Review

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Biomarkers in Medicine

The emergence of point-of-care blood-based biomarker testing for psychiatric disorders: enabling personalized medicine

For psychiatric disorders, repeated failures in converting scientific discoveries into novel drugs has precipitated a crisis and eroded confidence in drug discovery. This review describes how current and future innovations driven by application of biomarkers can help to re-initiate research in this area. This will have positive impact on the field of psychiatry and result in application of sensitive and specific biochemical tests in parallel with the traditional questionnaires for improved diagnosis. Furthermore, application of emerging biosensor tools will facilitate point-of-care testing by fusion of biochemical and clinical data. In this way, patient data will be comprised of past medical histories, biopatterns and prognosis information, resulting in personalized profiles or molecular fingerprints for patients with these conditions.

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Background

Psychiatric disorders are debilitating behavioral and mental health diseases which can strike at different ages and seriously impair medical health, quality of life, social well-being and productivity, with significant impact on society and the economy. According to the World health Organization (WHO), mental disorders will be the second leading cause of disability worldwide by 2020. Major depression alone affects approximately 20% of the people in Europe and socio-economic costs approximate 1% of the gross domestic product, with major costs also occurring outside the healthcare systems due to the knock-on effects on employment and related issues [1]. According to the Global Burden of Disease study, mental disorders such as depression account for over 12% of years lived with disability. This is compounded by the fact that current medications are efficacious in only around 50% of the cases and many of these can have serious side effects. Despite the urgent medical need, no clinically validated molecular biomarker lab-on-a-chip (LOC) point-of-care test exists for any psychiatric disorder. Perhaps counter-productively, pharmaceutical companies have cut research in this area over the past 10 years, due to the dearth of knowledge available for understanding these disorders and the consequential poor availability of surrogate markers to support drug discovery efforts. Therefore, there is now an urgent need for new technologies in biomarker-based approaches that are fast, cost-effective and simple to use for the designated operators. The biggest markets which should be targeted with these approaches are point-of-care use by primary care providers and clinicians to aid diagnosis, and pharmaceutical companies conducting large-scale clinical trials or testing new medicinal compounds. Diagnosis and treatment of major depression, for example, is often performed in primary care by general practitioners but

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with a low success rate as 70% of these patients are undertreated [2,3]. However, applying the correct therapeutic approach for each patient is paramount as is the speed with which this occurs. In line with this, patient stratification using biomarkers is essential for correct treatment. The application of accurate *in vitro* diagnostic LOC devices that can accurately classify patients according to the type of disorder and even identify disease subtypes will help to reduce duration of illness and improve compliance by placing the right patients on the right treatments as early as possible. This will change the overall paradigm from reactive medical care to a more efficient personalized medicine scheme (Figure 1). In addition, if earlier effective treatment is implemented this will also reduce patient referral to secondary aid such as hospital services, community groups and crisis teams. These are expensive services and any reduction in use of these services will help reduce the overall financial burden of psychiatric disease.

Severe neuropsychiatric disorders, such as schizophrenia, major depression and autism affect people of all ages and can be ameliorated only partially with prescription medicines. In conventional medical practice, the physician comes to a diagnosis based on observation of symptoms and appropriate neuropsychological testing and prescribes a specific treatment accordingly. However, when confronted by a wide array of sometimes fleeting and often subtle cognitive, behavioral and emotional disturbances, exact psychiatric diagnosis can be problematic and often even experienced psychiatrists, mental health workers and physicians can have difficulty in quick and precise clinical assessments. They can also access other data to help improve diagnostic accuracy such as observations regarding appearance and behavior of patients and well as their speech and thought content. A detailed history and chronology of self-reported symptoms, mental health history, social background and lifestyle is also employed to guide diagnostic formulation. The most systematic classifications are usually performed using questionnaires based on standard diagnostic criteria, guided by the International Classification of Diseases version 10 (ICD-10) and the Diagnostic and Statistical Manual of Mental Disorders (DSM). However, in reality, these diagnostic evaluations are often incomplete, unstructured, open-ended and prone to inaccuracy, all of which can lead to misdiagnosis of the patient.

One reason for the above difficulties is that the current diagnostic protocols for mental disorders do not normally take into account biological or pathophysiological measurements. However, recent advances are beginning to unravel the neurobiochemical nature of psychiatric disorders. Thus, molecular biomarkers are rapidly becoming the method of choice as the fundamental lesions involving diseases of the CNS are likely to be associated with complex perturbations and dysregulation of molecular expression profiles. Consequently, any understanding of these disorders should be accompanied with a systems level view of the dysregulated factors contributing to the pathogenesis and should include construction of disease- or symptom-specific molecular biomarker panels. The discovery of authenticated biomarkers could significantly enhance future mental healthcare if the resulting tests can be incorporated into standard operating systems and clinical decision making, as well as being established as fast, cost-effective, user friendly, point-of-care devices.

What kind of biomarker tests should be developed?

Although genomic studies are able to identify genes conferring susceptibility to a particular disease, the functional abnormalities are ultimately reflected in the proteome. Furthermore, the proteome reflects changes in a dynamic manner and can therefore give an assessment of health or disease states that closely approximate real time. With this concept in mind, recent years have seen the increasing use of proteomics as a tool for the discovery of biomarkers for diagnosis, monitoring disease progression, treatment response and for the identification of novel therapeutic targets [4]. It is also important to remember that analysis of CNS disorders is difficult as the brain is not readily accessible for invasive diagnostic purposes. Thus, sources such as cerebrospinal fluid, serum, plasma, saliva, urine and peripheral blood cells have been undergoing increasing scrutiny as these have a higher utility [5]. Serum or plasma present the most accessible fluids for proteome analysis although they are both inherently complex, with dynamic protein concentration ranges exceeding 10 orders of magnitude and the 22 most abundant proteins accounting for 99% of the proteome mass. In addition, proteomic platforms must cope with the vast number of post-translational modifications of these same proteins. It should be kept in mind that there is no universal platform which can capture the entire serum or plasma proteome and so combinations of methods will give a more complete picture in screening studies. However, for ease of use in the clinical environment and for point-of-care testing, a single method is optimal, which is simple to use by nonexperts, as well as being rapid and sensitive.

Clinical impact of biomarkers

In the foreseeable future, it is likely that increased biomarker testing by clinicians will lead to more extensive 'bio-'signatures in individuals that reflect the

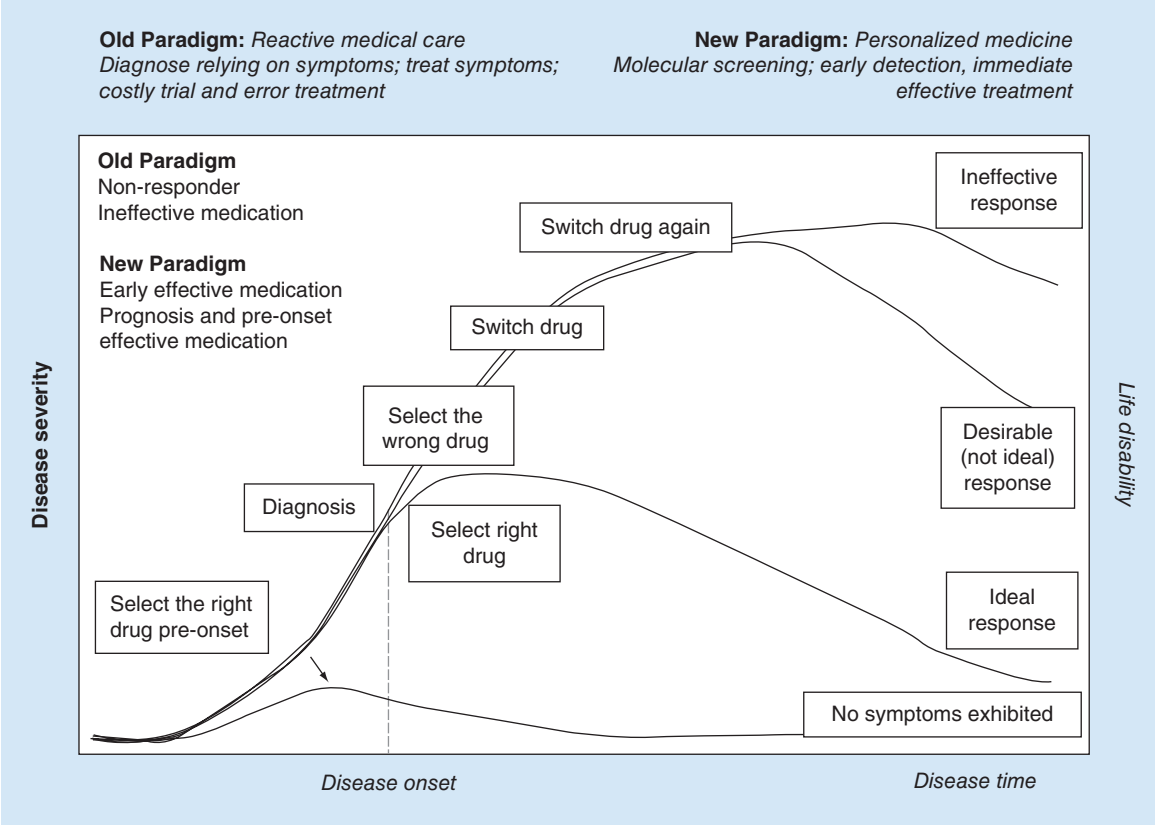

Figure 1. Comparison of the old and new paradigm of medical care as distinguished by the use of biomarkers for improved patient care.

molecular changes occurring in health or disease. An optimally developed diagnostic test should enhance the sensitivity of identifying patients suffering from a particular illness and the specificity of correctly identifying those individuals who are not. Ideally, biomarker tests should have sensitivities and specificities, which are both greater than 90% and should be highly reproducible to be of any real clinical utility. Currently, tests for schizophrenia and depression have almost reached these specifications, although they still require multiple stages of validation testing before they can be incorporated into clinical studies [6,7]. This is important as many so-called 'biomarker panels' have failed in these validation stages and have now disappeared from the scene. Also, the platforms involved in the biomarker discovery stages are typically comprised of multiple large components, requiring considerable operational expertise and they do not return results quickly enough to be useful in point-of-care scenarios. However, this is now changing.

Multiplex immunoassay

Blood serum and plasma samples contain many molecules such as hormones, growth factors and cytokines, which can only be detected using methods that are highly sensitive. One of the best methods to achieve this is the sandwich format of immunoassay [8,9]. In this approach, antibodies against a specific protein are linked into defined wells in a microtitre plate. Then the sample is added and the specific protein is captured by the antibody. Next, a fluorescently labeled secondary antibody against the same protein is added which also binds to the protein, creating a 'sandwich' configuration. Each well is then analyzed in a plate reader to determine the amount of bound labeled antibody, which is proportional to the amount of that protein in the sample. Such assays typically require 24 h for completion as an overnight incubation with the primary antibody is often required.

The same concept is employed in the standard multiplex immunoassay technique (Figure 2), although in this case two or more biomarkers can be measured simultaneously. Multiplex bead arrays emerged early in the new century such as those manufactured by the Luminex company [10]. This uses 5.6 micron polystyrene microspheres that have been loaded with different ratios of red and infrared dyes to give unique fluorescent signatures. A capture antibody is attached to a fluorescent bead surface such that each specific antibody is attached to a bead with a specific red/infrared signature. As each microsphere carries a unique signature, the detection system can identify the corresponding

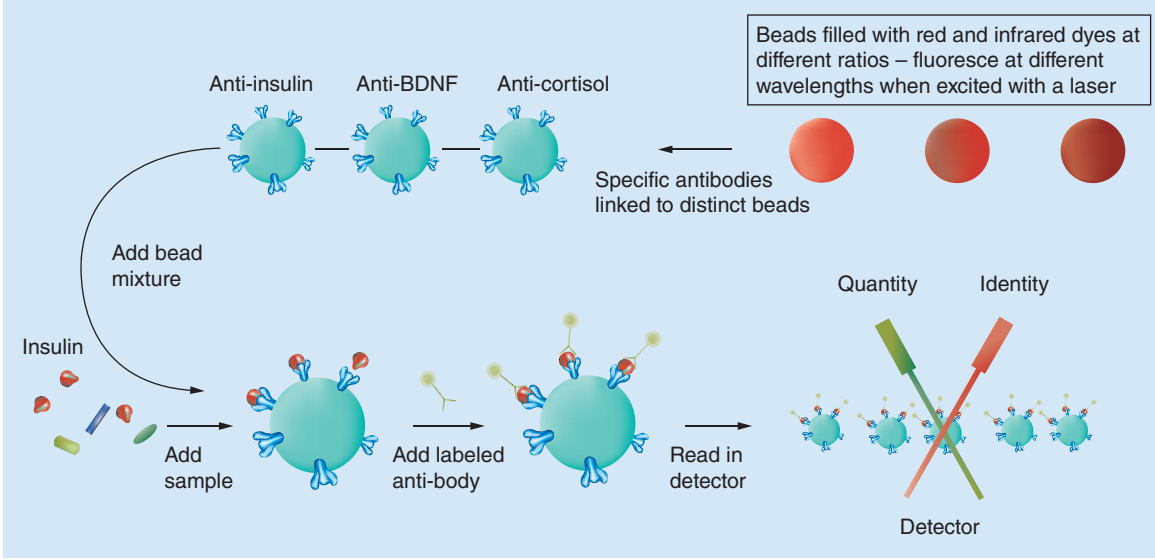

Figure 2. Overview of multiplex immunoassay technique. Samples are added to dye-coded microsphere-antibody conjugates that target specific biomolecules. Following incubation with a 2nd antibody containing a fluorescent label to form the 'sandwich' configuration, the mixtures are streamed through a flow cytometry instrument which uses lasers for identification of the antibody-microsphere conjugates and quantitation of the bound molecules.

antibody. Upon incubation with the sample, the target molecule binds to antibody-bead conjugate. Next, a fluorescently labeled detection antibody is added which binds to target molecule to give the 'sandwich' configuration and a flow cytometry-based reader provides identification and quantification of the molecule. The last step is achieved as the beads flow along a guided stream analyzed by two lasers. The lasers identify which analytes are present by measuring the unique fluorescent signature of each bead, and determine the quantity of target molecule by measuring the amount of fluorescence from the tagged second antibody associated with each bead. In the example shown, it would be possible to determine the concentrations of insulin, brain-derived neurotrophic factor and IL-1, simultaneously as each antibody-bead conjugate would have a distinct signature as it passes the quantitation laser.

The final step in the process is calculating the experimentally determined molecular levels of each analyte measured in the samples. As with most immunoassay methods, this is achieved by plotting the readings for each sample along with standard curves of known analyte concentrations to derive that of the target molecule. The corresponding concentration determined for that sample can then be adjusted by the appropriate dilution factor to calculate the absolute concentration of the molecule of interest. Thus far this method has been used in the study of multiple psychiatric disorders including schizophrenia [6,11–13], bipolar disorder [14,15], major depressive disorder [11,16,17] and autism spectrum conditions [18,19]. Recently, the method was used to identify a signature for schizophrenia in individuals several years before the onset of overt symptoms and actual diagnosis [20]. However, as stated above, such tests still require years of validation testing, preferably using both retrospective and prospective sample cohorts, before they can be deemed of any practical use in the clinical setting.

Finally, although the speed of the multiplex immunoassay technique is moderate (a single sample can be analyzed in ~24 h), this method is not user friendly since it requires considerable expertise to carry out the assay. Furthermore, the platform is slightly cumbersome as it consists of a medium-sized table-top reader and sample requirements are medium to high since it requires 25–350 µl of serum or plasma (50–700 µl blood).

Plasmonic biosensing

A newer method called plasmonic biosensing has emerged as a potential alternative to fluorescence-based methods for rapid, real-time detection of biological molecules [21,22]. This method is known for its rapid sensing response, low reactivity to external interferences, high stability in heterogeneous environments and low-invasiveness in patient sampling. Plasmonic biosensing is based on localized surface plasmon resonance (LSPR) such that surface binding of biomolecules is detected in real-time via a shift in photon absorbance and scatter behavior of oscillating electrons on metallic nanoparticles (Figure 3). Thus, the integration of miniature LSPR biosensors into microfluidic chips is a promising approach toward for point-of-care biomarker screening.

Recently, Chen and colleagues described the development of an LSPR approach for parallel high-throughput detection of multiple cytokine biomarkers in very low quantities of serum samples [23]. The LSPR microarray device was fabricated using one-step microfluidic patterning, followed by antibody conjugation to gold nanorods. The authors then used dark-field imaging optics which enabled quantitative measurements of the cytokines over the concentrations range of 10–10,000 pg/ml, using only 1 µl of serum. The parallel on-chip assays were completed within 40 min and this included loading, incubation and washing of eight different samples, and ten-times repeated detections for each sample. The potential applications using this system include detection of a wide variety of biomarkers of human diseases and for patient stratification purposes in personalized medicine approaches. The rapid throughput time also makes this a prime candidate for point-of-care testing, although further work is required.

Credit card-sized devices for user-friendly rapid testing

Multiplex antibody-based biomarker tests have now been developed on *in vitro* diagnostic devices which are approximately the size of a credit card. A novel innovative approach toward development of a lab-on-chip system for point-of-care *in vitro* diagnostics was described in 2012 and has been rapidly developing ever since [24]. This platform offers the possibility of inexpensive on-site analysis. It also features modularity, which allows adaptation to various assay types and formats. Thus far, immunoassay-based microcards have been developed for detection of protein markers such as prostate-specific antigen using either electrochemical or optical read-outs. This format has several advantages over existing systems, including the fact that it is user friendly (no expertise is required for operation), and it is rapid with a low cost. The test protocol involves application of blood drops to the card, and then the card is inserted into a small table top analyzer/reader to deliver the diagnosis as a final 'score' in less than 15 min (Figure 4). A device such as this can be used directly in a doctor's office, with the result obtained with in that short time frame. Thus, one of the anticipated major benefits will be the rapid turnover time as this will help to cut down on waiting times for the results of lab tests which can often take several weeks using standard methods. Furthermore, these devices can contain a universal serial bus (USB) port for connection to a computer and transmission of data to a smartphone or a wearable device through incorporation of near-field-communication modules. However, it should be noted that all of the above delivery methods still require the development of validated biomarker panels as well as regulatory approval by the appropriate agencies before they can be used in research or clinical studies.

Other benefits: cost savings for the healthcare systems

The medical consequences and associated stigma of mental illnesses can create a vicious cycle of discrimi-

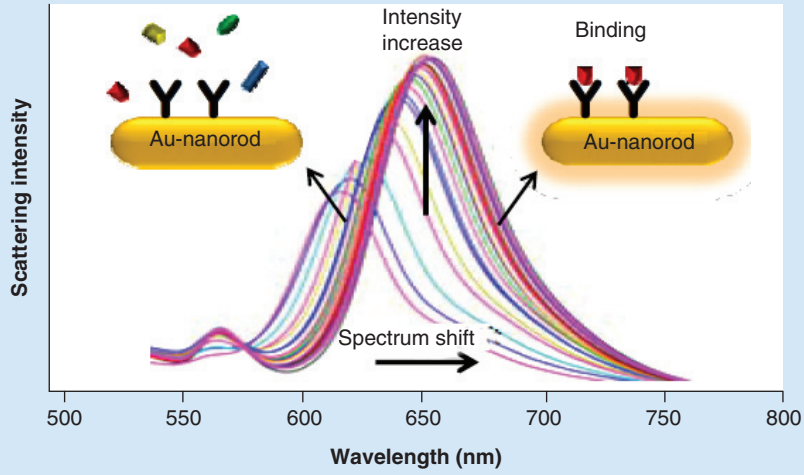

Figure 3. Principle of localized surface plasmon resonance miccroarray analysis. Au nanorod microarrays are integrated in a microfluidic chip with parallel microfluidic detection channels with inlet and outlet ports for reagent loading and washing. Specific antibodies are conjugated to the Au nanorods. The sample is applied and binding of the targeted molecules induces a redshift and scattering intensity change, which is imaged using dark-field microscopy and quantitated.
Au: Gold.

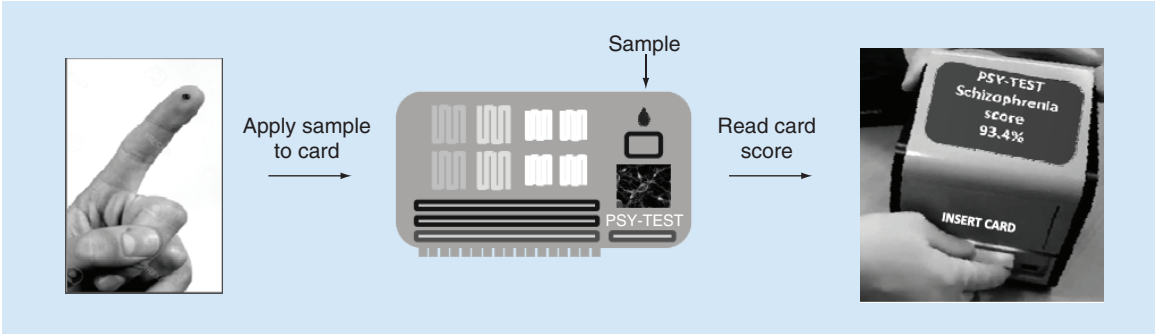

Figure 4. Lab-on-a-chip work flow.

nation, social isolation, unemployment, drug abuse, long-term institutionalization or homelessness. User friendly devices employing validated biomarker signatures for early diagnosis would help to alleviate or circumvent these issues by reducing misdiagnosis, allowing early intervention, enabling healthcare professionals to decide on the best treatment course and reducing the duration of untreated illness as well as ameliorating disease severity. As described above, one of the primary markets which should be targeted to achieve these results is point-of-care. Diagnosis and treatment of major depression is often performed in primary care by general practitioners. However, this has a poor success rate in identifying such diseases as around 70% of patients with psychiatric disorders who visit a general practitioner are under treated [25,26]. Thus, applying the best available therapeutic approach for each patient is paramount and this could be achieved through patient stratification using point-of-care biomarker tests. Another future targeted market could be the pharmaceutical companies conducting large-scale clinical trials and testing new drugs. Improved stratification of patients would help to enhance trial outcomes by ensuring that the right patients are enrolled in these studies from the beginning.

The deployment of point-of-care diagnostic tests will help to meet the need of providing effective therapies at an earlier stage in the disease. It is expected that economic benefits will be seen at individual and societal levels via improved health, productivity and reduced healthcare costs. The cost of depression alone in the European Union was ~€43 billion in 2010 and €17 billion (40%) of this was due to indirect costs [27]. Assuming that a point-of-care test could increase the number of successfully diagnosed and treated patients by twofold, national healthcare savings of ~€8.5 billion/year would be realized for the indirect costs alone. The actual savings are likely to be even higher considering that many other disorders can also present with depressive symptoms. For example, over 95% of major depression patients present at their clinics with cognitive symptoms which can negatively impact quality of life and ability to function professionally and socially [27–29] and longer periods of untreated disease can lead to increased debilitation [30].

The successful application of point-of-care testing will help to reduce duration of illness and improve compliance by placing the right patients on the right treatments as early as possible. This would be a considerable improvement with a huge positive impact on the lives of patients as current strategies typically involve a waiting period to determine if a medication or dosage is effective [31]. However, it can sometimes take several weeks to attain response and many months to achieve remission after initial treatment, as in the case of antidepressants. To compound this problem, nonresponding patients can be subjected to trial-and-error testing, during which medication doses are adjusted and/or drugs switched or tested in combination. The response rates to most psychiatric medications are around 50% and many patients are treatment resistant or suffer from severe side effects and around 25% of patients stop taking medication [32,33].

The need for point-of-care devices

Point-of-care testing employs a spectrum of devices ranging from large table-top instruments to implanted, wearable and handheld modules. Most of the current handheld systems consist of a disposable reagent prefilled strip incorporated into a cartridge or a cassette to facilitate addition of the sample and to house the conduction of the test, and the resulting signal is interpreted visually or measured using an inexpensive reader or meter. The point-of-care-type tests that are already in existence can be used to measure blood gases and ions, markers of heart attack, cholesterol, markers of diabetes, clotting availability of blood, pregnancy, certain infectious diseases and the presence/levels of alcohol and some drugs of abuse. Such devices usually consist of a test strip or pad, lateral flow devices, cards, slides, flow-through tubes and cassettes with built-in meters and displays. Perhaps the most familiar example is the Clearblue Digital Pregnancy Test® by SPD Swiss Precision Diagnostics GmbH (Geneva, Switzerland),

which provides a pregnant/not pregnant readout and an indication of the number of weeks since conception.

The *in vitro* diagnostic market revenue is expected to reach US$75 billion by 2020. The USA is currently the largest market in the world at US$24 billion each year. Growth in this area reflects patient preferences for being seen in a doctor's office or clinic rather than a central laboratory. Since psychiatric disorders are becoming an increasing problem and will lead to the highest burden by the year 2020, more innovative LOC devices and connectivity solutions will be required that use low sample volumes, with less labor and time spent carrying out the test, and lower cost. In addition, large consumer market companies such as Apple and Google indicate growing interest in the diagnostic market, since opportunities exist to connect an app result with a diagnostic answer provided by smart software. Likewise, pharmaceutical companies now consider that low-cost and time-effective biomarkers are essential for decision-making in clinical trials and drug discovery efforts.

Mobile phone apps & neuropsychiatry

An important requirement of developing point-of-care devices revolves around the use of mobile communication and the internet so that data can be organized, interpreted, evaluated and formatted for simple presentation to the end users. This would allow testing using real-time, multiplexed sensors, combined with artificial intelligence and mobile communication systems. This is of high relevance to mental disorders, since these are long-term debilitating diseases that afflict people worldwide and require constant monitoring and treatment. There is also a movement toward the use of networked computing to aid disease prediction, diagnosis, prognosis and monitoring of medication compliance. The envisaged product involves creation of bioprofiles or biomarker fingerprints at the individual level that fuse genomics, proteomics and imaging data with patient histories.

In the 21st century, mobile phones have become nearly ubiquitous. At the end of 2011, there were a staggering 6 billion mobile phone subscriptions, equating to around 87% of the world population [34]. A rapid convergence in technology has led to emergence the smartphone, which combines basic voice and text messaging functions with computation technology that can support applications (apps), sensing and wireless internet access and connectivity with other devices. These features combined with their widespread usage make them an attractive platform for delivery of health promotion and disease management [35]. Basic mobile phone-based interventions have already shown promise and have led to improved outcomes in a variety of health conditions, diseases and habit controls. For example, the San Francisco-based company Ginger.io (CA, USA) [36] provides an app-based mental health program for people with depression and anxiety. This app allows individuals to easily analyze their behavioral patterns over time, adopt coping strategies designed by medical experts and even receive mental health support if required. In general, a recent review of trials involving healthcare interventions delivered by cell phones showed improvements in 61% of outcomes [37]. These included better attendance of patients at medical appointments, more rapid diagnosis and treatment, and improved communication, along with behavioral improvements such as smoking cessation and improved medication compliance, and clinical enhancements including improved blood sugar control, decreased symptoms of asthma and lower stress levels.

Some apps have already been developed which use molecular biomarkers to deliver the measured readout. For example, Niculescu *et al.* have developed biomarker-based apps for prediction of suicidality [38]. They propose that the eventual incorporation of these tests into healthcare assessments can lead to early disease intervention, allowing preventive lifestyle modifications or pharmacological treatment. Recent trends have seen the development of multiplex immunoassay based tests on a hand-held and cost-effective cell phone-based colorimetric microplate reader platform, which uses 3D-printed optomechanical attachments to illuminate each well on a 96-well plate via a light-emitting-diode optical fiber array [39]. The captured images are transmitted to centralized servers for processing and generation of diagnostic results, which can be delivered to the user within 1 min. Thus far, this mobile platform has been tested successfully in a clinical microbiology laboratory using mumps, measles and herpes simplex I and II virus immunoglobulin tests. As with all biomarker-related tests, considerable further work is required to ensure that only the most robust and reliable tests are developed on these platforms. In a similar manner, tests for other diseases such as psychiatric disorders should be developed and become available in the not so distant future.

Personalized medicine

This review has addressed the point that biomarker signatures identified in patient blood samples can be used to improve their lives by enabling personalized medicine approaches. This will allow persons to be treated based on their individual biomarker profiles rather than as one of many with a particular disease using a standard blockbuster drug. Most recently, such programs are now in their infancy in psychiatry as treatment of the frequent comorbidities in the patients.

In these cases, comorbidities can be identified by biomarker profiling as a means of stratifying individuals based on those who are likely to benefit most from the treatment. For example, the recent finding that some schizophrenia patients show more prominent changes in hormones and others show greater alterations in immune-related molecules [12] suggests that it may be possible to distinguish subgroups of patients based on the molecular profiles and then treat them accordingly to ease symptoms. However, it should be borne in mind that the following approaches may be valid only for specific subgroups of patients.

Targeting insulin resistance

It is now established that 30–50% of first onset schizophrenia patients have high insulin levels or increased insulin resistance [40–43] and many patients show similar effects after treatment with second-generation antipsychotics [44]. Thus, it is likely that schizophrenia patients who have high levels of insulin might benefit from add-on treatment with drugs that improve insulin action. One investigation described the use of the insulin-sensitizing agents metformin and rosiglitazone as a means of correcting the antipsychotic-induced insulin resistance often associated with this class of drugs and this did not hinder the psychiatric benefits [45]. This makes possible studies using biomarker testing for perturbed insulin signaling to stratify patients or monitor treatment responses or side effects (Figure 5). An approach along these avenues is already being tested for relief of memory deficits in patients with Alzheimer's disease and mild cognitive impairment using insulin-sensitizing agents as an alternative therapy [46]. One clinical trial of patients with mild Alzheimer's disease accompanied by Type 2 diabetes mellitus revealed that patients who were given pioglitazone tended to have improved cognition scores along with increased cerebral blood flow in specific brain regions, compared with patients who received a placebo [47]. In addition, drugs targeting other hormonal signaling pathways

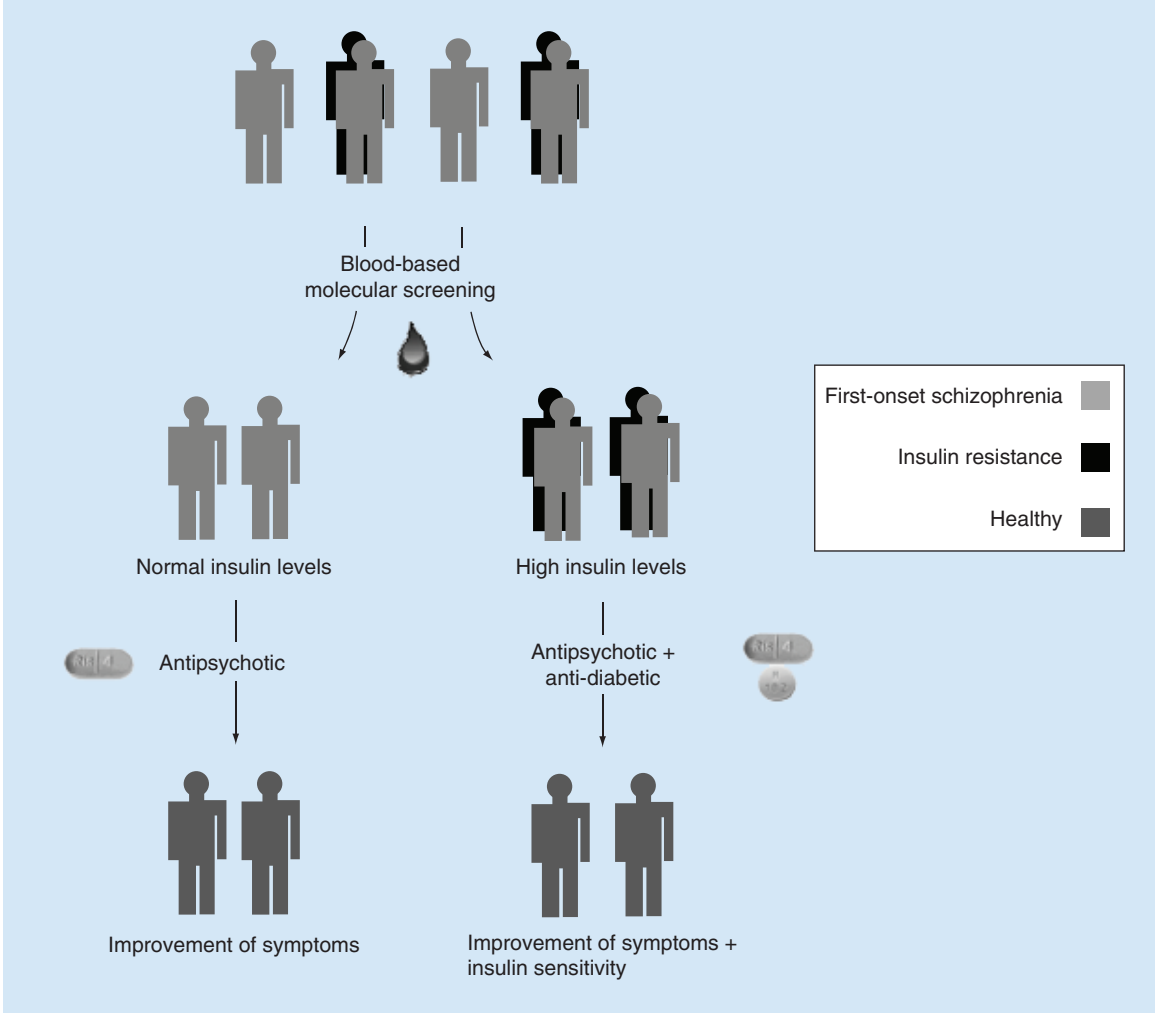

Figure 5. Personalized medicine strategy for targeting psychiatric patients who also suffer with insulin resistance.

have been tested as a novel means of treating specific symptoms of schizophrenia. For example, the adrenal steroid dehydroepiandrosterone was used as an add-on treatment in medicated schizophrenia patients, which led to an improvement in negative symptoms [48].

Targeting inflammation

It has also been possible to use anti-inflammatory drugs to treat those patients with perturbed immune or inflammatory biomarker profiles. This has been shown in studies that tested the use of COX-2 inhibitors as a treatment for schizophrenia symptoms [49,50]. Again, this also led to improved negative symptoms with a greater improvement found after treatment of recent onset patients with amisulpride and celecoxib compared with those treated with amisulpride and a placebo [50]. This suggests that initial screening of patients for biomarkers related to altered immune function, followed by treatment with an antipsychotic in combination with an anti-inflammatory drug, could lead to improved patient outcomes. As another example, aspirin given in combination with a traditional antipsychotic treatment resulted in reduced symptoms associated in patients with schizophrenia spectrum disorders [51] In addition, a recent meta-analysis of schziophrenia clinical studies showed that co-administration of nonsteroidal anti-inflammatory drugs (such as ibuprofen, naproxen and aspirin) with standard antipsychotics was effective for alleviating psychiatric symptoms as well as comorbid inflammation [52].

Biomarkers for prediction of response

Studies have shown that measurement of basic physical parameters such as waist circumference and body mass index, as well as circulating levels of triglycerides and high-density lipoproteins could be used to predict whether or not a patient will develop antipsychotic-induced metabolic syndrome or other insulin resistant states [53]. Thus, determination of these characteristics would be useful for stratification of patients who would be likely to benefit from adjunctive antidiabetic treatments, by helping to minimize metabolic side effects. Measurement of other molecular biomarkers may also prove to be useful for this purpose. A proteomic study carried out in 2012 found that schizophrenia patients with higher levels of serum prolactin have a better outcome in response to long-term treatment with antipsychotics [54]. Another serum biomarker profiling study carried out around the same time found that prolactin levels, along with those of fatty acid binding protein, ferritin, C-reactive protein, myoglobin, complement factor H and IL-16 could be used to predict an improvement in positive symptoms of schizophrenia following antipsychotic treatment and measurement of insulin and matrix metalloproteinase 2 levels could be used for prediction of improvements in negative symptoms [55]. The same study also found that low levels of the hormones insulin and leptin and high levels of the inflammatory growth factor TGF-β might be useful as a biomarker pattern for prediction of whether or not subjects would suffer from imminent relapse. Further work in this area will help to bring about the validation and deployment of the most robust molecular biomarker tests in the form of user friendly point-of-care devices.

Conclusion

There has been a recent shift toward the development of biomarker tests on rapid bench-top or handheld devices so that they can be used as point-of-care devices. However, there are still many challenges ahead. Most of these relate to the fact that we have only just skimmed the surface of the ocean of biomarkers that are likely to be useful for diagnostic purposes. This is mainly due to the fact that most biomarkers have a complex nature and are exceedingly low in abundance, and therefore are still not technologically accessible. Delving further into these 'deeper' biomarkers will most likely require the development of more reproducible and sensitive methods, as well as appropriate user friendly platforms to facilitate point-of-care testing. Furthermore, emerging biomarker candidates will have to be tested and retested before they can be considered for use in the clinic. Of course, this will require development of new technologies and a massive integration of these and existing ones. However, there is now reason to be optimistic that technological and interdisciplinary advancements in this area will help to usher the study of psychiatric disorders fully into the 21st century and thereby improve treatment and outcomes of those individuals who are afflicted by these debilitating disorders.

Future perspective

This review has described recent advances using blood-based proteomic biomarker tests which can be used for improved diagnosis and classification of individuals with psychiatric disorders, with the ultimate goal of providing more informed treatment strategies and, therefore, better patient care. The use of multiplex proteomic approaches also provides a means of deciphering the vast array of molecular pathways affected in specific psychiatric conditions as well as in different subtypes of the diseases. Eventually, this could lead to a more holistic view of the perturbed biological pathways in psychiatric diseases, which have now been established to affect multiple organ systems of the body and not just the brain. This can be seen by the identification of

distinctive proteomic biomarker profiles in body fluids such as cerebrospinal fluid, serum and plasma, as well as cells such as peripheral blood cells [56,57] and skin fibroblasts [58], and tissues such as postmortem pituitary glands [59,60]. Many patients show distinct patterns of circulating proteins and small molecules which are indicative of a dysfunctional immune or inflammatory system with elevated levels of inflammatory cytokines. Others have metabolic abnormalities such as altered HPA axis function, high insulin levels or insulin resistance. The finding that schizophrenia patients can have distinct changes in one or more of these pathways suggests that this disorder at least may be comprised of different biomarker-defined subtypes. However, it is possible that these different patterns do not represent distinct subtypes but may be simply different manifestations of the same underlying disorder. Nevertheless, better classification of patients based on such molecular profiles would enable stratification prior to treatment and this could be particularly useful for suggesting the type of add-on therapy required to achieve the best possible patient outcomes. Furthermore, the use of multiplex biomarker tests on credit card sized or handheld devices which are capable of distinguishing schizophrenia subtypes may be useful for rapid identification of patients who are most likely to respond to specific psychiatric medications either alone or in combination with other drugs. It obvious that such an approach could result in more effective treatment of patients with fewer side effects and, thus, a decreased proportion of patients who decide to discontinue medication because of severe side effects.

Stratification of individuals with psychiatric disorders using proteomic biomarker profiles to assign the right treatments to the right patients fits the remit of the movement toward personalized medicine approaches. This approach has already seen increasing use in the field of cancer treatment. For example, the measurement of HGF receptor 2 overexpression in breast cancer can be used to identify those who are most likely to benefit from treatment with Trastuzumab (also known as Herceptin), which was developed by Genentech (CA, USA) in the 1990s [61]. Similar efforts in other diseases such as psychiatric disorders would support the personalized medicine movement by helping to deconvolute the complexity of these diseases which have heretofore been based only on symptom characterization using the new paradigm which correctly identifies and targets the affected biological pathways.

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Executive summary

Background

- There is an urgent need for point-of-care clinical testing to improve diagnosis and treatment of individuals with psychiatric disorders.

What kind of biomarker tests should be developed?

- As blood-based test would be most amenable to clinical testing, platforms utilizing proteomic markers would be most appropriate.
- The highly dynamic nature of the proteome, compared with the genome and transcriptome, means that proteomic-based tests offer the closest real-time assessment of health or disease states.

Clinical impact of biomarkers

- Several proteomic blood-based biomarker panels have now been developed but all require extensive validation testing before they can be developed further for clinical use.
- To ensure maximum impact, biomarker test panels will require extensive testing and retesting before final deployment on point-of-care platforms.

Mobile phone apps & neuropsychiatry

- The emerging platforms most amenable to point-of-care testing incorporate multiplex immunoassay arrays embedded in lab-on-a-chip devices or those linked with smart phone apps.

Personalized medicine

- The ease of use and rapid testing protocols associated with these devices means that they can enable personalized medicine approaches in psychiatric disorders.
- Possible applications include point-of-care tests for early and accurate detection of disease, disease subtype classification and treatment prediction or response.

Future perspective

- This will lead to validated point-of-care tests that can be used in conjunction with traditional psychiatric evaluations to ensure the right patients receiving the right treatment in shortest possible time for the best possible patient outcomes.

conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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References

Papers of special note have been highlighted as:
• of interest; •• of considerable interest

1 Olesen J, Gustavsson A, Svensson M, Wittchen HU, Jönsson B. CDBE2010 study group. European Brain Council. The economic cost of brain disorders in Europe. *Eur. J. Neurol.* 19, 155–162 (2012).

• **The cost is a high burden on society and the healthcare services and reflects the current mediocre diagnosis and treatment of psychiatric patients.**

2 Mitchell AJ, Vaze A, Rao S. Clinical diagnosis of depression in primary care: a meta-analysis. *Lancet* 374, 609–619 (2009).

3 Bet PM, Hugtenburg JG, Penninx BW, Balkom Av, Nolen WA, Hoogendijk WJ. Treatment inadequacy in primary and specialized care patients with depressive and/or anxiety disorders. *Psychiatry Res.* 210, 594–600 (2013).

4 Martins-de-Souza D, Harris LW, Guest PC, Turck CW, Bahn S. The role of proteomics in depression research. *Eur. Arch. Psychiatry Clin. Neurosci.* 260, 499–506 (2010).

5 Taurines R, Dudley E, Grassl J *et al.* Proteomic research in psychiatry. *Psychopharmacology* 25, 151–196 (2011).

6 Schwarz E, Guest PC, Rahmoune H *et al.* Identification of a biological signature for schizophrenia in serum. *Mol. Psychiatry* 17, 494–502 (2012).

7 Papakostas GI, Shelton RC, Kinrys G *et al.* Assessment of a multi-assay, serum-based biological diagnostic test for major depressive disorder: a pilot and replication study. *Mol. Psychiatry* 18, 332–339 (2013).

• **The first study to identify a blood-based biomarker panel for depression.**

8 Salmon SE, Mackey G, Fudenberg HH. "Sandwich" solid phase radioimmunoassay for the quantitative deterimination of human immunoglobulins. *J. Immunol.* 103, 129–137 (1969).

9 Salmon SE, Smith BA. Sandwich solid phase radioimmunoassays for the characterization of human immunoglobulins synthesized *in vitro*. *J. Immunol.* 104, 665–672 (1970).

10 Khan SS, Smith MS, Reda D, Suffredini AF, McCoy JP Jr. Multiplex bead array assays for detection of soluble cytokines: comparisons of sensitivity and quantitative values among kits from multiple manufacturers. *Cytometry B Clin. Cytom.* 61, 35–39 (2004).

11 Domenici E, Willé DR, Tozzi F *et al.* Plasma protein biomarkers for depression and schizophrenia by multi analyte profiling of case-control collections. *PLoS ONE* 5, e9166 (2010).

12 Schwarz E, van Beveren NJ, Ramsey J *et al.* Identification of subgroups of schizophrenia patients with changes in either immune or growth factor and hormonal pathways. *Schizophr. Bull.* 40, 787–795 (2014).

13 van Beveren NJ, Schwarz E, Noll R *et al.* Evidence for disturbed insulin and growth hormone signaling as potential risk factors in the development of schizophrenia. *Transl. Psychiatry* 4, e430 (2014).

14 Haenisch F, Alsaif M, Guest PC *et al.* Multiplex immunoassay analysis of plasma shows prominent upregulation of growth factor activity pathways linked to GSK3β signaling in bipolar patients. *J. Affect. Disord.* 156, 139–143 (2014).

15 Haenisch F, Alsaif M, Guest PC *et al.* Multiplex immunoassay analysis of plasma shows differences in biomarkers related to manic or mixed mood states in bipolar disorder patients. *J. Affect. Disord.* 185, 12–16 (2015).

16 Podlipný J, Hess Z, Vrzalová J, Rosolová H, Beran J, Petrlová B. Lower serum levels of interleukin-6 in a population sample with symptoms of depression than in a population sample without symptoms of depression. *Physiol. Res.* 59, 121–126 (2010).

17 Stelzhammer V, Haenisch F, Chan MK *et al.* Proteomic changes in serum of first onset, antidepressant drug-naive major depression patients. *Int. J. Neuropsychopharmacol.* 17, 1599–1608 (2014).

18 Schwarz E, Guest PC, Rahmoune H *et al.* Sex-specific serum biomarker patterns in adults with Asperger's syndrome. *Mol. Psychiatry* 16, 1213–1220 (2011).

19 Ramsey JM, Guest PC, Broek JA *et al.* Identification of an age-dependent biomarker signature in children and adolescents with autism spectrum disorders. *Mol. Autism* 4, 27 (2013).

20 Chan MK, Krebs MO, Cox D *et al.* Development of a blood-based molecular biomarker test for identification of schizophrenia before disease onset. *Transl. Psychiatry* 5, e601 (2015).

•• **The first large-scale study to show that biomarkers for schizophrenia were present in at-risk individuals before disease onset.**

21 Homola J. Surface plasmon resonance sensors for detection of chemical and biological species. *Chem. Rev.* 108, 462–493 (2008).

22 Mariani S, Minunni M. Surface plasmon resonance applications in clinical analysis. *Anal. Bioanal. Chem.* 406, 2303–2323 (2014).

23 Chen P, Chung MT, McHugh W *et al.* Multiplex serum cytokine immunoassay using nanoplasmonic biosensor microarrays. *ACS Nano* 9, 4173–4181 (2015).

24 Schumacher S, Nestler J, Otto T *et al.* Highly-integrated lab-on-chip system for point-of-care multiparameter analysis. *Lab. Chip* 12, 464–473 (2012).

•• **A breakthrough study showing the development of credit card-sized lab-on-a-chip platforms to enable point-of-care testing.**

25 Stewart WF, Ricci JA, Chee E, Hahn SR, Morganstein D. Cost of lost productive work time among US workers with depression. *JAMA* 289, 3135–3144 (2003).

26 Greer TL, Kurian BT, Trivedi MH. Defining and measuring functional recovery from depression. *CNS Drugs* 24, 267–284 (2010).

27 OECD Publishing.*Sick on the Job?: Myths and Realities about Mental Health and Work, Mental Health and Work.* Organisation for Economic Cooperation & Development. ISBN-10: 9264124519 (2012).

28 Mitchell AJ, Vaze A, Rao S. Clinical diagnosis of depression in primary care: a meta-analysis. *Lancet* 374, 609–619 (2008).

29 Bet PM, Hugtenburg JG, Penninx BW, Balkom Av, Nolen WA, Hoogendijk WJ. Treatment inadequacy in primary and specialized care patients with depressive and/or anxiety disorders. *Psychiatry Res.* 210, 594–600 (2013).

30 Mental well-being: For a smart, inclusive and sustainable Europe. EC Report. http://ec.europa.eu

31 Fochtmann LJ, Gelenberg A. Guideline watch: practice guideline for the treatment of patients with major depressive disorder. www.pshpgeorgia.com

32 Papakostas GI, Fava M. Does the probability of receiving placebo influence clinical trial outcome? A meta-regression of double-blind, randomized clinical trials in MDD. *Eur. Neuropsychopharmacol.* 19, 34–40 (2009).

33 Gaynes BN, Warden D, Trivedi MH, Wisniewski SR, Fava M, Rush AJ. What did STAR*D teach us? Results from a large-scale, practical, clinical trial for patients with depression. *Psychiatr. Serv.* 60, 1439–1445 (2009).

34 mobiForge website. http://mobithinking.com

35 Klasnja P, Pratt W. Healthcare in the pocket: mapping the space of mobile-phone health interventions. *J. Biomed. Inform.* 45, 184–198 (2012).

36 Ginger.io. https://ginger.io

37 Krishna S, Boren SA, Balas EA. Healthcare via cell phones: a systematic review. *Telemed. J. E. Health* 15, 231–240 (2009).

38 Niculescu AB, Levey DF, Phalen PL *et al.* Understanding and predicting suicidality using a combined genomic and clinical risk assessment approach. *Mol. Psychiatry* 20, 1266–1285 (2015).

•• **A breakthrough study describing the development of biomarkers combined with a mobile phone app for suicidality.**

39 Berg B, Cortazar B, Tseng D *et al.* Cellphone-based hand-held micro-plate reader for point-of-care testing of enzyme-linked immunosorbent assays. *ACS Nano* 9, 7857–7866 (2015).

•• **A study describing a useful interface of biomarker plates with mobile phone app readouts.**

40 Ryan MC, Collins P, Thakore JH. Impaired fasting glucose tolerance in first-episode, drug-naive patients with schizophrenia. *Am. J. Psychiatry* 160, 284–289 (2003).

41 Spelman LM, Walsh PI, Sharifi N, Collins P, Thakore JH. mpaired glucose tolerance in first-episode drug-naive patients with schizophrenia. *Diabet. Med.* 24, 481–485 (2007).

42 Guest PC, Wang L, Harris LW *et al.* Increased levels of circulating insulin-related peptides in first-onset, antipsychotic naïve schizophrenia patients. *Mol. Psychiatry* 15, 118–119 (2010).

43 Guest PC, Schwarz E, Krishnamurthy D *et al.* Altered levels of circulating insulin and other neuroendocrine hormones associated with the onset of schizophrenia. *Psychoneuroendocrinology* 36, 1092–1096 (2011).

44 Pramyothin P, Khaodhiar L. Metabolic syndrome with the atypical antipsychotics. *Curr. Opin. Endocrinol. Diabetes Obes.* 17, 460–466 (2010).

45 Bahtiyar G, Weiss K, Sacerdote AS. Novel endocrine disrupter effects of classic and atypical antipsychotic agents and divalproex: induction of adrenal hyperandrogenism, reversible with metformin or rosiglitazone. *Endocr. Pract.* 13, 601–608 (2007).

• **Shows that antipsychotic-induced insulin resistance can be ameliorated in schizophrenia patients by cotreatment with antidiabetic agents.**

46 Sato T, Hanyu H, Hirao K, Kanetaka H, Sakurai H, Iwamoto T. Efficacy of PPAR-gamma agonist pioglitazone in mild Alzheimer disease. *Neurobiol. Aging* 32, 1626–1633 (2011).

47 Landreth G, Jiang Q, Mandrekar S, Heneka M. PPARgamma agonists as therapeutics for the treatment of Alzheimer's disease. *Neurotherapeutics* 5, 481–489 (2008).

48 Nachshoni T, Ebert T, Abramovitch Y *et al.* Improvement of extrapyramidal symptoms following dehydroepiandrosterone (DHEA) administration in antipsychotic treated schizophrenia patients: a randomized, double-blind placebo controlled trial. *Schizophr. Res.* 79, 251–256 (2005).

49 Müller N, Riedel M, Schwarz MJ. Psychotropic effects of COX-2 inhibitors – a possible new approach for the treatment of psychiatric disorders. *Pharmacopsychiatry* 37, 266–269 (2004).

50 Akhondzadeh S, Tabatabaee M, Amini H, Ahmadi Abhari SA, Abbasi SH, Behnam B. Celecoxib as adjunctive therapy in schizophrenia: a double-blind, randomized and placebo-controlled trial. *Schizophr. Res.* 90, 179–185 (2007).

51 Müller N, Krause D, Dehning S *et al.* Celecoxib treatment in an early stage of schizophrenia: results of a randomized, double-blind, placebo-controlled trial of celecoxib augmentation of amisulpride treatment. *Schizophr. Res.* 121, 118–124 (2010).

52 Bumb JM, Enning F, Leweke FM. Drug repurposing and emerging adjunctive treatments for schizophrenia. *Expert Opin. Pharmacother.* 16, 1049–1067 (2015).

53 Jin H, Meyer J, Mudaliar S *et al.* Use of clinical markers to identify metabolic syndrome in antipsychotic-treated patients. *J. Clin. Psychiatry* 71, 1273–1278 (2010).

54 Shrivastava A, Johnston M, Bureau Y, Shah N. Baseline serum prolactin in drug-naive, first-episode schizophrenia and outcome at five years: is it a predictive factor? *Innov. Clin. Neurosci.* 9, 17–21 (2012).

55 Schwarz E, Guest PC, Steiner J, Bogerts B, Bahn S. Identification of blood-based molecular signatures for prediction of response and relapse in schizophrenia patients. *Transl. Psychiatry* 2, e82 (2012).

- **Identifies a blood-based molecular biomarker panel for prediction of response to antipsychotics in first onset schizophrenia patients.**

56 Wang L, Lockstone HE, Guest PC *et al.* Expression profiling of fibroblasts identifies cell cycle abnormalities in schizophrenia. *J. Proteome Res.* 9, 521–527 (2010).

57 Herberth M, Koethe D, Cheng TM *et al.* Impaired glycolytic response in peripheral blood mononuclear cells of first-onset antipsychotic-naive schizophrenia patients. *Mol. Psychiatry* 16, 848–859 (2011).

58 Freudenreich O, Brockman MA, Henderson DC *et al.* Analysis of peripheral immune activation in schizophrenia using quantitative reverse-transcription polymerase chain reaction (RT-PCR). *Psychiatry Res.* 176, 99–102 (2010).

59 Stelzhammer V, Alsaif M, Chan MK *et al.* Distinct proteomic profiles in post-mortem pituitary glands from bipolar disorder and major depressive disorder patients. *J. Psychiatr. Res.* 60, 40–48 (2015).

60 Krishnamurthy D, Harris LW, Levin Y *et al.* Metabolic, hormonal and stress-related molecular changes in post-mortem pituitary glands from schizophrenia subjects. *World J. Biol. Psychiatry* 14, 478–489 (2013).

61 Demonty G, Bernard-Marty C, Puglisi F, Mancini I, Piccart M. Progress and new standards of care in the management of HER-2 positive breast cancer. *Eur. J. Cancer* 43, 497–509 (2007).