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Immunologic, Metabolic and Genetic Characteristics of Prediabetes Stage of Insulin-Dependent Diabetes mellitus

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The screening for individuals at high risk for IDDM development was done in 292 siblings of IDDM patients. Children were screened for the presence of anti-islet antibodies (ICA) and anti-glutamate decarboxylase 65-kD antibodies (GADAbs). The level of GADAbs was evaluated by microradioimmunoprecipitation method with the use of GAD antigen synthesised in vitro and labelled with ^{35}S. ICA were detected by indirect immunofluorescence. The presence of ICA and/or GADAbs was observed in 26/292 (8.9%) children. This group of 26 children was further studied for genetic, immunologic and metabolic markers of prediabetes stage. The presence of ICA was observed in 15/26 (57.7%) subjects and GADAbs were present in 23/26 (88.5%) of children in the studied group. Both autoantibodies were found in 12/26 (46%) of children. GADAbs alone were observed in 11/26 (42%) and ICA alone in 3/26 (11.5%). The presence of IA2 antibodies was found in 25% of children in studied group. Anti-insulin antibodies were observed in 3/26 (11.5%). The endocrine capacities of pancreas were evaluated by first-phase insulin response (FPIR) after intravenous glucose injection and by oral glucose tolerance test (OGTT). The values of FPIR below the 10th percentile were observed in 4/26 (36.4%) of children. These findings were associated with lower fasting levels of C-peptide. In OGTT, we observed no abnormalities in the studied group. In order to assess the genetic predisposition to IDDM, 10 children were genotyped for DRB1, DQA1 and DQB1 gene alleles by the PCR-SSO method. In all except one the IDDM predisposing alleles were found. During 2 years follow-up, one child of the studied, high-risk group developed clinically overt insulin-dependent diabetes mellitus.

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HLA-DQ Modulate the Protective Effect of HLA-DR in IDDM in the Polish Population

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Susceptibility and resistance to insulin-dependent diabetes mellitus (IDDM) are strongly associated with alleles of HLA class II DR and DQ genes. We have studied HLA DRB1, DQA1, DQB1 allele and haplotype distribution in 192 IDDM children and 181 unrelated healthy individuals from the region of Lodz in central Poland by the polymerase chain reaction and hybridisation with allele-specific oligonucleotide probes. In this study, we summarised findings concerning protective effect of HLA-DR and -DQ loci in the Polish population.

Analysis of protective effect of some allelic variants of HLA class II genes indicates the similarity of the Polish population with other studied populations. The DRB1*1501 allele showed the strongest protection from IDDM in the Polish population (OR = 0.06, $p < 0.0001$). A weaker protective effect was conferred by DRB1*07 (OR = 0.4, $p < 0.0001$), DRB*11 (OR = 0.2, $p < 0.0001$), DRB1*13 (OR = 0.3, $p < 0.0001$) and DRB1*14 (OR = 0.05, $p = 0.003$).

Like in most populations studied so far, the DRB1*1501-DQB1*0602 haplotype confers a strong protection from IDDM (OR = 0.03, $p < 0.0001$). However, some interesting findings could be observed which are unusual in other populations. Contrary to other populations the protective effects as strong as for DRB1*1501-DQB1*0602 haplotye was associated also with other haplotypes. The protective effect of DRB1*07 was associated with the presence of DQB1*0201 on the same haplotype in other populations. In the Polish population a much stronger protective effect of DRB1*07 was associated with DQB1*0303 (OR = 0.07, $p = 0.0007$) than DQB1*0201 (OR = 0.5, $p < 0.005$). Similarly, the protection conferred by DRB1*13 was higher when this allele was present together with DQB1*0301 (OR = 0.04, $p = 0.0007$) than with DQB1*0603 (OR = 0.2, $p = 0.0006$). Haplotye DRB1*14-DQA1*0101-DQB1*0503 has very strong negative correlation with IDDM in the Polish population (OR = 0.05, $p = 0.003$).

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Aspects of Lipohyperthrophy in Insulin-Dependent Diabetic Children

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The aim of the study was to identify the factors related to the appearance of lipohypertrophy in children with IDDM.

Fifthy diabetic children (under 18 years of age) were consecutively selected. Their clinical, metabolic and therapeutic data were analysed and their injection technique was practically verified independently by at least two of the authors. The patients were divided into two groups according to the presence of lipohypertrophy (group A, n = 41) or its absence (group B, n = 9).

The two groups did not significantly differ by age (group A 13.78 ± 3.16 years; group B 14.88 ± 2.40) or sex (group A 20 girls – 48.78%; group B 6 girls – 66.6%). In contrast, the duration of diabetes was significantly different: 6.47 ± 3.56 years in group A and 2.31 ± 3.05 in B, 8 patients (19.51%) in group A had diabetes for more the 10 years as compared to none in group B and 26 (63.41%) had diabetes for 2–10 years as compared to 3 (33.3%). Possibly related, the metabolic control was better in group B (HbA1c 7.54 ± 1.3%) as compared to group A (HbA1c 9.72 ± 1.86). There was also no difference regarding the number of injections per day or the instrument used (syringe/pen). Patients used to follow a rotating system for injection in 65.85% of the cases in group A as compared to 88.88% in group B. 18 patients (43.9%) in group A injected rapid and prolonged action insulin in different places, as compared to 3 (33.3%) in group B. At direct inspection only 22 patients in group A (53.65%) used a correct technique, whereas 8 of 9 (88.9%) did so in group B.

Conclusion: Lipohypertrophy appeared as a frequent complication in insulin-treated children (82%). The rotation of the injection site and a correct technique of injection seem to avoid or delay its appearance stressing the role of education in treatment.

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Thyroid Disorders in Diabetic Children and Adolescents

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Thyroid function was investigated in 33 children and adolescents (29 females, 4 males) with IDDM. Mean age at diagnosis was 8.2 ± 3.4 years (range 0.1–13.0 years). Five patients were pubertal when first seen. Duration of follow-up ranged between 5 and 10 years.

Thyroid gland abnormalities were encountered in 16 of the patients during follow-up, with enlargement of the gland in 13 and development of thyroid antibodies unaccompanied by thyroid gland enlargement in 3. All patients with thyroid enlargement were negative for thyroid antibodies. TSH was elevated in 4 of the goitrous patients, 2 of them being clinically hypothyroid. The remaining 9 patients were euthyroid. Increased TSH levels were also noted in 2 of the 3 patients with antibody positivity.

In the group with thyroid enlargement, the goiter developed 1–3 years after diagnosis in 6 patients who were pubertal or nearing puberty, whereas in 7 prepubertal patients, development of the goiter occurred 5–7 years after diagnosis, at a time coinciding with or preceding puberty. Similarly, the development of thyroid antibodies was also found to occur at the same time as the development of pubertal signs.

No differences were noted in Hba1c levels between IDDM patients with or without thyroid disorders.

These findings indicate that development of thyroid disorders in IDDM patients is associated with pubertal changes. Duration or severity of the diabetic state do not appear to influence the development of thyroid pathology.

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GAD_{65} and ICA512/IA2 Autoantibodies as Disease Markers: Natural History and Relationship to Glycemic Control in Young IDDM Patients

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Circulating autoantibodies (Ab) to islet autoantigens, glutamic acid decarboxylase (GAD_{65}) and tyrosine phosphatase ICA512/IA-2, measured in combination, have been proposed as predictive markers of IDDM. To analyse their potential for screening children with these markers, we examined their sensitivity in identifying IDDM in a population of 63 children at diagnosis of IDDM (mean ± SD age 7.5 ± 4 years) (group A). We also examined the natural history of the autoantibodies amongst childhood diabetics in 91 adolescent patients with IDDM (age 14.7 ± 1.6 years; mean duration of IDDM 7 ± 3.5 years) (group B). 42 normal adolescent subjects (age 14.6 ± 1.8 years) without family history of IDDM were the control group. GAD_{65} and ICA512/IA-2 Ab were detected in 56 and 63% of group A and the prevalences were not different in relation to clinical onset with ketoacidosis, or family history of IDDM. Both Ab were associated with ICA ($p < 0.05$) and indices were higher in ICA-positive children ($p < 0.05$). 81% had at least one Ab, but the Ab occurred together in only 38%. 68% of ICA-negative patients had at least one of the two Ab. There was no correlation with levels of HbA1C or C peptide. GAD_{65} and ICA512/IA-2 Ab were present in 44 and 45% ($p < 0.05$ and $p < 0.05$ vs. group A, respectively) of group B and occurred together in 21%. Only ICA512 Ab were significantly associated with ICA ($p < 0.05$) and mean ICA512 index was higher in ICA-positive than in ICA-negative patients ($p < 0.005$). In this cohort, levels of ICA512 Ab negatively correlated with levels of HbA1c ($p < 0.005$) and with daily insulin requirement ($p < 0.05$). Mean ICA512 Ab index was significantly higher amongst the group A ($p < 0.0001$), and levels declined with diabetes duration ($p < 0.05$). At diagnosis, levels of GAD_{65} Ab positively correlated with age ($p < 0.05$), and in adolescent patients levels did not decline with diabetes duration.

Our findings indicate that positivity for either GAD_{65} and ICA512 Ab is a highly sensitive marker of IDDM in the paediatric age group, identifying a group of patients with absent ICA immunofluorescence, and that the two Ab appear to evolve in opposite directions. The persistence of ICA512 Ab possibly represents a marker of better glycemic control and less insulin requirement, indicating residual β-cell function and conferring clinical relevance to these autoantibodies.

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Low Systemic Expression of BCL-2 in Young Adults with Early Diabetic Nephropathy

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Bcl-2 gene product has been shown to regulate apoptotic cell death and its expression is reduced in newly diagnosed adults with type 1 (insulin-dependent) diabetes (IDDM).

The aim of this study was to assess the relationship between lymphocyte bcl-2 expression and albumin excretion rate (AER) in young adults (age 18–24 years) with IDDM as compared to healthy subjects. Bcl-2 was measured in lymphocytes from 11 IDDM patients with AER less than 20 μg/min (group A), 8 IDDM patients with AER greater than 20 μg/min (group B) and 5 controls (group C); the three groups were accurately matched for sex, age and body mass index; diabetics were also matched for age at onset, duration of the disease, insulin requirement and metabolic control (HbA1c values).

Venous blood samples were obtained after an overnight fasting. Lymphocytes were isolated from whole blood by Ficoll technique. Bcl-2 was determined using Western blotting and quantified by densitometric image analysis; results were expressed in arbitrary units of

optical density, as percent of controls. In group B, bcl-2 was markedly reduced (29.7 ± 12.0%) when compared with both group A patients ($p = 0.03$) and controls ($p = 0.007$); diabetics without microalbuminuria showed bcl-2 values lower than controls (54.1 ± 36.1%, $p = 0.04$). In conclusion, young patients with onset of diabetes during childhood have low levels of bcl-2 protein; furthermore, very low systemic expression of this antiapoptotic protein is constantly associated with early kidney disease in IDDM young adults, independent of glycemic control and duration of diabetes.

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Sodium-Lithium Countertransport Activity May Help to Predict Incipient Nephropathy in Adolescents and Young Adults with IDDM

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Some but not all cross-sectional studies have suggested that sodium-lithium countertransport (NaLiCT) may be linked to incipient or overt diabetic nephropathy. In this study we examined the ability of NaLiCT to predict incipient nephropathy in IDDM. NaLiCT was measured 8 years ago in 170 normoalbuminuric diabetic adolescents and young adults (age 12–23 years; duration of diabetes longer than 7 years). Participants were clinically examined at baseline and biennally thereafter. Microalbuminuria (MA) was defined as an albumin excretion rate (AER) between 20 and 200 μg/min in 2 of 3 timed overnight urine samples. During 8 years, 18 (10.5%) (10 males) patients developed MA; no patient developed ON. The risk of developing microalbuminuria was higher in children with increased NaLiCT (using 300 μmol/erythrocytes × h as the cut-off point) compared with those with normal NaLiCT at the beginning of the study (18.98 vs. 3.29%, $p < 0.01$; sensitivity 96.7%; specificity 57.9%). Gender did not influence predictive value, sensitivity and specificity. NaLiCT was not significantly correlated with HbA1 or IDDM duration. Multivariate analyses show that the independent predictors of MA are HbA1, waist-to-hip ratio, and triglycerides for females, and HbA1 and hypertension for males. The results suggest that NaLiCT may be one of the predictors and risk factors for incipient diabetic nephropathy and may help to identify normotensive, normoalbuminuric adolescents and young adults with IDDM predisposed to develop persistent microalbuminuria and incipient diabetic nephropathy.

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Geographic Variation in the Incidence of IDDM in Children under 5 Years in NSW, Australia

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Aim: To compare the regional incidence of IDDM in children aged <5 years (population 428,000) in the state of NSW over the period 1992–1996.

Methods: Standardised incidence rates of IDDM for NSW and regional incidence rates were calculated using the NSW Children's Diabetes Register and the Australian Bureau of Statistics 1991 and 1996 population census data. The relative change in IDDM incidence over time was calculated from logarithms of incidence using linear regression, where the regression coefficient is the change per year expressed as a percentage. Confidence intervals were calculated assuming a Poisson distribution. Chi-squared analysis and the Cochrane-Armitage trend test (StatXact statistical software) were used to compare regional incidences.

Results: The overall mean incidence was 12.1 per 100,000 in children under 5 years (95% CI 9.2–15.9) compared with the age standardised incidence of 19.1 (95% CI 16.9–21.8) for the entire state of NSW. The average rise in boys and girls aged 0–4 was 5.5 and 2.9% per year, respectively, compared with an average rise of 3.2% per year for the overall group of children aged 0–14 years. The incidence in children aged 0–4 years across the 12 statistical subdivisions of NSW ranged from 2.4 (95% CI 0.1–13.1) to 30.7 per 100,000 (95% CI 0.8–171) and there was a significant variation in incidence by region ($\chi^2 = 125.5$, 11 d.f., $p = 0.0005$). Standardised incidence ratios ranged from 20 to 253% in 0- to 4-year-olds compared with 71–131% for the total group. When the 12 regions were ranked by population density (total population per km^2), a significant trend in incidence was found ($p = 0.005$), with a positive correlation between incidence and population density ($r^2 = 0.27$).

Conclusion: Along with the rising incidence rates of IDDM in under-5-year-olds, there is a significant regional variation in incidence.

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Does Dietary Protein Intake Influence the Early Stages of Diabetic Nephropathy in Childhood and Adolescence?

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The progression of both diabetic and nondiabetic renal disease is slowed by restriction in dietary protein intake (DPI). However, the role of DPI in the onset and early progression of diabetic nephropathy (DN) is not known.

Objective: This study was undertaken to examine the relationship between DPI and known markers of early DN, namely kidney volume (KV), glomerular filtration rate (GFR), and albumin excretion rate (AER), in children and adolescents with type 1 diabetes (DM).

Methods: Subjects included 145 children and adolescents (72 males; mean ± SD age 13.2 ± 3.5 years) with DM of 5–10 years duration, divided into 3 groups (prepubertal, midpubertal and postpubertal) depending on pubertal duration. Each subject collected three 24-hour urine specimens for measurement of urinary urea, creatinine and AER. GFR was estimated from creatinine clearance and standardized for body surface area (BSA). KV was measured by ultrasound and corrected for BSA. DIP was calculated by the formula: (UUN + NUN) × 6.25, where UUN = urinary urea nitrogen, NUN = nonurea nitrogen (31 mg/kg/day). Blood was drawn for measurement of creatinine, HbA1c and red-cell sodium-lithium countertransport (NaLiCT).

Results: Mean DPI in the entire cohort was 1.22 ± 0.04 g/kg/day (mean ± SE), and was higher in males than females (1.41 vs. 1.02 g/kg/day, respectively, $p < 0.0001$). DPI was higher in prepubertal than in mid- and postpubertal subjects (1.49, 1.12, 0.98 g/kg/day, respectively). In univariate analyses, DPI was related to GFR ($p < 0.0001$), but not AER, KV, HbA1c or NaLiCT. Multiple regression analysis was performed with DPI as the dependent variable, and age, pubertal duration, HbA1c, NaLiCT, BSA, GFR, AER and KV as independent variables: DPI was significantly positively associated with GFR ($p < 0.0001$) and male sex ($p < 0.0001$), and negatively with BSA ($p = 0.0013$) and age ($p = 0.01$).

Conclusion: In this cross-sectional study, we have failed to demonstrate a relationship between DPI and early markers of DN, other than GFR. The possibility that DPI may be implicated in the progression of DN cannot be ruled out without either a prospective study or an intervention trial.

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Observed Mother-Daughter Interactions among Teenage Girls with Type 1 Diabetes and Eating Disturbances

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Eating disturbances are common and persistent among adolescent girls with type 1 diabetes (DM). Disturbed family interactions have been linked both with eating disturbances (ED) in nondiabetic girls and with impaired metabolic control in teens with DM. However, little is known about the links between the quality of family functioning, diabetes, adjustment, and eating disturbances among diabetic girls.

Objectives: This study examined if and how: (1) disturbed family interactions are linked with ED among DM girls, and (2) ED mediate the impact of family functioning on metabolic control.

Methods: 102 adolescent girls with DM [$\overline{X}$ + SD age = 15.1 ± 2.2 years; DM duration = 7.1 ± 3.9 years; age of illness onset = 8.0 ± 4.0 years; Hba1c = 8.7 ± 1.4%] were classified as non- (n = 42), mildly (n = 36), and highly eating disturbed (n = 24) using the Eating Disorder Inventory and the Diagnostic Survey for Eating Disorders. Family functioning was assessed by observing mothers and daughters engaging in two 7-min problem-solving tasks (one diabetes-related and one a general parent-teen dilemma) and was rated using a macroanalytic coding scheme [The Intimacy and Autonomy Coding System for Parent-Adolescent Transactions, Maharaj et al., 1996]. Metabolic control was assessed by HbA1c levels.

Results: Repeated measures mancova illustrated that interactions among mothers and eating-disturbed daughters are significantly more impaired than among non-disturbed girls, and results illustrated a continuum where greater levels of dysfunction are linked with greater eating pathology. In both observed scenarios, highly disturbed dyads showed poorer perspective-taking ($p = 0.0005$) and conflict resolution ($p = 0.0005$); a more negative style of presenting concerns ($p = 0.0005$), and mothers more rejecting of their attempts ($p = 0.0005$); and lower levels of affective connection ($p = 0.0005$), with less emotional attunement ($p = 0.0005$) and more negative emotional expression ($p = 0.0005$). Regression analyses revealed that the presence and severity of an ED mediates the impact of family dimensions on metabolic control ($p = 0.0004$).

Conclusion: Results suggest that effective treatment of females with DM may require attention to the quality of family functioning as a critical factor associated with both eating disturbances and diabetes-related adjustment among teenage girls.

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Multiple Sclerosis in a Girl with Type 1 Diabetes

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The association between type 1 diabetes and multiple sclerosis (MS) is unusual, expecially in young patients. F.M.D., born in 1971, developed type 1 diabetes in 1983. Organ- and non-organ-specific autoantibodies were absent. In 1992, screening of microangiopathic complications revealed background retinopathy. In February 1993, the girl was admitted with sudden visual decrease at the right eye, diplopia (due to sixth cranial nerve palsy) and peripheral facial palsy with inability to close the eyelid. CT of the brain was normal. Vitamin B orally was prescribed and symptoms slowly disappeared within 8 months. Then metabolic control gradually worsened, and in 1996 subclinical peripheral polyneuropathy was also found. In July 1997, the girl complained of retroorbital pain and visual loss at the right eye. Neurological evaluation showed hyporeflexia. Cortical response of visual evoked potentials was absent at the left eye, while the response showed normal latency and reduced amplitude at the right eye. Brain magnetic resonance showed multiple small areas of increased T2 signal in white matter strongly suggestive for demyelinating lesions. Cerebrospinal fluid examination showed high IgG index (2.72; n.v. <0.7), and oligoclonal banding at the isoelectrofocusing, expression of intrathecal immunoglobulin synthesis. Treatment included intravenous pulses of metylprednisolone, with careful glycemic control. After 3 months visual impairment disappeared. Visual evoked potentials showed prolonged latency in the left eye, low amplitude with normal latency in the right eye. This form of MS can be defined as 'relapsing-remitting', therefore treatment with β-IFN from recombinant DNA would be suggested.