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REVIEW ARTICLE

Biomarkers in neonatology: the new ''omics'' of bronchopulmonary dysplasia

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Abstract

Bronchopulmonary dysplasia (BPD) is a complex disorder resulting from gene–environmental interactions. An improved understanding of the pathogenesis of this most common chronic lung disease in infants has been made by utilizing animal models and correlating with human data. Currently, while some (vitamin A, caffeine) pharmacotherapeutic options are being utilized to ameliorate this condition, there is still no specific or effective treatment for BPD. It would be helpful for prognostication and targeted potential novel therapeutic strategies to identify those babies accurately who are at risk for developing this disease. A reliable biomarker would have the capacity to be detected in the initial phase of the disease, to allow early interventions to avoid or minimize the detrimental effects of the disease. This review will focus on human studies performed with the ''omic'' techniques, specifically genomics, epigenomics, microbiomics, transciptomics, proteomics and metabolomics, and summarize the information available in the literature, as it pertains to biomarker identification for BPD. Using ''omics'' technologies, investigators have reported markers that have the potential to be used as biomarkers of BPD: SPOCK2, VEGF −624C > G, VEGF −460T > C, mast cells specific markers, miR-219 pathway, miR-152, −30a-3p, −133b, −206, −7, lactate, taurine, trimethylamine-N-oxide, gluconate, myoinositol and alterations in surfactant lipid profile.

Keywords

Bronchopulmonary dysplasia, metabolomics, prematurity, preterm newborn, proteomics

History

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Introduction

Bronchopulmonary dysplasia (BPD) is the most common chronic lung disease in infants [1]. The pathogenesis of this disorder is multifactorial, arising from a complex interaction between genetic and environment factors [1]. The foundation of this disease is a genetically pre-disposed [2] immature lung which is exposed to multiple environmental risk factors: ante-/post-natal infections, hyperoxia and ventilator-induced baro-/volu-/atelecto-trauma [3]. This results in injury during critical stages of lung development secondary to initiation and escalation of inflammatory processes, mediated, among other molecules, by a multitude of cytokines [4,5]. The end-result is a lung with characteristic pathological features of impaired alveolarization and dysregulated vascularization [3]. While there is no specific or effective treatment, it is still important to identify those babies who are predisposed to develop BPD in order to target testing of preventive strategies or early therapies.

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As BPD is multifactorial and involves different molecular pathways, a number of biomarkers have been proposed to identify babies at risk for developing this disease [6]. An effective or useful biomarker has to be reliable and should have the capacity to be detected in the initial phase of the disease or even before the disease has developed, to allow early interventions to avoid or minimize the detrimental effects of the disease.

Some biomarkers have been identified in maternal, cord or peripheral blood and urine [6]. A limitation of these biomarkers is that the concentration of these molecules can be influenced by an inflammatory state in other parts of the body, hence these are not lung specific. In fact, the currently available biomarkers are neither universally accepted nor used routinely in the clinical setting to identify those babies pre-disposed to develop the disease. Bronchoalveolar lavage fluid (BALF) has also been analyzed to find some specific biomarkers of BPD, as it reflects more accurately target organ-specific alterations in molecular mediators.

Biomarker studies can either select pre-defined biomarker(s) measured by specific methods or apply an ''unbiased'' approach involving use of indiscriminate detection platforms.

The study of particular biochemical profiles of the organism's response in temporal sequence of the clinical evolution of BPD can improve timing, sensitivity and specificity of diagnosis and potentially improve treatment strategies. The ''omic'' approach can help to define the profile of babies with and without BPD and identify some compounds that are specific to the host response during the pathogenesis of the disease and subsequently, these can be potentially used as biomarkers.

This review will focus only on human studies performed with the new ''omic'' technologies that are promising approaches to identify novel biomarkers for BPD: genomics, epigenomics, microbiomics, transcriptomics, proteomics and metabolomics.

"Omics" of preterm birth

As mentioned above, an immature lung is the foundation for development of BPD. Since a premature infant would be likely to have an immature lung, the ability to detect biomarkers that would pre-dispose to preterm birth could potentially allow us to identify infants before and/or soon after birth who would be considered high-risk for the development of BPD.

Genetic variance is commonly detected as alterations in the DNA nucleotide sequence (single nucleotide polymorphisms, SNPs). Several gene polymorphisms have been found to predispose to preterm birth, arising both from the maternal and fetal side [7]. Other genes have been found to pre-dispose to preterm premature rupture of the membranes (pPROM) and consequently to preterm delivery [8]. Capece et al. [9] performed a systematic literature review of studies that investigated genetic factors involved in both preterm delivery and pPROM. A total of 248 SNPs in 102 genes were found to be statistically significantly associated with spontaneous preterm birth with intact membranes, and 39 SNPs in 32 genes were found to be statistically significantly associated with pPROM.

Proteomics is the large-scale study of the structure and function of proteins of a cell or organism. The goal of proteomics is to provide a snapshot of all proteins in a fluid, tissue or organism. It is possible to observe quantitative and qualitative changes in the protein content of a sample even in the absence of changes at the mRNA level due to processes occurring in the cell such as proteolysis or reversible post-translational modifications (e.g. phosphorylation) that can alter the function of the proteins. If some proteins change consistently in patients with a specific disease compared to patients without the disease, these proteins can be considered as candidate biomarkers of that specific disease.

Proteomics has been used to try to find a biomarker of preterm delivery, but existing studies have a wide heterogeneity in the type of samples and technology used. A systematic review performed by Kacerovsky et al. [10] reports a total of 64 deregulated proteins (of amniotic fluid, cervicovaginal fluid, placental tissue and maternal serum specimens), but only five were confirmed in more than one study (14-3-3 protein sigma, annexin A5, protein S100-A8, protein S100-A12 and inter-α-trypsin inhibitor heavy chain H4).

Metabolomic profiling of amniotic fluid was able to distinguish patients who delivered at term from patients who delivered preterm. A decrease in carbohydrates was associated with preterm delivery in the presence or absence of inflammation whereas an increase in amino acid metabolites was a unique feature of preterm labor with inflammation [11]. In another study, altered metabolites were related to liver function, fatty acid, coenzyme A and histidine metabolism [12].

Microbiomics

The microbiome is defined as the collective genomes of the microbial community that colonizes the body. In fact, the human body contains over 10 times more microbial cells than human cells and the whole bacterial genome contains at least 100 times as many genes than our own genome [13].

Modern techniques of DNA sequencing has allowed researchers to detect the whole microbiome, because these techniques conduct the analysis of genetic material harvested directly from microbial communities without the need to culture the microbes.

It has been demonstrated that the mode of delivery influences the lung microbiota: vaginally delivered infants acquire bacterial communities resembling their own mother's vaginal microbiota (*Lactobacillus*, *Prevotella* or *Sneathia*) and C-section infants acquire mostly skin microbiota (*Staphylococcus*, *Corynebacterium* and *Propionibacterium*) [14].

Most lung microbiome studies have been performed using BALF samples. In preterm infants, the lung microbiome has not yet been defined, although some attempts have been made [15]. Studies performed to define the lung microbiome suggest that the lung of preterm infants is colonized already at birth, suggesting that the amniotic environment is not sterile. Lohmann et al. [16] found that Acinetobacter was predominant at birth but no specific bacterial pattern was associated with development of BPD, although neonates developing BPD had a reduced diversity of the lung microbiome.

Genomics and epigenomics

It is becoming more and more evident that there are underlying genetic factors involved in the risk of developing BPD, as highlighted in the review by Shaw et al. [17]. This article reviews the extant literature on genetic factors influencing the risk of BPD and describes a variety of approaches, including studies involving familial aggregation, twins, candidate genes and genome-wide association studies (GWAS) [17].

Besides detection of SNPs during lung development, epigenetic mechanisms, such as DNA base modifications (methylation, alteration of histones resulting in chromatin modification) and the action of non-coding RNA (mircoRNAs or miRs), can control genetic expression by activating or silencing the information embedded in the genome [18].

Epigenetics is also influenced by the environment (infections, inflammation and oxidative stress), accounting for different outcomes even in the presence of the same epigenetic alterations.

Some SNPs have been associated with BPD, such as certain polymorphisms of surfactant proteins: Weber et al. [19] found an increased frequency of the SP-A1 polymorphism $6A^6$ in infants with BPD whereas Rova et al. [20] found an increase of the SP-B intron 4 deletion in BPD patients compared with controls.

Few studies have been conducted to identify genetic variants involved in BPD. Carrera et al. [21] performed an exome sequencing pilot study on 26 severely affected BPD preterm newborns and identified the top candidate genes as nitric oxide synthase 2 (NOS2), matrix metallopeptidase 1 (MMP1), C-reactive protein (CRP), lipopolysaccharide binding protein (LBP) and the toll-like receptor (TLR) family.

Hadchouel et al. [22] performed a GWAS prospectively evaluating 418 premature neonates (gestational age [GA] <28 weeks). Polymorphisms associated with BPD were confirmed in an independent replication population of 213 neonates. SPOCK2 gene was identified as the most significant polymorphism associated with BPD.

Contrariwise, Wang et al. [23] did not identify any SNPs associated with BPD in their GWAS analysis. In their study, the authors analyzed 1726 very low birth weight infants (VLBW; GA <30 weeks) but they did not identify any SNPs associated with BPD at the genome-wide significance level (5×10^{-8}) and none of the 27 SNP identified in previous studies reached statistical significance [including SPOCK2, vascular endothelial growth factor A (VEGFA), superoxide dismutase 2 (SOD2), toll-interleukin 1 receptor (TIR) domain containing adaptor protein (TIRAP), mannose-binding lectin (MBL2), surfactant protein D, interleukin 18 (IL18), superoxide dismutase 3 (SOD3), MMP16 and selectin L (SELL)].

Fujioka et al. compared 55 Japanese neonates with a GA <32 weeks and BPD to 42 controls. Genomic DNA was extracted from umbilical cord, cord blood and buccal mucosa and six VEGF phenotypes were determined by DNA sequencing. The $-634C>G$ genotype was significantly associated with BPD [24]. Kwinta et al. [25] found a statistical correlation between the $-460T>C$ VEGF gene polymorphism and BPD in a Polish population of 181 preterm infants.

Pathway-based approaches, which consider a biological pathway and analyze all the interactions contributing to alter that specific pathway, help understanding GWAS data in many complex diseases in which there are multiple interactions between genetics and environment.

A genome-wide transcriptional profiling study performed by Bhattacharya et al. [26] analyzed RNA isolated from lung tissue obtained at autopsy from 11 BPD cases and 17 age-matched controls; the isolated RNA was interrogated using microarray. The authors found that a total of 159 genes were differentially expressed in BPD tissues compared to controls. Further pathway analysis indicated previously identified (DNA damage regulation of cell cycle) as well as novel pathways: three of the top five most highly induced genes were mast cell-specific markers, suggesting an increased mast cell accumulation in pulmonary tissue of BPD patients.

Ambalavanan et al. [27] combined the use of GWAS with a pathway-based approach to increase the understanding of the role of genetic susceptibility to BPD. A genome-wide scan was conducted on 1.2 million genotyped SNPs, and an additional 7 million imputed SNPs, using a DNA repository of extremely low birth weight infants. Genome-wide association and gene set analysis was performed for BPD or death, severe BPD or death and severe BPD in survivors. Among 751 neonates that were analyzed, none of the SNPs were significant at the genome-wide significance level ($p < 10^{-8}$). Of the approximately 8000 pathways analyzed, the authors identified approximately 75 at false discovery rate <0.1 that were associated with BPD/death, severe BPD/death and severe BPD in survivors, but the overlap in these different pathways was limited to only three pathways, suggesting that the difference in clinical phenotype between mild and severe BPD is also manifest at the genomic level.

The pathway ''microRNA-19 or miR-19'' was the top pathway for BPD/death, although not all targets of miR-219 were dysregulated (perhaps because most genes are regulated by multiple miR as well as by other factors, as transcription factors, long, non-coding RNA and DNA methylation), whereas the top pathway associated with severe BPD/death and in survivors with severe BPD was phosphorus oxygen lyase activity.

Umbilical cord samples were used to perform an expression profiling study to discover molecular signatures associated with an increased risk of developing BPD in 54 preterm infants born at less than 28 weeks. Chromatin remodeling and histone acetylation pathways, appeared to be differentially regulated in umbilical cord tissues of the subsequently BPD-affected neonates [28].

The fact that all these epigenetics data were not confirmed by the different studies performed may be due to the different ethnicity of the population studied, which may account for a different genetic predisposition.

Transciptomics

The transcription of genes into RNA is the first stage of gene expression. The transcriptome is the complete set of RNA transcripts produced by the genome. High-throughput transcriptomic technologies such as genome-wide gene expression microarrays and next-generation RNA sequencing are becoming common in medical research to better define the molecular pathway of many complex diseases.

In particular, miRs are 21–25 nt long non-coding RNAs that are involved in various biological processes, including cell proliferation and cell death. They inhibit protein expression at the post-transcriptional level; the dysregulation of messenger RNA and as a consequence of proteins by miRs can contribute to the pathogenesis of various diseases [29].

It has been demonstrated that miRs are differentially regulated during lung development, as highlighted in the recent review of Herriges et al. [30]. However, little is known about how they regulate lung development. In principle, miRs that peak early in lung development during the pseudoglandular stage generally promote proliferation and inhibit differentiation. The miR17–92 cluster is one of the most widely studied; expression of this cluster is high early in development and promotes proliferation, and as expected the loss of this cluster leads to severely hypoplastic lungs.

In contrast, miR17–92 overexpression in the lung induces hyperproliferation and inhibits differentiation of lung progenitor cells.

A recent study aimed to identify aberrations in miR expression associated with BPD in preterm infants [31]. The expression profiles of 365 miRs were investigated in the peripheral blood of 30 VLBW infants at 36 weeks postmenstrual age (15 subjects with BPD and 15 controls). A down-regulation of miR-152 and miR-30a-3p and an up-regulation of miR-133b and miR-7 were found in the neonates with BPD compared to the neonates without BPD [31].

Zhang et al. [32] studied the influence of miR-206 on the development of BPD. They observed a reduction in the expression of miR-206 in blood samples of BPD patients compared with controls. The overexpression of miR-206 significantly induced cell apoptosis, reduced cell proliferation, migration and adhesion abilities, whereas the inhibition of miR-206 expression had the opposite effect. The authors assert that miR-206 acts through up regulation of fibronectin 1, an extracellular matrix glycoprotein that is involved in cell adhesion and migration processes. The up-regulation of fibronectin 1 could play a role in extracellular matrix remodeling and, thus, contribute to the progression of BPD [32].

Proteomics

In one paper, the authors analyzed BALF samples collected at different days of life from 12 preterm babies who later developed BPD, to get a complete molecular description of the disease progression [33]. Babies were grouped according to their maturity (23–25 weeks versus 26–29 weeks) and BPD severity (severe versus mild). Although in this study, the authors could not define a cluster of proteins to identify babies pre-disposed to the development of BPD, they could identify some molecular differences between younger and older patients and between severe versus mild BPD patients. In the BALF of younger babies (23–25 weeks), they found an increase of several serum proteins (serum albumin, serotransferrin and clusterin) and a reduced abundance of calcium signaling-related proteins. When comparing patients with older gestational ages (26–29 weeks), there was a reduction of calcyphosine (CAPS), calcium and integrin binding protein 1 (CIB1) and chloride intracellular channel 1 (CLIC1) in severe versus mild BPD patients. The hypothesized effect of CAPS is to regulate essential down-stream cellular functions like proliferation, differentiation and surfactant secretion. CIB1 acts as a calcium sensor, its decrease may lead to a less active calcium signaling, as expected in lung dysfunction. Furthermore, it directly interacts with integrins, negatively regulating their activation.

Metabolomics

Metabolomics is the emerging field of ''omics''; it consists of the quantitative analysis of a large number of low molecular mass metabolites (<2000 Da) found in a specific cell, organ or organism, involving substrates or products of a defined metabolic pathway. This analysis allows one to identify changes in the composition of metabolites caused by the interaction between specific pathophysiological states, gene expression and environment [34]. Metabolites analyzed can be peptides, lipids, organic acids, vitamins, minerals, drugs, amino acids, nucleic acids, carbohydrates, fatty acids, hormones, drugs and any other chemical with a molecular weight <2000 Da.

Metabolomic studies can be performed using two techniques: the targeted and the non-targeted approach. The first approach consists of investigating some pre-identified and expected metabolites. This technique can be only used when the pathophysiological state of a disease is already well known or at least partially understood. The non-targeted approach conversely consists of investigating all metabolites detectable in a sample to capture as much information as possible, providing a functional fingerprint of the pathological state that is being investigated.

Metabolites can be identified and quantified using different technologies including nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS). Each method is typically able to identify or characterize 50–5000 different metabolites or metabolite ''features'' at a time, but currently it is not possible to analyze the entire range of metabolites by a single analytical method [35].

As for proteomic studies, metabolomic studies require a comparison between normal patients and patients with the disease. The application of clinical metabolomics in the area of respiratory diseases in pediatrics (asthma, pneumonia and bronchiolitis) has been described by Atzei et al. in a recent review [36]. The most commonly used samples in preterm infants are urine or BALF [37].

Biomarkers in BPD: tracheal aspirates

Fabiano et al. [38] conducted a preliminary study to analyze metabolic profiles on the BALF of preterm infants complicated by respiratory distress syndrome (RDS). In this study, 12 BALF samples collected at birth prior to surfactant administration, post-surfactant administration during mechanical ventilation and at extubation time-points, were analyzed by NMR and gas chromatography (GC)-MS. The analysis identified 25 metabolites of whom 10 had a known molecular structure and were overexpressed in BALF collected during mechanical ventilation after surfactant administration compared to BALF collected prior to surfactant administration. They were: undecane, decanoic acid, dodecanoic acid, hexadecanoic acid, octadecanoic acid, hexadecanoic acid methyl ester, 9-octadecanoic acid, tetracosanoic acid, myristic acid and phosphate. The remaining metabolites which had an unknown structure were reported as being under investigation [38].

Urine

Urine metabolomic analysis has the disadvantage of being distant from the tissue of interest. Furthermore, kidney diseases may alter the excretion of metabolites. Nevertheless, urine samples have been used for metabolomics analysis because they have the advantage of being non-invasive and easy to collect.

In a recent paper, Fanos et al. compared the urinary metabolic profile at birth in 36 newborns with a gestational

age below 29 weeks and birth weight below 1500 g. Neonates were divided in two groups: the first group (18 cases) consisted of newborns who later developed the disease and the second group (18 controls) consisting of newborns not affected by BPD [39]. They found five discriminant metabolites: lactate, taurine, trimethylamine-N-oxide (TMAO), myoinositol (which increased in BPD patients) and gluconate (which was decreased). The increase in urinary lactate in the BPD group suggested an involvement of anaerobic respiration. Taurine and TMAO have a fundamental biological role for osmoregulation and membrane stabilization. In addition, taurine also plays important roles in calcium homeostasis, renal cell cycle and apoptosis, nerve cell activity and detoxification. Thus, the authors conclude that BPD is probably related to an abnormal development of the vascular tree and the pneumocytes. Taken together, these results suggest that BPD is congenital disease, deriving from genetics plus intrauterine epigenetics. These data were confirmed by the studies of Baraldi et al., performed on the amniotic fluid of 10 mothers of preterm newborns who developed BPD versus 11 who did not develop BPD. The analysis of the amniotic fluid revealed the presence of metabolic profiles characterizing the development of BPD [40].

Volatile compounds

Exhaled breath condensate (EBC) is increasingly used in the attempt to obtain a non-invasive assessment of airway inflammation and oxidative stress in chronic lung disease. Recently, measurement of volatile organic compounds (VOCs) in EBC has been termed ''breathomics'' [41]. EBC is a biofluid collected noninvasively by cooling the air expired during tidal breathing [42]. Exhaled air contains a complex mixture of VOCs in the gas phase and non-volatile compounds derived from condensed water vapor and aerosol particles. The standard for detecting individual molecular compounds in VOCs is GC-MS. The advantage of exhaled air analysis is its non-invasive nature.

Regarding BPD, in a recent study, Carraro et al. [43] studied the metabolomics profile of EBC of 20 adolescents with BPD and 15 healthy controls. The EBC samples were analyzed using a metabolomic approach based on MS. The obtained spectra showed a discrimination between cases of BPD and healthy controls. The analysis identified an altered complex lipid profile in the adolescents with BPD; the biomarkers identified in BPD patients were lyso-phosphatidylcholine, platelet activating factor (PAF), the unsaturated phosphatidylcholine and a plasmenyl-phosphatidylserine.

Table 1. Different patterns of biomarkers of BPD.

Samples	Biomarker	Levels associated with increased risk of BPD
Genomics/epigenomics		
Blood [22]	SPOCK 2	↑
Umbilical cord/cord blood/buccal mucosa [24]	VEGF −624C > G	↑
Blood [25]	VEGF −460T > C	↑
Blood [27]	miR-219 pathway	↑
Blood [31]	miR-133b, miR-7	↑
	miR-152, miR-30a-3p	↓
Blood [32]	miR-206	↓
Proteomics		
BALF [33]	CAPS, CIB1, CLIC1	↓
Metabolomics		
Urine [39]	Taurine, TMAO, myoinositol, lactate	↑
	Gluconate	↓
Breathomics		
Exhaled breath condensate [43]	Lyso-phosphatidylcholine, PAF, unsaturated phosphatidylcholine, plasmenyl-phosphatidylserine	↑

BPD: bronchopulmonary dysplasia; CAPS: calcyphosine; CIB1: calcium and integrin binding protein; CLIC1: chloride intracellular channel 1; PAF: platelet activating factor; TMAO: trimethylamine-N-oxide.

The results of the metabolomics analysis of these EBC suggest that the lungs of survivors of BPD are characterized by long-term metabolic abnormalities. The authors attribute this different lipid composition to an altered surfactant composition in patients with BPD, which may persist far beyond infancy. They speculate that the dysfunction of surfactant could contribute to the persistent airflow limitation found in adolescent survivors of BPD.

The collection of EBC can be performed even in ventilated infants [44]; however, in preterm infants, it is not used routinely because of the technical difficulties of sample collection. Recently, an electronic nose was used for measuring VOCs directly in TA fluids from preterm neonates [45]. Electronic noses can detect the combined molecular composition of gas mixtures using a semi-selective electronic sensor array, resulting in the recognition of volatile odor patterns that can be regarded as a fingerprint of a particular clinical condition (breathprint). In their study, the authors collected TA from 28 intubated preterm infants, then 250 μL of the sample was heated to 37 °C and the snout of an electronic nose was placed above the surface of the TA for 10 s. In the 12 neonates who later developed BPD, the smellprints differed from the neonates without BPD [46].

A summary of the biomarkers has been shown in Table 1.

Conclusion

In the neonatal period, BPD manifests as a severe respiratory disease, with a significant impact on the quality of life of affected children. Even as they grow up, despite an improvement in their overall health, they continue to have a persistent airflow limitation and may experience a significant decline in lung function during adulthood [47].

Characterizing the mechanisms involved in the genesis and progression of BPD could have a significant impact on the outcome of affected children. Our current understanding suggests that targeting individual pathways is unlikely to be sufficient. We need to better understand the basic mechanisms of neonatal lung development, injury and repair to identify novel targets for intervention. In addition, research focused on genetic determinants of BPD may lead to identification of specific patient genetic and epigenetic characteristics.

In fact, it would be helpful for prognostication and targeted potential novel therapeutic strategies to identify those babies at risk for developing this disease. The identification of a panel of biomarkers coming from a combination of genomics, epigenomics, microbiomics, transcriptomics, proteomics and metabolomics, detected in the initial phase of the disease, would allow early interventions to avoid or minimize the detrimental effects of the disease.

Once a molecular pattern has been found to be associated with a specific pathological condition, the identification of these clusters of substances could lead to the development of a rapid test, allowing its use in the clinical setting. This may prove very useful in stratifying patients by risk category in future new interventions.

The most promising fluid to analyze seems to be BALF/TA as it reflects directly what is going on in the lung. Nonetheless, it is invasive and babies need to be ventilated to allow sample collection. As nasal ventilation is increasingly used in preterm neonates, sample access could be an issue. Further studies could clarify if the use of nasopharyngeal secretions could reflect TA specimens and therefore be used to evaluate the same panel of biomarkers.

In conclusion, the aim of biomarker identification should be theranostics that tailors optimized therapies based on a biomarker profile of each individual patient, thus reducing side-effects and improving response to treatment. This field promises to increase the quality of clinical care by identifying the right drug for the right patient at the right time.

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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