Type 1 Diabetes: Clinical and Experimental

M. Loredana Marcovecchio and Francesco Chiarelli

Department of Paediatrics, University of Chieti, Chieti, Italy

This 2014 chapter on type 1 diabetes (T1D) includes 19 articles, selected from a large number of very relevant basic research and clinical articles published in the last 12 months.

One of the central topics of this year's chapter is prediction of T1D based on presence of autoantibodies and their combination in risk scores as well as on genetic scores. Interesting data supporting the role of coxsackievirus B1 and other environmental factors (gliadin) in the pathogenesis of T1D are also described, as well as several new findings on factors that regulate β -cell proliferation. Another main topic of the chapter is T1D complications, investigated by very elegant experimental studies, describing new concepts in diabetic retinopathy and in the cerebrovascular complications of diabetic ketoacidosis. In addition, we describe two recent reviews summarizing the state of the art on autonomic and peripheral neuropathy in children and adolescents with T1D.

Progress in T1D treatment has also been highlighted, mainly in relation to the implementation of the artificial pancreas system in the home setting and the application of other systems to prevent hypoglycemia.

We hope that the chapter will provide a useful update on key topics on T1D and that you will enjoy reading it!

New hormones

Cross-talk between the bone and the pancreas

Osteocalcin promotes β -cell proliferation during development and adulthood through Gprc6a

Wei J, Hanna T, Suda N, Karsenty G, Ducy P

Department of Genetics and Development, College of Physicians and Surgeons, Columbia University, New York, NY, USA

Diabetes 2014;63:1021–1031

Background: Expanding β -cell mass through β -cell proliferation is considered a potential therapeutic approach to treat β -cell failure in diabetic patients.

Methods and Results: Osteocalcin, a bone-derived hormone, regulates β -cell replication in a cyclin D1-dependent manner by signalling through the Gprc6a receptor expressed in these cells. Mice lacking Gprc6a only in the β -cell lineage are glucose-intolerant due to an impaired ability to produce insulin. Of note, this regulation occurs during both the perinatal peak of β -cell proliferation and in adulthood.

Conclusions: These findings underscore osteocalcin/Gprc6a signalling as a major regulator of β -cell endowment that can become a potential target for β -cell-proliferative therapies.

In recent years the bone has emerged as a new endocrine organ and osteocalcin as one of key mediators of this function. Osteocalcin is an osteoblast-derived protein acting as a hormone that stimulates insulin sensitivity, insulin secretion and also energy expenditure [1]. This recent study aimed to gain further insight into the osteocalcin-mediated cross-talk between the bone and the pancreas, by looking at the potential effect of osteocalcin in the regulation of β -cell proliferation during perinatal life as well as during adulthood.

Osteocalcin acts on the β cell through the Gprc6a receptor, therefore inhibition of this receptor represents an experimental way to explore osteocalcin effects on β cells. Using this approach, the key findings of the study were that inactivation of Gprc6a led to reduced β -cell proliferation associated with a decrease of cyclin D1 gene expression during perinatal life, when the peak β -cell proliferation

generally occurs, and this effect continues during adulthood. β -Cell proliferation was the main process affected, whereas there was no effect on β -cell differentiation.

It is well established that β cells keep their potential to replicate, although at a very low rate under physiological conditions, involving around 0–1.2% of the β cells in adults [2]. However, factors such as osteocalcin that are able to stimulate β -cell proliferation might be a key starting point for the development and progress of β -cell-regenerative therapy, an appealing research area in the field of diabetes.

New mechanisms

New triggers of pancreatic autoimmune responses

Exosomes released by islet-derived mesenchymal stem cells trigger autoimmune responses in NOD mice

Rahman MJ, Regn D, Bashratyan R, Dai YD

Division of Immune Regulation, Torrey Pines Institute for Molecular Studies, San Diego, CA, USA

Diabetes 2014;63:1008–1020

Background: Exosomes are secreted, nano-sized membrane vesicles that contain potent immunostimulatory materials. The aim of this study was to investigate whether primary islet cells can produce exosomes.

Methods and Results: Cells from the islet of Langerhans of non-obese diabetic (NOD) mice were isolated and cultured in vitro. These cells released fibroblast-like, fast-replicating cells that express mesenchymal stem cell (MSC) markers, including CD105 and stem-cell antigen-1. These islet MSC-like cells released highly immunostimulatory exosomes that could activate autoreactive B and T cells endogenously primed in NOD mice. Serum exosome levels and exosome-induced interferon- γ production were positively correlated with disease progression at the early prediabetic stage. Immunohistological analysis of pancreata showed that CD105+ cells are restricted to the peri-islet area in normal islets but penetrate into the β -cell area as lymphocyte infiltration occurs. Immunization with exosomes promoted expansion of transferred diabetogenic T cells and accelerated the effector T-cell-mediated destruction of islets.

Conclusions: Exosomes could be the autoantigen carrier with potent adjuvant activities and may function as the autoimmune trigger in NOD mice.

Exosomes are small vesicles, variable in size from 30 to 200 nm, generated and released by many cells. They are produced as a result of inverse membrane budding into the lumen of the endocyte compartment and subsequent fusion with plasma membrane. Their release is induced by stimuli, such as stress or tissue damage [3]. During recent years, exosomal proteomics has been an emerging area of research in several fields, including diabetes.

Using the NOD mouse model, Rahman et al. showed that exosomes released from fibroblast-like cells expressing MSC markers may act as a trigger of the autoimmune response. These exosome-producing cells were located at the periphery of normal islets in prediabetic NOD mice and penetrated into β -cells' areas following lymphocyte infiltration. Exosomes produced by these cells stimulated production of proinflammatory cytokines, enhanced the maturation of antigen-presenting cells and expanded autoreactive T and B cells, thus contributing to the immune-mediated destruction of pancreatic β cells.

Overall these findings suggest that exosomes can initiate local autoimmune response and provide a new insight in the events related to the development of autoimmune diabetes.

Beta1 integrin is a crucial regulator of pancreatic β -cell expansion

Diaferia GR, Jimenez-Caliani AJ, Ranjitkar P, Yang W, Hardiman G, Rhodes CJ, Crisa L, Cirulli V

Department of Experimental Oncology, European Institute of Oncology (IEO), Milan, Italy

Development 2013;140:3360–3372

Background: Development of the endocrine compartment of the pancreas, as represented by the islets of Langerhans, occurs through a series of highly regulated events. Cellular interactions with the extracellular matrix mediated by receptors of the integrin family are postulated to regulate key functions in these processes.

Methods and Results: Ablation of the $\beta 1$ integrin gene in developing pancreatic β cells reduced their ability to expand during embryonic life, during the first week of postnatal life, and thereafter. Mice lacking $\beta 1$ integrin in insulin-producing cells showed a dramatic reduction of the number of β cells to only approximately 18% of wild-type levels, although these mutant mice were not diabetic. β cells lacking $\beta 1$ integrin showed a normal expression of β -cell markers, normal architectural organization within islet clusters, and a normal ultrastructure. Global gene expression analysis revealed that ablation of this extracellular matrix receptor in β cells inhibits the expression of genes regulating cell cycle progression.

Conclusions: This study highlights $\beta 1$ integrin receptors as crucial positive regulators of β -cell expansion.

Integrins are a family of α/β heterodimeric receptors with a key role in mediating the recognition of extracellular matrix components, which is followed by the activation of signalling between the inside and the outside of the cells. These receptors have been suggested to have a key role in the development of the endocrine compartment of the pancreas [4].

Diaferia et al. show that $\beta 1$ integrin is specifically required for β -cell proliferation in vivo. Using a mouse model, they show that deletion of $\beta 1$ integrins in the β cells impairs their proliferation during late embryogenesis as well as during postnatal development, leading to a relevant decrease in β cells. Surprisingly, the remaining β cells were phenotypically normal and were able to compensate for the depletion in their overall number by increasing their insulin secretion. The specific defect in $\beta 1$ integrin did not affect differentiation or secretory function of the β cells, in support of a specific effect on cell proliferation. Overall these findings suggest that $\beta 1$ integrin might be a potential therapeutic target for expanding β cells in subjects with diabetes and/or a modulator of β cells cultured for transplantation. Future studies should explore these potential therapeutic applications of $\beta 1$ integrin.

Islet microenvironment, modulated by vascular endothelial growth factor-A signaling, promotes β cell regeneration

Brissova M, Aamodt K, Brahmachary P, Prasad N, Hong JY, Dai C, Mellati M, Shostak A, Poffenberger G,

Aramandla R, Levy SE, Powers AC

Division of Diabetes, Endocrinology, and Metabolism, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA

marcela.brissova@vanderbilt.edu

Cell Metab 2014;19:498–511

Background: Pancreatic islet endocrine cell and endothelial cell (EC) interactions mediated by vascular endothelial growth factor-A (VEGF-A) signaling are important for islet differentiation and the formation of highly vascularized islets.

Methods and Results: To dissect how VEGF-A signaling modulates intra-islet vasculature, islet microenvironment, and β -cell mass, VEGF-A production by β cells was transiently increased in mouse models. This was followed by a dramatic increase in intra-islet ECs but also by β -cell loss. After withdrawal of the VEGF-A stimulus, β -cell mass, function, and islet structure normalized due to the proliferation of preexisting β cells. Bone marrow-derived macrophages recruited to the site of β -cell injury

were key players in β -cell proliferation, which was independent of pancreatic location and circulating factors.

Conclusions: Identifying signals implicated in the proliferation of adult, terminally differentiated β cells will improve strategies aimed at β -cell regeneration and expansion.

Pancreatic islets contain a dense vascular structure and VEGF-A, produced by islet endocrine cells, appears to be essential for its development and maintenance [5].

Brissova et al. performed a series of elegant experiments to assess the specific mechanisms implicated in VEGF-A effects on the intra-islet vasculature, islet microenvironment, and β -cell mass. Their main finding was that a transient increase in β -cell VEGF-A leads to activation of islet endothelial cells and, at the same time, is associated with β -cell loss. After removal of the VEGF-A stimulus, there was a vivid β -cell proliferation from preexisting β cells, leading to islet regeneration. The regeneration required bone marrow-derived macrophages and was independent of the pancreatic location or circulating factors.

This study finds important clues as to the mechanisms that regulate β -cell proliferation and regeneration, specifically highlighting that VEGF-A production in adult β cells is essential for islet vascular homeostasis and maintenance of normal β -cell mass. In line with the findings of the previous two studies from Wei et al. and Diaferia et al., VEGF-A is another key factor, similarly to osteocalcin and $\beta 1$ integrin, which could have a potential application for strategies directed at preserving and/or regenerating pancreatic β cells.

Important for clinical practice Improving prediction of T1D

Seroconversion to multiple islet autoantibodies and risk of progression to diabetes in children

Ziegler AG, Rewers M, Simell O, Simell T, Lempainen J, Steck A, Winkler C, Ilonen J, Veijola R, Knip M, Bonifacio E, Eisenbarth GS

Institute of Diabetes Research, Helmholtz Zentrum München, and Forschergruppe Diabetes, Klinikum rechts der Isar, Technische Universität München, Neuherberg, Germany

anette-g.ziegler@helmholtz-muenchen.de

JAMA 2013;309:2473–2479

Background: T1D usually has a preclinical phase characterized by circulating islet autoantibodies. The aim of this study was to determine the rate of progression to T1D after islet autoantibody seroconversion.

Methods: Participants were children recruited and followed up in three prospective cohort studies in Colorado ($n = 1,962$), Finland ($n = 8,597$), and Germany ($n = 2,818$), examining children genetically at risk for T1D for the development of insulin autoantibodies, glutamic acid decarboxylase 65 (GAD65) autoantibodies, insulinoma antigen 2 (IA2) autoantibodies, and diabetes. Follow-up assessment in each study was concluded by July 2012. The primary analysis was the diagnosis of T1D in children with two or more autoantibodies. The secondary analysis was the diagnosis of T1D in children with one autoantibody or no autoantibodies.

Results: In 585 children with multiple islet autoantibodies, progression to T1D at 10-year follow-up, after islet autoantibody seroconversion, was 69.7% (95% CI 65.1–74.3), and in 474 children with a single islet autoantibody was 14.5% (95% CI 10.3–18.7). Risk of diabetes in children who had no islet autoantibodies was 0.4% (95% CI 0.2–0.6) by the age of 15 years. Progression to T1D in the children with multiple islet autoantibodies was faster for those who had islet autoantibody seroconversion when aged younger than 3 years versus children 3 years or older (hazard ratio (HR) 1.65; 95% CI 1.30–2.09; $p < 0.001$); for those with the HLA genotype DR3/DR4-DQ8 versus other HLA genotypes (HR 1.35; 95% CI 1.09–1.68; $p = 0.007$) and for girls versus boys (HR 1.28; 95% CI 1.04–1.58; $p = 0.02$).

Conclusions: The majority of children with multiple islet autoantibody seroconversion progressed to T1D over the next 15 years.

The identification of children at high risk of T1D is an area of intense research, because it represents the starting point for preventive strategies.

In the natural history of T1D [6], the appearance of disease-related autoantibodies, or seroconversion, is the first laboratory manifestation of β -cell injury, and measuring autoantibodies over time has been suggested as a valuable means to follow the progression towards T1D onset.

This study by Ziegler et al. reports data on risk of developing T1D from the time of seroconversion, in three longitudinal birth cohorts of children who have high genetic risk of T1D, either recruited from the general population (in Colorado and Finland) or offspring of parents with T1D (in Germany). The findings were similar between the three studies, highlighting the strong positive predictive value of multiple autoantibodies (2 or more) on the risk of developing T1D, with 70% of children developing the disease within 10 years and 84% within 15 years. Therefore, children with multiple islet autoantibodies represent a very high-risk population. Future preventive strategies for T1D should either aim to prevent the appearance of multiple autoantibodies or try to stop or, at least, delay the progression from seroconversion to T1D.

Other factors should be taken into account when assessing the risk of T1D, such as the higher risk if seroconversion occurs before age 3 years, female sex, and DR3/DR4-DQ8 genotype. Additional variation in risk might be related to autoantibody types and titers, and these questions need further investigations.

The prediction of type 1 diabetes by multiple autoantibody levels and their incorporation into an autoantibody risk score in relatives of type 1 diabetic patients

Sosenko JM, Skyler JS, Palmer JP, Krischer JP, Yu L, Mahon J, Beam CA, Boulware DC, Rafkin L, Schatz D, Eisenbarth G

Division of Endocrinology, University of Miami, Miami, FL, USA

jsosenko@med.miami.edu

Diabetes Care 2013;36:2615–2620

Background: The aim of this study was to assess whether a risk score that incorporates levels of multiple islet autoantibodies can enhance the prediction of T1D.

Methods: TrialNet Natural History Study participants (n = 784) were tested for three autoantibodies (GADA, IA-2A, and mIAA) at their initial screening. Samples from those positive for at least one autoantibody were subsequently tested for ICA and ZnT8A. An autoantibody risk score (ABRS) was developed from a proportional hazards model that included levels of each of the 5 autoantibodies together with their designations of positivity and negativity.

Results: The ABRS was highly predictive of T1D (hazard ratio [95% CI] 2.72 [2.23–3.31], $p < 0.001$). Receiver-operating characteristic curve areas (95% CI) for the ABRS revealed good predictability (0.84 [0.78–0.90] at 2 years, 0.81 [0.74–0.89] at 3 years, $p < 0.001$ for both). The composite of levels from the 5 autoantibodies was predictive of T1D before and after an adjustment for autoantibodies positivity or negativity ($p < 0.001$). Similar findings were obtained after excluding ICA from the risk score model. The combination of the ABRS and the previously validated Diabetes Prevention Trial-Type 1 Risk Score (DPTRS) predicted T1D more accurately (0.93 [0.88–0.98] at 2 years, 0.91 [0.83–0.99] at 3 years) than either the DPTRS or the ABRS alone ($p \leq 0.01$ for all comparisons).

Conclusions: The incorporation of multiple autoantibody levels into an autoantibody risk score is highly predictive of T1D.

This study follows very nicely the previous article from Ziegler et al., as it represents a further step in better characterizing children at risk of T1D by incorporating multiple autoantibodies in a risk score. The autoantibody risk score (ABRS) was based on a model that included positivity/negativity and level of each of the 5 assessed autoantibodies (GADA, IA-2A, mIAA, ICA, ZnT8A), and it was tested in a population of 784 children (95 developing T1D) who were relatives of T1D patients.

The ABRS score was highly predictive of risk of developing T1D after 2 years and also after 3 years, although the predictive ability was higher at 2 years, probably due to a larger number of subjects at that time point. A further advance was to assess the combination of the ABRS with a previously developed score, the Diabetes Prevention Trial-Type 1 Risk Score (DPTRS) [7], based mainly on metabolic parameters (baseline C-peptide and C-peptide levels during OGTT, glucose levels during OGTT) along

with BMI and age. Indeed, the combination of the two scores, which reflect different stages of T1D history, significantly improved prediction, suggesting that they should be used jointly. More specifically, the ABRs should be considered a predictor for longer-term risk, given that autoantibodies appear early in the natural history of T1D, whereas the DPTRs is more relevant to short-term risk, as it comprises subclinical metabolic alterations, which appear more closely to the overt manifestation of the disease. Two secondary findings were that the inclusion of anti-insulinoma autoantibodies (ICA) did not improve the score, which is in contrast with the belief that ICA is a major autoantibody in T1D prediction. Also of interest was the marked risk difference between subjects positive or negative for ZnT8, suggesting the importance of specific types and levels of autoantibodies. These secondary findings suggest additional questions for future studies, together with further studies to assess the validity of the ABRs in the general population.

A strategy to find gene combinations that identify children who progress rapidly to type 1 diabetes after islet autoantibody seroconversion

Bonifacio E, Krumsiek J, Winkler C, Theis FJ, Ziegler AG

Center for Regenerative Therapies Dresden, Technische Universität Dresden, Dresden, Germany

ezio.bonifacio@crt-dresden.de

Acta Diabetol 2014;51:403–411

Background: T1D has a preclinical phase including seroconversion to autoimmunity and subsequent progression to diabetes. The aim of the study was to apply a gene combination approach to identify combinations that contribute either to islet autoimmunity or to the progression from islet autoantibodies to diabetes onset.

Methods: Twelve T1D susceptibility genes (*INS*, *ERBB3*, *PTPN2*, *IFIH1*, *PTPN22*, *KIAA0350*, *CD25*, *CTLA4*, *SH2B3*, *IL2*, *IL18RAP*, *IL10*) were assessed in a cohort of children of parents with T1D prospectively followed from birth. The most predictive combination was subsequently applied to a smaller validation cohort.

Results: The combinations of genes only marginally contributed to the risk of developing islet autoimmunity, but significantly modified the risk of progression to diabetes in islet autoantibody-positive subjects. The greatest discrimination was provided by risk allele scores of 5 genes, *INS*, *IFIH1*, *IL18RAP*, *CD25*, and *IL2* genes, which identified 80% of islet autoantibody-positive children who progressed to T1D within 6 years of seroconversion and discriminated between high-risk (63% within 6 years; 95% CI 45–81) and low-risk (11% within 6 years; 95% CI 0.1–22; $p = 4 \times 10^{-5}$) antibody-positive children. Risk stratification by these 5 genes was confirmed in a second cohort of islet autoantibody children.

Conclusions: This study suggests that genetic variants may affect the rate of β -cell destruction once autoimmunity has initiated and may help to identify islet autoantibody-positive subjects with rapid progression to T1D.

Over the last years, genome-wide association studies have identified several genetic variants associated with T1D [8]. However, apart from the HLA locus, the other genetic variants identified show only small effects on T1D susceptibility when taken individually. Therefore, there is great interest in developing risk scores including several T1D gene variants, which could help in predicting risk of seroconversion or the rate of progression from seroconversion to T1D.

In this context, the study by Bonifacio et al. represents a novel attempt to combine multiple T1D susceptibility loci in a prediction score. The authors selected 12 T1D susceptibility genes, which were tested in 288 children in the BABYDIET study. These children were at high risk of T1D, being born to a mother or father with T1D and carrying high-risk HLA genotypes. In this study population, a risk score based on 5 genes (*INS*, *IFIH1*, *IL18RAP*, *CD25*, and *IL2*) emerged to be highly predictive of progression to T1D within 6 years, whereas the ability to predict seroconversion was low. These results are important as they not only suggest that prediction based on a combination of risk genes is possible but also highlight that some genes may be more specifically implicated in the β -cell destruction once autoimmunity has occurred than on the induction of autoimmunity.

The genetic score was validated in a second sample of children from the BABYDIET study. It is now essential to test whether the same score is also applicable to the general population, not only in relatives of T1D at high risk, and to investigate also the value of other T1D susceptibility genes in the context of genetic risk scores.

Coxsackievirus B1 is associated with induction of β -cell autoimmunity that portends type 1 diabetes

Laitinen OH, Honkanen H, Pakkanen O, Oikarinen S, Hankaniemi MM, Huhtala H, Ruokoranta T, Lecouturier V, Andre P, Harju R, Virtanen SM, Lehtonen J, Almond JW, Simell T, Simell O, Ilonen J, Veijola R, Knip M, Hyoty H
Vactech Ltd, Tampere, Finland

Diabetes 2014;63:446–455

Background: Enteroviruses are among the suspected environmental triggers of T1D, and there is increasing interest in possible vaccines against these viruses. The aim of this study was to identify enterovirus serotypes that could be involved in the initiation of the T1D process.

Methods: Neutralizing antibodies against 41 different enterovirus types were assessed in a unique longitudinal sample from a large prospective birth-cohort study. 183 case children testing persistently positive for at least two diabetes-predictive autoantibodies were compared with 366 autoantibody-negative matched control children.

Results: Antibodies against coxsackievirus B1 (CVB1) were associated with an increased risk of β -cell autoimmunity. This risk was strongest when infection occurred a few months before autoantibodies appeared and was attenuated by the presence of maternal antibodies against the virus. Two other coxsackieviruses, B3 and B6, were associated with a reduced risk, with an interaction pattern, suggesting immunological cross-protection against CVB1.

Conclusions: These results confirm a role for the group B coxsackieviruses in the pathogenesis of T1D. The clustering of the risk and protective viruses to this narrow phylogenetic lineage supports the biological plausibility of this phenomenon.

Virus antibody survey in different European populations indicates risk association between coxsackievirus B1 and type 1 diabetes

Oikarinen S, Tauriainen S, Hober D, Lucas B, Vazeou A, Sioofy-Khojine A, Bozas E, Muir P, Honkanen H, Ilonen J, Knip M, Keskinen P, Saha MT, Huhtala H, Stanway G, Bartsocas C, Ludvigsson J, Taylor K, Hyoty H

Department of Virology, University of Tampere, Tampere, Finland

Diabetes 2014;63:655–662

Background: Enteroviruses have been linked to T1D in various studies. The aim of this study was to assess the association between specific enterovirus subtypes and T1D.

Methods: Type-specific antibodies against the group B coxsackieviruses (CVBs), were measured in 249 children with newly diagnosed T1D and 249 control children matched according to sampling time, sex, age, and country, recruited in Finland, Sweden, England, France, and Greece between 2001 and 2005 (mean age 9 years; 55% male).

Results: Antibodies against CVB1 were more frequent among T1D children than among controls (odds ratio 1.7; 95% CI 1.0–2.9), whereas other CVB types did not differ between the groups. CVB1-associated risk was not related to HLA genotype, age, or sex. Finnish children had a lower frequency of CVB antibodies than children in other countries.

Conclusions: These findings support previous studies that suggested an association between CVBs and T1D, and highlight the possible role of CVB1 as a diabetogenic virus type.

Environmental factors are believed to be key contributors to the increasing incidence of T1D over the last decades. Viral infections are among the most suspected environmental factors and in particular coxsackievirus subtypes of the enterovirus family [9].

These two studies, published in the same issue of *Diabetes* in February 2014, provide key support for a causal association between coxsackievirus and T1D.

Laitinen et al. report a case-control study nested within the DIPP study cohort (Type 1 Diabetes Prediction and Prevention Project), which was established in Finland in 1994 and has followed prospectively (until the onset of T1D or age of 15 years) all infants at high risk of T1D. The main finding, from the assessment of neutralizing antibodies against different enteroviruses, was a significant

association between the presence of antibodies against the serotype 1 of Coxsackievirus group B (CBV1) and risk of autoimmunity. Anti-CBV1 antibodies were more frequent in cases than in controls: 59 versus 50.1%, corresponding to an odds ratio of 1.5. The risk effect was particularly evident when CBV1 infection occurred few months before the appearance of autoantibodies. Another important finding was the lower prevalence of neutralizing antibodies against two other serotypes of coxsackievirus B, CBV3 and 6, in the cases compared to controls. Also of interest was the evidence of an interaction between CBV1, CBV3 and CBV6. This was highlighted by a phylogenetic evaluation showing a close origin between the three serotypes as well by the observation that when antibodies to CBV1 were found in the simultaneous presence of antibodies against CBV3 or CBV6, there was no increased risk of T1D. This suggests a potential cross-protection induced by CBV3 or CBV6 against CBV1.

A similar association between CBV1 and risk of T1D was reported in the cross-sectional study by Oikarinen et al., which was performed in five European countries in children at the onset of T1D. The results of these two studies, together with previous observations indicating the capacity of CBV1 to induce insulinitis both in animal and human models, make this serotype a likely environmental factor in the pathogenesis of T1D, and a potential target for preventive strategies.

New hopes A gliadin-based vaccine to prevent T1D

Prevention or early cure of type 1 diabetes by intranasal administration of gliadin in NOD mice

Funda DP, Fundova P, Hansen AK, Buschard K

The Bartholin Institutttet, Rigshospitalet, Copenhagen, Denmark, and Department of Immunology and Gnotobiology, Institute of Microbiology, v.v.i., Academy of Sciences of the Czech Republic, Prague, Czech Republic
PLoS One 2014;9:e94530

Background: The dietary plant protein fraction has been associated with high penetrance of T1D, whereas gluten-free diets prevent T1D in animal models. The aim of this study was to assess whether intranasal administration of gliadin or gluten may arrest the diabetogenic process.

Methods and Results: Intranasal administration of gliadin to 4-week-old NOD mice significantly reduced the diabetes incidence and lowered insulinitis. Intranasal gliadin also rescued a fraction of prediabetic 13-week-old NOD mice from progressing to clinical onset of diabetes compared to OVA-treated controls. Vaccination with intranasal gliadin led to an induction of CD4+Foxp3+ T cells and even more significant induction of γ/δ T cells in mucosal, but not in non-mucosal lymphoid compartments. This prevention strategy was characterized by an increased proportion of IL-10 and a decreased proportion of IL-2, IL-4 and IFN- γ -positive CD4+Foxp3+ T cells, and IFN- γ -positive γ/δ T cells, preferentially in mucosal lymphoid organs.

Conclusions: Intranasal vaccination with gliadin may represent a novel and safe strategy for prevention or even early cure of T1D.

This study explored another potential environmental factor implicated in T1D. Animal studies support a role of diet in the pathogenesis of T1D; in NOD mice a gluten-free diet prevents diabetes while a cereal-based diet promotes diabetes. In infants, the amount, timing and mode of introduction have been shown to affect the diabetogenic potential of gluten, and some studies have suggested that a gluten-free diet may preserve β -cell function [10]. However, other human studies have not confirmed such findings and therefore the potential role of gluten in T1D pathogenesis remains an open question.

Using a mouse model, Funda et al. showed that a 4-week administration of intranasal gliadin reduced the incidence of T1D by 56% and blocked the insulinitis process. Of interest, CD4+Foxp3 T cells and γ/δ T cells were increased in the mucosal lymphoid compartments, in particular in the site of gliadin application, in the draining lymph nodes, as well as in gut and pancreas lymphoid areas. These accompanying changes in components of the immune system could reflect bystander suppression as

a preventive mechanism associated with intranasal administration of gliadin. No effect was seen with administration of gluten, probably due to lower concentrations of gliadin or to different absorption mechanisms of the two substances. Overall these results open a new area to be further explored not only in animal models but also in humans, with the aim of testing the potential value of a gliadin-based vaccine for the prevention of T1D.

New paradigms

A tissue-specific regulation of retinal protein synthesis

mTORC1-independent reduction of retinal protein synthesis in type 1 diabetes

Fort PE, Losiewicz MK, Pennathur S, Jefferson LS, Kimball SR, Abcouwer SF, Gardner TW
Kellogg Eye Center, Departments of Ophthalmology and Visual Sciences, University of Michigan, Ann Arbor, MI, USA
Diabetes 2014 (Epub ahead of print)

Background: Poorly controlled diabetes has long been known as a catabolic state with profound loss of muscle and fat body mass due to a simultaneous reduction in protein synthesis and enhanced protein degradation. By contrast, retinal structure is largely maintained during diabetes in spite of reduced Akt activity and enhanced cell death, probably reflecting a different regulation of retinal protein turnover compared to other insulin-sensitive tissues.

Methods and Results: Ins2^{Akita} mice (carrying a dominant mutation in the Insulin 2 gene leading to spontaneous diabetes with a rapid onset) and streptozotocin-induced diabetic rats exhibited marked reductions of retinal protein synthesis, concomitant reduction of retinal protein degradation, but preserved retinal mass and protein content. The reduction in protein synthesis was dependent on both hyperglycemia and insulin deficiency, but protein degradation was only reversed by normalization of hyperglycemia. The reduction in protein synthesis was associated with reduced protein translation efficiency, mainly related to a reduction of mTORC2 complex activity but surprisingly not with reduced activity of the mTORC1/S6K1/4E-BP1 pathway.

Conclusions: These findings reveal distinctive responses of diabetes-induced retinal protein turnover compared to muscle and liver that may provide new means to improve diabetic retinopathy.

Diabetic retinopathy, a microvascular complication of T1D, is the leading cause of visual loss in adults. Early subclinical changes within the retina can occur soon after T1D onset even in children and adolescents, and often these alterations worsen during puberty [11].

The authors performed a series of elegant in vivo and ex vivo studies to assess the metabolic basis of the early stages of diabetic retinopathy, with a particular focus on the rates of local protein synthesis and degradation. The study showed that, under the catabolic state of insulin deficiency, protein synthesis is reduced in the retina, similarly to other insulin-sensitive tissues, such as the muscle and liver. In contrast, retinal protein degradation is also reduced, a finding discordant with the increase in the same process in the muscle and liver. Of interest, peri-ocular administration of insulin or systemic improvement of glucose levels restored protein synthesis, whereas only improved glycemic control influenced protein degradation. Whereas in other insulin-sensitive tissue mTORC1 is the main mediator of protein synthesis regulation, retinal protein synthesis appears to be mTORC1-independent, but is influenced by mTORC2. These different patterns between the retinal versus the muscle and liver likely reflect differences in tissue characteristics. Whereas the muscle and liver are mainly energy reservoirs, regulating nutrients and energy supplies to other organs, the retina is a highly metabolic active tissue, without a reservoir function.

Understanding the tissue-specific mechanisms regulating protein synthesis and degradation in the retina is of high importance as it could guide new approaches to limit the retinal damage already apparent during early stages of diabetes.

CXCL1/CXCL8 (GRO α /IL-8) in human diabetic ketoacidosis plasma facilitates leukocyte recruitment to cerebrovascular endothelium in vitro

Omatsu T, Cepinskas G, Clarson C, Patterson EK, Alharfi IM, Summers K, Couraud PO, Romero IA, Weksler B, Fraser DD

Centre for Critical Illness Research, London, ON, Canada

Am J Physiol Endocrinol Metab 2014;306:E1077–E1084

Background: Diabetic ketoacidosis (DKA) in children is associated with intracranial vascular complications, possibly due to leukocyte-endothelial interactions. This study assessed whether DKA-induced inflammation promotes leukocyte adhesion to activated human cerebrovascular endothelium.

Methods: Plasma was obtained from children with T1D either in acute DKA or in an insulin-controlled state (CON) and was used to assess plasma levels of 21 inflammatory analytes.

Results: DKA was associated with increased circulating levels of CXCL1 (GRO α), CXCL8 (IL-8), IL-6, IFN- α 2, and reduced levels of CXCL10 (IP-10) compared with CON. These plasma analyte measurements were then used to create physiologically relevant cytokine mixtures (CM). Human cerebral microvascular endothelial cells (hCMEC/D3) were stimulated with either plasma (DKA-P or CON-P) or CM (DKA-CM or CON-CM) and assessed for polymorphonuclear leukocyte (PMN) adhesion. Stimulation of hCMEC/D3 with DKA-P or DKA-CM increased PMN adhesion to hCMEC/D3 under 'flow' conditions. PMN adhesion to hCMEC/D3 was suppressed with neutralizing antibodies to CXCL1/CXCL8 or their hCMEC/D3 receptors CXCR1/CXCR2. DKA-P, but not DKA-CM, initiated oxidative stress in hCMEC/D3.

Conclusions: DKA induces inflammation in children associated with changes in circulating cytokines/chemokines. Increased CXCL1/CXCL8 stimulates PMN adhesion to hCMEC/D3, potentially contributing to DKA-associated cerebrovascular complications.

DKA is an acute life-threatening complication of T1D and can be associated with several cerebral complications, such as cerebral hemorrhage, stroke, and vasogenic edema, which contribute to the overall morbidity and mortality. Therefore, there is a great interest in understanding the mechanisms implicated in the pathogenesis of cerebrovascular complications of DKA. It seems that DKA is an inflammatory status, characterized by an increase in several acute inflammatory mediators. Animal studies have suggested that DKA can elicit systemic inflammation associated with cerebrovascular endothelial dysfunction.

To further explore the role of inflammation and the link with cerebral endothelial dysfunction and to see if similar phenomena occur in humans, Omatsu et al. performed a series of investigations using plasma derived from children with T1D and DKA, and from children with T1D but metabolically stable. Plasma from children with DKA showed a distinct inflammatory profile. Of particular interest was the finding that stimulation of human cerebral microvascular endothelial cells with plasma from the same children or with cytokines mixtures led to an increase in adhesion to endothelial cells, which was mainly mediated by the cytokines CXCL1/CXCL8.

These findings highlight a new mechanism related to inflammatory cytokines, and in particular to CXCL1/CXCL8, implicated in cerebral endothelial dysfunction in the context of DKA, and the importance of targeting inflammation as a potential way to prevent cerebral consequences associated with DKA.

The majority of patients with long-duration type 1 diabetes are insulin microsecretors and have functioning β cells

Oram RA, Jones AG, Besser RE, Knight BA, Shields BM, Brown RJ, Hattersley AT, McDonald TJ
Diabetologia 2014;57:187–191

Background: Recently developed ultrasensitive assays allow very low levels of C-peptide, under 5 pmol/l, to be detected in patients with long-standing T1D. The aim of this study was to assess the prevalence of low-level detectable C-peptide in long-duration T1D and whether it responds to a meal stimulus.

Methods: A mixed-meal tolerance test was performed in 74 volunteers with long-duration (>5 years) T1D, i.e. with median (interquartile range) age at diagnosis 16 (9–23) years and diabetes duration of 30 (19–41) years. Fasting and stimulated serum C-peptide levels were assessed using an electrochemoluminescence assay (detection limit 3.3 pmol/l), along with urinary C-peptide:creatinine ratio (UCPCR).

Results: Post-stimulation serum C-peptide was detectable at very low levels (>3.3 pmol/l) in 54 of 74 (73%) patients. In these patients, C-peptide either increased ($n = 43$, 80%) or remained unchanged ($n = 11$) in response to a meal, with no indication of levels falling ($p < 0.0001$). With increasing T1D duration, absolute C-peptide levels fell although the numbers with detectable C-peptide remained high (68%, i.e. 25 of 37 patients with >30 years' duration). Similar results were obtained for UCPCR.

Conclusions: Most patients with long-duration T1D continue to secrete very low levels of endogenous insulin, which increase after meals. This reflects the presence of a small number of still functional β cells that escape immune attack or undergo regeneration.

This study from Oram et al. reverses the classical dogma of the natural history of T1D that after 1–2 years from the onset of the disease the pancreas is no longer capable of producing any insulin. Since the beginning of the 20th century there have been reports of histological findings of β cells in patients even with long-standing diabetes. However, such findings had been denied mainly due to lack of assessment of the functionality of the detected β cells [12]. During recent years, following the implementation of more sensitive assays for C-peptide, there have been observations suggesting that patients with long-standing T1D might still secrete insulin.

In this recent study, detectable C-peptide levels were found in 73% of patients with T1D duration over 5 years after stimulus and in 66% at baseline. The low-level C-peptide was able to respond to stimuli, as indicated by 80% of patients responding to the mixed meal. Thus, not only there was a residual C-peptide secretion years after the onset of T1D, but the residual β cells seem to be able to respond to stimuli.

T1D patients with residual C-peptide have been defined as 'insulin microsecretors' and the next step is to clarify the role of this residual endogenous production of insulin. In view of data suggesting a protective role of residual C-peptide against hypoglycemia and vascular complications [13], the current study should lead to future investigations aiming at exploring whether low levels of C-peptide are also related to lower complication rates. In addition, the study findings question the current choice of including only recent-onset T1D patients in secondary prevention trials, given that long-term insulin microsecretors might benefit from therapies to preserve residual β -cell mass/function.

CpG methylation changes within the IL2RA promoter in type 1 diabetes of childhood onset

Belot MP, Fradin D, Mai N, Le Fur S, Zelenika D, Kerr-Conte J, Pattou F, Lucas B, Bougneres P
INSERM U986 and Department of Pediatric Endocrinology, Bicetre Hospital, Pole I3E, Paris Sud University, France
PLoS One 2013;8:e68093

Background: None of the genetic variants in *IL2RA* associated with T1D have an apparent functional effect. The aim of this study was to assess whether epigenetic variation could play a role at this locus.

Methods: The methylation of 6 CpGs located within the proximal promoter of *IL2RA* was assessed in 252 T1D patients and 286 age-matched controls.

Results: DNA methylation at two CpGs -373 and -456 was slightly but significantly higher in T1D than in control subjects (40.4 ± 4.6 vs. 38.3 ± 5.4 , $p = 1.4^{E4}$; 91.4 ± 2.8 vs. 89.5 ± 5.3 , $p = 1.8^{E-6}$), while other CpG showed comparable methylation levels. Among 106 single nucleotide polymorphisms (SNPs) located in the neighboring 180 kb region, 28 SNPs were associated with DNA methylation at CpG-373. Sixteen of these SNPs were known to be associated with T1D.

Conclusions: These data suggest that the effect of *IL2RA* risk alleles on T1D may be partially mediated through epigenetic changes.

IL2RA encodes the α chain of the interleukin-2 (IL2) receptor complex, also known as CD25. *IL2RA* is constitutively expressed on regulatory T cells and can be induced on other T cells. Several polymorphic variants in *IL2RA* are associated with T1D and also other autoimmune diseases [14]. However, no T1D-associated SNP has been reported in the regulatory regions implicated in the modulation of the expression of *IL2RA*. In T1D, epigenetic phenomena, such as DNA methylation, histone modifications, and microRNA dysregulation, have been associated with altered gene expression [15].

In the present study, performed in a pediatric population, differences in the methylation of two CpG promoter regions of *IL2RA* were detected between children with and without T1D. Methylation levels were also associated with 16 SNPs previously associated with T1D, with a predominant effect of two SNPs located in intron 1. These findings suggest potential short distance genetic-epigenetic interactions within a gene.

Overall these data suggest that some SNPs might influence T1D risk through an effect on DNA methylation. However, there are certainly other genetic/environmental factors that regulate CpG methylation in the *IL2RA* promoter region, given that the identified SNPs explained only a small variance in the methylation at the two CpGs.

Follow-up on two 2013 papers

Further insights into the gut microbiota

Compromised gut microbiota networks in children with anti-islet cell autoimmunity

Endesfelder D, Zu Castell W, Ardisson A, Davis-Richardson AG, Achenbach P, Hagen M, Pflueger M, Gano KA, Fagen JR, Drew JC, Brown CT, Kolaczowski B, Atkinson M, Schatz D, Bonifacio E, Triplett EW, Ziegler AG
Scientific Computing Research Unit and Institute of Diabetes Research, Helmholtz Zentrum München, and Forschergruppe Diabetes, Klinikum rechts der Isar, Technische Universität München, Germany
anette-g.ziegler@helmholtz-muenchen.de
Diabetes 2014;63:2006–2014

Background: The gut microbiome seems to play a role in the pathogenesis of autoimmune disorders such as T1D. The aim of this study was to determine whether differences were present in early composition of the gut microbiome in children who developed anti-islet cell autoimmunity.

Methods: Microbiomes were repeatedly assessed in 298 stool samples prospectively collected up to age 3 years from 22 case children who developed anti-islet cell autoantibodies and from 22 matched control children who remained islet autoantibody-negative during follow-up.

Results: The microbiome changed markedly during the first year of life, and was affected by breastfeeding, food introduction, and birth delivery mode. No differences between anti-islet cell autoantibody-positive and -negative children were found in bacterial diversity, microbial composition, or single genus abundances. In contrast, relevant alterations in microbial interaction networks were observed at age 0.5 and 2 years in the children who developed anti-islet cell autoantibodies.

Conclusions: The findings suggest a role of the microbiome in the pathogenesis of anti-islet cell autoimmunity and T1D.

Our previous YB chapter included two papers on the potential role of the gut microbiota in T1D. The first was an elegant experimental study in mice showing that alterations in the gut microbiome composition in early life might suppress autoimmunity in animals at high risk for T1D [16]. The second was a case-control study assessing fecal composition in children with and without islet autoantibodies, and showed some critical differences in microbiota composition between the two groups [17].

This article by Endesfelder et al. reports the largest yet study assessing the early gut microbiota composition in children at risk of T1D, from birth to the age of 3 years. Previous data suggested that the bacterial composition of the gut flora evolves over time, reaching a composition typical of adult life by age 3 years. Therefore, assessing earlier changes in relation to the development of autoimmunity could unveil potential triggers for T1D. In contrast to previous studies, the present study found no differences in the diversity or community composition of the gut microbiome between children who developed autoantibodies and those who were autoantibody-negative. However, some intriguing differences in the interaction networks within the gut microbiome emerged between the two study groups. This might suggest that the interaction between gut bacteria might be altered in children who develop autoantibodies. Another key finding was the identification of other early life factors that can influence gut composition: cesarean section, duration of breastfeeding, and solid food introduction.

The main lesson is that the gut microbiome needs to be explored, starting from birth, in terms of its composition and interactions between the different species, without forgetting to consider other interactions, such as those between the gut microbiome, diet and other environmental factors.

Clinical trials

Preventing severe hypoglycemia

Effect of sensor-augmented insulin pump therapy and automated insulin suspension versus standard insulin pump therapy on hypoglycemia in patients with type 1 diabetes: a randomized clinical trial

Ly TT, Nicholas JA, Retterath A, Lim EM, Davis EA, Jones TW

Department of Endocrinology and Diabetes, Princess Margaret Hospital for Children, Perth, WA, Australia

JAMA 2013;310:1240–1247

Background: Sensor-augmented insulin pumps with automated low-glucose insulin suspension may help to avoid major hypoglycemic events. The aim of the study was to determine the incidence of severe and moderate hypoglycemia using a sensor-augmented pump with low-glucose suspension compared to a standard insulin pump therapy.

Methods: 95 patients with T1D were randomized to insulin pump only or with automated insulin suspension for 6 months between December 2009 and January 2012 in Australia. The primary outcome was the combined incidence of severe (hypoglycemic seizure or coma) and moderate hypoglycemia (an event requiring assistance for treatment). In a subgroup, counter-regulatory hormone responses to hypoglycemia were assessed using the hypoglycemic clamp technique.

Results: 95 patients were randomized, 49 to the standard-pump (pump-only) therapy and 46 to the low-glucose suspension group. The mean (SD) age was 18.6 (11.8) years, duration of diabetes, 11.0 (8.9) years, and duration of pump therapy, 4.1 (3.4) years. The baseline rate of severe and moderate hypoglycemic events in the pump-only group was 20.7 versus 129.6 events per 100 patient-months in the low-glucose suspension group. After 6 months of treatment, the event rates decreased from 28 to 16 in the pump-only group versus 175 to 35 in the low-glucose suspension group. The adjusted incidence rate per 100 patient-months was 34.2 (95% CI 22.0–53.3) for the pump-only group versus 9.5 (95% CI 5.2–17.4) for the low-glucose suspension group. The incidence rate ratio was 3.6 (95% CI 1.7–7.5; $p < 0.001$). There was no change in HbA_{1c} in either group. Counter-regulatory hormone responses to hypoglycemia were unchanged. There were no episodes of diabetic ketoacidosis or hyperglycemia with ketosis.

Conclusions: Sensor-augmented pump therapy with automated insulin suspension reduced the combined rate of severe and moderate hypoglycemia in patients with T1D.

Hypoglycemia is an important acute complication of T1D and alarmingly up to 75% of episodes occur at night in children. Fear of hypoglycemia represents a key barrier to good glycemic control [18].

Sensor-augmented pump therapy with an automatic insulin suspension or low-suspension function is a new system, based on the principle of stopping insulin infusion automatically for up to 2 h when sensor glucose goes below a specific threshold, which potentially could reduce the duration and frequency of relevant episodes of hypoglycemia. In this paper, Ly et al. report the first randomized clinical trial of sensor-augmented pump therapy, recruiting a selective group of 95 high-risk young T1D patients with impaired awareness of hypoglycemia and therefore at increased risk of severe and moderate hypoglycemia. Sensor-augmented insulin pump therapy with insulin suspension reduced episodes of severe hypoglycemia during a 6-month period and reduced the overall time spent with low glucose values.

There were no changes in counter-regulatory hormone responses to hypoglycemia, possibly because the system reduced the magnitude and duration of hypoglycemia but did not avoid completely these episodes.

The possibility that the low-suspension pump can reduce severe episodes of hypoglycemia in young people could have a relevant impact in terms of quality of life and better long-term prognosis. However, the present trial presented some issues which need further assessments. Firstly, the study population was very selective, being composed by young patients with shorter diabetes duration than patients with hypoglycemia unawareness recruited to other studies. Secondly, the higher baseline rate of hypoglycemia in the low-suspension group compared to the control group could represent a potential bias.

Clinical trials

Successful testing of the artificial pancreas at home

Overnight closed-loop insulin delivery in young people with type 1 diabetes: a free-living, randomized clinical trial

Hovorka R, Elleri D, Thabit H, Allen JM, Leelarathna L, El-Khairi R, Kumareswaran K, Caldwell K, Calhoun P, Kollman C, Murphy HR, Acerini CL, Wilinska ME, Nodale M, Dunger DB
rh347@cam.ac.uk

Diabetes Care 2014;37:1204–1211

Background: To evaluate feasibility, safety, and efficacy of overnight closed-loop insulin delivery in free-living youth with T1D.

Methods: Overnight closed loop was evaluated at home by 16 pump-treated adolescents with T1D aged 12–18 years. Over a 3-week period, overnight insulin delivery was directed by a closed-loop system, and on another 3-week period sensor-augmented therapy was applied, in a random order. The primary endpoint was time when adjusted sensor glucose was between 3.9 and 8.0 mmol/l from 23:00 to 07:00 h.

Results: Closed loop was constantly applied for at least 4 h on 269 nights (80%); sensor data were collected for at least 4 h on 282 control nights (84%). Closed loop increased time spent with glucose in target by a median 15% (interquartile range -9 to 43; $p < 0.001$). Mean overnight glucose was reduced by a mean 14 (SD 58) mg/dl ($p < 0.001$). Time when glucose was <70 mg/dl was low in both groups, but nights with glucose <63 mg/dl for at least 20 min were less frequent during closed loop (10 vs. 17%; $p = 0.01$). Despite lower total daily insulin doses, overall 24-h glucose was reduced by a mean 9 (SD 41) mg/dl ($p = 0.006$) during closed loop.

Conclusions: This study shows that unsupervised home use of overnight closed loop in adolescents with T1D is safe and feasible. Glucose control was improved during the day and night with fewer episodes of nocturnal hypoglycemia.

Over the last years the closed-loop technology, or 'artificial pancreas', has made remarkable progress. The validity and safety of the system has been tested in adults and children with T1D, in pregnant women with T1D and, more recently, also in adults with type 2 diabetes. Most studies have been short-term and performed in a hospital setting. Last year's *Yearbook* chapter described the successful use of the closed-loop system within a diabetic camp setting [19], the first outside-hospital application of such system. However, in that and a subsequent outside-hospital study [20], real-time telemonitoring and on-site supervision by clinical research staff was present. Recently, an interim analysis of data on a 4-night home implementation of the closed-loop system was reported [21], with encouraging results.

The current study by Hovorka et al. is novel in that it covered a long period of 21 days in the home setting. The study was performed in a relatively small number of adolescents with T1D ($n = 16$) and used an open-label randomized cross-over design. The study tried to reflect real-life conditions, so patients followed unrestricted diets and normal school and sporting activities, without any telemonitoring or clinical supervision. Under these real-life conditions, the closed-loop system increased time in the target glucose range by 15%, reduced overnight glucose levels by 14 mg/dl and reduced frequency of episodes of hypoglycemia. The system was safe, without major adverse events. These results are encouraging although further studies are required to assess the system in larger number of subjects, younger age groups and for even longer time periods.

Reviews

Focus on diabetic neuropathy in children and adolescents

Screening for peripheral neuropathies in children with diabetes: a systematic review

Hirschfeld G, von Glischinski M, Blankenburg M, Zernikow B

German Paediatric Pain Centre, Children's Hospital Datteln, Datteln, Germany

Pediatrics 2014 (Epub ahead of print)

Background: The aim of this study was to summarize the evidence regarding the diagnostic accuracy of the Semmes-Weinstein monofilament and the Rydel-Seiffer tuning fork to detect diabetic peripheral neuropathy (DPN) in children and adolescents with T1D compared with the gold standard nerve conduction studies.

Methods: Based on PubMed and secondary searching, 72 articles were selected for review. Studies were included if they (1) assessed DPN with the gold standard nerve conduction studies, (2) used non-invasive screening for DPN (monofilament, tuning fork, or biothesiometer), and (3) were performed in the relevant population (children with diabetes). Five articles met these criteria.

Results: Diagnostic accuracies were heterogeneous for the different screening methods. Sensitivities ranged from 1 to 19% for the tuning fork (3 studies), from 61 to 80% for the biothesiometer (2 studies), and from 19 to 73% for the monofilament (2 studies).

Conclusions: Data show extremely low diagnostic utility for standard screening methods (tuning fork and 10 g monofilament) but acceptable utilities for biothesiometry and finer (1 g) monofilaments. Evidence on diagnostic utility should be used to inform national and international guidelines on diabetes management.

Autonomic neuropathy in young people with type 1 diabetes: a systematic review

Tang M, Donaghue KC, Cho YH, Craig ME

School of Women's and Children's Health, University of New South Wales, Kensington, NSW, Australia

Pediatr Diabetes 2013;14:239–248

Summary: Autonomic neuropathy is an under-recognized complication of diabetes, affecting several organ systems. Its clinical manifestations include: orthostatic hypotension, exercise intolerance, gastroparesis, diarrhea, constipation, and urinary incontinence. The most severe consequences include hypoglycemia unawareness and cardiovascular dysfunction. While overt autonomic neuropathy is rare in childhood and adolescence, subclinical signs of autonomic dysfunction are common, can be found soon after diabetes diagnosis and can accelerate during puberty. Risk factors for autonomic neuropathy in young people include diabetes duration, poor glycemic control, and presence of aldose reductase gene polymorphisms.

Diabetic neuropathy is a microvascular complication that has received less attention in the pediatric age group compared to nephropathy and retinopathy. Based on studies using objective measures of nerve dysfunction, the prevalence of abnormalities in children with diabetes is reported to be 28–58%. These two reviews highlight the current state of the art on both autonomic neuropathy and peripheral neuropathy in children and adolescents.

The first review relates to the diagnosis of DPN in children and adolescents. A systematic review of the literature was performed to assess the diagnostic utility of non-invasive screening tests for DPN. The main finding was that despite the widespread use of non-invasive screening for DPN, their diagnostic utility has been tested in only few studies and the results provide little support for the DPN screening procedures used.

The second review offers a comprehensive overview on the frequency and manifestation of autonomic dysfunction in children and adolescents with diabetes. Although overt manifestations of autonomic neuropathy are rare in children and adolescents, early subclinical signs can occur, similarly to other vascular complications, such as nephropathy and retinopathy. Also in the context of autonomic neuropathy it is of paramount importance to determine the gold standard test to assess signs of this complication in young people with diabetes.

Better measures and diagnosis of both peripheral and autonomic neuropathy associated with diabetes are key to the collection of more accurate data on the prevalence of these conditions, and represent the starting point to develop and implement preventive and treatment strategies.

References

1. Ferron M, Lacombe J: Regulation of energy metabolism by the skeleton: osteocalcin and beyond. *Arch Biochem Biophys* 2014 DOI: 10.1016/j.abb.2014.05.022.
2. Ackermann AM, Gannon M: Molecular regulation of pancreatic β -cell mass development, maintenance, and expansion. *J Mol Endocrinol* 2007;38:193–206.
3. Thery C, Ostrowski M, Segura E: Membrane vesicles as conveyors of immune responses. *Nat Rev Immun* 2009;9:581–593.
4. Parnaud G, Hammar E, Rouiller DG, Armanet M, Halban PA, Bosco D: Blockade of $\beta 1$ integrin-laminin-5 interaction affects spreading and insulin secretion of rat β -cells attached on extracellular matrix. *Diabetes* 2006;55:1413–1420.
5. Brissova M, Shostak A, Shiota M, Wiebe PO, Poffenberger G, Kantz J, Chen Z, Carr C, Jerome WG, Chen J, Baldwin HS, Nicholson W, Bader DM, Jetton T, Gannon M, Powers AC: Pancreatic islet production of vascular endothelial growth factor-A is essential for islet vascularization, revascularization, and function. *Diabetes* 2006;55:2974–2985.
6. Atkinson MA, Eisenbarth GS: Type 1 diabetes: new perspectives on disease pathogenesis and treatment. *Lancet* 2001;358:221–229.
7. Sosenko JM, Skyler JS, Mahon J, Krischer JP, Beam CA, Boulware DC, Greenbaum CJ, Rafkin LE, Cowie C, Cuthbertson D, Palmer JP: Validation of the diabetes prevention trial-type 1 risk score in the TrialNet Natural History Study. *Diabetes Care* 2011;34:1785–1787.
8. Torres JM, Cox NJ, Philipson LH: Genome-wide association studies for diabetes: perspective on results and challenges. *Pediatr Diabetes* 2013;14:90–96.
9. Eringsmark Regnell S, Lernmark A: The environment and the origins of islet autoimmunity and type 1 diabetes. *Diabet Med* 2013;30:155–160.
10. Antvorskov JC, Josefsen K, Engkilde K, Funda DP, Buschard K: Dietary gluten and the development of type 1 diabetes. *Diabetologia* 2014 (Epub ahead of print).
11. Cho YH, Craig ME, Donaghue KC: Puberty as an accelerator for diabetes complications. *Pediatr Diabetes* 2014;15:18–26.
12. Faustman DL: Why were we wrong for so long? The pancreas of type 1 diabetic patients commonly functions for decades. *Diabetologia* 2014;57:1–3.

13. Steffes MW, Sibley S, Jackson M, Thomas W: Beta-cell function and the development of diabetes-related complications in the diabetes control and complications trial. *Diabetes Care* 2003;26:832–836.
14. Wang J, Wicker LS, Santamaria P: IL-2 and its high-affinity receptor: genetic control of immunoregulation and autoimmunity. *Semin Immunol* 2009;21:363–371.
15. Stankov K, Benc D, Draskovic D: Genetic and epigenetic factors in etiology of diabetes mellitus type 1. *Pediatrics* 2013;132:1112–1122.
16. Markle JG, Frank DN, Mortin-Toth S, Robertson CE, Feazel LM, Rolle-Kampczyk U, von Bergen M, McCoy KD, Macpherson AJ, Danska JS: Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. *Science* 2013;339:1084–1088.
17. De Goffau MC, Luopajarvi K, Knip M, Ilonen J, Ruotula T, Harkonen T, Orivuori L, Hakala S, Welling GW, Harmsen HJ, Vaarala O: Fecal microbiota composition differs between children with β -cell autoimmunity and those without. *Diabetes* 2013;62:1238–1244.
18. Bohme P, Bertin E, Cosson E, Chevalier N: Fear of hypoglycaemia in patients with type 1 diabetes: Do patients and diabetologists feel the same way? *Diabet Metab* 2013;39:63–70.
19. Phillip M, Battelino T, Atlas E, Kordonouri O, Bratina N, Miller S, Biester T, Stefanija MA, Muller I, Nimri R, Danne T: Nocturnal glucose control with an artificial pancreas at a diabetes camp. *N Engl J Med* 2013;368:824–833.
20. Kovatchev BP, Renard E, Cobelli C, Zisser HC, Keith-Hynes P, Anderson SM, Brown SA, Chernavvsky DR, Breton MD, Farret A, Pelletier MJ, Place J, Bruttomesso D, Del Favero S, Visentin R, Filippi A, Scotton R, Avogaro A, Doyle FJ, 3rd: Feasibility of outpatient fully integrated closed-loop control: first studies of wearable artificial pancreas. *Diabetes Care* 2013;36:1851–1858.
21. Nimri R, Muller I, Atlas E, Miller S, Kordonouri O, Bratina N, Tsioli C, Stefanija MA, Danne T, Battelino T, Phillip M: Night glucose control with Md-Logic artificial pancreas in home setting: a single blind, randomized crossover trial-interim analysis. *Pediatr Diabetes* 2014;15:91–99.