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The Emerging Science of Precision Medicine and Pharmacogenomics for Parkinson's Disease

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Abstract

Current therapies for Parkinson's disease are problematic because they are symptomatic and have adverse effects. New drugs have failed in clinical trials due to inadequate efficacy. At the core of the problem is trying to make one drug work for all Parkinson's disease patients, when we know this premise is wrong because (a) Parkinson's disease is not a single disease, and (b) no two individuals have the same biological make up. Precision medicine is the goal to strive for, but we are only at the beginning stages of building the infrastructure for one of the most complex projects in the history of science, and it will be a long time before Parkinson's disease reaps the benefits. Pharmacogenomics, a cornerstone of precision medicine, has already proven successful for many conditions, and could also propel drug discovery and improve treatment for Parkinson's disease. To make progress in pharmacogenomics of Parkinson's disease, we need to change course from small inconclusive candidate gene studies to large-scale rigorously planned genome-wide studies that capture the nuclear genome and the microbiome. Pharmacogenomics studies must use homogenous subtypes of Parkinson's disease, or apply brute force of statistical power to overcome heterogeneity which will require large sample sizes achievable only via internet-based methods and electronic databases. Large-scale pharmacogenomics studies, together with biomarker discovery efforts, will yield the knowledge necessary to design clinical trials with precision, to alleviate confounding by disease heterogeneity and inter-individual variability in drug response, two of the major impediments to successful drug discovery and effective treatment.

Keywords

Parkinson's disease; Precision Medicine; Pharmacogenomics; Pharmacogenetics; Microbiome

The challenge

Comprehensive treatment for Parkinson's disease (PD) would entail prevention before onset of symptoms, disease modifying treatment to stop the progressive neurodegeneration for those who develop disease, and therapies to alleviate symptoms that are already present

without creating further complications. We are far from this goal. Currently, there is no preventative or disease modifying treatment. We only have drugs that ease some of the symptoms, but they also cause unwanted complications.

Current drugs

Levodopa (L-DOPA) and other dopaminergic drugs used today ease the motor symptoms but have limited effects on non-motor features, which are common and involve autonomic and sensory systems, sleep, mood, behavior, and cognition. None of the current drugs can slow or stop neurodegeneration or halt clinical progression of symptoms. As the disease progresses, drug efficacy decreases, requiring higher and more frequent doses, which increase the risk of developing more serious side effects (see ¹⁻³ for review). There is wide inter-individual variation in both the efficacy and toxicity of dopaminergic drugs^{4, 5}. L-DOPA and dopamine agonists can cause psychosis and other behavioral problems, and about half of patients develop dyskinesia within the first five years of using L-DOPA ⁶. The COMT-inhibitor tolcapone, which is used to ameliorate the motor complications of L-DOPA, can itself cause gastrointestinal problems⁷ and liver toxicity ⁸. We do not understand and therefore cannot predict the inter-individual variability in drug efficacy and toxicity.

Disease modifying and neuroprotective drugs

Many compounds with promising disease modifying potential have been pursued for treatment of PD, but so far, none have been approved for clinical use ¹. The most common cause for failing regulatory approval is lack of sufficient efficacy in clinical trial ^{1, 9}, a problem that may arise from several sources. In some cases, the pre-clinical evidence for the drug may be misleading due to inaccuracy or irrelevance of animal models to human disease. Another source of impediment is the limitations in the design of clinical trial. Although clinical trials try to enroll patients at early stages of disease, it may still be too late because about 80% of dopaminergic neurons are already gone by the time disease is diagnosed and virtually all are gone within the first four years of disease¹⁰. Efforts are underway to identify biomarkers for early detection¹¹. Even if the pre-clinical (animal model) data for the drug are robust, and, the trial is enriched with very early disease, the drug could still fail if trial design is too simplistic for the complexities of the disease. There is extensive heterogeneity in the pathogenesis of PD as evidenced by dozens of genes and several environmental risk factors that are known so far. Consider for example PD due to α -synuclein over expression vs. *LRRK2*G2019S mutation vs. head trauma vs. pesticide exposure, and add to the mix the genotypic combination of over 20 PD-associated susceptibility loci: a drug that works for one may not work for other subtypes. Even if the drug is targeted to a downstream pathway where different etiologies might converge (e.g., dopamine loss), or a mechanism that might be key to neurodegeneration irrespective of the cause (e.g., inflammation), there will still be inter-individual variability related to the target pathway, as well as inter-individual variability in drug response. Clinical trials often ignore genetics, they enroll patients irrespective of the genetic heterogeneity in disease etiology or the genetic heterogeneity in drug response, and measure efficacy as an average for all patients. A drug that may be effective for some patients would be missed because the effect would be diluted in the mixed population.

Precision Medicine & Pharmacogenomics

Precision medicine strives to promote health, disease prevention and effective treatment by embracing the unique biological, environmental and life style characteristics of each individual¹². President Obama announced the Precision Medicine Initiative (PMI) in 2015¹² and NIH awarded \$55 million in fiscal year 2016 to build the infrastructure (<https://www.nih.gov/research-training/allofus-research-program>). The federal initiative and NIH allocation of funds are now at risk of severe cuts or elimination due to the recent drastic shift in political attitudes towards advancement of science¹³.

In NIH's own words, PMI is one of the most ambitious research programs in history. The initiative intends to enroll 1 million individuals, obtain consent to access their clinical data through electronic medical records, collect blood and urine to generate genetic and other biological information, and longitudinally gather health, lifestyle and environmental data via mobile apps in real time. Research participants will be enrolled both directly via mobile apps, and through existing programs, including the Million Veteran Program, which has already enrolled 500,000 US veterans to study genes and health (<http://www.research.va.gov/MVP/>). PMI is currently building the infrastructure which includes the network of Health Care Provider organizations, a Participant Technologies Support Center, a Biobank, and a Data and Research Support Center. It is hoped that PMI can eventually partner with similar programs across the world. PMI holds great promise for revolutionizing data collection and accelerating discovery. Our concern is the wait time for PD to reap the benefits.

To fully realize the PMI vision, the program would entail obtaining and analyzing print outs of the genomes (nuclear, mitochondrial and microbiome), transcriptomes, epigenomes, proteomes, metabolomes, physiomes and exposomes, for the past and in real-time. The multi-scale data will be used to first discover the predictive algorithms across the population, and then translate the findings to public health and clinical practice¹⁴. Unimaginable only a few years ago, the vision is technically within reach: comprehensive omics assessment tools are available, so are wearable biosensors and smart phone apps that can track physiological metrics and environmental elements from brain waves to quality of the drinking water. While we have the advanced technology, we still face several challenges. One is securing public's interest to take an active role in research and in management of their own health care, as well as their trust to share their personal data. Cost is an issue for the research component, for translation, and for implementation in clinical practice. Data analysis and interpretation will be particularly challenging for a complicated disease like PD because formulating prevention and treatment will entail identifying and deciphering the interactive effects of many yet unknown factors from across the multi-scale data. While precision medicine in its full scale may not be in the immediate future for PD, pharmacogenomics, a cornerstone of precision medicine¹⁵, has already proven successful for many conditions and drugs^{16, 17}, and is gaining momentum in PD.

Pharmacogenomics is the study of how genes affect a person's response to medication, so that treatment can be tailored to the genetic makeup of each person to achieve maximum benefit with minimum side effects. For drugs that are on the market, genotype will enable

healthcare providers to quickly and accurately determine suitability of each drug for each person and devise a personalized treatment plan. For new investigational drugs, predictive genotyping can help select the appropriate patient groups for clinical trials, enriching for positive responders, hence boosting the chances of successful drug development. To ensure that sufficient DNA is available for pharmacogenetics clinical trials, the drug regulation agencies in the US (FDA) and Europe (EMA) strongly encourage collection and banking of DNA samples from all participants in all arms of all clinical trials¹⁸. Pharmacogenomics, although a relatively new field, has already produced genetic predictors of drug response that are being used in the clinic for treatment of cancer, heart disease, pain, depression, autoimmune disease, immunosuppression and HIV infection¹⁶.

Candidate gene studies: Pharmacogenetics

Pharmacogenetics (as opposed to pharmacogenomics) refers to the study of candidate genes, one or a few genes at a time, in relation to drug response. The most obvious and extensively studied candidates for pharmacogenetics in general are the cytochrome P450 family, which metabolize over 50 known drugs¹⁹, and the major histocompatibility complex genes, *HLA*, for immunologically mediated drug response²⁰. Examples of successful candidate gene studies that are FDA approved and in clinical use include *CYP2D6* genotyping for administering tamoxifen for treatment of cancer and for prescribing codeine for pain management²¹, and *HLA-B* genotyping to predict hypersensitivity to Abacavir for treatment of HIV-infection²². For the list of actionable pharmacogenetic markers see reference 16¹⁶.

Candidate gene association studies are hypothesis-driven, and can be very powerful if the investigator happens to pick the right gene to study. Ironically, successful discoveries via candidate gene studies are rare. Most candidate gene studies have produced inconsistent results. The problem often arises from the inherent bias of hypothesis-driven study: the investigator chooses a gene that, based on known biology, would offer an attractive explanation for the outcome that is being tested. In turn, when data arrives, a positive result seems more believable because of the biological plausibility, and statistical rigor becomes less important. The genetics community recognized over a decade ago that candidate gene studies were flooding the literature with irreproducible results. In response, measures were taken to ensure rigor. Genetics journals require sufficiently large sample sizes to ensure power, multiple testing correction, and independent replication as pre-requisite for publication. Unfortunately, studies that are conducted in clinical and epidemiological setting (which most pharmacogenetics studies are) rarely adopt the recommended criteria for rigor. Continued publication of small studies adds confusion, instead of clarity, to an already messy literature. Pharmacogenetic studies of PD exemplify this problem.

There have been over 50 papers on PD pharmacogenetics and all have produced inconsistent results for all genes investigated². There are several review articles on pharmacogenetics of PD, one in particular² presents an easy viewing of the results from each of the 50+ studies for every gene that was tested. Briefly, most studies examined polymorphisms in PD drug metabolism and action: dopa decarboxylase (*DDC*) which transforms L-DOPA to dopamine, dopamine transporters (*DAT1* or *SLC6A3*), dopamine receptors (*DRD1*, *DRD2*, *DRD3*, *DRD4*, *DRD5*), and monoamine oxidase (*MAO-B*) and catechol-O-methyltransferase

(*COMT*) which degrade dopamine. Some found evidence for association of a genetic variant with a measure of drug response, others did not. Positive findings were dubious because each study often tested numerous genetic variants against various drug response outcomes, tallying up the number of tests without correcting for multiple testing, which is a common source of false positive findings. Negative findings were also inconclusive because most studies were too small and underpowered. To further complicate the problem, it is difficult to synthesize the collective literature on any gene (i.e. Meta-analysis) because studies varied in the patient populations that were studied and the outcomes that they measured. Here I will use the two most recently published studies (not captured in published reviews) for illustration.

Moreau et al. recently reported a study entitled “Polymorphism of the dopamine transporter type 1 gene modifies the treatment response in Parkinson’s disease”²³. The study makes an exciting case: they show data that variation in a highly plausible gene (*SLC6A3*, dopamine transporter 1) is associated with efficacy of L-DOPA and methylphenidate for motor symptoms, with impressive looking statistics ($P \sim 0.0001$), and propose “Our results suggest that to better relieve gait disorders, it may be worth increasing the dose of L-DOPA in patients harboring the *SLC6A3* rs3836790 6/6 or *SLC6A3* rs28363170 10/10 genotypes”. That a well-known gene with extreme biological plausibility would have high impact for treatment of PD is so compelling that it could easily overshadow the limitations of the study. But are data compelling enough to put patients on high doses of L-DOPA? Let us look at the study design: sample size was 28 patients for methylphenidate response and 61 patients for L-DOPA response. In this small cohort, 6 variants in four genes (*DDC*, *SLC6A3*, *COMT*, *MAOB*) were tested for response to 2 drugs (L-DOPA, methylphenidate), in two conditions (OFF and ON), with 4 outcomes each (UPDRS III score, number of steps, completion time, freezing of gait), without multiple testing correction or replication. Authors state that the sample size was too small to correct for multiple testing, which insinuates that the seemingly impressive P values were not significant after correction. They also call for replication in independent cohorts, when there were already at least 5 published studies of *SLC6A3* and response to L-DOPA with conflicting outcomes², and another negative study has since followed²⁴.

This latest study by Masellis et al.,²⁴ entitled “Dopamine D2 receptor gene variants and response to rasagiline in early Parkinson’s disease: a pharmacogenetic study”, raises the bar for rigor in study design and execution. The study was leveraged on a recently completed placebo-controlled clinical trial of rasagiline, an MAOB inhibitor, which had banked DNA and clinical data on 692 study participants (largest N in comparable studies). Patients were newly-diagnosed and untreated at the start of the study, and rasagiline was used as monotherapy (avoiding confounding by multiple medications). The primary outcome was the change in total UPDRS score from baseline to weeks 12, 24 and 36 after start of rasagiline or placebo. Standard rigorous quality control practices for genetic association were followed. Statistical analysis appropriately included assessment of assumptions and covariates in model building, controlling for the placebo-effect, *a priori* power calculations, and post-analysis multiple testing correction. Response to rasagiline was associated with variation in a non-coding regulatory sequence within the *DRD2* gene: one genotype resulted in a reduction in UPDRS score at 12 weeks, the alternate genotype did not. (The association

of *SLC6A3* with L-DOPA in the abovementioned study²³ did not replicate). While by far the most rigorous of pharmacogenetic studies of PD to date, the authors point out two limitations for their study: lack of genome-wide analysis and lack of an equally well-defined dataset for replication.

Genome-wide studies: Pharmacogenomics

The unbiased, hypothesis-free nature of genome-wide studies makes it possible to identify the most significant markers, be it a well-known gene or one that was unsuspected. Take for example drug-induced hepatotoxicity, which was long believed to be linked to cytochrome P450 genes and there are over a thousand publications on cytochrome P450 and hepatotoxicity. Then came GWAS on drug-induced hepatotoxicity conducted for three different drugs: Flucloxacillin (antibiotic)²⁵, Lumiracoxib (anti-inflammatory)²⁶ and Ximelagatran (anticoagulant)²⁷, and all revealed *HLA* polymorphisms (not cytochrome P450) as the most significant predictor of hepatotoxicity. Another example is the discovery of interleukin 28B gene as the predictor of drug response in treating hepatitis C in three independent GWAS conducted in three different populations^{28–30}. Despite the clear advantages of genome-wide studies, there have been far fewer pharmacogenomics studies than candidate gene studies, because they cost more, require larger sample sizes, and demand a more sophisticated study design and execution.

There have been two pharmacogenomics studies for PD. They were both hypothesis-free genome-wide association studies (GWAS), conducted on the same dataset (1,458 persons with PD and 931 healthy controls from the NeuroGenetics Research Consortium), in search of variants that interact with the protective effects of caffeine³¹ and smoking³² on PD risk. Smoking/nicotine and caffeine were chosen for these studies because they are robustly associated with reduced risk of PD in epidemiological studies^{33, 34}, have shown neuroprotective properties in animal models^{35, 36}, and have been pursued in clinical trials for treatment of PD with variable results^{37–39}.

GWAS of a gene-caffeine interaction on PD risk identified a block of variants that mapped to the *GRIN2A* gene³¹. *GRIN2A* encodes the NMDA receptor 2A subunit that binds glutamate. The finding was genome-wide significant after multiple testing correction, and has been replicated in several studies with some evidence for heterogeneity (summarized in⁴⁰). The variants that rose to significance in the GWAS mapped inside *GRIN2A* and are known to associate with methylation levels of *GRIN2A*, which suggests a regulatory role in expression levels of the glutamate receptor⁴¹. While the *GRIN2A* gene was identified based on statistical significance alone, with no hypothesis and no prior evidence for a genetic link with PD, there was already extensive literature on the role of *GRIN2A* in the central nervous system^{42–44} and the potential relevance of its function in PD^{45–47}. The existing evidence-based knowledge can be mined to formulate and test the mechanism of neuroprotection by coffee and the mediating effect of *GRIN2A*. One potential unifying mechanism would be the following: Over activation of glutamate receptor, encoded by *GRIN2A*, causes a massive influx of calcium and triggers a cascade of signaling that ends in neuronal death⁴⁸. When cells are stressed, adenosine levels increase, and activated adenosine receptors induce glutamate release, which then feeds back and exacerbates glutamate toxicity. Caffeine is an

adenosine receptor antagonist; it counteracts adenosine, reduces glutamate levels, and prevents the neurodegeneration that ensues from activation of glutamate receptors⁴⁹. Two studies have carried forward *GRIN2A* evaluation into pharmacogenetic studies. A phase 3 placebo-controlled clinical trial of creatine for the treatment of PD reported a deleterious interaction between caffeine and creatine which was dependent on the *GRIN2A* genotype⁵⁰. The second study reported an association between *GRIN2A* genotype and L-DOPA-induced dyskinesia⁵¹. Clinical trials of caffeine for treatment of PD have reported either beneficial effects^{37–39} or no effect on rate of disease progression⁵². Pharmacogenetics studies are underway to determine if the variable results of caffeine could be explained by *GRIN2A* genotype.

GWAS of a gene-smoking interaction on PD risk identified the *SV2C* gene³², which encodes the synaptic vesicle protein 2C. The finding was corroborated by genome-wide gene expression studies in a paraquat-induced *Drosophila* model of PD which revealed interaction between beneficial effect of nicotine and the *Drosophila* homologue of *SV2C* against paraquat toxicity³². Replication in humans has not yet been attempted. At the time of this GWAS finding, little was known about *SV2C* except that it was highly expressed in substantia nigra and that it might be involved in dopamine release. The discovery of *SV2C* in GWAS prompted functional studies to decipher its role in PD⁵³ and pharmacogenetic studies to test it as a candidate marker for drug response⁵⁴. Functional studies found that disrupted expression of *SV2C* is a unique pathological feature of PD; it causes reduced dopamine release in the dorsal striatum, reduced striatal dopamine content, disrupted α -synuclein expression, abnormal motor function in rodents, and alterations in neurochemical effects of nicotine⁵³. A clinical study examined *SV2C* and other biological variables as potential markers for L-DOPA dose response and adverse effects⁵⁴. The original GWAS had revealed a beneficial effect of *SV2C*rs30196_C allele in interaction with smoking, the lowest PD risk was seen in individuals who had two copies of C, and intermediate risk reduction in individuals with one C. A similar additive pattern was seen in the clinical study: presence of each C allele significantly reduced L-DOPA dose by ~76 mg/day, suggesting that individuals who have one or two copies of the rs30196_C allele require lower doses of medication⁵⁴.

Microbiome

Microbiome refers to the collective genomes of the microorganisms living within our body. Sometimes called the second genome, the microbiome is 100x larger than the human genome, and drives pathways that are essential to human health ranging from digesting food and producing vitamins, to metabolizing drugs and degrading toxins, maintaining structural integrity of the lining of the gut, maintaining the brain-gut axis equilibrium, and shaping and maintaining the immune system. The microbiome affects host biology partly through the activity of microbial genes, and partly by manipulating the expression of human genes (of note, microbiome influences expression and activity of cytochrome P450 genes⁵⁵). Dysbiosis of the gut microbiome has been noted in many disorders⁵⁶, including PD^{57–62}. The effect seen in PD was highly significant independently of the confounding effects of medications, diet, gastrointestinal symptoms (including constipation), sex, age and demographics⁵⁷.

In a study of nearly 4,000 individuals aimed at identifying variables with the most profound effects on the gut microbiome, medication was found to have the largest effect size on the total variance⁶³. The effect size on microbiome was larger for medication than it was for parameters that defined blood, bowel, diet, health, anthropometrics, or life style⁶³. There is a two-way interaction between drugs and microbiome⁶⁴. The microbiome facilitates the metabolism of prescription drugs (directly via microbial metabolites and indirectly by affecting host xenobiotic metabolism) and can therefore affect efficacy and toxicity of medications^{65, 66}. In turn, medications could alter the composition of the microbiome (antibiotics is the classic case), cause dysbiosis, and compromise health^{63, 67, 68}.

Two studies have examined PD medications in relation to the gut microbiome. The first study reported altered abundance of some taxa in individuals taking COMT-inhibitors⁶⁰. The second study confirmed the dysbiosis associated with COMT-inhibitors, and in addition, found significant changes as a function of treatment with anticholinergics, and a borderline significant difference between patients taking L-DOPA vs. untreated patients⁵⁷. The effects of COMT-inhibitor and anticholinergics on the microbiome were each significant independently of disease status (only patients were studied), other medications, diarrhea (a side effect of COMT-inhibitors), sex, age, diet, and demographics⁵⁷. The studies were small and exploratory and the findings were by no means conclusive. But there is enough evidence from the general literature on drug-microbiome interaction, and the early data on PD to warrant sufficiently powered and properly designed studies to explore the dual interaction of microbiome and PD drugs. Does the pre-treatment inter-individual variability in microbiome determine efficacy and toxicity of medication? Does the medication change the microbiome and cause adverse effects? Depending on the answers, microbiome may prove to be a useful biomarker for drug development and pharmacogenomics-guided treatment.

Moving forward

How do we address the scattered candidate gene data on efficacy and toxicity of various PD drugs? One option is for the groups to come together, agree on a focused study design (patient selection criteria, polymorphism, medication, and outcome), pool their data, and if they have enough analytic power for discovery and replication, re-test the candidate genes. The other option is to switch to hypothesis-free genome-wide studies and let the truly significant markers emerge on their own merit.

What if the best pharmacogenetic markers are in unsuspected regions of the genome? Yet another reason to switch to hypothesis-free genome-wide studies. GWAS must be anchored on carefully defined homogenous subtypes of PD, or have very large sample sizes to overcome heterogeneity with the brute force of statistical power.

How do we optimize patient selection for new drug development? There are two elements here, the patients and the drugs. (i) For patients, we need to pay attention to genetic and environmental heterogeneity in the etiology of PD and stratify accordingly. Use biomarkers that may differentiate patient groups and predict disease progression and outcome for this purpose. The Parkinson's Progression Marker Initiative (PPMI) is a large-scale effort sponsored by Michael J Fox Foundation dedicated to this mission¹¹. A group of renowned

movement disorder specialists recently challenged the PPMI's design, and instead propose that the next decade be spent on an exploratory, unbiased, non-hypothesis-driven approach with a reverse analytic plan to that applied in PPMI ⁶⁹. The dialogue is a good sign that the leaders of the field are becoming increasingly engaged and applying different approaches to tackle the problem. (ii) For drugs, it would be helpful to find biomarkers for efficacy and toxicity. As recommended by the FDA, DNA should be banked for all subjects in every clinical trial, towards gaining large sample sizes for gene-drug studies. I propose that in addition to DNA, we bank stool samples for microbiome studies, collect a standard set of key demographic, environmental and behavioral variables, and obtain consent to re-contact the subjects as new unforeseen information emerges from PPMI and other independent investigations.

How do we amass the enormous sample sizes that these studies will require? One model is to utilize and expand on the existing health care systems, connecting patients, health care providers and researchers through an electronic infrastructure that includes medical records, an extensive research database, and a biobank. The Institute for Human Genetics at University of California at San Francisco and the Kaiser Permanente Health Care in Northern California pioneered this model in 2007. Under the umbrella of a genomics project, they amassed medical, genetic and environmental data on over 200,000 Kaiser members. This massive dataset includes all conditions and treatments (PD included), a readily available resource to explore interactions between treatment and outcome. The data are available to researchers via Kaiser and a subset of it is on the NIH database on genotype and phenotype (dbGaP).

Another model for generating large datasets in a reasonably short time and relatively low cost is via web-based research. This was pioneered by the biotech company, 23andMe, for a GWAS of PD ⁷⁰. 23andme amassed a cohort of 3,500 individuals with PD and 30,000 controls for their publication, and the sample size has continued to grow. Research participants shipped a saliva sample for genotyping and filled out questionnaires online. Despite non-conformity to epidemiological standards for data collection, but adhering to genetic standards for rigor, the study succeeded to replicate all six PD susceptibility genes that were known at the time, and discovered two previously unknown genes that were later validated ⁷⁰. What 23andMe did in a matter of years would have taken several decades and tens of millions of dollars if done conventionally. More recently, Noyce et al ⁷¹ demonstrated feasibility of conducting longitudinal studies of PD online. They too recruited subjects online using emails in advertising campaign in the UK. Participants were prompted by email to return to the website and complete annual follow-up tests. They had high retention rate (79%–90% per year with 64% completing the entire 3-year study). Since the aim of the study was to test an algorithm to predict the development of PD, individuals who reported conversion to PD were followed by phone and then a home visit to confirm PD diagnosis according to the standard UK Brain Bank criteria.

Online studies are feasible, reliable, generate troves of data fast and inexpensively, and can be scaled upward to meet any sample size requirement, enabling a well-powered and comprehensive exploration of the many factors that affect treatment response. They are ideal for genome-wide searches for biomarkers of drug efficacy and toxicity, because the sample

size can be large enough to afford power for discovery and replication, while testing multiple drugs and many outcomes in stratified groups of patients. They will generate robust, replicated pharmacogenetic candidates along with the formula of the specific patient group, drug, and outcome that should be targeted. The findings can be validated in conventional, but *precisely-designed*, observational studies or clinical trials, and then moved into clinical practice, ultimately leading to a significant improvement in overall patient care.

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