P-1

High incidence of type 1 diabetes mellitus in children under 15 years on the Avalon Peninsula, Newfoundland and Labrador, Canada

Joseph Curtis, Leigh Newhook, Donna Hagerty, Marie Grant, Tracey Bridger
Janeway Children's Health and Rehabilitation Centre

Background: The incidence of type 1 diabetes mellitus (TIDM) is known to be increasing internationally. A few geographically diverse areas, including the province of Newfoundland and Labrador (NL), have documented unusually high incidences of TIDM. We have now studied the incidence of the disease in one region of NL, where more than a third of the population lives.

Methods: All new diagnoses of TIDM in children aged <15 years from the Avalon Peninsula (AP) in NL were documented prospectively over the 15 years from 1987-2001. Recently, the charts of these patients were reviewed to ensure that the documentation as to diagnosis was accurate. The annual incidence rates were calculated per 100,000 population.

Results: The overall incidence of TIDM on the AP from 1987-2001 was 35/100,000/yr (confidence interval 31.1-39.5). In the 5-year periods 1987-1991, 1992-1966, 1997-2001, the incidence was 31, 32, and 43/100,000/year respectively. The incidence was >40 per 100,000 in 4 years of the 1997-2001 period. During this period, the annual incidence ranged from 37-47/100,000.

Conclusions: The high incidence of TIDM on the AP in children <15 years reflects the incidence of TIDM in the province of NL as a whole and is close to the reported incidence of TIDM for the same age group in Finland and Sardinia. The AP incidence in the last 5 yrs of the study, however, shows a marked upper deviation from the already high levels of earlier years. We postulate that the high incidence of TIDM on the AP may be due to a lower than usual degree of genetic heterogeneity in an ethnically stable population which arose from a small number of founder families.

P-2

Type 1 diabetes in New Zealand children and adolescents: Incidence, prevalence and mortality

Brian Darlow, Jinny Willis, Russell Scott, Chris Florkowski
Christchurch Hospital, Christchurch, New Zealand

Background: Type 1 diabetes results from the autoimmune destruction of pancreatic beta cells. Epidemiological studies from many populations and geographical contexts reveal increases in the incidence of type 1 diabetes with time. This study in Canterbury (New Zealand) determined the incidence of type 1 diabetes from 1970 to the present, the prevalence mid-way through the study period and mortality rates at 9 and 15 years follow-up.

Methods: Prospective ascertainment of incident cases aged 0-19 years commenced in 1982. Cases presenting 1970-82 were ascertained from hospital records. The prevalence of type 1 diabetes in the population was determined in January 1994 via a community survey based in retail pharmacies. The prevalence cohort was followed up at 9 and 15 years to establish vital status.

Results: Incidence rates ranged from 2.40 to 26.59 cases/100,000 person years over the three decades, increasing by 0.59 cases/100,000 per year or 5% annually. The prevalence of type 1 diabetes mid-way through the observation period was 0.3 and 1.8 per 1,000 for the 0-9 and 10-19 year age groups respectively. At follow-up after 15 years, the standardised mortality difference, or excess mortality, for individuals diagnosed with diabetes before age 30 was 8.4 for females and 9.9 for males. While cardiovascular disease accounted for the highest absolute mortality rate, the standardised mortality rates for hypoglycaemia and for renal failure were 7.52 and 35.74 respectively.

Conclusion: The incidence rate of type 1 diabetes is increasing significantly over time. A diagnosis of diabetes is associated with considerable excess mortality.

P-3

Romania remains a low-incidence country for childhood type 1 diabetes

Viorel Serban, Corina Hogea, Cleo Simionescu, Iulia Dunca,
for ONROCAD Study Group
County Hospital, Timisoara, Romania

Background: Over the last decade, establishing the incidence of type 1 DM in children and its trends has been a constant and important preoccupation of Romanian pediatricians/diabetologists. Data up to 2001 indicated that Romania belongs to the group of countries with low incidence for type 1 DM, however with an ascending trend.

Methods: Data processed have been collected from two different sources. The first from the members of the ONROCAD Study Group, pediatricians and diabetologists from the 41 districts of Romania and Bucharest, the capital. For each new case of diabetes they filled in special files including name of the patient, age, date of first insulin injection and address. All files were centralised and processed at the ONROCAD subgroup of Timisoara. The second source was the Clinical Center for Evaluation and Rehabilitation of Children and Adolescents "Cristian Serban" Buzias, where children with type 1 DM from all over Romania are admitted for assessment and treatment.

Results: In 2002, in Romania (with a pediatric population of 4,098,080 between 0 and 14 years and 5,759,858 between 0 and 18 years), 200 new cases of type 1 DM have been diagnosed in the age group 0-14 years, and 283 new cases in the age group 0-18 years. The resulting incidence was: 4.88/100,000 for age group 0-14 years and 4.91/100,000 for age group 0-18 years (Table). Sex-specific incidence showed that boys were more affected than girls (5.6/100,000 vs 4.18/100,000 for age group 0-18 yrs).

Age group	Incidence (No. of cases/100,000)		
	General	**Sex specific**	
		Girls	**Boys**
0-14 years	4.88	4.34	5.39
0-18 years	4.91	4.18	5.60

Conclusions: Incidence of type 1 DM in children in Romania remains low in 2002, and the ascending trend recorded in the previous decade (from 3.57/100,000 between 1992-1995 to 5.66/100,000 in 2001) is not confirmed. The incidence trend needs to be closely followed up in the years to come.

P-4

**Incidence of IDDM remains very low
during the last two decades in the Republic of Macedonia**

Mirjana Kocova, Natalija Angelkova, Ljiljana Kojic
Medical Faculty, Skopje, Republic of Macedonia

Background: Although the incidence of type 1 diabetes has been studied by several international study groups, data from the region of the Balkans are still limited. Variations between countries in this region are reported, but no long-term study has been conducted to show the trends in type 1 diabetes incidence. After the first report on the lowest incidence in Europe for type 1 diabetes in children, registration and follow-up of this incidence has been continued in the Republic of Macedonia for a total of 18 years. This is the first study for one entire country from the Balkan region with a long-term follow-up of diabetes incidence.

Methods: Data were collected and analysed according to the EURODIAB methodology.

Results: The overall age-adjusted incidence of type 1 diabetes in children 0-14 years old during the period 1985-2002 was 3.44 (95% CI 3.03-3.89). It is still the lowest in Europe. The overall incidence increased steadily, during the last 18 years reaching statistical significance (p for trend <0.001). Trend for type 1 diabetes incidence was different between different age groups. The incidence was very low in the age group 0-4 years and it did not change significantly over time. For the age groups 5-9 and 10-14 years trend analysis showed increase, but, except for boys in the age group 5-9 years (p=0.0014), it was not statistically significant. Thus, the increasing trend in overall incidence is mostly due to the boys in this age group.

Conclusion: Further studies are needed to clarify the factors contributing to the very low incidence of type 1 diabetes in Macedonia.

P-5

**Epidemiological, clinical and biological heterogeneity
of type 1 diabetes diagnosed under age 15 years**

Sylvie Tenoutasse, Ilse Weets, Frans Gorus, Dominique Beckers,
Kristina Casteels, Marina Coeckelberghs, Rita Craen, Jean De Schepper,
Marie-Christine Lebrethon, Raoul Rooman, Harry Dorchy
*HUDERF, Belgian Diabetes Registry, AZ-VUB, Brussels,
UZ Gasthuisberg, Leuven, Kinderziekenhuis Paola, Antwerpen,
UZ Gent, Gent, CHR Citadelle, Liège, UZA, Antwerpen, Belgium*

Background: Severity of clinical manifestations and frequency of the HLA-DQ2/DQ8 genotype tend to be lower at onset of type 1 diabetes in adulthood than in childhood.

Aims: To study age-dependent heterogeneity of type 1 diabetes diagnosed under age 15 years.

Methods: Antibody-positive diabetic children (n=952) were consecutively recruited nationwide and 3 age categories (A: 0-4 years, n=193; B: 5-9 years, n=313; C: 10-1 years, n=446) were compared in terms of epidemiological, clinical and biological data at diagnosis. Islet cell autoantibodies (ICA, IAA, GADA and IA-2A), random C-peptide and HLA-DQ were determined centrally.

Results: The annual incidence (n/10^5/year) of type 1 diabetes determined in the Antwerp district was 6.9 in group A, 11.9 in group B and 16.8 in group C (p<0.001). The prodromal phase increased with increasing age at diagnosis (p=0.002). The 3 groups did not differ in M/F ratio (1.1), age- and sex-adjusted BMI and prevalence of ketonuria. The insulin needs tended to decrease with age (p=0.04). Median random C-peptide (A: 0.29 µg/L; B: 0.36 µg/L; C: 0.55 µg/L; p<0.001) and GADA prevalence (A: 74%, B: 75%, C: 85%, p=0.001) increased with age at diagnosis, whereas DQ2/DQ8 (A: 42%, B: 36%, C: 29%, p=0.003) and IAA prevalence (A: 88%, B: 70%, C: 57%, p<0.001) decreased.

Conclusions: In antibody-positive type 1 diabetic patients under age 15 years, there is a marked epidemiological, clinical and biological heterogeneity according to age at diagnosis.

P-6

Some epidemiological data of diabetes mellitus type 1
in children admitted to U.H.C. of Tirana, Albania (1990-2003)

Lindita Grimci, P. Hoxha, Z. Ylli, A. Ylli, L. Dodaj
University Hospital Centre of Tirana, Tirana, Albania

Background: Diabetes mellitus type 1 (DM1) is a disease developing new expressivity. It is becoming more frequent at very young ages, changing seriously their family's life and their own lives. This is why we wanted to evaluate the morbidity of DM1 in children treated in our hospital.

Methods: We studied patients admitted to our hospital from 1990-2003. The following parameters were specified: personal information data of the patient, age at time of diagnosis, season, expression of DKA. We divided them into 3 age groups: 0-4 years, 5-9 years and 10-15 years, to evaluate the frequency of this disease at different ages. The information about the total population is taken from the official Annual Statistics of the Ministry of Health of Albania.

Results: There were 166 new cases during this period from age 0 to 14 years old, with mean age 8.5 ± 3.8 yr (range 8 months-14 years old). The male/female ratio was 1.2:1. Winter was the slightly preferred season for a new established diagnosis, but without significant differences from the other seasons. Looking at the three age groups, there was an increased number of younger children, with 15% in the 0-4 year group, 40.4% in 5-9 year olds, and 44.6% in 10-15 year old children, compared with our previous study (1987), in which the corresponding percentages were 11%, 29.5% and 59.5%. Since 1999, we have seen children younger than 2 years old (the youngest was 11 months old), and the mean age of the 0-4 year group in 2001 was 2.66 ± 0.57 years, and in 2002 it was 2.12 ± 0.94 years (with 3 very young children of 18 mo, 15 mo and 18 mo in 2002).

Conclusions: This higher frequency at younger age in recent years deserves further investigation relating to predisposing factors.

P-7

Worldwide epidemiological surveys of month of birth support the viral etiology of childhood onset type 1 diabetes

Zvi Laron, Hadas Lewy
Schneider Children's Medical Center, Sackler School of Medicine,
Tel Aviv University, Israel

Background: The worldwide increase in the incidence of childhood auto-immune diabetes (T1DM) and its consequences have become a public health problem. We conducted collaborative epidemiological studies on large populations of childhood onset T1DM and the general populations in Europe (n=5234), N. America (n=4307), Australasia (n=2765), New Zealand (n=275) and Israel (n=1868). We analyzed seasonality of birth (MOB) compared to total live births.

Methods: The methods used were Cosinor rhythm analysis and t-test between seasons of MOB and total live births.

Results: The main conclusions are: a) The seasonality of MOB of children who subsequently develop T1DM differed from that in the general population; b) Within these findings differences between males and females were observed in several populations. In most European countries studied, New Zealand and Israel, the peak of the yearly rhythm of MOB of children with T1DM was in spring or summer whereas in the US and Sydney with mixed ethnic populations the results varied but remained different from the total live births. The peak of the clinical disease onset in most populations occurs in late autumn or winter coinciding with the yearly epidemics. Our findings thus support the theory that the first trigger for beta-cell destruction occurs in genetically susceptible individuals already in utero being caused by enteroviruses transmitted by the pregnant mother to the fetus during the yearly viral epidemics in autumn and winter. Comparing the MOB seasonality in children with T1DM between the two sexes we found in several populations rhythmicity only in the males and none in the females as exemplified for Ireland in the figures.

Conclusion: These findings may indicate less susceptibility in females than in males as was also found in studies measuring enterovirus antibody titers in pregnant mothers in the general population.

P-8

Genetic predisposition to DM 1 in the Greek-Cypriot population

Charis Stylianou, Nitsa Apsiotou, Stavros Stavrou, Matheos Demetriades,
Alina Kyriacou, Pavlos A Costeas, Nicos Skordis

Karaiskakion Foundation, Nicosia, Cyprus

Background: Genetic linkage of diabetes mellitus type 1 (DM1) with certain HLA alleles is still of scientific interest. The aim of this study is to identify the association of HLA alleles with DM1 in Greek Cypriots.

Methods: 101 DM1 patients with age of onset less than 15 years were HLA typed using PCR/SSOP and PCR/SSP methods and compared to 209 controls randomly selected from a population of healthy volunteer donors. Statistical analysis was performed using the SPSS statistical package.

Results: The results are shown in the table below:

HLA	DM1 %	CTL %	Odds Ratio (95% CI)	Fisher's test
DR4	66	18	8.735 (5.076-15.033)	P<0.001
DR3	50	13	6.741 (3.839-11.837)	P<0.001
DR5	9	56	0.079 (0.38-0.166)	P<0.001
DR2	25	46	0.385 (0.227-0.653)	P<0.001
DR3 or 4	92	30	27.266 (12.486-59.542)	P<0.001
DQ2	73	27	7.230 (4.237-12.337)	P<0.001
DQ3	49	54	0.802 (0.499-1.289)	P<0.214
DQ2 or 3	95	73	7.278 (2.819-18.792)	P<0.001

High resolution testing of the DR4 and DR3 alleles revealed the predominant presence of the DRB1*0403 (0% vs 36%), similar frequency of the DRB1*0402 in both groups (19% vs 14%) and that DRB1*0301 was the only DR3 allele detected. The DQB1 alleles were nearly exclusively DQB1*0201 and DQB1*0302.

Conclusions: DM1 in our population seems to be linked to HLA: DRB1*0301 and DRB1*0405 which are associated with DQB1*0201 and DQB1*0302. A negative association and possible protection from DM1 of DR5 and DR2 antigens is also considered. Further studies on the HLA and other genes are needed to uncover the immunogenetic basis of DM1.

P-9

**Association of insulin gene polymorphism
with HLA DQB1 risk genotypes in type 1 diabetes in Greece**

Maria Dokopoulou, Evangelos Bozas, Andriani Vazeou, Ioanna Thymelli,
Peristera Paschou, Jorma Ilonen, Christos Sp Bartsocas
*Pediatric Research Laboratory, Faculty of Nursing, University of Athens,
Athens, Greece; Turku University, Turku, Finland*

Background: The insulin gene (INS) has a central role in the pathogenesis of type 1 diabetes (T1D) and is considered as a non-HLA candidate susceptibility gene (IDDM2). The purpose of the study is to evaluate the role of a single nucleotide polymorphism (SNP) of the insulin gene promoter in T1D in the Greek population and its relation to HLA DQB1 genotypes.

Methods: The single nucleotide polymorphism (SNP) of insulin gene promoter MSPI (INS) -2221 was analyzed in 132 T1D patients versus 145 controls previously typed for HLA DQB1 alleles. Genotyping was performed by PCR followed by hybridization using time resolved fluorometry method. The results were statistically analyzed by χ^2-test.

Results: No statistically significant differences in the frequencies of polymorphisms (CT, CC, TT) in the region of the insulin gene were found between T1D patients and controls. However, a higher frequency of the CT genotype associated with HLA DQB1*0201,x was found in T1D patients compared with the controls (20.5% vs 3.4%, p=0.001, OR 7.2). Further, the frequency of the CC genotype associated with HLA DQB1*0302,x genotype was increased in T1D patients compared with the controls (8.3% vs 0.7%, p=0.001, OR=13.1). Both the combination of CT genotype with HLA DQB1 *0201,x genotype and of CC with HLA DQB1*0302,x increased the OR of HLA genotypes alone (OR 7.2 vs 4.96 for HLA DQB1 *0201,x and OR 13.1 vs 3.9 for HLA DQB1*0302,x).

Conclusion: The combined presence of SNP genotypes (CT, CC) of the insulin gene promoter with HLA DQB1 moderate risk genotypes enhances the susceptibility effect for T1D.

P-10

CTLA-4 gene polymorphism confers susceptibility to type 1 diabetes in Greece: Analysis of association with HLA genotypes

Evangelos Bozas, Maria Dokopoulou, Andriani Vazeou, Ioanna Thymelli,
Peristera Paschou, Jorma Ilonen, Christos Sp Bartsocas
*Pediatric Research Laboratory, Faculty of Nursing, University of Athens,
Athens, Greece; Turku University, Turku, Finland*

Background: HLA-DQB1 is a known genetic locus which conveys increased risk for type 1 diabetes (T1D). Recent data have shown that the cytotoxic T lymphocyte associated antigen 4 (CTLA-4) exon 1 polymorphism (49 A/G) is correlated to T1D. The purpose of the study is to evaluate whether CTLA-4 49 A/G polymorphism confers genetic risk for T1DM in the Greek population and whether its correlation to HLA DQB1 alters the risk of the disease.

Methods: The CTLA-4 exon-1 polymorphism (49 A/G) was analyzed in 144 T1D patients vs 161 control subjects previously typed for HLA-DQB1 alleles. Genotyping was performed by PCR followed by hybridization using time resolved fluorometry method.

Results: AG genotype had the highest frequency among T1D patients (59.63 vs 40.94%, p=0.001, OR=2.13) whereas AA genotype was shown to be protective as its frequency was higher among the control subjects compared with the T1D patients (49.71 vs 27.33%, p=0.001, OR=0.38). The combination of CTLA-4 genotype AG with HLA DQB1*0201,x genotype (AG,*0201,x) was increased in T1D patients compared with controls (26.4% vs 5%, p=0.001, OR=6.85). Its OR was higher than that of HLA DQB1*0201,x genotype alone (4.96). Similarly, the frequency of the AG,*0302,x genotype was higher in T1D patients than in controls (9.0% vs 1.2%, p=0.001) and its OR was higher compared to the OR of HLA DQB1*0302,x alone (7.9 vs 3.9). AA,*0201,x genotype abolished the risk of HLA DQB1*0201,x for T1D.

Conclusions: In a population at moderate risk for T1D due to the presence of HLA DQB1*0201,x and *0302,x genotypes, the CTLA-4 AG genotype increases the risk for T1D. The protective CTLA-4 AA genotype decreases the risk for T1D in a moderate risk population positive for the HLA DQB1*0201,x genotype.

P-11

Association studies of *CTLA-4* and *ICOS* gene polymorphisms with type 1 diabetes in the Polish population

Izabela Kubryn, Wojciech Mlynarski, Krzysztof Andrzejewski,
Agnieszka Heinrich, Jerzy Bodalski
Institute of Pediatrics, Medical University of Lodz, Poland

Background: The cell-specific cell surface receptors CD28 and CTLA-4 are important regulators of the immune system. A new member of this family of co-stimulatory molecules, ICOS, is induced on the T-cell surface. The genes of CTLA-4 and ICOS, located near the IDDM12 locus, are candidate genes for type 1 diabetes. Many autoimmune diseases have been linked to markers near the CTLA4 gene. However, as all 3 genes are closely linked and have related functions, the findings could be due to variations in CD28 or ICOS. Determining the genomic structure of the ICOS gene showed single nucleotide polymorphism (SNP). This study evaluated the association of the polymorphism of ICOS and A/T +49 CTLA-4 gene with type 1 diabetes.

Methods: The study population consisted of 177 type 1 diabetes patients compared to 110 controls. ICOS typing for alleles T and C was performed by PCR and hybridisation with SSO probes. Genotyping of an A/G substitution in exon 1 of the CTLA-4 gene by PCR-RFLP analysis with ItaI digestion.

Results: Genetic analysis showed association of the A/G polymorphism of CTLA-4 with type 1 diabetes, but no association of the T/C polymorphism in the ICOS gene. The allele G frequency of this CTLA-4 gene polymorphism was higher in IDDM patients (54%) than in the controls (38%, $p<0.0003$, OR=1.94 [1.36-2.77]). Genotype GG was more frequent in diabetics (32%) than in controls (12.7%). The ICOS-C and ICOS-T allele frequencies were 9.6% vs 10.4% and 90.4% vs 89.6%. There were no differences in genotype frequency of T/C ICOS gene polymorphism. Linkage disequilibrium between the 49A/G and T/C polymorphisms was $D´=0.5$. The estimated frequency of haplotype CTLA-4*G-ICOS*T in patients was significantly higher than in controls (46% vs 32%; $p<0.003$; OR=0.47 [0.29-0.77]).

Conclusions: Haplotype CTLA-4*G-ICOS*T may be associated with genetic predisposition to type 1 diabetes. Further studies on the significance of the polymorphism in the CTLA-4 and ICOS genes will help us to understand the mechanism of the association between the genes of co-stimulatory molecules and type 1 diabetes.

P-12

The -23 HphI *INS* polymorphism in clinical course of type 1 diabetes in Polish children

Wojciech Mlynarski, Agnieszka Zmyslowska, Izabela Kubryn,
Olga Wegner, Agnieszka Szadkowska, Jerzy Bodalski
Institute of Pediatrics, Medical University of Lodz, Poland

Background: Investigations of genetic predisposition to type 1 diabetes (T1D) have suggested a strong association of the -23 HphI *INS* polymorphism with the disease. Given the key role of insulin deficiency in T1D pathogenesis, polymorphism of the insulin gene may also be related to the clinical course of T1D. The aim of this study was to examine the role of the -23 HphI *INS* mutation in genetic predisposition and clinical outcome of T1D in Polish children.

Methods: The prevalence of this polymorphism was studied in 130 patients with T1D aged 1-17.2 years (mean 10.4 yr), M/F 67/63, and in 181 healthy control subjects using PCR amplification and digestion with HphI restriction enzyme. Plasma C-peptide levels (radioimmunoassay) and fasting glycaemia were examined at the onset of disease and after 10, 30, 60, 90, 180, 360 days and after 2 and 3 years of T1D. At the same time points the insulin require\-ment, HbA_{1c} level and body mass index (z-score) normalized by age and sex were estimated.

Results: The pp genotype was observed in 71% of individuals with T1D and in 55% control subjects (OR [95%Cl] = 2 [1.2-3.2], $p < 0.01$). Carriers of the pp genotype were characterised by higher exogenous insulin requirements at the onset of diabetes than the pa and aa carriers (1.13 ± 0.5 U/kg vs 0.59 ± 0.3 U/kg, $p < 0.01$). Diabetic patients with pp genotype also had lower z-score values at onset of disease in comparison with other diabetic subjects (-0.49 ± 1.06 vs 0.006 ± 0.7, $p < 0.05$).

Conclusions: The -23 HphI *INS* polymorphism may be associated not only with the predisposition to T1D but also with the clinical course of the disease. In particular, it may be related to endogenous insulin deficiency.

P-13

Mortalin – a putative candidate gene for T1DM

Jesper Johannesen, Angeles Pie, Zenia Marian Larsen,
Ole Peter Kristiansen, Henrik Vissing, Allan Ertmann Karlsen,
Flemming Pociot, Jørn Nerup, DSGD and DIEGG
Steno Diabetes Center, Copenhagen, Denmark

Background: Mortalin has been identified by 2D-protein gel analysis and found to be up-regulated in isolated rodent islets exposed to cytokines. In a rat model system, previously characterized as being strain-dependent cytokine responsive, we observed strain-dependent mortalin expression in the isolated islets. As mortalin has furthermore been associated with cellular senescence, we consider the gene encoding for mortalin at 5q31.1 a putative candidate gene for cytokine induced beta-cell destruction.

Methods and Results: We have scanned the human mortalin gene for polymorphisms and identified three novel polymorphisms at the cDNA level in positions 272, 977 and 1962 (Genbank: L15189). Sequence data generated by the human genome project have enabled us to establish gDNA based typing assays for the three SNPs. Neither the SNPs considered individually nor constructed haplotypes were found to be in linkage by (E)TDT in a Danish T1DM population comprising 257 Danish Caucasoid families, in total 1,143 individuals. Furthermore, we tested the polymorphic D5S500 dinucleotide marker located less than 2.3 cM from the mortalin gene at 5q31.1 without finding linkage to T1DM.

Conclusion: The mortalin gene has been considered a putative T1DM candidate gene, but linkage has not been established either for the three novel identified polymorphisms or constructed haplotypes nor for a VNTR located close to the mortalin gene at 5q31.1 in Danish T1DM family material.

P-14

MHC class II-peptide chimeras for identification and down-regulation of diabetogenic T cells

Ioana Preda, Robert C McEvoy, Sunil Kumar, Robert Rapaport,
Teodor-Doru Brumeanu, Sofia Casares
Mount Sinai School of Medicine, New York, NY,
Children's Hospitals and Clinics, St Paul, MN, USA

Background: Type 1 diabetes is a T cell-mediated autoimmune disease. At present, there is no effective therapy to prevent or cure this disease. Also, identification of prediabetic individuals by sensitive tests able to detect autoreactive T cells in blood is highly desirable. We showed that soluble, dimeric MHC-II/peptide chimeras (DEF) are powerful anti-diabetogenic reagents. Administration of DEF chimeras to prediabetic mice prevented the onset of disease or reversed the disease in animals already diabetic. The anti-diabetogenic effects of DEF rely on the stimulation of IL-10-secreting T regulatory type 1 (Tr1) cells in pancreas, and anergy of autoreactive T cells in spleen.

Methods: Human DEF chimeras made up of the HLA-DR*0401 and expressing diabetogenic peptides of human GAD65 and proinsulin have been generated. These chimeras were used to evaluate their immunomodulatory effects on peptide-specific CD4 T cells from blood of HLA-DR-matched diabetic patients, as well as for quantification of these cells by FACS.

Results: Human DEF chimeras were shown to polarize the differentiation of peptide-specific autoreactive CD4 T cells toward the IL-10-secreting Tr1 phenotype in vitro. Autoreactive CD4 T cells were also detected by FACS in blood of diabetic patients, and their frequency correlated with the levels of IL-10 secretion upon in vitro stimulation with DEF chimeras.

Conclusions: MHC class II-peptide (DEF) chimeras are suitable reagents to quantify autoreactive CD4 T cells in blood, as well as powerful T cell immunomodulators. This approach has potential for the early diagnosis of the disease, and as an antigen-specific therapy in type 1 diabetes.

Predictive models of type 1 diabetes
for clinical use in siblings of affected children
Samy Mrena, Pekka Laippala, Kaisa Savola, Petri Kulmala,
Helena Reijonen, Jorma Ilonen, Hans K Åkerblom, Mikael Knip,
and the Childhood Diabetes in Finland Study Group
Universities of Tampere, Oulu, Turku, and Helsinki, Finland

Background: Both HLA-conferred disease susceptibility and autoantibodies have turned out to be useful in the prediction of type 1 diabetes in first-degree relatives of patients with newly diagnosed diabetes. In this study we set out to design predictive models for type 1 diabetes and test their utility in the prediction of type 1 diabetes in siblings of affected children.

Methods: The initial study cohort comprised 701 unaffected siblings of children with newly diagnosed type 1 diabetes, out of whom 93 tested positive for at least one diabetes-associated autoantibody (ICA, IAA, GAD and/or IA-2 antibodies) close to the time of diagnosis in the index case. An IVGTT was performed in 83 out of the 93 antibody-positive children. Thirty-two out of the 93 antibody-positive siblings (34%) progressed to clinical diabetes over a mean follow-up period of 14 years.

Results: Two models were constructed. The more extensive model including 10 variables [initial age, age of index case, IA-2 antibody positivity and titre, IAA positivity and titre, HLA-DR conferred susceptibility and protection, first phase insulin response (normal/reduced) and glucose elimination rate (normal/reduced)] explained 97.4% of the variation in age at diagnosis in the 21 progressors with all variables available. The simpler model based on four variables (initial age, ICA positivity, IA-2 antibody titre and HLA-DR defined predisposition) explained 85.4% of the variation in the age at onset in the 32 progressors with informative data. The two models were tested among all 93 initially autoantibody-positive siblings. The simpler model providing a predictive value similar to that of the more extensive one identified a higher proportion (56%; $p<0.05$) of those siblings who presented with overt diabetes before the age of 10 years than among those diagnosed between the age of 10 and 20 years (29%) or after the age of 20 (18%).

Conclusion: The designed models may be useful in the prediction of type 1 diabetes in siblings of children with newly diagnosed disease, particularly in those manifesting overt diabetes before the age of 10 years.

P-16

Heterogeneity of type 1 diabetes in Polish patients

Joanna Bodalska-Lipinska, Leszek Markuszewski,
Krystyna Wyka, Agnieszka Zmyslowska, Izabela Kubryn, Jerzy Bodalski
Institute of Paediatrics, Medical University of Lodz, Poland

Background: The purpose of this study was to find the differences between various forms of type 1 diabetes.

Methods: 158 children (group 1: mean age = 11 yr) and 69 elderly patients with recent onset type 1 diabetes (group 2: 36.1 ± 8.8 yr) were studied. Based on the results of immunological tests for the presence of antibodies (ICA, GAD and IA2) we selected patients who were positive for at least one autoantibody (group 1A: 119/158 and 2A: 47/69) and patients who had none of the examined antibodies (1B: 39/158; 2B: 22/69). Characteristics of all patients also included age, BMI, frequency of ketonuria, fasting blood glucose (FBG), plasma C-peptide fasting (Cp0) and after glucagon (Cp6), HbA_{1C}. ICA were measured by indirect immunofluorescence, GADab and IA-2ab by radioimmunoprecipitation.

Results: There was no statistical difference between the frequency of all antibodies in 1A. In 2A the frequency of ICA (30/69, 43%) and GADab (40/69, 58%) was similar, but the frequency of GADab was higher than the frequency of IA-2ab (19/69, 28%) ($p < 0.01$). There was no statistical difference between the frequency and levels of ICA and GADab in patients of both groups. IA2 was more frequent (57% vs 28%; $p < 0.01$) and at higher levels in group 1A than in group 2A (55 vs 5 AU; $p < 0.02$). In both groups the coexistence of one, two and three autoantibodies was found with similar frequency. In 1A we observed lower Cp0 level (0.18 vs 0.36 pmol/ml; $p < 0.001$), higher FBG (408 vs 360 mg/dl; $p < 0.05$), and higher HBA_{1C} (12.1 ± 2.8 vs 10.1 ± 3.1; $p < 0.001$) than in 2A. Similarly, Cp0 levels were lower in 1B compared to group 2B (0.2 vs 0.58 pmol/ml, $p < 0.003$), but FBG and HbA_{1C} were similar. We observed no statistical difference comparing levels of FBG, Cp0 and HBA_{1C} between ab(+) and ab(-) diabetic children. Children ab(+) were older than ab(-) children (11 vs 5 yr; $p < 0.02$). Among adult diabetics, ab(+) patients had significantly lower Cp0 concentrations (0.36 vs 0.58 pmol/ml; $p < 0.002$). Age, BMI and levels of Cp6, FBG and $HbA1_c$ were similar in 2A and 2B groups.

Conclusions: The autoimmune process is more frequent and intensive in children than in adults. C-peptide secretion is lower in ab(+) patients than in ab(-) diabetics.

P-17

**Islet autoantibodies in cord blood from patients
who developed type 1 diabetes mellitus at 15-30 years of age**
Maria Elfving, Bengt Lindberg, Lennarth Nyström, Göran Sundkvist,
Åke Lernmark, Sten-A Ivarsson and the DISS Study Group
*Department of Pediatrics, Lund and Malmö, Department of Epidemiology,
Umeå, Department of Endocrinology, Malmö, Sweden*

Background: Islet cell autoantibodies are early markers for type 1 diabetes. In an earlier study we found an increased prevalence of autoantibodies in cord blood of children who developed type 1 diabetes below the age of 15 years. The aim of this study was to determine whether islet autoantibodies were present at birth in young adults who developed type 1 diabetes at 15-30 years of age.

Methods: Cord blood sera from 30 patients who developed type 1 diabetes between 15 and 25 years of age and sera from 320 randomly selected control children were tested for islet cell antibody (ICA), autoantibodies against the 65 kDa isoform of glutamic acid decarboxylase (GADA), autoantibodies against islet cell antigen-2 (IA-2A) and autoantibodies against insulin (IAA).

Results: The young adults, median age 18 years, who developed type 1 diabetes did not differ from controls in the prevalence of any of the four islet autoantibodies. This is in contrast to our previous findings that children who developed type 1 diabetes below 15 years of age had an increased prevalence of cord blood islet autoantibodies.

Conclusions: Our present data suggest that, in contrast to children, pre- and perinatal risk factors are less likely to be involved in the development of type 1 diabetes in young adult people.

Familial clustering of beta-cell autoimmunity
in initially non-diabetic children

Marika Kukko, Anna Toivonen, Antti Kupila, Sari Korhonen,
Päivi Keskinen, Riitta Veijola, Suvi Virtanen, Jorma Ilonen,
Olli Simell, Mikael Knip

JDRF Center for Prevention of Type 1 Diabetes in Finland

Background: Type 1 diabetes is characterized by familial aggregation. We set out to explore whether also beta-cell autoimmunity which usually precedes the manifestation of overt diabetes by months and years shows familial clustering.

Methods: Tests for six *HLA DQB1* alleles and islet cell autoantibodies (ICA) were performed on 5,847 children from 2,286 families. The *DQB1* genotypes were classified into those conferring high (**02/0302*), moderate (**0302/x*; where $x \neq *02, *0301$ or **0602*), low (**0301/0302, *02/0301, *02/x, *0302/0602*; where x indicates **02* or a non-defined allele) or decreased risk (*DQB1*x/x, DQB1*0301/x, DQB1*02/0602, DQB1*02/0603, DQB1 *02/0604, DQB1*0602/x, DQB1*0603/x, DQB1*0604/x*). When a child was observed to be positive for ICA, all his/her samples available were also tested for insulin autoantibodies (IAA), antibodies to glutamic acid decarboxylase (GADA) and antibodies to the IA-2 protein (IA-2A). Similarly all sibs of ICA-positive children were tested for all four autoantibody specificities.

Results: Forty-four families were observed to have at least two children positive for at least one autoantibody. This proportion (1.9%) was almost five times higher than the expected one (0.4%; $p < 0.00001$). In 12 (27.3%) families the children with autoantibodies had the same autoantibody profile. There were no significant differences in the distribution of the HLA risk genotypes between the families with at least two autoantibody-positive children and the other families ($p = 0.085$).

Conclusions: Beta-cell autoimmunity defined by the appearance of diabetes-associated autoantibodies demonstrates familial aggregation. Families with two or more children with signs of beta-cell autoimmunity could be a valuable population for characterizing the pathogenetic process in type 1 diabetes.

P-19

Secretion function of pancreatic beta-cells
at prediabetic stage and in clinical type 1 diabetes

Jerzy Bodalski, Krystyna Wyka, Agnieszka Zmyslowska,
Joanna Bodalska-Lipinska, Wojciech Mlynarski

Institute of Pediatrics, Medical University of Lodz, Poland

Background: Type 1 diabetes (T1D) is caused by an autoimmune beta-cell destruction. Changes in beta-cells are related to disorders of secretion of proinsulin, insulin and C-peptide. The aim of the study was an evaluation of secretion of these hormones at the prediabetic stage and at onset of clinical T1D.

Methods: 30 children with autoantibodies (at prediabetic stage) (mean age 10.6 ± 4.2 years) and 115 diabetic patients at the clinical onset of disease (mean age 12.9 ± 5.1 years) were studied. 50 healthy children (mean age 10.6 ± 3.4 years) were analysed as control subjects. The levels of proinsulin, insulin and C-peptide were measured by radioimmunoassay.

Results: Higher proinsulin levels were observed in T1D patients (15.9 pmol/l) than in control children (8.6 pmol/l, $p < 0.0002$). In subjects at prediabetic stage insulin levels (20.1 µU/ml) were higher in comparison to healthy children (7.9 µU/ml, $p < 0.00001$). Individuals both at the prediabetic stage and at onset of T1D had C-peptide levels (0.6 pmol/ml and 0.2 pmol/ml, respectively) significantly different from those of control subjects (0.4 pmol/ml, $p < 0.002$ and $p < 0.05$, respectively). The relation between proinsulin and insulin (PRO/INS) as well as between proinsulin and C-peptide levels (PRO/P-C) were analysed. The PRO/INS ratio was lower in children at prediabetic stage than in the control group ($p < 0.00001$). By contrast, a higher PRO/P-C ratio was observed in diabetic patients compared with the controls ($p < 0.00001$).

Conclusions: The results showed changes in hormone secretion during the development of T1D. At the prediabetic stage increase of proinsulin secretion and its conversion products was observed while at the clinical onset of T1D increase of proinsulin secretion may be associated with decreased insulin secretion. This suggests disorders of conversion of the hormones at this stage.

P-20

"Honeymoon phase" in children with type 1 diabetes:
Frequency, duration and affecting factors

Majedah Abdul-Rasoul, Hessa Habeb, Maha Alkholy
Farwania Hospital, State of Kuwait

Background: The honeymoon period in type 1 diabetes is characterized by reduced insulin requirements, while maintaining good glycemic control. The clinical significance of this period is the potential possibility of pharmacological intervention during this period to slow down or stop the ongoing destruction of the remaining B cells.

Methods: 68 diabetic children, less than 12 years of age, diagnosed during the period of 1999-2002 were prospectively studied to assess the frequency, duration of partial remission (honeymoon) as well as the factors influencing that remission. Patients were characterized by age, sex, duration of symptoms before diagnosis, ketoacidosis, and blood sugar at admission. The honeymoon period was defined as an insulin requirement <0.5 U/kg/d.

Results: Partial remission occurred in 43 (63.2%), being complete in three. None of the four children <3 years of age remitted. The length of time until remission was 35 ± 20 (mean$\pm$SD) days. The duration of remission was 7.8 ± 4.8 months. Remission rate was higher in those aged >5years than <5 (excluding those less than 3). Similar rates were found in boys and girls. DKA at presentation and long duration of symptoms before diagnosis were associated with lower rate of remission ($p<0.001$, $p<0.001$, respectively). The children with remission had significantly lower blood sugar and higher pH at presentation ($p<0.001$, $p<0.001$, respectively.). The mean HbA1c beyond the honeymoon period was significantly lower in children who had partial remission ($7.1\pm1.1\%$ vs $8.3\pm1.5\%$, $p<0.05$).

Conclusion: Young age and severe disease are associated with decreased residual B cell function, reflected by a lower incidence of partial remission. This is important to consider in the research regarding therapies that will have potential use to induce long term and complete remission at disease onset or shortly after.