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Association of rs 3807337 polymorphism of CALD1 gene with diabetic nephropathy occurrence in type 1 diabetes — preliminary results of a family-based study

Związek polimorfizmu rs 3807337 genu *CALD1* z nefropatią cukrzycową w przebiegu cukrzycy typu 1 — wstępne wyniki badania rodzin

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Abstract

Introduction: The worldwide growing burden of diabetes and end-stage renal disease due to diabetic nephropathy has become the reason for research looking for a single marker of chronic kidney disease development and progression that can be found in the early stages of the disease, when preventive action delaying the destructive process could be performed. The aim of the study was to investigate the influence of rs3807337 polymorphism of the *caldesmon 1 (CALD1)* gene located on the long arm of chromosome 7 encoding for protein that is connected with physiological kidney function on development of diabetic nephropathy.

Material and methods: There was an association study of rs3807337 polymorphism of the *CALD1 gene* in parent-offspring trios by PCR-RFLP method. Ninety-nine subjects: 33 patients with diabetic nephropathy due to type 1 diabetes and 66 of their biological parents, were examined. The mode of alleles transmission from heterozygous parents to affected offspring was determined using the transmission disequilibrium test.

Results: The allele G of rs3807337 polymorphism of the *CALD1* gene was transmitted to affected offspring significantly more often than expected for no association.

Conclusions: The obtained results suggest that the genetic variability of the *CALD1* gene may influence the development of diabetic nephropathy in type 1 diabetes, but further studies involving larger studied groups of patients are needed. **(Endokrynol Pol 2017; 68 (1): 13–17)**

Key words: *diabetic nephropathy; type 1 diabetes; CALD1; gene polymorphism*

Streszczenie

Wstęp: Znaczne rozpowszechnienie występowania cukrzycy i schyłkowej niewydolności nerek na tle cukrzycy na całym świecie sprawia, że poszukuje się obecnie pojedynczego markera rozwoju i progresji przewlekłej choroby nerek, który mógłby być wykrywany na wczesnych etapach choroby, gdy możliwe byłoby jeszcze podjęcie działań prewencyjnych w celu opóźnienia destrukcyjnego procesu chorobowego. Celem badania było sprawdzenie czy istnieje związek polimorfizmu rs3807337 genu kaldesmonu 1 (CALD1) zlokalizowanego na ramieniu długim chromosomu 7, który koduje białko związane fizjologicznie z funkcją nerek, z rozwojem nefropatii cukrzycowej.

Materiał i metody: Przeprowadzono badanie związku polimorfizmu rs3807337 genu CALD1 w układzie rodzice–dziecko przy użyciu metody PCR-RFLP. Przebadano 99 osób w tym 33 pacjentów chorujących na nefropatie cukrzycową w przebiegu cukrzycy typu 1 i 66 ich biologicznych rodziców. W celu oceny sposobu przekazywania alleli od heterozygotycznego rodzica do dotkniętego chorobą dziecka wykorzystano test nierównowagi transmisji (TDT, *transmission disequilibrium test*).

Wyniki: Allel G polimorfizmu rs3807337 genu CALD1 był przekazywany chorym dzieciom istotnie częściej niż w przypadku braku związku.

Wnioski: Uzyskane wyniki sugerują, że zmienność genetyczna genu CALD1 może wywierać wpływ na rozwój nefropatii cukrzycowej w przebiegu cukrzycy typu 1, ale niezbędne są dalsze badania na większej populacji osób. **(Endokrynol Pol 2017; 68 (1): 13–17)**

Słowa kluczowe: *nefropatia cukrzycowa; cukrzyca typu 1; CALD1; polimorfizm genu*

Introduction

Incidence of type 1 diabetes increases continuously worldwide [1–3] the epidemiology of T1DM among Korean youth has not been reported since 2001. We investigated the incidence of T1DM in Korean children and adolescents from 2012 to 2014 and compared it with data from 1995 to 2000. PATIENTS AND METHODS: Data were obtained from the National Health Insurance Service (NHIS). Diabetic nephropathy (DN) is one of the most serious complications in patients with type 1 diabetes associated with an increased risk

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of progression to end-stage renal disease (ESRD) and one of the major predictors of premature death [4, 5] the association between rapid GFR decline and renal hyperfiltration is not well described in Type 1 diabetes. We hypothesized that renal hyperfiltration (estimated glomerular filtration rate, eGFR \u2265 120 mL/min/1.73 m(2). DN is a major cause of ESRD in many places in the world, and its incidence has increased in the last decade [5] normoalbuminuric after\u2265 15 years of T1D; and 60 were DN Cases, 25 with proteinuria and 35 with ESRD.\n\nINTERVENTION(S). The development and progression of diabetic nephropathy have been delayed since introduction of renin-angiotensin system inhibitors and improvements in glycaemia and blood pressure control; however, a significant proportion of patients with diabetes develop nephropathy even though their glycaemic control is good [6]. The environmental risk factors linked to the occurrence and progression of DN, which have been recognised up to date, are not sufficient enough for identification of groups of people at higher risk of the disease development early enough to stop or slow down the disease onset and progression as well as to develop new and efficient treatment methods. Peak incidence of diabetic nephropathy appears after 15–20 years of disease duration and declines afterwards, meaning that the cumulative incidence of diabetic nephropathy is not more that 30%. It is therefore obvious that is it not only hyperglycaemia itself that leads patients to end-stage renal disease but also some patients are predisposed to nephropathy development despite glycaemic control [7] DKD aggregates in families and has higher incidence rates in African, Mexican, and American Indian ancestral groups relative to European populations. The Family Investigation of Nephropathy and Diabetes (FIND). There is strong evidence coming from epidemiological and family studies indicating that a genetic predisposition to diabetic nephropathy exists, but unfortunately no evident causative genes have been recognised to date [8, 9] which determines most of ACE interindividual variance, was proposed as a genetic marker for diabetic nephropathy. A substitution (M235T). In a view of the foregoing there are a lot of expectations put on genetic studies [10] Embase and Cochrane Library on 1 October 2011, and eligible investigations were identified and synthesized using the meta-analysis method. Results were expressed using odds ratios (OR). Due to the hypothesis that common genetic variants predispose to common diseases, there is a rise in interest in a single nucleotide polymorphism (SNP) [11]. One of the approaches for association studies of complex traits is called the transmission/disequilibrium test (TDT) [12]. The test examines the transmission of a particular molecular variant (allele) from heterozygous parents to affected offspring, and the observed transmission is compared with the transmission expected for no association (i.e. random transmission of 50:50% for two-allele systems) [12,13] several studies that have sought to show linkage with IDDM by testing for cosegregation in affected sib pairs have failed to find evidence for linkage. As means for identifying genes for complex diseases, both the association and the affected-sib-pairs approaches have limitations. It is well known that population association between a disease and a genetic marker can arise as an artifact of population structure, even in the absence of linkage. On the other hand, linkage studies with modest numbers of affected sib pairs may fail to detect linkage, especially if there is linkage heterogeneity. We consider an alternative method to test for linkage with a genetic marker when population association has been found. Using data from families with at least one affected child, we evaluate the transmission of the associated marker allele from a heterozygous parent to an affected offspring. This approach has been used by several investigators, but the statistical properties of the method as a test for linkage have not been investigated. In the present paper we describe the statistical basis for this \"transmission test for linkage disequilibrium\" (transmission/disequilibrium test [TDT]). There is emerging evidence that actin cytoskeleton may be an important target for injury in diabetic nephropathy triggered by high glucose, and this implies the development of diabetic glomerulosclerosis, which in turn is the pathological hallmark of diabetic nephropathy [14]. Caldesmon is a cytoskeletal protein of smooth muscle and modified smooth muscle cells, such as mesangial cells, which promotes assembly or disassembly of actin stress fibres and inhibits cell contractility [15, 16]. There has been one study performed that found association of –579 A > G single nucleotide polymorphism (SNP) in the promoter region of caldesmon gene with type 1 DN in the Northern Ireland population, confirmed in an independent sample from the Republic of Ireland [17]. Additionally, it has been proven recently that the caldesmon gene is over-expressed in type 1 diabetic nephropathy [18]. It is suggested that disruption of actin cytoskeletal structure in mesangial cells exposed to high glucose can be associated with glomerular hyporesponsiveness to vasopressor stimuli, which can be potentially responsible for vasodilatation of the afferent arteriole, intraglomerular hypertension, glomerular dilatation, and consequential hyperfiltration, which can be observed in early stages of diabetic nephropathy [19, 20]. Baring mentioned information in mind it is reasonable that caldesmon could be a good candidate gene for diabetic nephropathy. Since caldesmon is a protein that is over-expressed in type 1 diabetic patients with

diabetic nephropathy, it possibly plays an important role in diabetic nephropathy onset, and there was only one study performed to date that confirmed the potential connection of its SNP with diabetic nephropathy, which is why it seems important to continue studies of this polymorphism.

The aim of the presented study was to evaluate the association of rs3807337 polymorphism of the *CALD 1* gene with diabetic nephropathy occurrence in type 1 diabetic patients. The gene of this protein was chosen due to its important role in kidney function and limited data of previously performed studies. The presented study represents preliminary data where we have examined only a small number of patients already presenting the end stage of renal disease due to diabetic nephropathy, but we plan to enlarge the number of patients enrolled in order to have better statistical power of the studied sample and additionally include patients at stage 3 chronic kidney disease (CKD) to examine a potent association of studied polymorphism with kidney function (estimated glomerular filtration rate) over the next five years (rate of progression of renal function loss).

Material and methods

The studied polymorphism was genotyped in a group of 33 family trios: offspring affected with diabetic nephropathy, and their both parents.

Selection of family trios

Subjects for the study were recruited from the Dialysis Centre in Zabrze, Poland. The main inclusion criteria were the existence of type 1 diabetes, end stage renal disease due to diabetic nephropathy, and patients thought to have both parents alive. There were 33 patients with diabetic nephropathy due to type 1 diabetes mellitus included. All patients and their biological parents gave written informed consent, and the study was carried out in accordance with the Declaration of Helsinki, and the protocol was approved by the University Ethics Committee.

Diagnosis of underlying aetiology of CKD

Diagnosis was made after examination of urinary albumin excretion rate (obtaining in two out of three measurements an outcome of 30–299 mg albumin in 24-hour urine collection or ≥ 300 mg of albumin in 24-hour urine collection) and analysis of clinical history (lack of other clinical, laboratory, or radiological evidence of nondiabetic kidney or urinary tract disease). Urinary albumin was measured using a commercially available kit. The urine collection procedure was performed from 8:00 p.m. to 8:00 p.m. during three consecutive days.

DNA Analysis

From all patients and their biological parents, genomic DNA was isolated from peripheral blood leukocytes. Genotyping was performed in a blinded fashion.

Genotyping of rs3807337 polymorphism of the *CALD1 gene.*

Genomic DNA was extracted from frozen whole blood samples containing EDTA as an anticoagulant by use of the MasterPure TM DNA purification kit (Epicentre Technologies) and resuspended in TE. Primers (Epicentre Technologies) used for amplification were as follows: forward 5′ GTG GGG AAG GCT TCT TAC CAG AG - 3′; and reverse: 5′ - AGA CCC GGG TCC TGT CTC CCT C- 3′, where C is a changed nucleotide (TIP MOLBIOL). PCR amplification was performed in a total volume of 25 μl, which contained 200 ng of genomic DNA. Reaction mixture contained: 2.5 mmol/L of each deoxynucleotide triphosphate, 10 pmol of each primer, 1.5 mmol/L magnesium chloride, 0.5 U of thermostable Taq DNA polymerase (DyNAzyme ™ II, Finnzymes) with buffer for it, and sterile water. PCR amplification consisted of an initial denaturation at 94°C for five minutes, followed by 35 cycles of denaturation at 94°C for one minute, annealing at 60°C for one minute, and extension at 72°C for one minute, and a final extension at 72°C for seven minutes. PCR products were digested with DdeI enzyme (Q-BIOgen) for the detection of rs3807337 polymorphism of the *CALD1 gene.* All samples were analysed on 2% agarose gel electrophoresis and visualised by ethidium bromide staining in UV light (Vilber Loumat transilluminator UV).

Other determinants and data processing

Anthropometric measurements (height and weight) were measured by standard methods. Serum creatinine was measured by Jaffe's method (Cobas Integra 800, Roche Diagnostics). All patients had BMI calculated as weight/height^2 (kg/m^2), and the glomerular filtration rate (GFR) per 1.73 m^2 was estimated according to MDRD: 186 × {serum creatinine (mg/dL)} – 1.154 × {age (years)} – 0.203 × (0.742 if woman) [27].

Statistics

All statistical calculations were performed using Microsoft Office Excel 2015 and Statistica 12.5, StatSoft Inc. (USA). Shapiro-Wilk test for normality was used. Descriptive statistics for continuous parameters of normal distribution were arithmetic means ± SD (standard deviation), or geometric means (interquartile range) for continuous data that did not have normal distribution. Categorical variables were absolute value and percentage. Accordance with Hardy-Weinberg was tested with Pearson 2 test with Yates correction. In the TDT, the observed transmission of alleles from heterozygous

Table I. *TDT for transmission frequency of A and G allele of rs3807337 polymorphism of CALD1 gene in type 1 diabetic patients with diabetic nephropathy*

Tabela I. *Test nierównowagi transmisji częstości przekazywania alleli A i G polimorfizmu rs3807337 genu CALD1 wśród pacjentów chorujących na cukrzycową chorobę nerek w przebiegu cukrzycy typu 1*

	A allele transmitted	A allele not transmitted	G allele transmitted	G allele not transmitted	Total number of transmitted alleles	$\chi 2$	p
Observed	8	18	18	8	26	3.12	0.049
Expected	13	13	13	13			

parents to affected offspring was compared with an expected proportion of 50% transmission for an allele not associated with the phenotype, and McNemar's test was used for the comparisons.

The two-tailed statistical significance was set at $P < 0.05$.

Results

All of the 33 type 1 diabetic patients enrolled in the study were on maintenance haemodialysis (eGFR enrolment 10.4 (8.7–15.6) mL/min/1.73m$^{2)}$. There were 48.5% of female patients. The average age of study population was 38.0 (32.0–48.0) years, the average BMI value was 22.0 (18.0–24.0) kg/m^2. The genotype distributions of studied polymorphism were in Hardy-Weinberg equilibrium. The allele G of rs3807337 polymorphism of the CALD1 gene was transmitted to affected offspring significantly more often than expected for no association ($P = 0.049$) (Table I). Genotype AA, AG, and GG distribution of rs3807337 polymorphism of the CALD1 gene among 33 patients with type 1 diabetes was 10 (30%), 18 (55%), and 5 (15%), respectively, and allele A and allele G distribution was 38 (58%) and 28 (42%), respectively.

Discussion

On the basis of the performed analyses, allele G of rs3807337 polymorphism of the *CALD1* gene, previously named –579A > G, has been proven to be associated with the occurrence of the DN among type 1 diabetic patients. Data regarding studies of influence of genetic variations of the caldesmon gene on diabetic nephropathy are very limited. We found only one study (a case-control one), performed by Conway et al., examining the association of polymorphism of this candidate gene with diabetic nephropathy development in type 1 diabetes, and our family-based association study confirms their findings [17]. Evidence also exists that there are some proteins differentially expressed in cultured fibroblast in patients with type 1 diabetes with established nephropathy, compared to the ones without that complication and, typically, altered protein classes consist of the actin-associated protein, including caldesmon [21]. This information is consistent with the results of in vivo and in vitro studies pointing out the importance of genes that regulate the actin filament organisation in diabetic nephropathy development. This in turn is in line with the theory that actin cytoskeleton is a major target for injury triggered by high glucose concentration in diabetic nephropathy and contributes to diabetic glomerulosclerosis onset [14, 19]. Typical changes in early stages of diabetic nephropathy consist of afferent arteriole vasodilatation, intraglomerular hypertension, and renal hyperfiltration [6]. Milioni et al. have proven recently that caldesmon gene is overexpressed in fibroblast from type 1 diabetic patients with diabetic nephropathy compared to the ones without nephropathy and those from controls, and they concluded that the association between the caldesmon gene and susceptibility to diabetic nephropathy is consistent with a key role of this protein in diabetic nephropathy development, and they suggest that variation in this gene as well as gene protein could serve as a marker of diabetic nephropathy, but further and larger studies are necessary [18].

Molecular medicine, where genetic linkage studies, gene expression experiments, and candidate studies are performed, offer the possibility of revealing the genetic basis of complex diseases such as diabetic nephropathy. If it transpires that major genes involved in diabetic nephropathy susceptibility become identified, intensive multifactorial treatment could be targeted early enough in predisposed individuals, and maybe more novel therapeutic agents could be developed [29].

A major limitation of our study at the time was the sample size, but this is only a preliminary data report and the obtained outcome encourages us to enrol more patients with time and at earlier stages of CKD, in order to observe their kidney function over time and examine the association of studied SNP not only with development but also with progression of renal function loss.

Conclusions

In conclusion, the obtained outcome of our study suggests that in addition to non-genetic risk factors, rs3807337 polymorphism of *CALD1* gene alone or with interaction of other polymorphisms may play a role in predisposition to DN occurrence in patients with type 1 diabetes, but further, larger studies are necessary to confirm the preliminary data.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Informed consent was obtained from all individual participants included in the study.

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