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Research Article

Evidence-Based Prioritization and Scoring of Genomic Loci Interacting with Pioglitazone Therapeutic Response in Diabetic Patients

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Abstract

Extensive literature shows multiple genetic loci's involvement in regulating pioglitazone's action in diabetic patients; most of the available literature lacks replication, and it is difficult to separate true positives from false-positive loci. To overcome the inconsistency in pharmacogenetics and pharmacogenomics data, we performed evidence-based semi-automated prioritization and scoring of candidate genes of pioglitazone in diabetic patients. We developed a Python script and searched with MeSH terms of "Pioglitazone (PIO)" and synonyms," and (i) built a complete library of published data (22,532 publications), (ii) extracted sentences containing pioglitazone-gene pair by using Python 3.6, (iii) annotated each sentence for its relevance, (iv) developed evidence scoring algorithm based on pharmacogenomics relatedness, frequency, and consistency. From the literature, we have identified that the gene encoding peroxisome proliferator-activated receptor *gamma* showed the strongest evidence of mediating the pioglitazone response. Additionally, polymorphisms in genes affecting the pharmacokinetics were from the Cytochrome enzyme family, i.e., *Cyp2C8*, *Cyp3A4*, and *Cyp2C9*, and pharmacodynamics were protein tyrosine phosphokinase (*Ptprd*), Lipoprotein (*Lpl*), *Adiponectin receptor 2* (*Adipora2*), and PPARG coactivator 1 alpha (*Ppargc1*). Furthermore, drug-induced adverse drug reactions were associated with polymorphisms in the Solute carrier family 12 member 1 (*Scl12a1*) and Aquaporin 2 (Aqp2) genes. We have prioritized candidate gene variants and their response to pioglitazone in diabetic patients. Our results showed PPAR-α receptors/ PPARG as one of the most robust evidence of therapeutic response to pioglitazone and highlighted other variants in different pathways. However, we further warrant validation of our results in a large drug response GWAS dataset.

Keywords: Genetic polymorphism, PPAR, pioglitazone, gene-drug interaction, Type 2 diabetes, genome-wide association studies

1. Introduction

Pioglitazone is the most used thiazolidinedione and acts as an insulin sensitizer by activating the Peroxisome Proliferator-Activated Receptor-γ (PPARγ). Pioglitazone is approved to manage type 2 diabetes mellitus (T2DM), but its use in other therapeutic areas is increasing due to multiple effects (Kawaguchi-Suzuki and Frye 2013). Pioglitazone [PIO] is the only Thiazolidinediones (TZD) member currently being used after troglitazone withdrawal due to hepatotoxicity and rosiglitazone due to the high risk of myocardial infarction (Watkins and Whitcomb 1998; Kung and Henry 2012).

There remains uncertainty about the exact mechanism of action of TZDs, but the present-

day consensus is that TZDs target the transcription factor peroxisome proliferator-activated receptor-γ (PPARγ) to improve insulin sensitivity (Cariou, Charbonnel, and Staels 2012).

Ligand-dependent transactivation of PPARγ causes heterodimerization with the retinoid-X receptor followed by recognition of peroxisome proliferator response elements (PPREs) that stimulate transcription of genes involved in metabolic homeostasis (Desvergne and Wahli 1999) [**Fig: 1].** The effectiveness of PIO as an antihyperglycemic agent was demonstrated in randomized controlled trials (Cariou, Charbonnel, and Staels 2012)**.** PIO monotherapy lowered HbA1C by ~1% in T2DM patients (Aronoff et al. 2000; Baba 2001; Herz et al. 2003; Rajagopalan, Iyer, and Khan 2005; Yamanouchi et al. 2005).

TZDs improve insulin sensitivity, which may reduce insulin doses or eliminate the need for insulin in some patients (Yau et al. 2013). In addition to T2DM, PIO use has increased in other therapeutic areas such as non-alcoholic fatty liver disease, atherosclerosis, inflammation, infertility, and cancer due to the broad spectrum of effects secondary to PPARγ activation. At the same time, more undesirable off-target effects have been identified(Desvergne and Wahli 1999; Cariou, Charbonnel, and Staels 2012). Despite being one of the most commonly used antidiabetic drugs in T2D; Pioglitazone has shown variable effectiveness in patients (Umpierrez and Dagogo-Jack 2006). Studies that have made direct assessments have shown a 30-40% of lack of response among individuals taking pioglitazone as monotherapy (Bozkurt et al. 2007; Kahn et al. 2006).

The presence of definite gene variants in the pioglitazone pathways is responsible for variable drug response and increased risk of unwanted effects in individuals treated with the drug. (Nolan et al. 1994; Buchanan et al. 2000, 2002).

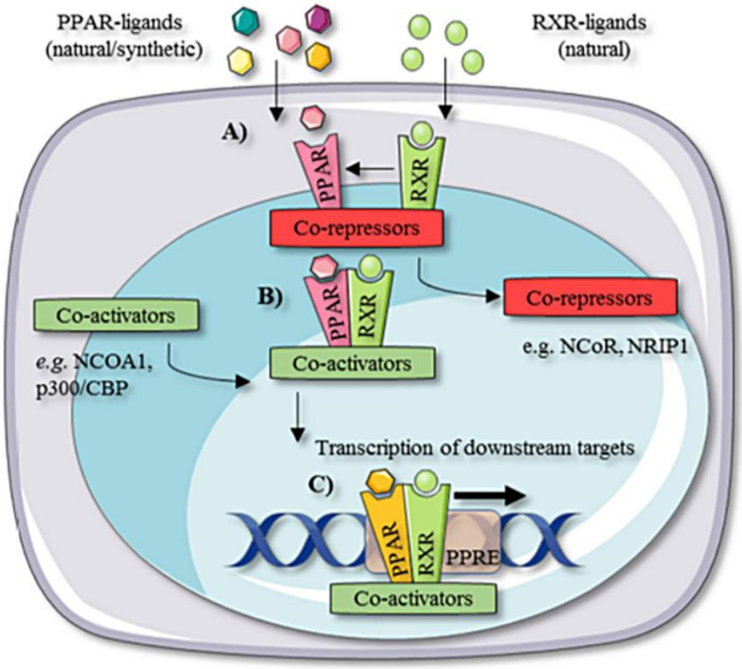

Figure 1. Mechanism of peroxisome proliferator-activated receptor (PPAR) activation and downstream gene transcription. (A) The PPAR receptor forms a heterodimer with a retinoid X receptor (RXR) upon the binding of an activating ligand. (B) PPAR-RXR heterodimerization causes the release of corepressors and the recruitment of coactivators. (C) The PPAR-RXR heterodimer binds to PPAR-responsive elements on the DNA, transcribing downstream target genes.

As the drug is metabolized by the CYP2C8 enzyme, variation in this gene may cause an increase or decrease in the drug's metabolism, leading to an increase or no effect in the drug response(Nolan et al. 1994). Similarly, treatment with pioglitazone has been particularly associated with variable adverse drug reactions in individuals, such as edema, bone fracture, and cardiovascular complications (Eckland and Danhof 2000; Hanefeld and Belcher 2001). In the kidney, the *AQP2* gene encodes aquaporin, which works as a water channel. Polymorphisms in the gene *AQP2 (rs296766)* alter this activity and lead to pioglitazone-induced edema (Chang et al. 2011; Knepper et al. 1996). *SLC12A1 rs12904216* polymorphism also takes part in pioglitazone-induced edema (Bozkurt et al. 2007; Ji et al. 2008). Moreover, the administration of pioglitazone inhibits or/and increases the expression of several genes. Expression of *TNFA* and *IL6* genes is inhibited by pioglitazone, while expression of glucose transporter genes *GLUT1* and *GLUT4* is increased by pioglitazone (Cariou, Charbonnel, and Staels 2012; Kahn et al. 2006; Hounoki et al. 2008; Himelfarb et al. 2011; Hofmann, Lorenz, and Colca 1991; Young et al. 1995).

Reviewing the vast literature on gene-pioglitazone, concluding a fair overview is difficult. Many factors like study design, population characteristics, and analytic strategies impose challenges to such studies. Therefore, to facilitate this process, automated approaches to integrate evidence from multiple sources, cataloging the levels of evidence, validating in a real-world dataset, and using this to prioritize genes for follow-up are increasingly favored. Here, in this article, we provide a semi-automated approach to prioritize genes that showed clinical evidence of interacting with pioglitazone based on the strength of evidence from published studies.

2. Data collection and cleaning

2.1. Selection and download of articles

Articles containing the reference to pioglitazone and gene were identified through the Gene Ontology database GO PubMed and retrieved from PubMed®/Medline databases. Overall, 22,532 articles by using pioglitazone and its related terms were identified, and after removing duplicates, 12,164 articles proceeded for further evaluation**. [Fig: 2].** PubMed IDs (PubMed Articles Identifiers) of selected articles were collected in notepad and then used to download full-text articles automatically by using Endnote and NCBI Batch Entrez. Both tools allow us to access articles from different journals that are open access. The complete references were downloaded and imported to Endnote. University of Dundee subscription was used to download full-text articles. Most of the time, published articles are found in PDF forms, making them convenient, multi-dimensional, and secure. This is why PDFs are hard to edit; sometimes, extracting information from them is challenging.

Henceforth, PDFs were converted to the more liable text format by using Nitro pro PDF converter software ("Nitro Pro Release Notes," n.d.) (https://www.gonitro.com/ accessed from 1 January to 30 April 2018).

The irrelevant or unwanted information, such as the names of authors and research institutes, emails, and references, are given for each article that was removed before sentence extraction.

1.1. Gene and drug dictionary construction

Different naming conventions exist for drugs and genes. Similarly, the biomedical literature has used diverse names for the drug pioglitazone and its respective genes. Therefore, a comprehensive dictionary for the drug-containing generic name, brand names, synonyms, and International Union of Pure and Applied Chemistry (IUPAC) names of pioglitazone were compiled from drug cards of

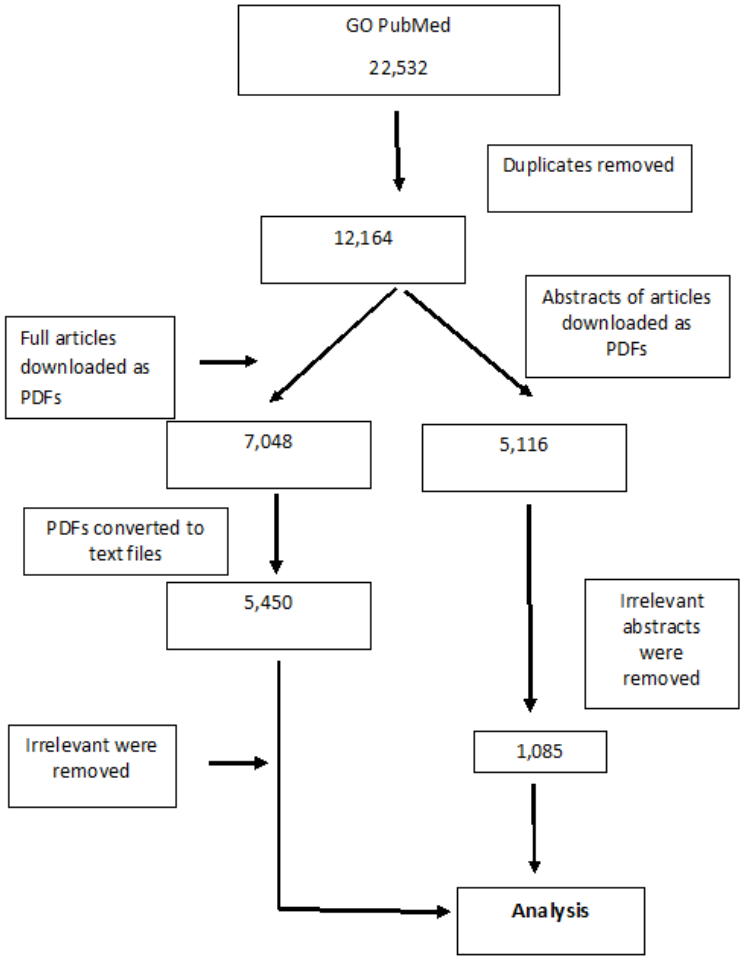

Figure 2: Flow-chart summarizing screening and selection of articles.

the Drug Bank (www.drugbank.ca/). Similarly, a dictionary containing gene names by extracting gene synonyms from NCBI Gene (www.ncbi.nlm.nih.gov/gene/), UCSC Genome Brower (www.genome.ucsc.edu/), SymAtlas (www.biogps.org/), Google (www.google.com/) and Gene Cards (www.genecards.org/) /) was constructed. All these databases were accessed from (1 January to 30 April 2018).[see Table 1a & b]

1.2. Sentence segmentation

Sentence extraction comprises text segmentation, tokenization, and named entity recognition. For this purpose, Python programming software and its NLTK (Natural Language Toolkit) package were used, freely available at https://www.python.org/ (accessed from 1 January to 30 April 2018). The total number of sentences present in the document was collected and then processed for tokenization. Gene and drug names were tagged using a Perl-based markup algorithm that uses a set of hashes and regular expressions. Sentences that contain a drug and a gene (i.e., a gene-drug dyad) were extracted from the corpus of each article (e.g., from titles, abstracts, or the main body of texts). Entities are given particular tags using a Python-based markup algorithm that uses a set of built-in functions and NLTK package set to recognize the terms from the whole set of articles. Finally, those sentences which contain the drug or gene name are extracted from the data, including the titles. To avoid obscurity, every sentence was saved with

Table 1(a): Different drug names and its synonyms used to construct dictionary for pioglitazone.

Chemical names	5-[[4-[2-(5-ethylpyridin-2- yl)ethoxy]phenyl] methyl]-1,3- thiazolidine-2,4- dione, 5-{4-[2-(5- ethylpyridin-2- l)ethoxy]benzyl} -1,3-thiazolidine-2,4-dione
Brand names	Actos, Accel-Pioglitazone, Pioglitazone-Accord-Pioglitazone Actavis, Pioglitazone-Teva, PMS- pioglitazone, Ratio- pioglitazone, Ran- pioglitazone, Dom- pioglitazone, Mint-pioglitazone, Jamp-pioglitazone, Act Pioglitazone, Glustin, Sandoz, Pioglitazone, Ach-pioglitazone, Glidipion, Auro-pioglitazone, Apo- pioglitazone, Pro-pioglitazone, Teva- pioglitazone, Torrent- pioglitazone, Van-pioglitazone, Mylan- pioglitazone, PHL- pioglitazone, Zym-pioglitazone
Generic names	Pioglitazone HCl, Pioglitazone Hydrochlori de

the title, PMID, and distinctive number to identify.

1.3. Annotation of extracted sentences

This analysis was based on an assumption in which it was anticipated that drug-gene pairs clustered together in a sentence repeatedly. Each sentence was then manually annotated to create a connection between pioglitazone and genes according to the annotation guideline established by the gene-drug interaction Corpus and Comparative Evaluation by DIEGO lab (http://diego.asu.edu/, accessed on 10th of May 2018). The effect is assumed to be articulated with interaction words that give evidence of an interaction. These words, such as effect, influence, or action," explain the action of a gene on clinical outcomes, pharmacodynamics, and pharmacokinetics of the drug. Moreover, the drug's effect, influence, or action on gene expression is also included in the interaction. Therefore, each ought to contain one interaction word to be considered for annotation.

2.5 Annotation categories

Main annotations are used to ensure the presence and absence of an interaction between genes and a drug. It can be direct and indirect and explicit and inferred. Three categories of data about interactions were used in this analysis: direct explicit, indirect explicit, and indirect inferred. Only direct and explicit interactions were taken forward for further analysis. Besides interactions, several annotation subcategories were used if they were found in sentences, such as; amplified interaction, decreased interaction, hypothetical interaction, speculation, and negation. Contrary to decreased interaction, negation means the absence of interaction and is commonly stated as "not," "never," and other negative words in the sentence. [see examples in Table 2]

2.6. Developing an evidence Scoring Algorithm

Evidence scoring algorithm was developed based on pharmacogenomics relatedness, frequency, and consistency of evidence for co-occurring gene- pioglitazone pairs. Each gene was assigned a score depending on the strength of evidence of that gene's interaction with pioglitazone (DiStefano and Watanabe 2010). Henceforth, each gene was given a score from 1 to 7 (1 representing the strongest evidence of showing the effect to 7, the weakest evidence of showing no effect on drug response). The word "consistency" explains the effect of the gene on drugs or drugs on the gene and is found more often than the opposite effect. That says if 1000 sources of evidence show that the effect of the gene increases the clinical outcome of the drug and 500 sources show no effect, then based on consistency, the

Table 1 (b): Different Genes and their aliases used to construct gene dictionary

Genes	Aliases
ABCA1	ABC-1;ABC1;CERP;HDLDT1;TGD
ACE2	-
ACKP3	CMKOR1; CXC-R7; CXCR-7; CXCR7; GPR159; RDC-1;RDC1
ADIPOQ	ACDC; ACRP30; ADIPQTL1; ADPN; APM-1; APM1;GBP28
adipoq	30kDa; APN; Acd; Acrp30; Ad; GBP28; adipo; apM1
ADIPOR1	ACDCR1; CGI-45; CGI45;PAQR1;TESBP1A
adipor1	2810031L11Rik; B30ACDCR1; CGI-45; Paqr1
AGER	RAGE; SCARJ1
Ahsg	Aa2-066; pp63
Akt1	Akt; LTR-akt; PKB; PKB/Akt; PKBalpha; Rac
Akt1	Akt
Alox5	LOX5A
Apoe	AI255918; Apo-E
App	Abeta
CCND1	BDPLT10; CHDS7; FAT; GP3B; GP4; GPIV; PASIV;SCARB3
CD36	BDPLT10; CHDS7; FAT; GP3B; GP4; GPIV; PASIV;SCARB3
Cdk5	AW048668; Crk6
CDKN1A	CAP20; CDKN1; CIP1; MDA-6; P21; SDI1; WAF1; p21CIP1
CDKN1B	CDKN4; KIP1; MEN1B; MEN4; P27KIP1
CFD	ADIPSIN; AND; DF; PFD
CIDEC	CIDE-3; CIDE3; FPLD5; FSP27
CISD1	C10orf70; MDS029; ZCD1; mitoNEET
Col3a1	-
Ctgf	CTGRP
CYP2C8	CPC8;CYPIIC8; MP-12/MP-20
Dpysl2	AI851130; Crmp2; DRP2; Musunc33; TOAD-64; Ulip2

EDN1	ARCND3; ET1; HDLCQ7; PPET1; QME
Gsk3b	-
GYS1	GSY; GYS
Hif1a	MOP1
Igf1	IGF
IGF2BP2	IMP-2; IMP2; VICKZ2
IGFBP3	BP-53; IBP3
Igfbp5	IGF-BP5
Il12a	IL-12p35; Il-12a; Ll12a; p35
Il6	ILg6; Ifnb2
JUN	AP-1; AP1; c-Jun; p39
Klf4	GKLF
LANCL2	GPR69B; TASP
Mapk14	CRK1; CSBP; CSPB1; Csbp1; Csbp2; Exip; Hog; Mxi2; Prkm14; Prkm15; RK; Sapk2A; p38; p38Hog; p38alpha
NCEH1	AADACL1; NCEH
Nfkb1	NF-KB1; NF-kappaB; NF-kappaB1; p105; p50; p50/p105
Nkx2-1	AV026640; Nkx2.1; T/EBP; Titf1; Ttf-1
Nkx2-1	TTF1; Titf1
Nos2	MAC-NOS; NOS-II; Nos-2a; i-NOS; iNOS; Nos2
Nos2	Nos2a; iNos
Nos3	2310065A03Rik; Nos-3; eNOS; ecNOS
NOS3	eNOS
Pemt	PEAMT; PEMT2; PLMT; Pempt; Pempt2
Perkcg	-
Pkd1	PC1; mFLJ00285
PPARG	CIMT1; GLM1; NR1C31; PPARG2; PPARgamma; PPARG
Pparg	Nr1c3; PPAR-gamma; PPAR-gamma2; PPARgamma;PPARgamma2
Pparg	-

PPARG	NR1C3
PPARGC1A	LEM6; PGC-1(alpha); PGC-1alpha; PGC-1v; PGC1; PGC1A; PPARGC1
Ppargc1a	A830037N07Rik; Gm11133; PGC-1; PPARGC-1-alpha; Pgc- 1alpha; Pgc1; Pgco1; Ppargc1
Prkcg	PKC; PKCI; Prkc; Prkcc; RATPKCI
Prkcq	Pkcq
Ptgs2	COX-2; Cox2
PTPRD	HPTP; HPTPD; HPTPDELTA; PTPD; RPTPDELTA
RBP4	MCOPCB10; RDCCAS
REST	GINGF5; HGF5; NRSF; WT6; XBR
Serpine1	PAI-1; PAI1; Planh1
Sgk1	Sgk
SLC1A2	EAAT2; EIEE41; GLT-1; HBGT
SLC2A5	GLUT-5; GLUT5
Socs3	Cis3; Cish3; EF-10; Ef10; SSI-3; Ssi3
Srebf1	ADD1; SREBP1; bHLHd1
TERT	CMM9; DKCA2; DKCB4; EST2; PFBMFT1; TCS1; TP2; TRT; hEST2; hTRT

evidence that outweighs it is considered.
Studies were categorized into three groups: Type I describes the highest level of Pharmacogenomics (PGx) evidence, followed by type II and type III with decreased and weakest evidence. [see Table 3]

3. Results

3.1. Data Retrieval and Extraction

We identified 22,532 articles using pioglitazone and its related terms; after removing duplicates, 12,164 articles proceeded for further evaluation. Further, by removing irrelevant articles, the analysis was done on 7,043 articles, from which 5,450 were downloaded as full-text PDFs and while the remaining 1,085 were downloaded as abstracts and were directly exported to a single notepad file from Endnote. Those journals which contributed more than 20 articles were taken for further analysis, so 54 journals contributing to full-text articles and 21 journals contributing to abstracts were considered [**Figure: 2**]. Sentences were extracted from the corpus in python NLTK using our generic code, a total number of 1927 sentences were extracted, and after removing irrelevant sentences, 680 and 163 sentences from full-text articles and abstracts were removed and evaluated for pioglitazone-gene interaction. These sentences were then annotated and assigned an evidence score according to the annotation and scoring system. Twenty-three genes were identified that directly or indirectly interact with pioglitazone

Table 2: Some examples of sentences from corpus with their annotations

Annotation	Example Sentences	Impact
DE	Pioglitazone improves glycemic control in people with Type 2 diabetes by improving insulin sensitivity through its action at PPAR gamma 1 and PPAR gamma 2.	CR
DE	To test the hypothesis that genetic variation in PPARG associates with variability in pioglitazone response, we conducted a meta-analysis to synthesize the currently available data on the PPARG (p.Pro12Ala) polymorphism.	PD
DE	Pioglitazone inhibits androgen production in NCI-H295R cells by regulating gene expression of CYP17 and HSD3B2.	GE
IE	The antidiabetic agent pioglitazone increases expression of glucose transporters in 3T3-F442A cells by increasing messenger ribonucleic acid transcript stability.	GE
A	Retn C-420G (rs 1862513) polymorphism is linked with a stronger glucose-lowering effect of pioglitazone	PD

CR: Clinical Relevance, **PD:** Pharmacodynamics, **GE:** Gene Expression, **DE:** Direct, Explicit, **ID:** Indirect Explicit, **A:** Affirmative

response from identified articles.

3.2. *Genes related to clinical outcomes as a consequence of pioglitazone treatment*

We have identified a total of nine genes that showed strong evidence to alter the clinical outcomes of pioglitazone in patients with T2DM. These genes were found to be clinically relevant and assigned evidence score '1'. These included *PPARG, PTPRD, ADIPOQ, ADIPOR2,* and *PTPRD* genes were strongly associated with clinical outcomes of pioglitazone with the highest number of evidence. PPAR-γ encoding gene *PPARG* is a target gene for pioglitazone and was found to affect response with 82 evidence of affirmative while 56 evidence favors decreased interaction. *PTPRD* gene was found in sentence corpus with 21 pieces of evidence, of which 17 were affirmative, and two decreased interactions. The functionality of protein tyrosine phosphatase receptor type D (*PTPRD*) gene polymorphism has not yet been cleared. Considering that it is located in an intron, the polymorphism may affect mRNA splicing or may be in linkage disequilibrium with other functional polymorphisms. Contrary to *PPARG* and *PTPRD* genes, the *ADIPOQ gene* encodes important adipokine involved in controlling fat metabolism and insulin sensitivity, with direct anti-diabetic, anti-atherogenic and anti-inflammatory activities. It also stimulates AMPK phosphorylation and activation in the liver and the skeletal muscle, enhancing glucose utilization and fatty-acid combustion.

Adiponectin receptor 2 (*ADIPOR2*) is another gene studied along with *ADIPOQ* polymorphism to explain Pioglitazone response variability.

Table 3: Evidence code assignments based on strength of evidence for gene-drug interaction

Evidence Score	Evidence Code Definition		Assessment of outcomes
	Study Category	Study Objectives/Findings	
1	Clinical outcome studies	* Consistent effect of genetic variant on drug of interest	Clinically relevant
2	PK or PD Study	* Consistent effect of genetic variant on drug of interest	Clinically relevant
3	Molecular or Cellular functional studies	* Consistent effect of genetic variant on drug of interest	Potential clinical relevance
4	Clinical outcome studies	Inconsistent effect on drug of interest	Clinical relevance unknown
5	PK or PD Study	Inconsistent effect on drug of interest	Clinical relevance unknown
6	Molecular or cellular functional studies	Inconsistent effect on drug of interest	Clinical relevance unknown
7	Clinical outcome studies, PK or PD study	Demonstrates no effect of the genetic variant on drug response	Clinical relevance unsupported

The *ADIPOR2* gene is highly expressed at critical sites involved in glucose metabolism, and activation of this receptor is known to increase fatty acid oxidation and adiponectin-mediated glucose utilization. Further investigations are needed to understand the involvement of the *ADIPOR2 gene* in the Pioglitazone response. Though, if any polymorphism in *ADIPOR2* can affect the functionality of the receptor, it may still be possible that the polymorphism could influence response to PIO therapy.

Methylene-tetrahydrofolate reductase (MTHFR) and the LPL gene favor the cardioprotective action of pioglitazone. RETN gene also showed indirect, explicit interaction when it comes to clinical relevancy. Similarly, IGF2BP2 genes have affirmative interaction with pioglitazone by improving its response. These genes interact with pioglitazone either on its binding site or in the mechanism pathway. [see Table 4]

3.3. Genes affecting Pharmacokinetics/ Pharmacodynamics of Pioglitazone

Genes affecting bioavailability, clearance, and metabolism of pioglitazone were also identified, and as these genes did not consistently affect clinical outcomes of the drug, they were assigned an evidence score of '2'. Cytochrome P450 family members CYP2C8 (Cytochrome P450 family 2 subfamily C member 8), CYP3A4 (Cytochrome P450 family subfamily A member 4), CYP2C9 were found to have interaction in pioglitazone metabolism. Besides this, 16 other genes (PPARG, PTPRD, ADINOPOQ, ADINOPOR1, ADINOPOR2, LPL, AQP2, APOH, MTHFR, ACE, SLC12A1, PKM2, RETN, TNFA, IL6, and VCP2) were associated with the pharmacodynamics of the drug with different frequencies. [see Table 5]

Table 4: Shows the list of identified genes affecting clinical outcomes of pioglitazone.

Genes with Annotation and Scoring					
Gene	**Annotation**	**Sub Annotation**	**Impact**	**Outcome**	**Evidence score**
PPARG	DE	A	CR	C	1
PTPRD	IE	DI	CR	C	1
LPL	IE	A	CR	C	1
RETN	IE	A	CR	C	1
IGF2BP2	IE	HI	CR	C	1
MTHFR	DE	A	CR	C	1
ACE	DE	A	CR	C	1
ADIPOR2	DE	A	CR	C	1
ADIPOQ	DE	A	CR	C	1

3.4. Genes with potential clinical relevance

Pioglitazone may affect the molecular and cellular functions of those genes that encode proteins, and these genes were assigned a score 'of 3' because they may have clinical value. These genes include Glucose transporter genes GLUT1 and GLUT4, Cytochrome P450 member *CYP11B*1, *SERCA* (Sarco-endoplasmic reticulum Ca2+-ATPase) gene, *LPL gene*, *AT1R*, *TXS, TXR, Cd36, ACKR3, REST*, *ABCA1,* and *PPARGC1* gene. [see Table 6]

4. Discussion

Although there are a number of publications relating to the PK/PD and clinical outcomes of pioglitazone, no database concisely summarizes the mechanisms describing gene-drug interactions. Most of the time, specific evidence of interactions is found deep in the literature and hard to conclude. In this paper, we emphasize interactions between pioglitazone and genes that impact the drug's clinical outcomes, PK, and/or PD using a comprehensive semi-automated text-mining strategy.

Despite being one of the most commonly used drugs in T2D; PIO has shown variations in effectiveness and adverse effects. The mode of action, kinetics, and risk outline of pioglitazone create a long list of genetic targets that may contribute to different drug responses and risks.

Table 5: shows the clinically relevant genes of PK/PD of the drug with their evidence scores.

Genes	Annotation	Sub-Annotation	Impact	Effect	Score
PTPRD	IE	A	CR	PD	2
ADIPOR2	E	DI	CR	PD	2
AQP2	DE	dI	CR	PK/PD	2
SLC12A1	DE	dI	CR	PK/PD	2
APOH	IE	Spec	CR	PK/PD	2
ADORA1	IE	Spec	CR	PK/PD	2
PKM2	IE	Spec	CR	PKPD	2
NPY	IE	Spec	CR	PK/PD	2
GYSI	HI	Spec	CR	PK/PD	2
CCL2	HI	Spec	CR	PK/PD	2
GHRH	HI	Spec	CR	PK/PD	2

Variability in response may be assessed as a response, less response, or no response. Studies that have made direct assessments have shown a 30-40% lack of response among individuals taking pioglitazone as monotherapy (Auwerx 1999). Understanding the link between genetic variation and the glucose-lowering response of drugs may help improve efficacy and lower adverse effects, disease burden, and cost of treatment.

In this study, a total number of 23 genes were prioritized that were found to interact directly or indirectly with pioglitazone response. Seven genes PPARG, PTPRD, ADIPOQ, ADIPOR2, LPL, RETN, IGF2BP2, MTHFR, and ACE genes were found to be associated with the clinical outcomes of pioglitazone. PPAR-γ activation leads to an insulin signaling cascade by inducing several genes, such as GLUT4 and CAP, thereby, insulin sensitivity in patients with T2DM is improved (Namvaran et al. 2011). Genetic polymorphism in PPARG that encodes PPAR-γ2 plays an important role in variability in pioglitazone response. A missense polymorphism in PPARG (Pro12Al) is associated with variable pioglitazone-induced antihyperglycemic effect (Li et al. 2008; Ruscica, Steffani, and Magni 2012). The PTRD SNP rs17584499 of Protein tyrosine phosphate receptor type D is found to affect PD of the pioglitazone in the Chinese population, and a potent drug response alteration is noticed with ADIPOR2 and ADIPOQ variants (Groenemeijer et al. 1997).

Table 6: This table shows clinically relevant genes of pioglitazone with frequency and evidence score.

Gene	A	DI	Freq.
PPARG	56	82	138
CYP2C8	50	6	56
PTPRD	17	4	21
TNFA	10	2	12
IL6	8	2	10
SLC12A1	0	4	4
IGF2BP2	2	1	3
MTHFR	2	0	2
ACE	2	0	2
RETN	2	0	2
ADIPOR2	0	2	2
AQP2	0	1	1
APOH	1	0	1
ADORA1	1	0	1
PKM2	1	0	1
GYSI	1	0	1
CCL2	1	0	1
GHRH	1	0	1

ADIPOQ (C-11377G) genotype and ADIPOR2 (G795A) genotype substantially affects the pioglitazone-induced reduction in glycated hemoglobin HbA1C in the Chinese and Iranian population of T2DM (Wang et al. 2007; Eckland and Danhof 2000). LPL (Leptin) gene encodes hepatic lipase and the breakdown of triglycerides. Variation in the LPL gene attributed to the influence effect of pioglitazone on macrovascular complications in T2DM (Wang et al. 2007). LPL (S447X) genotype favors the effects of pioglitazone by enhancing the synthesis and secretion of LPL and is shown to decrease the risk of cardiovascular complications compared to the LPL S447S genotype (Scheen 2007). CYP2C8 and CYP3A4 genes encoding CYP2C8 and CYP3A4 enzyme of the Cytochrome P450 enzyme family, respectively, were found to be related to the PK of pioglitazone. Pioglitazone is metabolized 60% by CYP2C8 and less than 20% by CYP3A4 (Tornio et al. 2008). Two non-synonymous polymorphisms of CYP2C8 *3 at rs11572080: G>A and rs10509681: A>G have been found to alter the oral clearance of pioglitazone (Chang et al. 2011; Wei et al. 2010).

In the kidney, the AQP2 gene encodes aquaporin, which works as a water channel. Polymorphisms in the gene AQP2 (rs296766) alter this activity and lead to pioglitazone-induced edema. In addition, SLC12A1 rs12904216 polymorphism also takes part in

pioglitazone-induced edema (Sandouk, Reda, and Hofmann 1993; Chang et al. 2011).

Administration of pioglitazone inhibits the expression of TNFA and IL6 genes by activation of PPAR-γ receptors (Cariou, Charbonnel, and Staels 2012). TNFA and IL6 genes encode tumor necrosis factor TNFα and interleukin-6, which are important in differentiating monocyte into osteoclasts, and their reduction leads to osteoclastogenesis inhibition (Kapoor, Tan-Koi, and Teo 2016) increasing the risk of fracture in patients taking pioglitazone (Kahn et al. 2006). Unlike this, the expression of glucose transporter genes GLUT1 and GLUT4 is increased by pioglitazone.

The pharmacogenetic studies of drug response help to predict optimal drug dosage, identify patients' risk of drug-induced toxicity or adverse side effects and generate considerable cost savings for the health systems. Genetic profiles of individual patients can be used to identify patients who will respond to medication. This forms the cornerstone for personalizing the treatment of diseases such as cancers, metabolic disorders, and infectious diseases. Likewise, recent studies have uncovered numerous genetic linkages with drug-induced toxicity and/or side effects, where knowledge of the individual genetic profile can indicate the likelihood of an undesirable outcome and direct the practitioner towards alternative medications if necessary. Moreover, assigning all the patients with expensive alternatives to acquire unnecessary expenses to the healthcare system while the freehand use of the cheaper alternatives can result in a rise in morbidity rate and a conforming loss in quality of life due to the side effects. Understanding the individual genetic profiles of the patients means costlier alternatives can be assigned to only those who cannot tolerate the cheaper medications, which can produce significant cost savings for the healthcare system.

The literature contains numerous GWAS that reveals the fundamental genetics of an individual. Based on the strength of genomic data, genes associated with pharmacokinetics and pharmacodynamics of the drug will surely provide novel and useful information interactions which will help to treat each person accordingly to take a step forward towards personalization of treatment. However, validation in large studies in different populations and careful interpretation of pharmacogenetics and pharmacogenomics information are necessary before it could apply to patients. Still, missing genotypes, genetic heterogeneity, small effect size, low allele frequency, or complex genetic architectures remain a challenge. Moreover, SNP-level annotation and studying the pathways underlying genes encoding proteins and population-specific variability can help further assess drug response.

5. Conclusion

To overcome the inconsistency in the genetic association studies of drug response, we described a semi-automated test mining and evidence-scoring algorithm that could help extract and bring together useful information on pioglitazone interaction with genes from the bulk of available scientific literature. We have prioritized and annotated genes encoding PPAR-α receptors. PPARG, showed the strongest evidence of mediating pioglitazone response. Additionally, genes affecting the pharmacokinetics of pioglitazone, including the CYP2C9 gene, showed significant pharmacokinetic variability. The proof of genes affecting the pharmacodynamics of pioglitazone existed for PPTRD, LPL, Retn, Adiponectin, ADINOPORA2, and PPARGC1, whereas MTHFR and ACE genes contributed to the cardioprotective response of the drug. Furthermore, drug-induced adverse drug reactions were associated with polymorphisms in SCL12A1 and AQP2 genes. Moreover, before considering them in clinical and public health policies, we warrant validating and replicating

these candidate genes in high-powered and multi-ethnic cohorts.

Conflict of Interest

The authors declare that they have no competing interests.

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Study Approval

NA

Consent Forms

NA.

Authors Contribution

NS conceptualized the study, A, AJS, and MIA helped in the literature review and analysis, ZU and MI helped write the first draft, A did the experimental analysis, and NS supervised the whole project and wrote the final manuscript.

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