Promise of Pharmacogenomics for Drug Discovery, Treatment and Prevention of Parkinson's Disease. A Perspective

Haydeh Payami · Stewart A. Factor

Published online: 21 November 2013

© The American Society for Experimental NeuroTherapeutics, Inc. 2013

Abstract Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by a heterogeneous array of motor and non-motor features. Anti-PD drugs that are in use target only the motor symptoms, may lose efficacy over time, and can cause serious adverse effects such as dyskinesia and psychosis. There are currently no preventative or disease modifying treatments. All attempts to develop disease modifying drugs have failed. Pharmacogenomics (PGx) has the potential to change the way new drugs are developed and the way drugs are prescribed. By using genetic markers that correlate with, and can therefore predict drug response, clinical trials can be designed to be enriched with individuals who are most likely to benefit from the drug, maximizing drug's efficacy, minimizing its adverse effects, and boosting the odds of successful drug discovery. Clinical application of PGx will help physicians to quickly and accurately determine the right drugs and the right doses for individuals, avoiding the lengthy trial and error approaches and adverse effects. In combination with known protective factors such as nicotine and caffeine, PGx may enable development of personalized methods for PD prevention and, by extension, care.

Keywords Parkinson's disease · Pharmacogenomics · Treatment · Prevention · Drug discovery

Electronic supplementary material The online version of this article (doi:10.1007/s13311-013-0237-y) contains supplementary material, which is available to authorized users.

H. Payami (✉)
New York State Department of Health, Division of Genetics,
Wadsworth Center, Albany, NY 12208, USA
e-mail: hpayami@wadsworth.org

S. A. Factor
Department of Neurology, Emory University School of Medicine,
Atlanta, GA 30322, USA

The Challenge

Current Drugs Most pharmacological approaches to the treatment of PD are symptomatic and target the nigrostriatal dopaminergic pathway [1]. Loss of dopamine, once considered the cause of PD, is now questioned as the initial event in PD pathogenesis and is certainly not the only pathway that is important. The gold-standard drug is l-dopa, a precursor of dopamine, which crosses the blood brain barrier and is converted to dopamine. Other drugs are used as monotherapy or in combination with l-dopa to enhance its efficacy including dopamine receptor agonists, catechol-O-methyltransferase (COMT) inhibitors and monoamine oxidase (MAO) inhibitors [1]. Dopaminergic treatments provide symptomatic relief of motor problems for the majority of patients, but they have limited impact on non-motor symptoms of PD which are very frequent and include olfaction, digestive, sleep, psychiatric and cognitive dysfunction. Dopaminergic drugs do not slow disease progression, and they can have serious adverse effects.

There is wide inter-individual variation in response to anti-PD drugs. While some patients are maintained on l-dopa for many years, others do not receive the expected benefit [2]. Similarly, some patients experience minimal toxicity to higher doses while others develop troublesome side effects to low doses [3]. Up to 50 % of patients develop drug-induced motor complications within 5 years of initiating l-dopa treatment [4]. L-dopa can also cause psychosis and impulse control disorders. Dopamine agonists can cause psychosis, excessive daytime sleepiness, compulsive behavior, and impulse control disorders. The COMT-inhibitor tolcapone, used to modify side effects of l-dopa, can itself cause liver toxicity [5].

Drug Development The last major breakthrough in PD drug development was the emergence of l-dopa nearly half a century ago for improvement of symptoms. However, attempts to develop disease-modifying, neuroprotective drugs have not

been as successful [6]. Several promising new families of drugs are being evaluated for treatment of PD, including adenosine A2A receptor antagonists, neurotrophic factors, glutamate antagonists, and transdermal nicotine. None has been approved for general use in PD in the United States, and often the reason is insufficient efficacy.

Drugs do not Work Equally for Everyone Drug efficacy varies for individuals based on both intrinsic (genes, sex) and extrinsic (drug interactions) factors. Clinical trials pay careful attention to drug interactions and medical history, but 99.8 % of them ignore the role of genetics [7]. Consider a clinical trial that is composed of a mixture of patients (as most are); some subjects may have a genotype that enables them to benefit from the drug, while others may have genotypes that make the drug ineffective or even harmful. By ignoring genetics, efficacy is measured on average, and the average may not be statistically significant, the trial fails, and a drug that might have helped a portion of patients is abandoned.

The same applies to drugs that are approved for PD. Because of the unpredictability of drug response, physicians rely on trial and error to determine the right drug combinations and the proper doses for each patient, which is a process that can take a long time, may not even reach maximum efficacy and may result in adverse events.

Pharmacogenomics

PGx is the science and clinical application of genetics to pharmacological treatments, i.e., determining if inter-individual differences in drug efficacy and toxicity are due to genetic differences, identifying the genes that influence drug response, and using the genotype to predict outcome so that benefit is maximized and side effects averted.

The US Food and Drug Administration (FDA), The European Medicines Agency (EMA) and Japan's Pharmaceuticals and Medical Devices Agency (PMDA) have been promoting the application of PGx in drug development for a decade [8]. NIH, the major medical research funding agency in the United States and the home for the National Center for Advancing Translational Science, considers PGx essential in translational science and a cornerstone of personalized medicine [9]. PGx should be an integral part of study design of clinical trials. At a minimum, genetic material should be obtained and banked so that as new information becomes available, closed trials can be re-evaluated and abandoned drugs may be resurrected.

Candidate Gene Studies Pharmacogenetics (as opposed to pharmacogenomics) refers to the study of candidate genes, often one at a time, in relation to drug response.

Pharmacogenetics is hypothesis-driven and can be very powerful if the investigator happens to pick the right gene to study.

Polymorphisms (DNA variations) that affect drug absorption, distribution, metabolism and excretion (ADME) can result in clinically significant variability in the efficacy and toxicity of drugs [10,11]. Variations in expression levels (RNA) of ADME genes could also be critical in drug response [12], although data on ADME gene expression is only emerging. The most extensively studied ADME genes are the cytochrome P450 (*CYP450*) family [13]. In clinical use are *CYP2D6* for administering tamoxifen and codeine for treatment of cancer and pain management [14], as well as *CYP2C9* and vitamin K epoxide reductase complex subunit 1 (*VKORC1*) to avoid risk of hemorrhagic complications of the anticoagulant, warfarin [15]. Of closer relevance to PD is the use of tetrabenazine for treatment of Huntington's disease whose dosing is based, only in part, on *CYP2D6* genotype [16] because the predictive value of this genotype is not high enough to accurately predict outcome by itself [17].

Major histocompatibility complex genes, *HLA*, are critical for immunologically mediated drug response [18]. The most common class of adverse reactions is drug-induced hypersensitivity, which is often characterized by rash, hepatitis and fever. *HLA* has been associated with hypersensitivity to a wide range of drugs including aspirin, non-steroidal anti-inflammatory drugs, antipsychotics, antibiotics, antiepileptics, and anti-HIV drugs [18]. *HLA-B*57:01* is an FDA-approved PGx marker for treatment of HIV-infection with Abacavir. Patients are genotyped for *HLA-B*57:01* prior to initiating treatment; those who test negative will not be expected to develop drug-induced hypersensitivity (negative predictive value is 100 %) [19].

Candidate Genes for PD: Past The literature on pharmacogenetics of PD consists of association studies with genes whose products *directly* interact with l-dopa, dopamine, or dopamine agonists, namely, *COMT*, *MAO-A*, *MAO-B*, dopamine transporter (*DAT*), and dopamine receptors (*DRD1*, *DRD2*, *DRD3*, *DRD4*, *DRD5*). There are excellent reviews that discuss the individual studies [10,20,21]. In brief, studies were small and underpowered, and as would be expected from a series of small studies, results were mostly negative and the few that yielded positive results were not replicated. Adding to the confusion, drug of interest varied across studies (l-dopa, entacapone, tolcapone), as did the definition of drug response (drug dose, time to peak response, duration and magnitude of response, daytime sleepiness, sudden onset of sleep, l-dopa induced dyskinesia, hallucinations). The disparity in study designs, the small sample sizes and the lack of replication make it nearly impossible to determine, from existing data, whether and which dopamine-related polymorphisms are important for dose determination and if they could potentially be

useful for avoiding side effects or predicting a positive response.

Candidate Genes for PD: Now and in the Future The era of testing a few candidate genes at a time has passed. If we are to pursue candidate genes, we ought to cast a wide net that captures all known and potentially relevant genes, and, which allows in-depth investigation of all types of variations including coding polymorphisms which affect the structure of the molecule, and non-coding regulatory elements that alter its expression levels. The “relevant genes” might include, but are not limited to (1) the dopamine metabolism pathway, which is in fact far more complex than the few genes tested so far [22], (2) genes involved in protein degradation, oxidative stress, mitochondrial function, immune response, inflammation, lysosomal trafficking and other pathways that have come to light through genetic discoveries of the last decade [23,24], and (3) genes that affect the risk of developing PD. At the molecular level, PD could be caused by a mutation in any of a number of genes including *SNCA*, *LRRK2*, *PARK2* and *DJ1* (reviewed in [23]). For these genetic forms, an ideal treatment would have to be individualized to the molecular mechanism causing the disease. Idiopathic PD also has a strong but very complex genetic component [25–30]. Although the dozens of PD susceptibility genes identified to date seem to be working in different pathways—though it is possible that the pathways share a step or two, or that they converge on one point that will lead to PD—there is sufficient disparity in the varied functions of these genes to suggest that different drugs may work differently for different genotypes. In sum, candidate gene studies for PD need to be much larger in scope and size than the early studies. Technically, this can be easily accomplished with custom-built genotyping, sequencing and expression microarrays. It will however require well-orchestrated collaborative studies, with laser-sharp questions, carefully defined drug-response phenotypes, and firm plans for validation. This is an ambitious goal but it is doable. In less than two decades, PD researchers transformed the understanding of the disease from being purely environmental to one of the most complex and well-dissected genetic disorders. With such phenomenal track record and the collaborations that were forged in the process, the way is already paved for PGx studies.

Genomics Pharmacogenomics refers to genome-wide association studies (GWAS) of drug response. Genetic markers spanning the genome are tested for association with a drug-response phenotype. Defined broadly, genetic markers include single nucleotide polymorphisms, copy number variations, epigenetic markers, gene expression intensities, and whole genome sequence data. Technology is sufficiently advanced

for mid to high throughput analyses at reasonable costs. The cost of whole genome sequencing is also dropping rapidly.

The main advantage of pharmacogenomics is that there is no a-priori hypothesis as to the genomic location or the function of genes that might be involved. This makes it possible to uncover previously unsuspected genetic factors with important ties to disease or therapy.

In fact, GWAS for drug response has yielded astounding results (reviewed in [7,8,31]). Unlike standard GWAS, which often require sample sizes in tens of thousands (because the effects sizes of genetic association with disease risk are very small, i.e., odds ratios, $OR < 1.3$), GWAS for drug response have often succeeded with fewer than 100 patients because the genetic associations with drug response are turning out to be very strong. An early triumph of the genomic approach was the discovery in four populations—U.S. whites, African Americans, Japanese and Australians—of the association of the interleukin 28B gene with the efficacy of interferon alpha treatment for hepatitis C with some ORs approaching ~30 [32–34]. Similarly, *SLC01B*, a liver specific member of the organic anion transporter family, was found to be associated with methotrexate toxicity in children with leukemia ($OR \sim 16$) [35], and also with skeletal myopathy associated with cholesterol lowering drug simvastatin ($OR \sim 17$) [36]. Drug-induced hepatotoxicity, which is a common reason for terminating clinical trials and withdrawing drugs from the market, was assumed to be linked to *CYP450* polymorphisms—a belief firmly grounded on the knowledge that *CYP450* enzymes metabolize over 90 % of all drugs. Hence, research on drug-induced hepatotoxicity had primarily been focused on *CYP450* as evidenced by 1,198 papers that we identified with a single PubMed search with the term “cytochrome p450 hepatotoxicity”. However, when GWAS were conducted, it was discovered that *HLA* alleles were largely responsible for drug-induced hepatotoxicity. A GWAS that included only 51 cases uncovered $OR \sim 81$ for association of *HLA-B*57:01* with liver toxicity due to Flucloxacillin [37]. For Lumiracoxib, a Cox-2 selective inhibitor anti-inflammatory drug that was withdrawn from market because of hepatotoxicity, a GWAS with only 41 cases uncovered *HLA-DRB1*15:01* as the genetic underpinning of the toxicity ($OR \sim 5$) [38]. Similarly, hepatotoxicity induced by Ximelagatran, an anticoagulant that was tried as a replacement for warfarin but was abandoned because of liver damage, was mapped to *HLA-DRB1*07:01* ($OR \sim 4$) [39].

Pharmacogenomics Studies in PD There has not yet been a GWAS that was designed specifically to assess response to PD drugs (either those in use or in development). Nor has there been a GWAS for prevention.

We conducted two genome-wide gene–drug interaction studies for PD; in one [40] the drug was caffeine, in the other

[41] the drug was nicotine. Nicotine and caffeine are neuroprotective in animal models [42,43] and are robustly associated with reduced risk of PD in humans in epidemiological studies [44,45]. We were interested in identifying the genetic variations that were associated with enhanced or diminished effects of smoking/nicotine and caffeine on PD risk reduction, with the hope that they would be useful as PGx markers for prevention and treatment. We found that the caffeine effect is associated with polymorphisms in *GRIN2A*, which encodes the NMDA glutamate receptor subunit 2A [40], and that the nicotine effect is associated with polymorphisms in *SV2C* which encodes the synaptic vesicle protein 2C [41]. The significance of *GRIN2A* and *SV2C* in the central nervous system, the etiology of PD, and their intermediary roles in the effects of caffeine and nicotine on neurotransmission have been well-documented [46–50]. Thus it was astounding to identify genes with such strong biological plausibility via hypothesis-free GWAS. In fact, in genome-wide expression studies in a *Drosophila* model of PD, we identified the *SV2C* homologue as the single most significant gene in the protective effect of nicotine against paraquat-induced toxicity [41]. Our results suggest (1) *GRIN2A* genotype is associated with efficacy of caffeine in protecting against PD and (2) *SV2C* genotype is associated with response to nicotine, where allele dosage correlates with benefit, no response, or possibly harm (the latter was unexpected). More specifically, we found that drinking more than the median amount of caffeinated coffee was associated with 32 % risk reduction overall, but when *GRIN2A* genotype was taken into account, one genotype that is present in about 1/4 of individuals was associated with 65 % risk reduction ($P=2 \times 10^{-5}$), whereas 3/4 of individuals showed 22 % risk reduction ($P=0.02$). Smoking cigarettes was associated with an average 19 % risk reduction, but varied by *SV2C* genotype from 56 % risk reduction in 1/4 of subjects, no significant effect in the remaining majority, and a possible 200 % increase in risk in a rare genotype that is present in 2 % of the population. These findings provide testable hypotheses for prevention and treatment.

The inverse association of smoking and caffeine with PD may be an opportunity for devising prevention strategies, although the adverse health consequences of these drugs are serious and must be carefully considered against potential benefit. To test these hypotheses will require enrolling large populations of healthy individuals, obtaining DNA samples (blood, saliva, etc.), and then following them over time to determine whether and how well genotype and drug (caffeine, smoking) interaction can predict PD incidence. The epidemiological component of such a study would be extremely demanding both in time and cost. A first logical step might be to test the hypotheses in web-based studies, as has been pioneered by 23andMe [28], whereby tens of thousands of middle-aged individuals would be enrolled and followed online for several years. Volunteers send a sample of saliva to a

central laboratory for genotyping and record their health status and smoking and caffeine intake periodically online. If results support genotype-specific protection for caffeine and smoking, then the hypothesis can be properly tested and validated in PGx clinical trials for prevention, using drugs that are similar to but do not confer the toxicity of coffee and smoking.

GRIN2A and *SV2C* may also be useful for stratifying patients in treatment trials of PD. A2A receptor antagonists and nicotine have been examined as potential PD drugs; both seem safe and possibly beneficial but efficacy has been disappointing. It will be easy and inexpensive to test whether *GRIN2A* and *SV2C* genotype predict drug response in ongoing or closed trials if DNA is available. The adenosine A2A antagonist story has been particularly interesting in this realm. Caffeine does not only decrease risk of PD but also has symptomatic effects [51]. The development of adenosine A2A selective antagonists, which have biological plausibility since these receptors occupy the striatum and colocalize with D2 and MGLuR5 receptors, lead to great anticipation that these drugs would provide similar if not better efficacy than caffeine on a background of superior safety and tolerance. Preclinical studies of two such agents, istradefylline and preladenant, demonstrated antiparkinsonian effects and dyskinesia prevention [52–55]. However, istradefylline was the subject of four phase 3 trials in ~1500 advanced PD patients and demonstrated only modest decrease in off times with an increase in dyskinesia. This ultimately led to a non-approvable letter from the FDA [56]. The second generation agent preladenant has greater potency, affinity and selectivity and in a phase 2 study demonstrated greater antiparkinsonian efficacy than the first generation agent without an increase in dyskinesia [57]. However, three phase 3 trials in 2,276 subjects were reported as negative in a recent press release. The sponsor has since chosen to stop development. With nearly 4,000 subjects there is more than an adequate number to perform proof of principle pharmacogenetics study examining whether *GRIN2A* genotype impacts drug response but, to our knowledge, this has not yet been done.

Cost and Sample Size Does including genetics in clinical trials add an unaffordable expense? And will stratification by genotype dictate that much larger sample sizes be enrolled? These two assumptions are the basis for a not uncommon misconception that PGx is too expensive to ever become a reality for PD. Ideally, the first step should be genome-wide studies to find markers that associate with drug response, and then the markers can be used as candidates and validated in additional studies. It will cost \$250 to \$650 per person, depending on the choice of the array, to perform genome-wide genotyping. This cost is accepted and considered justified, even when sample sizes are in tens of thousands as is the case for standard GWAS for risk (<http://www.ncbi.nlm.nih.gov/gap>).

For PGx, however, GWAS with sample sizes of few hundred individuals have been successful because the effect sizes appear to be quite large for genes that affect drug response as previously described [32–39]. Therefore, GWAS for PD drug-response will probably cost far less than what has been spent on GWAS of PD risk. Once a gene is identified via GWAS, it will cost less than \$1 per person to genotype it. For example, if DNA were saved for the 4,000 participants of the A2A agonist trials, it would cost about \$4,000 to conduct a proof of concept PGx study for the *GRIN2A* markers. Stratification by relevant genotypes can increase power and reduce the cost of trials. In the case of testing a candidate gene, genotyping can be done prior to enrollment, as an inclusion/exclusion criteria, to exclude non-responders and enrich the trial with individuals who are most likely to benefit. If 75 % of persons in a trial have the *GRIN2A* genotype associated with no response, at less than \$1 per genotype, it is more cost effective to identify and exclude them than to carry them through the trial which would triple the high cost of the trial and dilute the signal for drug efficacy. Once validated and approved by regulatory agencies, the genetic test can be used in the clinical setting to personalize treatment, the cost of which will vary by the type of the test.

Conclusion

It is mystifying that there has not been more on PGx of PD. Rationale is strong. There are no technical obstacles. Required sample sizes are very reasonable. Cost of genotyping is almost negligible compared to the cost of a clinical trial. Why then has genetics not been brought into PD treatment development? The resistance to embrace PGx is not unique to the PD community. A recent study reported that only 0.2 % of trials registered on clinicaltrials.gov had a PGx component [7]. Moreover, 80 % of the trials that included PGx were sponsored by academic institutions while only 20 % were sponsored by the industry.

PGx is fundamental to translational science and personalized medicine. FDA and NIH have made PGx a high priority, making strong recommendations that genetics be incorporated in drug development and patient therapy. GWAS for drug response has been surprisingly efficient, often requiring much smaller sample sizes than traditional GWAS because effect sizes are large. PGx is the most promising near-future opportunity for improving the current PD treatments, and boosting the development of new symptomatic and potentially disease modifying drugs.

Funding

HP is supported by a grant from the National Institutes of Neurologic Disorders and Stroke (R01NS067469). SAF is

supported by the Sartain Lanier Foundation. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

- Factor SA. Current status of symptomatic medical therapy in Parkinson's disease. *Neurotherapeutics* 2008;5(2):164–80.
- Sweet RD, McDowell FH. Five years' treatment of Parkinson's disease with levodopa. Therapeutic results and survival of 100 patients. *Ann Intern Med* 1975;83:456–63.
- Fahn S, Oakes D, Shoulson I, et al. Levodopa and the progression of Parkinson's disease. *NEJM*. 2004;351(24):2498–508. doi:10.1056/NEJMo033447.
- Ahlskog JE, Muentner MD. Frequency of levodopa-related dyskinesias and motor fluctuations as estimated from the cumulative literature. *Mov Disord* 2001;16(3):448–58.
- Olanow CW. Tolcapone and hepatotoxic effects. *Tasmar Advisory Panel. Arch Neurol* 2000;57:263–7.
- Hart RG, Pearce LA, Ravina BM, Yalthro TC, Marler JR. Neuroprotection trials in Parkinson's disease: systematic review. *Mov Disord* 2009;24:647–54. doi:10.1002/mds.22432.
- Burt T, Dhillon S. Pharmacogenomics in early-phase clinical development. *Pharmacogenomics* 2013;14:1085–97. doi:10.2217/pgs.13.81.
- Maliepaard M, Nofziger C, Papaluca M, et al. Pharmacogenetics in the evaluation of new drugs: a multiregional regulatory perspective. *Nat Rev Drug Discov* 2013;12(2):103–15.
- Collins FS. Reengineering translational science: the time is right. *Sci Transl Med*. 2011;3:90
- Chan A, Pirmohamed M, Comabella M. Pharmacogenomics in neurology: Current state and future steps. *Ann Neurol* 2011;70:684–97.
- Li J, Zhang L, Zhou H, Stoneking M, Tang K. Global patterns of genetic diversity and signals of natural selection for human ADME genes. *Hum Mol Genet*. 2011;20(3):528–40.
- Schroder A, Klein K, Winter S, et al. Genomics of ADME gene expression: mapping expression quantitative trait loci relevant for absorption, distribution, metabolism and excretion of drugs in human liver. *Pharmacogenomics J*. 2013;13(1):12–20.
- Ingelman-Sundberg M. Pharmacogenetics of cytochrome P450 and its applications in drug therapy: the past, present and future. *Trends in Pharmacological Sciences* 2004;25:193–200.
- McLeod HL. Cancer pharmacogenomics: early promise, but concerted effort needed. *Science* 2013;339:1563–6.
- Yang J, Chen Y, Li X, et al. Influence of CYP2C9 and VKORC1 genotypes on the risk of hemorrhagic complications in warfarin-treated patients: A systematic review and meta-analysis. *Int J Cardiol*. 2013;168(4):4234–4. doi:10.1016/j.ijcard.2013.07.151.
- Guay DR. Tetrabenazine, a monoamine-depleting drug used in the treatment of hyperkinetic movement disorders. *Am J Geriatr Pharmacother* 2010;8:331–73.
- Mehanna R, Hunter C, Davidson A, Jimenez-Shahed J, Jankovic J. Analysis of CYP2D6 genotype and response to tetrabenazine. *Mov Disord* 2013;28:210–5.
- Pavlos R, Mallal S, Phillips E. HLA and pharmacogenetics of drug hypersensitivity. *Pharmacogenomics*. 2012;13:1285–306.
- Mallal S, Phillips E, Carosi G, et al. HLA-B*5701 screening for hypersensitivity to abacavir. *NEJM*. 2008;358:568–79.
- Arbouw ME, Guchelaar HJ, Egberts TC. Novel insights in pharmacogenetics of drug response in Parkinson's disease. *Pharmacogenomics* 2010;11:127–9.

21. Kalinderi K, Fidani L, Katsarou Z, Bostantjopoulou S. Pharmacological treatment and the prospect of pharmacogenetics in Parkinson's disease. *Int J Clin Pract*. 2011;65:1289–94.
22. Meiser J, Weindl D, Hiller K. Complexity of dopamine metabolism. *Cell Commun Signal* 2013;11:34.
23. Hardy J. Genetic analysis of pathways to Parkinson disease. *Neuron* 2010;68:201–6.
24. Trinh J, Farrer M. Advances in the genetics of Parkinson disease. *Nat Rev Neurol*. 2013;9(8):445–54.
25. Satake W, Nakabayashi Y, Mizuta I, et al. Genome-wide association study identifies common variants at four loci as genetic risk factors for Parkinson's disease. *Nat Genet*. 2009;41:1303–7.
26. Hamza TH, Zabetian CP, Tenesa A, et al. Common genetic variation in the HLA region is associated with late-onset sporadic Parkinson's disease. *Nat Genet* 2010;42:781–5.
27. Simon-Sanchez J, van Hilten JJ, van de Warrenburg B, et al. Genome-wide association study confirms extant PD risk loci among the Dutch. *Eur J Hum Genet*. 2011;19:655–61.
28. Do CB, Tung JY, Dorfman E, et al. Web-based genome-wide association study identifies two novel loci and a substantial genetic component for Parkinson's disease. *PLoS Genet* 2011;7:e1002141.
29. Lill CM, Roehr JT, McQueen MB, et al. Comprehensive research synopsis and systematic meta-analyses in Parkinson's disease genetics: the PDGene database. *PLoS Genet*. 2012;8(3):e1002548.
30. Pankratz N, Beecham GW, DeStefano AL, et al. Meta-analysis of Parkinson's disease: identification of a novel locus, RIT2. *Ann Neurol*. 2012;71:370–84.
31. Harper AR, Topol EJ. Pharmacogenomics in clinical practice and drug development. *Nat Biotechnol*. 2012;30:1117–24.
32. Tanaka Y, Nishida N, Sugiyama M, et al. Genome-wide association of IL28B with response to pegylated interferon-[alpha] and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009;41:1105–9.
33. Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature*. 2009;461(7262):399–401.
34. Suppiah V, Moldovan M, Ahlenstiel G, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009;41(10):1100–4.
35. Trevino LR, Shimasaki N, Yang W, et al. Germline genetic variation in an organic anion transporter polypeptide associated with methotrexate pharmacokinetics and clinical effects. *J Clin Oncol* 2009;27:5972–8.
36. Group SC, Link E, Parish S, Armitage J, et al. SLC1B1 variants and statin-induced myopathy—a genomewide study. *NEJM* 2008;359(8):789–99.
37. Daly AK, Donaldson PT, Bhatnagar P, Shen Y, Péér I, Floratos A et al. HLA-B*5701 genotype is a major determinant of drug-induced liver injury due to flucloxacillin. *Nat Genet*. 2009;41:816–9.
38. Singer JB, Lewitzky S, Leroy E, et al. A genome-wide study identifies HLA alleles associated with lumiracoxib-related liver injury. *Nat Genet*. 2010;42:711–4.
39. Kindmark A, Jawaaid A, Harbron CG, et al. Genome-wide pharmacogenetic investigation of a hepatic adverse event without clinical signs of immunopathology suggests an underlying immune pathogenesis. *Pharmacogenomics J*. 2008;8:186–95.
40. Hamza TH, Chen H, Hill-Burns EM, et al. Genome-wide gene-environment study identifies glutamate receptor gene GRIN2A as a Parkinson's disease modifier gene via interaction with coffee. *PLoS Genet*. 2011;7(8):e1002237.
41. Hill-Burns EM, Singh N, Ganguly P, et al. A genetic basis for the variable effect of smoking/nicotine on Parkinson's disease. *Pharmacogenomics J* 2012; doi:10.1038/tpj.2012.38
42. Quik M, Parameswaran N, McCallum SE, Bordia T, Bao S, McCormack A et al. Chronic oral nicotine treatment protects against striatal degeneration in MPTP-treated primates. *J Neurochem*. 2006;98:1866–75.
43. Chen JF, Xu K, Petzer JP, Staal R, Xu YH, Beilstein M et al. Neuroprotection by caffeine and A(2A) adenosine receptor inactivation in a model of Parkinson's disease. *J Neurosci*. 2001;21:RC143.
44. Herman MA, Takkouche B, Caamano-Isorna F, Gestal-Otero JJ. A meta-analysis of coffee drinking, cigarette smoking, and the risk of Parkinson's disease. *Ann Neurol* 2002;52:276–84.
45. Powers K, Kay D, Factor S, et al. Combined effects of smoking, coffee and NSAIDs on Parkinson's disease risk. *Mov Disord* 2008;23:88–95.
46. Dorus S, Vallender EJ, Evans PD, et al. Accelerated evolution of nervous system genes in the origin of Homo sapiens. *Cell* 2004;119:1027–40.
47. Caudle WM, Zhang J. Glutamate, excitotoxicity, and programmed cell death in Parkinson disease. *Exp Neurol* 2009;220:230–3.
48. Higley MJ, Sabatini BL. Competitive regulation of synaptic Ca²⁺ influx by D2 dopamine and A2A adenosine receptors. *Nat Neurosci* 2010;13:958–66.
49. Turner TJ. Nicotine enhancement of dopamine release by a calcium-dependent increase in the size of the readily releasable pool of synaptic vesicles. *J Neurosci* 2004;24:11328–36.
50. Sawcer S, Hellenthal G, Pirinen M, et al. Genetic risk and a primary role for cell-mediated immune mechanisms in multiple sclerosis. *Nature* 2011;476:214–9.
51. Postuma RB, Lang AE, Munhoz RP, Charland K, Pelletier A, Moscovitch M et al. Caffeine for treatment of Parkinson disease: a randomized controlled trial. *Neurology* 2012;79:651–8.
52. Smith Y, Wichmann T, Factor SA, DeLong MR. Parkinson's disease therapeutics: new developments and challenges since the introduction of levodopa. *Neuropsychopharmacol* 2012;37:213–46.
53. Kanda T, Jackson MJ, Smith LA, et al. Adenosine A2A antagonist: a novel antiparkinsonian agent that does not provoke dyskinesia in parkinsonian monkeys. *Ann Neurol* 1998;43:507–13.
54. Bibbiani F, Oh JD, Petzer JP, et al. A2A antagonist prevents dopamine agonist-induced motor complications in animal models of Parkinson's disease. *Exp Neurol* 2003;184:285–94.
55. Hodgson RA, Bedard PJ, Varty GB, et al. Preladenant, a selective A(2A) receptor antagonist, is active in primate models of movement disorders. *Exp Neurol* 2010;225:384–90.
56. Duno R, Deeks ED. Istradefylline: first global approval. *Drugs*. 2012;73:875–82.
57. Hauser RA, Cantillon M, Pourcher E, Micheli F, Mok V, Onofrij M et al. Preladenant in patients with Parkinson's disease and motor fluctuations: a phase 2, double-blind, randomised trial. *Lancet Neurol*. 2011;10:221–9.