

Variation in TCF7L2 influences therapeutic response to sulfonylureas: A GoDARTs study.

Received for publication 30 March 2007 and accepted in revised form 18 May 2007.

Additional information for this article can be viewed in an online appendix at <http://diabetes.diabetesjournals.org>.

Ewan R Pearson^{1*} PhD, Louise A Donnelly^{2*} BSc, Charlotte Kimber³, Bsc., Adrian Whitley³, Bsc., Alex S F Doney¹ PhD, Mark I McCarthy⁴ MD, Andrew T Hattersley⁵ DM, Andrew D Morris¹ MD, Colin N A Palmer³ PhD

Affiliations

1. Division of Medicine & Therapeutics, University of Dundee, UK.
2. Health Informatics Centre, University of Dundee, UK.
3. Population Pharmacogenetics Group, Biomedical Research Centre, University of Dundee, UK.
4. Oxford Centre for Diabetes, Endocrinology & Metabolism, Oxford, UK
5. Institute of Biomedical Sciences, Peninsula Medical School, UK

* These people contributed equally to this work

Corresponding Author

Ewan Pearson
Division of Medicine & Therapeutics,
Ninewells Hospital & Medical School,
Ninewells Avenue,
Dundee,
DD1 9SY,
UK
Email e.pearson@chs.dundee.ac.uk

Abstract word count 250

Article word count 2169

References 25

Tables 3

Figures 1

Objective. There is considerable inter-individual variation in sulfonylurea response in type 2 diabetes (T2DM). *TCF7L2* variants have been identified to be strongly associated with T2DM risk, probably due to decreased beta cell function. We hypothesized that variation in *TCF7L2* would influence response to sulfonylureas but not metformin. We studied the effect of *TCF7L2* rs12255372 and rs7903146 genotypes on glycaemic response.

Research Design & Methods. The DARTS/MEMO collaboration database includes prescribing, biochemistry and clinical phenotype of all patients with diabetes within Tayside, Scotland from 1992. Of these, *TCF7L2* genotype was determined in 4469 patients with T2DM recruited to GoDARTS between 1997 and July 2006. 901 incident sulfonylurea users and 945 metformin users were identified. A logistic regression was used with treatment failure defined as an HbA1c >7% within 3 to 12 months after treatment initiation. Covariates included *TCF7L2* genotype, BMI, gender, age diagnosed, drug adherence and drug dose. HbA1c pre-treatment was available in a subset of patients (sulfonylurea n=579, metformin n=755).

Results. Carriers of the risk allele were less likely to respond to sulfonylureas with an OR (95%CI) for failure of 1.95 (1.23-3.06), p=0.005, comparing rs12255372 T/T v G/G. Including the baseline HbA1c strengthened this association (OR 2.16 (1.21-3.86), p=0.009). A similar, although slightly weaker, association was seen with rs7903146. No association was seen between metformin response and either SNP, after adjustment for baseline HbA1c.

Conclusions. *TCF7L2* variants influence therapeutic response to sulfonylureas but not metformin. This study establishes that genetic variation can alter response to therapy in type 2 diabetes.

Sulfonylureas are widely used to treat type 2 diabetes (T2DM). There is considerable inter-individual variation in the hypoglycemic response to sulfonylureas. Physiological studies have shown response is in part predicted by stimulated C-peptide, as a marker of endogenous beta-cell reserve (1; 2). Therefore variation in sulfonylurea response may be explained by variation in genes involved in regulating beta-cell function. Apart from in some monogenic forms of diabetes (3-5) there has been limited success in pharmacogenetic studies of sulfonylurea response: The results for the effect of the E23K variant of *KCNJ11* (6-8) on response are conflicting; and although in physiological studies variants in *ABCC8* (encoding SUR1) have been shown to influence insulin secretory response to intravenous tolbutamide (9) the impact of these variants on glycemic response has not been studied.

Recently, two intronic SNPs within the transcription-factor-7-like 2 (*TCF7L2*) gene, rs12255372 and rs7903146, were found to contribute substantially to the risk of T2DM (10). This finding has been robustly replicated in a number of studies across multiple populations (11-23) and has been shown to influence progression to diabetes (11; 18; 20). *TCF7L2* is involved in the Wnt signaling pathway, yet the mechanism linking this with diabetes is not known. *TCF7L2* is expressed in the mature and developing pancreatic beta-cells (17), and insulin secretion is reduced in those with the risk alleles (11; 13; 16) suggesting a predominant direct or indirect role of *TCF7L2* on beta-cell function.

Given the robust influence of *TCF7L2* variants on risk of T2DM, and their probable role in beta-cell function we hypothesized that carriers of the diabetes

risk alleles at rs12255372 and rs7903146 would have a poorer hypoglycemic response to sulfonylureas due to decreased beta-cell function compared with individuals lacking these alleles. On the other hand these variants would have minimal impact on metformin response, which acts predominantly by improving insulin action rather than secretion. Therefore, we studied the influence of variation within *TCF7L2* on the early response to sulfonylureas and metformin in 1846 patients with T2DM in Tayside, Scotland.

RESEARCH DESIGN & METHODS

Study Subjects. Patients were identified from an ongoing study of the Genetics of Diabetes Audit and Research Tayside (GoDARTS) (4469 cases) recruited in Tayside between 1st October 1997 and 1st July 2006 who could be linked to the MEMO (Medicines Monitoring Unit) databases. The DARTS/MEMO collaboration includes validated prescribing, biochemistry and phenotypic historical data from 1992 to present (24) and prospective longitudinal data is collected on each person with T2DM recruited into the genetic study.

For this study, patients were selected to have T2DM on the basis of an age of diagnosis after the age of 40, with no progression to insulin within 6 months of diagnosis. Patients were excluded who were diagnosed with diabetes after 90 years of age. Prescription data were available between January 1992 and April 2004. All incident users of sulfonylureas and metformin were identified; to be eligible all study participants had to have received no diabetes treatment for at least six months prior to their index prescription for sulfonylurea or metformin, and were thus considered treatment-naïve. We

therefore identified 1168 patients who subsequently encashed at least two sulfonylurea prescriptions, and 1263 patients who encashed at least two metformin prescriptions. The study was approved by the Tayside Medical Ethics Committee and informed consent was obtained from all subjects.

Definition of response. For inclusion in the study, patients were required to have at least one HbA1c recorded within 3 to 12 months after commencing sulfonylureas (n=901) or metformin (n=945). A “treat to target” approach was taken, with failure defined as the failure to reach an HbA1c \leq 7% within the 3 to 12 month period after incident drug prescription. In a further analysis, the HbA1c within the 6 months prior to commencing sulfonylureas (n=579) or metformin (n=755) was included as a covariate. Where HbA1c was measured more than once prior to treatment, the HbA1c nearest to drug initiation was taken. The HbA1c was DCCT aligned.

Drug adherence and dose. We used population-based drug dispensing records to calculate the % maximum possible adherence for each patient (25). Dose was expressed as a % of the maximal prescribed dose in the British National Formulary (to allow comparison between sulfonylurea drugs) (26)..

Determination of Body Mass Index. The BMI (average) was taken as the mean of the BMI measures recorded throughout the study period. The BMI at diagnosis was taken as the mean of the BMI measures within one year either side of diagnosis. The BMI at treatment initiation was taken as the mean of the BMI measures within one year either side of treatment initiation.

Genotyping. We genotyped rs12255372 and rs7903146 of *TCF7L2*

using TAQMAN allelic discrimination assays as previously described (22).. Both variants were in Hardy Weinberg equilibrium and were in tight LD as previously reported ($R^2=0.9$). Genotyping success rate for each SNP was ~98% and duplicate genotyping concordance was >99%.

Statistical analysis. Comparison of baseline characteristics by genotype was by ANOVA. Genotype frequencies were analysed by Chi Square test for trend (1degree of freedom). A logistic regression analysis was used to investigate response, with failure of treatment (minimum HbA1c after starting treatment >7%) as the dependent variable. Covariates were selected if there was a significant difference in baseline characteristics by genotype, or if there was a simple correlation with response with $p<0.1$. With respect to genotype a co-dominant model was assumed, with the GG (rs12255372) and CC (rs7903146) genotype as the reference.

RESULTS

The baseline characteristics of incident sulfonylurea and metformin users according to genotype at rs12255372 and rs7903146 is shown in table 1. In keeping with previous studies, those with two copies of the T allele of rs12255372 or rs7903146 (TT) had a lower BMI at diagnosis of diabetes in both the sulfonylurea and metformin treated groups. This was more marked in the metformin-treated group who were more obese and could be seen in BMI at treatment initiation and in a BMI averaged over the whole study period.

Across the whole cohort, 42% of sulfonylurea users and 49% of metformin users did not achieve a target HbA1c <7% within 1 year of treatment initiation. The genotype frequencies at rs12255372 and rs7903146 according to

the early therapeutic response to sulfonylureas or metformin are shown in table 2. Genotype influenced response to sulfonylureas, with more treatment failure in the TT homozygotes of either SNP. 57% of the TT homozygotes failed to reach target compared to only 40% of the GG individuals. The heterozygote group displayed an intermediate failure rate and this corresponded to a per-allele odds ratio for treatment failure of 1.28 (p=0.014) for rs12255372, and 1.27 (p=0.017) for rs7903146. There was no significant effect of genotype on metformin response.

A logistic regression was used to account for baseline differences and other confounding factors (table 3). For sulfonylurea response, the rs12255372 TT homozygotes were more likely not to be treated to target as the GG homozygotes (OR 1.94); with a slightly weaker association with rs7903146 (OR 1.73). The per-allele OR for treatment failure was 1.28 (p=0.02) for rs12255372 and 1.26 (p=0.03) for rs7903146. For metformin there was also an increase in treatment failure by genotype, although this only achieved statistical significance when comparing TT versus CC for rs7903146 (OR 1.58, p=0.046).

Because *TCF7L2* is likely to be influencing baseline glycemic control, a further logistic regression analysis was done on 579 sulfonylurea treated and 755 metformin treated patients in whom the HbA1c was known within 6 months prior to treatment initiation (table 3). The baseline characteristics of this subgroup are shown in supplementary tables 1 and 2. Including the HbA1c pre-treatment in the model abolished any effect of genotype on treatment response to metformin. However, inclusion of pre-treatment HbA1c strengthened the association between sulfonylurea response and genotype at

rs1225372 (TT vs GG OR 2.16) and at rs7903146 (TT vs CC OR 1.90). This can also be seen if the both cohorts are analysed together using treatment (sulfonylurea or metformin) and pre_treatment HbA1c as covariates: In a logistic regression, there was a significant interaction between treatment and genotype (rs1225372 TT vs GG p=0.04)

In a complementary approach, a linear regression model was used with the minimum HbA1c achieved within the year following sulfonylurea initiation as the dependent variable (supplementary table 3). Using this model the predicted HbA1c on treatment by genotype at rs1225372 was GG 7.0 (CI 6.86 to 7.14) versus TT 7.33 (7.06 to 7.60); p=0.032. Similar results were seen for rs7903146.

Finally, to include the time taken to achieve target HbA1c <7% we used Cox proportional hazards to analyse the effect of genotype on response. The Kaplan-Meier plots for response by rs1225372 genotype, adjusted for pre-treatment HbA1c are shown in figure 1, with the TT group more likely not to achieve target than the GG group (HR 1.54, p=0.03) for sulphonylurea treatment but with no effect of genotype on metformin response (p=0.82).

DISCUSSION

We show that variation in *TCF7L2* influences initial treatment success with sulfonylurea therapy in patients with T2DM. This is seen for both SNPs that have been reported to be associated with diabetes risk, and is in addition to the effect of dose, adherence, sex and baseline glycemia (determined by HbA1c pre-treatment). With respect to rs1225372 the 12% of the diabetic population with two copies of the T allele are twice as likely not to achieve an HbA1c below 7% within 1 year of

treatment initiation than the 42% of the population with two copies of the G allele, even accounting for baseline difference in pre-treatment HbA1c. This results in the majority of TT (57%) homozygotes not achieving target HbA1c, an absolute difference of 17% compared to the GG homozygotes.

There was a weak association between metformin treatment success and *TCF7L2* genotype, however this effect was abolished by inclusion of pre-treatment HbA1c as a covariate in the model. While this could reflect a reduction in power or suggest that metformin may be less effective in the TT homozygotes, it may reflect an effect of these variants on glycemia rather than a genotypic influence on response per se. In support of this we have recently shown that in the overall Go-DARTs population the rs7903146 TT homozygote cases and controls had a higher HbA1c, with the TT homozygote cases being more likely to require oral medication or insulin than the CC homozygotes (22).

The association between *TCF7L2* variants and sulfonylurea response, but not metformin response supports the growing body of evidence that *TCF7L2* is involved in direct or indirect (e.g. incretin mediated (10)) regulated regulation of beta-cell function (11; 13; 16). Detailed physiological and pharmacokinetic studies of patients selected on the basis of their *TCF7L2* genotype are required to further investigate the mechanism for decreased sulfonylurea response in the TT homozygotes.

In line with previous studies, we show an association of genotype on BMI (11; 20; 22; 23). This is particularly striking in the more obese metformin treated group. Because retrospective data is available following recruitment in our

study, it is possible to investigate the association between genotype and BMI at diagnosis of diabetes. The BMI difference by genotype is largest at diagnosis, with the effect reducing by treatment initiation or when averaged over the whole study period. This is in keeping with the *TCF7L2* risk variants effecting beta-cell function, causing diabetes to present at a lower level of obesity or insulin resistance.

There are limitations to this study. This is an observational study rather than a randomized interventional study and is therefore prone to prescriber bias. However, the prescriber would have been blind to genotype, except where the genotype impacted on a clinical phenotype that might influence management. In this study we were able to account for differences in BMI, sex and baseline HbA1c. Furthermore the dose prescribed, and a measure of adherence, based on prescription encashment, could also be accounted for. The potential difficulties with an observational study are highlighted by our finding that those who received a higher starting dose of sulfonylurea were less likely reach the HbA1c target. This probably reflects an anticipated lack of response, by the prescriber, on the basis of diabetes severity or other unmeasured factors. A further limitation is that this study was designed to look at the early response to therapy between three and twelve months after initiation. Three months after treatment initiation might be too early to see a full therapeutic effect, especially of metformin, and this short period may explain why the drug doses were relatively low. However, the decision to study early response was taken to minimize individual patient and physician factors that are likely to confound the effect of genotype on longer term response or failure, which

would be better studied in the context of a carefully controlled clinical trial.

To date, pharmacogenetics studies on therapeutic response within diabetes have been limited. The most important example of genetic aetiology impacting on clinical diabetes management has been in monogenic diabetes (3; 5). We believe this current study is a robust example of pharmacogenetics within an unselected polygenic type 2 population. Compared to the monogenic examples, the effect size of *TCF7L2* variation on response is modest, although a two-fold greater likelihood of treatment failure in the 12 % of the population who are TT homozygote at rs1225372 is striking. This highlights strong parallels between type 2 diabetes pharmacogenetics and genetics of disease risk, with individual risk alleles contributing only little to overall risk. However, with an increasing number of clear risk genes for type 2 diabetes resulting from whole genome association studies (21) it may be possible to study the effect of combined genotypes on response, as has been done for type 2 diabetes risk (27),

which may in due course allow genotype to contribute to response prediction in the clinical management of patients. We believe that this study is a “proof of principle” that common genetic variation can influence therapeutic response in type 2 diabetes, and that given robust candidate genes and a well characterized therapeutic response phenotype, large population biobanks can be used successfully in population pharmacogenomic studies.

ACKNOWLEDGEMENTS

We would like to thank the patients for taking part in this study, and the research nurses who recruited them. We acknowledge Diabetes UK, The Wellcome Trust, the Scottish Executive Chief Scientist’s Office for funding this work. ERP holds an NHS Education for Scotland Clinician Scientist fellowship. CNAP and ADM are supported by the Scottish Executive Chief Scientist’s Office as part of the *Generation Scotland* initiative.

REFERENCES

1. Blaum CS, Velez L, Hiss RG, Halter JB: Characteristics related to poor glycemic control in NIDDM patients in community practice. *Diabetes Care* 20:7-11, 1997
2. Hermann LS, Schersten B, Bitzen PO, Kjellstrom T, Lindgarde F, Melander A: Therapeutic comparison of metformin and sulfonylurea, alone and in various combinations. A double-blind controlled study. *Diabetes Care* 17:1100-1109, 1994
3. Pearson ER, Starkey BJ, Powell RJ, Gribble FM, Clark PM, Hattersley AT: Genetic cause of hyperglycaemia and response to treatment in diabetes. *Lancet* 362:1275-1281, 2003
4. Sagen JV, Raeder H, Hathout E, Shehadeh N, Gudmundsson K, Baevre H, Abuelo D, Phornphutkul C, Molnes J, Bell GI, Gloyn AL, Hattersley AT, Molven A, Sovik O, Njolstad PR: Permanent neonatal diabetes due to mutations in KCNJ11 encoding Kir6.2: patient characteristics and initial response to sulfonylurea therapy. *Diabetes* 53:2713-2718, 2004
5. Pearson ER, Flechtner I, Njolstad PR, Malecki MT, Flanagan SE, Larkin B, Ashcroft FM, Klimes I, Codner E, Iotova V, Slingerland AS, Shield J, Robert JJ, Holst JJ, Clark PM, Ellard S, Sovik O, Polak M, Hattersley AT: Switching from insulin to oral sulfonylureas in patients with diabetes due to Kir6.2 mutations. *N Engl J Med* 355:467-477, 2006
6. Gloyn AL, Hashim Y, Ashcroft SJ, Ashfield R, Wiltshire S, Turner RC: Association studies of variants in promoter and coding regions of beta-cell ATP-sensitive K-channel genes SUR1 and Kir6.2 with Type 2 diabetes mellitus (UKPDS 53). *Diabet Med* 18:206-212, 2001
7. Sesti G, Laratta E, Cardellini M, Andreozzi F, Del Guerra S, Irace C, Gnasso A, Grupillo M, Lauro R, Hribal ML, Perticone F, Marchetti P: The E23K variant of KCNJ11 encoding the pancreatic beta-cell adenosine 5'-triphosphate-sensitive potassium channel subunit Kir6.2 is associated with an increased risk of secondary failure to sulfonylurea in patients with type 2 diabetes. *J Clin Endocrinol Metab* 91:2334-2339, 2006
8. Hansen L, Echwald SM, Hansen T, Urhammer SA, Clausen JO, Pedersen O: Amino acid polymorphisms in the ATP-regulatable inward rectifier Kir6.2 and their relationships to glucose- and tolbutamide-induced insulin secretion, the insulin sensitivity index, and NIDDM. *Diabetes* 46:508-512, 1997
9. Hansen T, Echwald SM, Hansen L, Moller AM, Almind K, Clausen JO, Urhammer SA, Inoue H, Ferrer J, Bryan J, Aguilar-Bryan L, Permutt MA, Pedersen O: Decreased tolbutamide-stimulated insulin secretion in healthy subjects with sequence variants in the high-affinity sulfonylurea receptor gene. *Diabetes* 47:598-605, 1998
10. Grant SF, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadóttir A, Styrkarsdóttir U, Magnusson KP, Walters GB, Palsdóttir E, Jonsdóttir T, Gudmundsdóttir T, Gylfason A, Saemundsdóttir J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Gudnason V, Sigurdsson G, Thorsteinsdóttir U, Gulcher JR, Kong A, Stefansson K: Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* 38:320-323, 2006
11. Florez JC, Jablonski KA, Bayley N, Pollin TI, de Bakker PI, Shuldiner AR, Knowler WC, Nathan DM, Altshuler D: TCF7L2 polymorphisms and

- progression to diabetes in the Diabetes Prevention Program. *N Engl J Med* 355:241-250, 2006
12. Damcott CM, Pollin TI, Reinhart LJ, Ott SH, Shen H, Silver KD, Mitchell BD, Shuldiner AR: Polymorphisms in the transcription factor 7-like 2 (TCF7L2) gene are associated with type 2 diabetes in the Amish: replication and evidence for a role in both insulin secretion and insulin resistance. *Diabetes* 55:2654-2659, 2006
 13. Melzer D, Murray A, Hurst AJ, Weedon MN, Bandinelli S, Corsi AM, Ferrucci L, Paolisso G, Guralnik JM, Frayling TM: Effects of the diabetes linked TCF7L2 polymorphism in a representative older population. *BMC Med* 4:34, 2006
 14. Scott LJ, Bonnycastle LL, Willer CJ, Sprau AG, Jackson AU, Narisu N, Duren WL, Chines PS, Stringham HM, Erdos MR, Valle TT, Tuomilehto J, Bergman RN, Mohlke KL, Collins FS, Boehnke M: Association of transcription factor 7-like 2 (TCF7L2) variants with type 2 diabetes in a Finnish sample. *Diabetes* 55:2649-2653, 2006
 15. Zhang C, Qi L, Hunter DJ, Meigs JB, Manson JE, van Dam RM, Hu FB: Variant of transcription factor 7-like 2 (TCF7L2) gene and the risk of type 2 diabetes in large cohorts of U.S. women and men. *Diabetes* 55:2645-2648, 2006
 16. Saxena R, Gianniny L, Burtt NP, Lyssenko V, Giuducci C, Sjogren M, Florez JC, Almgren P, Isomaa B, Orho-Melander M, Lindblad U, Daly MJ, Tuomi T, Hirschhorn JN, Ardlie KG, Groop LC, Altshuler D: Common single nucleotide polymorphisms in TCF7L2 are reproducibly associated with type 2 diabetes and reduce the insulin response to glucose in nondiabetic individuals. *Diabetes* 55:2890-2895, 2006
 17. Cauchi S, Meyre D, Dina C, Choquet H, Samson C, Gallina S, Balkau B, Charpentier G, Pattou F, Stetsyuk V, Scharfmann R, Staels B, Froguel G, Froguel P: Transcription factor TCF7L2 genetic study in the French population: expression in human beta-cells and adipose tissue and strong association with type 2 diabetes. *Diabetes* 55:2903-2908, 2006
 18. Humphries SE, Gable D, Cooper JA, Ireland H, Stephens JW, Hurel SJ, Li KW, Palmen J, Miller MA, Cappuccio FP, Elkeles R, Godsland I, Miller GJ, Talmud PJ: Common variants in the TCF7L2 gene and predisposition to type 2 diabetes in UK European Whites, Indian Asians and Afro-Caribbean men and women. *J Mol Med* 84:1-10, 2006
 19. Groves CJ, Zeggini E, Minton J, Frayling TM, Weedon MN, Rayner NW, Hitman GA, Walker M, Wiltshire S, Hattersley AT, McCarthy MI: Association analysis of 6,736 U.K. subjects provides replication and confirms TCF7L2 as a type 2 diabetes susceptibility gene with a substantial effect on individual risk. *Diabetes* 55:2640-2644, 2006
 20. Cauchi S, Meyre D, Choquet H, Dina C, Born C, Marre M, Balkau B, Froguel P: TCF7L2 variation predicts hyperglycemia incidence in a French general population: the data from an epidemiological study on the Insulin Resistance Syndrome (DESIR) study. *Diabetes* 55:3189-3192, 2006
 21. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, Boutin P, Vincent D, Belisle A, Hadjadj S, Balkau B, Heude B, Charpentier G, Hudson TJ, Montpetit A, Pshzhetsky AV, Prentki M, Posner BI, Balding DJ, Meyre D, Polychronakos C, Froguel P: A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 445:881-885, 2007

22. Kimber CH, Doney AS, Pearson ER, McCarthy MI, Hattersley AT, Leese GP, Morris AD, Palmer CN: TCF7L2 in the Go-DARTS study: evidence for a gene dose effect on both diabetes susceptibility and control of glucose levels. *Diabetologia* 50:1186-1191, 2007
23. Helgason A, Palsson S, Thorleifsson G, Grant SF, Emilsson V, Gunnarsdottir S, Adeyemo A, Chen Y, Chen G, Reynisdottir I, Benediktsson R, Hinney A, Hansen T, Andersen G, Borch-Johnsen K, Jorgensen T, Schafer H, Faruque M, Doumatey A, Zhou J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Sigurdsson G, Hebebrand J, Pedersen O, Thorsteinsdottir U, Gulcher JR, Kong A, Rotimi C, Stefansson K: Refining the impact of TCF7L2 gene variants on type 2 diabetes and adaptive evolution. *Nat Genet* 39:218-225, 2007
24. Morris AD, Boyle DI, McMahon AD, Greene SA, MacDonald TM, Newton RW: Adherence to insulin treatment, glycaemic control, and ketoacidosis in insulin-dependent diabetes mellitus. The DARTS/MEMO Collaboration. Diabetes Audit and Research in Tayside Scotland. Medicines Monitoring Unit. *Lancet* 350:1505-1510, 1997
25. Donnan PT, MacDonald TM, Morris AD: Adherence to prescribed oral hypoglycaemic medication in a population of patients with Type 2 diabetes: a retrospective cohort study. *Diabet Med* 19:279-284, 2002
26. Donnelly LA, Doney AS, Hattersley AT, Morris AD, Pearson ER: The effect of obesity on glycaemic response to metformin or sulphonylureas in Type 2 diabetes. *Diabet Med* 23:128-133, 2006
27. Weedon MN, McCarthy MI, Hitman G, Walker M, Groves CJ, Zeggini E, Rayner NW, Shields B, Owen KR, Hattersley AT, Frayling TM: Combining information from common type 2 diabetes risk polymorphisms improves disease prediction. *PLoS Med* 3:e374, 2006

Table 1. Baseline demography

Patients Started on SULFONYLUREAS	All	GG	rs1225372		ANOVA	CC	rs7903146		ANOVA
			GT	TT			CT	TT	
N	901	382	415	104		380	409	112	
Age at Rx (years)	63.8 (9.6)	63.8 (9.6)	63.9 (9.8)	63.7 (8.9)	0.99	63.8 (9.8)	64.1 (9.6)	62.9 (8.7)	0.53
Age at diagnosis (years)	61.3 (9.6)	61.5 (9.4)	61.3 (9.8)	60.9 (9.7)	0.84	61.6 (9.6)	61.3 (9.7)	60.2 (9.3)	0.40
BMI average (kg/m ²)	28.3 (4.6)	28.3 (4.5)	28.4 (4.5)	27.6 (4.7)	0.26	28.4 (4.6)	28.3 (4.6)	27.7 (4.4)	0.37
BMI at diagnosis (kg/m ²) ^a	28.1 (4.6)	27.9 (4.6)	28.5 (4.8)	26.8 (3.5)	0.020	28.1 (4.7)	28.4 (4.7)	27.0 (3.5)	0.049
BMI at start of treatment ^b	28.0 (4.5)	28.0 (4.6)	28.2 (4.6)	27.0 (3.3)	0.09	28.2 (4.8)	28.1 (4.6)	27.1 (3.3)	0.13
Sex (% male)	58	57	59	63	0.55	58	57	63	0.63
Dose (% maximum)	28 (15)	28 (14)	29 (16)	29 (15)	0.75	28 (15)	29 (15)	28 (14)	0.84
Adherence (%)	80 (18)	80 (18)	79 (18)	83 (15)	0.092	79 (18)	80 (17)	82 (15)	0.31
METFORMIN	All	GG	GT	TT	ANOVA	CC	CT	TT	ANOVA
N	945	434	420	91		422	424	99	
Age at Rx (years)	60.0 (9.5)	60.4 (9.7)	59.5 (9.0)	61.2 (10.5)	0.17	60.5 (9.6)	59.5 (9.2)	60.6 (9.9)	0.24
Age at diagnosis (years)	57.7 (9.4)	58.2 (9.5)	56.9 (8.9)	59.2 (10.4)	0.041	58.3 (9.4)	57.0 (9.1)	58.4 (9.9)	0.11
BMI average (kg/m ²)	32.8 (5.6)	33.4 (5.7)	32.4 (5.4)	31.4 (5.5)	0.002	33.3 (5.7)	32.6 (5.4)	31.2 (5.5)	0.003
BMI at diagnosis (kg/m ²) ^c	33.1 (6.0)	34.0 (6.3)	32.4 (5.6)	31.8 (5.6)	<0.001	34.0 (6.3)	32.6 (5.6)	31.5 (5.5)	0.001
BMI at start of treatment ^d	32.9 (5.8)	33.6 (5.9)	32.5 (5.6)	31.3 (5.7)	0.001	33.5 (6.0)	32.6 (5.5)	31.2 (5.6)	0.001
Sex (% male)	52	47	56	48	0.026	49	54	53	0.28
Dose (% maximum)	19 (7)	19 (7)	19 (6)	19 (5)	0.38	19 (7)	19 (7)	19 (7)	0.50
Adherence (%)	92 (12)	91 (13)	92 (11)	92 (11)	0.26	91 (14)	92 (11)	93 (11)	0.16

Data are mean (SD) except sex which is percent male. ^a N=643; ^b N=800; ^c N=761; ^d N=893

Table 2. Genotype frequencies by treatment response

Failure to reach target?	rs1225372				rs7903146				
	SULFONYLUREA	GG	GT	TT	P-value	CC	CT	TT	P-value
No N (%)		230 (60)	246 (59)	45 (43)	0.006	232 (60)	236 (58)	43 (47)	0.035
Yes N (%)		152 (40)	169 (41)	59 (57)		148 (40)	173 (42)	59 (53)	
	METFORMIN	GG	GT	TT	P-value	CC	CT	TT	P-value
No N (%)		225 (52)	213 (51)	42 (46)	0.61	229 (54)	207 (49)	44 (44)	0.12
Yes N (%)		209 (48)	207 (49)	49 (54)		193 (46)	217 (51)	55 (56)	

Table 3. Logistic regression analysis with treatment failure as the dependent variable

	rs1225372				rs7903146				
	Without Baseline HbA1c (n=901)		With Baseline Hba1c (n=579)		Without Baseline HbA1c (n=901)		With Baseline Hba1c (n=579)		
SULFONYLUREA	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value	
TCF7L2 genotype									
GT	1.06 (0.79 to 1.43)	0.69	1.14 (0.78 to 1.68)	0.50	CT	1.13 (0.84 to 1.52)	0.42	1.23 (0.84 to 1.81)	0.29
TT	1.94 (1.23 to 3.06)	0.005	2.16 (1.21 to 3.86)	0.009	TT	1.73 (1.11 to 2.70)	0.015	1.90 (1.09 to 3.33)	0.024
Sex (Male=1; Female=0)	0.68 (0.51 to 0.90)	0.008	0.60 (0.41 to 0.87)	0.007		0.68 (0.51 to 0.91)	0.009	0.60 (0.42 to 0.87)	0.007
Age Diagnosed	0.99 (0.98 to 1.01)	0.30	0.99 (0.97 to 1.00)	0.13		0.99 (0.98 to 1.01)	0.34	0.99 (0.97 to 1.00)	0.15
Dose (per 5% of maximum)	1.20 (1.14 to 1.27)	<0.001	1.18 (1.10 to 1.27)	<0.001		1.21 (1.14 to 1.27)	<0.001	1.18 (1.11 to 1.27)	<0.001
Adherence (per 5%)	1.06 (1.02 to 1.11)	0.003	1.04 (0.99 to 1.10)	0.13		1.07 (1.02 to 1.11)	0.001	1.05 (0.99 to 1.11)	0.12
Hba1c pre-treatment (%)	-	-	1.38 (1.24 to 1.52)	<0.001		-	-	1.30 (1.18 to 1.44)	<0.001
METFORMIN (n=945)									
TCF7L2 genotype									
GT	1.06 (0.80 to 1.39)	0.70	0.96 (0.70 to 1.33)	0.81	CT	1.26 (0.95 to 1.67)	0.10	1.17 (0.85 to 1.61)	0.34
TT	1.41 (0.89 to 2.25)	0.15	1.09 (0.63 to 1.91)	0.75	TT	1.58 (1.01 to 2.50)	0.047	1.19 (0.69 to 2.02)	0.53
Sex (Male=1; Female=0)	0.98 (0.75 to 1.29)	0.91	1.10 (0.81 to 1.49)	0.54		0.98 (0.75 to 1.28)	0.89	1.09 (0.81 to 1.48)	0.57
Age Diagnosed (years)	0.99 (0.98 to 1.01)	0.32	0.99 (0.97 to 1.01)	0.19		0.99 (0.98 to 1.01)	0.39	0.99 (0.97 to 1.01)	0.23
BMI average (kg/m ²)	1.01 (0.98 to 1.03)	0.84	1.00 (0.97 to 1.01)	0.91		1.01 (0.98 to 1.03)	0.59	1.00 (0.98 to 1.03)	0.82
Dose (per 5% of maximum)	1.29 (1.19 to 1.41)	<0.001	1.31 (1.18 to 1.45)	<0.001		1.29 (1.19 to 1.41)	<0.001	1.31 (1.17 to 1.45)	<0.001
Adherence (per 5%)	1.01 (0.95 to 1.06)	0.84	0.98 (0.92 to 1.04)	0.56		1.00 (0.95 to 1.06)	0.91	0.98 (0.92 to 1.04)	0.51
HbA1c pre-treatment	-	-	1.38 (1.24 to 1.52)	<0.001		-	-	1.38 (1.24 to 1.53)	<0.001

Legend to Figure 1.

Kaplan-Meier Plots showing the proportion of patients, by genotype at rs1225372, who achieve a target HbA1c <7% after being initiated on treatment with a sulfonylurea (panel A) or metformin (panel B).

