European Review for Medical and Pharmacological Sciences 2020; 24: 469-477

Assessment of pharmacogenomic SLCO1B1 assay for prediction of neuromuscular pain in type 2 diabetes mellitus and cardiovascular patients: preliminary results

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Abstract. – OBJECTIVE: At present, several strategies for preventing neuromuscular pain in Type 2 Diabetes Mellitus (T2DM) have been investigated. Recently, findings on genetic variants associated with adverse events to statin-based therapy have been reported. The study aimed at measuring whether Pharmacogenomics (PGx) profile can affect neuromuscular pain in patients carrying T2DM and cardiovascular diseases. An extensive panel of 5 polymorphisms on 4 candidate genes, previously validated as significant markers related to Sulphonylureas and Glitinides (SU-G) plus Simvastatin neuromuscular toxicity, is herein analyzed and discussed.

PATIENTS AND METHODS: We genotyped 76 T2DM patients carrying cardiovascular discrasia undergone anti-diabetic and anti-cholesterolemic polypharmacy. 35 subjects out of the total received concurrent SU-G and Statin-based therapy. Candidate variants consisted of drug transporters, such as *Solute Carrier Organic 1B1 (SLCO1B1) Val174Ala ATP-binding cassette subfamily B member (ABCB1), subfamily C member 8 (ABCC8)*, and drug biotransformers of Cytochrome P450 Family (CYP) including *CYP2C9*2 CYP2C9*3 CYP2C8*3*, and *CYP3A4*22*. Moreover, we also focused on an early outline evaluation of the genotyping costs and benefits.

RESULTS: 6 out of 35 patients treated with SU-G plus statins (17.1% experienced adverse neuropathy events). Pharmacogenomics analysis showed a lack of any correlation between candidate gene polymorphisms and toxicity, except for the *SLCO1B1 T521C* allele; 14.3% of patients had a high risk for grade >2 neuromuscular pain (Odds Ratio [OR] 2.61.95% CI 0.90-7.61, *p*=0.03).

CONCLUSIONS: The clinical polymorphism effectiveness outlined therein will be assured by diagnostic improvements suitable for driving treatment decisions. In light of our experimental results and literature data, the analysis of the *SLCO1B1* T521C variant will allow clinicians to take advantage from a better treatment planned for their patients in order to minimize neuromuscular pain and maximize benefits.

Key Words:
Pharmacogenetics, Genotyping methods, SLCO1B1, Simvastatin, Repaglinide Neuromuscular pain.

Introduction

Numerous physicians discern the importance of genetic variants in drug response and support the use of genetic test to plan tailored cure. In practice, Pharmacogenomics and Pharmacogenetics (PGx) testing can stratify patients who have little chance to take advantage from costly treatments and who develop adverse events at standard doses. This process encourages both more individualized and alternative treatments and presumably a delay reduction for the patient[1]. For these reasons, PGx tests represent attractive options for Type 2 Diabetes Mellitus (T2DM) patients receiving

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polypharmacy because of neuro and cardiovascular co-morbidities[2-4]. Current therapies for T2DM include lifestyle changes and the use of several classes of oral antidiabetic drugs like sulphonylureas-Glinides (SU-G), biguanides, thiazolidinediones (TZDs), Glycogen-Like peptide (GLP-1) analogs, Sodium-Glucose transport Protein-1 (SGTP-1) inhibitors and insulins[5]. The effects of these drugs depend on the extent of drug absorption/transport into cells (i.e., intermembrane solute carriers) and metabolism, primarily cytochrome P450 (CYP) family. The incidence of polymorphisms in these genes has a crucial role in the efficacy/toxicity and drug-drug interaction[6-8]. At the best of our knowledge, during clinical managements, we noticed a singular muscle pain of T2DM in a subset of patients with cardiovascular comorbidities receiving concurrent therapies based on statins and SU-G. Discrimination must be made between muscle pain and Diabetic Poly-Neuropathy pain (DPN). Muscle pain is an asymmetrical "stocking-glove" numbness, causing a loss of deep tendon reflexes and a burning-tingling,while DPN is a microvassels deregulation affecting sensory nerves. Aiming at investigating the probable genetic predisposition to muscular pain, we performed a genotyping panel to interrogate the well-known genetic variants related to statins and SU-G (primary Repaglinide)[9]. Recently, the well-known synonymous Single Nucleotide Polymorphisms (SNPs) Solute Carrier Organic 1B1 (*SLCO1B1*) codon Val174Ala (T521C) were associated with musculoskeletal pain in patients treated with Simvastatin[10]. Similarly, in healthy volunteers, the *SLCO1B1*1B/*1B* genotype was associated both to lower plasma concentrations and to a higher clearance of Repaglinide, in agreement with the increased transport activity of the encoded enzyme[11]. Several trials have been carried out in an effort to recognize strategies for pain reductions (i.e., supplements with neuroprotective agents), although these attempts have led to modest achievements[12,13]. Besides, to a large extent, inter-individual variability in neuromuscular pain remains unsolved. Recently, many PGx studies have reported several SNPs associated with the same adverse drug response, but, to date literature does not report any investigation concerning the PGx study between SU-G and statins[14]. Moreover, several studies and meta-analyses have reported that neurotoxicity related to the anti-diabetics treatment can be predicted by identyfing gene polymorphisms involved in transports and biotransformation[15]. *SLCO1B1* encodes the organic anion-transporting polypeptide (OATP1B1), with primary function as hepatic uptake of a variety of xenobiotics, including taxane during breast cancer therapy[16]. A well-known SNP in the *SLCO1B1* gene rs4149056 521 T>C, Val174Ala is known to affect transport action of OATP1B1 protein, leading to a serum modification in the level of numerous drugs, including statins[17]. In addition, this SNP of *SLCO1B1* gene has been correlated to familial diabetic predisposition[18].

In the light of this scientific suggestion, we have validated a genotyping panel assay covering not only the well-known *SLCO1B1 Val174Ala* rs4149056, but also other candidate genes, including drug transporters *ATP-binding cassette subfamily B member 1 (ABCB1, Alias MDR1), ATP-binding cassette subfamily C member 8 (ABCC8), drug transformers* of Cytochrome P450 Family (CYP) including *CYP2C9*2 CYP2C9*3 CYP2C8*3,* and *CYP3A4*22*. The assessment of these SNPs could provide valuable predictive results on both acquired and heritable adverse reactions in TD2M patients treated with SU-G and Simvastatin[19,20]. If the detection and the predictive value of these SNPs on the previously cited genes are regularly incorporated into clinical procedures, there will be an advantage in terms of optimizing an individualized therapy. However, an accurate description that PGx tests suggest that supplementary value, in terms of proportional costs and benefits, is still uncompleted. At present, the literature lacks of policy and trials exploring the pharmacoeconomic impact of genetic test in patients who receive polypharmacy for T2DM and cardiovascular disease[21]. Nevertheless, the optimum choice of genotyping platforms is fundamental in terms of cost-efficacy studies on PGx[22].

The aim of this pilot study is to establish a validated genotyping panel assay for preventing neuromuscular pain in T2DM patients to plan SU-G and statin-based therapy.

Patients and Methods

Patient Selection

Patients were recruited from a single centre Institute for Study and the Cure of Diabetes (ISCD) "Abetaia" of Casagiove (CE), Italy. This retrospective work was performed in agreement with the Ethical values according to the Declaration of Helsinki; informed consent documentation was reviewed and approved by the Independent Ethics Committee of ASL (CE). In total, 76 diabetic

patients (27 males and 49 females) were enrolled, 35 of whom received Repaglinide-based therapy (Table I).

All patients had a diagnosis of diabetes and cardiovascular co-morbidities. They are currently treated with anti glycaemic therapy. The dose, the schedule, and the duration of diabetes were not considered for genotyping.

The sample included 37 patients aged < 55 years and 39 aged ≥ 55 years, who were disjointedly analyzed in relation to risk factors for neuromuscular pain. Patients were separated in cases and control cohort: the first cohort consisted of subjects with SU-G plus statins based-therapy, while the second one brought together patients not following that treatment. Neuromuscular pain for any grade was also individually registered by a survey administered for all patients.

The inclusion criteria took into consideration patients with a diagnosis of T2DM with cardiovascular co-morbidities. All enrolled patients were without comorbidity causative additional peripheral neuropathy (i.e., treatment with platinum and/or fluoropyrimidines).

The assessment of neuromuscular pain involved the use of a survey based on individual symptom narration, since it did not include International Consensus Guidelines. The presence of asymmetrical "stocking-glove" numbness, the loss of deep tendon reflexes, and burning-tingling were evaluated in all patients.

Pharmacogenetic Assay

Genomic DNA was extracted from peripheral blood (within routine glucose level control procedure) in accordance with the manufacturer's protocol for the Ampli-DNA extraction kit (Dia-Chem, srl, Naples, Italy). The genotyping test was achieved using the TaqMan probe-based chemistry allelic discrimination assay in the OneStep platform (Life Technologies, Monza, Italy), following the manufacturer's protocols (Ampli-CYP and Ampli-statin, Dia-Chem, srl, Naples, Italy). The panel test included the *SLCO1B1, ABCB1, ABCC8, CYP2C8*3, CYP2C9*2, CYP2C9*3, CYP3A4*22,* polymorphisms.

Statistical Analysis

Differences according to age, gender, and adverse events, in particular for neuromuscular pain, between SU-G/statin users and the control cohort were calculated using the Chi-square test. Univariate analyses were performed to coincide with the two arms: the unadjusted logistic regression meth-

Table I. Distribution variables of the case/control cohort: T2DM patients SU-G+Statin users (case cohort n=35) *vs.* no SU-G+Statin (control cohort n=41). Univariate analysis.

	Control cohort n (%)	Case cohort SU-G +St users n (%)	*p*-value*	OR (95% CI)**
Age			0.06	
<55	15 (36.6)	22 (62.9)		1
≥55	26 (63.4)	13 (37.1)		0.42 (0.16-1.06)
Gender			0.04	
Male	18 (43.9)	9 (25.7)		1
Female	23 (56.1)	26 (74.3)		2.75 (1.04-7.29)
Medicines			nd	
-Sulphonyl ureas-glinides	44	35		
-Thyazolidines	24	28		
-GLP1 inhibitors	8	11		
-Insulin	31	2		
-Statins	59	35		
-Other	18	15		
DPN			0.003	
No	29 (70.7)	12 (34.3)		1
G1a & G1b	11 (26.8)	18 (51.4)		4.09 (1.49-11.18)
G2a & G2b	1 (2.4)	5 (14.3)		12.5 (1.32-118.47)
Neuromuscolar pain[$]			0.02	
No	37 (90.2)	24 (68.6)		1
Yes	4 (9.8)	11 (31.4)		4.35 (1.24-15.25)
CHD+AICAR	6.2±0.36[#]	0.62±0.07[#]		

Abbreviation: DPN, Diabetic Polyneuropathy. St, Statins. *Chi-Square test; **Crude odds ratio logistic regression were adjusted for age and gender. [$]Not correlated to DPN. It is based on individual anamnestic record.

od was used to assess crude odds ratios (ORs) and 95% confidence intervals (CIs). Logistic regression models adjusted for age and gender were used to calculate adjusted ORs and 95% CI for each Gene Variants risk factors. All analyses were performed using SPSS for Windows, version 23.0 (IBM Corp., Armonk, NY, USA). A two-sided *p*-value <0.05 was considered statistically significant.

Results

Patient Reports

Thirty-five T2DM patients (26 females and 9 males) who were administered SU-G/statin therapy were clustered in the case-cohort. 5 subjects out of the total (14.3%) were grade 2a/b diabetic polyneuropathy (DPN); while eleven patients (31.4%) experienced recurrent neuromuscular pain (Table I), 22 subjects of the sample were under 55 years old and 13 were over the age of 55 years. The control cohort was characterized by 41 cases who did not receive SU-G/Statin; all of them following concurrent therapy for cardiovascular co-morbidity. It is noteworthy that the SU-G/statin users experienced more cases with DPN with grade 1 and 2 than the control, 18+5 (65.7%) and 11+1 (29.2%), respectively (p=0.003). Neuromuscular pain, recorded by the individual anamnestic report, was remarkable in 11 out of 35 subjects (31.4%) of the case cohort in contrast with 4 out of 41 (9.8) cases of control cohort (p=0.02).

Genotyping Assay

Several criteria were considered to select gene variants for the pharmacogenomic panel tests: ì) searching the whole standardized polymorphisms acknowledged to influence the pharmacokinetics/pharmacodynamics of SU-G and Statins (www.pharmgkb.org)[23]; ìì) reviewing current researches, particularly trials including polymorphisms related to adverse events and polypharmacy[15,24]; ìíì) identifying issues related to the impact of genotyping testing which might provide likely answers concerning the incorporation of PGx markers in clinical practice.

The genotypes of all genes analyzed in this study are summarized in Table II.

Table II. Distribution of genetic polymorphism according to risk factors (any grade of muscular pain).

Gene variants	Control cohort n=41 (%)	SU-G +St users n=35 (%)	*p*-value*	OR (95% CI)**
SLCO1B1 Val174Ala rs4149056			0.03	
"CC"	34 (82.9)	23 (65.7)		1
"CT"	7 (17.1)	7 (20.0)		1.52 (0.5-4.91)
"TT"	0	5 (14.3)		n.d.
"TT+CT"		12 (34.3)		2.61 (0.90-7.61)
ABCB1 Iso1145Iso rs1045642			0.50	
"CC"	15 (36.6)	13 (37.1)		1
"CT"	23 (56.1)	18 (51.4)		0.57 (0.20-1.66)
"TT"	3 (7.3)	4 (11.5)		1.67 (0.26-10.67)
ABCC8 Arg1273Arg rs1799859			0.40	
"AA"	15 (36.6)	19 (54.3)		1
"GA"	22 (53.7)	14 (39.6)		0.56 (0.21-1.47)
"AA"	4 (9.8)	2 (6.1)		0.44 (0.07-2.76)
CYP2C9*2 Arg144Cys rs1799859			0.70	
"AA"	25 (70.0)	22 (62.9)		1
"AG"	16 (30.0)	13 (37.1)		1.25 (0.44-3.60)
CYP2C9*3 Ile359Leu rs1799853			0.70	
"AA"	29 (70.7)	26 (74.3)		1
"AG+GG"	12 (29.3)	9 (25.7)		0.60 (0.20-1.83)
CYP2C8*3 Lys399Arg rs10509681			0.19	
"TT"	28 (75.7)	25 (71.4)		1
"CT"	6 (16.2)	10 (28.6)		1.61 (0.49-5.36)
"CC"	3 (8.1)		0	n.d.
CYP3A4*22 intron 6 rs35599367			0.30	
"CC"	38 (92.7)	30 (85.7)		1
"CT"	3 (7.3)	5 (14.3)		2.17 (0.48-9.79)

*Chi-Square test; ** Crude odds ratio logistic regression were adjusted for age and gender.

SLCO1B1 Val174Ala rs4149056 genes were separated into two groups: CC *vs.* TT/CT genotypes. OR for any grade of neuromuscular pain was 2.61 (95% CI: 0.90-7.06, p=0.03). Other candidate genes were found not statistically significative.

The *ABCB1 3435C>T* rs1045642 (Iso1145Iso) genotype of SU-G/statin users was divided into two groups: TT allele *vs.* CT+CC alleles (4 cases, 11.5%). The OR for every toxicity grade was 1.67 (0.26-10.67, p=0.50), when compared with CT+CC (medium and low risk, respectively) allele genotype.

The *ABCC8-3C>-3T* rs1799859*(Arg1274Arg)* genotype was divided into two groups: GG allele *vs.* GA and AA alleles (2 cases, 6.1%). OR for every muscular pain grade was 0.44 (95% CI: 0.07-2.76, p= 0.40), when compared with GA+ GG (medium and low risk respectively) allele genotype.

*CYP2C9*2 rs1799853 (Arg144Cys)* was divided into two groups: AA allele *vs.* AG genotypes. OR for any grade neuromuscular was 0.70 1.25 (95% CI: 0.44-3.60, p= 0.70).

*CYP2C9*31057910 (Ile359Leu)* was divided into two groups: AA allele *vs.* AG/GG genotypes. OR for any grade neuromuscular was 0.60 (95% CI: 0.20-1.83, p= 0.70).

*CYP2C8*3* rs10509681 (*Lys399Arg)* genotype was divided into two groups: TT allele *vs.* CT alleles (8 cases, 25.8%). OR for every neuromuscular grade was 1.61 (95% CI: 0.49-5.36, p= 0.19), towards CT (medium risk) allele genotype.

*CYP3A4**22 rs35599367 intron 6 gene was divided into two groups: CC allele *vs.* CT genotypes, no homozygous for TT were detected. OR for any neuropathy grade was 2.17 (95% CI: 0.48-9.79, p= 0.30), towards of CT genotype.

Discussion

Predictive genetic signatures allow physicians to better adopt an appropriate therapy. Furthermore, the clinical value of the selected *SLCO1B1* SNP in anti-T2DM therapy is partially limited by: (i) the restricted diffusion of genotyping in routine diagnostics procedures; (ii) the lack of tangible confirmation that PGx evidence expands health; and (iii) the cost-effectiveness of testing, which is still an open query[25].

The aim of our pilot study is to perform a validated PGx panel assay for preventing neuromuscular pain. We validated an inexpensive genotyping test using the TaqMan "allelic discrimination platform" including the homogeneous detection of *SLCO1B1 T521C* polymorphism, and other candidate genes. As previously shown, identifying the polymorphisms described above may enable to plan personalized therapy in patients who receive SU-G and anti-cholesterol drugs[26]. In our investigation, we evaluated additional SNPs, i.e., *ABCB1 (alias MDR1), ABCC8, CYP2C9*2 CYP2C9*3*, *CYP2C8*3, CYP3A4*22,* but we did not observe a relationship with neuromuscular adverse events, except for *SLCO1B1 T521C*, whether for TT or CT alleles (p=0.03).

The *SLCO1B1* encodes for a transmembrane receptor protein, called OATP1B1, involved in the removal of anionic compounds from blood into hepatocyte. *SLCO1B1* is an extremely polymorphic gene; its best well-known non-synonymous variant rs4149056, T521C codon Val174Ala, has been confirmed to enhance Repaglinide bioavailability in both T2DM and healthy subjects of Caucasian and Asian ethnicity[27,28]. In the same gene, another non–coding SNP (rs4149015) has been found to be related with an increased glucose–lowering effect of Repaglinide[29,30].

The gene *CYP2C8* encodes for a metabolizing enzyme able to reduce Repaglinide levels. In subjects carrying dyplotype CYP2C8*3 rs11572080 (Arg139Lys) and rs10509681 (Lys399Arg) called ultrarapid metabolizer (UM), the drug bioavailability is reduced[31,32]. By divergence, hypoglycemic events were reported in a small cohort of T2DM patients CYP2C8*3 who experienced undergoing treatment with SU (Glimepiride, Gliclazide, or Glipizide)[33]. In our case, we accounted statistically insignificant relationship among neuromuscular (0 case) and SNP for the *CYP2C8 CC* genotype (OR: 1.61, 95% CI: 0.49-5.36, p=0.19), compared to the CT (medium-risk) genotype. Polymorphism in *CYP2C8 (CYP2C8*3* allele) has been shown to be associated with reduced plasma concentration of Repaglinide[32]. Genetic polymorphism in hepatic-uptake transporter *SLCO1B1* is another independent determinant of the pharmacokinetics of Repaglinide (Figure 1). The AUC of Repaglinide is markedly increased in homozygous carriers of *SLCO1B1 521T>C* than in subjects carrying 521TT genotype[27]. Since Repaglinide is selective for pancreatic β cells and its clinical efficacy depends on functioning β cells and glucose, it does not appear to affect skeletal or cardiac muscle. It is a possible explanation of synergistic neuromuscular pain only when it was co-administered with Statins[33]. Statin pre-

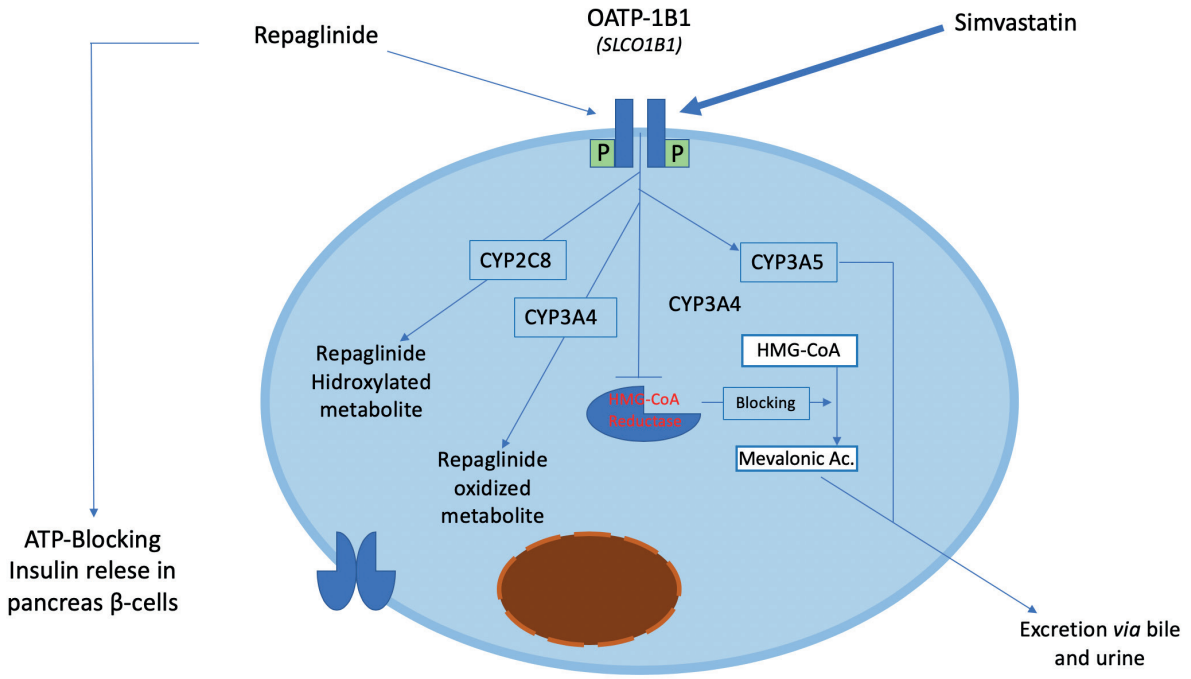

Figure 1. Pharmakodinamic pathway of Repaglinide and Simvastatin. *SLOC1B1* coding for carrier transporter of both Repaglinide and Simvastatin. Repaglinide is metabolized by oxidation and hydrolyzation by CYP2C8 and CYP3A4, respectively. Simvastatin blocking HMG-CoA reductase inhibits cholesterol synthesis.

scriptions are the ordinary practice ensuring the control of cholesterolemia in patients with cardiovascular risk. The myopathy in patients with *SLCO1B1 CC* genotype who receive Simvastatin is documented[17]. Cases of myopathy, including rhabdomyolysis, have been reported with HMG-CoA reductase inhibitors coadministered with colchicine, and caution should therefore be exercised when prescribing these two medications together[34]. An association between statin and incidence of breast cancer was recently described[16].

The strictly related *CYP2C9* enzyme is the major responsible for SU-G breakdown[35]. The non–synonymous *CYP2C9**2 and *CYP2C9**3 variants rs1799853 (Arg144Cys) and rs1057910 (Ile359Leu) respectively have been reported to determine lower–*CYP2C9* catalytic activity resulting in reduced SU-G clearance[26]. Nevertheless, no data was found concerning *CYP2C9**2 and *CYP2C9*3* in relation to increased risk of hypoglycemia in healthy volunteers and T2DM patients who received SU[35]. Since it has been recently demonstrated that CYP2C9 catalytic deficiency might be neutralized by the effects of polymorphisms in the CYP oxidoreductase (*POR*) gene (it modulates their activity), these data should be carefully interpreted[36,37]. Indeed, it has been reported that a subset of patients carrying *CYP2C9**2 and *3 alleles (extrapolated from the GoDART database) was linked with an augmented risk of hypoglycemic and enhanced activity to SU-G in patients carrying the POR*1/*1 wild-type genotype[38].

It is noteworthy that this evidence on *CYP450* translates into clinical routine increased odds of moderate to severe hypoglycemic events during treatment with SU-G[39].

Several observational types of research using genome-wide association studies (GWASs) have focused on SNPs related to PGx of SU-G and statins in the ABC transport proteins[40]. They include additional candidate gene *ABCB1*, two SNPs (rs1045642, and rs2032586)[41], and *ABCC8* gene.

The ABCC8 ATP-binding cassette transporter sub-family C member 8, together with KIR6.2 transporters encoded by potassium voltage-gated channel subfamily J member 11 (*KCNJ11gene*), acts as a K-ATP channel protein. Several SNPs within the *ABCC8* locus have been associated with inter individual variability in response to SU-G treatment. The intronic polymorphism rs1799854 (exon 16 −3C → −3T) is in linkage disequilibrium (LD) with the variant rs1801261

(Thr759Thr). It has been associated with reduced insulin secretion after tolbutamide infusion in non diabetic relatives of T2DM patients[42]. T2DM patients on SU-G treatment carrying the rs1799854 C/C genotype exhibited significantly lower-HbA1c levels compared to patients with T/T genotype and improved insulin sensitivity determined by HOMA index in response to Repaglinide, with respect to T carriers[43]. Other studies describe the synonymous SNP *ABCC8* rs1799859 (Arg1273Arg). T2DM patients carrying the G/G genotype, in treatment with SU-G had significantly higher-HbA1c levels compared with the patients with A/A genotype[43]. Furthermore, the capability of *ABCC8* Ala1369Ser to interfere with SU-G therapy is controversial since this SNP is in strong LD with the non-synonymous variant Lys23Glu in *KCNJ11*, and it suggests the presence of a molecular discriminatory specificity for the genetic variation at K-ATP channels. Undeniably, when compared with *ABCC8-KCNJ11* wild-type haplotype carriers, 1369Ala and 23Lys haplotype were shown to increase sensitivity to gliclazide[44]. Both *ABCC8* rs1799854 and rs1799859 polymorphisms resulted remarkably associated with the increased level of triglycerides after SU-G therapy[45].

Conclusions

The clinical usefulness of the *SLCO1B1* will be assured by diagnostic improvements suitable for driving treatment decisions. In particular, genotyping patients for *SLCO1B1 T521C* gene will probably help physicians to select subjects who are most susceptible to neuromuscular pain. We hypothesize that higher muscular pain in T2DM patients carrying *SLCO1B1 T521C* whether for TT or CT alleles haplotype has reduced SU-G and statins bioavailability (p=0.03).

There have been certain restrictions in our research: i) these PGx signatures require to be confirmed in multiple studies with a larger number of patients. The small sample size could produce false-positive results, or might overestimate the magnitude of the association[45]; ii) our genotyping data are limited to a Caucasian population; iii) definition of the grading of neuromuscular pain is based on anamnestic information without a consensus guidelines; iv) we did not adjust our data for multiple calculations (i.e., several types of cardiovascular disease) due to a small number of cohort samples; v) selection of the gene variants were based on the recent findings in GWAS, limited to significant correlations between PGx profile and SU-G/statins therapy. However, with regard to the gene variants investigated in this study, the single endpoint was to evaluate the usefulness of a PGx assay suitable for application in clinical practice, with particular attention to the so-called "frail patients"[46].

Promisingly, it can be expected that there will be an improvement for routine diagnostics in PGx test concerning SU-G/statins. PGx assay is useful because it is low cost (about 50,00€/patients) and it is suitable for the most clinical laboratories with Real Time-PCR equipment. In addition, no high genomic expertise needs to perform genotype results[47].

Finally, clinicians, geneticists, pharmacists, and laboratory managers should join in evaluating the benefits and limits, particularly regarding costs and applicability of the pharmacogenomic tests likely suitable for routine clinical practice integration.

Conflict of Interests

The Authors declare that they have no conflict of interests.

Acknowledgements

The authors are grateful to Dr. O. Catapano from the "Italian Association of Pharmacogenomics and Molecular Diagnostics" for the invaluable bibliography research, and Dr Monaco Francesco for his expert assistance in the supervision of the manuscript.

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