論文の内容の要旨

Genetic susceptibility for diabetic nephropathy and retinopathy in Type II diabetic patients

-Searching for genetic markers-

2型糖尿病患者における糖尿病性腎症と網膜症の遺伝的susceptibilityの検討

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Introduction. Since insulin and oral antihyperglycemic agents are available, not diabetes per se, however, diabetic complications, such as nephropathy, and retinopathy, which evolve from long-term hyperglycemia, are responsible for excess morbidity and mortality in diabetic patients. Diabetic retinopathy leads frequently to blindness, and diabetic nephropathy accounts for about one third of the newly-introduced patients to the hemodialysis program in Japan. While the patients experience a steadily decreasing quality of life, the country has to cope with a huge financial burden for the social welfare and health care system.

Epidemiological studies showed, that more or less half of the patients seem to be protected from these complications during their life-time, while others develop severe retinopathy and/or nephropathy despite tremendous efforts to control blood sugar level, and hypertension. Growing evidence from epidemiological and family studies suggests, that hyperglycemia is only a pre-requisite, whereas genetic susceptibility is the trigger for the development and/or progression of complications. The impact of the metabolic milieu might be dependent on the extent of genetic predisposition. Discovering genetic risk factors would give us the tool to screen for patients at high risk, who need intensified treatment to prevent/postpone the onset of diabetic

retinopathy and nephropathy.

Diabetic nephropathy is associated with rising blood pressure, and increasing incidence of coronary artery disease (CAD), due to endothelial cell dysfunction and atherogenic changes of rheological factors, and lipid-profile. Characteristic features of diabetic retinopathy are microaneurysms, excessive vascular permeability, vaso-obliteration, and neoformation of retinal capillaries as well as proliferation of fibrous tissue. Elevated blood pressure contributes to the worsening of both complications.

Objective. Based on this rational, it was hypothesized, that the methylenetetrahydrofolate reductase (MTHFR) gene polymorphism with impact on atherosclerotic vascular changes through hyperhomocysteinemia, may cause predisposition to diabetic nephropathy and retinopathy. Therefore the MTHFR genotype was evaluated in Japanese Type II diabetic patients, and Danish Type I diabetic patients. In order to estimate, whether there is a causal connection between the MTHFR genotype and hyperhomocysteinemia, the homocysteine (tHcy) plasma values were measured in patients and controls with normal kidney function.

Furthermore, the endothelial cellular nitric oxide synthase (ecNOS) gene polymorphism, which is involved in the regulation of blood pressure, and endothelial function, as well as the polymorphism of platelet activating factor acetylhydrolase (PAFah), which is suggested to prevent atherogenic changes, were studied in Japanese Type II diabetic patients. The association of diabetic nephropathy, retinopathy, and hypertension with MTHFR-, ecNOS-, and PAFah gene polymorphism was evaluated in cross-sectional studies.

Research design and methods . *Patients:* The study population was based on Japanese (in birth and lineage) Type II diabetic patients, healthy controls and non-diabetic patients with end-stage renal disease with 20 to 70 years of age. Clinical data of each individual were obtained from hospital records and by personal questioning, following a questionnaire concerning ethnic origin, family history of diseases, history and treatment of diabetes and other diseases (especially of the kidney and renal tract). Danish Type I diabetic patients were attending the outpatient clinic at Steno Diabetes Center. Examined were all records of albuminuric patients with less than 70 years of age. Type I diabetic patients with persistent normoalbuminuria (urinary albumin excretion rate (UAE) less than 30 mg/24 h) were matched for sex,

age and duration of diabetes. Diabetic nephropathy was diagnosed clinically based on the following criteria: persistent dip-stick positive proteinuria (greater than 200 mg/24 h) in at least two out of three consecutive 24-h urine collections, or end-stage renal failure, presence of retinopathy and no clinical or laboratory evidence of renal tract or kidney disease other than diabetic glomerulosclerosis. Microalbuminuria was defined as the average of three measurements of urinary albumin to urinary creatinine ratio between 20 to 200 mg/g creatinine in proteinuria-negative patients. The diagnosis of diabetic retinopathy was assessed by fundus photography after pupillary dilatation. Coronary artery disease and hypertension were diagnosed according to WHO criteria. The investigation of blood chemistry was performed in the morning after an overnight fast. The data were kindly provided by the laboratories of the hospitals, where the patients were treated. The experimental design was approved by the ethical committee, and all patients and controls gave their written informed consent.

Genotype analysis: Genomic DNA was extracted from peripheral blood leukocytes of each individual. The presence of a mutation was identified by digestion with *restriction enzymes* (restriction endonuclease *HinfI* for MTHFR genotype detection, *MaeII*, for PAFah genotype detection), which cut at the mutation sites. The results have been made visible by agarose gel electrophoresis and ethidium bromide fluorescence in reference to a molecular weight marker.

Statistical analysis: Non-continuos variables, genotype distribution and allele frequency of the study groups were compared with the chi-square test and Fisher's exact test. Odds ratio (OR), and multiple regression analysis were performed, where appropriate. A two-sided p value < 0.05 was regarded as significantly different.

Results. The MTHFR gene polymorphism is an independent genetic risk factor, which contributes to diabetic retinopathy, and a 2.55 higher OR of progression to diabetic nephropathy in Japanese Type II diabetic patients. However, there was no association of the MTHFR gene polymorphism with diabetic nephropathy, micro-(retinopathy) and macrovascular disease in Danish Type I diabetic patients, which may be due to ethnic difference and different type of diabetes. Although, hyperhomocysteinemia was not correlated to the genotype, there was an association of high tHcy plasma level with diabetic nephropathy and hypertension in Type II diabetic patients. The ecNOS4a-allele was associated with a 2.87 higher OR of progression to diabetic nephropathy than homozygotes for the wild-type allele. No association,

however, was found between ecNOS4a-allele and diabetic retinopathy or hypertension. PAFah gene polymorphism, which was reported to be associated with cardio-vascular disease, was not associated with either of the diabetic complications, however, one cannot exclude the influence by survivors on the study outcome.

Conclusion. The study of Japanese Type II diabetic patients revealed, that among the evaluated gene polymorphisms, ecNOS and MTHFR gene polymorphism are strong predictors for diabetic nephropathy, and MTHFR is a risk factor for diabetic retinopathy as well. The negative results of Danish Type I diabetic patients demonstrate ethnic differences of genetic markers. These study results may serve as bases for further investigations in large cohort studies in different ethnic populations. Whether there is a causal connection between the ecNOS- or MTHFR genotypes and diabetic nephropathy or retinopathy remains to be elucidated in prospective trials.