



Review

Pharmacometabolomics-aided Pharmacogenomics in Autoimmune Disease



Theodora Katsila*, Evangelia Konstantinou, Ioanna Lavda, Harilaos Malakis, Ioanna Papantoni, Lamprini Skondra, George P. Patrinos

University of Patras, School of Health Sciences, Department of Pharmacy, University Campus, Rion, Patras, Greece

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ABSTRACT

Inter-individual variability has been a major hurdle to optimize disease management. Precision medicine holds promise for improving health and healthcare via tailor-made therapeutic strategies. Herein, we outline the paradigm of “pharmacometabolomics-aided pharmacogenomics” in autoimmune diseases. We envisage merging pharmacometabolomic and pharmacogenomic data (to address the interplay of genomic and environmental influences) with information technologies to facilitate data analysis as well as sense- and decision-making on the basis of synergy between artificial and human intelligence. Humans can detect patterns, which computer algorithms may fail to do so, whereas data-intensive and cognitively complex settings and processes limit human ability. We propose that better-informed, rapid and cost-effective omics studies need the implementation of holistic and multidisciplinary approaches.

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1. Introduction

Tailor-made therapeutics and cost-effective disease management will only become possible when inter-individual variability is addressed. Indeed, the interplay of genomic and environmental influences (diet, lifestyle, polypharmacy, toxins, gut microbiome) forms a data-intensive and cognitively complex context that offers great intervention opportunities, but also presents challenges. In the current era of big

data, a major challenge is that of merging several high throughput datasets coming from various platforms and tools to generate and/or test hypotheses with regard to disease heterogeneity or mechanisms for variation in drug response.

Herein, we outline the paradigm of “pharmacometabolomics-aided pharmacogenomics” in autoimmune disease. We envisage addressing the interplay of genes and the environment by merging pharmacometabolomic and pharmacogenomic data with information technologies. The latter will not only facilitate data analysis, but also sense- and decision-making via the synergy between artificial and human intelligence. We feel that this synergy is of fundamental importance, if rapid and efficient data processing is anticipated. Notably,

* Corresponding author at: University of Patras, School of Health Sciences, Department of Pharmacy, University Campus, Rion, GR-26504 Patras, Greece.
E-mail address: thkatsila@upatras.gr (T. Katsila).

humans can detect patterns, which computer algorithms may fail to do so (Agrawal et al., 2012). On the other hand, data-intensive and cognitively complex processes limit human ability. We propose that better-informed, rapid and cost-effective omics studies need the implementation of holistic and multidisciplinary approaches.

2. Pharmacogenomics in Precision Medicine

Pharmacogenomics, the prediction of the outcome of a drug or xenobiotic intervention in an individual based on an analysis of that individual's genetic profile (Everett et al., 2013), is an integral part of precision medicine (personalized evidence-based medicine that addresses patient-to-patient variability) that explores how a person's genome relates to drug efficacy/toxicity as well as disease phenotype. The ultimate goal of pharmacogenomics is an efficacious patient-tailored therapeutic strategy that minimizes toxicity and ensures treatment efficacy taking into account the genetic basis of inter-individual variability following drug administration (Roden and George Jr, 2002; Ma and Lu, 2011) (Table 1). Today, FDA-approved drug labeling may contain information on genomic biomarkers that relate to (i) drug exposure and clinical response patient-to-patient variability, (ii) risk for adverse reactions, (iii) genotype-specific dosing, (iv) mechanisms of drug action as well as (v) polymorphic drug target and disposition genes.

In 2009, one of the first pharmacogenomics successes took place. All the protein-coding DNA of a very ill 4-year old boy was sequenced and data were used to determine a causative gene mutation of his life-threatening gut inflammation, leading to an ultimately effective treatment (Katsnelson, 2013). Since then, the prospect of sequencing whole exomes or genomes for less than \$1000 has reshaped our thinking about genetic testing approaches (Hayden, 2014). Recently, Mizzi et al. (2014) analyzed whole-genome sequences of 482 unrelated individuals of various ethnic backgrounds to obtain their personalized pharmacogenomics profiles. In a continuous effort to define genotype-to-phenotype associations, pharmacogenomics research has focused on large-scale analyses (Genome Wide Association studies). At the same time single nucleotide polymorphism (SNP) studies aim to characterize functional differences in similar gene outputs (Monte et al., 2012).

Today, recent technological advances have dramatically improved the prospect of applying broadly the concept of precision medicine. Large-scale databases, omics technologies, cellular assays, epigenetics, informatics as well as imaging technologies converge towards optimum disease prevention, diagnosis and treatment. Hence, disease classification is refined, often accompanied by diagnostic, prognostic and treatment implications (Collins and Varmus, 2015). Successful examples include the FDA-approved use of chip technologies for the detection of variations in patients' *CYP2D6*, *CYP2C19* and *UGT* genes that are of fundamental importance in drug metabolism (Swen et al., 2007). Notwithstanding, clinical pharmacogenomics depends on validated actionable genomic data that will inform diagnosis, prognosis or treatment (Lander, 2015). We feel that forthcoming trials are needed to demonstrate pharmacodynamic associations with genomic variants (Caraco

et al., 2008; Mega et al., 2011). Notably, the currently available screening tests are characterized by unacceptable positive and negative predictive values (less than a 50% success rate in terms of safety and efficacy prediction), implying that inter-individual variability is not exclusively shaped by genomics. Indeed, combining screening for genomic variants in the *CYP2C19* and *VKORC1* genes – the most effective pharmacogenomics screen to date – resulted in a mere 41% prediction of the variability in warfarin doses (Namazi et al., 2010).

3. Pharmacometabolomics in Precision Medicine

Pharmacogenomics does not consider environmental influences on drug pharmacokinetics (absorption, distribution, metabolism, excretion) and/or pharmacodynamics – neither the role of gut microbiome (Walter and Ley, 2011; Gurwitz, 2013; Li and Jia, 2013). More recently, in an alternative, but yet complementary discipline, pharmacometabolomics aims to predict and/or evaluate drug metabolism (Everett, 2015; Nicholson et al., 2011).

Pharmacometabolomics is the later term used synonymously with pharmacometabonomics, the prediction of the outcome of a drug or xenobiotic intervention in an individual based on a mathematical model of preintervention metabolite signatures (Clayton et al., 2006). Historically, Jeremy Everett and Jeremy Nicholson first defined metabonomics in 1999, during the course of collaboration between Pfizer R&D, UK and Imperial College London (Nicholson et al., 1999). Pharmacometabonomics was established in 2000 as a result of inconsistent findings among subgroups of animal models. The hypothesis of Clayton et al. was that post-dose drug metabolism and safety were related to pre-dose metabolic profile differences (Clayton et al., 2006).

Pharmacometabolomics is based on metabolic phenotypes (metabotypes), which are considered as the net result of genetic, physiological, chemical, and environmental influences (Holmes et al., 2008; Everett et al., 2013). Metabolic profiles refer to a huge list of chemical entities, both endogenous and exogenous, such as peptides, amino acids, nucleic acids, carbohydrates, fatty acids, organic acids, vitamins, hormones, drugs, food additives, phytochemicals, and toxins (Wishart et al., 2007). Surprisingly enough, even though the overall number of endogenous metabolites has been reported to be extremely high (~100,000), the major metabolites relevant for clinical diagnostics and/or drug development have been estimated at 1400–3000 molecules (Xu et al., 2009). What is also rather interesting to note is that metabolites are not just end- or by-products, as they can regulate gene expression and/or affect cell biology. Betaine, for example, is an osmolyte (protects from environmental stress) and methyl donor (participates in methionine cycle in human liver and kidneys) (Craig, 2004; Friesen et al., 2007), while it is a positive regulator of mitochondrial respiration and cytochrome c oxidase activity (Lee, 2015).

In the presence or absence of chemometrics, a pre-dose metabotype can assist modeling and prediction of inter-individual drug responses (Nicholson et al., 2011). Similarly, Kaddurah-Daouk et al. (2007) investigated the lipid profiles of 50 patients with schizophrenia, before and after olanzapine-, risperidone- and aripiprazole-treatment. Pre- and

Table 1
The genetic basis of inter-individual variability in drug response.

Genetics	Effect	Phenotype (active drug)	Phenotype (pro-drug)
Poor metabolizer	Metabolic rate: slower than normal	High toxicity risk	Lack of efficacy
Extensive metabolizer	Metabolic rate: normal	Expected drug effect	
Rapid metabolizer	Metabolic rate: faster than normal	Lack of efficacy	High toxicity risk
Therapeutic targets	Drug efficacy (pharmacodynamics)		High toxicity risk
Drug transporters	Drug disposition (pharmacokinetics)		High toxicity risk
Modulators (drug action)	Metabolic rate: faster than normal		Reduced efficacy

post-treatment profiles were compared resulting in the identification of baseline lipid alterations that correlated with acute treatment response (Kaddurah-Daouk et al., 2007). Clayton et al. (2009) demonstrated a clear connection between a pre-dose urinary metabolite profile of an individual, and the metabolic fate of a standard dose of acetaminophen. It was reported that in individuals with high bacterially mediated p-cresol generation, competitive O-sulfonation of p-cresol reduces the effective systemic capacity to sulfonate acetaminophen, implying that the effects of microbiome activity should be an integral part of pharmaceutical development and of personalized healthcare (Clayton et al., 2009). Another study has shown that pre-treatment metabolotypes could be predictive of sertraline response (acute treatment) in patients with major depressive disorder (Kaddurah-Daouk et al., 2011). This is the principle of “metabotype-based pharmacokinetics/pharmacodynamics”. Everett (2015) provides a comprehensive overview.

Metabotype data coupled to metagenomic measurements (information on gut microbiota) can enhance our knowledge about the complex interactions between the host and its gut microbiota (Kaddurah-Daouk et al., 2014) as well as their role in modulating host physiology, gut microbiome-related disorders, and metabolism of xenobiotics (Li and Jia, 2013). Even though a “core microbiome” has been proposed among different individuals and family members (Qin et al., 2010; Rajilić-Stojanović et al., 2007), the entire composition of the gut microbiota is highly variable in humans and associated with a variety of diseases (obesity, inflammatory bowel disease, diabetes, nonalcoholic fatty-liver disease, Crohn’s disease, and colorectal cancer) (Li and Jia, 2013). Remarkably, O’keefe et al. (2015) demonstrated that changes in the food content of fiber and fat affected profoundly the colonic microbiota as well as the metabolome of individuals from high- and low-risk cancer populations (within 2 weeks) (O’keefe et al., 2015). Additionally, metabolic networks have been analyzed to shed light on correlations between metabolites (considering even gut microbiota) and disease. Such an approach was employed for the first time during the urinary metabolomics profiling of Italian autistic pediatric patients and their healthy siblings (Noto et al., 2014). The analysis of such metabolic networks, which are indicated as nodes and edges, is crucial for the identification of the main routes connecting the entities of interest within the metabolic pathways in question. For instance, MetaMapR (<http://dgrapov.github.io/MetaMapR/>) generates richly connected metabolic networks via the integration of enzymatic transformations with metabolite structural similarity, mass spectral similarity and empirical associations. Overall, such approaches are defined as “metabotype-based subtyping”.

Especially in neonatology and pediatrics, there is a great potential for pharmacometabolomics studies to rationalize therapeutic use in infants and children. Drug pharmacokinetics differs substantially from those in adults and so far, a dosage approximation becomes necessary, as many drugs are not specifically approved for pediatric use. Today, very few – if any – pharmacometabolomics studies have been conducted in pediatric patients. Relevant reviews on the matter have been recently published (Mussap et al., 2013; Katsila and Patrinos, 2015).

Unfortunately, both “metabotype-based pharmacokinetics/pharmacodynamics” and “metabotype-based subtyping” approaches are currently of little utility to the practicing clinician, as reproducibility issues need to be overcome (Simó et al., 2011). Furthermore, it has been shown that even small changes in physiology can have significant impact on the metabotype (Johnson and Gonzalez, 2012). Only if clinicians focus on larger stable metabolic signals, transient metabolic associations that do not represent causation could be diminished. Polypharmacy is rather challenging, too (Nicholson et al., 2012).

4. Pharmacometabolomics-aided Pharmacogenomics

The idea of the constructive coupling of omics technologies is not new. Pharmacogenomics and pharmacometabolomics complement each other and thus, reinforce the identification of clinically relevant

associations. Instead of traditional tag SNP genotyping, genotype imputation could determine genomic variants of interest in pathways identified during pharmacometabolomics studies (Abo et al., 2012; Suhre et al., 2011). This strategy accelerates and broadens the scope of the analysis of pharmacogenomic candidate genes. Similarly, a wider survey becomes possible, reducing the need of genotyping prior to replication. In this context, Ji et al. (2011) explored citalopram/escitalopram treatment biomarkers, following a metabolomics analysis in plasma, according to which (i) glycine was reported to be negatively associated with treatment outcome leading to tag SNP genotyping for genes encoding glycine synthesis and (ii) rs10975641 (*GLDC*) was defined as a response biomarker in major depressive disorder patients.

When untargeted analysis is considered, pharmacometabolomics also serves as a tool to shape hypothesis, as multiple analytes are quantified simultaneously and pharmacometabolomic modeling appears not to be limited by prior understanding or hypotheses. Hence, pharmacometabolomic modeling can be a powerful hypothesis-generating scenario. Of course, an adequate monitoring of the analytical quality is always mandatory, if a cluster of metabolites associates to a well-defined physiopathological condition. In the context of a pharmacometabolomics-aided pharmacogenomics strategy, the breakthrough of metabotype-based findings is patient or therapy profiling that addresses genomic and environmental influences in a rather pathway-targeted way, even if the mechanism in question (disease, drug efficacy/toxicity) is not completely known. Konstanyowicz et al. (2012) reported that children with autistic spectrum disorders (ASD) demonstrated 3-fold greater plasma oxalate levels as well as 2.5-fold greater urinary oxalate concentrations compared with healthy individuals. As the authors commented “whether hyperoxalemia and hyperoxaluria may be involved in the pathogenesis of ASD in children or this is the outcome of an impaired renal excretion or an extensive intestinal absorption, or both, or whether oxalate crosses the blood brain barrier and disturbs CNS function in the autistic children remains unclear” (Konstanyowicz et al., 2012). Taking into account that the *SLC26* gene family encodes anion exchangers and channels transporting a broad range of substrates, including oxalate (Alper and Sharma, 2013), genetics is expected to play a role. In any case, a low oxalate diet has resulted in the ease of ASD symptoms (improvements in expressive speech, reduced obsessive behavior) (Konstanyowicz et al., 2012), allowing disease handling on the basis of metabolotypes. Pre-dose metabolotypes can be also predictive of a post-dose phenotype (drug toxicity or efficacy), addressing environmental influences as well as the role of gut microbiome. This is the exact information that pharmacogenomics fails to achieve patient-tailored treatment. An overview of the human pharmacometabolomics, metabolomics, metabolomics, metagenomics and pharmacometabolomics-aided pharmacogenomics studies reported in the literature to late 2015 is depicted in Supplementary Table 1.

What we envisage is merging omics and information technologies beyond data mining and analysis. Instead, we propose the synergy between artificial and human intelligence to (i) acquire pharmacometabolomic and pharmacogenomic data and thus, address the interplay of genomic and environmental influences, (ii) facilitate collaborative data analysis and (iii) guide sense- and decision-making towards rapid and efficient data output. A “one-stop-shop” crowd-sourced, cloud- or web-based platform (a standard around which a system can be developed) where the informatics community and/or biomedicine scientists could explore and validate such an approach could pave the way for better-informed and cost-effective studies. Omics data demand strict filtering as well as thorough analysis and interpretation. At the same time, biomedicine scientists need to efficiently and effectively collaborate and make decisions. For this, large-scale volumes of complex multi-faceted data need to be meaningfully assembled, mined and analyzed. Tsiliki et al. (2014) presented an innovative web-based collaboration support platform that adopts a hybrid approach on the basis of the synergy between artificial and human intelligence.

5. The Paradigm of Autoimmune Disease

Autoimmune disease results from the incapacity of the immune system to discern endogenous substances from xenobiotics and affects 5% of the population in Western countries (Sinha et al., 1990). The etiology of autoimmune disease is currently unclear, perplexing differential diagnosis, patient stratification and decision-making in the clinic. Although a genetic component has been described, disease occurrence has been also associated with several environmental factors, gut microbiota, infections as well as gender bias (Shoenfeld and Isenberg, 1989). Overall, disease management options are limited and ultimately fail to protect patients from disease symptoms due to its chronic nature.

Considering the role of the environment and gut microbiome in the pathobiology of the disease (Li and Jia, 2013; Wilson, 2009), a “pharmacometabolomics-aided pharmacogenomics” strategy could be of great benefit, as pharmacogenomics alone fails to address environmental influences. Taking into account host–microbiome interactions as well as the implications of gut microbiota for nutrition, data-intensive and cognitively complex settings and processes that limit human ability are anticipated. Can we delineate inter-individual variability towards differential diagnosis? Can we highlight the disease mechanisms in question to assist disease management? We consider immune disease as a model that presents multiple challenges, which could only be met by a multidisciplinary strategy built on the synergy of artificial and human intelligence. Some potential case scenarios are presented below.

Celiac disease is a complex chronic immune-mediated disorder of the small intestine. Gluten has been identified as the environmental trigger of the disease (Di Sabatino and Corazza, 2009). Today, the presence of *HLA-DQ2* and *HLA-DQ8* coupled to a positive biopsy and serological antibodies upon a gluten-containing diet is used for diagnosis. However, the *HLA-DQ2* and *HLA-DQ8* genes are necessary, but not sufficient for the development of celiac disease. To date, a few studies report differential metabolotypes between healthy individuals and celiac patients. In 2009, a metabolic signature of celiac disease was defined, according to which differential serum levels of glucose and ketonic bodies suggested alterations of energy metabolism, whereas alterations of gut microbiota were also evident following urine data analysis (Bertini et al., 2009). In agreement with our view that the gut microflora of the small bowel is altered in celiac patients or presents peculiar species with their own microbial metabolome, Di Cagno et al. (2011) extensively explored the duodenal and fecal microbiota of celiac children, performing a molecular, phenotype as well as metabolome characterization.

Rheumatoid arthritis is another chronic inflammatory disorder that typically affects the lining of joints, causing a painful swelling that can eventually result in bone erosion and joint deformity (Longo et al., 2015). Anti-tumor necrosis factor (anti-TNF) therapies are highly effective in rheumatoid arthritis. Yet, many patients exhibit only a partial or no therapeutic response. Kapoor et al. (2013) investigated the possibility a pre-dose patient's metabolotype could predict responses to anti-TNF agents. Findings were rather informative. Another metabolomic analysis identified serum biomarkers to evaluate methotrexate treatment in patients with early rheumatoid arthritis (Wang et al., 2012).

Autoimmune hepatitis is one of the most common chronic liver diseases caused by the activation of host's immune system against its own hepatocytes (Hadzic and Hierro, 2014). Notably, wrong diagnosis is a key issue as there are no accurate biomarkers to discriminate autoimmune hepatitis from other diseases that have similar symptoms, such as drug induced liver disease. Moreover, a recent study revealed that nonalcoholic steatohepatitis shares the same autoimmune antibodies with autoimmune hepatitis (Czaja, 2013). In 2014, Wang et al. successfully identified nine metabolites that serve as disease biomarkers for its diagnosis and distinguish between similar or overlapping liver diseases (accuracy of 93%) (Wang et al., 2014).

Systemic lupus erythematosus is a chronic inflammatory disease characterized by multi-system involvement and diverse clinical presentation. Interestingly, the metabolic disturbances that underlie the disease are currently unknown. In a thorough study, Wu et al. (2012) compared the metabolotypes of patients against their healthy counterparts to show that disease metabolome exhibited profound lipid peroxidation, reflective of oxidative damage.

In all cases, although extremely limited, (pharmaco)metabolomics, (pharmaco)metabonomics and (pharmaco)genomics have been applied, suggesting that the omics and information technologies coupling that we propose is feasible.

6. A “Pharmacometabolomics-aided Pharmacogenomics” Workflow

A “pharmacometabolomics-aided pharmacogenomics” workflow includes sample acquisition and preparation, analysis (NMR or mass spectrometry technologies), data processing and data analysis (targeted and non-targeted) (Mussap et al., 2013). For those familiar with (pharmaco)metabolomics approaches, such an outline is not new. What we propose at this point is the use of information technologies for in-depth data mining, analysis, and argumentation.

Tools such as the human metabolome database (<http://www.hmdb.ca>) and/or MetaboAnalyst (www.metaboanalyst.ca) are fundamental for initial data processing and interpretation. An integrative genes/metabolites analysis of confirmed metabolite identifiers will be facilitated by applying MAGENTA (<http://www.broadinstitute.org/mpg/magenta/>) to all curated biological pathways, such as: KEGG (<http://www.genome.jp/kegg/>), GO (<http://www.geneontology.org>), Reactome (<http://www.reactome.org>), Panther (<http://www.pantherdb.org>), Biocarta (<http://www.biocarta.com>) and Ingenuity (<http://www.ingenuity.com/>) databases, allowing statistical filtering and gene set enrichment. If kinome is of interest, ReKINect that has been recently reported and validated could be employed (Creixell et al., 2015). Data visualization and analysis will be further supported by applications and web services, such as BioGRID (Stark et al., 2006), BDNB (Birkland and Yona, 2006), BioMart (Guberman et al., 2011), OncoPrint (Rhodes et al., 2004), GenePattern (<http://www.broadinstitute.org/cancer/software/genepattern/>), PyMOL (<https://www.pymol.org/>), MetaMapR (<http://dgrapov.github.io/MetaMapR/>), the UCSC Genome Browser (Rosenbloom et al., 2013; Karolchik et al., 2009) or even networks (caBIG, <http://cabig.cancer.gov>; BIRN, <http://www.nbirn.net>) and projects (Genotype-Tissue Expression Project) (Lonsdale et al., 2013) that enable sharing of data and resources.

As the next step and on the basis of candidate pathways of interest, untyped SNP genotypes may be imputed with the software package MaCH 1.0 (Li et al., 2010; Biernacka et al., 2009; Nothnagel et al., 2009) to merge candidate pathway data with pharmacogenomics, cost-effectively and possibly, with broader gene coverage than that of routine tag SNP genotyping. Quality control measures will be also employed (MaCH “Rsq”) to define the correlation between imputed and true genotypes (Li et al., 2010). Data validation and replication will occur by routine genotyping (PCR, Sanger sequencing). *In silico* tools, such as those provided by RD-Connect (<http://rd-connect.eu/>), CRAVAT (Douville et al., 2013), SIFT (Sim et al., 2012) and PROVEAN (Choi and Chan, 2015) at the gene level will further assist on data interpretation.

Various statistical approaches will be employed at several steps throughout the proposed workflow (the R project for statistical computing, <https://www.r-project.org/>), including those that are mostly used for (pharmaco)metabolomics and pharmacogenomics studies – principal component analysis (PCA) and partial least square discriminant analysis (PLS-DA). PCA, a statistical method for element reduction through an orthogonal transformation, is an unsupervised method that can be used to identify specific structures in a dataset (clusters, anomalies or trends that exist between the observations). For this, PCA is employed to identify patients who respond to treatment from those who do not. On the contrary, PLS-DA is a supervised method. This

supervised analysis will define the important variables – the main metabolites responsible for the separation among the groups in question.

Data mining, analysis, collaboration and decision-making in such diverse data-intensive and cognitively complex settings will be performed via the Dicode approach, supporting artificial and human intelligence. The envisioned architecture combines batch (a series of non-interactive tasks is executed all at one time) and stream (continuous computation that occurs as data is flowing through the system) processing (Karacapilidis, 2014), ensuring rapid and efficient outcomes.

7. Conclusions

The paradigm of autoimmune disease illustrates the great need to delineate disease mechanisms towards tailor-made therapeutics and differential diagnosis. Considering the interplay among genomic and environmental influences (diet, lifestyle, polypharmacy, toxins, gut microbiome) as well as host–gut microbiota interactions, a data-intensive and cognitively complex setting arises that limit human ability. We feel that a “pharmacometabolomics-aided pharmacogenomics” strategy coupled to information technologies that are built on the synergy of artificial and human intelligence will be of great benefit, as autoimmune disease pathobiology remains unclear, perplexing differential diagnosis, patient stratification and decision-making in the clinic. We propose that better-informed, rapid and cost-effective omics studies need the implementation of holistic and multidisciplinary approaches.

8. Outstanding Questions

Herein, we consider immune disease as a model that presents multiple challenges, which could only be met rapidly and cost-effectively via a multidisciplinary strategy – a “pharmacometabolomics-aided pharmacogenomics” approach coupled to information technologies that is built on the synergy of artificial and human intelligence. It is now the time to implement new working practices to turn information growth into knowledge growth and hence, better informed decisions. Can we delineate inter-individual variability towards differential diagnosis? Can we highlight the disease mechanisms in question to assist disease management? What are the host–microbiome interactions? Do post-dose drug metabolism and safety relate to pre-dose metabolotypes and how?

9. Search strategy and Selection Criteria

Data for this review were identified by searches of PubMed and references from relevant articles using the search terms “pharmacogenomics”, “pharmacometabolomics”, “pharmacometabonomics”, “collaborative informatics”, and “precision medicine”. Only articles published in English between 2006 and 2015 were included (except for those introducing pharmacometabonomics, metabolomics and autoimmune disease for the first time).

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Authors' Contributions

GPP conceived and designed the study. TK and GPP drafted the manuscript. Literature search has been performed by TK, EK, IL, HM, IP and LS.

Competing Interests

The authors declare that there are no conflicts of interest.

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