2710-PO

Validation of the Association between PSMA6 -8 C/G Polymorphism and Type 2 Diabetes Mellitus in Chinese Dongxiang and Han Populations

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Abstract Objective: The aim of our study was to validate the association between the previously reported -8 C/G variant in the proteasome subunit alpha type-6 gene (PSMA6) and the risk of type 2 diabetes mellitus (T2DM) in the Dongxiang and Han populations from north-western China. Method: Genotyping of *PSMA6* gene -8 C/G polymorphism was detected in nondiabetic control subjects and patients with T2DM in these populations, using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique. Result: The frequency of CG + GG genotype of *PSMA6* was obviously different from C/C genotype in the T2DM groups and control groups in the Chinese Dongxiang and Han populations (OR = 1.494, 95% CI: 1.182-1.889, P = 0.001; OR = 1.477, 95% CI: 1.210-1.802, P = 0.000, respectively). In Dongxiang population, the FPG, HOMA-IR, SBP and TC levels of the CG + GG genotype were markedly higher than that of the CC genotype, in control group (all $P < 0.05$) respectively. Furthermore the DBP level of the CG + GG genotype was markedly higher than that of the CC genotype in both T2DM group and control group (all $P < 0.05$) respectively. Conclusion: Our investigation suggests that the -8 C/G variant in the *PSMA6* gene may be associated with T2DM and diabetes-related metabolic traits in Chinese Dongxiang and Han populations.

2711-PO

Study of Resistin Gene Polymorphism in the Elderly Type 2 Diabetes Mellitus Han Patients in Northeast China

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The purpose of this study was to investigate the relationship between resistin gene polymorphism and the risk of type 2 diabetes mellitus (T2DM) in elderly Han people in Northeast China. 115 elderly patients and 99 normal controls in Han people in Northeast China were included. The single nucleotide polymorphism (SNP) in resistin gene was found in 20 elderly patient using PCR direct sequencing method. Then these SNPs were genotyped by PCR direct sequencing. Five SNPs (g.-638G>A, g.-537A>C, g.-420C>G, g.-358G>A, g.-238G>A) were detected in the intron reginos, but the allele frequencies of these SNPs were not significantly different between type 2 diabetic patients and the control group(P>0.05). So we concluded no association of these SNPs in resistin gene with the risk of T2DM was found in elderly Han people in Northeast China.

2712-PO

Comparison of Genome-Wide Variation between Kuwaiti and HapMap Populations

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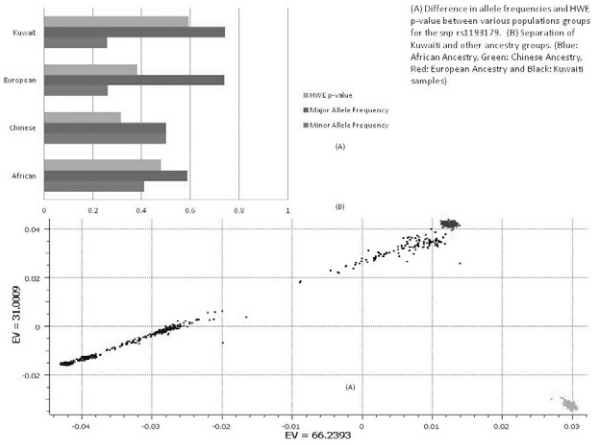
Variations in gene allele frequencies can contribute to differences in the prevalence of complex diseases and differential drug responses among populations. Our analysis with GWAS data showed clear clusters between Kuwaitis and south Asian Group. Therefore, we compared genetic variations frequencies, population substructures and Hardy Weinberg Equilibrium (HWE) between Kuwaiti and other HapMap populations. Allele frequencies were measured for 551345 single nucleotide polymorphisms across subjects. Allele frequencies in the Kuwaiti population (N = 122) were highly correlated with allele frequencies in HapMap populations of European ancestry (CEU (N = 112) and TSI (N=102)) (Spearman's $r^2 = 0.98$). The correlation was lower between Kuwaiti and other ancestry populations ($r^2 < 0.85$). (Chinese Ancestry (CHB (N = 137), CHD (N = 113) and JPT (N = 113)), (African Ancestry (ASW (N = 53), LWK (N=110), MKK (N=150) and YRI (N=147)). Principal component Analysis revealed no population sub-structures among the Kuwaiti subjects but revealed clear clusters of various ancestry groups among HapMap population. A particular SNP of interest to diabetes community is rs1193179 that is associated with *Type II Diabetes Mellitus* in French case-control cohort. Our analysis showed similar allele frequencies (and hence similar risk) for this SNP in European and Kuwaiti population but with different HWE p-values; Cochran-Armitage association test after Bonferroni correction for multiple testing showed -log10 P values ≥ 10 for Chinese and African population, but -log10 P value of 0.024 for European population.



IMMUNOLOGY

2713-PO

Effect of Intensive Diabetes Treatment on the Association Between Periodontal Condition and Glycohemoglobin

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It is well known that diabetic subjects with poor control are at high risk for periodontal disease. But it is unclear whether diabetes treatment improves periodontal condition. The aim of this study was to prospectively assess the effect of intensive diabetes treatment on periodontal disease by evaluating the association between glycohemoglobin (A1c) and variables indicating periodontal condition. We assessed 36 diabetic patients (age: 53.1 ± 13.4 years; 19 men and 17 women) with poor control, who were admitted to our hospital for intensive treatment and education for diabetes management. Scaling and mechanical tooth cleaning were performed, and the patients were given oral hygiene instructions during the course of hospitalization. All the patients were treated as outpatients after discharge. Body mass index (BMI), A1c level and periodontal conditions of the patients were recorded at baseline and 6 months after hospitalization. The variables used for evaluating periodontal conditions included percentage of bleeding on probing (BOP) and plaque control record (PCR). After 6 months, significant reduction was noted in A1c level (10.7 ± 2.2% vs 7.4 ± 1.5%; $p < 0.001$) and the BOP rate (%BOP) (40.6 ± 27.8% vs 23.8 ± 20.7%; $p < 0.001$). However, no significant change was observed in the BMI and the PCR rate (%PCR). Spearman's rank correlation analysis showed positive correlation between ΔA1c and Δ%BOP ($r = 0.388$; $p = 0.019$), while Δ%PCR also tended to be positively associated with Δ%BOP ($r = 0.321$; $p = 0.057$). Furthermore, a stronger correlation between ΔA1c and Δ%BOP was observed among 13 non-smoking patients ($r = 0.757$; $p = 0.003$), whereas no significant correlation was found among 23 smoking patients ($r = 0.020$; $p = 0.927$). Our results suggested that improvement in the A1c level by intensive diabetes treatment might lead to a decrease in the BOP rate, which is a marker of gingival inflammation. PCR rate, which is a marker of oral hygiene status, and a smoking habit could also affect the BOP rate.

2714-PO

A Preliminary Report on Serum Monocyte Chemoattractant Protein-1 (MCP-1) as a Biomarker in Diabetes and Periodontitis

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Role of Serum MCP-1 as a biomarker of periodontitis is well studied. However its role in diabetes with periodontitis is not known. This study was conducted to determine the presence and concentration of serum MCP-1 in diabetic patients with and without periodontitis, and also correlate with glycemic status. A prospective study was conducted with 37 adult diabetic patients (excluding hemoglobinopathies, hypertension, renal and hepatic failure, cardiovascular diseases and pregnancy) grouped into well-controlled diabetes (HbA1c<7%) without periodontitis (n=7) (group I), well-controlled diabetes with periodontitis (n=15) (group II) and uncontrolled diabetes (HbA1c ≥7%) with periodontitis (n=15) (group III). Blood samples were collected and a quantitative assay (ELISA) was done to estimate MCP-1 concentration in

serum samples. All 37 serum samples tested positive for the presence of MCP-1. Mean serum MCP-1 concentration was highest (482.3pg/ml) in group III, lowest (149.3pg/ml) in group I and 398.8pg/ml in group II. Correlation and regression analysis was done between HbA1c and serum MCP-1. A significant positive correlation (p value<0.001) was observed (Figure). Serum MCP-1 increased by 37.278pg/ml for every 1% rise in HbA1c. Serum MCP-1 levels were raised in group II and group III than group I irrespective of their glycemic status. But in group III the levels are markedly increased than group II. With an HbA1c range of 6.5-6.9% (group II), the serum MCP-1 values cluster around 380-410pg/ml. Elevated levels of serum MCP-1 (>500pg/ml) in three subjects correspond to HbA1c values more than 12.2% (group III). To our knowledge this is the first study to document serum MCP-1 levels in diabetic patients with periodontitis. Glycemic status influence serum MCP-1 and lack of glycemic control contributes to increased serum MCP-1 levels. Thus serum MCP-1 may serve as a biomarker of inflammation and disease progression in diabetes with periodontitis.

2715-PO

The Autoantibody of ZnT8 is Related With TRAb in Patients With Graves' Disease

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Graves' disease (GD) and type 1 diabetes are the most common autoimmune endocrine disease. Znic transporter 8 (ZnT8) is one of the most important autoantigen of type 1 diabetes but it has also been reported to be expressed in thyroid tissues. To investigate the correlativity of thyroid-stimulating hormone (TSH) receptor antibody (TRAb), ZnT8 antibody (ZnT8A) and glutamic acid decarboxylase antibody (GADA) in patients with GD we detected the levels of TRAb, ZnT8A and GADA by radioimmunoprecipitation methods from the serum of 51 GD patients without diabetes. The antigens of GAD65 and ZnT8 marked with ^{35}S, were incubated in serum and precipitated by protein A sepharose and counted by the liquid scintillation counter. TRAb were detected by thyrotrophic receptors autoantibody Radioreceptor assay kit. Pearson's correlation coefficients were used to calculate the correlations between the autoantibodies. The results showed that the positive rates of TRAb, ZnT8A and GADA in the patients of GD are 90.2%,15.7% and 5.9% respectively. There was a mild positive linear correlation between TRAb and ZnT8A index ($P<0.05$) and this correlation is increased in TRAb positive patients ($P<0.01$). From the results we can conclude that the positive rate of TRAb is related with ZnT8A level in the patients with GD. Combined detection of ZnT8 and TRAb level may benefit to the diagnosis and the prognosis of GD.

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2716-PO

Inflammatory Cytokine Transcription Profile in Renal Transplant Recipients With Glucose Metabolism Disorders

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Type 2 diabetes (T2D) is a chronic inflammatory disease promoted by changes in immune system function especially in the course of chronic kidney disease. We tested the hypothesis that systemic pro- and anti-inflammatory cytokines are associated with prediabetes and T2D but the profile of pro/anti - inflammatory cytokine expression may be modulated by immunosuppressive therapy in renal transplant recipients. The expression profile of pro-inflammatory cytokines IL-1beta, TNF-α, and the anti-inflammatory cytokine IL-10 were assessed in blood mononuclear cells of 83 patients (53 men and 31 women; aged 45.6 ± 12.1 years) after kidney transplantation (in mean time of 5.3 ± 2.3 years) receiving standard scheme of immunosuppressive therapy. Quantification of IL-1beta, TNF-α and IL-10 mRNAs was performed by real time QRT-PCR technique. Upon the results of the oral glucose tolerance test 14 patients with impaired fasting glucose/impaired glucose tolerance, 11 patients with T2D, and 58 control patients with euglycemia (fasting serum glucose <100 mg/dl), respectively, were identified. Two-sided t-Student test after normalization was applied (statistical signification with <0,05 and power more than 0.8) to compare cytokine profiles of study groups. The mRNA levels of pro - inflammatory cytokines were higher (mean 1,80E+06 copies/1ug total RNA for IL1beta, 7,15E+04 for TNF alfa) than anti-inflammatory one (mean 7,30E+03 copies/1ug total RNA for IL10) in all study groups. There was no statistical significant differences (p>0,05) in expression profile of analyzed gene between T2D, prediabetes and control groups. Although, according to other data, patients with T2D had higher level of serum inflammatory cytokine, in our study prediabetes and type 2 diabetes could not be distinguished by expression profile of those systemic cytokines in renal transplant recipients treated by immunosuppressive therapy. We are planning further, larger studies to verify of this finding.

2717-PO

WITHDRAWN

2718-PO

Diabetes Mellitus Clinico-Pathological Syndromes

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Diabetes Mellitus (DM) is as syndrome resulting from a variety of pathogenetic mechanisms. We have previously characterized two major pattern of beta cell pathology in childhood onset DM, termed pattern A (pA) and B(pB), characterized by the presence and absence respectively of insulin negative islets (pseudo-atrophic islets). In this study we analyzed these patterns in relation to the presence of anti-islet auto antibodies (as determined by radioassay to the islet autoantigens GAD65, ICA512 and Znt8) and clinical diagnosis of Type 1 (T1DM) or Type 2 (T2DM) in organ donors with DM obtained through the JDRF sponsored nPOD program and autopsy cases collected at the University of Colorado Denver. The 4 following groups were thus defined by the combination of clinical diagnosis and beta cell pathology pattern: 1) T 1DM pA; 2) T2DM pA; 3) T 1DM pB and 4) T 2DM pB. These four clinical pathological syndromes had different mean relative insulin positive cell area (RIPCA) by morphometrical analysis (T1DM pA mean RIPCA = 0.2 %, and standard deviation of 0.57%; T2DM pA mean RIPCA = 1.05 %, and standard

For author disclosure information, see page 797.

deviation of 1.48 % ; T1DM pB mean RIPCA = 1.77 %, and standard deviation of 1.66 %; T2DM pB mean RIPCA = 0.9 %, and standard deviation of 1.18 %). T 1DM pB was distinguished from T1DM pA by lack of islet autoantibodies (1/9 antibody positive vs. 20/52), higher mean age of onset (vs. 12.49 yrs 21.67 yrs) and higher prevalence of non- white non-Hispanic ethnicity (7/9 vs. 8/53). T1DM p B could be distinguished from Type 2 pattern B by lack of amyloidosis (0/9 vs. 5/7) and, surprisingly, higher IPRCA. The smallest group consisted of patient with T2DM and pattern A; in these subjects, the mean R IPCA was significantly higher than in subjects with T1DM pA; only two of these subjects were tested for islet autoantibodies and one was positive. These data showed that a combination of clinical diagnosis and pathological data, allow distinction of four diabetic syndromes with different clinical - pathological features and presumably different pathogenesis.

Supported by: JDRF

2719-PO

The Pancreatic Beta-Cell Surface-Specific Monoclonal Autoantibody IC2 for Noninvasive Beta-Cell Imaging and Inhibition of the NKT Immune Regulatory Function

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The monoclonal autoantibody IC2 derived from a BB-rat IgM-kappa hybridoma exhibits unique specificity for pancreatic beta-cells, and thus has potential for assessing beta cell mass in humans and other species. We have cloned the VH and VL domains of the IC2 heavy and light chain genes, sequenced their cDNA and produced smaller recombinant formats in eukaryotic: recombinant scFv, chimeric Fab and IgG1, and a diabody-Gaussia luciferase fusion protein. Three independent proof-of-concept experiments demonstrating IC2 beta-cell specific homing in vivo has been obtained by SPECT, BLI, and radiotracer in vivo biodistribution. The in vivo imaging trials are now further extended to FMT-NIR imaging and photoacoustic imaging in animal models. The IC2 monoclonal autoantibody has no diabetogenic effect and does not inhibit insulin release in vitro. Recently, we have observed that pancreatic beta-cell lines express surface CD1d, and it has be known since the late 1980s, that IC2 binds to selective lipids in immunoTLC, which fits to the expected glyco and phospholipid expression on beta-cells. Based on these data, we tested the functional binding of IC2 to CD1d-lipid complexes, which gave reproducible strong binding and inhibition in NKT hybridoma cell cytokine release assays and microscale thermophoresis. We hypothesized that an anti-idiotypic antibody (monoclonal or polyclonal) might restore the normal CD1d-lipid stimulated NKT activity by blocking the NKT idiotype specific binding site of IC2 to the CD1d-lipid prediabetic in BB-rats and thereby restore normal immune tolerance regulation and avoid autoimmunity to occur. IC2 is world-wide the only explicit pancreatic beta-cell surface specific biomarker and may have multiple applications in diabetes research for preventive interventions restoring normal immune tolerance in autoimmune diabetes and noninvasive imaging of residual functional beta-cell mass.

Supported by: JDRF

2720-PO

True Insulin Allergy: Could this be Exacerbated by Co-Exiting Medications Causing Mast Cell Irritability Locally at the Injection Sites?

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True insulin allergy is rare (<1%). Allergic reactions can be antibody mediated (usually systemic) or mast cell mediated (usually local). Allergic reactions to insulin pose a difficult diagnostic and therapeutic challenge. A 58 year-old man with a 16-year history of type 2 diabetes and $HbA1_c$ of 69mmol/mol required insulin to optimise his glycaemic control. He had also been treated with Simvastatin (15 years) and Perindopril (5 years). He was commenced on Humalog Mix25 but within 2 weeks developed symptomatic and painful erythematous wheals around the injection sites. Novomix30 resulted in a similar reaction. Insulin was stopped, oral hypoglycaemic agents were re-commenced and skin-prick testing performed. Results showed a 1mm wheal to Levemir and Lantus, 3mm to Insuman and 4mm to Novorapid and Humalog (control 1mm, 6mm histamine). Anti-insulin antibodies were detected to bovine, porcine and human insulin. In view of this, Lantus was commenced but this resulted in local wheals at the injection within 1 week. Further skin-prick tests showed 1mm to Insulatard and Actrapid, 2mm to Humulin I, Humulin-M3, Hypurine Porcine and Hypurine Bovine insulins. Allergen-specific IgE antibodies to human, porcine and bovine insulin were again mildly elevated. Whilst the patient has evidence of mild insulin allergy (IgE antibodies), the local reactivity and urticaria suggest that this may have been exacerbated by possible local allergic (mast cell instability) effects related to statins and ACE inhibitors. These co-existing therapies have now been discontinued and the patient will be rechallenged with Lantus after a period of 12 months.

TRANSPLANTATION

2721-PO

WITHDRAWN

2722-PO

Novel Approach to Test for Immune Protecting Properties of Semipermeable Membranes in Pancreatic Islet Transplantation

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Despite being first investigated over 50 years ago, the concept of transplanting pancreatic islets in immunoprotecting devices, that prevent cell migration, but allow transfer of nutrients and proteins, is not completely understood. Since this could be a viable curative option to restoring metabolic homeostasis in people with diabetes, without the need of immunosuppressants, it is crucial, to understand the exact mechanisms of graft protection by encapsulation. Here, we used a novel approach to test the immunoprotective properties of TheraCyte ™ devices. We transplanted encapsulated, neonatal, pancreatic tissue from (i) genetically engineered RIP-LCMV and (ii) control B6 mice, into RIP-LCMV recipients. In this strain, infection with LCMV induces insulitis, and, after viral clearance, leads to a massive CTL response against the viral glycoprotein that is transgenically expressed on β cells, thus resembling an autoimmune attack. 80% of the transplanted mice maintained euglycemia up to 6 months after transplantation and subsequent infection with LCMV virus, whereas 100% of the infected control mice developed full blown diabetes. Histological analysis of the grafts showed good, long term survival, and function of the transplanted cells. However, immunfluorescent staining for CD8 showed, that CD8 positive cells surrounded the encapsulation membrane of devices, containing transgenic RIP-LCMV islets, but not of those, containing control B6 islets. We hypothesize, that protein antigen from the encapsulation device, was able to pass through the membrane pores, and be presented by host antigen-presenting cells. Further investigations are ongoing, to decipher, which of the routes, by which transplanted islets may activate harmful mechanisms, are efficiently blocked by a semipermeable membrane, and which are not. Elucidating this aspect will help, understanding general aspects of graft rejection in organ transplantation.

2723-PO

Diabetes and Waist Circumference Are Associated With Baseline Carotid Intima Media Thickness (CIMT), While Only Cholesterol and Inflammation Are Associated With Progression after Kidney Transplantation

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Causes for cardiovascular disease (CVD) progression after kidney transplant (KTX) have not been well studied. In a longitudinal, observational trial, we evaluated the association of baseline CIMT with survival after KTX and the association of CVD risk factors with baseline and change in carotid intima media thickness (CIMT) over time. All KTX recipients ≥ 6 mo after KTX with GFR >30 ml/min were invited to participate (n=342; 57%M/43%F). Of these, 24% had type 1, 11% type 2, and 15% post-transplant diabetes, while 50% had no diabetes. Mean age was 52.6±0.6y (± SEM) and time since KTX was 5.92±0.33y (0.5-33.8y). Better overall survival was observed in patients with baseline CIMT <0.6mm versus ≥0.6mm (p=0.0013) with estimated 3-year overall survival rate of 97% vs. 57%. Many risk factors were analyzed in univariate analysis: age, gender, race/ethnicity, graft type, smoking, diabetes, blood pressure, BMI, waist circumference (WC), lipids, A1C, GFR, 25-OH-vitamin D, PTH, urine albumin, highly sensitive C reactive protein (hsCRP), homocysteine, hemoglobin, and uric acid. Race/ethnicity, time

since transplant, and diabetes, were included in multivariate analysis with significant ($p \leq 0.15$) univariate factors (age, smoking, urine albumin, cholesterol, hsCRP, hemoglobin, and WC). Of these, age ($p<0.0001$), race/ethnicity ($p=0.0002$), diabetes status ($p<0.0001$), and WC ($p<0.0001$) were independently associated with baseline CIMT, while only hsCRP ($p=0.0086$) and cholesterol ($p=0.03$) were independently associated with CIMT progression over time. In conclusion, baseline CIMT is associated with patient survival after KTX, and age, diabetes, WC more than BMI, and race/ethnicity are independently associated with baseline CIMT. However, only hsCRP, suggesting inflammation, and cholesterol were independently associated with CVD progression after KTX.

2724-PO

Digital Image Analysis (DIA) for Pancreatic Islet Mass and Purity Measurement Better Meets cGMP Requirements in Clinical Islet Transplantation

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Although the mass of transplanted pancreatic islet (IEQ) is the most important factor deciding about the clinical success, the manual islets count is still commonly used for IEQ and purity estimation. Such count can be easy biased, therefore has wide variation and is hardly verified since samples are discarded afterwards. We report our application using a DIA system, which objectively measures IEQ and purity as well as allows saving sample image for records and verification. Our DIA method includes digital picture of the entire islet sample, calibration of the surface area utilizing software Image Pro-plus and calculate IEQ and purity by a custom designed excel template. Five trained technicians participated in the study measuring human islets IEQ and purity in the same samples by both manual count and DIA.

		Inter-technician variation (ITV)			Inter-sample variation (ISV)		
		Manual	DIA	p	Manual	DIA	p
High purity sample 150 µL (n=10)	IEQ Mean ±SD	295 ± 29	282 ± 27	NS	295 ± 47	282 ± 50	NS
	CV% ± SD	10 ± 3	10 ± 5	NS	16 ± 2	18 ± 3	NS
	Purity Mean ±SD	78 ± 7	76 ± 4	NS	78 ± 6	76 ± 5	NS
	CV% ± SD	8 ± 2	5 ± 1	0.001	7 ± 1	7 ± 2	NS
High purity sample 100 µL (n=10)	IEQ Mean ±SD	168 ± 17	157 ± 12	NS	168 ± 41	157 ± 44	NS
	CV% ± SD	10 ± 2	8 ± 2	NS	24 ± 3	28 ± 2	NS
	Purity Mean ±SD	77 ± 7	75 ± 3	NS	77 ± 7	75 ± 6	NS
	CV% ± SD	9 ± 4	4 ± 2	0.006	10 ± 5	7 ± 1	NS
Low purity sample 100 µL (n=10)	IEQ Mean ±SD	97 ± 8	88 ± 8	NS	97 ± 18	88 ± 17	NS
	CV% ± SD	8 ± 3	10 ± 3	NS	19 ± 1	19 ± 3	NS
	Purity Mean ±SD	31 ± 4	38 ± 5	0.001	31 ± 4	38 ± 6	0.009
	CV% ± SD	14 ± 7	13 ± 4	NS	14 ± 1	15 ± 2	NS

IEQ showed no significant difference between both methods. The coefficients of variation (CV) in ISV were under 30% and in ITV under 10% in manual count and DIA. In low purity samples there was significant difference in purity measurement between both methods. Comparison of purity CV showed significant differences in high purity levels of ITV between two methods. We conclude that DIA of islet mass (IEQ) is as reliable as manual count. DIA purity is based on objective, digital and calibrated area measurements; therefore it is more accurate than the traditional subjective estimation. DIA stores a permanent record of the islet sample so allows verification by quality control thus complies better with the cGMP requirements than manual count.

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2725-PO

Impact on Morbidity and Mortality of Intensified Treatment of Hyperglycemia in Patients Undergoing Liver Transplant

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Several studies have shown an association between hyperglycemia and poor prognosis in hospitalized patients. There are few studies which analyze the benefit of the intensive insulin therapy in the field of organ transplantation. The aim was to study the impact of intensive insulin therapy on in-hospital morbidity and mortality in patients undergoing liver transplantation. Longitudinal observational study of a cohort of 94 patients undergoing liver transplantation between March 2010 and February 2011 (Group A), in which a protocol of intensive insulin therapy was established. Morbidity and mortality outcomes were compared with a retrospective series of 92 patients transplanted between January and December 2005 (Group B), who had a looser glycemic control based on an insulin sliding scale. Variables with significant associations were adjusted for confounders (sex, age, BMI, MELD index, etiology of cirrhosis, immunosuppressive therapy). Data are presented as mean (standard deviation) or percentage. There were no significant differences in baseline characteristics between the groups. The protocols for the immunosuppressive therapy induction and antibiotic prophylaxis were the same in both groups. The mean blood glucose during the first 10 days was 141.9 (21.0) mg/dl in group A and 189.6 (45.4) mg/dl in group B ($p<0.001$). The in-hospital mortality rate was 1.1% in group A and 4.4% in group B ($p=0.162$). The average length of hospital stay was 19.1 (13.1) days in group A and 24.2 (22.0) days in group B ($p=0.058$). The infection rate was 27.7% in group A and 41.1% in group B ($p=0.055$). The rate of acute graft rejection was 5.3% in group A and 14.3% in group B ($p=0.040$). Quick index at tenth day was 81.4% (11.3) in group A and 73.4% (14.6) in group B ($p<0.001$). The intensive insulin therapy improves the mean blood glucose during the hospital stay after liver transplantation, and seems to have a positive impact on hospital morbidity in patients undergoing liver transplantation.

2726-PO

Astragalus Polysaccharides Treat Type 1 Diabetes Mellitus in Nonobese Diabetic Mice Transplantated by Bone Marrow Mesenchymal Stem Cells and Its Possible Mechanisms in Immunology

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Astragalus Polysaccharides treat T1DM in Nonobese Diabetic mice transplantated by Bone Marrow Mesenchymal Stem Cells and its Possible Mechanisms in Immunology Backgroud: Bone marrow mesenchymal stem cells (BM-MSC) is one type of stem cells. Astragalus Polysaccharide (APS) has been reported to have immunomodudulatory activities. Our experiment investigated that APS treated T1DM in NOD/Lt mice transplantated by BM-MSC and its possible mechanisms. Methods: Twenty-four spontaneous TIDM NOD/Lt mice were divided into 4 groups. APS group, BM-MSC group, APS plus BM-MSC group, T1DM control group. After 3 weeks' treatment,mice were sacrificed. Proportion of regulatory T cells in the spleen contentand expession of insulin in pancreas were measured. Results: Compared with control group, 3 intervention groups had more stable blood glucose. a higher proportion of regulatory T cells in the spleen content and more insulin expressed, especialy in the BM-MSC plus APS group. Conclusion: The results indicated that the combined group was more effective than two single intervention group, the mechanism may be that they can prevent the further destruction mediated by autoimmune.

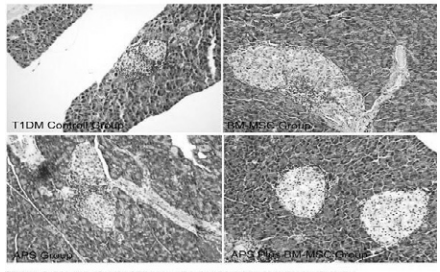

Figure 1:Morphology of islets stained with hematoxylin and eosin 20× magnification

For author disclosure information, see page 797.

2727-PO

Hepatic Hematoma: A Rare Complication after Islet Cell Transplantation

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Percutaneous transhepatic portal access is a minimally invasive technique for islet cell transplant (ICT). Bleeding and portal-vein thrombosis are rare complications. Fifty year-old female with T1DM and severe hypoglycemia unawareness received ICT under immunosuppression with thymoglobulin/sirolimus+tacrolimus. After 1st-infusion 50% reduction in insulin requirement was achieved. Second infusion was performed under same protocol by US/fluoroscopically guided percutaneous-intraportal technique. No significant elevation in portal pressure was observed during ICT. Heparinization and catheter tract sealing protocols were performed. Patient complained of significant abdominal pain during the procedure, without any post-procedure US bleeding evidence. Hemoglobin&hematocrit remained stable. Three days after, she presented to emergency room with severe abdominal pain/nausea/vomiting. An abdominal-CT demonstrated large subcapsular&intrahepatic hematoma with active extravasation of contrast consistent with ongoing arterial bleeding (Hgb:7.5g/dl,Hct:21.5). She required 5-units of PRBC-transfusion. Hepatic arteriogram demonstrated pseudoaneursym and AV-fistula arising from right hepatic artery. Successful coil embolization resulted in stabilization of hemorrhage. Pain management by opiate analgesics was started. Control-CT revealed 14.6x14.4x5.8cm hematoma at 1st-month which continued to cause significant pain likely due to capsular distention. Drainage was offered but patient refused. The hematoma gradually shrank; 5.0x2.5cm at 6th-months, 3.7x1.8cm at one year. Liver size was normal and characteristics with no evidence of steatosis. She achieved insulin independence at 6th-month post-ICT. Most post-ICT bleedings are venous in nature, but in this instance it was the result of a laceration of a hepatic artery that became evident several days after procedure,which was repaired during selective arteriogram. The hematoma resolved with conservative management.

2728-PO

To Investigate How Islet Transplantation Influences Diet, Exercise Habits and Body Composition

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A retrospective study was performed in 33 (14M, 19F) IT recipients using dietary recording, physical activity questionnaire, anthropometric measurements and body composition analysis. Data were collected before transplantation and every 6 months thereafter. Data were grouped by gender (M, F) and eras (pre-IT, 0-3y, 4-6y and 7-10y after IT). Preliminary analysis shows significant reduction in BMI 0-3y after IT (23.68±2,18kg/m^2 to 22.07±2.94kg/m^2, P<0.05). Significant increment between 0-3y and 4-6y was observed in waist circumference (WC), BMI and weight (79.44±7.58cm, 22.75±3.11kg/m^2, 67.43±14kg, P<0.03). WC continues rise till the 10y (86.33±9.45cm P<0.05). Quantity of physical activity performed did not change significantly during time (5.3±5.6 h/wk, 98% cardiovascular of total practiced). Between pre-IT and 0-3y, there was a 19% reduction in protein consumption (P<0.05) and a 39% increase in calories from saturated fat (P<0.05). Tendency of reduction in carbohydrates intake between pre-IT and 0-3y with constant augmentation till 10y was observed (ns). When analyzed by gender, male show WC and BMI reduction between pre-IT and 0-3y; BMI, WC and weight rise at 4-6y and continuous BMI rise till 10y (P<0, 05). They showed reduction in calories intake in the 0-3y period (P<0.05) and increase in the 7-10y (P<0.05) when compared to pre-IT. Female group considerably increase WC at 7-10y in comparison to other periods (P<0.01). A persistent increase in monosaccharide intake (P<0.05) and calories from saturated fat (P<0.05) was observed. Islet transplantation appears to be associated with a significant decreased BMI early on. The subsequent weight gain and waist circumference increase could be a result of chronic immunosuppressive therapy and/or a voluntary change on eating habits. Interestingly, this population knows the importance of exercise, might be related to the understanding of their diabetes management. Constant monitoring of nutritional parameters is needed after IT.

2729-PO

Beneficial Effect of Alpha-1 Antitrypsin Supplementation for Unpurified Islets: Studies on Islet Auto-Transplant Preparations

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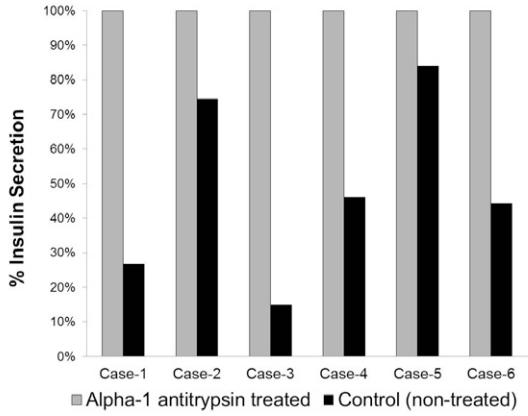
Unpurified islets are commonly transplanted during autologous islet transplantation (IAT), with contaminating acinar cells co-transplanted as impurities. We recently reported the finding of a degrading effect of acinar cell proteases on insulin, resulting in a dysfunctional environment for islets before and after allo-transplantation. We also showed that supplementation of clinical grade alpha-1-antitrypsin (A1AT) in allo islet culture results in restoration of insulin levels and reduced islet loss. In this current work, we extend this study to unpurified islets isolated from chronic pancreatitis (CP) pancreases after overnight culture. CP pancreases (for IAT, n=6) were digested using our standard islet isolation protocol. Islet count samples collected during isolation were cultured with and without A1AT (0.5mg/ml). After overnight culture, samples of culture supernatants were collected to measure the basal insulin accumulation in medium. The insulin release per islet was calculated to compare the effect of A1AT treatment with controls. The numbers of islets in digest counting samples varied from 77-370. The purities of the islet preparations were <5% while the total tissue digest volume ranged from 3.5-40cc. The basal insulin release into the culture medium varied widely (ranges 0.4μU to 24.7μU/IEQ). In the control group, insulin accumulation was lower (average 48%; range 16-75%) compared to the treatment group (Figure). A1AT treatment resulted in higher insulin accumulation in culture medium after overnight culture by blocking acinar cell protease insulin cleavage.

Basal insulin release into the culture medium after overnight culture of islet samples (islet counting samples) from islet auto-transplant preparations



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2730-PO

WITHDRAWN

2731-PO

The Effect of Liraglutide on Tolerance Induction Using Co-Stimulation Blockade

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Islet transplantation is performed in type 1 diabetes mellitus patients as an effective treatment option. However, there are several problems: One of these problems is side effect by life-long immunosuppressive agents, such as infectious diseases, malignant neoplasm. As the solution for this problem, transplantation tolerance is researched by several researchers. We previously reported that the induction of donor-specific immune tolerance with co-stimulation blockade was possible, and that permanent islet graft survival was achieved in mice. In the meantime, Liraglutide, a long-acting human glucagon-like peptide 1 analog, is emerging therapeutic option for type 2 diabetes mellitus patients through the stimulation of insulin secre-

tion, suppression of glucagon secretion and slower gastric emptying. Previous studies showed the beneficial effect by this agent on transplanted islets in human. In this study, the effect of Liraglutide on tolerance induction by the treatment with donor-specific transfusion (DST) plus anti-154mAb (MR1) in mice was examined. The BALB/c (H2d) mice treated with a C57BL/6 (H2k) DST+MR1 were given C57BL/6 skin grafts. The half of the recipients was received 300μg/kg i.p. twice a day of Liraglutide from 7days before induction to the end of all experiments. There were no significant differences on the percentages of $CD4^+CD25^+Foxp3^+$ T cells between before induction and before transplantation. The killing effects to allogeneic cells in the recipients using in-vivo cytotoxicity assay at the time of transplantation were comparable between Liraglutide injected group and no treatment group. The allogeneic skin graft MSTs of these two groups were statistically same. The combination of the tolerance induction treatment using co-stimulation blockade and Liraglutide can be the promising option for islet transplantation, because of the no effect on immune systems and positive effects on transplanted islets of Liraglutide.

2732-PO

WITHDRAWN

2733-PO

Mechanical Immune Isolation System Using Nanofilter for Pancreatic Islet Transplantation

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Pancreatic islet transplantation has recently emerged as an alternative therapy for cure of type 1 diabetes and some type 2 diabetes patients. But immune rejection is still a serious problem that we have to overcome. To minimize both cellular and humoral immune attack on the islet graft, w planned to make a filter to block entrance of immune cells and immunoglobulins while permitting pass of glucose and insulin via the filter. The filter was made of PEO-functionalized straight nanochannel array based on a self-organized porous alumina for a novel biofilter with antifouling, superior immunoprotection and high permeability of nutrients. We performed function tests for adhesion of immune cells on the surface, blocking of immune cells and passing of nutrients. We succeeded in making PEO-coated biofilters with pore size from 14 to 100 nm. The biofilter inhibited adhesion of immune cells on the surface of the filter. Ig-G was effectively blocked by the filter with pore size less than 18nm. In test of passing of glucose, glucose passed through biofilter with pore size more than 26 nm more effectively. We concluded that PEO-coated biofilter with 18~26 nm sized pores could be used for islet transplantation. We think that such the filter based islet transplantation could be applied in a form of vessel type or subcutaneous type.

INSULIN ACTION—INSULIN RESISTANCE IN VITRO

2734-PO

ECM Molecules Increases Myostatin Activity During the Myoblast Differentiation

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Skeletal muscle is the most abundant tissue and the major protein reservo ir in the human body. Muscle mass usually results from a dynamic balance between protein synthesis and degradation. Myostatin is hyperexpressed in several conditions associated with muscle atrophy. We investigated the interaction between specific ECM molecules and myostatin, in order to bet ter understand the mechanisms by which myostatin is modulated in the ECM during the myoblast differentiation. Myostatin expression was gradu ally increased during differentiating myoblast to myotube. During myoblast differentiation in ECM-coated dish, myostatin expression was significantly increased by ECM molecules. Myostatin expression was increased with angiotensin II, TNF-a, and FFA in myotubes. Low-dose insulin (1-10nM) was increased myostatin, myogenin, and myostatin receptor activin R2b, but high-dose insulin (>1uM) was suppressed in myotubes. Only high-dose insulin treatment in ECM-coated dish was arrest differentiating myoblast to myotube. However, in myotubes, increased myostatin by ECM did not suppressed by high-dose insulin. These results suggest that ECM molecules might be modulate myostatin activity even if role of insulin to suppress in muscle cells.

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INSULIN ACTION—METABOLISM

2735-PO

WITHDRAWN

2736-PO

Green Tea Polyphenols Improves Glucose Metabolism Through Enhancing Insulin-Stimulated Glucose Uptake and Glycogen Synthesis

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Green tea polyphenols (GTP) have been found to be beneficial to patient with type 2 diabetes. In this study, we investigated the effects and mechanisms of GTP on glucose metabolism in vitro. Liquid Chromotography-Mass Spectrometry (LC-MS) was carried out to quantify the active polyphenolic components of GTP. The green tea catechins that were identified included (-)-epigallocatechin gallate, (-)-epigallocatechin, (-)-epicatechin gallate and (-)-epicatechin. Methylthiotetrazole (MTT) assay on GTP-treated 3T3-L1 cells confirmed that 10-8-10-4 M GTP did not cause toxicity in cells. Results indicate that 24 hr GTP treatment induced a dose-dependent increased in glucose uptake significantly, whereby 10-5 M GTP increased glucose uptake by 2 fold ($p<0.05$) and 10-4 M GTP increased glucose uptake by almost 3 fold ($p<0.01$). Furthermore, we observed increased protein expressions of IRS-1, AKT and GLUT4 with GTP treatment, suggesting that GTP actions involve in the insulin-signaling pathway. GTP-treated 3T3-L1 cells up-regulated glycogen synthesis in a dose-dependent manner. In the presence of 100 nM insulin, 10-5 M and 10-4 M GTP increased glycogen synthesis by approximately 1.5- fold ($p<0.01$). Further to this, similar results were obtained in HepG2 cells where 10-5 M GTP also brought about a significant increase in the presence of high glucose ($p<0.01$). We observed that 10-5 M GTP increased phosphorylation of both GSK3b and GS ($p<0.05$) in HepG2 cells exposed to both normal (5 mM) and high (30 mM) levels of glucose. This also indicates that a significant increase in phosphorylation of GSK3b and GS leads to a consequent down-regulation of GSK3b and up-regulation of GS expression with 10-5 M GTP treatment. These findings demonstrate the potential of GTP to be used as a glucose-lowering agent for type 2 diabetes.

2737-PO

WITHDRAWN

2738-PO

Akt Regulates Hepatic Metabolism by Suppressing a FoxO1-Dependent Global Inhibition of Adaptation to Nutrient Intake

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Considerable data support the idea that FoxO1 drives the liver transcriptional program during fasting, that its insulin-dependent inactivation by Akt after feeding suppresses hepatic glucose output and that this process is dysfunctional in type 2 diabetes mellitus. Consistent with this model, mice with deletion of hepatic Akt1 and Akt2 were glucose intolerant, insulin resistant, and defective in transcriptional response to feeding in liver. These metabolic defects were normalized upon concomitant liver-specific deletion of FoxO1. Surprisingly, in the absence of both Akt and FoxO1, mice adapted appropriately to both the fasted and fed state, and insulin suppressed hepatic glucose production normally. These data are inconsistent with the canonical model of hepatic metabolism in which Akt is an obligate intermediate for insulin's actions. Global gene expression analysis revealed that, in control animals, Akt maintained suppression of FoxO1-dependent gene expression,

For author disclosure information, see page 797.