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Research Paper

DESCRIPTION OF A FACILE, RAPID, AND INEXPENSIVE METHOD TO PROFILE FOR THE ORGANIC CATION TRANSPORTER (OCT1) DEL420 VARIANT

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Pharmacogenomics provides enormous potential for significantly improving the medication response and therapeutic outcomes in patients, but little clinical realization of that potential to date. One of the reasons for this is the lack of facile, rapid and cost-effective methods to perform the genotyping. State of the art facilities require enormous start-up funding, significant space, and multiple personnel. Many research-interested institutions do not have such a facility and so are limited in the ability to perform pharmacogenomics studies. Type 2 diabetes mellitus represents a significant burden to healthcare and is associated with high morbidity and mortality. Metformin, a common first line treatment for Type 2 diabetes, is processed by organic cation transporters (OCT). Previous study has shown that an inactivating mutation of OCT1, del420, has been linked to metformin response. Thus detection of this variant in patients may aid in their therapeutic drug management and enhance their treatment outcomes. We report here a facile, rapid and inexpensive method to detect this OCT1 del420 variant.

Keywords: Pharmacogenomics, Organic cation transporters, Biotechnology, Individualized drug therapy, Metformin, Type 2 diabetes mellitus

INTRODUCTION

The field of pharmacogenomics provides enormous potential for significantly improving the medication response and therapeutic outcomes in individuals. Although genotyping has identified many variants, the translation of these results to meaningful clinical usage remains very limited. Newer, high throughput technologies have continued to identify genetic variants associated with human disease conditions. However, DNA chips are not yet available for many applications and more traditional genotyping approaches will

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likely remain important (Pinto et al., 2010). Many researchers interested in pharmacogenomics still do not have access to high throughput technologies. Our group is interested in improving the outcomes of patients with type 2 diabetes, and we researched the known genetic variants thought to contribute to variability in response to metformin. The organic cation transporter, OCT1, is responsible for transporting metformin into hepatocytes (Wang et al., 2002) and is needed for the hepatic glucose lowering effect of metformin (Shu et al., 2007). Polymorphisms of OCT1 have been described with variable effects on metformin response (Shu et al., 2007; Becker et al., 2009). One variant involves the deletion of three base pairs causing the loss of a methionine (residue 420) in the predicted 9th transmembrane spanning domain (Shu et al., 2003). This 420del variant has been shown to have impaired metformin transport activity in vitro (Shu et al., 2007). Several loss-of-function polymorphisms, including 420del, have been shown to affect the glucose-lowering response of metformin in healthy subjects (Shu et al., 2007). These data provide evidence that 420del may be contributing to variable patient responses to metformin.

Upon inspection of the 420del sequence we observed that it contained a 6 base palindrome (TCATGA) that matched the recognition site for BspH1 restriction enzyme, and that there were no other BspH1 sites within at least ~ 100 bases in either direction. We reasoned that utilization of this restriction site could provide a quick and easy method to discriminate between non-variant and variant forms. We report here the development of this assay that allows for easy determination of homozygous non-variant OCT1, homozygous variant 420del, and heterozygous variant 420del without the use of DNA sequencer or real-time PCR amplification.

MATERIALS AND METHODS

Participants: Human volunteers were solicited for participation and twelve responded. The mean age was 40.5 years (range 25-48) and there were 3 males and 9 females. This study received Institutional Review Board approval.

Genomic DNA Extraction: Buccal cell collection and DNA extraction was performed according to manufactures instructions (Isohelix Swab and Isohelix DNA extraction kits respectively). The quality of the extracted DNA was assessed using Isohelix DNA Quality Check Kit per the manufacturer's instructions. We routinely obtained ~2 μg/swab.

OCT1 Primers: OCT1-specific primers GGCCTTCATAGCCCTCATCAC (forward primer) and GACAAAGGTAGCACCTCATCT (reverse primer) were synthesized by Sigma-Genosys and designed to generate a 227 base pair (bp) PCR product that encompasses the 420del region near the middle of the product. The derived PCR product would contain one BspH1 restriction site that would generate two bands of 138 and 89 bp. This restriction site would be absent from any PCR product amplified from 420del DNA.

PCR Amplification: Optimal conditions for the PCR reaction were determined to be 2 mM $MgCl_2$, 1 unit Polymerase (New England Biolabs), 200 μM dNTPs, 0.4 μM forward primer, 0.4 μM reverse primer, 1X reaction buffer (20mM Tris HCl pH 8.8, 10 mM $(NH_4)_2SO_4$, 10mM KCl, 2 mM $MgSO_4$, 0.1% Triton X100), and 5 μL genomic DNA (~ 100 ng). The PCR amplification conditions were: 1 x [5 min at 95° C], 33 x [0.5 min at 95 °C,

0.5 min at 57°C, 0.75 min at 75°C], and 1 x [7 min at 75 °C]. After amplification, primers were removed using QIAquick PCR Purification Kit (Quiagen).

Restriction Enzyme Digestion: Purified PCR products (50 μl, ~ 200 ng DNA) were digested with 263 U/ml BspH1 in a reaction volume of 57.5 μl with final concentrations of 20 mM Tris acetate pH 7.9, 10mM magnesium acetate, 50mM potassium acetate, and 1mM DTT at 37°C for a minimum of 15 h. Aliquots were run on a 1.9 % agarose gel containing 0.5 μg/ml ethidium bromide.

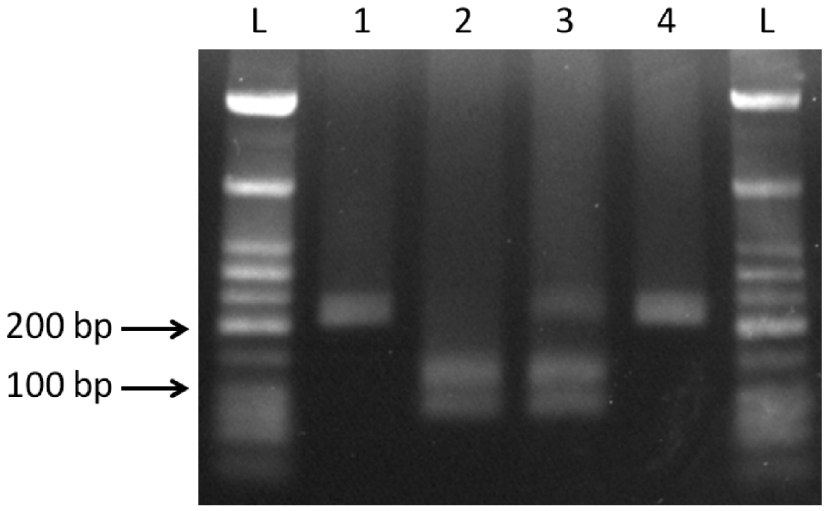
DNA Sequencing: DNA sequencing of PCR products was performed by DNASU Sequencing Facility in The Biodesign Institute at Arizona State University.

RESULTS AND DISCUSSION

Control PCR Products: The quality of the extracted genomic DNA was satisfactory as assessed by the manufacturer's quality control kit (data not shown). The OCT1-specific primers were used to amplify homozygous non-variant DNA (obtained from a participant and confirmed by sequencing). In Figure 1, lane 1, the results of this PCR reaction showed one band migrating at the expected size of 227 bp. A similar size PCR product would be generated from 420del genomic DNA; it would be three base pairs smaller than the non-variant PCR product, but this small size difference would not be detected under these agarose gel conditions. Therefore lane 1 simulates the results that would be obtained from *digested* PCR products from homozygous 420del variants; lacking the restriction site due to the deletion they would run essentially as undigested PCR product. To control for effects of BspH1 digestion reaction conditions on migration of bands, the 227 bp PCR product was incubated overnight in enzyme reaction conditions minus BspH1 (Lane 4). Digestion of the 227 bp PCR product with BspH1 produced two bands at 138 and 89 bp (Lane 2), simulating the results that would be obtained from digested PCR products from homozygous non-variants. To simulate a heterozygous individual, equal amounts of undigested and digested PCR products were mixed prior to electrophoresis, resulting in all three bands (89 bp, 138 bp and 227 bp) being present (Lane 3). Thus, these conditions showed patterns that allow for correct identification of homozygous non-variants, homozygous 420del variants, and heterozygous individuals.

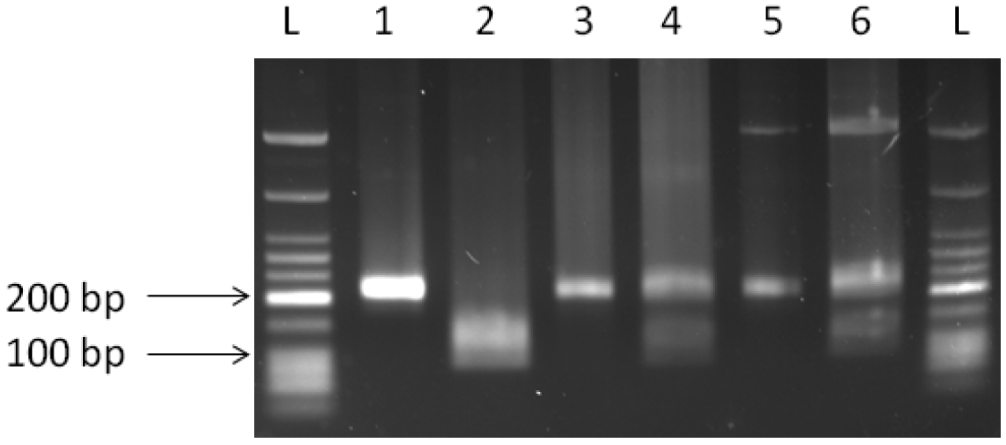
Experimental PCR Products: Participant DNA was amplified with the OCT1-specific primers and a PCR product of the expected size of 227 bp was generated (data not shown). After digestion of the PCR product with BspH1, all but two samples were determined to be homozygous non-variant (data not shown). The remaining two samples were determined to be heterozygous. Figure 2 shows the undigested and BspH1-digested PCR products of these two variants, in addition to a non-variant. The 227 bp PCR product from the non-variant (lane 1) is completely eliminated with BspH1 digestion and generates the expected doublet at 138 and 89 bp (lane 2). The 227 bp PCR product from the heterozygous variants (lane 3, 5) were still visible after BspH1 digestion, in addition to the appearance of the doublet (lanes 4, 6). This was not likely the result of incomplete restriction enzyme digestion, as additional enzyme and increased reaction time did not alter the results. Furthermore, these results were confirmed by DNA sequencing of PCR products.

Figure 1: Control PCR Products Using Non-Variant Genomic DNA*: Lane 1 - PCR Product, Lane 2 – BspHI-Digestion of PCR Product, Lane 3 – Mixture BspHI-Digested and Undigested PCR Product, Lane 4 – PCR Product in Digestion Reaction Buffer (no enzyme), L – Ladder



Note: * Confirmed by sequencing.

Figure 2: Experimental Heterozygous Variants: Lanes 1, 3, 5 - PCR Product, Lanes 2, 4, 6 – BspHI Digestion of PCR Product, Lanes 1-2 – Non-Variant Participant DNA*, Lanes 3-6–Heterozygous Variant Participant DNA*; L – Ladder



Note: * Confirmed by sequencing.

Time and Cost: The entire procedure from DNA extraction to the results were routinely accomplished in 24 h, and was easily done by one person. We estimate that to perform 25 samples it would cost ~230$ in enzymes, reagents, kits and other supplies. Only standard laboratory equipment is needed.

Significance: This method facilitates the study of OCT1 420del variant, which has been implicated in several disease states. Metformin

is a commonly used drug to treat type 2 diabetes mellitus, and OCT1 was shown to be responsible for transporting metformin into hepatocytes, and for the hepatic glucose lowering effect of metformin (Wang et al., 2002; Shu et al., 2007). The 420del variant has been shown to have impaired metformin transport activity *in vitro* (Shu et al., 2007), and to increase metformin renal clearance in healthy human volunteers (Tzvetkov et al., 2009). However, Zhou and colleagues reported that there was no clinically evident reduction in the ability of metformin to decrease A1C in diabetic patients expressing the 420del variant (Zhou et al., 2009). Further studies are needed to validate the 420del as a predictor of clinical metformin response (Zolk, 2009).

This method may be useful in the study of other disease states such as polycystic ovary syndrome in which polymorphisms of OCT1 (including 420del) have been shown to block the positive response of metformin (Gambineri et al., 2010). Additionally, OCT1 polymorphisms have been implicated in interactions involving drugs other than metformin. The tyrosine kinase inhibitor erlotinib (approved for non-small cell lung carcinoma) was shown to potently inhibit OCT1, and the 420del variant was even more sensitive to erlotinib inhibition than non-variant OCT1 (Minematsu and Giacomini, 2010). Similarly, OCT1 420del has been shown to be more sensitive than reference OCT1 to drug inhibition by various drugs including prazosin, chlorpromazine, and quinidine, with IC50 values up to13 times lower (Ahlin et al., 2011).

CONCLUSION

We have described a facile, rapid, and inexpensive method to perform genetic profiling for the OCT1 420del variant that is able to distinguish between homozygous non-variant, homozygous variant, and heterozygous variant. This method will facilitate the study of this important drug transporter, and help in the translation of pharmacogenomics data to meaningful clinical applications.

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REFERENCES

1. Ahlin G, Chen L, Lazorova L, Chen Y, Ianculescu A G, Davis R L, Giacomini K M and Artursson P (2011), "Genotype-Dependent Effects of Inhibitors of the Organic Cation Transporter, OCT1: Predictions of Metformin Interactions", *Pharamacogenomics J.*, Vol. 11, pp. 400-411.
2. Becker M L, Visser L E, van Schaik R H N, Hofman A, Uitterlinden A G and Stricker B H C (2009), "Genetic Variation in the Organic Cation Transporter 1 is Associated with Metformin Response in Patients with Diabetes Mellitus", *Pharmacogenomics J.*, Vol. 9, pp. 242-247.
3. Gambineri A, Tomassoni F, Gasparini D I, Di Rocco A, Mantovani V, Pagotto U, Altieri P, Sanna S, Fulghesu A M and Pasquali R (2010), "Organic Cation Transporter 1 Polymorphisms Predict the Metabolic Response to Metformin in Women with the Polycystic Ovary Syndrome", *J. Clin. Endocrinol. Metab.*, Vol. 95, pp. E204-208.
4. Minematsu T and Giacomini K M (2010), "Interactions of Tyrosine Kinase Inhibitors

with Organic Cation Transporters and Multidrug and Toxic Compound Extrusion Proteins", *Mol. Cancer Ther.*, Vol. 10, pp. 531-539.

5. Pinto A, Di Francia R, Frigeri F, Berretta M, Cecchin E, Orlando C and Pinzani P (2010), "Decision Criteria For Rational Selection of Homogeneous Genotyping Platforms for Pharmacogenomics Testing in Clinical Diagnostics", *Clin. Chem. Lab. Med.*, Vol. 48, pp. 447-459.
6. Shu Y, Leabman M K, Feng B, Mangravite L M, Huang C C, Stryke D, Kawamoto M, Johns S J, DeYoung J, Carlson E, Ferrin T E, Herskowitz I and Giacomini K M (2003), "Evolutionary Conservation Predicts Function of Variants of the Human Organic Cation Transporter, OCT1", *Proc. Natl. Acad. Sci.*, Vol. 100, pp. 5902-5907, USA.
7. Shu Y, Sheardown S A, Brown C, Owen R P, Zhang S Z, Castro R A, Ianculescu A G, Yue L, Lo J C, Burchard E G, Brett C M and Giacomini K M (2007), "Effect of Genetic Variation in the Organic Cation Transporter 1 (OCT1) on Metformin Action", *J. Clin. Invest.*, Vol. 117, pp. 1422-1431.
8. Tzvetkov M V, Vormfelde S V, Balen D, Meineke I, Schmidt T, Sehrt D, Sabolic I, Koepsell H and Brockmoller J (2009), "The Effects of Genetic Polymorphisms in the Organic Cation Transporters OCT1, OCT2, and OCT3 on the Renal Clearance of Metformin", *Clin. Pharmacol. Ther.*, Vol. 86, pp. 299-306.
9. Wang D S, Jonker J W, Kato Y, Kusuhara H, Schinkel A H and Sugiyama Y (2002), "Involvement of Organic Cation Transporter 1 in Hepatic and Intestinal Distribution of Metformin", *J. Pharmacol. Exp. Ther.*, Vol. 302, pp. 510-515.
10. Zhou K, Donnelly L A, Kimber C H, Donnan P T, Doney A S, Leese G, Hattersley A T, McCarthy M I, Morris A D, Palmer C N and Pearson E R (2009), "Reduced-Function SLC22A1 Polymorphisms Encoding Organic Cation Transporter 1 and Glycemic Response to Metformin: a GoDARTS Study", *Diabetes*, Vol. 58, pp. 1434-1439.
11. Zolk O (2009), "Current Understanding of the Pharmacogenomics of Metformin", *Clin. Pharmacol. Ther.*, Vol. 86, pp. 595-598.