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Increased frequency of G-protein β_3-subunit 825 T allele in dialyzed patients with type 2 diabetes

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Background. A polymorphism (C825T) in exon 10 of the gene encoding the β_3 subunit of heterotrimeric G proteins (GNβ3) has recently been described, and the T allele was found to be associated with late-onset hypertension. Because hypertension is a known risk factor for the development of clinically manifest progressive renal disease, we examined the C825T polymorphism in older hemodialysis patients suffering from nondiabetic renal disease or type 2 diabetes with presumed diabetic nephropathy, respectively, and in older healthy controls.

Methods. Genotyping was performed by polymerase chain reaction, followed by restriction enzyme analysis.

Results. The study showed that the frequency of the T allele in the nondiabetic patients on dialysis (0.232) was significantly ($P < 0.03$) lower than in older healthy controls (0.293). In contrast, the frequency was significantly ($P < 0.02$) higher in older patients with type 2 diabetes on dialysis. No significant change in T-allele frequency was noted in older patients with type 2 diabetes without microangiopathy (0.286). The odds ratios for patients with type 2 diabetes on dialysis versus nondiabetic patients on dialysis were 3.24 (1.3 to 7.9, $P < 0.00079$) for TT/CC and 1.82 (1.07 to 3.09, $P < 0.02$) for CT/CC. The respective odds ratios for patients with type 2 diabetes on dialysis versus controls were 2.05 (1.07 to 3.9, $P < 0.028$) for CT/CC and 1.216 (0.79 to 1.87; $P < 0.37$) for CT/CC.

Conclusion. The data do not support a role of the hypertension-associated T allele in the genesis of dialysis-dependent end-stage renal failure in general, but are compatible with a specific role of the T allele in the development or progression of diabetic nephropathy.

Several observations point to genetic predisposition of hypertension as an important factor determining the presence of clinically manifest renal disease. This is true in IgA glomerulonephritis, in which Schmid et al and Boulahrouz et al found a higher frequency of hypertension in parents of propositi than in parents of control individuals [1, 2]. Evidence for a role of genetic predisposition to hypertension was also provided in patients with type 2 diabetes and nephropathy. Strojek et al examined the offspring of patients with type 2 diabetes with or without nephropathy [3], and found that the offspring of parents with type 2 diabetes plus nephropathy had significantly higher blood pressure values.

Recently, Siffert et al described a polymorphism of the β_3 subunit of G proteins, which was associated with late-onset hypertension [4]. The rationale for examining the G protein was based on previous findings that in immortalized B cells of patients with essential hypertension, the pH-dependent activation kinetics of the sodium proton exchanger, which is a G-protein controlled process, were increased [5]. This was also specifically true in patients with type 1 diabetes and diabetic nephropathy [6].

Based on these observations, we reasoned that the hypertension-associated T allele of this polymorphism should be enriched in a population of older dialysis patients with or without type 2 diabetes. Our study was carried out to test this hypothesis.

METHODS

Patients

All available patients with the onset of hemodialysis treatment after the age of 60 years were recruited from the dialysis centers in Heidelberg, Heilbronn, Mannheim-Käfertal, Villingen-Schwenningen, and Wiesloch (Table 1). Of these patients, 125 had nondiabetic renal disease, and 130 had type 2 diabetes according to National Diabetes Data Group criteria [7]. Of the nondiabetic patients, 29 had glomerulonephritis, 22 autosomal dominant polycystic kidney disease (ADPKD), 9 analgesic nephropathy, 9 urological problems, and 56 were of unknown or other causes. In addition, patients (*a*) with

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Table 1. Characteristics of our study population

	Elderly patients on dialysis without type 2 diabetes	Elderly patients on dialysis with type 2 diabetes	Elderly patients with type 2 diabetes without microangiopathy (microalbuminuria, retinopathy[a])	Controls
N	125	130	77	357
Age *years*[b]	71 ± 7.2	70.8 ± 7.0	64.3 ± 9.7	56.7 ± 4.4
Gender distribution *female/male*	59/56	58/72	44/33	141/216
Duration of hemodialysis *years*	5.99 ± 5.36	3.28 ± 2.44	—	—

[a] Data given as mean ± standard deviation
[b] Known duration of type 2 diabetes > 5 years

a known duration of diabetes of more than five years, (*b*) who were above age 50 years, or (*c*) who were without evidence of microalbuminuria or retinopathy were recruited from the diabetes outpatient clinic in Heidelberg. Random controls were all available blood donors above age 50 from the Department of Transfusion Medicine, Essen, Germany. None of these individuals were on any medication.

The study was approved by the local ethics committee, and all patients gave written informed consent.

DNA genotyping

From each patient, 10 ml of ethylenediaminetetraacetic acid (EDTA) blood were drawn for isolation of genomic DNA according to standard procedures. Genotyping was performed by polymerase chain reaction (PCR), followed by restriction enzyme analysis of the resulting products [4]. PCR was performed with 200 ng of genomic DNA and primers GP-1 (sense, 5-TGACCCACTTG CCACCCGTGC-3′) and GP-2 (antisense, G′-GCAG-CAGCCAGGGCTGGC-3′) in a reaction volume of 50 μl. Reaction conditions were per cycle as follows: denaturation at 94°C for 45 seconds, annealing at 60°C for 45 seconds, and elongation at 72°C for 45 seconds. All together, 35 cycles were performed. Reaction products were digested with the restriction enzyme BseDI (MBI Fermentas) and were analyzed on 1.7% agarose or 2% metaphor agarose gels, stained with ethidium bromide, and visualized under ultraviolet illumination. The undigested PCR product (TT genotype) gave a band of 268 bp in size. The completely digested PCR product (CC genotype) generated two bands of 152 bp and 116 bp, respectively. Thus, heterozygotes (CT genotype) produced three bands of 268 bp, 152 bp, and 116 bp.

Statistical analysis

Data are given as mean and standard deviation (SD). Genotype distributions, allele frequencies, and odds ratios (ORs) were compared between groups using the chi-squared test.

RESULTS

Table 2 shows the genotype distribution, and Table 3 lists the allele frequency in the study population. All groups complied to the Hardy Weinberg equilibrium, with the exception of the group of older patients with type 2 diabetes and no evidence of microangiopathy.

The T allele was significantly less frequent in nondiabetic older patients on dialysis than in the older blood donors (Table 3), but it was significantly more frequent in patients with type 2 diabetes on dialysis, but not in patients with type 2 diabetes without microangiopathy.

The ORs for dialysis-dependent patients with type 2 diabetes versus nondiabetic patients on dialysis were 3.24 (1.3 to 7.9, $P < 0.00079$) for TT/CC, 1.82 (1.07 to 3.09, $P < 0.02$) for CT/CC, and 2.05 (1.24 to 3.37, $P < 0.0047$) for (TT + CT)/CC. The ORs for dialysis-dependent patients with type 2 diabetes versus controls were 2.05 (1.07 to 3.9, $P < 0.028$) for TT/CC, 1.22 (0.79 to 1.87, $P < 0.37$) for CT/CC, and 1.36 (0.9 to 2.03, $P < 0.14$) for (TT + CT)/CC.

DISCUSSION

The study was designed to examine the working hypothesis that the hypertension-associated T allele of the Gβ3 polymorphism [4] is associated with progressive nephropathy leading to end-stage renal failure in older patients. The results in nondiabetic patients clearly do not support this hypothesis. Indeed, the T allele was found less frequently in this population, and this may be due to the association of the T allele with hypertension [4], particularly low renin hypertension and elevated diastolic blood pressures [8], and thus presumably higher cardiovascular mortality, which is blood pressure related in dialysis patients [9]. This point is relevant because the average duration of dialysis was rather long.

In contrast, however, the unanticipated result was a higher gene frequency of the T allele found in patients with type 2 diabetes who were on maintenance hemodialysis. Although no specific information on the activity

Table 2. Genotypes of our study population

	Elderly patients on dialysis without type 2 diabetes	Elderly patients on dialysis with type 2 diabetes	Elderly patients with type 2 diabetes without microangiopathy (microalbuminuria, retinopathy)	Controls
N	125	130	77	357
CC	75 (60%)	55 (42.3%)	34 (44.2%)	178 (49.9%)
CT	42 (33.6%)	56 (43%)	42 (54.5%)	149 (41.7%)
TT	8 (6.4%)[a]	19 (14.6%)[bc]	1 (1.3%)	30 (8.4%)

CC, CT, and TT are defined in the **Methods** section.
[a] $P < 0.147$ vs. control
[b] $P < 0.089$ vs. control
[c] $P < 0.006$ vs. type 2 without microangiopathy

Table 3. Allele frequencies of our study population

	Elderly patients on dialysis without type 2 diabetes	Elderly patients on dialysis with type 2 diabetes	Elderly patients with type 2 diabetes without microangiopathy (microalbuminuria, retinopathy)	Controls
N	125	130	77	357
C	0.768	0.638	0.714	0.707
T	0.232[a]	0.362[bc]	0.286	0.293

[a] $P < 0.002$ vs. control
[b] $P < 0.001$ vs. control
[c] $P < 0.0003$ vs. type 2 without microangiopathy

of G-protein–controlled transport processes in type 2 diabetes with nephropathy is currently available, it is of note that Pietruck et al found higher G-protein activation in lymphoblasts grown from patients with type 1 diabetes and nephropathy compared with type 1 diabetic patients without nephropathy [6]. In view of the high cardiovascular mortality in type 2 diabetes [10], the finding in this population of dialysis-dependent diabetic patients is particularly noteworthy. One might speculate that the association of the T allele with diabetes type 2 is so strong that it overrides any potential survival disadvantage from hypertension and excess cardiovascular mortality. We emphasize that the duration of dialysis was less in diabetic compared with nondiabetic patients on dialysis. We tried to collect a further group of older type 2 diabetic patients without evidence of microangiopathy. This group was small, and the genotype distribution was not in Hardy Weinberg equilibrium, possibly as a result of selection. The inclusion criterion of no retinopathy and microalbuminuria after five years of known duration of type 2 diabetes must have led to selective exclusion of patients with hypertension. In view of these interpretational difficulties, we were unable to state whether the data point to an association of type 2 diabetes with the T allele, a finding that is suggested by unpublished data (manuscript submitted for publication) or whether the T allele is specifically associated with diabetic nephropathy.

These results may be of interest in view of increasingly strong evidence for important familial clustering, presumably genetic determination, of end-stage renal failure, particularly in diabetic patients [11, 12].

These results provide an interesting working hypothesis, but we emphasize that the hypothesis needs to be confirmed by further studies.

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