Mitochondrial genotype associated with occurrence of type 1 diabetes mellitus
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It has been proposed that accumulation of mitochondrial DNA mutations in somatic cells contributes to aging and to degenerative diseases because the DNA genotypes influence oxidative damage of its own. Recently it was reported that Mt5178A (a C-to-A transversion at nucleotide position 5178 within the NADH dehydrogenase subunit 2 gene) is related to longevity, while the individuals with Mt5178C is more susceptible to adult-onset diseases than those with Mt5178A. On the other hand, oxidative stress plays an essential role in the destruction of pancreatic ß-cells by infiltrating inflammatory cells in type 1 diabetes. To evaluate the effect of the mitochondrial DNA variations on occurrence of type 1 diabetes, we analyzed the frequencies of Mt5178A/Mt5178C by PCR-RFLP with Alu I in 385 (156 males, 229 females) Japanese type 1 diabetic patients who were selected randomly among follow-up patients in the Diabetes Center of our University. We also studied DNA of 163 (65 males, 98 females) healthy controls who had no abnormality in glucose and lipid metabolism. The frequency of Mt5178A was significantly lower in type 1 diabetic patients (119/385, 31.0%) than in healthy controls (66/163, 40.5%) (p = 0.03, odds ratio 1.52 (95% CI 1.04-2.22)). This finding suggests that the individuals with Mt5178A is less susceptible to type 1 diabetes than those with Mt5178C. As Cann et al. mentioned that Mt5178A is relatively rare among the global population, the high frequency of Mt5178A among the Japanese population 40.5% may be relevant to the fact that the incidence of type 1 diabetes in Japan is relatively low in the world.

Natural history of hyperglycaemia in children not due to type-1 diabetes
Salardi S, Zucchin S, Ragni L, Mainetti B, Cacciari E, First Pediatric Clinic, University of Bologna, Italy.

We followed 35 children and adolescents over a 2-19 yrs period (2-5 yrs in 25 cases and 10 yrs in 15 cases) who were referred for evaluation of fasting hyperglycaemia (≥ 110 mg/dl in absence of type-1 diabetes) found on: routine screening (19 cases); screening for family history of hyperglycaemia or diabetes (9 cases); during acute illness (7 cases). Obesity was present only in 5 patients. 15 patients (none with obesity) had at least one first-degree relative with hyperglycaemia or diabetes mellitus, 15 patients (4 with obesity) had second or third-degree relatives with diabetes and finally 5 patients (1 with obesity) had no family history for diabetes. A fter 2 years from diagnosis hyperglycaemia was no longer present in 9 cases (1 in the group with first-degree relative; 1 was obese), was confirmed in 15 cases (7 in the group with first-degree relative; 1 was obese) and worsened (fasting blood glucose and/or OGTT) in 11 cases (7 in the group with first-degree relative; 3 had obesity). All patients of the latter group were given oral hypoglycaemic therapy when glycaemia reached 130 mg/dl and/or HbA1c 6% (normal range 3.8-5.8%) and this happened within 2 yrs from diagnosis in 10 cases and after 3 yrs in 1 case. 2 out of the 11 treated patients required the administration of insulin after 3 and 6 yrs from diagnosis. On the contrary, follow-up at 5 and 10 yrs did not show significant changes in the 24 patients without treatment.

In conclusion: 1) the tendency towards a worse glycaemic control with the need of therapy always occurred in our patients within 3 years from diagnosis and no case showed a progression beyond that limit; 2) the group requiring treatment included both about half of the patients with first-degree related affected and 3 out of the 5 obese patients, but also 1 patient who was thin and with family history negative for diabetes; 3) the patients, usually non obese, with a weaker family history for diabetes or even without affected relatives, are difficult to be classified; most of them show a milder metabolic abnormality.

Decreased membrane polarity of polymorpho-nuclear leukocytes from diabetic children
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Polymorphonuclear leukocytes (PMN) from diabetic children have been shown to be abnormal in chemotaxis, adherence, phagocytosis, killing and respiratory burst. All these activities are the result of complex events mediated by plasma membrane of PMN. Chemical and physical events that take place within the membrane allow the cells to carry out their specific function. The polarity properties of the membrane bilayer are strongly influenced by its composition and dynamics. Different modes of organization of the compositionally and functionally differentiated domains correspond to different functional states of the bilayer. Membrane polarity of PMN obtained from 22 children with insulin-dependent diabetes mellitus (IDDM) (age range between 6.6 and 14.4 years; mean 10.4 ± 2.2) and healthy controls were studied using fluorescence technique. Membrane polarity was studied by measuring the steady-state fluorescence emission and excitation spectra of 2-dimethylamino-6-lauroyl naphthalene (Laurdan), which is known to be incorporated at the hydrophobic-hydrophilic interface of the bilayer, displaying spectral sensitivity to the polarity of its surroundings. Laurdan shows a marked steady-state emission blue shift in nonpolar solvents, with respect to polar solvents. PMN were isolated and immediately used for fluorescence measurements. Measurements were performed on an LS 50B spectrophotometer (Perkin-Elmer Ltd. U.K) as previously described (Photochem Photobiol 1993;57:438-441). Our results show a blue shift of Laurdan emission spectra in PMN from the IDDM group with respect to the control (maximal intensity from 458nm to 454nm). These data indicate a decrease in membrane polarity. The observed changes in plasma membrane could be on the
basis of the alterations in the functional activities of PMN in children with IDDM.

**P48 Lipid tracking in diabetic patients**
Chueca M, Oyarzábal M, Sola A, Aliaga M, Echarte G, Aizpun M, Mondela I., Paediatric Endocrinology Unit, ‘Virgen del Camino’ Hospital, Pamplona, Spain

Objectives:
2. To assess the influence of diabetes and particularly metabolic control on lipid values.
3. To identify early groups with lipidic risk values and/or a family history of the disease.

Material and methods

I. Diabetic Population
80 patients 41M / 39F
Present age 17.54 ± 2.88 years
Age at onset 9.25 ± 3.67 years
Evolution time 7.95 ± 3.99 years
Anual HbA1c 8.23% ± 0.6
Evolution time 7.73%(92 vs 96)

Complications-risk markers
Microalbuninuria 10 intermittent
Retinopathy 10 (8 slight, 2 lasertherapy)
Limited joint 16
Mobility (LJM) 11
Family history 11
dyslipemia, CV disease 29
Smoking

II. Lipid and lipoprotein measurements in diabetics 1992–1996 Results:

<table>
<thead>
<tr>
<th>Lipid values</th>
<th>1992</th>
<th>1996</th>
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<tbody>
<tr>
<td>Chol (mg/dl)</td>
<td>176 ± 29.73</td>
<td>160 ± 27.2</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>65.3 ± 25.29</td>
<td>57.4 ± 21.7</td>
</tr>
<tr>
<td>HDL-C (mg/dl)</td>
<td>56 ± 12.5</td>
<td>48.3 ± 11.5</td>
</tr>
<tr>
<td>LDL-C (mg/dl)</td>
<td>109.5 ± 26.7</td>
<td>96.2 ± 30.2</td>
</tr>
<tr>
<td>Apo-A (mg/dl)</td>
<td>150.7 ± 33</td>
<td>138.9 ± 27.4</td>
</tr>
</tbody>
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<table>
<thead>
<tr>
<th></th>
<th>1992</th>
<th>1996</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo-B (mg/dl)</td>
<td>87.4 ± 28.6</td>
<td>77.7 ± 17.1</td>
</tr>
<tr>
<td>Apo-A / Apo-B</td>
<td>1.93 ± 0.97</td>
<td>1.88 ± 0.56</td>
</tr>
<tr>
<td>LDL / HDL</td>
<td>2 ± 0.6</td>
<td>2.1 ± 0.8</td>
</tr>
<tr>
<td>Chol / HDL</td>
<td>3.31 ± 0.7</td>
<td>3.36 ± 0.8</td>
</tr>
<tr>
<td>Lp [a] (mg/dl)</td>
<td>20.54 ± 19.3</td>
<td>19.4 ± 24.6</td>
</tr>
</tbody>
</table>

Metabolic control in 1996 improved significantly in girls.

Complications: no significant differences are observed in lipid values of patients with complications and/or risk markers (retinopathy, microalbuminuria, LJM).

Lipidic risk: The most evident improvement and that which is associated with best metabolic control is observed in the group with cholesterol > 200 mg/dl.

Conclusions:
1. Longitudinal follow-up of patients between 1992-1996 shows improvement in some lipid values (chol and TG) associated with better metabolic control; however, worsening of HDL-Chol counteracts this satisfactory evolution with the negative influence of smoking being observed in our patients.
2. HDL-Chol worsened in patients with a family history as in the rest of the patients study, and moreover, presents worse LDL-C and Lp [a] values. These patients form a special group in whom hygienic-dietetic and/or pharmacologic measures should be applied early.

**P49 Decreased magnesium levels in serum and erythrocytes of young type 1 diabetic subjects. Relationships with glycated haemoglobin levels (HbA1c) and subclinical complications**
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Paediatric studies on magnesium depletion in type 1 diabetic patients are scarce, and there are no data on erythrocyte magnesium content (EMC) which is important since 99% of total magnesium (Mg) are intracellular. Moreover, in the paediatric studies there are no data on the relationships between hypomagnesaemia and HbA1c levels or subclinical complications. Therefore the aim of the present study is to communicate our experience in that field.

Serum Mg levels, EMC, magnesuria, and HbA1c were determined in 118 type 1 diabetic subjects (105 boys and 83 girls) aged 19 ± 8 years (mean ± SD) with a diabetes duration of 11 ± 8 years, and in 96 controls. Mg was measured by colorimetric calmagite kits and HbA1c using a HPLC method. We searched for subclinical retinopathy (fluorescein angiography), neuropathy (conduction velocities in the limbs), nephropathy (microalbuminuria and β2-microglobulinuria) in patients aged > 12 years with a diabetes duration > 3 years\(^a\).

The mean ± SD Mg serum concentration was 1.8 ± 0.2 mg/dl in the diabetic population and 2.0 ± 0.2 mg/dl in the controls (Mann Whitney: p < 0.001). The mean EMC was 5.0 ± 0.5 mg/dl in the patients, vs 5.3 ± 0.2 mg/dl in the controls (p < 0.001). In 14% of the patients serum Mg levels were less than −2 SD below the normal mean, while 6% of the diabetic subjects had EMC less than −2 SD under the normal mean. In the diabetic patients, serum Mg levels were positively correlated with EMC (r = 0.19; p < 0.01) and negatively with age (r = −0.24; p < 0.01), duration of diabetes (r = −0.19; p < 0.05), HbA1c (r = −0.16; p < 0.05). EMC levels were negatively correlated to magnesuria (r = −0.31; p <
whose mean HbA1c level reaches 147% of the upper normal level. Type 1 diabetic subjects with the poorest glycemic control are related to age or diabetes duration.

In conclusion, lower serum Mg levels and EMC were found in type 1 young diabetic patients. Hypomagnesaemia is related to age, duration of diabetes, bad glycemic control, and presence of subclinical complications. Mg depletion should be searched for, even in the paediatric population and supplementation in Mg should be considered.

Reference:

P50
Relationships between glycated haemoglobin levels (HbA1c) and eradication of Helicobacter pylori (HP) infection in young type 1 diabetic subjects
M. Scallion1, H. Dorchy2, S. Cadranet3, Clinics of Gastroenterology4 and Diabetology5, University Children’s Hospital Queen Fabiola, Brussels, Belgium

In a preliminary study, we have shown that HP-positive diabetic children, adolescents and young adults had higher HbA1c levels than our total diabetic population1,2. The aim of the present study was to examine the relationships between HbA1c levels and eradication of HP infection. A total of 47 patients (mean ± SEM age of 18 ± 1 years, and diabetes duration of 9 ± 1 years) with HP infection, proven by histology, culture and 13C-urea breath test (13C UBT), were included in the study during a 6 month period after a bitherapy based on a bacterial antibiogram. HP eradication was controlled 2 months later by 13C UBT. HbA1c levels, measured by an HPLC method at diagnosis, 2 and 6 months after treatment, were expressed as % of normal values, the upper normal limit being 100%.

Eradication of HP infection was obtained in 32/47 patients (68%). Age and diabetes duration were not different in the 2 groups, neither was the the ratio immigrants/non immigrants. HbA1c levels were significantly higher in HP-non eradicated patients than in HP-eradicated subjects at diagnosis (147 ± 10% vs 136 ± 5%; p = 0.004), 2 months after treatment (147 ± 8% vs 136 ± 4%; p = 0.008), while the difference was less significant 6 months after treatment (143 ± 8% vs 135 ± 4%; p = 0.06). In both groups, HbA1c levels were not different at diagnosis and 2 or 6 months after treatment, and were unrelated to age or diabetes duration.

In conclusion, eradication of HP infection is less efficient in type 1 diabetic subjects with the poorest glycemic control whose mean HbA1c level reaches 147% of the upper normal limit.

Reference:

P51
Incidence and some risk factors of late complications by diabetic children in Latvia
Iveta Dzivite, Children’s Endocrinology centre, children’s hospital of Latvian medical academy

The goal of this study was to check the incidence of late complications by diabetic children in Latvia and to evaluate the role of some risk factors in development of them.

In conclusion, lower serum Mg levels and EMC are found in type 1 young diabetic patients. Hypomagnesaemia is related to age, duration of diabetes, bad glycemic control, and presence of subclinical complications. Mg depletion should be searched for, even in the paediatric population and supplementation in Mg should be considered.

Reference:
It has been suggested that cell adhesion molecules be involved with the early development of vascular endothelial dysfunction in diabetes mellitus. Thus the aim of this study was to investigate the plasma levels of tetranectin (TN), cP-selectin and vascular-cell adhesion molecule-1 (VCAM-1) in IDDM children without vascular complications and to compare their relationship to glaucametric control. TN, cP-selectin and VCAM-1 were determined by ELISA in the plasma of 69 IDDM children (mean age 103 yrs, diabetes duration 52 y) and compared with those of 35 sex, age and BMI matched healthy controls. Patients were divided into subgroups A and B according to their levels of HbA1c (HbA1c > 6.8% and HbA1c > 6.8% respectively). Median plasma levels of TN in children with IDDM (14.9 mg/l, interquartile range: 12.5–18.1) were higher than those of controls (13.4 mg/l, interquartile range: 10.4–16.3, p = 0.02). Median plasma levels of cP-selectin and VCAM-1 were also significantly increased in patients (301 ng/ml (218–401), p = 0.004 and 997 ng/ml (814–1222), p = 0.0015 respectively) than in controls (201 ng/ml (135–243) and 724 ng/ml (602–864) respectively). A trend towards higher cP-selectin levels was observed in subgroup B compared to subgroup A. As regard VCAM-1 no statistically significant difference was found between subgroup A and B. However TN levels were higher in subgroup B than in subgroup A (median value 16.3 mg/l vs. 13.6 mg/l, p = 0.012), indicating TN elevation with the worsening of glaucametric control. Likewise a longitudinal follow-up of 10 IDDM children revealed a positive correlation between TN and HbA1c in each patient. In conclusion, these findings provide evidence for adhesion molecules elevation in IDDM, even in disease stages without apparent vascular damage.

P53

Renal sodium and dopamine handling in diabetic children with family history of essential hypertension

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Diabetic nephropathy (DN) develops only in a subset of patients with type 1 diabetes mellitus (IDDM). It has been suggested, that diabetic patients with a genetic trait for essential hypertension (EH) are susceptible for DN. Since sodium handling is altered both in DM and EH, and dopamine (DA) is the main natriuretic hormone, the aim of our study to assess the difference in sodium and DA excretion after sodium challenge in diabetic patients with and without a genetic trait for essential hypertension. Eight diabetic children with (FH+) and right patients without (FH-) a family history of hypertension have been studied. Age, duration of diabetes, metabolic control (HbA1C) and body mass index were comparable in the two groups. All patients underwent a 3 day sodium challenge by ingesting 8 g NaCl per day beside a sodium and carbohydrate fixed diet. Blood pressure was recorded by ambulatory blood pressure monitoring, and urinary DA excretion was measured by HPLC before and after the sodium load. Systolic and diastolic blood pressure were unaltered and comparable in the two groups before and after sodium challenge. After sodium load urinary sodium excretion was significantly higher (p < 0.03) in FH+ group (212 ± 47 mmol/day) compared to FH− group (146 ± 51 mmol/day). Natriuresis was accompanied by significantly (p < 0.005) higher urinary DA excretion in the FH+ group (2.48 ± 0.7 μmol/die) compared to the FH− children (1.48 ± 0.3 μmol/die).

Conclusion: Diabetic children with a family history of EH fail to increase urinary sodium and DA excretion following high salt diet. The importance of his phenomenon in the development of DN is unknown.

PS4

Analysis of association of insertion/deletion (I/D) polymorphism of angiotensin I-converting enzyme (ACE) with diabetic nephropathy and retinopathy in the group of Moscow children

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The association of insertion/deletion (I/D) polymorphism of angiotensin I-converting enzyme (ACE) with diabetic microangiopathy remains controversial. To assess the association of the ACE genotypes with the development of diabetic nephropathy (DN) or retinopathy (DR) we studied I/D polymorphism in a group of children and adolescents with IDDM (n = 72). In comparison with 168 healthy adults from Moscow the I allele was more frequent (49.3% vs. 36%, p = 0.005). Patients were divided into 3 groups: patients with early (less than 10 yr after the diagnosis) onset of DR – A (n = 46), patients with early onset of DN – B (n = 36), patients without these complications (the duration of diabetes more than 10 yr) – C (n = 22). There was no difference in frequency of I and D alleles in these groups. Genotype ID was less frequent in the C group (p < 0.005). Early onset of microangiopathy was associated with the higher frequency of other complications: cataract, delayed puberty and short stature, necrobiosis lipoidica, limited joint mobility (LJM). HbA1c (%) was higher, too (p < 0.001). The results are shown in table 1.

<table>
<thead>
<tr>
<th>I/D</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>I/D</td>
<td>47,8%</td>
<td>52,2%</td>
<td>50,4%</td>
</tr>
<tr>
<td>II/D/D</td>
<td>19,5%</td>
<td>65,6%</td>
<td>16,7%</td>
</tr>
<tr>
<td>HbA1c</td>
<td>13,5%</td>
<td>0,037</td>
<td>14,1%</td>
</tr>
<tr>
<td>DN</td>
<td>67,4%</td>
<td>100%</td>
<td>0</td>
</tr>
<tr>
<td>DR</td>
<td>100%</td>
<td>83%</td>
<td>0</td>
</tr>
<tr>
<td>LJM</td>
<td>43%</td>
<td>50%</td>
<td>22,7%</td>
</tr>
<tr>
<td>Delayed puberty and short stature</td>
<td>21,7%</td>
<td>19,4%</td>
<td>9%</td>
</tr>
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Cataract

Necrobiosis lipoidica

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
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<tbody>
<tr>
<td>21,7%</td>
<td>27,7%</td>
<td>0%</td>
</tr>
<tr>
<td>10,8%</td>
<td>13,8%</td>
<td>0%</td>
</tr>
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</table>
Bone density in type 1 diabetes

O. V. Remizov, I. V. Shirokova, T. L. Kouraeva, L. N. Scherbatcheva, V. A. Peterkova, National Endocrinological Research Centre, Paediatric Division, Moscow, Russia

Measurements of quantitative ultrasonic velocity (SoS) in patients with type 1 diabetes were carried out to evaluate the bone status. 85 children and adolescents (44 b., 41 g.) with type 1 diabetes of duration from 5 month to 15 yrs. aged 6–15 yrs. and the mean HbA1c 9.4 ± 1.6% (ref. < 6.8%) were enrolled in this study. SoS was measured at the right mid tibia using a M yiрид ultrasound system (SoundScan 2000TM). The results compared with our age- and sex- matched reference values, expressed as Z-score and made correction for the bone age (that was assessed according to the method of Greulich and Pyle).

We found a reduced bone density (SDs between –1.5 and –2.1) was correlated with diabetes duration (p < 0.03), age (p < 0.03), delayed puberty (p < 0.05), glycated haemoglobin level (p < 0.05), but not sex, limited joint mobility and microalbuminuria. None of the patients with diabetes duration less than 5 yrs. (n = 22) had decreased bone density at the investigated site. But decreased bone density was established in 15.9% (n = 10) of the patients after as long as 5 yrs. of disease. While making correction for the bone age we obtained these results. It is to be pointed out that measurement of SoS without the correction shown a reduction of bone density in 22.2% of the patients (n = 14). In regard to correlation between glycated haemoglobin level and low bone density we suppose that it may well as being due to deteriorated glycaemic control in teenage years when decreased bone density is seen frequently.

In conclusion: This study indicates that osteopenia can developed in children and adolescents with long- standing diabetes. Furthermore, it seems to reflect one of the initial metabolic changes of bones in diabetes patients that might account in part for the increased prevalence of food problems in the future. Thus, regular monitoring of bone density can be recommended for long-term diabetes children, adolescents and young adults.