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Pilot study of an association between a common variant in the non-muscle myosin heavy chain 9 (MYH9) gene and type 2 diabetic nephropathy in a Taiwanese population

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Abstract: Nowadays diabetic nephropathy (DN) is the most common cause of end-stage renal disease (ESRD). Recent studies have demonstrated that the myosin, heavy chain 9, non-muscle (MYH9) gene is associated with ESRD in African Americans. In this study, we tested the hypothesis that a common single nucleotide polymorphism rs16996677 in the MYH9 gene may contribute to the etiology of DN in type 2 diabetes (T2D) in a Taiwanese population with T2D. There were 180 T2D patients diagnosed with DN and 178 age- and sex-similar T2D without DN controls. Single locus analyses showed no significant main effects of MYH9 rs16996677 on the risk of DN in T2D. The results suggest that the rs16996677 SNP in MYH9 may not contribute to the risk of DN in T2D in Taiwanese T2D patients.

Keywords: diabetic nephropathy, end-stage renal disease, single nucleotide polymorphisms, type 2 diabetes

Introduction

End-stage renal disease (ESRD) is the fifth stage of chronic kidney disease when the kidneys permanently fail to work.[1,2] The number of ESRD patients treated through dialysis or transplantation has continued to grow worldwide.[1,2] Nowadays diabetic nephropathy (DN) is the leading reported cause of ESRD.[1,2] DN is a common microvascular complication of diabetes (for type 1 and type 2),[3] and type 2 diabetes (T2D) is characterized by insulin insensitivity and pancreatic beta-cell dysfunction.[4,5] More and more genetic variants associated with ESRD are being discovered using candidate gene approaches, family linkage studies, gene expression profiling, and genome-wide association studies.[6–8] The identification of genetic variants that predispose to ESRD will enhance our understanding of the pathophysiology of renal disorders, thereby potentially leading to novel tailored therapies for treatment and prevention.[7,8]

The myosin, heavy chain 9, non-muscle (MYH9) gene encodes the protein non-muscle myosin heavy chain, class II, and isoform type A, which is abundantly expressed in the kidney, liver, and platelets.[9,10] Recently, the MYH9 gene has received much attention. Some studies have suggested that the MYH9 gene is associated with non-diabetic ESRD,[11] hypertension-associated ESRD,[12,13] and T2D-associated ESRD[14] in African Americans. In addition, MYH9 has been linked with idiopathic and human immunodeficiency virus-associated focal segmental glomerulosclerosis in African Americans.[12] It has also been shown that there is an association between MYH9 and albuminuria was

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in hypertensive African Americans.[15] Moreover, a common single nucleotide polymorphism (SNP) rs16996677 in MYH9 has been found to be highly significantly associated with non-diabetic ESRD,[11] hypertension-associated ESRD,[13] and T2D-associated ESRD[14] in a population of African Americans. Furthermore, it has been reported that MYH9 rs16996677 was not associated with kidney failure by contrasting the T2D-associated ESRD cases with T2D lacking nephropathy controls.[14]

The previous findings[11,13,14] mainly reported of the association studies of the MYH9 rs16996677 polymorphism and ESRD in African Americans. In this work, we focused on T2D-associated ESRD due to DN and tested the hypothesis that the rs16996677 polymorphism in the MYH9 gene may contribute to the etiology of DN in T2D amongst Taiwanese T2D individuals.

Materials and methods

Patients

The patients were partially original to the previous study by Wu et al[16] and are described in detail elsewhere.[16] Briefly, there were 358 Taiwanese patients with T2D who were recruited from the Tri-Service General Hospital in Taipei, Taiwan, in 2002. The case group comprised 180 T2D patients with DN and the control group comprised 178 T2D patients without DN. All the recruited patients fulfilled the following four inclusion criteria: (1) The patient had been diagnosed with diabetes for more than 5 years; (2) The age was between 30 and 75 years; (3) The fasting plasma glucose was greater than 126 mg/dl; (4) The glycated haemoglobin was greater than 6%. We then further classified the study subjects as DN or T2D without DN groups according to three surrogate endpoints, comprising urinary albumin to creatinine ratio, blood urea nitrogen, and serum creatinine.

Before conducting the study, approval was obtained from the Internal Review Board of the Tri-Service General Hospital and the approved informed consent form was signed by each subject.

Laboratory methods

Table 1 provides detailed information on the selected SNP, which includes its chromosome position, commercial assay identifier, allelic variants, and the minor allele frequency.

DNA was isolated from blood samples using a QIAamp DNA blood kit following the manufacturer's instructions (Qiagen, Valencia, CA). To extract DNA, we used 200 µl of blood which was further solved in 200 µL of distilled water.[16] Before PCR reaction, part of the extracted DNA was diluted into a concentration of 10 µg/µL. The qualities of isolated genomic DNAs were checked using agarose gel electrophoresis and the quantities determined using spectrophotometry.

All SNP genotypings were performed using the Taqman SNP genotyping assay (Applied Biosystems Inc. Foster City, CA). The primers and probes of SNPs were from the ABI Assay on Demand kit. Reactions were carried out according to the manufacturer's protocol. The probe fluorescence signal detection was performed using the ABI Prism 7900 Real-Time PCR system.

HapMap database

In this work, we utilized the HapMap database[17] to provide a comparison between a Taiwanese population and the five populations (African ancestry in Southwest USA [ASW], Utah residents with Northern and Western European ancestry from the CEPH collection [CEU]; Han Chinese in Beijing, China [CHB]; Japanese in Tokyo, Japan [JPT]; Yoruba in Ibadan, Nigeria [YRI]) of the HapMap database in terms of allele frequencies.

Statistical analysis

The categorical data were analyzed using the chi-square test. Furthermore, we compared differences for continuous variables using the Student's t-test. In addition, genotype frequencies were evaluated for Hardy-Weinberg equilibrium using a χ^2 goodness-of-fit test. The criterion for significance was set at $P < 0.05$ for all tests. Data are presented as mean ± standard deviation.

Results

Table 2 describes the demographic and clinical characteristics of the study population. As shown in Table 2, unrelated DN cases and T2D without DN controls had a similar gender distribution ($P = 0.1116$). In addition, the distribution of age in the two groups was well-matched ($P = 0.1008$). All SNPs were evaluated for their contribution to DN in the complete

Table 1 Chromosome position, commercial assay identifier, nucleotide variation, and minor allele frequency of the selected SNP

Gene	SNP	Chromosome	Position	Commercial assay identifier	Major/minor allele	MAF[a]
MYH9	rs16996677	22	35057219	C_33480390_10	G/A	0.001

Notes: [a]MAF in this study.
Abbreviations: MAF, minor allele frequency; SNP, single nucleotide polymorphism.

Table 2 Demographic and clinical characteristics of study subjects

Characteristic	DN (case)	T2D without DN (control)	*P* value
n	180	178	
Age, years	58.20 ± 10.08	56.54 ± 8.99	0.1008
Gender, M/F	94/86	78/100	0.1116

Notes: Data are presented as mean ± standard deviation.
Abbreviations: DN, diabetic nephropathy; T2D, type 2 diabetes.

sample population, including 180 DN subjects and 178 T2D without DN subjects.

Table 3 shows the genotype and allele distributions of the rs16996677 SNP in the DN (case) and T2D without DN (control) groups for the complete sample population. No association between MYH9 rs16996677 and DN was detected by the allelic or genotypic test ($P = 0.3156$ and 0.3153, respectively). In addition, genotype frequency distribution for rs16996677 was in Hardy-Weinberg equilibrium (Table 3; $P = 0.9788$).

Furthermore, Figure 1 demonstrates the allele frequencies of the rs16996677 SNP in the MYH9 gene for the five populations of the HapMap database and the Taiwanese population collected in this study. We compared the allele frequencies between the Taiwanese population and each of the five populations of HapMap (that is, Taiwanese versus ASW, Taiwanese versus CEU, Taiwanese versus CHB, Taiwanese versus JPT, and Taiwanese versus YRI). There was a significant difference between Taiwanese and ASW populations ($P < 0.0001$) as well as between Taiwanese and YRI populations ($P < 0.0001$). However, we did not find a significant difference between Taiwanese and CEU populations ($P = 0.3883$), between Taiwanese and CHB populations ($P = 0.7216$), or between Taiwanese and JPT populations ($P = 0.7216$).

Discussion

Our study is the first to date to have examined whether the MYH9 rs16996677 polymorphism is associated with the risk of DN amongst Taiwanese T2D individuals. In this study, MYH9 rs16996677 was not associated with DN in T2D (in either allelic or genotypic tests) using single locus analyses. Similarly, Freedman et al compared the T2D-associated ESRD patients with T2D lacking nephropathy controls and found no association between MYH9 rs16996677 and kidney failure among African Americans.[14] In contrast, Freedman et al further reported a significant association between MYH9 rs16996677 and T2D-associated ESRD by comparing T2D-associated ESRD cases with non-diabetic non-nephropathy controls in the same African American study.[14] In a previous Taiwanese study, Wu et al also suggested that the SNP rs1044498 in the ectonucleotide pyrophosphatase/phosphodiesterase 1 (ENPP1) gene displayed a statistically significant difference in the risk of DN in T2D patients.[16] In addition, they indicated a potential gene-gene interaction between ENPP1 and the growth hormone secretagogue receptor (GHSR) gene using both the generalized multifactor dimensionality reduction method and logistic regression models.[16] On the other hand, hypertension is a chronic disorder in which the blood pressure is elevated,[18] and hypertension is also the cause of ESRD.[1,2] In a population of African Americans, Kao et al showed that MYH9 rs16996677 was highly significantly associated with non-diabetic ESRD and hypertension-associated ESRD.[11] Furthermore, another study replicated these results by finding significant associations with MYH9 rs16996677 in non-diabetic ESRD and hypertension-associated ESRD cases among African Americans.[13]

The MYH9 gene is approximately 110 kb with 41 exons in a region of chromosome 15, and the MYH9 gene product, myosin-IIA, is responsible for moving actin filaments in cells.[19,20] Mutations in MYH9 are associated with several Mendelian conditions and autosomal dominant disorders, such as Epstein syndrome, Fechtner syndrome, May-Hegglin anomaly, Sebastian syndrome, and an autosomal dominant form of deafness.[21] Kopp et al conducted an admixture-mapping linkage-disequilibrium genome scan and detected an association with MYH9 in African Americans with idiopathic and human immunodeficiency virus-associated focal segmental

Table 3 Distributions of genotypes and alleles between the diabetic nephropathy (DN) cases and type 2 diabetes (T2D) without DN controls

Gene	SNP	Allele (1/2) and genotype (11/12/22)	DN	T2D without DN	Association (*P* value)				
					Allele (1/2)	Genotype (11/12/22)	Genotype (11 + 12/22)	Genotype (11/12 + 22)	HWE
	rs16996677	G/A	353/1	356/0	0.3156	1.0000	1.0000	0.3153	0.9788
		GG/GA/AA	176/1/0	178/0/0					

Abbreviations: DN, diabetic nephropathy; HWE, Hardy-Weinberg equilibrium; SNP, single nucleotide polymorphism; T2D, type 2 diabetes; MYH9, myosin, heavy chain 9, non-muscle gene.

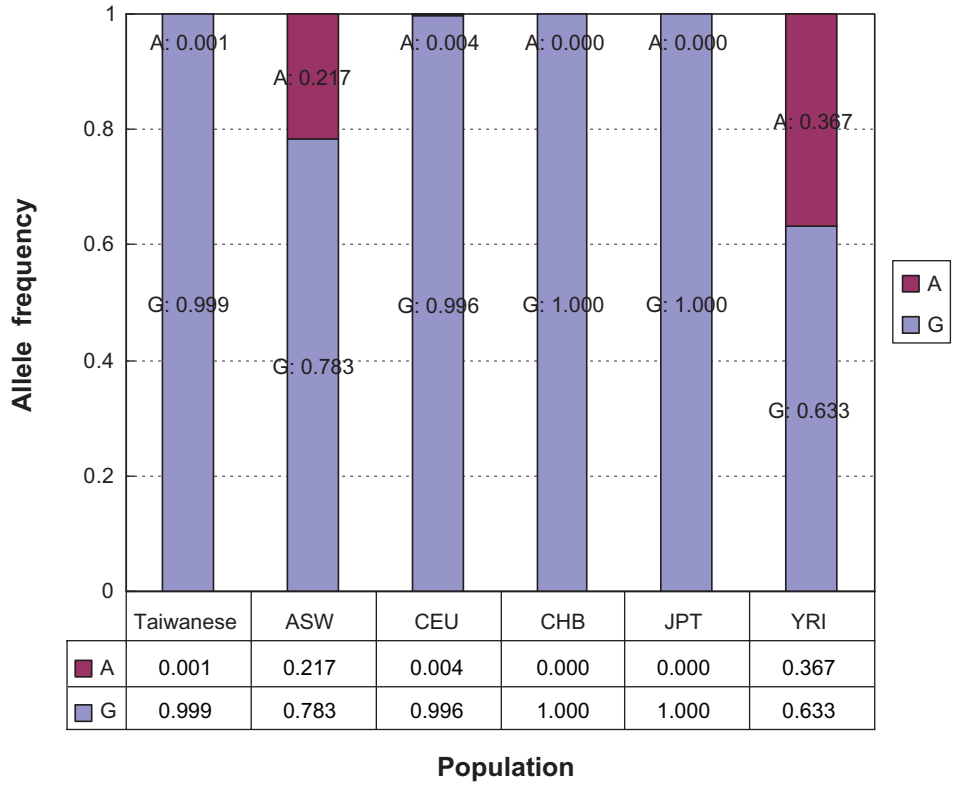

Figure 1 The allele frequencies of the rs16996677 SNP in the MYH9 gene for the five populations of the HapMap database and the Taiwanese population collected in this study.

Abbreviations: ASW, African ancestry in southwest USA; CEU, Utah residents with northern and western European ancestry from the CEPH collection; CHB, Han Chinese in Beijing, China; JPT, Japanese in Tokyo, Japan; MYH9, non-muscle myosin heavy chain 9; YRI, Yoruba in Ibadan, Nigeria.

glomerulosclerosis.[12] They also revealed that the MYH9 was significantly associated with non-diabetic forms of ESRD (predominantly ESRD from hypertensive nephrosclerosis), but not with T2D-associated ESRD.[12] Kao et al replicated these findings by identifying a highly significant association between MYH9 and several non-diabetic etiologies of ESRD among African Americans but not diabetic ESRD.[11] Another study in African Americans further reported that significant associations were detected between MYH9 variants and non-diabetic patients with ESRD (including chronic glomerulonephritis-associated ESRD and hypertension-associated ESRD).[13] In a population of African Americans, Freedman et al also demonstrated that MYH9 was associated with T2D-associated ESRD by comparing T2D-ESRD cases with non-diabetic non-nephropathy controls.[14] As mentioned previously, Freedman et al did not find an association between MYH9 rs16996677 and kidney failure among African Americans by contrasting the T2D-associated ESRD patients with T2D non-nephropathy controls.[14] However, they identified three SNPs, including rs4821480, rs2032487 and rs4821481, that were associated with kidney failure in T2D in the same African American study.[14]

Moreover, we observed that allele frequencies for the MYH9 rs16996677 SNP in this Taiwanese study were in accordance with HapMap database entries[17] in the populations of European ancestry (that is, CEU) and Asian ancestry (that is, CHB and JPT). However, we did not find a correlation in allele frequency between our study population and HapMap database entries[17] in the populations of African ancestry (that is, ASW and YRI). In addition, MYH9 rs16996677 was not polymorphic among CHB and JPT. This rs16996677 SNP also had only one Taiwanese participant with the heterozygous genotype and none with the rare homozygous genotype.

One limitation of this study is that the contributions of other markers in the MYH9 gene should be further examined in future work. As discussed previously, the selected MYH9 rs16996677 variant was one of the most mentioned SNPs in previous reports.[11,13,14] In the current pilot study, we assumed that an SNP might be a good candidate to investigate the genetic role of the implicated gene if the SNP has been investigated in several studies.[16] Second, we focused on single locus analyses without considering epistatic models in this study. It is the essential role of gene-gene interactions to define

a trait implicating complex disease-related mechanisms, particularly when each involved variant only has a minor marginal effect.[22–24] Several statistical methods such as logistic regression and the generalized multifactor dimensionality reduction method have been applied to SNP association studies for detecting gene-gene interactions associated with a number of complex diseases.[25–28] In addition, future research using pattern recognition approaches[29–31] is needed in order to model associations between gene variants and ESRD. Third, these findings may not be suitably generalized to other populations.[16,27] Large ethnically-matched studies would be necessary to know if such an association exists in non-Taiwanese subjects. In future work, we will attempt to recruit more DN and T2D patients as a replication group in a large chronic renal disease and ESRD project for facilitating further analyses.

In conclusion, our study has tested the association between a common SNP rs16996677 in the MYH9 gene and DN in Taiwanese T2D subjects based on single-locus analyses. Our findings did not support the hypothesis that the MYH9 rs16996677 SNP contributes to the risk of DN in T2D. Independent replications in large sample sizes are needed to confirm the role of the polymorphisms found in this study for DN in T2D.

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Disclosures

The authors have no conflicts of interest to disclose.

References

1. Grassmann A, Gioberge S, Moeller S, Brown G. ESRD patients in 2004: Global overview of patient numbers, treatment modalities and associated trends. *Nephrol Dial Transplant*. 2005;20(12):2587–2593.
2. Meguid El, Nahas A, Bello AK. Chronic kidney disease: The global challenge. *Lancet*. 2005;365(9456):331–340.
3. Remuzzi G, Schieppati A, Ruggenenti P. Clinical practice. Nephropathy in patients with type 2 diabetes. *N Engl J Med*. 2002;346(15):1145–1151.
4. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: Principles of pathogenesis and therapy. *Lancet*. 2005;365(9467):1333–1346.
5. Hattersley AT, Pearson ER. Minireview: Pharmacogenetics and beyond: The interaction of therapeutic response, beta-cell physiology, and genetics in diabetes. *Endocrinology*. 2006;147(6):2657–2663.
6. Freedman BI, Bostrom M, Daeihagh P, Bowden DW. Genetic factors in diabetic nephropathy. *Clin J Am Soc Nephrol*. 2007;2(6):1306–1316.
7. de Borst MH, Benigni A, Remuzzi G. Primer: Strategies for identifying genes involved in renal disease. *Nat Clin Pract Nephrol*. 2008;4(5):265–276.
8. Conway BR, Maxwell AP. Genetics of diabetic nephropathy: Are there clues to the understanding of common kidney diseases? *Nephron Clin Pract*. 2009;112(4):c213–c221.
9. Lalwani AK, Goldstein JA, Kelley MJ, Luxford W, Castelein CM, Mhatre AN. Human nonsyndromic hereditary deafness DFNA17 is due to a mutation in nonmuscle myosin MYH9. *Am J Hum Genet*. 2000;67(5):1121–1128.
10. Even-Ram S, Yamada KM. Of mice and men: Relevance of cellular and molecular characterizations of myosin IIA to MYH9-related human disease. *Cell Adh Migr*. 2007;1(3):152–155.
11. Kao WH, Klag MJ, Meoni LA, et al. MYH9 is associated with nondiabetic end-stage renal disease in African Americans. *Nat Genet*. 2008;40(10):1185–1192.
12. Kopp JB, Smith MW, Nelson GW, et al. MYH9 is a major-effect risk gene for focal segmental glomerulosclerosis. *Nat Genet*. 2008;40(10):1175–11784.
13. Freedman BI, Hicks PJ, Bostrom MA, et al. Polymorphisms in the nonmuscle myosin heavy chain 9 gene (MYH9) are strongly associated with end-stage renal disease historically attributed to hypertension in African Americans. *Kidney Int*. 2009;75(7):736–745.
14. Freedman BI, Hicks PJ, Bostrom MA, et al. Non-muscle myosin heavy chain 9 gene MYH9 associations in African Americans with clinically diagnosed type 2 diabetes mellitus-associated ESRD. *Nephrol Dial Transplant*. 2009;24(11):3366–3371.
15. Freedman BI, Kopp JB, Winkler CA, et al. Polymorphisms in the nonmuscle myosin heavy chain 9 gene (MYH9) are associated with albuminuria in hypertensive African Americans: The HyperGEN study. *Am J Nephrol*. 2009;29(6):626–632.
16. Wu LS, Hsieh CH, Pei D, Hung YJ, Kuo SW, Lin E. Association and interaction analyses of genetic variants in ADIPOQ, ENPP1, GHSR, PPAR{gamma} and TCF7L2 genes for diabetic nephropathy in a Taiwanese population with type 2 diabetes. *Nephrol Dial Transplant*. 2009;24(11):3360–3366.
17. The International HapMap Consortium. The International HapMap Project. *Nature*. 2003;426(6968):789–794.
18. Carretero OA, Oparil S. Essential hypertension. Part I: definition and etiology. *Circulation*. 2000;101(3):329–335.
19. D'Apolito M, Guarnieri V, Boncristiano M, Zelante L, Savoia A. Cloning of the murine non-muscle myosin heavy chain IIA gene ortholog of human MYH9 responsible for May-Hegglin, Sebastian, Fechtner, and Epstein syndromes. *Gene*. 2002;286(2):215–222.
20. Marini M, Bruschi M, Pecci A, et al. Non-muscle myosin heavy chain IIA and IIB interact and co-localize in living cells: Relevance for MYH9-related disease. *Int J Mol Med*. 2006;17(5):729–736.
21. Singh N, Nainani N, Arora P, Venuto RC. CKD in MYH9-related disorders. *Am J Kidney Dis*. 2009;54(4):732–740.
22. Lin E, Hwang Y, Liang KH, Chen EY. Pattern-recognition techniques with haplotype analysis in pharmacogenomics. *Pharmacogenomics*. 2007;8(1):75–83.
23. Lin E, Hwang Y, Chen EY. Gene-gene and gene-environment interactions in interferon therapy for chronic hepatitis C. *Pharmacogenomics*. 2007;8(10):1327–1335.
24. Lin E, Hsu SY. A Bayesian approach to gene-gene and gene-environment interactions in chronic fatigue syndrome. *Pharmacogenomics*. 2009;10(1):35–42.
25. Lou XY, Chen GB, Yan L, et al. A generalized combinatorial approach for detecting gene-by-gene and gene-by-environment interactions with application to nicotine dependence. *Am J Hum Genet*. 2007;80(6):1125–1137.
26. Lin E, Hong CJ, Hwang JP, et al. Gene-gene interactions of the brain-derived neurotrophic-factor and neurotrophic tyrosine kinase receptor 2 genes in geriatric depression. *Rejuvenation Res*. 2009;12(6):387–393.

27. Lin E, Pei D, Huang YJ, Hsieh CH, Wu LS. Gene-gene interactions among genetic variants from obesity candidate genes for nonobese and obese populations in type 2 diabetes. *Genet Test Mol Biomarkers*. 2009;13(4):485–493.
28. Lin E, Chen PS, Chang HH, et al. Interaction of serotonin-related genes affects short-term antidepressant response in major depressive disorder. *Prog Neuropsychopharmacol Biol Psychiatry*. 2009;33(7): 1167–1172.
29. Lin E, Hwang Y, Wang SC, Gu ZJ, Chen EY. An artificial neural network approach to the drug efficacy of interferon treatments. *Pharmacogenomics*. 2006;7(7):1017–1024.
30. Lin E, Huang LC. Identification of significant genes in genomics using Bayesian variable selection methods. *Advances and Applications in Bioinformatics and Chemistry*. 2008;1:13–18.
31. Lin E, Hwang Y. A support vector machine approach to assess drug efficacy of interferon and ribavirin combination therapy. *Mol Diagn Ther*. 2008;12(4):219–223.