

The regulatory subunit p85 α of the enzyme phosphoinositide 3-kinase is a critical component in insulin action. In mice reduced expression levels of p85 α increase insulin sensitivity, and in humans variation within the p85 α gene (*PIK3R1*) is associated with insulin resistance and an increased susceptibility for developing type 2 diabetes (T2D). The aim of the present study was to investigate if the *PIK3R1* associated with glycemic response to 26 weeks of oral antidiabetic therapy in patients with T2D. 290 T2D patients were randomized, parallel, double-blind to monotherapy with either 1 or 2 mg/day of the dual acting PPAR- γ/α agonist ragaglitazar (n=165) or 2 g/day metformin (n=63) or 20 mg/day glyburide (n=62). At 26 weeks, the mean HbA_{1c} % and fasting plasma glucose (FPG) of the ragaglitazar treatment groups (1 and 2 mg/day) had dropped by approximately 1.1 HbA_{1c} %, and 2 mmol/l, compared to 0.7 HbA_{1c} % and 0.4 mmol/l for glyburide and 0.9 HbA_{1c} % and 0.9 mmol/l for metformin. Haplotypes were generated by genotyping (MALDI-TOF) 7 single nucleotide polymorphisms covering the coding region and the 5'upstream region of the *PIK3R1*. General linear regression models were run with Box-Cox transformed variables (HbA_{1c}, FPG) of treatment as the endpoints, and with haplotypes h1, h2, h3, and hx as the markers of interest, adjusting for age, sex, treatment group, BMI, ethnicity, diastolic BP, the Met326Ile snp, and the baseline level of the endpoint. Haplotyping classified the 290 T2D patients into 4 common haplotypes: h1, 1111112, 25%; h2, 1112112, 13%; h3, 1112111, 10%; hx, 52%. A general test showed that these haplotypes had effect on FPG (p=0.002) and HbA_{1c} (p=0.031) in patients randomized to ragaglitazar only. In pair-wise comparisons haplotypes h1, h2, h3 dropped 0.55, 0.93 and 0.73 mmol/l less in FPG (p=0.013, 0.0007 and 0.018) whereas h2 and h3 also dropped 0.27 and 0.43 HbA_{1c} % less than the hx reference haplotype (p=0.038 and 0.012).

The present study indicates that variation in the *PIK3R1* gene may regulate the glycaemic response to antidiabetic PPAR- γ/α agonist therapy in patients with T2D.

Introduction

Activation of phosphoinositide 3-kinase (PIK3) plays a central role in insulin signal transduction and expression levels of the p85 α regulatory subunit (PIK3R1) have in mice models been shown to regulate insulin sensitivity negatively (1-3). In humans, the *PIK3R1* has been investigated by single nucleotide polymorphism (SNP) analyses as a candidate gene for inherited insulin resistance and type 2 diabetes, but a possible genetic role in these common phenotypes remains controversial (4, 5). On the other hand, studies have shown that PIK3 plays an active role in restoring insulin sensitivity by PPAR γ agonist (thiazolidinedione, TZD) treatment of patients with type 2 diabetes (6, 7). The aim of the present study, therefore, was to investigate if variation in the *PIK3R1* associated with glycemic response (fasting plasma glucose, FPG; and HbA_{1c}) to 26 weeks of antidiabetic therapy with a dual PPAR γ/α agonist ragaglitazar (8) in patients with type 2 diabetes.

Association of Common Haplotypes of the p85 α Subunit of Phosphoinositide 3-kinase Gene (*PIK3R1*) with Glycemic Response to 26 Weeks of Treatment with an Insulin Sensitizing PPAR- γ/α Agonist in Type 2 Diabetic Patients

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Materials and methods

545 patients with type 2 diabetes, who consented to give DNA, were randomized in a double-blind, parallel, active-controlled trial to monotherapy for 52 weeks with either:

- 1 mg/day of the dual acting PPAR- α/γ agonist Ragaglitazar (n = 167)
- 2 mg/day of the dual acting PPAR- α/γ agonist Ragaglitazar (n = 178)
- 20 mg/day Diabeta™ (glyburide) (n = 99)
- 2000 mg/day Glucophage™ (metformin) (n = 110)

After 26 weeks of treatment the trial was stopped prematurely, at which time 290 patients had completed this visit:

- n = 165 patients randomized to 1 or 2 mg/day of Ragaglitazar
- n = 135 patients randomized to either 20 mg/day Diabeta™ or 2000 mg Glucophage™

Haplotypes were generated by genotyping (MALDI-TOF) 7 single nucleotide polymorphisms, that were not in strong linkage disequilibrium, covering the coding region and the 5'-upstream region of the *PIK3R1* (tables 1-3).

Table 1. *PIK3R1* SNP identity (Genaisance Inc) and distribution

SNP ID (number in row)	Nucleotide change	Number of alleles
659886485 (1)	C/T (Tyr73Tyr)	807/280
659886489 (2)	A/C	706/342
659886595 (3)	A/C	939/197
659886608 (4)	G/A	725/383
659886629 (5)	A/G	793/241
659886702 (6)	A/G	1023/109
659886707 (7)	G/A (Met326Ile)	912/218

Table 2. SNP-Hap estimate of haplotype distribution in *PIK3R1* at baseline

Haplotype alleles	Number (frequency)	Cumulative
1111112 (h1)	284 (0.25)	284 (0.25)
1112112 (h2)	162 (0.14)	446 (0.39)
1112111 (h3)	103 (0.09)	559 (0.48)
nnnnnnn (hx)	591 (0.52)	1140 (1.00)

Average posterior probability of haplotypes 0.85

Table 3. SNP-Hap estimate of haplotype distribution in *PIK3R1* at 26 weeks

Haplotype alleles	Number (frequency)	Cumulative
1111112 (h1)	132 (0.25)	132 (0.25)
1112112 (h2)	67 (0.13)	199 (0.38)
1112111 (h3)	51 (0.10)	250 (0.48)
nnnnnnn (hx)	285 (0.52)	535 (1.00)

Results and conclusion

- Haplotypes of *PIK3R1* had an overall effect on fasting plasma glucose (p = 0.002) and HbA_{1c} (p = 0.031) in response to 26 weeks of treatment with the dual PPAR α/γ agonist ragaglitazar
- Three haplotypes h1, h2, and h3 (together constituting 48% of the trial population) associated with a 0.55; 0.93; and 0.73 mM smaller reduction in fasting glucose compared to a reference haplotype hx (14 pooled haplotypes with frequency <8%, and constituting the remainder 52% of the trial population, figure 1)
- Two haplotypes h2 and h3 also associated with 0.27; and 0.43 smaller reduction in HbA_{1c}% compared to the reference haplotype hx (figure 2)
- The effects of *PIK3R1* haplotypes were independent of the Met326Ile variant, which alone did not have any effect on treatment response
- The present study indicates that variation in the *PIK3R1* gene contributes to the inter-individual difference in therapeutic response to the insulin-sensitizing efficacy of PPAR γ agonist treatment in patients with type 2 diabetes

Statistics

General linear regression models were run with Box-Cox transformed variables: HbA_{1c}, FPG, body weight, and BMI after 26 weeks of treatment as endpoints including most frequent haplotypes h1, h2, h3, hx, and Thr504Ala as markers of interest, and adjusting for age, sex, treatment, BMI, ethnicity, diastolic blood pressure, and the baseline value of the endpoint in the model formula: Response~AGE + Race + SEX + TREATCD + BMI + BPDIAB + baseline + h1 + h2 + h3 + hx + Met326Ile

References

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Figure 1. Estimates for *PIK3R1* haplotypes on FPG at baseline and after 26 weeks of treatment with ragaglitazar

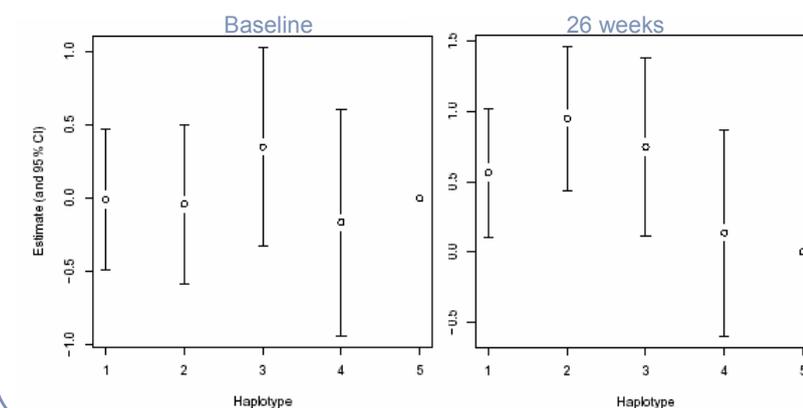


Figure 2. Estimates for *PIK3R1* haplotypes on HbA_{1c} at baseline and after 26 weeks of treatment with ragaglitazar

