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Multidrug and Toxin Extrusion Protein (MATE1) Gene Polymorphism and Therapeutic Effects of Metformin in Type 2 Diabetes Mellitus in Egypt

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Abstract

Background: Individual variability in drug efficacy and drug safety is a major challenge in current clinical practice, drug development, and drug regulation. Studies of pharmacogenetics have provided ample examples of causal relations between genotypes and drug response to account for phenotypic variations of clinical importance in drug therapy. Metformin is widely used to treat type 2 diabetes and to delay or prevent its onset in people at high risk. Metformin is actively transported into the liver, where it exerts its primary action, and then is actively transported from the liver into bile and blood stream. Elimination from the liver is mediated in part by the multidrug and toxin extrusion protein (MATE1), a membrane-bound transporter protein. **The aim of the work** was to analyze the frequency of MATE gene prevalence among the Metformin non responder Egyptian Diabetic patients and to demonstrate the role of rs2289669 polymorphism on Metformin response. **Patients and methods:** This study included 44 newly diagnosed type 2 diabetic patients. All the patients were educated for life style modification regarding diet and exercise and were given metformin alone with regular follow up of their fasting blood glucose monthly and at the end of study HbA1c was repeated. Patients were divided into 2 groups (responders and non responders).Genomic DNA extraction and analysis using PCR followed by sequencing analysis of rs2289669 polymorphism in the gene encoding MATE1 (SLC47A1) was done for each patient. **Conclusion**: The study concluded that rs2289669 G>A polymorphism was associated with an increased glucose-lowering effect of metformin .

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1 Introduction

Individual variability in drug efficacy and drug safety is a major challenge in current clinical practice, drug development, and drug regulation. Studies of pharmacogenetics (PhGs) have provided ample examples of causal relations between genotypes and drug response to account for phenotypic variations of clinical importance in drug therapy. The convergence of (PhGs) and human genomics has dramatically accelerated the discovery of new genetic variations that potentially underlie variability in drug response, giving birth to (PhGs). In addition to the rapid accumulation of knowledge on genome- disease and genome-drug interactions, there arises the hope of individualized medicine. For example, knowledge on genetic determinants of disease pathogenesis and drug action, especially those of complex disease and drug response, is not always available. Relating the many gene variations from genomic sequencing to clinical phenotypes may not be straightforward. It is often very challenging to conduct large scale, prospective studies to establish causal associations between genetic variations and drug response or to evaluate the utility and cost-effectiveness of genomic medicine. Overcoming the obstacles holds promise for achieving the ultimate goal of effective and safe medication to targeted patients with appropriate genotypes [1]. Metformin, an oral glucose-lowering drug, is widely used to treat type 2 diabetes and to delay or prevent its onset in people at high risk of the disease [2]. Metformin acts primarily by inhibiting glucose production in the liver, but it also reduces gastrointestinal glucose absorption and improves glucose utilization. Although metformin is considered a first-line treatment for type 2 diabetes, approximately one-third of patients fail to respond. Preliminary evidence suggests that this may be due to certain single-nucleotide polymorphisms (SNPs) in genes that mediate metformin transport. Metformin is actively transported into the liver, where it exerts its primary action, and then is actively transported from the liver into bile and blood stream. Elimination from the liver is mediated in part by the multidrug and toxin extrusion protein (MATE1), a membrane-bound transporter protein [3]. Thus, genetic changes that affect MATE1 activity could influence the concentration and, presumably, effectiveness of metformin in the liver cells [4]. Studies have found that rs2289669 polymorphism in the gene encoding MATE1 (SLC47A1) may affect the clinical effectiveness of Metformin.

2 Aim Of The Work

The aim of this study is to study the frequency of MATE gene polymorphism (rs2289669) among the Metformin non responder in Egyptian diabetic patients and to demonstrate the role of rs2289669 polymorphism on Metformin response.

3 Subjects And Methods

3.1 Subiedts

This study was conducted on newly diagnosed type 2 diabetic patients, referred to Fayoum university hospital out-patient clinics, in Fayoum Governorate, Egypt. Sample size was calculated using www.survey system.com, and accordingly 44 patients were carefully selected, following strict inclusion and exclusion criteria. age range was between 30 and 60 years. patients having HbA1c between 7.5% and 8.5% were recruited, excluding those showing severely uncontrolled diabetes with HbA1c more than 9.0%. Type 1 diabetic patients were not included. Patients presenting with renal impairment, elevated liver enzymes, symptomatic heart failure, moderate or severe anemia were excluded. None of our patients had concomitant hemoglobinopathies. None of the female patients were pregnant at anytime during the interval of the study. All patients gave a written informed consent and approval was granted by our institutional medical ethics committee.

Following clinical assessment all patients were referred to the laboratory for investigations including fasting and postprandial blood glucose, glycemic state by HbA1c, serum creatinine, serum AST and ALT, creatinine and lipids. also genomic DNA extraction for further direct sequence analysis for detection of the rs 2289669 polymorphism in the SLC47A1 gene.

The patients were then instructed for life style modification and were given metformin alone in doses ranging from 500 mg to 2550 mg daily then followed up for 3 months (NICE clinical guidelines 2009). At the end of this 3 month period of compliant therapy patients were re-evaluated by repeating their fasting and postprandial blood glucose as well as their glycemic state by HbA1c.

As there is no clinical phenotype that usefully predicts response to metformin, the patients were classified into metformin responder group (group1) and metformin non-responder group (group 2) according to their HbA1c results. Metformin responders were those whose HbA1c decreased by more than 0.5% following 3 months compliant treatment while non-responders were those whose HbA1c decreased by less than 0.5% were those whose HbA1c decreased by less than 0.5%.

3.2 Methods

3.2.1 Sample collection

For collection of samples patients were instructed to fast and following a 10 hour fast, 10 ml venous blood sample was drawn and divided into four sample tubes: 2ml in sterile EDTA vacutainer for DNA extraction, 4ml in SST for blood chemistry, 2ml in a separate EDTA vacutainer for HbA1c analysis, and 2ml in a sodium fluoride vacutainer for blood glucose analysis.

At the end of the follow up period a further 4ml venous blood was taken for the glucose and HbA1c analysis as stated before.

3.2.2 Blood chemistry assay

Blood chemistry was done on Beckman Synchron CX9.

3.2.3 Molecular assay

DNA extraction was done using Invisorb Spin Blood Mini Kit (Catalog No K0721). DNA amplification for the initial PCR was done using a set of forward and reverse primers from Invitrogen and Dream Taq PCR Master Mix from Qiagen (Catalog No K1081) using a Perkin Elmer 9600 thermal cycler . The total reaction volume was 50 µl consisting of 25µl master mix, 1µl of each primer with working concentration of 5 pmole and 5µl of DNA template. Forward primer sequence was (5' ACC TGG GCT CCT GGT GAG TCA GTC 3') while the reverse primer sequence was (5' ACC CGG ACA CTG GCA GCC ACA CTG 3') (Tzvetkov et al., 2009). PCR conditions were optimized as follows: an initial denaturation at 95℃ for 5 min, 35 cycles of denaturation (95℃ for 30 sec), annealing (60℃ for 30 sec), extention (72℃ for 3 min) and a final extension at 72℃ for 10 min. Confirmation of the presence of the PCR product was done by ethidium bromide agarose gel electrophoresis, using a 100 base-pair DNA ladder from Ferrmentes and a SYNGENE GVM 20X UV transilluminator. Quantification of the PCR product was done using Nanodrop ND-100 U/VIS spectrophotometer from Nanodrop technologies. PCR product purifiaction was then done using the QIAquick purification kit from Qiagene (Catalog no 281040). This was followed by the cycle sequencing step using a sequencing inner primer from Invitrogen (5' CCA TCC CCC GAC ACC CAG AGG AGG A 3') (Tzvetkov et al., 2009) with a working concentration of 10 pmole, and BigDye ® Terminator Cycle sequencing Ready Reaction Kit v 3.1. The sequencing primer was used for the forward sequencing while the reverse PCR primer was used for the reverse. The reaction composition included 4µl of the BigDye kit mix, 1 µl of either the forward or the reverse primer, and 1.5 µl of the PCR product template in a final reaction volume of 10 µl. The cycle sequencing was done using Perkin Elmer 9600 thermal cycler with conditions recommended by Applied Biosystems as follows: denaturation at 95℃ for 30 sec, annealing at 50℃ for 15 sec, and extension at 60℃ for 4 min for 25 cycles. Purification was done using Centri-Sep ™ columns (Invitrogen ™, cat No CS-901 100 pack). Sequence analysis of the cycle sequencing product was done on the ABI PRISM 310 (Applied Biosystems (ABI), Jena Lab, Germany). The sequences obtained were analyzed with Auto-assembler software and sequences were compared with those available in the GenBank database.

4 Results

The study was conducted on 44 newly diagnosed type 2 diabetic patients. The patients were classified into 2 groups according to HbA1c results metformin responder group and metformin non responder group. We considered that metformin responders were those whose HbA1c decreased by more than 0.5% following 3 months of patient compliant therapy while Metformin non responders were defined as those patients whose HbA1c decreased by < 0.5% following 3 months of patient compliant therapy.

The difference in sex distribution between responders and non responders was not statistically significant. While the difference in age was statistically significant (p=0.05).

Table 1: Age, gender, HbA1c changes in different groups

		Group		P - value
		responders	Non responders	
Gender	Females N %	14 63.6%	11 50.0%	0.36
	Males N %	8 36.4%	11 50.0%	
Age in years (Mean ± SD)		43.95±4.47	46.9± 5.2	0.05
HbA1c (before) % (Mean ±SD)		7.97±0.33	8.2±0.29	0.09
HbA1c (after) %(Mean ±SD)		7.16±0.47	8.05±0.26	0.001
HbA1c change % (Mean ±SD)		-0.81±0.17	-0.16±0.17	0.001
p value		<0.001	0.08	

HbA1c = Hemoglubin A1c

The laboratory data before treatment were; the mean fasting glucose level was 160.95± 33.6 (137 -176 mg/dl) while the mean postprandial glucose level was 221.1 ± 48.3 (187 to 271 mg/ dl). The mean HbA1c was 8.15%. (7.5 to 8.5) with and both liver and kidney functions were normal.

Comparison between results of HbA1c before and after treatment with metformin showed very high significant difference within the responders group with where the HbA1c% was 7.97±0.33 before treatment while after treatment it was 7.16±0.47(p value <0.001). As regards non responders HbA1c % showed less decrease from 8.2±0.29 to 8.05±0.26 after treatment. The decrease of HbA1c % in responders was (0.81+0.17) versus (-0.16+0.17) in non responders and the difference between both groups was statistically highly significant (p -value <0.001).

Regarding genotype frequencies in responders and non responders it was found that The AA genotype in our patients had a frequency of 12/44 (27.3%) where two of them were non responders and ten were responders. The AG genotype frequency was 16/44 patients (36.4%), six of them were non responders and ten were responders .while the GG genotype frequency was 16/44 patients (36.4%), fourteen of them were non responders and two were responders. Statistical analysis reveals a highly significant increase in AA & AG genotypes frequency in responders than non responders group as (p -value <0.001,OR=0.05, 95% CI =0.01).

Allelic Distribution among Responders and Non responders:

- Among the two groups, G allele (wild type) frequency was 14 in the responders group representing (31.8%) of the group, while in the non responders it was 34 alleles (70.8%).
- As regards the A variant allele group, frequency was 30 in the responders group forming 68.2% of the group and 10 (22.8%) in the non responders group.

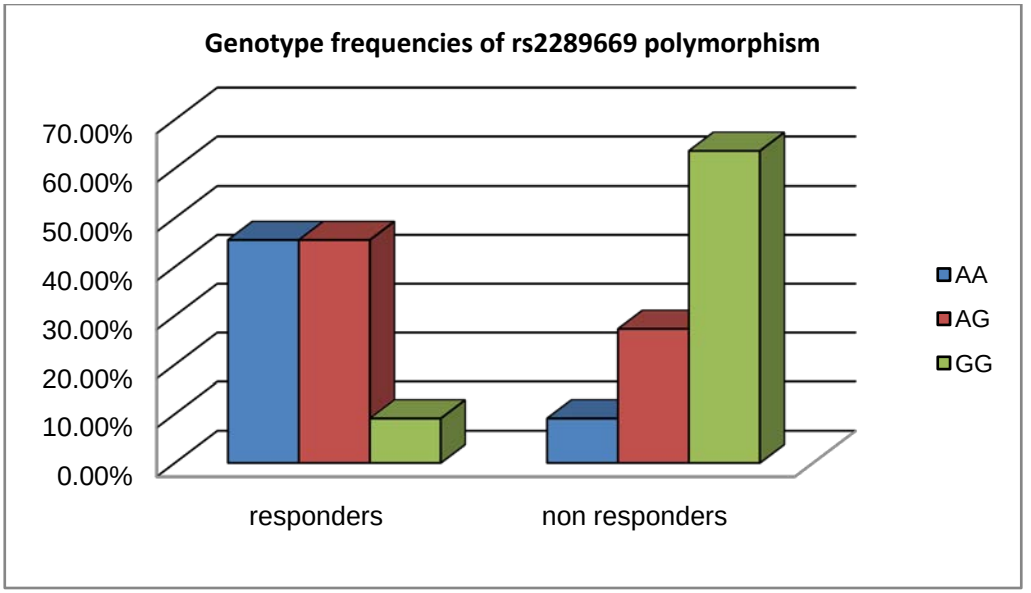

Figure 1 Genotype frequency of rs2289669 G>A polymorphism in both groups

- Statistical analysis revealed very high significant increase in A allele frequency in responders when compared to the non responders group with a p value <0.001(OR=0.137, 95% CI =0.05-0.35).

Table 2: genotype frequencies of rs2289669 G>A polymorphism in both groups:

group	**Genotype frequency**			**Odd ratio**	**95% Confidence interval**	**P-value**
	Responders	**Non-responders**	**Total**			
AA genotype: No (%) AG genotype: Number (%) GG genotype: Number (%)	10/22 (45.5%) 10/22 (45.5%) 2/22 (9.1%)	2/22 (9.1%) 6/22 (27.3%) 14/22 (63.6%)	12/44 (27.3%) 16/44 (36.4%) 16/44 (36.4%)	0.05	0.01-0.3	<0.001
Allele frequencies						
G (wild allele) Number (%)	14/44 (31.8%)	34/44 (77.2%)		0.137	0.05-0.35	<0.001
A (mutant allele) Number (%)	30/44 (68.2%)	10/44 (22.8%)				

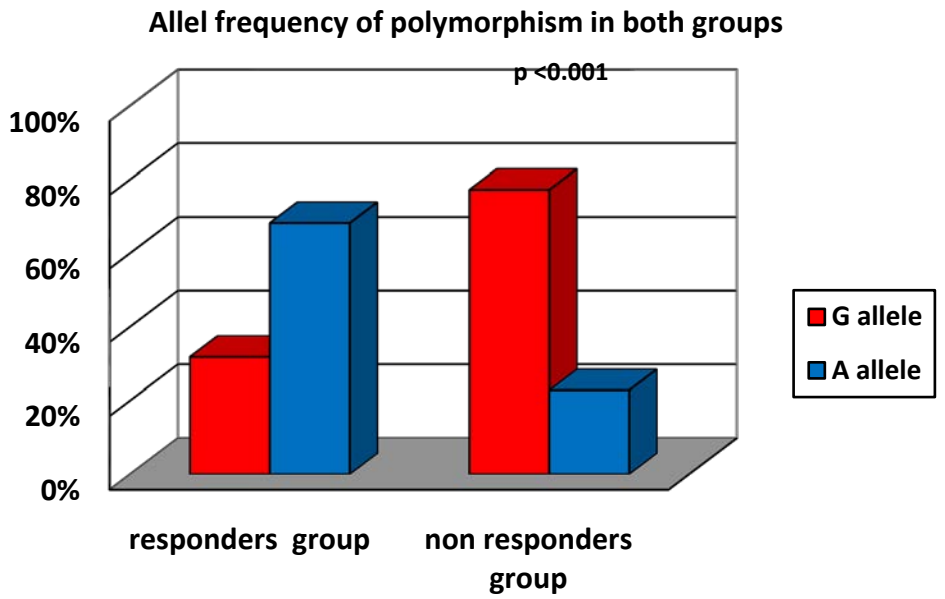

Figure 2: Allele frequency of polymorphism in responders and non responders groups.

The change in HbA1c was significantly different between GG and AG genotypes (p=0.012), and very highly significant between GG and AA genotypes (p=0.001). The decrease of HbA1c was highest in AA genotype (-0.71±0.26) followed by AG genotype (-0.57±0.35), and lowest found in GG genotype (-0.23±0.3).

Table 3: Comparison of change in HbA1c in different genotypes of responders and non responders:

	GG	AA	AG
HbA1c change Mean ±SD	-0.23±0.3	-0.71±0.26	-0.57±0.35
P - value	P = 0.23	p=0.001	p=0.012

HbA1c = Hemoglubin A1c

5 Discussion

Pharmacogenomics is an approach that has evolved from pharmacogenetics and promises a new drug selection process, which takes into account variations in an individual's genetic makeup to optimize pharmacokinetics and pharmacodynamics to ultimately increase drug efficacy and safety profile. In other words, it involves creating genetically tailored drug regimens to optimize an individual's response [5]. It is estimated that about

95% of the variability in drug response is due to genetic differences; accounting for these differences then would be highly beneficial, not only for the health care industry, but for patients themselves, decreasing the burden of treatment failures and adverse events on society[6]. A primary focus of individualized medicine is to provide the physician with tools that aid in selecting the most effective treatment regimen for a patient, while decreasing the possibility for adverse events and complications related to the recommended medical care [7]. Establishing the relation of the many SNPs and other gene variations derived from genomic sequencing to clinical phenotypes of drug therapy may not be straightforward. Large scale, population based, and multiparameter prospective clinical studies are often very challenging but much needed for establishing causal associations between genetic variations and drug response phenotypes and for evaluating the utility and cost-effectiveness of genotyping and individualized medicine. Economic, ethical, social, and regulatory issues associated with genomic medicine are also complex and challenging [1].

The pharmacogenomics approach has already supplied researchers with candidate genes and their translational ramifications on drug response in many complex disease states. A popular focus of several GWAS (genomic wide association studies) and candidate gene analysis has been dedicated to understanding the implications of genetic variation in individuals diagnosed with type 2 diabetes mellitus [8]. A great deal of progress has been made in the form of establishing a genetic explanation for etiological mechanisms by which T2D develops and interindividual variability in response to standard therapy [9].

Type 2 diabetes is usually part of the “metabolic syndrome”, which is associated with other risk factors from early in the disease process including abdominal obesity, hypertension, dyslipidemia, Although macrovascular disease is the major cause of morbidity and mortality in type 2 diabetes, microvascular complications are often present when diabetes is diagnosed, even in people with no symptoms [10]. In treating Type 2 diabetes metformin is recommended as first line therapy in most guidelines. Metformin can be used as monotherapy alone, or added to other therapies. However, current prescribing practice is for metformin to be used first line so the results of pharmacogenetic studies may be valuable for daily clinical use [11]. There is considerable variability in glycaemic response to metformin. No clinical phenotype usefully predicts response yet there has been many pharmacogenetic investigation of metformin [12].Several drug transporters are involved in the distribution and excretion of metformin. Multidrug and toxin extrusion (MATE) transporter protein family was identified, assigned as the SLC 47 family [13]. The SLC47A1 gene, with gene location 17p11.2, encodes the MATE1 transporter. Metformin is one of the substrates of this transporter [14]. MATE1 is located in the bile canalicular membrane in the hepatocyte and in the brush border of the renal epithelium and is responsible for the final step of metformin excretion through the bile and urine [3].

The co-localization of OCT1 and MATE1 in the hepatocyte and OCT2 and MATE1 in the renal epithelium suggests that MATE1 may have an important influence on the pharmacokinetics of metformin. The intrahepatic uptake of metformin by OCT1 is an essential step in the glucoselowering effect, while the excretion out of the hepatocyte into the bile by MATE1 probably averts this. The uptake in the renal epithelium by OCT2 and subsequent excretion by MATE1 are two consecutive steps in the tubular secretion of metformin [15]. The Wellcome Trust Case Control Consortium 2 study (WTCCC2), a GWA study of 15 complex traits and disorders, was carried out as the first GWA study on metformin response in patients with type 2 diabetes, using a large Scottish observational

genetic cohort of European ancestry. The second replication cohort was 1113 UK patients prospectively treated with metformin in the UKPDS (UK Prospective Diabetes) cohort [16] was the first epidemiological study assessing the role of MATE1 in metformin response.

The aim of the current study was to screen MATE gene among the Metformin treated Egyptian Diabetic patients and to assess the association between the rs2289669 G>A single nucleotide polymorphism (SNP) in the SLC47A1 gene and the Hb A1c lowering effect of metformin.

Our newly diagnosed diabetic patients were instructed for life style modification regarding eating and exercise and were given metformin alone, while Becker et al did not exclude sulfonylurea users. This combination of drugs is considered as potential determinant affecting the change in A1C level [15].

The dose of metformin and follow up were done according to recommended clinical guidelines. The maximum dose (2550 mg daily) was reached gradually over weeks to minimize risk of gastro-intestinal (GI) side effects [11]. Beckr et al stated that low doses of metformin (741 mg) were used in their study and that may be due to the high average age of the study population (77 years), and physicians are prudent to prescribe high doses of metformin in this elderly population because of potential adverse effects, so the average decrease in A1C level was less than what would be expected when recommended doses are used.

The mean age in our study was 45.4±5.0 years and this allowed the use of recommended doses. According to NICE guidelines 87, HbA1c was measured after 3 months to assess the response and the need of drug change. As no clinical phenotype usefully predicts response to metformin, patients were divided into two groups. We considered that responders are the patients whose HbA1c decreased by more than 0.5 % and non responders, whose HbA1c decreased by less than 0.5 %. Becker et al [2009] stated that measurement of A1C was done both in the period of 90 days before the first prescription of metformin and in the period between 30 and 120 days following the first prescription of metformin. This difference in measurement time adds to the effect on metformin response assessment. The frequency of genotypes in our study showed highly significant increase in AA & AG genotypes in responders than non responders group (P-value <0.001). The AA genotype was demonstrated in 44 of our patients with frequency of 12/44 (27.3%) where ten of them were responders and two were non responders. The AG genotype frequency was 16/44 patients (36.4%), six of them were non responders and ten were responders .while the GG genotype frequency was 16/44 patients (36.4%), fourteen of them were non responders and two were responders. there was a high significant increase in AA & AG genotypes frequency in responders than non responders group as p value <0.001(OR=0.05, 95% CI =0.01-0.3) There was a high significant increase in A allele frequency in responders when compared to the non responders group with a p value <0.001(OR=0.137, 95% CI =0.05-0.35). The glucose lowering effect of metformin was stronger in AA genotype (-0.71+0.26) followed by AG genotype (-0.57+0.35), and the lowest decrease was found in GG genotype (-0.23+0.3).The change in HbA1c was significantly different between GG and AG genotypes (p=0.012), and highly significant between GG and AA genotypes (p=0.001). The rs2289669 G>A polymorphism was associated with an increased glucose-lowering effect, implying that the gene with the A allele benefit from metformin as regard to HbA1c % decrease. In agreement with our results Becker et al found higher decrease with the AA genotype (-0.87) followed by GA genotype (-0.59) and lowest in GG genotype (-0.28). Becker et al [15]found an

association between the SNP rs2289669 in the SLC47A1 gene, encoding the MATE1 transporter, and the glucose-lowering effect of metformin. In incident metformin users the decrease in A1C level was 0.3% larger per copy of the A allele. These results suggest that MATE1 may have an important role in the pharmacokinetics and pharmacodynamics of metformin. Kathleen et al [17] provided evidence supporting what Becker et al reported about association of variants in the metformin transporter gene SLC47A1 with weaker metformin response, defined as the reduced ability of metformin to lower diabetes incidence. They identified a number of nominal associations with diabetes incidence or metformin response in several compelling candidate genes. In agreement with our findings Tkáč et al [18] found that patients with diabetes that are homozygous for A-allele of SLC47A1 had a twofold reduction in HbA1c % in comparison with the patients carrying G-allele (GG+GA:0.55±0.09% versus AA: 1.10 ± 0.18%, p = 0.018).
They concluded that SLC47A1 rs2289669 genotype was significantly associated with the reduction in HbA1c % after 6 months. The reduced efflux of metformin in the renal brush border due to an impaired MATE1 transporter will lead to an increase in metformin plasma levels and possibly to a larger decrease in glucose levels. Similarly, a reduced efflux from the hepatocyte will lead to higher metformin levels in the hepatocyte and a stronger inhibition of the gluconeogenesis, resulting in lower glucose levels. This SNP is located in an intron, and the SNP does not code for an amino acid change. Most likely, the SNP rs2289669 is in linkage disequilibrium with a SNP causing the reduced MATE1 functioning, although we cannot exclude that it has a direct effect, e.g., by affecting gene expression [15].

6 Conclusion

In Egyptian population the frequency of genotypes AA & AG was increased more in responders than non responders group comparing with GG genotype. The rs2289669 G>A polymorphism of the MATE gene was associated with an increased glucose-lowering effect of metformin.

7 Recommendations

Integration of genomics, proteomics, metabolomics, and epigenetics in genome-wide association studies is likely to facilitate identification of predisposing genetic factors associated with type 2 diabetes mellitus and metformin response. The reasonable cost of the rs2289669 G>A polymorphism of the MATE gene sequencing in our study (about 60 US dollars per test) is encouraging to include it in workup of newly diagnosed diabetic patients. This may be a step forward in the application of individualized medicine.

Conflict of interest

Mohamed Mashahit . Noha Abdelghafar, Ghada Ezzat & Lamia Mansour had no Conflict of interest

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