells known to regulate autoimmunity in other settings (CD4+CD25+Foxp3+ as well as CD4+CTLA4+ cells), which was supposed to contain Treg cells involved in the long-term remission. Therefore, these data suggest that depletion of B cells may be useful in modifying the progression of diabetes and numerous therapeutic interventions have been tested, mostly in animal models, but also in humans. In this regard, rituximab (Rituxan; Genentech) [36], a humanized anti-CD20 mAb that induces B-cell depletion and is FDA-approved for B-cell lymphoma therapy, is currently being assessed in a clinical trial in T1D. It is hoped that this new treatment may help in prevention or therapy of T1D.

New Mechanisms

A mutation in the TMD0-L0 region of sulfonylurea receptor-1 (L225P) causes permanent neonatal diabetes mellitus

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Background: In this study, Masia et al. have examined the molecular mechanisms underlying permanent neonatal diabetes mellitus (PNDM) in a patient with a heterozygous de novo L225P mutation in the L0 region of the sulfonylurea receptor (SUR)1, the regulatory subunit of the pancreatic ATP-sensitive K⁺ channel (K_{ATP} channel).

Methods: By patch-clamp experiments on excised membrane patches and by macroscopic Rb-flux experiments in intact cells, the effects of L225P on the properties of recombinant K_{ATP} channels in transfected COS cells were evaluated.

Results: L225P-containing K_{ATP} channels were significantly more active in the intact cell than in wild-type channels. In excised membrane patches, L225P increased channel sensitivity to stimulatory Mg nucleotides without altering intrinsic gating or channel inhibition by ATP in the absence of Mg²⁺. The effects of L225P were abolished by SUR1 mutations that prevent nucleotide hydrolysis at the nucleotide-binding folds. L225P did not alter channel inhibition by sulfonylurea drugs, and, consistent with this, the patient responded to treatment with oral sulfonylureas.

Conclusion(s): L225P underlies K_{ATP} channel overactivity and PNDM by specifically increasing Mg-nucleotide stimulation of the channel, consistent with recent reports of mechanistically similar PNDM-causing mutations in SUR1. The mutation does not affect sulfonylurea sensitivity, and the patient is successfully treated with sulfonylureas.

During the last decades, several molecular mechanisms underlying permanent neonatal diabetes mellitus (PNDM) have been thoroughly studied. Among these, mutations in KCNJ11 and ABCC8 gene, encoding the Kir6.2 and SUR1 subunit of the pancreatic K_{ATP} channel involved in regulation of insulin secretion, account for one-third to one-half of the PNDM cases [37, 38]. ATP-sensitive potassium (K-ATP) channels regulate the flux of K⁺ ions across the cell membranes and couple cell metabolism to electrical activity. These channels are octameric complexes of 4 pore-forming Kir (KCNJ11) and 4 regulatory sulfonylurea receptor (SUR) subunits (ABCC8). The K-ATP channels play multiple physiological roles in the glucose metabolism regulation, especially in the pancreatic β cells where they regulate insulin secretion, in response to increases in ATP concentrations. Activating mutations in the pancreatic K-ATP channel leads to cell underexcitability and suppression of insulin secretion and may determine permanent and transient forms of neonatal diabetes mellitus. However, the majority of neonatal diabetes mellitus (NDM)-associated K-ATP mutations are localized to Kir6.2, while recent studies have now described novel mutations in SUR1 [39]. In this study, Masia et al. describe a patient with PNDM due to a novel heterozygous SUR1 mutation (L225P) located in L0. Furthermore, by performing a patch-clamp experiment on excised membrane patches and by macroscopic Rb-flux experiments in intact cells, they showed a significant L225P-dependent increased stimulation of recombinant K-ATP channels by Mg nucleotides and increased activity in the intact cell. Continuous improvement in the knowledge of molecular mechanisms involved in NDM is paramount to better define prognosis and treatment of these children. In fact, precise definition of channel activity defects is responsible for different phenotypes and different responses to insulin or sulfonylureas.

Enhanced thymic selection of FoxP3⁺ regulatory T cells in the NOD mouse model of autoimmune diabetes

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Background: FoxP3⁺CD4⁺ regulatory T cells (Tregs) play a key role in the maintenance of peripheral self-tolerance, and it has been suggested that diabetes-susceptible non-obese diabetic (NOD) mice are defective in the generation and number of Tregs.

Results: Fetal thymic organ cultures on the NOD background required 3- to 10-fold more antigen than corresponding cultures on the B6 background for optimal induction of Tregs, but once the threshold for induction was reached the NOD background yielded close to 10-fold more Tregs. This increased selection of Tregs was also found in non-transgenic NOD mice in fetal through adult stages. This trait did not map to the MHC, *idd3*, or the chromosome 3 (Chr3) regions that control clonal deletion, but mainly to two regions on Chr1 and Chr11. Thus, NOD mice do not have a global defect in the generation or maintenance of Tregs; if anything, they show the opposite.

Conclusion(s): These results show that thymic selection of Tregs is under genetic control.

Naturally-occurring regulatory T cells (Tregs) are emerging as key regulators of immune responses to self tissues and infectious agents [38]. New insights have been gained into the cell types and the cellular events that are regulated by Tregs. Indeed, Tregs have been implicated in the control of initial activation events, proliferation, differentiation and effector function. However, the mechanisms by which Tregs disable their cellular targets and especially how immature thymocytes are selected into this alternative lineage are not well understood, therefore this question remains of central interest. Previous reports showed that NOD mice have a generalized defect in their ability to generate an effective number of Tregs, with a percentage of CD4CD2 T cells lower compared with that of other autoimmunity-resistant mouse strains; this study has demonstrated that the thymic selection of Tregs is under genetic control. Furthermore, Feuerer et al. showed an unexpected perspective on Tregs in the autoimmunity-prone NOD mouse, with more effective Tregs selection in the thymus than in the reference diabetes-resistant strain. Tregs activity is emerging as a novel opportunity for intervention in patients with T1D and other autoimmune diseases [40]. A complete characterization of the complex mechanisms regulating its production and action will help to fine tune therapeutic strategies in order to promote or constrain specific arms of Treg suppression.

Robust associations of four new chromosome regions from genome-wide analyses of type 1 diabetes

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Background: The Wellcome Trust Case Control Consortium (WTCCC) primary genome-wide association (GWA) scan on seven diseases, including the multifactorial autoimmune disease T1D, shows associations at $p < 5 \times 10^{-7}$ between T1D and six chromosome regions: 12q24, 12q13, 16p13, 18p11, 12p13 and 4q27.

Methods: In this study, Todd et al. have validated these and six other top findings in 4,000 individuals with T1D, 5,000 controls and 2,997 family trios independent of the WTCCC study.

Results: An unequivocal association of 12q24, 12q13, 16p13 and 18p11 was shown ($p_{\text{(follow-up)}} \leq 1.35 \times 10^{-9}$; $p_{\text{(overall)}} \leq 1.15 \times 10^{-14}$), leaving eight regions with small effects or false-positive associations. In addition, from a GWA study of non-synonymous SNPs, Todd et al. have documented evidence for chromosome 18q22 ($p_{\text{(overall)}} = 1.38 \times 10^{-8}$). Several regions, including 18q22 and 18p11, showed association with autoimmune thyroid disease.

Conclusion(s): This study increases the number of T1D loci with compelling evidence from 6 to at least 10.

Many studies have shown that human leukocyte antigen (HLA) class II loci on chromosome 6q21.3 are most strongly associated with diabetes risk. Nevertheless, because HLA class II loci may account for nearly 41% of the familial aggregation of T1D, during the past years several studies have been looking for the detection of new T1D susceptibility loci. In particular, five loci have been demonstrated: the gene encoding insulin (INS) on 11p15, CTLA4 on 2q33, PTPN22 on 1p13, the interleukin-2 receptor α chain (IL2RA, also known as CD25) region on 10p15 and, most recently, the IFIH1 (also known as MDA5) region on 2q24. Anyway, the improved availability of large genetic analyses has postulated the presence of newer genetic susceptibility loci. Based on data evidenced from two genome-wide analysis studies (a non-synonymous SNP (nsSNP) case-control and the Wellcome Trust Case Control Consortium study), in this study, Todd et al. were able to demonstrate a robust association of four new chromosome regions in genotyped samples from 4,000 affected individuals and 5,000 controls and 2,997 parent-child trios. In particular, 12q24, 12q13, 16p13, 18p11 were linked to the genetic susceptibility. Furthermore, they postulated that the new candidates and the known T1D loci account for approximately 48% of familial clustering of T1D, compared with an estimated 41% for the MHC region alone. This study gives relevant improvements in the characterization of genetic-related risk of T1D. Improved understanding of the pathogenesis of T1D has completely changed our view of this disease in the past 25 years from an acute disease, to a chronic, autoimmune process. Information on genetic and serologic markers has increased our ability to identify individuals at risk. A complete characterization of T1D-related genetic risk factors will soon be a possible reliable tool in the daily detection of patients with an increased genetic susceptibility for diabetes who need close observation and counseling.

A genome-wide scan for type 1 diabetes susceptibility genes in nuclear families with multiple affected siblings in Finland

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Background: A genome-wide search for genes that predispose to T1D using linkage analysis was performed using 900 microsatellite markers in 70 nuclear families with affected siblings from Finland, a population expected to be more genetically homogeneous than others, and having the highest incidence of T1D in the world. Furthermore, Finland has the highest proportion in Europe of cases (10%) carrying none of the highest risk HLA haplotypes that include DR3 or DR4 alleles.

Results: In addition to the evidence of linkage to the HLA region on 6p21 (nominal $p = 4.0 \times 10^{-6}$), significant evidence of linkage in other chromosome regions was not detected with a single-locus analysis. The two-locus analysis conditional on the HLA gave a maximum lod score (MLS) of 3.1 (nominal $p = 2 \times 10^{-4}$) on chromosome 9p13 under an additive model; MLS of 2.1 (nominal $p = 6.1 \times 10^{-3}$) on chromosome 17p12 and MLS of 2.5 (nominal $p = 2.9 \times 10^{-3}$) on chromosome 18p11 under a general model.

Conclusion(s): The results obtained by genome scan in this study confirm the primary contribution of the HLA genes also in the high-risk Finnish population, and suggest that non-HLA genes also contribute to the familial clustering of T1D in Finland.

Recently, the availability of new methods to perform a whole-genome association study using a large number of single nucleotide polymorphisms (SNPs) has been offering additional information on the genetic-related risk determining T1D. Although the observation of familial clustering of T1D suggests that genetic factors are involved in the etiology of T1D, observational studies have clearly demonstrated that the penetrance of the type 1 susceptibility genes is low [41]. Finland is known to have a high degree genetically homogeneous population, compared with most other countries. Finnish patients with T1D have a high frequency of non-DR3, non-DR4-positive *HLA* haplotypes (approx. 10%), suggesting the possibility that non-*HLA* loci; perhaps a reasonable penetrance and population allelic frequency due to founder effects exist in Finland. In this study, by using 900 microsatellite markers in 70 nuclear families with affected siblings from Finland, a genome-wide search for genes that predispose to T1D using linkage analysis was performed. Qiao et al. have confirmed the primary contribution of the *HLA* genes in the high-risk Finnish population. Nevertheless, it has been demonstrated that there are non-*HLA* loci with suggestive evidence of linkage to three chromosome regions: 9p13, 17p12 and 18p11. The worldwide availability of new methods to carry out a whole-genome association study using a large number of single nucleotide polymorphisms (SNPs) represents an important element which will possibly give relevant information about ethnic-related influences on genetic susceptibility for T1D.

New ABCC8 mutations in relapsing neonatal diabetes and clinical features

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Background: Activating mutations in the *ABCC8* gene that encodes the sulfonylurea receptor 1 (SUR1) regulatory subunit of the pancreatic islet ATP-sensitive K^+ channel (K_{ATP} channel) cause both permanent and transient neonatal diabetes. Recently, Polak et al. have described the novel mechanism where basal Mg-nucleotide-dependent stimulatory action of SUR1 on the Kir6.2 pore is increased.

Results: In this study, Vaxillaire et al. have identified six new heterozygous *ABCC8* mutations, mainly in patients presenting the transient form of neonatal diabetes (6 of 8), with a median duration of initial insulin therapy of 17 months (range 0.5–38.0). Most of these mutations map to key functional domains of SUR1. Whereas Kir6.2 mutations are a common cause of permanent neonatal diabetes and in a few

cases associate with the DEND (developmental delay, epilepsy, and neonatal diabetes) syndrome, SUR1 mutations are more frequent in transient (52%) compared with permanent (14%) neonatal diabetes cases screened for ABCC8. Although ketoacidosis is frequent at presentation, SUR1 mutations associate mainly with transient hyperglycemia, with possible recurrence later in life.

Conclusion(s): One-half of the SUR1 neonatal diabetic patients presented with de novo mutations. In some familial cases, diabetes is not always present in the adult carriers of SUR1 mutations, supporting variability in their clinical expressivity that remains to be fully explained.

Until 1995, the etiology of 'neonatal diabetes' was totally unknown. In about a decade, mutations in 8 different genes (IPF1, EIF2AK3, GK, FOXP3, KCNJ11, ABCC8, PTF1A and GLIS3) have been discovered in patients with the permanent form of the disease, and 3 genetic abnormalities (defects in the paternally imprinted chromosomal region 6q24 and 'mild' activating mutations in KCNJ11 or ABCC8) have been detected in subjects with transient neonatal diabetes [37, 39]. The complete characterization of molecular mechanisms involved in neonatal diabetes has been increasing in the last years. In the present study, Vaxillaire et al. were able to demonstrate and characterize six novel *ABCC8* mutations in 8 patients, with the vast majority of subjects presenting with a transient form of neonatal diabetes. Furthermore, a characteristic phenotype was shown to be associated with a different mutation. This study characteristically demonstrates the importance of novel mutations in defining the genotype-phenotype relationship in patients with ND. Therefore, *ABCC8* sequencing in additional groups of patients with diabetes, not restricted to neonatal diabetes, should be useful to help in defining the clinical spectrum associated with different forms of mutations.

Nephropathy in type 1 diabetes is diminished in carriers of HLA-DRB1*04: the genetics of kidneys in diabetes (GoKinD) study

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Background: In this study, Cordovado et al. have examined whether known genetic risk factors for T1D (HLA-DRB1, -DQA1, and -DQB1 and insulin locus) play a role in the etiology of diabetic nephropathy.

Methods: Genetic analysis of HLA-DRB1, -DQA1, -DQB1 and the insulin gene (INS) was performed in the Genetics of Kidneys in Diabetes (GoKinD) collection of DNA (European ancestry subset), which includes case patients with T1D and nephropathy (n = 829) and control patients with T1D but not nephropathy (n = 904). The availability of phenotypic and genotypic data on GoKinD participants allowed a detailed analysis of the association of these genes with diabetic nephropathy.

Results: Diabetic probands who were homozygous for HLA-DRB1*04 were 50% less likely to have nephropathy than probands without any DRB1*04 alleles. In heterozygous carriers, a protective effect of this allele was not as clearly evident; the mode of inheritance therefore remains unclear. This association was seen in probands with both short (<28 years, p = 0.02) and long (≥28 years, p = 0.0001) duration of diabetes. A1C, a marker of sustained hyperglycemia, was increased in control probands with normoalbuminuria, despite long-duration diabetes, from 7.2 to 7.3 to 7.7% with 0, 1, and 2 copies of the DRB1*04 allele, respectively. This result is consistent with a protective effect of DRB1*04 that may allow individuals to tolerate higher levels of hyperglycemia, as measured by A1C, without developing nephropathy.

Conclusion(s): These data suggest that carriers of DRB1*04 are protected from some of the injurious hyperglycemic effects related to nephropathy. Interestingly, DRB1*04 appears to be both a risk allele for T1D and a protective allele for nephropathy.

Diabetic nephropathy represents the main cause of long term morbidity and mortality in T1D patients [42]. Epidemiological studies have estimated that about 1 in 3 European Americans with T1D develops severe nephropathy that leads to end-stage renal diseases (ESRD). Genetic factors play an important role in several diabetic complications, particularly diabetic nephropathy. In fact, only approximately one-half of patients with poor glycemic control develop diabetic nephropathy, whereas some patients do so despite relatively good control. In addition, although the majority of individuals with diabetes will develop background diabetic retinopathy, only one-third of individu-

als with diabetes will develop overt kidney disease. Furthermore, the factors that initiate overt nephropathy are unclear, but consistent familial aggregation of kidney disease (albuminuria, creatinine clearance, and ESRD) and renal histological changes have been observed. The familial clustering of overt diabetic nephropathy and diabetic ESRD has widely been observed in multiple racial and ethnic groups, with the earliest reports of familial aggregation of diabetic kidney disease in patients with T1D. Family members with diabetes, even in the absence of clinical nephropathy, demonstrate similar patterns of glomerular involvement. Renal biopsy material from sibling pairs with T1D was evaluated, and evidence of familial concordance in the percentage of the glomerulus occupied by mesangial matrix and the patterns of injury (presence of thickened glomerular basement membrane) were observed. In addition, consistent evidence across multiple studies also supports familial aggregation of albuminuria, GFR, and creatinine clearance. Analyzing data of a large population of 1,879 individuals with long-term T1D from the Genetics of Kidneys in Diabetes (GoKinD) study, approximately one-half had nephropathy (defined as persistent proteinuria or ESRD) and the other half did not. In this study, Cordovado et al. evaluated whether well-known T1D predisposing genes (HLA genes) determine an increasing risk for developing diabetes-related complications. They were able to demonstrate an enrichment of the DRB1*04 alleles in long-duration T1D with normoalbuminuria despite long duration of diabetes suggesting that these alleles are associated with a reduced risk of nephropathy. A complete understanding of the genetic predisposing factors will be helpful in defining patients with diabetes at increased risk for developing micro- and macrovascular complications. In this respect, the technology for carrying out large-scale molecular genetic studies is rapidly improving and in the near future it will be possible to combine the power and the ease of association studies with comprehensive examination of the genome (whole genome association – WGA).

New Hormones

Soluble insulin receptor ectodomain is elevated in the plasma of patients with diabetes

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Background: Insulin binds to the α -subunit of the insulin receptor ($IR\alpha$) and subsequently exerts its effects in the cells. The soluble ectodomains of several receptors have been found to circulate in the plasma. Therefore, in this study it has been hypothesized that soluble human insulin receptor (hIR) ectodomain (α -subunit and a part of β -subunit) may exist in the plasma of diabetic patients.

Methods: By a two-step purification followed by immunoblotting and gel-filtration chromatography, the authors were able to identify soluble hIR ectodomain in human plasma. Furthermore, they established an hIR α -specific enzyme-linked immunosorbent assay and measured the plasma $IR\alpha$ levels in patients with diabetes. Furthermore, this phenomenon was investigated in streptozotocin-induced diabetic hIR transgenic mice.

Results: Soluble hIR α , but not intact hIR β or whole hIR, exists in human plasma. The plasma $IR\alpha$ levels were significantly higher in type 1 (2.00 ± 0.60 ng/ml; $n = 53$) and type 2 (2.26 ± 0.80 ; $n = 473$) diabetic patients than in control subjects (1.59 ± 0.40 ng/ml; $n = 123$ ($p < 0.001$ vs. control)). Plasma $IR\alpha$ levels were positively correlated with blood glucose level and 10–20% of the insulin in plasma bound to hIR α . In the in vivo experiments using diabetic hIR transgenic mice, hyperglycemia was confirmed to increase the plasma hIR α level and the half-life estimated to be approximately 6 h.

Conclusion(s): According to these results it has been proposed that the increased soluble IR ectodomain level appears to be a more rapid glycemic marker than A1C or glycoalbumin.

The actions of insulin are initiated by hormone binding to its transmembrane glycoprotein cell surface receptors. These receptors are disulfide-linked homodimers, consisting of two identical extracellular

α -subunits that bind insulin, and two identical transmembrane β -subunits that have intracellular tyrosine kinase activity. From the N-terminus to C-terminus, the receptor ectodomain monomer consists of a leucine-rich repeat domain (L1), a cysteine-rich region (CR), a second leucine-rich repeat domain (L2) and three fibronectin type III domains (FnIII-1, FnIII-2 and FnIII-3), the second of which contains the large insert domain (ID). As shown in several molecular pathways, circulating levels of cleaved-derived fraction of ectodomain of receptors for several cytokines and growth factors have been found in plasma. Furthermore, these fractions have been shown to play an important and direct role in the hormonal biology [43]. In this study the presence of soluble hIR ectodomain (α -subunit and a part of β -subunit), but not intact hIR β or whole hIR, has been demonstrated in human plasma. Furthermore, patients with diabetes showed significantly elevated plasma hIR α levels compared with control subjects, measured using newly established hIR-specific ELISA systems. Plasma hIR α level positively correlated with blood glucose, glycoalbumin, and A1C levels. In addition, comparable amounts of plasma insulin appeared to bind to hIR α . Finally, hyperglycemia was confirmed to induce hIR α release in STZ-induced diabetic mice transgenically expressing hIR. These results represent an important progress in understanding the complex pathways of the insulin-IR complex. In fact, cleaved-derived fractions of ectodomain of receptors for several cytokines and growth factors have been shown to relevantly affect the biological effect of its own molecules. Therefore, novel studies are needed in order to fully characterize the function of hIR ectodomain and whether its concentration might influence glucose homeostasis as well as glucose-related complications.

Reviews

Proteomics and diabetic nephropathy

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Background: Proteomic methods have found broad applications in kidney disease research and more specifically in diabetic nephropathy (DN) research.

Results: Proteomic methods such as two-dimensional gel electrophoresis have been used to gain insight into glomerular and tubular nephropathies including DN. At the protein level, differences in high-abundant proteins in DN have been shown to reflect primarily differentially posttranslationally modified plasma proteins. Higher-sensitivity proteomic methods (e.g., liquid chromatography-mass spectrometry) have pushed the boundaries on the known urinary proteome to include more than 1,500 proteins. These same high-sensitivity methods have been applied toward profiling urinary peptides, which has resulted in methods to diagnostically screen urine to differentiate between type-2 diabetes mellitus and T1D urine, normal versus microalbuminuria, or by angiotensin II receptor blocker treatment. Proteomic methods are used to show response to insulin gene therapy in an animal model or alterations in the renal cortex mitochondrial proteome with the development of the diabetic phenotype.

Conclusion(s): Proteomic methods continue to aid in the discovery of new mechanisms of diabetic pathology and understanding of the etiology of diabetic complications, in particular diabetic kidney disease.

In patients with T1D, chronic hyperglycemia directly determines the development and progression of multiorgan damages, especially retinopathy and nephropathy [27]. Despite the relevant improvement in the knowledge of the underlying mechanism involved in diabetes-related disease [31] and the established protective role of tight metabolic control [1, 2], a large amount of patients with diabetes develops ocular and kidney complications. Therefore, the identification of novel biomarkers which are linked to micro- and macrovascular complication risks and which may add knowledge to the predictive value of established markers of disease (such as HbA1c, microalbuminuria, fundus ophthalmoscopy) are needed in order to enable early detection of subjects at increased risk. In fact, only through identification of the high-risk population and novel biomarkers will it be possible to evaluate preventive and therapeutic strategies. In this prospective study, proteomic analysis has offered

encouraging results and will possibly give relevant help in daily care of patients with diabetes. In fact, the proteomic method is a technique whereby biological samples are analyzed systematically with the intent of identifying, quantifying and discerning the function of conditionally unique proteins or peptides. Furthermore, recent proteomic advances have been driven by development in a set of core technologies including separation sciences, mass spectrometry and computer-assisted data analyses that accommodate an extremely large amount of information. In this paper the main applications and goals of proteomic analysis have been evaluated with major relevance to diabetic kidney disease. In the next few years the increasing knowledge obtained by proteomic analysis might provide novel and useful information on diabetes-related etiology, pathogenesis, as well as on the mechanisms involved in the development of diabetes complications.

Food for Thought

Decorin deficiency enhances progressive nephropathy in diabetic mice

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Background: Decorin, a proteoglycan that inhibits active transforming growth factor- β , is increased in diabetic nephropathy, however its functional significance is unclear.

Methods: In this study, Williams et al. have used low-dose streptozotocin to induce T1D in wild-type (C57BL/6J Dcn(+/+)), Dcn(–/–), and Dcn(+/–) mice and studied the mice for up to 1 year of diabetes.

Results: It has been reported that the decorin gene dose had no effect on severity of diabetes, however the Dcn(–/–) diabetic mice died significantly earlier than non-diabetic controls (57 vs. 7.3% mortality). In contrast to wild-type diabetic mice, which failed to develop significant nephropathy, the Dcn(–/–) diabetic mice developed a significant increase in albuminuria and plasma creatinine and a concurrent decrease in circulating adiponectin levels. Interestingly, adiponectin levels at 6 months of diabetes were predictive of mortality in diabetic mice. Dcn(–/–) diabetic mice exhibited advanced glomerular lesions, including diffuse mesangial matrix accumulation and fibrin cap formation. By immunohistochemistry, Dcn(–/–) diabetic mice exhibited significant increases in glomerular transforming growth factor- β , type I collagen, macrophage infiltration, and Nox4.

Conclusion(s): These results showed that decorin is a natural protective factor against diabetic nephropathy and that the Dcn(–/–) diabetic mouse is a useful new model for studying progressive diabetic nephropathy.

Diabetic nephropathy develops in 15–25% of all patients with T1D and is one of the leading causes of end-stage renal failure [42]. Accordingly, development of diabetic nephropathy is associated with a considerable increase in morbidity and mortality. Although overt nephropathy develops after 10–15 years, early and possible glomerular changes are detectable as early as a few years after clinical onset of diabetes. Recent studies have suggested that small leucine-rich proteoglycans (SLRP) of the extracellular matrix play a major role in modulating the activity of growth factors and in regulating the deposition of collagens [44, 45]. Among them, decorin, a multifunctional extracellular proteoglycan, has been shown to neutralize and antagonize the pro-sclerotic effect of TGF- β , one of the most important growth factors implicated in diabetic nephropathy. Furthermore, because both decorin and TGF- β were up-regulated in diabetes, it has been speculated that TGF- β and decorin act in a negative feedback loop with each other [44, 45]. In this study, Williams et al. demonstrated that decorin hypoexpression is associated with an increased risk of diabetic nephropathy. In fact, by generating decorin knockout in mice, they showed a protective role of decorin in streptozotocin-induced diabetes mice. Furthermore, they showed a novel genetically-determined decorin deficiency model which might allow to discover new and relevant determinant mechanisms involved in diabetic nephropathy. The evidence that the drivers of matrix accumulation are an appropriate therapeutic target for diabetic nephropathy is now accepted. Understanding the various pathways involved in

stimulating growth factors and protein synthesis in the diabetic kidney is paramount in developing strategies to combat the development and progression of diabetic kidney involvement and disease.

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