RESEARCH LETTER

Lack of an association between *GHR* exon 3 polymorphism and diabetic nephropathy in the Genetics of Kidneys in Diabetes (GoKinD) population

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Abbreviations:

DN diabetic nephropathy
GH growth hormone

GHR growth hormone receptor

GoKinD Genetics of Kidneys in Diabetes

To the Editor: Growth hormone (GH) signalling via the GH receptor (GHR) forms the GH/GHR axis and plays an important role in metabolism. There is a genomic deletion of full-length exon 3 (d3 isoform) in the GHR gene. Previously, the GHRd3 isoform is found to be significantly associated with increased responsiveness to growth hormone [1]. Although the consensus is lacking in subsequent studies [2], GHRd3 has also been found to be associated with hypertension among stroke patients [3]. Recently, a report from our research group has demonstrated that the homozygosity for the GHRd3 allele may have the protective effect on the prevalence of type 2 diabetes [4]. Furthermore, evidence has suggested that the glomerular podocyte is a target for GH action, and the GH/GHR axis may play a role in the development of diabetic nephropathy (DN) [5, 6]. A recent study has shown that deficiency of GHR in mice (Ghr knockout) causes a reduction in systolic blood pressure and plasma renin levels, as well as an increase in aortic endothelial NO synthase (eNOS) levels

[7]. We thus hypothesise that *GHR* exon 3 polymorphism may be involved in the pathogenesis of DN.

To test this hypothesis, we have genotyped GHR exon 3 polymorphism in the participants selected from the Genetics of Kidneys in Diabetes (GoKinD) study [8]. This GoKinD cohort consists of 663 (351 male and 312 female) type 1 diabetes patients with DN (cases) and 622 (252 male and 370 female) patients without DN (controls). Among the patients, ~92% were of European descent, while ~8% were Americans of Black, Asian, Hispanic or Indian descent. All type 1 diabetes patients were diagnosed according to the World Health Organization criteria [9]. The patients with DN had persistent proteinuria or end-stage renal disease (not due to condition other than diabetes). The patients without DN had persistent normal albuminuria despite them having had type 1 diabetes for at least 15 years and never having been treated with ACE inhibitors. The detailed information of the GoKinD cohort is available at GoKinD database [10] and reference [8]. The study was approved by the local ethics committees and material transfer agreement was completed prior to the study. Genotyping of GHR exon 3 polymorphism was performed with a multiplex PCR protocol [1, 11] and the primers G1, G2 and G3 are recorded in GenBank with the accession number of AF155912. PCR variables were: initial denaturing at 94°C for 5 min; 35 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 90 s; and a final extension at 72°C for 10 min. Genotypes with GHRfI and/or -d3 alleles were detected by 1% agarose gel electrophoresis. For genotyping quality control in the present study, the patients with and without DN were distributed randomly across PCR plates. Successful genotype calls were ≥95% and plates were randomly genotyped twice for duplication accuracy, which was calculated to be 98%. Furthermore, the sample sizes in both cases (type 1 diabetes with DN, n=633) and controls (type 1 diabetes without DN, n=622), which produced 1,266 case alleles and 1,244 control alleles, were sufficiently

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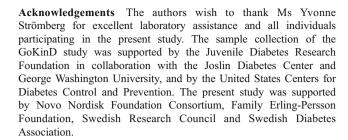
large to detect at least differences of 0.1 in allele frequency. Allele frequency and genotype distribution for GHR exon 3 polymorphism were tested for Hardy-Weinberg equilibrium. For differences between type 1 diabetes patients with DN and without DN, the model for comparing allele frequencies in 2×2 contingency table was tested, while the additive and relevant models for comparing genotype distributions were used. Tests for association between genotypes and quantitative traits were performed using Kruskal-Wallis analysis of ranks for traits with non-normal distributions, or alternatively, ANOVA for normally distributed traits. For association estimation, odd ratios and 95% confidence intervals were estimated from unconditional logistic regression models. The p value <0.05 was considered statistically significant. Analyses were carried out using STATISTICA version 7.0 (Tulsa, OK, USA).

Genotype distributions and allele frequencies of the GHR exon 3 polymorphism in the GoKinD population was in HWD; the results are summarised in Table 1. The frequencies of the GHRd3 allele in type 1 diabetes patients with DN and without DN were similar (25.5% and 25.6%, respectively, p=0.966). Distribution of three genotypes fI/fI, fI/d3 and d3/d3 between type 1 diabetes with DN and without DN were not significantly different (p=0.469, additive model). Although allele d3 frequency in female type 1 diabetes patients with DN (25.6%) was higher than in female type 1 diabetes patients without DN (24.2%), no significant association was found. Further analyses were done of the differences in phenotypes (quantitative traits) including HbA_{1c}, body mass index, creatinine, systatin, cholesterol, high-density lipoprotein, and systolic and diastolic blood pressures among type 1 diabetes patients with or without DN carrying with different genotypes. No statistically significant results were found. The present study thus indicates that GHRd3 allele frequency in the GoKinD type 1 diabetes patients (with and without DN) is similar to the frequency found in Swedish patients with type 2 diabetes (25.3%) [4]. There was no association between GHR exon 3 polymorphism and DN among type 1 diabetes patients in the GoKinD population.

Table 1 Genotype distribution and allele frequency of the *GHR* exon 3 polymorphism

Variable	T1D with DN (male/female)	T1D without DN (male/female)	p value
Genotypes			
fI/fI	359 (182/177)	344 (142/202)	0.469
fI/d3	270 (151/119)	238 (91/147)	
d3/d3	34 (18/16)	40 (19/21)	
Allele d3	0.255 (0.266/0.242)	0.256 (0.256/0.256)	0.966
frequency			

T1D, type 1 diabetes



Duality of interest The authors declare that there is no duality of interest associated with this study.

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