

Lack of Association between Arg144Cys Variant of CYP2C9 Gene and Therapeutic Response to Oral Agents in Type 2 Diabetes Patients

J. Hohendorff, S. Mrozinska, A. Plis, N. Nowak, T. Klupa and M.T. Malecki

Department of Metabolic Diseases, Jagiellonian University Medical College, Krakow, Poland

KEYWORDS Genetics. SNP. Rs1799853. Cytochrome P450. Secretagogues

ABSTRACT Sulfonylureas (SUs) used in type 2 diabetes (T2DM) treatment are mainly metabolized by the cytochrome p450 2C9 enzyme. The aim of the study was to verify whether the Arg144Cys polymorphism within the CYP2C9 gene may influence the length of time from T2DM onset to the insulin therapy initiation in T2DM patients in the Polish population. For the purpose of the study the researchers analyzed clinical data of 502 T2DM patients. The patients were genotyped for Arg144Cys using real-time PCR (polymerase chain reaction). The minor allele frequency was 10.6%. The mean time to insulin introduction was 7.9 +/- 5.9 years vs. 8.7 +/- 5.9 years for CC and CT/TT genotypes respectively, $p=0.3403$. The analysis did not show significant association between CYP2C9 variant and metabolic control as measured by the HbA1c% (glycated hemoglobin A1c), lipid profile or BMI (body mass index). In conclusion, the researchers did not find any association between the CYP2C9 gene Arg144Cys polymorphism and therapeutic response to oral agents in T2DM patients.

INTRODUCTION

The sulfonylureas (SUs) are one of the most important oral drugs that are used in the treatment of type 2 diabetes mellitus (T2DM). The therapeutic effectiveness of the SUs is limited by the time. The secondary failure (inability to maintain glycemic control) of sulfonylurea treatment leads to the necessity of intensification the therapy. It can be achieved by increasing doses/number of oral drugs or by introducing the insulin therapy. It was shown in previous reports (Qian et al. 2008; Lee et al. 2011) that the factors which determinate the period of successful management with SUs include duration of type 2 diabetes mellitus, BMI, SUs dose. SUs are metabolized by cytochrome p450 2C9 (Soga et al. 2004; Gao et al. 2011). The gene that encodes CYP2C9 is located on chromosome 10q24 (Inoue et al. 1994). It was confirmed that polymorphisms of CYP2C9 have an influence on activity of the enzyme (Xu et al. 2009). Recently it was suggested that therapeutic response to SUs can be conditioned by gene variant rs1799853 (Arg144Cys, CYP2C9*2). The presence on inactivated alleles can be related with long term

successful of SUs therapy because of poor metabolism of SUs.

Objectives

The aim of the study was to verify whether the Arg144Cys polymorphism within CYP2C9 gene may influence the length of the time from T2DM onset to the insulin therapy initiation in T2DM patients in the Polish population.

MATERIAL AND METHODS

Study Population

The study cohort consisted of 268 female and 234 male unrelated T2DM patients. All participants were Caucasian, residents of South Poland. The study was approved by the Ethical Committee of Jagiellonian University Medical College and was in accordance with the principles of the Declaration of Helsinki. Diagnosis of T2DM was made on the basis of WHO criteria (1999). Clinical data concerning age of diagnosis, diabetes duration, length of insulin-free therapy, HbA1c% (glycated hemoglobin A1c), lipid profiles, BMI (body mass index) were analyzed (in some cases not all data was available). Characteristic of the study group is shown in Table 1. Detailed data on type of oral therapy was not available. However, according to Polish Diabetes Association Recommendations most patients were on sulfonylureas being pre-

Address for correspondence:
Dr. Tomasz Klupa, M.D., Ph.D.
Department of Metabolic Diseases,
Jagiellonian University Medical College,
15 Kopernika Street, 31-501 Krakow, Poland,
Phone: +48 12 424 93 01
Fax: +48 12 421 97 86
E-mail: tomasz_klupa@yahoo.com

Table 1: Characteristic of the study group

	CC	CT/TT	<i>p</i> value
Female %	52.5 (211) ¹	57.0 (57)	0.4855
Age of diagnosis ² , years	48.9 +/- 9.4 (402)	47.9 +/- 9.8 (100)	0.3468
T2DM duration ² , years	11.4 +/- 7.2 (399)	12.2 +/- 7.9 (98)	0.4673
% insulin treatment	66.9 (267)	66.3 (65)	0.9933
BMI ² , kg/m ²	31.8 +/- 6.3 (397)	32.1 +/- 6.1 (98)	0.6562
total cholesterol ² , mmol/l	5.4 +/- 1.2 (287)	5.2 +/- 1.1 (75)	0.6944
LDL ² , mmol/l	3.0 +/- 1.0 (264)	2.9 +/- 1.0 (70)	0.8487
HDL ² , mmol/l	1.3 +/- 0.7 (274)	1.3 +/- 0.4 (73)	0.4123
TG ² , mmol/l	2.2 +/- 1.8 (285)	2.1 +/- 1.3 (75)	0.8806

1 (n) – number of cases 2 data shown as mean +/- SD

scribed as first choice monotherapy or in combined therapy with metformin.

Genotyping Methods and Quality Control

DNA of T2DM patients was isolated from peripheral blood lymphocytes using commercial DNA isolation kit (DNAzol Reagent, GIBCO) (Chomczynski et al. 1997). Genotyping for the Arg144Cys polymorphism within the CYP2C9 gene was performed using TaqMan assay (assay ID: C__25625805_10) on the 7900HT Fast Real-time PCR System (Applied Biosystems) according to the manufacturer's protocol. Results were analyzed with SDS Software v.2.3 (Applied Biosystems). The genotyping success rate was 85%. To exclude genotyping errors, we reexamined 20% samples for the SNP (single nucleotide polymorphism) and there were no mismatches.

Statistical Analysis

Statistical analyses were carried out using STATISTICA 9.0 PL Software (StatSoft). For Hardy-Weinberg equilibrium chi-square test was performed. The normality of data was assessed using Shapiro-Wilk test. The Student t-test was used for quantitative variables that were normally distributed and the Mann-Whitney U-test for variables that were not normally distributed. A logistic regression analysis was used to find whether CYP2C9 gene variants are associated with the time to insulin initiation.

RESULTS

The genotype distribution (CC-402, CT-94, TT-6) was in accordance with Hardy-Weinberg equilibrium. Minor T-allele had frequency of 10.6% and was almost the same as that reported in the HapMap database (European, 10.4%).

There were neither clinical nor biochemical heterogeneity between T-allele carriers and T-allele non-carriers found (Table 1). Therapeutic response to oral hypoglycemic agent therapy defined as length of time from T2DM onset to the insulin therapy initiation did not differ between study groups, 7.9 +/- 5.9 years vs. 8.7 +/- 5.9 years for CC and CT/TT genotypes respectively, $p=0.3403$. A logistic regression analysis did not show significant effects of T-allele on oral agents response ($p=0.4409$). The effect of the Arg144Cys polymorphism on the time of insulin-free therapy is shown in Figure 1 by the Kaplan-Meier method. Event time was defined as the time of insulin initiation. Patients who did not reach end point (constantly oral agents therapy) were censored (CC – 33.1%; CT/TT 33.7%) at time their last clinic visit.

DISCUSSION

In this retrospective study the researchers analyzed the effect of Arg144Cys (CYP2C9*2) polymorphism on the length of free-insulin therapy among 502 patients with T2DM using SUs. The researchers also hypothesized that carriers of this polymorphism may differ from wild type carriers with regard to age of diagnosis, duration of T2DM, BMI, HBA1c%, lipid profile. To the best of their knowledge this is the first study in which this correlation has been investigated in the Polish population. The influence of CYP2C9*2 polymorphism on clinical response to SUs is still not clearly explained. It is known that CYP2C9*3 polymorphism produces the greatest reduction of enzyme activity comparing with other variant alleles and this reduction within homozygous CYP2C9 *2/*2 and *3/*3 genotypes is more pronounced (Surrendiran et al. 2011). The results of previous studies have revealed reduced metabolism of glibenclamide among healthy human volun-

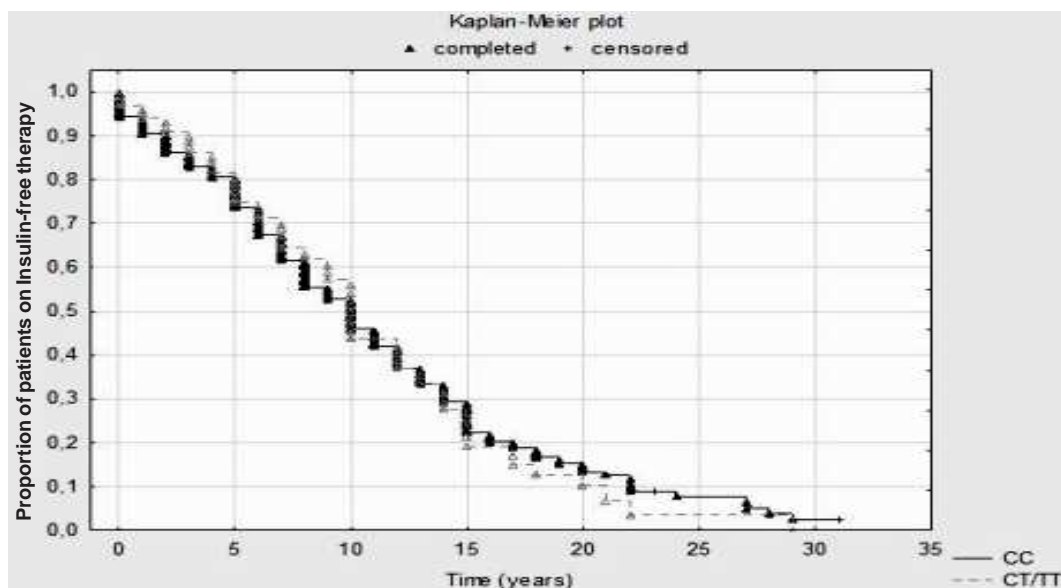


Fig. 1. Kaplan-Meier plot showing proportion of patients by genotype who are on insulin-free therapy

teers of German, Finnish and Chinese origin with $*3/*3$, $*2/*3$, $*1/*3$ variants of CYP2C9 (Kirchheiner et al. 2002; Lee et al. 2002; Niemi et al. 2002; Yin et al. 2005). Unfortunately the researchers have less information regarding the effect of the CYP2C9 genotype in T2DM patients. A previous study has found that there are no differences in the prescribed dose of tolbutamide users with the CYP2C9 $*2/*2$ genotype compared to wild-type patients (Becker et al. 2008). Another Dutch study (Swen et al. 2010) confirms, that there are no significant effects of the CYP2C9 $*2$ and CYP2C9 $*3$ alleles on time-to-stable dose in T2DM patients in primary care, however carriers of CYP2C9 $*3$ allele show a trend towards a stable glimeperide dose. In a study with a large Chinese cohort of 1073 T2DM patients a trend towards a 5% dose increase was observed for those treated with gliclazide as a monotherapy with none or one copy versus dose increase in carriers of two copies of CYP2C9 $*2$ or $*3$ (Zhou et al. 2010).

This study has shown no statistically significant differences between groups of variant. The researchers have confirmed that in patients of Caucasian origin genotyping for the CYP2C9 $*2$ allele has no clinical implications. Etiology and pathogenesis of T2DM remains not fully understood. Probably because of the genetically heterogeneous background of this dis-

ease the effect of CYP2C9 genotype on SUs response is not clearly explained either. However, the researchers cannot exclude that among T2DM patients there are ones in which the effect of CYP2C9 alleles may play a role of clinical relevance. One can also not exclude, that the effect of CYP2C9 variants on clinical response to SUs treatment may be significant only for some SUs, not for others. Recently positive findings concerning glimepiride (Yoo et al. 2011) and glibenclamide (Surrendiran et al. 2011) were published when our data were based on whole spectrum of different SUs used in Poland.

CONCLUSION

In conclusion, this analysis has not shown any association between the CYP2C9 gene Arg144Cys polymorphism and therapeutic response to oral agents in T2DM patients. Therefore, in our opinion genotyping for the CYP2C9 $*2$ allele has currently no clinical implication in the Polish population of T2DM patients.

ACKNOWLEDGMENTS

The study was supported by Jagiellonian University Medical College students grant.

REFERENCES

- Becker ML, Visser LE, Trienekens PH, Hofman A, van Schaik RH et al. 2008. Cytochrome P450 2C9 *2 and *3 polymorphisms and the dose and effect of sulfonylurea in type II diabetes mellitus. *Clin Pharmacol Ther*, 83(2): 288-292.
- Chomczynski P, Mackey K, Drews R, Wilfinger W 1997. DNAzol: A reagent for the rapid isolation of genomic DNA. *Biotechniques*, 22: 550-553.
- Gao L, He L, Luo J, Xu B, Yang J et al. 2011. Extremely low warfarin dose in patients with genotypes of CYP2C9*3/*3 and VKORC1-1639A/A. *Chin Med J (Eng)*, 124(17): 2767-2770.
- Inoue K, Inazawa J, Suzuki Y, Shimada T, Yamazaki H et al. 1994. Fluorescence in situ hybridization analysis of chromosomal localization of three human cytochrome P450 2C genes (CYP2C8, 2C9, and 2C10) at 10q24.1. *Jap J Hum Genet*, 39(3): 337-343.
- Kirchheiner J, Brockmoller J, Meineke I, Bauer S, Rohde W et al. 2002. Impact of CYP2C9 amino acid polymorphisms on glyburide kinetics and on the insulin and glucose response in healthy volunteers. *Clin Pharmacol Ther*, 71: 286-296.
- Lee CR, Pieper JA, Hinderliter AL, Blaisdell JA, Goldstein JA 2002. Evaluation of cytochrome P4502C9 metabolic activity with tolbutamide in CYP2C9 heterozygotes. *Clin Pharmacol Ther*, 72: 562-571.
- Lee YH, Lee BW, Chun SW, Cha BS, Lee HC 2011. Predictive characteristics of patients achieving glycaemic control with insulin after sulfonylurea failure. *Int J Clin Pract*, 65(10): 1076-1084.
- Niemi M, Cascorbi I, Timm R, Kroemer HK, Neuvonen PJ et al. 2002. Gliburide and glimepiride pharmacokinetics in subjects with different CYP2C9 genotypes. *Clin Pharmacol Ther*, 72(3): 326-332.
- Qian L, Zhang S, Xu L, Peng Y 2008. Endoplasmic reticulum stress in beta cells: Latent mechanism of secondary sulfonylurea failure in type 2 diabetes. *Med Hypotheses*, 71(6): 889-91.
- Soga Y, Nishimura F, Ohtsuka Y, Araki H, Iwamoto Y et al. 2004. CYP2C polymorphisms, phenytoin metabolism and gingival overgrowth in epileptic subjects. *Life Sci*, 74(7): 827-34.
- Surrendiran A, Pradhan SC, Agrawal A, Subrahmanyam DKS, Rajan S et al. 2011. Influence of CYP2C9 gene polymorphism on response to glibenclamide in type 2 diabetes mellitus. *Eur J Clin Pharmacol*, 67: 797-801.
- Swen JJ, Wessels JA, Krabben A, Assendelft WJ, Guchelaar HJ 2010. Effect of CYP2C9 polymorphisms on prescribed dose and time-to-stable dose of sulfonylureas in primary care patients with type 2 diabetes. *Pharmacogenomics*, 11(11): 1515-23.
- WHO 1999. *Report of a WHO Consultation: Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications*. Geneva: WHO.
- Xu H, Murray M, McLachlan AJ 2009. Influence of genetic polymorphisms on the pharmacokinetics and pharmacodynamics of sulfonylurea drugs. *Curr Drug Metabol*, 10(6): 643-58.
- Yin OQ, Tomlinson B, Chow MS 2005. CYP2C9, but not CYP2C19 polymorphisms affect the pharmacokinetics and pharmacodynamics of gliburide in Chinese subjects. *Clin Pharmacol Ther*, 78: 370-377.
- Yoo HD, Kim MS, Cho HY, Lee YB 2011. Population pharmacokinetic analysis of glimepiride with CYP2C9 genetic polymorphism in healthy Korean subjects. *Eur J Clin Pharmacol*, 67(9): 889-98.
- Zhou K, Donnelly L, Burch L, Tavendale R, Doney AS et al. 2010. Loss-of-function CYP2C9 variants improve therapeutic response to sulfonylureas in type 2 diabetes: A Go-DARTS study. *Clin Pharmacol Ther*, 87(1): 52-56.