Hepatic glucokinase promoter polymorphism is associated with hepatic insulin resistance in Asian Indians.

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Abstract

Background: The role of glucokinase (GCK) in the pathogenesis of maturity-onset diabetes of the young is well established. However, its role in the common form of type 2 diabetes is far from convincing. We investigated the role of the G-to-A polymorphism in the hepatic GCK promoter on insulin sensitivity and beta cell function in 63 normotensive Asian Indians with normal glucose tolerance. As proposed by Matsuda and DeFronzo, hepatic insulin sensitivity (ISIH) and total body insulin sensitivity (ISIM) were estimated from the oral glucose tolerance test. Beta cell function was estimated using %B from the Homeostasis Model Assessment and insulingenic index (dI/dG).

Result: We identified 38 GG, 24 GA, and one AA subjects. The AA subject was pooled with the GA subjects during the analysis. No difference was noted in the demographic features between the two genotypic groups (GG vs. GA/AA). Compared to the GG group, the GA/AA group had a lower ISIH (p=0.002), a lower ISI_H (p=0.009), a higher %B (p=0.014), and a higher dI/dG (p=0.030). Multivariate analysis revealed that this polymorphism is an independent determinant for ISIH (p=0.019) and along with age, waist-hip ratio, gender, and diastolic blood pressure accounted for 51.5% of the variation of ISIH. However, this polymorphism was a weak, but independent determinant for ISI_H (p=0.089) and %B (p=0.083). Furthermore, it had no independent effect on dI/dG (p=0.135).

Conclusions: These data suggest that the G-to-A polymorphism in the hepatic GCK promoter is associated with hepatic insulin resistance in Asian Indians.

Introduction

Glucokinase (GCK) was originally proposed to be a glucose sensor and metabolic signal generator in pancreatic beta cells and hepatocytes [1]. The discoveries of a linkage and subsequent identification of mutated GCK genes [2,3] in families with maturity-onset diabetes of the young (MODY) provide the strongest evidence for a crucial role of GCK in the pathogenesis of MODY [1]. However, the structural mutations (missense, nonsense mutation, or mutations affecting the splicing mechanism) of GCK were only found in less than 1% of patients with type 2 diabetes [4]. Thus, the mutated GCKs do not play a key role in the pathogenesis of most forms of diabetes.

Nonetheless, some studies suggest that defective liver GCK may play a role in the pathogenesis of insulin resistance and type 2 diabetes [5]. In patients with type 2 diabetes who underwent elective cholecystectomy, hepatic GCK activity was decreased by about 50% in obese dia-
Hepatic insulin sensitivity (ISI_H) was estimated from the OGTT as described by Matsuda and DeFronzo [13]. Beta cell function (%B) was estimated from the HOMA [14] and dI/dG (the ratio of the incremental response in insulin to that of glucose during the first 30 minutes of the OGTT). The GA/AA group had a lower ISI_H (p=0.002) and ISI_M (p=0.009) than the GG group. This polymorphism accounted for 14.4% and 10.7% of the variations in ISI_H and ISI_M, respectively. In contrast, the GA/AA group had better beta cell function, based on %B and dI/dG, compared to GG group (Table 2). Demographic features and glycemic parameters by genotypes.

Multivariate analysis showed that this polymorphism was an independent determinant for ISI_H (p=0.019) and along with age, waist-hip ratio, gender, and diastolic blood pressure explained 51.5% of the variation in ISI_H (Table 3 Stepwise regression analysis of the estimated indices for insulin sensitivity and beta cell function). However, systolic blood pressure and body mass index had no impact on ISI_H. Since hepatic insulin sensitivity (ISI_H) correlated very well with the whole body insulin sensitivity (ISI_M, p < 0.0001, r^2=0.800), this polymorphism also had an independent but marginal impact on ISI_M (p=0.089). In contrast to hepatic insulin sensitivity, this polymorphism had less impact on beta cell function (9.5% and 7.5% of the variations in %B and dI/dG, respectively). Multivariate analyses showed that this polymorphism was weakly associated with %B (p=0.083), but not dI/dG (p=0.135).

**Discussion**

Our data show that the G-to-A polymorphism at the -258 nucleotide position of the hepatic GCK promoter is an independent determinant for ISI_H, but has only marginal impacts on ISI_M and %B, and no impact on dI/dG. Hepatic and whole body insulin sensitivities are well correlated to each other [13] and a better correlation between this polymorphism and ISI_H was observed than with ISI_M. This suggests that the primary impact of this polymorphism is on ISI_H. Since all the subjects were glu-
cose tolerant, their beta cell function will compensate for the prevailing insulin resistance to maintain plasma glucose concentration within a relatively narrow physiological range. The observed differences in %B and dI/dG between the two groups are most likely due to the compensatory increase of beta cell response to the differences in insulin sensitivity. This interpretation is consistent with the nature of this polymorphism, which occurs within the hepatic GCK promoter and not in the beta cell GCK promoter. Therefore, these results indicate that the polymorphism mainly affects hepatic insulin sensitivity.

There are two forms of GCK: liver and islet. Although each tissue has its own exon 1 and promoter, they share common exons 2-10 [8]. The transcript of islet GCK is regulated by glucose [15] while insulin is the key regulator for hepatic GCK transcription [9]. Although substantial work has been accomplished [16], the IRS has not been identified within the hepatic GCK promoter. In contrast, the IRS of PEPCK has been mapped out and studied extensively, which is positively regulated by insulin [10]. This polymorphism (G-to-A substitution) was not only located in a region, which is highly similar to the IRS of PEPCK, but also occurred at the base pair, which was conserved between PEPCK and hepatic GCK and also conserved between human and rat for both PEPCK and hepatic GCK (Figure 2). This suggests that this base pair may be very important in IRS. Transgenic mice with

| Table 1: Clinical characteristics of the studied subjects |
|----------------------------------------------------------|
| n | % | Mean | Standard error | Minimum | Maximum |
|---|---|------|----------------|---------|---------|
| n | 63 | 41 ± 1 | 19 | 68 |
| Gender | female | | | |
| Age | year | | |
| Body mass index | kg/m² | 23.72 ± 0.42 | 16.93 | 32.61 |
| Waist-to-hip ratio | cm/cm | 0.828 ± 0.010 | 0.0684 | 0.969 |
| Systolic blood pressure | mmHg | 110 ± 1 | 83 | 134 |
| Diastolic blood pressure | mmHg | 72 ± 1 | 58 | 82 |
| Oral glucose tolerance test | | | |
| Fasting plasma glucose | mmol/L | 5.07 ± 0.04 | 4.36 | 5.77 |
| Plasma glucose at 30 minutes | mmol/L | 8.08 ± 0.12 | 5.00 | 10.05 |
| Plasma glucose at 60 minutes | mmol/L | 8.35 ± 0.18 | 5.16 | 10.94 |
| Plasma glucose at 90 minutes | mmol/L | 7.43 ± 0.16 | 4.72 | 10.60 |
| Plasma glucose at 120 minutes | mmol/L | 6.57 ± 0.11 | 4.39 | 7.76 |

| Table 2. Demographic features and glycemic parameters by genotypes |
|---------------------------------------------------------------|
| GG | Mean (n) | 95% CI (%) | GA/AA | Mean (n) | 95% CI (%) |
|---|----------|-------------|-------|----------|-------------|
| Gender | F | 24 | 63% | 1.4 | 56% |
| Age | year | | | | |
| Body mass index | kg/m² | 23.13 (22.05, 24.27) | 24.09 (22.92, 25.32) |
| Waist-to-hip ratio | cm/cm | 0.814 (0.790, 0.839) | 0.840 (0.808, 0.873) |
| Systolic blood pressure | mmHg | 108 (105, 112) | 111 (107, 116) |
| Diastolic blood pressure | mmHg | 72 (69, 74) | 73 (70, 76) |
| Fasting plasma glucose | mmol/L | 5.03 (4.95, 5.13) | 5.13 (4.98, 5.28) |
| Fasting plasma insulin | pmol/L | 42 (37, 49) | 60 (50, 72) |
| 1/SI_R | pmol/L | 0.76 (0.66, 0.88) | 0.52 (0.43, 0.63) |
| 1/SI_M | pmol/L | 5.61 (4.87, 6.48) | 4.08 (3.33, 5.00) |
| % B_D | pmol/mmol | 78 (67, 92) | 105 (90, 124) |
| dI/dG | pmol/mmol | 111 (91, 135) | 170 (116, 230) |

* geometric means, (95% CI); ** p=0.003 for; *** p=0.002; **** p=0.009; ***** p=0.014; ****** p=0.030.
overexpressed PEPCK developed hyperinsulinemia [17]. Increased GCK gene copies in mice leads to increased hepatic glucose metabolism and, consequently, a lower plasma glucose concentration [18]. In addition, overexpression of human hepatic GCK in mice liver also results in decreased glucose concentration and reduced body weight [19]. Furthermore, mice that lack GCK only in the liver are only mildly hyperglycemic but display pronounced defects in both glycogen synthesis and glucose turnover rate during a hyperglycemic clamp [20]. Therefore, it is tempting to speculate that reduced expression of hepatic GCK could lead to hepatic insulin resistance as we observed in this study (seen in a lower ISI_H for the GA/AA subjects). Initially, glucose homeostasis is maintained by the compensatory hyperinsulinemia (as observed from the higher plasma insulin concentration for

Table 3. Stepwise regression analysis of the estimated indices for insulin sensitivity and beta cell function

| Dependent Variable | Covariate entered | Covariate removed | $r^2$ | p     |
|--------------------|------------------|------------------|------|-------|
| ISI_H              | GCK polymorphism |                  | 0.144| 0.002 |
| ISI_H              | Age              |                  | 0.515| < 0.001|
|                    | Waist-hip ratio  |                  |      | < 0.001|
|                    | Gender           |                  | 0.003|       |
|                    | GCK polymorphism |                  | 0.019|       |
|                    | Diastolic blood pressure |          | 0.052|       |
|                    | Systolic blood pressure |          | 0.316|       |
|                    | Body mass index  |                  | 0.597|       |
| ISI_M              | GCK polymorphism |                  | 0.107| 0.009 |
| ISI_M              | Age              |                  | 0.414| < 0.001|
|                    | Waist-hip ratio  |                  |      | 0.008 |
|                    | Gender           |                  | 0.029|       |
|                    | Systolic blood pressure |          | 0.085|       |
|                    | GCK polymorphism |                  | 0.089|       |
|                    | Body mass index  |                  | 0.360|       |
|                    | Diastolic blood pressure |          | 0.574|       |
| %B                 | GCK polymorphism |                  | 0.095| 0.014 |
| %B                 | Age              |                  | 0.437| < 0.001|
|                    | Gender           |                  | 0.003|       |
|                    | Systolic blood pressure |          | 0.026|       |
|                    | Waist-hip ratio  |                  | 0.035|       |
|                    | GCK polymorphism |                  | 0.083|       |
|                    | Body mass index  |                  | 0.189|       |
|                    | Diastolic blood pressure |          | 0.433|       |
| d/dG               | GCK polymorphism |                  | 0.075| 0.020 |
| d/dG               | Age              |                  | 0.130| 0.005 |
|                    | Waist-hip ratio  |                  | 0.011|       |
|                    | Gender           |                  | 0.100|       |
|                    | GCK polymorphism |                  | 0.135|       |
|                    | Diastolic blood pressure |          | 0.720|       |
|                    | Body mass index  |                  | 0.913|       |
|                    | Systolic blood pressure |          | 0.954|       |
the GA/AA subjects in this study) through an increase in insulin secretion by the pancreatic beta cells, which was also observed in this study (a higher %B and dI/dG for the GA/AA subjects). However, the cause-effect relationship between this polymorphism and insulin resistance remains to be elucidated.

**Laboratory methods:**
Genomic DNA was extracted from the peripheral lymphocytes as described previously [21]. A polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay was developed for the 174-base pair fragment containing nucleotide -411 to -238 of the liver GCK promoter [8]. Since the substitution occurs within a region that is not cut by any known restriction enzyme, we created a *de novo* restriction site by placing the reverse primer close to the site of variation and replacing one of the nucleotides in the reverse primer. By substituting T with A at nucleotide -256 within the reverse primer, a *de novo* ACC/I restriction site was created when the molecular variation of G-to-A substitution was present. The standard PCR reaction was a 10-µl reaction mixture containing 0.1 µg of genomic DNA, 1 pmole of each primer, 0.2 mM of dNTP, 2 mM of MgCl₂, 1X PCR buffer, and 0.25 U of Thermal stable Taq polymerase. The PCR was performed with an initial denaturation at 94°C for 5 minutes, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 62°C for 30 seconds, extension at 72°C for 30 seconds, and then a final extension at 72°C for 10 minutes. The forward primer was CAGACCT-GGATTGTATGAAATG and the reverse primer was GGCTGCTTGGCCACAGTA. The restriction digestion
was carried out in a 10 µl reaction containing 2.5 µl of PCR reaction and 0.1 U of AcjI in the buffer supplied by the vendor (Promega Inc., Madison, WI, USA) at 37°C for 3 hours. The reaction was resolved on a 8% acrylamide gel which was scored under a UV illuminator after staining with ethidium bromide. The wild type (G at nucleotide -258) was not cut by AcjI and was isolated as a larger fragment (173 bp), while the variant (A at nucleotide -258) was cut by AcjI to produce a smaller fragment (154 bp).

**Statistical analysis:**
Variables with skewed distributions were logarithmically transformed before analysis. They were body mass index, waist-hip ratio, insulin concentrations, %S, ISI, M, %B, and d/dg. Data were presented as means (or geometric means when appropriate) with 95% confidence intervals, unless otherwise specified. Two-sided t-tests or chi-square tests were used to evaluate the differences between the two groups. To examine the influence of multiple variables on either insulin sensitivity or beta cell function, multivariate analysis was performed with a backward stepwise option. The probability to enter or to remove was set at 0.10. A nominal P value of less than 0.05 was considered significant. SYSTAT 8.0 for Windows from SPSS, Inc. (Chicago, Illinois) was used for the statistical analyses.

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**References**
1. Matschinsky FP: Glucokinase as glucose sensor and metabolic signal generator in pancreatic beta-cells and hepatocytes. Diabetes 1990, 39:647-652
2. Hattersley AT, Turner RC, Permutt MA, Patel P, Tanizawa Y, Chiu KC, O’Rahilly S, Watkins PJ, Wainscoat JS: Linkage of type 2 diabetes to the glucokinase gene [see comments]. Lancet 1992, 339:1307-1310
3. Forguel P, Zouali H, Vionnet N, Velho G, Vaxillaire M, Sun F, Lesage S, Stoffer M, Takeda J, Passa P, et al: Familial hyperglycemia due to mutations in glucokinase. Definition of a subtype of diabetes mellitus. N Engl J Med 1993, 328:697-702
4. Chiu KC, Tanizawa Y, Permutt MA: Glucokinase gene variants in the common form of NIDDM. Diabetes 1993, 42:579-582
5. Caro JF, Triester S, Patel VK, Tapscoot EB, Frazier NL, Dohn GL: Liver glucokinase: decreased activity in patients with type II diabetes. Harm Metab Res 1995, 27:19-22
6. Brichard SM, Hengquin JC, Girard J: Phlorizin treatment of diabetic rats partially reverses the abnormal expression of genes involved in hepatic glucose metabolism. Diabetologia 1993, 36:292-298
7. Chiu KC, Go RC, Aoki M, Riggs AC, Tanizawa Y, Acton RT, Bell DS, Goldberg RL, Rosenman JM, Permutt MA: Glucokinase gene in gestational diabetes mellitus: population association study and molecular scanning. Diabetologia 1994, 37:104-110
8. Tanizawa Y, Matsutani A, Chiu KC, Permutt MA: Human glucokinase gene: isolation, structural characterization, and identification of a microsatellite repeat polymorphism. Mol Endocrinol 1992, 6:1070-1081
9. Magnusson MA, Andreone TL, Printz RL, Koch S, Granner DK: Rat glucokinase gene: structure and regulation by insulin. Proc Natl Acad Sci U S A 1989, 86:4838-4842
10. O’Brien RM, Lucas PC, Forest CD, Magnuson MA, Granner DK: Identification of a sequence in the PEPCK gene that mediates a negative effect of insulin on transcription. Science 1990, 249:533-537
11. Ferrannini E, Buzzigoli G, Bonadonna R, Giorico MA, Oleggini M, Grazidei L, et al: Insulin resistance in essential hypertension. N Engl J Med 1987, 317:350-357
12. Rossetti L, Giaccari A, DeFronzo RA: Glucose toxicity. Diabetes Care 1990, 13:610-630
13. Matsuda M, DeFronzo RA: Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 1999, 22:1462-1470
14. Levy JC, Matthews DR, Hermans MP: Correct homeostasis model assessment (HOMA) evaluation uses the computer program. Diabetes Care 1998, 21:2191-2192
15. Magnuson MA, Shelton KD: An alternate promoter in the glucokinase gene is active in the pancreatic beta cell. J Biol Chem 1996, 271:29113-29120
16. Iynedjian PB, Marie S, Wang H, Ginovici A, Nazaryan K: Liver-specific enhancer of the glucokinase gene. J Biol Chem 1996, 271:29113-29120
17. Valera A, Pujol A, Pelegrin M, Bosch F: Transgenic mice overexpressing phosphoenolpyruvate carboxykinase develop non-insulin-dependent diabetes mellitus. Proc Natl Acad Sci U S A 1994, 91:1915-1914
18. Niswender KD, Shiota M, Postic C, Cherrington AD, Magnuson MA: Effects of increased glucokinase gene copy number on glucose homeostasis and hepatic glucose metabolism. J Biol Chem 1997, 272:22570-22575
19. Harirhan N, Farrell D, Hagan D, Hillyer D, Arbeeny C, Sabrah T, et al: Expression of human hepatic glucokinase in transgenic mice liver results in decreased glucose levels and reduced body weight. Diabetes 1997, 46:11-16
20. Postic C, Shiota M, Niswender KD, Jetton TL, Chen Y, Moates JM, et al: Dual roles for glucokinase in glucose homeostasis as determined by liver and pancreatic beta cell-specific gene knock-outs using Cre recombinase. J Biol Chem 1999, 274:305-315
21. Chiu KC, Province MA, Permutt MA: Glucokinase gene is genetic marker for NIDDM in American blacks. Diabetes 1992, 41:843-849

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