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Association between CYP2C9 slow metabolizer genotypes and severe hypoglycaemia on medication with sulphonylurea hypoglycaemic agents

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Aims
The genetically polymorphic cytochrome P450 (CYP) enzyme CYP2C9 metabolizes most sulphonylurea oral hypoglycaemic agents. The aim of this study was to test the hypothesis that individuals with genotypes predicting low CYP2C9 activity may be at a higher risk of severe drug-associated hypoglycaemia.

Methods
In a case-control study, 20 diabetic patients admitted to the emergency department with severe hypoglycaemia during sulphonylurea drug treatment were compared with a control group of 337 patients with type 2 diabetes but without a history of severe hypoglycaemia. A large sample of 1988 healthy Caucasian subjects served as a second control group.

Results
The *CYP2C9* genotypes **3/*3* and **2/*3* that are predictive of low enzyme activity were more common in the hypoglycaemic group than in the comparison groups (10% *vs* <2%, respectively: odds ratio 5.2; 95% confidence interval 1.01, 27). Furthermore, the diabetic patient group with severe hypoglycaemia exhibited lower body mass indexes, higher rates of renal failure, were older compared with the diabetic group without severe hypoglycaemia, and were being treated with higher doses of glibenclamide.

Conclusions
These findings suggest that among other factors, individuals with genetically determined low CYP2C9 activity are at an increased risk of sulphonylurea-associated severe hypoglycaemia. Thus, genotyping might be a tool for the better prediction of adverse effects caused by oral hypoglycaemic agents.

Introduction

Severe sulphonylurea-associated hypoglycaemia is fatal in 1.4–10% of cases and necessitates long and costly hospital stays [1]. Moreover, the risk of hypoglycaemia may lead physicians to adjust blood glucose concentrations to above those identified as optimal for the prevention of microvascular and macrovascular complications. Thus, the identification and consideration of individual risk of hypoglycaemia may be of great importance for the optimization of treatment.

The cytochrome P450 (CYP) enzyme, CYP2C9, is largely responsible for the metabolism of oral hypoglycaemic agents such as tolbutamide, glibenclamide, glimepiride, glipizide, and nateglinide [2–5]. However,

the effect of functional *CYP2C9* polymorphisms on the risk of adverse drug reactions in oral hypoglycaemic therapy has not yet been studied in patients.

Two allelic variants leading to the amino acid substitutions Arg144Leu (*CYP2C9*2*) and Leu359Ile (*CYP2C9*3*) have a major influence on the activity of CYP2C9. The genotypes *CYP2C9*2/*3* and *CYPC9*3/*3* account for the slow metabolizer phenotype of many CYP2C9 substrates [6], and possibly oral hypoglycaemics [7]. Other rare *CYP2C9* alleles have been described (*CYP2C9*4* to CYP2C9*13) but their frequencies in Caucasians are less than 1%, and *CYP2C9*5* and the deletion *CYP2C9*6* have only been found in Africans. More recently, severe bleeding complications during warfarin therapy were reported in patients carrying *CYP2C9* genotypes predictive of low CYP2C9 activity [8], and the clinical relevance of *CYP2C9* polymorphisms also has also been shown for nonsteroidal anti-inflammatory drugs [9]. In the present case-control study, we aimed to test the hypothesis that *CYP2C9* genotypes *2/*3 and *3/*3 are risk factors for hypoglycaemia due to oral hypoglycaemic drug therapy in Caucasian patients as a result of impaired metabolism.

Methods

Two hundred and forty-four patients with severe hypoglycaemia, 35 (14%) of them being treated with sulphonylurea agents, were identified among the 36 258 patients who attended the medical emergency department of the Klinikum Lippe-Detmold, a large tertiary care hospital in East Westphalia, Germany, between 1 January 2000 and 31 December 2003. The protocol was approved by the Ethics Committee of the University of Münster School of Medicine and (for the comparison groups) by the Ethics Committee of the Charité University Hospital, Berlin. As the only hospital in the area, the one at Lippe-Detmold is responsible for the inpatient and outpatient management of all emergencies in the region. Severe hypoglycaemia was defined as a symptomatic event requiring treatment with intravenous glucose or glucagon intramuscularly or subcutaneously and was confirmed by a blood glucose measurement of <50 mg dl^{-1} (< 2.8 mmol l^{-1}). Twelve sulphonylurea-treated patients died within a period of 0.5 and 14 months after the hypoglycaemic event. The causes of death were unrelated to the severe hypoglycaemia. DNA analysis could not be performed in these 12 subjects or in three hypoglycaemic sulphonylurea-treated individuals who declined to participate in the study.

The remaining 20 type 2 diabetic patients with severe sulphonylurea-induced hypoglycaemia (initial blood glucose 35 ± 16 mg dl^{-1} (1.9 ± 0.8 mmol l^{-1})) were genotyped for *CYP2C9*2* and *CYP2C9*3*. These data were compared with a control sample of DNA from 337 Caucasian diabetic patients receiving oral hypoglycaemic drugs who did not have a history of severe hypoglycaemia, and to those from a large sample of 1988 healthy Caucasian subjects. All gave their informed consent for genotyping.

The patients with severe hypoglycaemia had been treated with the sulphonylurea drugs glimepiride ($n = 17$) and glibenclamide ($n = 3$). Four had also been treated with insulin. In the comparison group, 59 of the patients had similar exposure to glibenclamide and 56 to glimepiride. The mean ($\pm$ SD) daily dose of glibenclamide (9.3 ± 2 mg) was higher ($P = 0.02$) in the hypoglycaemic subjects, but the mean dose of glimepiride was the same as in the comparison group (2.4 ± 1.4) mg.

Results

The frequencies of the *CYP2C9* genotypes in diabetic patients with and without hypoglycaemia, and in healthy controls are shown in Table 1. Two (10%) of the 20 patients were carriers of the rare genotypes *CYP2C9*2/*3* and *CYP2C9*3/*3*. This incidence of low CYP2C9 activity genotypes was higher than observed in either the control population (2.1%) or the diabetic patients

Table 1
CYP2C9 genotypes in the populations studied

CYP2C9 genotypes	Cases ($n = 20$)	Diabetic controls ($n = 337$)	Healthy controls ($n = 1988$)
*CYP2C9*1/*1*	13 (65%)	224 (66.5%)	1293 (65%)
*CYP2C9*1/*2*	4 (20%)	62 (18.4%)	389 (19.6%)
*CYP2C9*2/*2*	0	5 (1.5%)	36 (1.8%)
*CYP2C9*1/*3*	1 (5%)	39 (11.6%)	229 (11.5%)
*CYP2C9*2/*3*	1 (5%)	5 (1.5%)	34 (1.7%)
*CYP2C9*3/*3*	1 (5%)	2 (0.6%)	7 (0.4%)

Table 2
Demographic factors (mean (95% confidence interval)) in the two groups of diabetic patients with and without hypoglycaemia

	BMI (kg m^{-2})	Age (years)	Duration of illness (years)	Frequency of hypertonia	Renal insufficiency	Comedication*
Cases (n = 20)	25 (23, 27)	74 (67, 77)	7.6 (4, 11)	65%	55%	5 (3, 6)
Diabetic controls (n = 337)	30 (29, 30)	65 (64, 66)	10.6 (10, 12)	70%	7%	4 (3, 4)
Significance	<0.001	<0.001	NS	NS	<0.001	NS

*Significance of differences between cases and controls was tested by the nonparametric Mann–Whitney test for the variables BMI, age, duration of illness, and comedication and by the chi-square test for the other variables. *Number of different drugs administered. Five of the cases with hypoglycaemia had comedications potentially inhibiting CYP2C9 (diclofenac, allopurinol (n = 2), indomethacin, and amiodarone (n = 2)), but the two cases with genetically predicted low CYP2C9 metabolic activity were not taking any CYP2C9 inhibitors.*

without history of severe hypoglycaemia (2.08%; 7 out of 337, $P = 0.028$). In a large data set obtained from 1988 healthy Caucasians who had taken part or intended to take part in clinical studies in Berlin, Germany, 41 had the *CYP2C9* genotypes **3/*3* and **2/*3*. Thus, the *CYP2C9* slow metabolizer genotype confers an odds ratio of 5.2 (95% confidence interval 1.01, 27) for a severe hypoglycaemic event during treatment with sulphonylurea oral hypoglycaemic agents. The remaining 18 patients did not show an overrepresentation of any other genotype, and no individual with the rare genotype *CYP2C9*2/*2* was found.

The demographic characteristics of the 20 cases in comparison with the diabetic patients without severe hypoglycaemia are depicted in Table 2. Patients with severe hypoglycaemia were on average 9 years older. There was a higher incidence of renal failure in the hypoglycaemia group, whereas the body mass index tended to be higher in the control group.

Discussion

This study highlights that, although not the most prominent risk factor, *CYP2C9* genotypes predictive of low enzyme activity (*CYP2C9*3/*3* and **2/*3*) should be considered as one but not the major risk factor for severe hypoglycaemia resulting from treatment with sulphonylurea oral hypoglycaemic agents.

Studies have revealed that the clearance of glibenclamide and glimepiride in *CYP2C9*3/*3* genotypes was only 20% of that in homozygous wild type subjects [4, 5]. Similarly, the *CYP2C9*2/*3* genotype has also been associated with significantly lower clearance of these drugs [5]. Compared with these substantial differences, the heterozygous genotypes with one wild-type allele (*CYP2C9*1/*3* and **1/*2*) showed only a slight decrease in the oral clearances of glimepiride or glibenclamide [4, 5], and were no more frequent in the hypoglycaemic patient group of the present study compared with the normal population. Additional cohort studies involving larger sample sizes are required to determine whether there is an increased risk of hypoglycaemia in the **1/*3* genotype.

In this study, the mean daily doses of glibenclamide were higher in the hypoglycaemic group compared with the diabetic patients without hypoglycaemia. Thus, in addition to the potentially lower clearance caused by the CYP2C9, poor metabolizer genotypes were exposed to more drug in the hypoglycaemic patients *per se*. Furthermore, comedication with agents that inhibit CYP2C9 might be an additional risk factor for hypoglycaemia by increasing the concentrations of the oral hypoglycaemic drugs that are metabolized by this enzyme. Five of the patients with hypoglycaemia were also taking drugs that potentially inhibit CYP2C9, namely diclofenac, allopurinol ($n = 2$), indomethacin, and amiodarone ($n = 2$). However two patients with a genetically predicted low CYP2C9 activity were not receiving any CYP2C9 inhibitors and were being treated with glimepiride at a mean daily dose that was the same as that in the diabetic patients without hypoglycaemia.

Age represents another important risk factor for hypoglycaemia. The mean age in the 337 diabetic patients without a history of hypoglycaemia was 65 years (95% confidence interval 64, 66) and that in the group of patients suffering from severe hypoglycaemia was 74 years (95% confidence interval: 67, 77).

Furthermore, in the patient group with hypoglycaemia, 55% also exhibited renal failure (in comparison with only 7% in the control group), another known risk factor for hypoglycaemia [1]. The sulphonylureas are highly protein bound, and higher unbound concentrations of these drugs in patients with renal insufficiency might additionally increase the risk of hypoglycaemia. One-year mortality within this group of hypoglycaemic patients was high (12/35, 34%), which confirms the finding that patients who require hospital admission for the treatment of severe sulphonylurea-induced hypoglycaemia represent a group with a poor long-term prognosis [10].

Genotyping of *CYP2C9* allelic variants is a relatively easy and inexpensive test that might help to prevent adverse reactions to CYP2C9 substrates, such as oral anticoagulants, non-steroidal anti-inflammatory drugs, and, as shown by our data, oral antidiabetics. As an additional perspective, other polymorphisms might be predictive of hypoglycaemia. Such genes may include those coding for the sulphonylurea receptor, and for adrenergic receptors and other systems involved in counter-regulation of hypoglycaemia.

Competing interests: None declared.

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