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DIABETES CLINICAL STUDIES

SAO060 SYSTEMIC TRANSCRIPTOME ANALYSIS FOR EXTRACTION OF SPECIFIC GENE CLUSTERS IN DIABETIC NEPHROPATHY-REDUCED EXPRESSION OF INSULIN REGULATED AMINOPEPTIDASE ANGIOTENSIN IV RECEPTOR (AT4/IRAP) IN DIABETIC RENAL TISSUE

Tadashi Konoshita[1], Mai Ichikawa[1], Tomoko Kimura[1], Satsuki Sato[1], Miki Fujii[1], Yasukazu Makino[1], Shigeyuki Wakahara[1] and Isamu Miyamori[1]
[1]Fukui University, Eiheiji, Fukui

Introduction and Aims: The renin-angiotensin system (RAS) plays pivotal roles on progression of diabetic nephropathy. However, much remains unknown about the molecular mechanisms involved. The aim of the study was to obtain novel insights into the processes by systemic transcriptome analysis by DNA microarray technology.
Methods: RNA was extracted from a small part of renal cortical biopsy specimens. A total number of 54,675 transcripts expression levels were analyzed systemically with GeneChip Human Genome U133 Plus 2.0 Array (Affymetrix). Specific gene clusters involved in diabetic nephropathy was analyzed by hierarchical clustering method. Extracted candidate genes were analyzed by real-time PCR method with LightCycler (Roche).
Results: Hierarchical clustering analysis with 18 samples showed cluster formation considerably distinct by each original renal condition. By a hierarchical clustering analysis with 3 samples each from minor abnormality and diabetic nephropathy, we divided the transcripts to 12 clusters. By ontology and pathway analysis from the 11th cluster, the renin-angiotensin system was selected as a diabetic nephropathy specific pathway implying the reduction of ACE2, C9orf3, ENPEP and AT4/IRAP, which are thought to exert the effects for degradation of angiotensin II. Further analysis of 78 subjects with real-time PCR method revealed a significant reduction of AT4/IRAP expression in diabetics renal tissues compared to non- diabetics ($p=0.01$).
Conclusions: Recently AT4 (angiotensin IV receptor) was identified as IRAP (insulin regulated aminopeptidase). The AT4/IRAP was originally identified from GLUT4 vesicles and thought to be involved in insulin sensitivity. On the other hands, AT4/IRAP is thought to degrade angiotensin III to angiotensin IV and to further degraded fragments. Thus, the results suggest that reduction of IRAP/AT4 in renal tissue might be involved in formation and progression of diabetic nephropathy.

SAO061 ALBUMINURIA AND RENAL FUNCTION AS RISK FACTORS FOR CARDIOVASCULAR EVENTS AND MORTALITY

Maria Svensson[1], Jan Cederholm[2], Björn Eliasson[3], Bjorn Zethelius[4] and Soffia Gudbjörnsdottir[3]
[1]Department of Nephrology, Sahlgrenska University Hospital, Gothenburg, Sweden, [2]Department of Public Health and Caring Sciences / Family Medicine and Clinical Epidemiology, Uppsala University, Uppsala, [3]Department of Medicine, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden, [4]Department of Public Health and Caring Sciences/Geriatrics, Uppsala University, Uppsala, Sweden

Introduction and Aims: The relationships between renal function, albuminuria, and cardiovascular (CV) outcomes and mortality are still unclear in type 2 diabetes (T2D).
Methods: We studied these and the role of co-existing congestive heart failure (CHF) in an population- based cohort of T2D patients (n=26145) followed for 5 years using data from the National Diabetes Register (NDR), cause of death and hospital discharge registers in Sweden.
Results: During follow-up 11% of patients experienced a CV event (coronary heart disease and/or stroke) of which 4% were fatal. Altogether 5% of the patients died, thus the all-cause mortality rate was 1.9%/year. . Increasing albuminuria at baseline was associated with higher risk of CV and all-cause mortality adjusted for clinical risk factors (no albuminuria and respectively. Risk for CV and all-cause mortality increased with presence of renal impairment (RI), but to a lesser extent (Table 1, Model 1). After adjusting for CHF, albuminuria but not RI was associated with increased risk of CV and all-cause mortality (Table 1, model 2).
Conclusions: Albuminuria and RI are independent risk factors for CV and mortality in T2D, albuminuria being the strongest one and relevant at all levels of RI. After adjustment for pre-existing CHF, RI was not a significant risk factor for non-fatal CV events but only for CV and all-cause mortality.

SAO061 **Table 1.** Hazard ratios (95% CI) for cardiovascular outcomes and all-cause by different degrees of albuminuria and renal impairment in all 26 145 patients with type 2 diabetes followed for 5 years.

	Events N	Model 1 [a] Hazard ratio (95% CI)	P-value	Model 2 [b] Hazard ratio (95% CI)	P-value
Fatal/non-fatal CHD	2109				
Normoalbuminuria		1.0		1.0	
Microalbuminuria		1.24 (1.11-1.38)	<0.001	1.23 (1.10-1.37)	<0.001
Macroalbuminuria		1.43 (1.24-1.66)	<0.001	1.37 (1.18-1.59)	<0.001
Renal impairment		1.13 (1.02-1.25)	0.02	1.09 (0.98-1.21)	0.1
Fatal/non-fatal CVD	2993				
Microalbuminuria		1.27 (1.16-1.39)	<0.001	1.26 (1.15-1.38)	<0.001
Macroalbuminuria		1.32 (1.16-1.49)	<0.001	1.27 (1.12-1.45)	<0.001
Renal impairment		1.09 (1.00-1.19)	0.05	1.06 (0.98-1.16)	0.2
All-cause mortality	2367				
Microalbuminuria		1.36 (1.23-1.50)	<0.001	1.35 (1.22-1.49)	<0.001
Macroalbuminuria		1.54 (1.35-1.76)	<0.001	1.45 (1.27-1.66)	<0.001
Renal impairment		1.17 (1.06-1.28)	0.002	1.11 (1.01-1.22)	0.02

[a]Model 1: Adjusted for age, sex, diabetes duration, HbA1c, systolic BP, BMI, total/HDL chol, TG, smoking, type of glucose lowering treatment, history of CVD, RI of albuminuria.
[b]Model 2: Adjusted as in Model 1 and also for history of CHF.

SAO062 10-YEAR CARDIOVASCULAR RISK IN TYPE 2 DIABETES IS PREDICTED BY MEASURABLE URINARY ALBUMIN EVEN IN THE NORMOALBUMINURIC RANGE:

Esteban Porrini[1], Piero Ruggenenti[2], Nicola Motterlini[3], Annalisa Perna[3], Aneliya Parvanova Ilieva[3], Ilian Petrov Iliev[3], Alessandro Roberto Dodesini[4], Antonio Bossi[5], Giuseppe Sampietro[6], Enrica Capitoni[7], Flavio Gaspari[3], Nadia Rubis[3], Giulia Gherardi[3], Bogdan Ene-Iordache[3] and Giuseppe Remuzzi[3]
[1]Hospital Universitario de Canarias, Tenerife, Spain, [2]Clinical Research Center for Rare Diseases "aldo & Cele Daccò", Mario Negri Institute for Pharmacological Research, [3]Clinical Research Center for Rare Diseases "aldo & Cele Daccò", Mario Negri Institute for Pharmacological Research, [4]Units of Diabetology Azienda Ospedaliera "ospedali Riuniti DI Bergamo", [5]Unit of Diabetology, Treviglio Hospital, [6]Epidemiological Observatory, "azienda Sanitaria Locale Della Provincia DI Bergamo", [7]Research Foundation, Azienda Ospedaliera "ospedali Riuniti DI Bergamo"

Introduction and Aims: Studies evaluating outcomes of subjects categorized according to normo, micro or macroalbuminuria, found that incidence of cardiovascular disease (CVD) is reduced in those with normoalbuminuria. However, in non-diabetic hypertensive patients CVD risk is associated with the level of albuminuria, even within the normoalbuminuric range. Whether and to which extent this applies also to subjects with diabetes is unknown.
Methods: In this extension of the BENEDICT-A trial (concluded in January 2004), 1208 hypertensive, normoalbuminuric type 2 diabetic patients originally randomized to either Trandolapril, Verapamil, their combination, or placebo, were all given ACE-inhibitor (ACEi) therapy after the study end and were followed with pre-planned clinical, laboratory and case record evaluations until December 31, 2008. Data about fatal events and causes of deaths were also obtained by the local Health District Registry. The outcome was a composite endpoint of sudden death; fatal/non-fatal acute myocardial infarction or stroke; unstable angina or transient ischemic attack; revascularization of coronary, carotid or peripheral arteries; and hospitalizations for heart failure. Treatment targets were HbA1C <7% and blood pressure (BP) <120/80 mmHg. Normoalbuminuria was defined as albuminuria <20 μg/min in two of three consecutive overnight urine collections. Relationships between baseline albuminuria and outcomes were evaluated by Cox proportional hazards regression. Age, gender, diabetes duration, smoking, previous CVD, BMI, creatinine, HbA1C, mean arterial pressure, triglycerides and uric acid levels, LDL/HDL cholesterol ratio, lipid lowering therapy and original randomization to ACEi,

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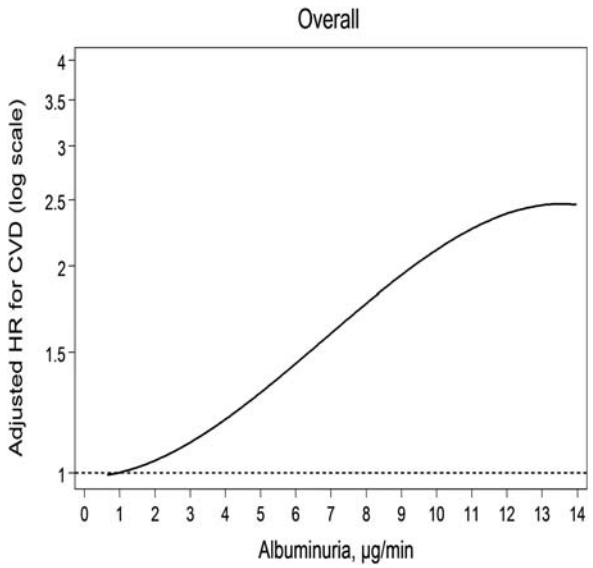
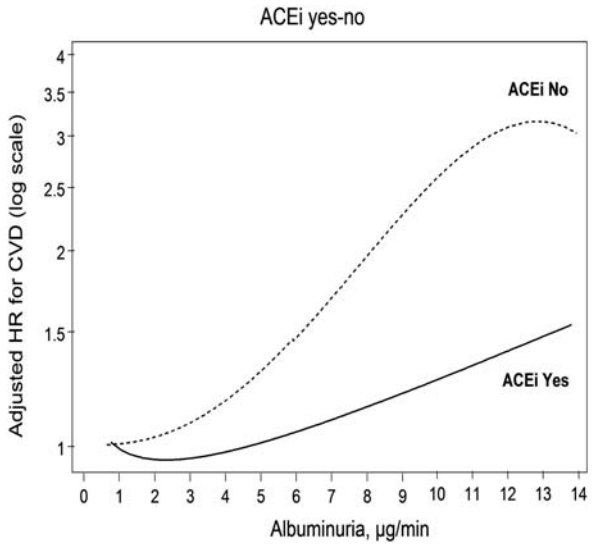
were considered as confounders. Baseline albuminuria was modelled with fractional polynomial algorithm to evaluate non-linear relationships between the variable and events. All continuous variables, including albuminuria, were kept continuous for the analyses.
Results: Of the 1208 BENEDICT-A patients, 1069 (87.4%) were available for analyses. Their median (range) baseline albuminuria was 5.2 (0.44 - 19.85) mg/min: it exceeded 14 mg/min in only 10% of subjects. Over a median (I.Q. range) follow-up time of 9.16 (6.16-9.86) years, there were 2.14 events/100 subjects/year. Of the 189 patients with endpoint events (25 fatal), 85 had been originally randomized to ACEi and 104 non-ACEi therapy (p=0.081). Albuminuria independently predicted events [HR (95% CI): 1.05 (1.02-1.08)]. Using second degree polynomial transformation, a no-threshold continuous non-linear relationship between albuminuria and CVD was observed. Significant risk [HR (95% CI): 1.04 (1.02-1.07] was already evident for albuminuria 1-2 µg/min versus <1 µg/min (reference value) (Figure 1). A similar continuous relationship was observed in subjects originally randomized to non-ACEi. Again, risk [HR (95% CI): 1.02 (1.01-1.04] was already evident for albuminuria 1-2 µg/min versus <1 µg/min (Figure 2). In those originally on ACEi therapy the event rate was uniformly low and independent of albuminuria, without thresholds for risk (Figure 2).



SAO062 **Figure 1**



SAO062 **Figure 2**

Conclusions: In normoalbuminuric type 2 diabetics, any incremental level of albuminuria is significantly associated with excess cardiovascular risk. The association is continuous, without risk thresholds, and is almost blunted with early ACEi therapy.

SAO063 EFFECT OF GLYCEMIC CONTROL ON GLOMERULAR HEMODYNAMIC MEASURED BY INULIN AND PARA-AMINOHYPURIC ACID CLEARANCE IN HUMAN—POOR GLYCEMIC CONTROL INDUCES INCREASED RESISTANCE OF EFFERENT ARTERIOLE

Akihiro Tsuda[1], Eiji Ishimura[2], Yoshiteru Ohno[2], Mitsuru Ichii[2], Shinya Nakatani[2], Katsuhito Mori[2] and Masaaki Inaba[2]
[1]Osaka City University Graduate School of Medine, [2]Osaka City University Graduate School of Medicine

Introduction and Aims: It has been reported that development and progression of diabetic nephropathy is associated with glomerular hypertension and glomerular hyperfiltration, which are induced by increased intrarenal rennin-aldosterone activation, atrial natriuretic peptide, and nitric oxide (Arima S, et al. Nephrol Dial Transplant 2003). Glomerular hypertension was demonstrated to be present not only in type 1 diabetes(Mogensen CE,et al. Semin Nephrol.1990, but also in type 2 diabetes (Jiten P, et al. Kidney Int 1992). However, precise glomerular hemodinamic abnormalities have not been demonstrated in human. In the present study, we examined glomerular hemodynamic by measuring both inulcin clearance (C-in) and para-aminohypuric acid clearance (C-PHA) simultaneously, and analyzed whether glycemic control indices affected glomerular hemodinamic abnormalities in human subjects.
Methods: Thirty-one subjects (age 55.4 ± 14.7 years; 15 males and 16 females; 21 diabetics and 10 non- diabetics), were enrolled in the present study. C-in and C-PAH were measured simultaneously according to the method reported by Horio, et al. (Clin Exp Nephrol 2009). C-in of all the subjects were more than 60 ml/min. According to Gomez formula (Guidi E, et al. Am J Hypertens 2001), resistance of afferent arteriole (R-a), resistance of efferent arteriole (R-e), glomerular hydrostatic pressure (P-glo) and glomerular filtration fraction (FF) were calculated. Association of these values with glycemic control indices was examined.
Results: 1) FF significantly and positively correlated with fasting plasma glucose (FPG), hemoglobin A1C (HbA1C) and glycated albumin (GA) ($r = 0.396$, $p = 0.0303$; $r = 0.587$, $p = 0.0007$; $r = 0.525$, $p = 0.0070$, respectively). 2) P-glo significantly and positively correlated with FPG, HbA1C and GA ($r = 0.572$, $p = 0.0008$; $r = 0.535$, $p = 0.0019$; $r = 0.540$, $p = 0.0053$, respectively). P-glo was significantly associated with FPG, HbA1C and GA after adjustment of age and serum albumin. 3) Although there was no significant correlation between R-a and glycemic control indices of FPG, HbA1C and GA, R-e significantly and positively correlated with HbA1C and GA ($r = 0.499$, $p = 0.0043$; $r = 0.592$, $p = 0.0018$, respectively). R-e was significantly associated with HbA1C and GA after adjustment of age.
Conclusions: These results directly demonstrates that poor glycemic control is associated with increased R- e, but not R-a, in human subjects. It is suggested that increased R-a causes increased P-glo, leading to increased FF. Thus, hemodynamic abnormalities in poor glycemic control is suggested to be related to glomerular hypertension in human subjects, leading to deterioration of glomeruli.

SAO064 TREATMENT OF DIABETIC NEPHROPATHY WITH TRIPTERYGIUM WILFORDII HOOK F: A PROSPECTIVE RANDOMIZED CONTROLLED CLINICAL TRIAL

Yongchun Ge[1], Honglang Xie[1], Shijun LI[1], Bo Jin[1], Jinhua Hou[1], Haitao Zhang[1], Mingjun Shi[1] and Zhihong Liu[1]
[1]Reasch Institute of Nephrology, Jinling Hospital, Nanjing University School of Medicine, Nanjing China

Introduction and Aims: The prevalence of diabetes mellitus (DM) is increasing worldwide, and diabetic nephropathy (DN) is the most common cause of end-stage renal failure. Angiotensin II Receptor Blockers (ARBs) can be used to attenuate the proteinuria in DN patients, but the efficacy is still not satisfactory. The present clinical trial aimed to evaluate the efficacy of *Tripterygium wilfordii* Hook F (TwHF) in treatment of type 2 DM induced nephropathy.
Methods: DN patients with a proteinuria level greater than 2.5 g/24h and serum creatinine less than 3 mg/dl were enrolled in this 6-month, prospective, randomized, controlled study.The patients randomly received treatment with 120 mg of TwHF extract tablets per day (40 mg thrice daily) for 3 months, followed by 60 mg per day (20mg thrice daily) for the rest of 3 months, or 160mg of Valsartan for 6 months. The primary efficacy measure was the reduction of 24-h urine protein from baseline at the end of study, and the secondary efficacy measure was the reduction of eGFR. The adverse events during the follow-up period were also evaluated.
Results: 65 DN patients were enrolled, 34 patients in TwHF group and 31 patients in Valsartan group. At the end of TwHF extract treatment, the urine protein was dramatically decreased (4.99 ± 2.25 g/24 h vs 2.99±1.81 g/24 h, $p < 0.01$), and the percentages of the decrease at months 1, 3 and 6 were 32.9%, 38.8% and 34.3%,

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respectively. In contrast, the proteinuria in the Valsartan group was not significantly attenuated, and the decrease of urine protein was 1.05%, 10.1% and -11.7% at months 1, 3 and 6, respectively. The mean decrease of eGFR in the Valsartan group was significantly greater than that in the TwHF group (26.4% vs 13.7%, $p < 0.05$). There was no significance in adverse events between two groups.
Conclusions: TwHF extract can reduce urine protein of patients with DN, and potentially slowdown the progression of DN. It is thus potentially a novel, effective and safe renoprotective drug for the treatment of DN. TwHF extract would play an important role in the treatment of DN with multi-drug regimens.

SAO065 DIABETES MELLITUS (DM) REDUCES SURVIVAL IN PATIENTS WITH RENAL CELL CARCINOMA (RCC): ROLE OF OGG1 AND TUBERIN.

Simona Simone[1], Marica Cariello[2], Antonio Vavallo[3], Antonia Loverre[4], Elena Ranieri[5], Michele Battaglia[3], Pasquale Ditonno[3], Loreto Gesualdo[6], G Grandaliano[7] and Giovanni Pertosa[1]

[1]Nephrology, Dialysis and Transplantation Unit, Deto, Univ. of Bari, Italy, [2]Nephrology, Dialysis and Transplantation Unit, Deto, Univ. of Bari,Italy, [3]Urology Unit, Deto, Univ. of Bari, Italy, [4]University of Bari, Bari, Italy, [5]Dialysis and Transplantation Unit, Dept. of Biomedical Sciences, Univ. of Foggia, Italy, [6]Dept. of Emergency and Organ Transplant, University of Bari, Italy, [7]Nephrology, Dialysis and Transplantation Unit, Dept. of Biomedical Sciences, University of Foggia

Introduction and Aims: DM represents a major concern for public health worldwide since its incidence and prevalence are constantly growing. Cancer has been recently recognized as one of the main cause of mortality in DM patients. A recent meta-analysis indicated that DM is associated with an increased risk of kidney cancer. 8-oxoG-DNA glycosylase (OGG1) is a specific DNA repair enzyme and its deficiency may increase the risk of cancer. In an animal model of DM, hyperglycemia leads to phosphorylation/inactivation of tuberin and downregulation of OGG1 via a redox- dependentAkt pathway activation in renal tubular epithelial cells. Aims of the study were to investigate whether DM may influence overall and cancer-specific survival in patients with RCC and to explore the hypothesis that the diabetic milieau may lead to increased tumor aggressiveness through tuberin inactivation and subsequent OGG1 downregulation.
Methods: We enrolled 462 patients treated with radical nephrectomy or nephron sparing surgery between 1979 and 2009 for unilateral sporadic RCC with (Group I, n=76) and without DM (Group II, n=386) with a median follow-up of 43 months. To test the association of DM with survival, Kaplan-Meier Method and multivariate Cox model were applied. We collected renal tissue sample from 13 patients of Group I and 13 of Group II to evaluate phospho-tuberin, OGG1 and phospho-S6p70protein expression by Immunofluorescence/immunohistochemical and immunoblotting.
Results: At the time of surgery, Group I showed a larger tumor size (p=.02), a higher percentage of necrosis (<.001) and metastases (<.001) compared with Group II. RCC cancer mortality, in the univariate analysis, was significantly associated with DM, tumor size, tumor grading, coagulative necrosis, TNM and UISS stage system. A significant excess risk emerged in multivariate analysis for DM patients compared with non-DM patients (HR 4.1; 95% CI; 2.6-6.4, p<.0001). We observed a significant increase (p<.05) in tuberin and S6p70 (p=.04) phosphorylation and a downregulation of OGG1 protein expression (p=.002) in Group I compared to Group II by immunoblotting. Immunohistochemical/Immunofluorescence analysis confirmed a reduction of OGG1 (p=.0006) and a significant up-regulation of tuberin phosphorylation in group I (p<.0001). Both normal kidney and tumor tissue samples of DM patients were characterized by a striking increase in nuclear 8-oxo-dG levels.
Conclusions: DM may significantly reduce overall and cancer specific survival in patients with kidney cancer and this observation might be at least partially explained at the molecular level by the reduced expression of OGG1.

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