Type 1 Diabetes: Clinical and Experimental

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Type 1 diabetes should be perceived as a chronic immune-mediated disease with a subclinical prodromal period characterized by selective loss of insulin-producing β cells in the pancreatic islets in genetically susceptible individuals. The past 12 months have provided new insights both into the pathogenesis and treatment of this disease. Further evidence has been provided that insulin may be the critical autoantigen in human type 1 diabetes, and the first data indicating that it may be possible to manipulate spontaneous β -cell autoimmunity not only in non-diabetic obese (NOD) mice but also in human disease have been generated. Nevertheless there are still issues remaining open both in relation to the development of type 1 diabetes and to its optimal treatment.

Mechanism of the year

Expanded T cells from pancreatic lymph nodes of type 1 diabetic subjects recognize an insulin epitope

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Background: In autoimmune diabetes pathogenic T cells are involved in the destruction of the insulin-producing β cells in the pancreatic islets. Identification of the autoantigens contributing to the triggering of this process is a crucial question.

Methods: The authors studied T cells from pancreatic draining lymph nodes of subjects affected by type 1 diabetes and unaffected control subjects. They cloned single T cells from such lymph nodes that are the site of islet cell-specific self-antigen presentation.

Results: Considerable T-cell clonal expansion was observed in pancreatic lymph nodes of patients with long-term type 1 diabetes but not from non-diabetic controls. The oligoclonally expanded T cells from affected HLA DR4-positive patients recognized the DR4-restricted insulin A 1–15 epitope.

Conclusion: These observations identify insulin-reactive, clonally expanded T cells from the site of pancreatic drainage in patients with long-term type 1 diabetes. This suggests that insulin may be the target autoantigen in autoimmune diabetes.

Prime role for an insulin epitope in the development of type 1 diabetes in NOD mice

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Background: The existence of any primary autoantigen has remained open in autoimmune diabetes. It is well established that a series of islet proteins are the target of autoimmunity in human type 1 diabetes and in animal models of autoimmune diabetes, but it is not clear whether any of the target molecules are essential for β -cell damage.

Methods: The authors studied NOD mice and generated insulin 1 and insulin 2 gene knockouts combined with a mutated proinsulin transgene in which tyrosine in position 16 on the B chain was replaced with

alanine. This mutation is known to inhibit the T-cell stimulation of a series of the major autoreactive T-cell clones in NOD mice.

Results: Female mice with only the modified insulin did not develop insulin autoantibodies, insulinitis or autoimmune diabetes, while mice with at least one copy of the native insulin gene did present with diabetes.

Conclusion: These results indicate that proinsulin is a primary autoantigen in NOD mice. They also imply that there are specific primary autoantigens in MHC-restricted organ-specific autoimmune disorder such as autoimmune diabetes.

These two papers support the role of insulin/proinsulin as the primary autoantigen in HLA DR4-restricted autoimmune diabetes. Several previous observations have pointed in the same direction, such as the finding that most T cells infiltrating NOD islets are insulin-reactive [1] and that insulin autoantibodies are the first or among the first autoantibodies to appear in young children with HLA-conferred disease susceptibility [2, 3]. If one accepts the proposed role of insulin, one may ask which antigen is the driving one in DR3-restricted diabetes, and how would one explain the additive effect of HLA DR3 and DR4 on diabetes risk. There are reasons to be cautious in the interpretation of the observations reported by Kent et al., one reason being that the 3 patients providing the tissues studied had had overt type 1 diabetes treated with exogenous insulin for 1.5, 15 and 29 years. It is interesting that the identified insulin epitope comprises the first 15 amino acids of the insulin A chain, a region harboring two of the three amino acid differences between human and bovine insulin, i.e. position A8 with threonine in human insulin and alanine in bovine insulin, and position A10 with isoleucine in the former and valine in the latter. This raises the potential role of bovine insulin playing the role of a driving dietary antigen in type 1 diabetes in parallel to the role of gluten in celiac disease [4]. That hypothesis is based on the idea that there is an environmental trigger of the diabetic disease process but, in addition, progression to overt type 1 diabetes requires continuous exposure to a driving antigen, i.e. bovine insulin, keeping up the immune-mediated attack against the insulin-producing β cells. Further research is definitely needed to define the role of insulin in the disease process in type 1 diabetes. The question remains whether insulin is the crucial autoantigen only in the initiation of the disease process, and whether other β -cell proteins become targets of the autoimmune response once the process has been set in motion.

New paradigms

Insulin resistance is a risk factor for progression to type 1 diabetes

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Background: Glucose homeostasis is determined by an interplay between insulin release and insulin action. In type 1 diabetes, autoimmune destruction of insulin-producing β cells results in decreased insulin secretion. However, the contribution of impaired insulin action, i.e. insulin resistance to the development of type 1 diabetes has barely been studied at all. The authors set out to assess whether insulin resistance was a risk factor for progression to type 1 diabetes.

Methods: Islet antibody-positive family members of patients with type 1 diabetes were observed for a median of 4.0 years. Insulin release was assessed based on first-phase insulin response (FPIR) to intravenous glucose. The homeostasis model assessment of insulin resistance (HOMA-R) was used for the estimation of insulin resistance. Subjects who progressed ($n = 43$) were compared to subjects who did not progress ($n = 61$) to diabetes, including 21 pairs matched for age, sex, islet antibodies and FPIR.

Results: Progressors had higher insulin resistance relative to their insulin release (HOMA-R:FPIR 0.033 vs. 0.013, $p < 0.0001$). According to Cox proportional hazards analysis, islet antibody number, FPIR, fasting plasma glucose, fasting serum insulin, HOMA-R and $\log(\text{HOMA-R:FPIR})$ were each predictive of progression to diabetes. However, $\log(\text{HOMA-R:FPIR})$; hazard ratio 2.57 per doubling, $p < 0.001$

was the only metabolic variable independently associated with progression. In the matched comparison, those who progressed to clinical disease had higher fasting glucose, fasting insulin, HOMA-R and HOMA-R:FPIR, both at baseline and during pre-clinical follow-up.

Conclusion: Islet antibody-positive family members who progress most rapidly to diabetes have a mild disturbance of their insulin-glucose homeostasis years before clinical disease presentation, characterized by increased insulin resistance for the level of insulin secretion. This observation indicates that steps reducing insulin resistance, such as weight loss and increased physical activity might delay the development of type 1 diabetes.

The blood glucose concentration reflects both insulin secretion and insulin sensitivity. Accordingly a person who is particularly insulin sensitive can be expected to survive longer on a reduced insulin secretion without developing hyperglycemia. This Australian study reports that insulin resistance is a risk factor for progression to type 1 diabetes in autoantibody-positive family members of affected patients. The observation illustrates that insulin resistance is involved not only in the development of type 2 diabetes but also in type 1 diabetes. It is well in line with the accelerator hypothesis presented by Wilkin [5] a few years ago indicating that insulin resistance is an important factor affecting the rising incidence of both type 1 and type 2 diabetes, the only differences between these two forms of diabetes being the pace of progression to overt disease and the fact that those who present with type 1 diabetes carry genetic susceptibility to autoimmunity. The finding raises the issue whether measures increasing peripheral insulin sensitivity should be recommended in subjects with signs of β -cell autoimmunity, i.e. positivity for diabetes-associated autoantibodies. Among children such measures could include encouragement to increased physical activity and optimal food intake supporting a stable weight-for-height during the period of childhood and pubertal growth. Insulin resistance in individuals with islet autoimmunity could have a genetic-constitutional basis, or could be secondary to the autoimmune disease process itself. The commonest association of insulin resistance is with obesity, and children who develop diabetes have been shown to be heavier both as infants and later in childhood.

New hope

Declining incidence of severe retinopathy and persisting decrease of nephropathy in an unselected population of type 1 diabetes – the Linköping Diabetes Complications Study

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Background: An earlier survey conducted over the last decades showed a reduced incidence of nephropathy but an unchanged incidence of severe retinopathy among patients with type 1 diabetes diagnosed in childhood and a disease duration of 20 years. The present study set out to investigate the incidence 5–10 years later in the same population.

Methods: All 269 patients, in whom type 1 diabetes was diagnosed in childhood between 1961 and 1985 in a district of southeastern Sweden, were studied. Ninety-one percent were monitored for retinopathy until at least 1997 and 95% were monitored for nephropathy. Severe retinopathy was defined as laser-treated retinopathy and nephropathy as persistent proteinuria. The data were analyzed with survival analysis and the patients divided into five cohorts according to the time of diagnosis of diabetes.

Results: A decrease was observed in the cumulative proportion of severe retinopathy ($p = 0.006$). After 25 years it was 47%, 28% and 24% in the cohorts diagnosed 1961–1965, 1966–1970 and 1971–1975, respectively. After 30 years it was 53% and 44% in the oldest cohorts. The cumulative proportion of nephropathy after a 25-year duration was 30%, 8% and 13% in the cohorts 1961–1965, 1966–1970

and 1971–1975, respectively. After 30 years, it was 32% and 11% for the two oldest cohorts ($p < 0.0001$).

Conclusion: Modern diabetes care has considerably decreased the cumulative incidence of both severe retinopathy and nephropathy in an unselected population of patients diagnosed with type 1 diabetes in childhood.

These are encouraging data showing that the efforts put into an improved care of young patients affected by type 1 diabetes do pay off. Both the cumulative incidence of severe retinopathy and nephropathy over a 25-year period were cut to about half within a time difference of 10 years in relation to the diagnosis of type 1 diabetes. An open issue is whether there is true prevention of these complications or a delay in their appearance. Continued follow-up will provide an answer to this question. A conspicuous feature in the study cohort, as in most other series of patients with type 1 diabetes, is a high mortality rate, close to 10%, with end-stage renal failure and coronary heart disease as the most common causes of death. Given the high mortality in coronary heart disease in patients affected by type 1 diabetes, it would be important to assess whether improved diabetic care is capable of reducing that severe complication. Whereas good glycemic control seems to be the main mechanism, other factors like smoking habits and blood pressure control may also be of importance.

Dietary manipulation of β -cell autoimmunity in infants at increased risk of type 1 diabetes: a pilot study

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Background: Early nutrition may modify the risk of later type 1 diabetes. This study aimed at assessing the feasibility of a dietary intervention trial with weaning to a highly hydrolyzed formula in infants at increased risk of type 1 diabetes and at determining whether weaning to such a formula decreases the cumulative incidence signs of β -cell autoimmunity, i.e. diabetes-associated autoantibodies, in early childhood.

Methods: The authors studied 242 newborn infants who had a first-degree relative with type 1 diabetes and carried risk-associated HLA-DQB1 alleles. After exclusive breastfeeding, the infants underwent a double-blind, randomized pilot trial of either casein hydrolysate (Nutramigen; Mead Johnson) or conventional cow's milk-based formula until the age of 6–8 months. During a mean observation period of 4.7 years, autoantibodies to insulin, anti-glutamic acid decarboxylase and insulinoma-associated antigen-2 were measured with specific radiobinding assays and islet cell antibodies (ICA) with conventional immunofluorescence.

Results: The feasibility of screening and identifying a cohort of first-degree infants with HLA-conferred disease susceptibility, enrolling them in a dietary intervention trial and following them for seroconversion to autoantibody positivity is established. The cumulative incidence of autoantibodies was somewhat smaller in the casein hydrolysate vs. control formula group, supporting the need for a larger well-powered study. After adjustment for duration of study formula feeding, life-table analysis showed a significant protection by the intervention from positivity for ICA ($p = 0.02$) and at least one autoantibody ($p = 0.03$).

Conclusion: These data suggest that it may be possible to manipulate spontaneous β -cell autoimmunity by dietary intervention in infancy in children with increased susceptibility to type 1 diabetes.

This is the first indication that it might be possible to manipulate the emergence of the early signs of β -cell autoimmunity and thereby the pathogenic process in human type 1 diabetes. Several hundred modalities have been shown to have a preventive effect on the disease process in NOD mice [6], but those applicable for clinical use have so far turned out to be ineffective in man. The observations by Åkerblom et al. hint to a role of the gut-associated lymphoid tissue, the body's largest immune organ, and its early programming. Postponing the exposure to dietary complex proteins, the first being cow's milk proteins in most infants in developed countries, may affect the programming of the

gut immune system in such a way that regulatory responses are favored. This pilot study has resulted in the initiation of a large-scale international intervention trial with the acronym TRIGR (Trial to Reduce IDDM in the Genetically at Risk) aimed at assessing whether weaning to a highly hydrolyzed formula decreases the cumulative incidence of persistent β -cell autoimmunity and clinical type 1 diabetes in young genetically susceptible children. The trial is well on its way and has by mid-2005 recruited about 70% of its target population, i.e. 2,032 infants with HLA-conferred susceptibility to type 1 diabetes and with at least one affected family member.

New concerns

Female children and adolescents with type 1 diabetes have more pronounced early echocardiographic signs of diabetic cardiomyopathy

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Background: This study set out to assess whether children and adolescents with type 1 diabetes have early echocardiographic signs of subclinical cardiac dysfunction and their possible relationship to gender, degree of metabolic control, and disease duration.

Methods: Eighty children and adolescents with stable type 1 diabetes and 52 age- and sex-matched control subjects were studied for systolic and diastolic blood pressure in supine and upright positions and echocardiographic parameters, including tissue Doppler measurements of the septal mitral annulus. A possible correlation was analyzed for age, gender, HbA1c, and diabetes duration with univariate and multivariate regression analysis.

Results: Female diabetic patients showed significantly larger left ventricular wall dimensions and signs of significant diastolic filling abnormalities on conventional and tissue Doppler echocardiography compared with female control subjects, suggesting delayed myocardial relaxation. Male diabetic patients only differed significantly from their control subjects for the isovolumetric relaxation time. As expected there were significant correlations between the measured parameters and both age and BMI standard deviation scores in the control group. This correlation was significantly weaker in the diabetic cohort; only a weak association was observed for diabetes duration and glycosylated hemoglobin levels.

Conclusion: Young diabetic patients already have significant changes in left ventricular dimensions and myocardial relaxation, with girls clearly being more affected. Tissue Doppler proved to provide additional information in the evaluation of ventricular filling in this population. Almost no relationship was observed between the cardiovascular changes and diabetes duration or metabolic control.

It is well established that adult patients with type 1 diabetes, women in particular, have increased cardiovascular morbidity and mortality. This phenomenon cannot be explained exclusively by an increased frequency of ischemic heart disease, but also systolic and diastolic dysfunction, i.e. 'diabetic' cardiomyopathy, seems to contribute to the increase. This report shows that children and adolescents with type 1 diabetes may have abnormalities in their left ventricular dimensions and myocardial relaxation indicative of diabetic cardiomyopathy. These abnormalities were clearly more common among girls than among boys. The reason for the gender difference remained open, but the authors considered it unlikely that the difference should be due to differences in body mass index or metabolic control. They speculate that hormonal factors may play a role, but do not discuss the role of female patients being more insulin-resistant than males. The lack of any association between metabolic control and the observed changes in left ventricular size and cardiac function implies that improved glycemic control would hardly abrogate these changes. We have to wait to for a longitudinal study to learn whether the observed changes are persistent or not, and whether they predict cardiac dysfunction in adult life.

Bone mineral acquisition in adolescents with type 1 diabetes

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Background: Children and adolescents with type 1 diabetes appear to be at risk for decreased bone mass, which may limit bone strength and enhance the risk of osteoporosis later in life. This study set out to assess whether bone mineral acquisition tracks in adolescents with type 1 diabetes.

Methods: The study comprised 42 adolescents, aged 12–18 years, with type 1 diabetes and a healthy regional referent series ($n = 199$). Measurements of tibia bone characteristics by peripheral quantitative computed tomography and spine and whole body by dual-energy X-ray absorptiometry (DEXA), anthropometrics, and lifestyle questionnaires were obtained during a 12-month period. Disease duration, insulin dose, renal function, and glycosylated hemoglobin (HbA1c) values for the previous 12 months were recorded.

Results: There were no differences in body size and maturation between the groups. Patients with type 1 diabetes had lower tibia, spine, and whole body bone characteristics but greater muscle mass (LBM) and lower bone mineral content (BMC)/LBM at baseline and 12 months. Annual gains for tibia cortical bone and whole body BMC/LBM were lower and inversely related to HbA1c levels ($R = -0.36$ to -0.51), whereas spine area and density and whole body LBM were greater and were predicted by pubertal-driven growth. Overall, the adolescents with diabetes had 8.5% less whole body BMC/LBM, suggesting that bone mineral deposition was not adequately adapted to muscle gains.

Conclusion: Adolescents with type 1 diabetes have reduced bone mass and bone size despite normal growth and maturation. Poor metabolic control appears to have an adverse impact on bone mineral acquisition.

The possible effects of type 1 diabetes on bone mass acquisition in the adolescent period have remained controversial. With sound methodology and a large enough cohort size, this work supports previous reports that adolescents with type 1 diabetes have lower bone mineral content than non-diabetic controls. In addition the study indicates that bone mineral acquisition was decreased in patients with type 1 diabetes over a 1-year follow-up period, and that it is inversely related to metabolic control. This provides an additional argument why everything should be done to maintain fair metabolic control in puberty, as 50–60% of peak bone mass is accrued during that period. Good metabolic control in puberty is a true challenge for both the patient and the diabetes team, however.

Concepts revised

The rising incidence of childhood type 1 diabetes and reduced contribution of high-risk HLA haplotypes

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Lancet 2004;364:1699–1700

Background: The incidence of childhood onset type 1 diabetes has increased globally during the second half of the 20th century. The authors set out to test the threshold model of genetic predisposition predicting that exposure of a population with similar genetic features to a more permissive environment will lead to a dilution of susceptibility genes in the affected portion of the population.

Methods: We compared the frequency of HLA class II haplotypes in 194 patients diagnosed more than 50 years ago and 582 age- and sex-matched individuals diagnosed between 1985 and 2002.

Results: The proportion of high-risk susceptibility genotypes was increased in the earlier cohort (47 vs. 35%; $p = 0.003$), especially in those diagnosed at age 5 years or younger.

Conclusion: This observation is consistent with the hypothesis that the increased incidence rate of type 1 diabetes is due to a major environmental effect.

This finding provides additional evidence for the crucial role of environmental factors in the rising incidence of childhood-onset type 1 diabetes in most developed countries after World War II. E.g. in Finland the annual incidence rate has increased 4.5-fold from 12/100,000 children under the age of 15 years in 1953 to 54/100,000 in 2003. The British report is in line with data reported from Finland showing that 18% of the patients with type 1 diabetes diagnosed in the time period 1990–2001 carried the high risk HLA class II genotype, while the corresponding proportion among those presenting with type 1 diabetes in the time interval 1939–1965 was 25% ($p = 0.007$) [7]. In that study the proportion of those with protective genotypes was higher in the younger cohort (13 vs. 6%; $p = 0.0004$). Taken together these findings indicate that the level of genetic susceptibility required to develop type 1 diabetes has decreased over time due to either a more permissive environment or an increased environmental load. There is no indication that HLA genes conferring increased susceptibility to type 1 diabetes would provide any survival benefit, which could be a possible confounding factor in studies such as this one.

Important for clinical practice

Disturbed eating behavior and eating disorders in preteen and early teenage girls with type 1 diabetes: a case-controlled study

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Background: Disturbed eating behavior is relatively common among teenage girls and young women with type 1 diabetes. The authors wished to compare the prevalence of eating disturbances in preteen and early teenage girls with type 1 diabetes to their non-diabetic peers.

Methods: The study cohort in this cross-sectional, case-controlled study included 101 girls with type 1 diabetes, ages 9–14 years, and 303 age-matched, female, non-diabetic control subjects. Participants completed a Children's Eating Disorder Examination interview. Socioeconomic status, body mass index, and diabetes-related variables were assessed. Groups were compared using χ^2 analyses.

Results: Girls with type 1 diabetes had experienced binge eating, the use of intense, excessive exercise for weight control, the combination of two disturbed eating-related behaviors, and subthreshold eating disorders more frequently than the control girls. There was no relationship between metabolic control and eating behavior in this study population.

Conclusion: Eating disturbances, though mostly mild, were significantly more prevalent in preteen and early teenage girls with type 1 diabetes. Screening and prevention programs for this high-risk group should begin in the preteen years.

There is growing evidence that disturbed eating behaviors are more frequent among young females with type 1 diabetes than among non-diabetic girls. Eating disturbances might reflect non-optimal psychosocial adaptation to type 1 diabetes. The present data show that eating disturbances are clearly more common already in preteen and early teenage girls with type 1 diabetes when compared to non-diabetic girls. One may discuss the role of compulsory meal plans that used to be an integrated part of patient care. Such meal plans leave little space for normal appetite regulation which may affect subsequent eating behavior. One might hope that the recent liberalization of the eating habits in children and adolescents with type 1 diabetes should favor normal eating habits. Eating disorders in girls with type 1 diabetes are associated with omission or decreased use of exogenous insulin readily leading to impaired metabolic control, and most teenage girls with poor metabolic control suffer from eating disturbances. Therefore screening for disturbed eating behavior should be implemented already in the preteen years in girls with type 1 diabetes to identify those individuals who are at greatest risk of developing severe eating disorders and to be able to intervene in such cases.

The impact of a decade of changing treatment on rates of severe hypoglycemia in a population-based cohort of children with type 1 diabetes

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Background: Insulin-induced hypoglycemia remains an important problem for patients with type 1 diabetes, in particular in children and adolescents. This study aimed at determining the impact of treatment changes on the incidence of severe hypoglycemia and its risk factors in a large population-based cohort of children with type 1 diabetes.

Methods: The cohort included 1,335 children (mean age at entry 9.5 ± 4.3 (mean $\pm$ SD, range 0–18) years), yielding 6,928 patient-years of data. The mean observation period was 4.7 ± 3.1 (range 0–10.7) years. Prospective assessment of severe hypoglycemia, defined as an event leading to loss of consciousness or seizure, and associated clinical factors and outcomes was made between 1992 and 2002. Patients were seen every 3 months. The negative binomial regression model was used to analyze the data.

Results: The total number of severe events recorded was 944. The incidence of severe hypoglycemia increased significantly by 29%/year for the first 5 years but appeared to level off subsequently. The overall average HbA1c decreased by 0.2%/year over the whole follow-up period. Lower HbA1c, younger age, higher insulin dose, male sex, and lower parental socioeconomic status were associated with an increased risk of severe hypoglycemia. A reduced rate of severe hypoglycemia was seen on pump treatment.

Conclusion: Severe hypoglycemia is still a major problem for children and adolescents with type 1 diabetes. Pump treatment may be associated with a decreased risk of severe hypoglycemia allowing simultaneously for improved metabolic control.

This is an interesting population-based study spanning an 11-year period from 1992 to 2002. The data show that there was a substantial increase in the incidence rate of severe hypoglycemia from less than 3/100 patient years in 1993 to about 20 in 1996. Subsequently the annual incidence remained at a level close to 15 episodes/100 patient years. The initial increase in the hypoglycemia rate was accompanied by a substantial reduction in average HbA1c levels, but the metabolic control continued to improve over the later 5-year period. When comparing different insulin treatment regimens the lowest rate of hypoglycemia was seen on pump treatment, but data were available only for the last 3 years of the follow-up and the number of patients on pump treatment remained low. The rates of severe hypoglycemia tended to be highest on treatment comprising rapid-acting insulin analogs. This study did not provide any data on hypoglycemia rates on long-acting insulin preparations. An increased hypoglycemia rate was observed in boys aged 13–18 years when compared to girls. This gender difference remained unexplained. A better metabolic control in boys could potentially contribute to the difference, as it is not clear, whether the gender comparison was adjusted for HbA1c.

Depressive symptoms predict hospitalization for adolescents with type 1 diabetes mellitus

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Background: Depressive disorders have been observed to be more common in patients with type 1 diabetes than in non-diabetic subjects. The authors set out to study the role of self-reported depressive symptoms in predicting hospitalization for complications of type 1 diabetes over a 2-year period.

Methods: The Center for Epidemiological Studies Depression Scale, a self-report measure of depressive symptoms, was completed by 231 adolescents affected by type 1 diabetes (age range 11–18 years). HbA1c levels were also analyzed to account for this known predictor of hospitalization.

Hospitalizations for medical complications that occurred up to 2 years after this assessment were recorded and analyzed with survival analysis.

Results: The odds ratio for prediction offered by the Center for Epidemiological Studies Depression Scale scores above the cutoff point (12 for boys and 22 for girls) was 2.58 (95% confidence interval 1.12–5.98) after adjusting for age, gender, socioeconomic status and metabolic control.

Conclusion: Adolescents with type 1 diabetes who show high levels of depressive symptoms are at increased risk for hospitalization for disease complications. Improved quality of life and positive health outcomes may be achieved through interventions aimed at improving the depressive symptoms in such subjects.

This study conveys an important message, i.e. that depressive symptoms in adolescents are associated with impaired metabolic control and more frequent hospitalization due to medical complications. According to these observations depression does not appear to affect the hospitalization rate entirely through impaired metabolic control, since the relationship between depressive symptoms and hospitalization did persist after controlling for baseline HbA1c. The study did not provide any details on the reasons for hospitalization, but the diagnosis codes that were monitored included poorly controlled type 1 diabetes and diabetic ketoacidosis. Clinical experience has implicated that frequently hospitalized teens often have dysfunctional families with lack of parental support and positive coping strategies. It is not surprising that such an environment is associated with mood disturbances in adolescents with type 1 diabetes. The next step would be a controlled clinical trial to assess whether interventions such as cognitive-behavioral therapy or family therapy would relieve the depressive symptoms and improve the health outcome in adolescents with type 1 diabetes.

Clinical trials, new treatments

Multiple injections or pumps: the never-ending saga

A randomized, prospective trial comparing the efficacy of continuous subcutaneous insulin infusion with multiple daily injections using insulin glargine

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Background: The efficacy of the available insulin analogs for multiple daily injection and continuous subcutaneous insulin infusion (CSII) therapy has not yet been established in children and adolescents with type 1 diabetes. This short-term study aimed at comparing the efficacy of CSII to multiple daily injections with glargine in decreasing HbA1c levels in pediatric and adolescent patients with type 1 diabetes.

Methods: The series comprised 32 young subjects with type 1 diabetes (age 8–21 years), who were randomly assigned to receive either multiple daily injection treatment with once-daily glargine and pre-meal/snack insulin aspart or CSII with insulin aspart. Dose titration in both groups was performed based on home self-monitored blood glucose measurements and HbA1c analyzed monthly. HbA1c, total daily insulin dose, self-monitored blood glucose levels, and adverse events were compared after 16 weeks of therapy.

Results: The subjects in the glargine group did not experience any significant improvement in metabolic control (HbA1c 8.2% at baseline vs. 8.1% at 16 weeks), whereas a conspicuous decrease was seen in HbA1c levels, from 8.1 to 7.2% after 16 weeks of therapy ($p < 0.02$ vs. baseline and $p < 0.05$ vs. glargine group) in subjects randomized to CSII. There was no change in total daily insulin dose in the glargine group, while the dose decreased significantly on CSII (1.4 units/kg at baseline vs. 0.9 units/kg at 16 weeks, $p < 0.01$). Both groups had similar basal doses and insulin-to-carbohydrate ratios. Fasting self-monitored blood glucose was similar in both groups, but lunch, dinner, and bedtime readings were significantly lower in the CSII group ($p < 0.01$).

Conclusion: This short-term study indicated that improved metabolic control was more achievable with CSII than with glargine-based multiple daily injection treatment. CSII offers an efficacious treatment modality to improve metabolic control in adolescents affected by type 1 diabetes.

This study is most welcome as a controlled clinical trial comparing multiple daily injection treatment with glargine as basal insulin to CSII therapy in children and adolescents with type 1 diabetes. The subjects on CSII experienced a significant improvement in their metabolic control over the first half of the 16-week trial, whereas no such change was observed in the group on multiple daily injections. This result is in disagreement with the observations reported by Weintrob et al. [9] who, in a randomized open crossover trial, compared CSII to multiple daily injections based on the use of NPH insulin as the basal insulin. In that study no changes over time or differences in metabolic control were observed between the two groups. This discrepancy may be due to a series of reasons including differences in study population, study design and basal insulin used. Both studies are short-term and cannot reliably assess the frequency of adverse events related to the two treatment modalities. Neither does the present study provide information on how persistent the improved metabolic control achieved over the first 8 weeks of CSII therapy will be. In addition it has to be taken into account that glargine insulin is a new insulin analog, and accordingly the experience of its use must have been limited in the clinical team performing the trial.

A randomized, controlled study of insulin pump therapy in diabetic preschoolers

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Background: The efficacy of CSII in the treatment of children with type 1 diabetes has remained controversial. The authors set out to compare metabolic control, safety, and parental satisfaction in preschool-aged children with type 1 diabetes randomized to treatment either with CSII or intensive insulin injection therapy.

Methods: The study cohort comprised 42 patients younger than 5 years of age who had been diagnosed with type 1 diabetes for at least 1 year. Children were randomly assigned to CSII ($n = 21$) or intensive insulin injection therapy ($n = 21$). The HbA1c level was analyzed at baseline, and at 3 and 6 months. Severe hypoglycemic events, meter-detected hypoglycemia, blood sugar variability, body mass index (BMI), and satisfaction with therapy were outcome measures.

Results: Five patients dropped out, and therefore 37 patients completed the 6-month trial. HbA1c levels dropped significantly in both groups (from 8.9 ± 0.6 to $8.6 \pm 0.6\%$ at the 3- and 6-month visits). At 3 months, children on CSII had a significantly lower HbA1c than the injection group (8.4 vs. 8.8%). By 6 months the 2 groups were similar (8.5 vs. 8.7%), however. No differences in pre-meal blood glucose variability were observed between groups. Children on CSII experienced an increase in the number of meter-detected episodes of hypoglycemia. CSII therapy was safe and well tolerated. No episodes of ketoacidosis were seen in either group, while one hypoglycemic seizure occurred in each group. Parents were satisfied with CSII, and 95% of the families continued on CSII beyond the study period.

Conclusion: Metabolic control was similar on pump treatment and on intensive insulin injection therapy in preschool children. The rationale for starting pump therapy in this age group should be based on patient selection and lifestyle preferences.

Here we have another randomized controlled study comparing the efficacy of CSII and intensive insulin injection therapy in the treatment of preschool-aged children with type 1 diabetes. In contrast to the trial reported by Doyle et al., this study showed no difference in metabolic control at the completion of the 6-month study, although there was a significant decrease in HbA1c levels during the active treatment period in both treatment groups. The two studies differ in several aspects. The study cohort from Yale comprised subjects older than 7 years of age (mean age 12.8 years), while this study included children under the age of 5 years (mean age 3.8 years). Glargine insulin was used as the basal insulin in the former study, whereas the other study used NPH insulin as basal insulin. It is,

however, difficult to believe that these differences explain the discrepant outcomes. The discrepancy between the two studies remains unexplained. This difference highlights the fact that results of a research study performed in a given setting cannot always be generalized. The difference between the studies may lie in the intensive education and monitoring offered to families in this study (education vs. pump). The present study also reports a slightly increased rate of meter-detected hypoglycemia on CSII, but this observation may be due to the increased frequency of home blood glucose measurements. Parental preference of treatment modality favored strongly CSII.

Flexible insulin therapy with glargine insulin improved glycemic control and reduced severe hypoglycemia among preschool-aged children with type 1 diabetes mellitus

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Background: Insulin treatment regimens now emphasize the importance of administering throughout the day insulin doses that are based on flexible food choices and focusing on improved metabolic control. A flexible multiple daily insulin regimen (premeal lispro plus bedtime glargine) has been reported to result in lower HbA1c levels and fewer hypoglycemic episodes than does a conventional multiple daily insulin regimen among school-aged children and adolescents with type 1 diabetes. This study aimed to determine the feasibility of flexible multiple daily insulin therapy for a group of preschool-aged children with type 1 diabetes who were transitioned from multiple daily insulin injections (premeal lispro plus ultralente insulin twice per day), by comparing BMI, total daily insulin requirements, HbA1c levels, and episodes of severe hypoglycemia.

Methods: The study cohort included 35 patients (17 female and 18 male patients, 4.8 ± 1.0 years of age) who had received multiple daily insulin therapy for at least 1 year before being transitioned to the flexible regimen. Data were collected over a 2-year period, during quarterly clinical visits.

Results: BMI remained unchanged on the flexible therapy, but 43% of the patients (6 girls and 9 boys) were overweight (BMI > 85th percentile for age) both before and after treatment. The total daily insulin requirement and bolus/basal insulin ratio increased significantly, and overall metabolic control improved after transition to the flexible therapy. The HbA1c levels dropped, however, only among normal-weight subjects but not among overweight subjects after the flexible therapy. The overall rate of severe hypoglycemia decreased significantly with the flexible regimen but again only among normal-weight children.

Conclusion: The use of flexible multiple daily insulin therapy with glargine among preschool-aged children with type 1 diabetes was associated with improved overall metabolic control and decreased frequency of severe hypoglycemia. These observations indicate that the flexible treatment may be a feasible therapeutic alternative to multiple daily insulin treatment among preschool children with type 1 diabetes. Excess body weight appeared to preclude a desirable therapeutic response.

This report suggests that flexible multiple daily insulin treatment with glargine as the basal insulin results in improved metabolic control and a decreased frequency of severe hypoglycemia in normal-weight preschool-aged children with type 1 diabetes. The reason why such a beneficial effect could not be observed in overweight children (43% of the patients) remains open, but the authors speculate that it could be due to a mismatch between the lispro bolus insulin dose and the actual meal carbohydrate intake in the overweight children. The present observations also indicate that the use of insulin analogs may lead to improved metabolic control without a concomitant increase in hypoglycemic episodes. One has, however, to remember that this study had no control group, and therefore the observations remain to be confirmed in a randomized controlled trial.

Dose-dependent effects of recombinant human insulin-like growth factor (IGF)-I/IGF binding protein-3 complex on overnight growth hormone secretion and insulin sensitivity in type 1 diabetes

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Background: Increased growth hormone (GH) secretion has been implicated in the pathogenesis of insulin resistance, and microangiopathic complications, and may be due to reduced circulating IGF levels. The authors examined the effects of recombinant human (rh)IGF-I [complexed in equimolar ratio with rhIGF binding protein (BP)-3 (rhIGF-I/IGFBP-3)] administration on overnight GH levels and insulin sensitivity in patients with type 1 diabetes.

Methods: Fifteen subjects, 13–24 years old (10 male), were given rhIGF-I/IGFBP-3 or placebo as a daily s.c. injection for 2 days. After the second injection overnight, insulin requirements for euglycemia were determined (04.00–08.00 h), followed by a 4-hour, 2-step (insulin, 0.6 and 1.5 mU/kg/min) hyperinsulinemic euglycemic [90 mg/dl (5 mmol/l)] clamp. The protocol was repeated on 3 occasions in random order in all patients. Seven subjects received placebo and rhIGF-I/IGFBP-3 (0.1 and 0.4 mg/kg/day), and 8 subjects received placebo and rhIGF-I/IGFBP-3 (0.2 and 0.8 mg/kg/day).

Results: Dose-dependent increases were observed in circulating IGF-I and IGFBP-3 concentrations after the injection. These were accompanied by significant decreases in mean overnight GH levels and GH pulse amplitude. Dose-dependent effects of rhIGF-I/IGFBP-3 were seen on overnight insulin requirements for euglycemia, with reductions of up to 41%. rhIGF-I/IGFBP-3 injection (0.4 and 0.8 mg/kg/day) improved insulin sensitivity.

Conclusion: These results indicate that the restoration of the IGF-I and IGFBP-3 concentrations in the peripheral circulation with rhIGF-I/IGFBP-3 suppresses GH secretion in adolescents with type 1 diabetes, resulting in reduced insulin requirements and increased insulin sensitivity.

Type 1 diabetes is a condition with secondary GH insensitivity with high circulating GH concentrations, reduced IGF-1 and IGFBP-3 but increased IGFBP-1 levels. These abnormalities in the GH/IGF-1 axis are likely due to the low insulin concentrations achieved in the portal vein. Extensive data has been accumulated on the deleterious effects of GH on glucose metabolism and insulin action. Portal hypoinsulinemia leads to decreased hepatic synthesis of IGF-1 and IGFBP-3 but increased production of IGFBP-1, and as a consequence decreased IGF-1 bioactivity. Accordingly this vicious circle might be broken by adjunct administration of IGF-1. Such treatment was, however, associated with a dose-dependent increased frequency of side effects, such as edema, jaw pain, arthralgia and early worsening of retinopathy [8]. The present study provides an alternative mode for the administration of IGF-1, i.e. injecting an IGF-I/IGFBP-3 complex. The results show that a 2-day course of this complex led to the desired effects on GH levels and insulin sensitivity: a dose-dependent reduction in overnight GH secretion and improved insulin sensitivity. The 2-day treatment was associated with occasional headache, which was as frequent on active treatment as on placebo. No other systemic adverse events were observed. Although these data are encouraging, more extensive controlled trials are needed to address long-term efficacy and safety of the IGF-1/IGFBP-3 complex.

Temporary preservation of β -cell function by diazoxide treatment in childhood type 1 diabetes

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Background: Diazoxide is an ATP-sensitive potassium channel opener that inhibits insulin secretion. The authors set out to study the effect of diazoxide on β -cell function and remission in children with newly diagnosed type 1 diabetes.

Methods: The study cohort included 56 newly diagnosed diabetic subjects (21 girls and 35 boys, age 7–17 years), who were randomized to a 3-month treatment with either diazoxide (5–7.5 mg/kg/day in divided doses) or placebo. The children were in addition treated with multiple daily insulin injections, and they were observed for 2 years.

Results: Diazoxide treatment decreased circulating C-peptide concentrations by approximately 50%. After stopping the treatment, basal and meal-stimulated C-peptide concentrations increased to a maximum at 6 months, followed by a decrease. The meal-stimulated C-peptide concentration was significantly higher at 12 months (0.43 ± 0.22 [mean $\pm$ SD] vs. 0.31 ± 0.26 nmol/l, $p = 0.018$) and tended to decrease less from diagnosis to 24 months in the diazoxide-treated patients (-0.05 ± 0.24 vs. -0.18 ± 0.26 nmol/l, $p = 0.064$). At 24 months, the meal-stimulated C-peptide concentrations were similar in the 2 groups. Side effects of diazoxide were frequently observed.

Conclusion: These data show that partial inhibition of insulin secretion for 3 months after the diagnosis of childhood type 1 diabetes postpones the period of remission and temporarily preserves residual β -cell function. The full potential of β -cell rest in clinical and preclinical type 1 diabetes should be evaluated using compounds with less side effects as well as study protocols optimized for sustained inhibition of insulin secretion.

Diazoxide is familiar to pediatric endocrinologists as a treatment option in infants with neonatal hyperinsulinism. In this study the drug was used for induction of β -cell rest in children with newly diagnosed type 1 diabetes. The results indicated that treatment with diazoxide for 3 months after disease presentation is associated with preserved residual β -cell function over several months and a postponed remission period. The suppression of β -cell function was partial and not always achieved. The overall effect of diazoxide was more modest than that achieved in an earlier study in young adult patients [10]. An intriguing observation was that the insulin dose did not increase during active treatment with diazoxide although the serum C-peptide concentrations were on average only half of those seen in the placebo-treated patients. This could be interpreted as diazoxide increasing peripheral insulin sensitivity. Almost 75% of the patients experienced side effects of the treatment with diazoxide, the most common one being increased hair growth on the face and on the arms, legs and back. Two patients developed severe edema during the first week of treatment. This study is interesting from a basic point of view, but one might question the rationale of maintaining β -cell mass if it is rendered ineffective by diazoxide.

Pancreatic β -cell function and immune responses to insulin after administration of intranasal insulin to humans at risk for type 1 diabetes

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Background: Mucosal administration of insulin retards development of autoimmune diabetes in the NOD mice. The authors conducted a double-blind crossover study in humans at risk for type 1 diabetes to assess whether intranasal insulin was safe, in particular did not accelerate β -cell destruction, and could induce immune effects consistent with mucosal tolerance.

Methods: The study cohort included 38 individuals, median age 10.8 years, with antibodies to one or more pancreatic islet antigens. They were randomized to treatment with intranasal insulin (1.6 mg) or a carrier solution, daily for 10 days and then 2 days/week for 6 months, before crossover. The primary outcome was β -cell function measured as first-phase insulin response (FPIR) to intravenous glucose at 0, 6, and 12 months and then yearly; the secondary outcome was immunity to islet antigens, measured monthly for 12 months.

Results: No local or systemic adverse effects were observed. Twelve subjects progressed to clinical type 1 diabetes after a median of 1.1 year. Of the remaining 26 participants, the majority had antibodies to two or three islet antigens and β -cell function that generally remained stable over a median follow-up of 3.0 years. Intranasal insulin was associated with an increased humoral immune response and a decreased cellular immune response to insulin.

Conclusion: This pilot study indicates that intranasal insulin treatment does not accelerate loss of β -cell function in subjects at risk for type 1 diabetes and induces immune changes consistent with mucosal tolerance to insulin. These observations justify a formal trial to assess whether intranasal insulin is immunotherapeutic and prevents or retards progression to clinical diabetes.

This pilot trial shows that treatment with intranasal insulin does not induce loss of β -cell function in subjects with increased risk for type 1 diabetes. The enhanced insulin antibody production and decreased T-cell reactivity to insulin during treatment can be interpreted as a switch of the Th1/Th2 balance in favor of non-destructive insulin-specific Th2 polarization or as induction of regulatory T cells. Both these phenomena would be beneficial in subjects with persistent signs of β -cell autoimmunity. The oral arm of the Diabetes Prevention Trial-Type 1 reported recently that daily administration of oral insulin did not delay or prevent diabetes in relatives at increased risk for progression to type 1 diabetes [11]. In a subgroup of relatives with high IAA levels there was, however, an indication of benefit from oral insulin. An intervention trial with daily intranasal administration of insulin in young children with genetic susceptibility and persistent signs of β -cell autoimmunity is ongoing in Finland [12]. This trial will provide an answer to the question whether nasal insulin is capable of preventing or delaying progression to clinical diabetes within the next 2 years.

New mechanisms and genes

Association of childhood type 1 diabetes mellitus with a variant of PAX4: possible link to β -cell regenerative capacity

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Background: β -Cell destruction is the crucial event in the development of type 1 diabetes. This is a consequence of an imbalance between autoimmune destruction and insufficient regeneration of islet cells. The authors set out to study the role of islet cell regeneration in the pathogenesis of type 1 diabetes, focusing on PAX4, a paired homeodomain transcriptional repressor that is involved in islet cell growth.

Methods: The study cohort comprised 379 children with type 1 diabetes and 1,070 controls from two distinct populations, and a cohort of children at risk who had not progressed to type 1 diabetes despite being positive for diabetes-associated autoantibodies. Genomic DNA analysis of PAX4 was carried out via direct sequencing of PCR-amplified fragments and allelic discrimination. The transrepression potential of the PAX4 variants was compared in β TC3 cells, and their influence on β -cell growth was analyzed.

Results: There was a significant difference between patients affected by type 1 diabetes and non-diabetic subjects in terms of the genotype distribution of the A1168C single nucleotide polymorphism (SNP) in PAX4. The C/C genotype was frequent among children with type 1 diabetes (73%) and rare among the controls (32%). Conversely, the A/C genotype was common among control subjects (62%) and antibody-positive children without type 1 diabetes (74%), but rare among patients with type 1 diabetes

(18%). The combination of PAX4A and PAX4C was observed to be functionally more active than PAX4C alone (the 'diabetic' variant). β Cells expressing PAX4A and PAX4C efficiently proliferated when stimulated with glucose, while cells expressing the PAX4C variant alone did not.

Conclusion: These results imply a link between β -cell regenerative capacity and susceptibility to type 1 diabetes. This observation could explain the fact that not all of the individuals who develop autoimmunity against β cells actually develop clinical disease. The C/C genotype of the A1168C polymorphism in PAX4 can be perceived as a predisposing marker that can help to identify subjects prone to develop type 1 diabetes.

PAX4 is a transcription factor belonging to the PAX family including several factors regulating early pancreatic development. The gene encoding PAX4 is located on the long arm of chromosome 7. This study presents a very attractive concept, i.e. that a PAX4 gene polymorphism (the C variant of the A1168C SNP) would be associated with type 1 diabetes by affecting the regenerative capacity of β cells detrimentally. According to the data presented, the C/C genotype would confer a 3.8-fold risk for type 1 diabetes compared to the A/C and A/A genotypes. This observation implies that a reduced regenerative capacity of the β cells should be linked to increased susceptibility to type 1 diabetes, in particular, as the authors also showed that β -like cells transfected with PAX4C had a blunted replication response to high glucose in vitro. It seems, however, strange that human genome-wide scans for genes conferring increased susceptibility to type 1 diabetes have not picked up a gene region with such a strong risk effect. We therefore decided to assess the association of the described PAX4 SNP with type 1 diabetes in two other populations, i.e. among Finns and Hungarians. In this context we observed that the genotype distributions in the Swiss and German populations analyzed by Bason-Laubert et al. were not in Hardy-Weinberg equilibrium, while they were so both in the Finnish and Hungarian cohorts. We did not see any association between the PAX4 SNP and type 1 diabetes in the Finnish and Hungarian populations [13]. We stratified the Finnish cohort according to age at diagnosis, gender, HLA genotypes, IDDM2 and CTLA4 genotypes, but no indications of any association between the PAX4 gene and type 1 diabetes could be detected. Deviation from Hardy-Weinberg equilibrium might be seen in multiple SNPs in a strong linkage disequilibrium block, and it may also be due to factors such as recent population migration or genetic drift. The most common reason for this phenomenon is, however, genotyping errors. This illustrates that findings on genetic disease association definitely requires replication before such associations can be considered solid.

Genetic association between a lymphoid tyrosine phosphatase (PTPN22) and type 1 diabetes

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Background: The lymphoid-specific phosphatase encoded by PTPN22 on the short arm of chromosome 1 has been reported to be involved in preventing spontaneous T-cell activation by dephosphorylating and inactivating T-cell receptor-associated Csk kinase. Recently, a missense mutation in PTPN22 was observed to be associated with type 1 diabetes. The authors set out to confirm the association between the reported PTPN22 polymorphism and type 1 diabetes.

Methods: Three hundred and ninety-six patients with type 1 diabetes and 1,178 control subjects of Caucasian descent from north central Florida were genotyped for the PTPN C1858T SNP resulting in the substitution of arginine with tryptophan in amino acid position 620 (R620W) in the lymphoid-specific phosphatase molecule.

Results: The homozygous T/T genotype encoding the 620W residue was associated with an increased risk for developing type 1 diabetes (odds ratio [OR] = 3.4, $p < 0.008$), and the heterozygous genotype C/T had an OR of 1.7 ($p = 6 \times 10^{-6}$). The C/C homozygous genotype was observed to protect against type 1 diabetes (OR = 0.5, $p = 6 \times 10^{-6}$). Furthermore, transmission disequilibrium analysis of 410 affected sibpair and simplex families of Caucasian descent indicated that the type 1 diabetes-associated T allele is transmitted more often (57.2%) than randomly expected ($p < 0.003$).

Conclusion: Together with previous observations of an association between PTPN22 and type 1 diabetes, these results provide evidence that lymphoid-specific phosphatase is involved in the disease process of type 1 diabetes.

The association between type 1 diabetes and the PTPN22 C1858T SNP was initially described by Bottini et al. in 2004 [14]. Subsequently this polymorphism was also reported to be associated with rheumatoid arthritis and systemic lupus erythematosus. The present study provided further evidence for the association between the PTPN22 polymorphism and type 1 diabetes derived from case-control studies by demonstrating significant distortion of transmission in a family study including 410 affected sibpair families. The association of the PTPN22 polymorphism with other autoimmune diseases suggest that the mechanism is not disease-specific. Lymphoid-specific phosphatase encoded by PTPN22 is known to be one of the strongest inhibitors of T-cell activation. It has been shown that the described mutation reduces the binding affinity of the molecule to its substrate and thereby weakens the inhibitory effect on T-cell activation. This could be the mechanism whereby the mutation confers susceptibility to type 1 diabetes and other immune-mediated disorders. This study illustrates that methodological and analytical improvements over the last few years have facilitated the possibilities to identify novel non-HLA genes conferring susceptibility to type 1 diabetes. Another example is the identification of a polymorphism in the gene encoding the small ubiquitin-like modifier 4 protein (SUMO4) on the long arm of chromosome 6 [15].

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