Progress toward an integrated understanding of Parkinson's disease [version 1; peer review: 2 approved]

Maxime W.C. Rousseaux¹,², Joshua M. Shulman¹-⁴, Joseph Jankovic³

¹Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, 1250 Moursund St, Houston, TX, 77030, USA
²Department of Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX, 77030, USA
³Parkinson's Disease Center and Movement Disorders Clinic, Department of Neurology, Baylor College of Medicine, 7200 Cambridge, Houston, TX, 77030-4202, USA
⁴Department of Neuroscience, Baylor College of Medicine, One Baylor Plaza, Houston, TX, 77030, USA

First published: 12 Jul 2017, 6(F1000 Faculty Rev):1121
Latest published: 12 Jul 2017, 6(F1000 Faculty Rev):1121
https://doi.org/10.12688/f1000research.11820.1

Abstract
Parkinson's disease (PD) is the second most common neurodegenerative disorder after Alzheimer's disease, affecting over 10 million individuals worldwide. While numerous effective symptomatic treatments are currently available, no curative or disease-modifying therapies exist. An integrated, comprehensive understanding of PD pathogenic mechanisms will likely address this unmet clinical need. Here, we highlight recent progress in PD research with an emphasis on promising translational findings, including (i) advances in our understanding of disease susceptibility, (ii) improved knowledge of cellular dysfunction, and (iii) insights into mechanisms of spread and propagation of PD pathology. We emphasize connections between these previously disparate strands of PD research and the development of an emerging systems-level understanding that will enable the next generation of PD therapeutics.

Keywords
Parkinson's disease, PD, neurodegenerative disorders, α-Synuclein, Parkinson's disease genetics

Open Peer Review
Approval Status ✓ ✓

Faculty Reviews are review articles written by the prestigious Members of Faculty Opinions. The articles are commissioned and peer reviewed before publication to ensure that the final, published version is comprehensive and accessible. The reviewers who approved the final version are listed with their names and affiliations.

1. Roger A Barker, University of Cambridge, Cambridge, UK
2. David G Standaert, Department of Neurology, The University of Alabama at Birmingham, Birmingham, USA

Any comments on the article can be found at the end of the article.
Corresponding author: Joseph Jankovic (josephj@bcm.edu)

Author roles: Rousseaux MWC: Writing – Original Draft Preparation, Writing – Review & Editing; Shulman JM: Writing – Original Draft Preparation, Writing – Review & Editing; Jankovic J: Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: Maxime W.C. Rousseaux and Joshua M. Shulman declare that they have no disclosures. Joseph Jankovic has received research or training grants and/or has served as a consultant for the Michael J. Fox Foundation for Parkinson's Research, Prothena Biosciences Inc, and Teva Pharmaceutical Industries Ltd.

Grant information: Maxime W.C. Rousseaux is supported in part by Grant No. PF-JFA-1762 from the Parkinson's Disease Foundation. Joshua M. Shulman is supported by the Huffington Foundation, the Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, and a Career Award for Medical Scientists from the Burroughs Wellcome Fund. Joseph Jankovic has received research and/or educational support from the Michael J. Fox Foundation for Parkinson's Research, Parkinson's Foundation, the Parkinson Study Group, Prothena Biosciences Inc, and Teva Pharmaceutical Industries Ltd. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2017 Rousseaux MWC et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Rousseaux MWC, Shulman JM and Jankovic J. Progress toward an integrated understanding of Parkinson's disease [version 1; peer review: 2 approved] F1000Research 2017, 6(F1000 Faculty Rev):1121
https://doi.org/10.12688/f1000research.11820.1

First published: 12 Jul 2017, 6(F1000 Faculty Rev):1121 https://doi.org/10.12688/f1000research.11820.1
Introduction

Parkinson’s disease (PD) is the most common movement disorder, affecting 2–3% of individuals over the age of 65. It is clinically characterized by a core set of motor manifestations, including tremor, slow movement (bradykinesia), increased muscle tone (rigidity), and gait and postural impairment as well as a variety of other motor and non-motor features, including cognitive impairment, depression, pain and other sensory symptoms, autonomic dysfunction, and others. PD is characterized pathologically by the loss of predominantly dopaminergic neurons, associated with intracellular, insoluble α-synuclein (α-Syn) aggregates, largely localized to cytoplasmic inclusions termed Lewy bodies and within neuronal processes termed Lewy neurites. Whereas current treatments can ameliorate the cardinal motor symptoms, no disease-modifying therapies exist. A large body of research has therefore focused on understanding the biological mechanisms that underlie disease onset and progression, with the goal of developing effective pathogenesis-targeted, disease-modifying therapies.

As this year marks the 200-year anniversary of the recognition of PD by James Parkinson1, we also celebrate the remarkable progress in understanding PD etiology and pathogenesis. Here, we review recent advances in the study of the genetics, cell biology, and pathology of PD, highlighting emerging areas of overlap. Where these areas were previously studied in isolation, results from these disparate strands of research are beginning to converge, providing a more unified understanding of PD pathogenesis. We argue that this integrative approach to PD, in which seemingly disconnected results are re-examined as components of a cohesive whole, is also creating exciting opportunities for clinical applications.

Functional genomics and the promise of personalized medicine

Although only a small minority of patients with PD have thus far been found to have responsible pathogenic gene mutations, genetic discoveries have nevertheless been a driving force in the elucidation of PD mechanisms. Following on the early success of studies of rare families with Mendelian forms of PD, more than 20 PD genes and variants have now been implicated, including from large-scale genome-wide association studies (GWAS) and, more recently, whole exome sequencing (WES) studies in population-based cohorts6–12. A common challenge with either approach entails definitive confirmation of the responsible genes and elucidation of implicated disease mechanisms. In GWAS, implicated genomic regions often contain several equally plausible gene candidates. Whereas WES studies usually single out specific gene candidates, the implicated alleles may be too rare to definitively confirm a causal link to PD based on currently available sample sizes. Medium- to high-throughput screening assays in cellular or animal models can connect promising candidate genes to PD-relevant biologic mechanisms, prioritizing a subset for further study. For example, Jansen et al.9 evaluated 27 promising candidate genes with homozygous or compound heterozygous loss-of-function alleles based on WES in 1,148 unrelated young-onset PD cases. Since nearly all of the gene candidates were observed only once in the cohort, the investigators probed each gene for roles in mitochondrial dynamics or α-Syn-mediated toxicity using cellular and fruit fly experimental models. Ultimately, five genes were supported by both functional data and additional human genetic analyses consistent with replication. Beyond accelerating the discovery of novel PD genes, related approaches are also revealing the function of many other established genes/variants, grouping discrete susceptibility loci into common pathways and thereby consolidating our understanding of PD pathogenesis. For example, the recently identified PD gene CHCHD2 may mediate its activity through a mitochondrial pathway like other recessive PD genes (see below)10–12. Moreover, VPS35 and EIF4G1, both implicated in autosomal dominant forms of PD13–17, were recently found to genetically interact with one another and converge on α-Syn toxicity in yeast, worm, and mouse models of synucleinopathy18.

By contrast, with the sequence-based discovery of rare variant PD risk factors, the susceptibility alleles identified by GWAS usually do not alter protein-coding regions, making functional follow-up more challenging. For example, one of the earliest discovered PD risk polymorphisms at the human MAPT locus may primarily impact alternative mRNA splicing19,20. In another recent study, Soldner et al. used human pluripotent cell-derived neurons containing an intronic PD-related variant in the gene encoding α-Syn (SNCA)21. The authors found that this common polymorphism, which is present in about half of the population, coincided with a distal enhancer element resulting in an approximately 10% increase in SNCA transcript levels. It was therefore suggested that a mild increase of α-Syn over the course of decades renders individuals susceptible to PD. This is consistent with the findings from rare families with SNCA locus multiplication. In these cases, individuals with SNCA gene multiplication present with clinical features typical of PD, including a similar age at onset to sporadic PD22–24, whereas individuals with SNCA gene triplication present with a more early onset, aggressive form of PD25. Thus, SNCA gene dosage may be an integral feature of PD pathogenesis. In the case of the more common SNCA polymorphism, the modest increase in α-Syn protein levels may interact with other genetic risk variants or environmental exposures to cause PD. For example, Goldman et al. found that head injury was significantly associated with increased PD risk, but only in the context of a disease-associated SNCA promoter polymorphism26.

One of the great hopes for advances in PD genetics is to realize goals for personalized medicine, including improved risk prediction and even targeted therapies. It has long been speculated that much of PD’s clinical heterogeneity may be genetically encoded26,27. For example, besides their potent impact on risk of PD28, GBA mutations have been reported by several groups to cause an earlier age-at-onset, more rapid progression, and an increased risk of cognitive impairment and dementia in carriers29–34. Moreover, additional studies have looked at the effect of allelic heterogeneity on modifying PD clinical presentations35–37. In the future, it may be important to couple such studies examining the clinical impact of selected allelic variants with experimental investigations to define functional consequences in well-defined cellular or animal models. Moreover, once characterized, these models can serve as a platform for testing putative “personalized” treatments38–42. For example, Sanofi Genzyme is currently supporting a study (MOVES-PD) of GZ/SAR402671, a glucosyleceramide synthase inhibitor, in PD patients.
carrying a GBA gene mutation (ClinicalTrials.gov, NCT02906020). Another study (Aim-PD) is examining the effects of oral Ambroxol, a glucocerebrosidase-modulating chaperone, in patients with PD (ClinicalTrials.gov, NCT02941822). Lastly, the use of biomarkers in stratifying clinical populations and understanding the biological underpinning of PD subtypes will be critically important when developing personalized medicine approaches. Specifically, profiling blood and CSF biomarkers may enhance disease subtyping based on clinical manifestations alone. This hypothesis is currently being tested in the Parkinson’s Progression Markers Initiative (PPMI) led by the Michael J. Fox Foundation for Parkinson’s Research141.

From genes to organelles and cellular homeostasis

The maintenance and function of cellular organelles, including mitochondria and lysosomes, are critical for functional neuronal integrity45–47. Interestingly, several previously identified PD genes such as PARK2, PARK6, and PARK7 (encoding Parkin, PTEN-induced putative kinase 1 [PINK1], and DJ-1, respectively) have been linked to mitochondrial function48–52. Since their initial discovery, a large body of work has elucidated a cellular pathway through which dysfunctional mitochondria can be recycled by way of autophagy or, more specifically, “mitophagy”53–59. Damaged mitochondria promote the phosphorylation of ubiquitin and Parkin by PINK1 and are subsequently degraded by the autophagosomal system60–62. Additionally, studies of these genes have provided insight into their mitochondrial functions in healthy and diseased contexts. For example, a recent study suggests that Parkin acts as an endogenous buffer for mitochondrial stress and its loss sensitizes dopaminergic neurons to mitochondrial mutations over time63,64. In addition, using a fly model, Vos and colleagues65 discovered a new pathway that could suppress the motor and biochemical abnormalities caused by PINK1 loss of function. Specifically, PINK1 genetically interacts with the enzyme responsible for the conversion of vitamin K1 into vitamin K265. Remarkably, supplementation of vitamin K2 could reverse PINK1 mutant phenotypes, suggesting a potential therapeutic approach. However, patient selection will be critical for potential clinical trials, as PARK6 mutations result in rare, autosomal recessive juvenile parkinsonism66, and it is uncertain whether a similar functional deficiency in vitamin K2 may apply in the general PD patient population. Identifying the subcellular impact of other PD genes may similarly lead to other targeted therapies.

Beyond discovering the primary targets of genetic abnormalities, it is essential to understand the subsequent cascade of cellular injury, such as how damaged mitochondria impact other cellular constituents, leading to neuronal dysfunction and death. In other words, discrete targets must be understood in the context of a cohesive, dynamic system. One example comes from recent studies that strongly implicate defects in the vesicular trafficking system67, which mediates cellular secretion and endocytosis as well as vesicle docking and fusion and is critically important for synaptic transmission, lysosomal degradation, and autophagy. Several PD genes, including VPS35, LRRK2, RAB7L1, GBA, and SNCA, have functions that converge on the vesicular trafficking system68–70. Leucine-rich repeat kinase 2 (LRRK2) and Rab-7-like protein 1 (RAB7L1), for example, act coordinately to regulate endolysosomal protein sorting via Rab GTPases, and several studies also support a key role for the cargo-shuttling retromer protein vacuolar protein sorting 35 (VPS35)71–73. In fact, the retromer is implicated as a critical downstream effector whose dysfunction may lead to neuronal toxicity and death74. Importantly, VPS35 may also play a role in mitophagy, perhaps via trafficking of mitochondria-derived vesicles to lysosomes75. The dense interconnections between PD genes and other regulators of vesicular trafficking were highlighted by recent work that combined an α-Syn protein–protein interaction network with suppressor-enhancer screening in yeast76,77. Based on these and other findings, chemical modulators of autophagy and/or the retromer, such as rapamycin (or similar “rapalogues”)78,79 and R5580,81, respectively, may be promising therapeutic avenues for targeting vesicular sorting defects in PD. However, since these pathways mediate essential functions in most tissues, successful dose titration to achieve selective action in the nervous system while minimizing potentially deleterious off-target effects is one anticipated challenge82. Nevertheless, these studies illustrate how the emerging, systems-based understanding of PD can highlight vulnerable “nodes” within complex cellular networks, creating promising therapeutic opportunities.

α-Syn toxicity and propagation: from cells to systems

The centrality of α-Syn in PD pathogenesis was established nearly two decades ago with the dual finding that (i) this protein is the principal constituent of the hallmark Lewy body pathology83 and (ii) SNCA gene mutations cause familial forms of PD84. Since then, α-Syn genomic locus multiplication22–24 or promoter polymorphisms that increase protein expression85 have also been confirmed as causal factors. Thus, intensive investigation has probed the relationship between α-Syn with PD pathogenesis, α-Syn was first described as a member of the synuclein family, which is associated with the synapse and the nucleus86. While studies on the physiological function of α-Syn suggest that it may play a role in synaptic transmission86 and aid in curving cellular membranes87, less is known regarding the mechanism(s) through which its gain-of-function causes neurodegeneration. Thus, one aspect of α-Syn research has focused on understanding how this protein causes toxicity within the cell. Studies in both animal models and human tissue have highlighted a role for α-Syn at the outer mitochondrial membrane88–91; the nucleus92–95; and the synapse96–99 as putative toxic mechanisms. Moreover, mechanisms involving proteostasis100–102 and lysosomal dysfunction103 that collectively lead to increased α-Syn levels are also tantalizing.

Given the direct relationship between α-Syn abundance and its role in PD pathogenesis and propagation, immunotherapy against α-Syn has emerged as one of the most promising therapeutic approaches for PD104. Results from ongoing and future immunotherapeutic trials by Prothena/Roche, Biogen, AFFiRiS, and other biotech companies will provide information on whether aggregated α-Syn is an important therapeutic target105. Nevertheless, caution is warranted given previous negative outcomes from immunotherapeutic trials of other neurodegenerative diseases, such as Alzheimer’s disease, characterized by protein aggregation (proteinopathies)106.
Another avenue for therapeutic intervention that is actively being investigated is based on the observation that hyperactivity of the non-receptor tyrosine kinase c-Abl contributes to α-Syn phosphorylation, accumulation, and neurodegeneration. This has led to preliminary investigation of Nilotinib, a c-Abl inhibitor previously approved for the treatment of chronic myeloid leukemia, as a potential therapeutic agent for PD. Further studies, however, are needed before this potent drug can be recommended as a symptomatic or disease-modifying therapy for PD. A single-center trial at Georgetown University (ClinicalTrials.gov, NCT02954978) and a multicenter trial (NILO-PD) (ClinicalTrials.gov, NCT02281474), sponsored by the Michael J. Fox Foundation for Parkinson’s Research and the Parkinson Study Group, are currently under way.

Since the seminal observation by Braak and colleagues that most idiopathic PD cases fit a relatively predictable, caudal to rostral pathologic staging progression, there has been great interest in understanding the mechanism by which α-Syn pathology may spread from the peripheral organs (e.g. the enteric or pericardial tissue) via the vagus nerve to the lower brainstem and eventually involve the neocortex. The intriguing possibility that α-Syn pathology can spread from cell to cell was suggested by observations of Lewy-like pathology in engrafted neurons from PD patients receiving fetal dopaminergic cell transplants. First studies indicated that aggregates of α-Syn could enter cells that express transgenic α-Syn and seed the formation of new aggregates. More recently, research findings have indicated that even synthetic, wild-type forms of α-Syn, if improperly folded and injected in the mouse brain, can induce the misfolding of otherwise normal endogenous α-Syn, thereby amplifying and propagating pathological oligomeric forms, properties consistent with those of prions (infectious proteinaceous agents). It appears that α-Syn can adopt a variety of different misfolded/aggregated oligomeric conformations that correspond to distinct profiles of toxicity in experimental assays. These findings raise the intriguing, though yet unproven, hypothesis that certain α-Syn "strains" may contribute to clinical and pathologic heterogeneity among PD and other synucleinopathies. Interestingly, Mao et al. recently identified lymphocyte-activation gene 3 (LAG3) as a candidate receptor for α-Syn oligomeric “seeds”, and genetic manipulation of LAG3 in cells and mouse models altered pathologic progression. These findings raise the possibility of diagnostic and therapeutic advance based on the detection of α-Syn strains in patient populations as well as potential pharmacologic blockade of propagation. It is also possible that neuroanatomic variation among key co-factors or receptors for α-Syn seeding or spread might contribute to the selective vulnerability of specific neuronal subpopulations in PD. Despite remarkable recent progress, the clinical relevance of α-Syn seeding and propagation (if any) remains to be fully understood. For example, autopsies from some PD patients receiving fetal grafts were devoid of pathology two decades following transplantation. Another recent study found that individuals receiving human growth hormone derived from human cadavers were at no greater risk of developing PD. The extent of pathological spread of α-Syn does not always follow a defined trans-synaptic pattern nor does the brain areas it affects fully correlate with clinical measures. Thus, a continued investigation into the mechanism through which α-Syn pathological assemblies form and how these are tied to toxicity is warranted.

Gastrointestinal dysmotility is a common early complaint in PD patients, and the enteric nervous system has been implicated as an early target of α-Syn pathology. Along with the growing interest in immunologic and inflammatory disease mechanisms, the gut microbiome has recently come under investigation in studies of both PD patients and animal models. Sampson and colleagues found that α-Syn transgenic mice living in a germ-free environment were less vulnerable to neurodegeneration, but when the mice were inoculated with fecal bacteria taken from patients with PD their motor function deteriorated. While this study suggests the possibility that the gut microbiome may influence PD manifestations, it will be important to define the specific microbial contributors to the disease as well as recapitulate these findings in other disease models before moving forward into humans. Beyond its potential role in disease modulation, the gastrointestinal tract might even be a trigger point for disease based on the findings of early enteric nervous system α-Syn pathology in some pathologic series. Long before the current excitement concerning α-Syn propagation, Braak et al. speculated about a possible gastrointestinal pathogen (or other enteric exposure) as an initiating event, followed by pathologic spread via the vagus nerve to its medullary dorsal motor nucleus where PD changes first appear in the brainstem. Following up on this provocative hypothesis, investigators recently found that subjects undergoing vagotomy (transsection of the vagal nerve for the treatment of peptic ulcer disease) are at a modest but significantly reduced risk of PD. While intriguing, the finding requires further confirmation, and animal model studies will be essential to definitively prove that the mechanism of protection is indeed based on the disruption of spread from the enteric to the central nervous system.

**Conclusion**

Recent advances have clearly enhanced our knowledge of the fundamental processes underlying PD pathogenesis. As connections are recognized among the disparate domains of PD inquiry, the broader patterns begin to emerge. As discussed above, we now recognize the cellular targets of many PD genes, such as how PARKIN and GBA regulate mitochondrial or lysosomal function, respectively. Additionally, the field has made progress toward understanding how dynamic interactions between such organelles impact overall cellular health, as in mitophagy. Lastly, studies of α-Syn illustrate the convergence of classical histopathologic analysis of PD with genetic investigations, and more recent investigations demonstrate how synucleinopathy not only impacts single cells or tissues but also may propagate throughout the nervous system. In sum, we are rapidly making progress toward a more cohesive model of PD pathogenesis, and this systems-level understanding is likely to accelerate therapeutic inroads. With the broad outlines of the "PD puzzle" now apparent, we predict that new insights can be more rapidly integrated within this framework. For example, forthcoming discoveries of new genetic risk loci can be understood within the context of known functional pathways within both neurons and other cell types, such as astrocytes or microglia. An integrated understanding of PD will
also enable more effective multi- and inter-disciplinary collaboration among scientists and clinicians, driving next-generation therapeutic trials targeting disease mechanisms and fulfilling the promise of personalized medicine. Advances in understanding the cellular mechanism underlying PD-related neurodegeneration will undoubtedly lead to better symptomatic and novel pathogenesis-targeted, disease-modifying therapies.\textsuperscript{136}

Abbreviations
\(\alpha\)-Syn, \(\alpha\)-synuclein; GWAS, genome-wide association study; LAG3, lymphocyte-activation gene 3; PD, Parkinson’s disease; PINK1, PTEN-induced putative kinase 1; VPS35, vacuolar protein sorting 35; WES, whole exome sequencing.

Competing interests
Maxime W.C. Rousseaux and Joshua M. Shulman declare that they have no disclosures. Joseph Jankovic has received research or training grants and/or has served as a consultant for the Michael J. Fox Foundation for Parkinson’s Research, Prothena Biosciences Inc, and Teva Pharmaceutical Industries Ltd.

Grant information
Maxime W.C. Rousseaux is supported in part by Grant No. PF-JFA-1762 from the Parkinson’s Disease Foundation. Joshua M. Shulman is supported by the Huffington Foundation, the Jan and Dan Duncan Neurological Research Institute at Texas Children’s Hospital, and a Career Award for Medical Scientists from the Burroughs Wellcome Fund. Joseph Jankovic has received research and/or educational support from the Michael J. Fox Foundation for Parkinson’s Research, Parkinson’s Foundation, the Parkinson Study Group, Prothena Biosciences Inc, and Teva Pharmaceutical Industries Ltd.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References
1. Poewe W, Seppi K, Tanner CM, et al.: Parkinson disease. Nat Rev Dis Primers. 2017; 3: 17013. PubMed Abstract | Publisher Full Text | F1000 Recommendation
2. Postuma RB, Berg D, Stern M, et al.: MDS clinical diagnostic criteria for Parkinson’s disease. Mov Disord. 2015; 30(12): 1591–601. PubMed Abstract | Publisher Full Text
3. Lotia M, Jankovic J: New and emerging medical therapies in Parkinson’s disease. Expert Opin Pharmacother. 2016; 17(7): 895–909. PubMed Abstract | Publisher Full Text
4. Parkinson J: An essay on the shaking palsy. 1817. J Neuropsychiatry Clin Neurosci. 2002; 14(2): 223–36; discussion 222. PubMed Abstract | Publisher Full Text
5. Jankovic J: Movement disorders in 2016: progress in Parkinson disease and other movement disorders. Nat Rev Neurol. 2017; 13(2): 76–8. PubMed Abstract | Publisher Full Text
6. Farlow JL, Robak LA, Heinick K, et al.: Whole-exome sequencing in familial Parkinson disease. JAMA Neurol. 2016; 73(1): 68–75. PubMed Abstract | Publisher Full Text | Free Full Text
7. Nalls MA, Pankratz N, Lill CM, et al.: Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson’s disease. Nat Genet. 2014; 46(9): 989–93. PubMed Abstract | Publisher Full Text | Free Full Text
8. Lill CM: Genetics of Parkinson’s disease. Mol Cell Probes. 2016; 30(6): 386–96. PubMed Abstract | Publisher Full Text
29. Thenganatt MA, Jankovic J: Reduction of mitochondrial function and interactions with alpha-synuclein modulates target gene expression. Proc Natl Acad Sci U S A. 2016; 113(1):398–406.

30. Chan-Palay V, Palczewski M, Palay I, Palay VL: Retinal ganglion cell loss in a mouse model of Parkinson's disease. J Neurosci. 1990; 10(12):4104–13.

31. Beitz C, Zill J, Schapira AH: Rapidly progressive parkinsonism due to homozygous GBA mutations. Neurology. 1996; 46(1):237–8.

32. Jansen IE, Ye H, Heetveld S, Veldman HD, van der Schouw Y, et al.: Mitochondrial DNA mutations in Parkinson's disease: a genome-wide linkage and sequencing study. Lancet Neurol. 2015; 14(3):274–82.

33. Chartier-Harlin MC, Dachsel JC, Vilariño-Güell C, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.

34. Trabzuni D, Wray S, Vandrovcova J, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.

35. Chartier-Harlin MC, Dachsel JC, Vilariño-Güell C, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.

36. Trabzuni D, Wray S, Vandrovcova J, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.

37. Chartier-Harlin MC, Dachsel JC, Vilariño-Güell C, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.

38. Trabzuni D, Wray S, Vandrovcova J, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.

39. Chartier-Harlin MC, Dachsel JC, Vilariño-Güell C, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.

40. Trabzuni D, Wray S, Vandrovcova J, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.

41. Chartier-Harlin MC, Dachsel JC, Vilariño-Güell C, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.

42. Trabzuni D, Wray S, Vandrovcova J, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.

43. Chartier-Harlin MC, Dachsel JC, Vilariño-Güell C, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.

44. Chartier-Harlin MC, Dachsel JC, Vilariño-Güell C, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.

45. Chartier-Harlin MC, Dachsel JC, Vilariño-Güell C, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.

46. Chartier-Harlin MC, Dachsel JC, Vilariño-Güell C, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.

47. Chartier-Harlin MC, Dachsel JC, Vilariño-Güell C, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.

48. Chartier-Harlin MC, Dachsel JC, Vilariño-Güell C, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.

49. Chartier-Harlin MC, Dachsel JC, Vilariño-Güell C, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.

50. Chartier-Harlin MC, Dachsel JC, Vilariño-Güell C, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.

51. Chartier-Harlin MC, Dachsel JC, Vilariño-Güell C, Zimprich A, Benet-Pagès A, Struhal W, et al.: Mitochondrial dysfunction in Parkinson's disease: a genome-wide association study. Nat Genet. 2015; 47(1):105–11.
72. Lashuel HA, Hirling H: "Parkinson's disease caused by mutations in DJ-1: a neuronal protein implicated in the regulation of mitochondrial quality control. Proc Natl Acad Sci U S A. 2007; 104(17): 7021–7026. PubMed Abstract | Publisher Full Text | Free Full Text

73. Wang S, Bellen HJ: "VPS35 causes mitochondrial dysfunction by recycling DLP1 complexes. Nat Med. 2016; 22(1): 64–69. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

74. Youle RJ, Narendra DP: "Mitochondria in neurodegenerative disease. Nat Rev Mol Cell Biol. 2011; 12(1): 9–14. PubMed Abstract | Publisher Full Text | Free Full Text

75. Lazarou M, Sliter D, Kane LA, et al.: "Ubiquitin kinase PINK1 recruits autophagy receptors to induce mitochondrial dysfunction. Nature. 2012; 494(7434): 203–208. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

76. Pickrell AM, Huang CH, Kennedy SR, et al.: "VPS35, the retromer complex and α-synuclein aggregation. J Biol Chem. 2016; 291(35): 21838–98. PubMed Abstract | Publisher Full Text | Free Full Text

77. F1000 Recommendation

78. Pickrell AM, Youle RJ: "The roles of PINK1, parkin, and mitochondrial fidelity in Parkinson's disease. Neuron. 2015; 85(2): 257–73. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

79. Yos M, Esposito G, Edirisinghe JN, et al.: "Vitamin K2 is a mitochondrial electron carrier that rescues pink1 deficiency. Science. 2012; 336(6086): 1306–10. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

80. Valente EM, Abou-Sleiman PM, Caputo V, et al.: "Hereditary early-onset Parkinson's disease caused by mutations in PINK1. Science. 2004; 304(5674): 1158–60. PubMed Abstract | Publisher Full Text | F1000 Recommendation

81. Aboilovich A, Gitler AD: "Defects in trafficking bridge Parkinson's disease pathology and genetics. Nature. 2016; 539(7628): 207–16. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

82. Wang S, Bellen HJ: "The retromer complex in development and disease. Development. 2013; 140(14): 2393–6. PubMed Abstract | Publisher Full Text | Free Full Text

83. McLelland G, Soubannier V, Chen CX, et al.: "Parkin and PINK1 function in a vesicular trafficking pathway regulating mitochondrial quality control. EMBO J. 2014; 33(4): 288–95. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

84. Lashiha EL, Hirling H: "Rescuing defective vesicular trafficking protects against alpha-synuclein toxicity in cellular and animal models of Parkinson's disease. ACS Chem Biol. 2006; 1(7): 420–6. PubMed Abstract | Publisher Full Text

85. MacLeod DA, Rhinn H, Kwakhe T, et al.: "RAB7L1 interacts with LRKK2 to modify intraneuronal protein sorting and Parkinson's disease risk. Neuron. 2013; 77(3): 425–39. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

86. Steger M, Tonelli F, Fl G, et al.: "Phosphoproteomics reveals that Parkinson's disease kinase LRKK2 regulates a subset of Rab GTPases. eLife. 2016; 5: e11813. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

87. Belina A, Rudenko IN, Kaganovich A, et al.: "Unbiased screen for interactors of leucine-rich repeat kinase 2 supports a common pathway for sporcadic and familial Parkinson disease. Proc Natl Acad Sci U S A. 2014; 111(7): 2626–31. PubMed Abstract | Publisher Full Text | Free Full Text

88. Williams ET, Chen X, Moore DJ: "VPS35, the retromer complex and Parkinson's disease. J Parkinsons Dis. 2013; 3(1): 219–33. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

89. Wang W, Wang X: "Lysosomal pH-dependent trafficking protects against α-synuclein toxicity in mammalian cells. J Cell Biol. 2017; 215(12): 2804–15. PubMed Abstract | Publisher Full Text | F1000 Recommendation

90. Cook G, Stetler C, Petrucelli L: "Disruption of protein quality control in Parkinson's disease. Cold Spring Harb Perspect Med. 2012; 2(5): a009423. PubMed Abstract | Publisher Full Text | Free Full Text

91. Valera E, Spencer B, Masliah E: "Immunotherapeutic approaches targeting amyloid-β, α-synuclein, and tau for the treatment of neurodegenerative diseases. Nat Rev Neurosci. 2015; 16: 449–56. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

92. MacLeod DA, Rhinn H, Kwakhe T, et al.: "RAB7L1 interacts with LRKK2 to modify intraneuronal protein sorting and Parkinson's disease risk. Neuron. 2013; 77(3): 425–39. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

93. Steger M, Tonelli F, Fl G, et al.: "Phosphoproteomics reveals that Parkinson's disease kinase LRKK2 regulates a subset of Rab GTPases. eLife. 2016; 5: e11813. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

94. Belina A, Rudenko IN, Kaganovich A, et al.: "Unbiased screen for interactors of leucine-rich repeat kinase 2 supports a common pathway for sporcadic and familial Parkinson disease. Proc Natl Acad Sci U S A. 2014; 111(7): 2626–31. PubMed Abstract | Publisher Full Text | Free Full Text

95. Williams ET, Chen X, Moore DJ: "VPS35, the retromer complex and Parkinson's disease. J Parkinsons Dis. 2013; 3(1): 219–33. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

96. Wang W, Wang X: "Lysosomal pH-dependent trafficking protects against α-synuclein toxicity in mammalian cells. J Cell Biol. 2017; 215(12): 2804–15. PubMed Abstract | Publisher Full Text | F1000 Recommendation

97. Cook G, Stetler C, Petrucelli L: "Disruption of protein quality control in Parkinson's disease. Cold Spring Harb Perspect Med. 2012; 2(5): a009423. PubMed Abstract | Publisher Full Text | Free Full Text

98. Valera E, Spencer B, Masliah E: "Immunotherapeutic approaches targeting amyloid-β, α-synuclein, and tau for the treatment of neurodegenerative diseases. Nat Rev Neurosci. 2015; 16: 449–56. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

99. MacLeod DA, Rhinn H, Kwakhe T, et al.: "RAB7L1 interacts with LRKK2 to modify intraneuronal protein sorting and Parkinson's disease risk. Neuron. 2013; 77(3): 425–39. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

100. Steger M, Tonelli F, Fl G, et al.: "Phosphoproteomics reveals that Parkinson's disease kinase LRKK2 regulates a subset of Rab GTPases. eLife. 2016; 5: e11813. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

101. Belina A, Rudenko IN, Kaganovich A, et al.: "Unbiased screen for interactors of leucine-rich repeat kinase 2 supports a common pathway for sporcadic and familial Parkinson disease. Proc Natl Acad Sci U S A. 2014; 111(7): 2626–31. PubMed Abstract | Publisher Full Text | Free Full Text

102. Williams ET, Chen X, Moore DJ: "VPS35, the retromer complex and Parkinson's disease. J Parkinsons Dis. 2013; 3(1): 219–33. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

103. Wang W, Wang X: "Lysosomal pH-dependent trafficking protects against α-synuclein toxicity in mammalian cells. J Cell Biol. 2017; 215(12): 2804–15. PubMed Abstract | Publisher Full Text | F1000 Recommendation

104. Cook G, Stetler C, Petrucelli L: "Disruption of protein quality control in Parkinson's disease. Cold Spring Harb Perspect Med. 2012; 2(5): a009423. PubMed Abstract | Publisher Full Text | Free Full Text

105. Valera E, Spencer B, Masliah E: "Immunotherapeutic approaches targeting amyloid-β, α-synuclein, and tau for the treatment of neurodegenerative diseases. Nat Rev Neurosci. 2015; 16: 449–56. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
disorders. Neurotherapeutics. 2016; 13(1): 179–89.
98. George S, Brundin P: Immunomodulation in Parkinson’s disease: micromanaging alpha-synuclein aggregation. J Parkinsons Dis. 2015; 5(3): 413–24. Published Abstract | Publisher Full Text | Free Full Text
99. Jankovic J, Godman I, Safirstein B, et al.: Results from a phase 1b multiple ascending-dose study of PRX002, an anti-alpha-synuclein monoclonal antibody, in patients with Parkinson’s disease. Neurodegener Dis. 2017; 17(1):1(567). [Abstract]
100. Wang Y: Alzheimer disease: lessons from immunotherapy for Alzheimer disease. Nat Rev Neuro. 2014; 10(4): 186–9. Published Abstract | Publisher Full Text
101. Brahmachari S, Ge P, Lee SH, et al.: Activation of tyrosine kinase c-Abi contributes to α-synuclein-induced neurodegeneration. J Clin Invest. 2016; 126(8): 2970–88. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
102. Pagani F, Hebron M, Valadez EH, et al.: Nilotinib effects in Parkinson’s disease and dementia with Lewy bodies. J Parkinsons Dis. 2016; 6(3): 503–17. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
103. Robledo I, Jankovic J: Media hype: patient and scientific perspectives on misleading medical news. Mov Disord. 2017. Published Abstract | Publisher Full Text
104. Braak H, Del Tredici K, Rüb U, et al.: Staging of brain pathology related to sporadic Parkinson’s disease. Neurobiol Aging. 2003; 24(2): 191–211. Published Abstract | Publisher Full Text
105. Surmeier DJ, Obsa JA, Halliday GM: Selective neuronal vulnerability in Parkinson disease. Nat Rev Neurosci. 2017; 18(2): 101–13. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
106. Hasegawa M, Nonaka T, Masuda-Suzukake M: α-Synuclein: experimental pathology. Cold Spring Harb Perspect Med. 2016; 6(9): pii: a024273. Published Abstract | Publisher Full Text
107. Luns E, Luk KC: Bent out of shape: α-synuclein misfolding and the convergence of pathogenic pathways in Parkinson’s disease. FEBS Lett. 2015; 589(24 Pt A): 3749–59. Published Abstract | Publisher Full Text | Free Full Text
108. Kordower JH, Chu Y, Hauser RA, et al.: Lewy body-like pathology in long-term embryonic nigral transplants in Parkinson’s disease. Nat Med. 2008; 14(5): 504–6. Published Abstract | Publisher Full Text
109. Li JY, Englund H, Holton JL, et al.: Lewy bodies in grafted neurons in subjects with Parkinson’s disease suggest host-to-graft disease propagation. Nat Med. 2008; 14(5): 501–3. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
110. Desplats P, Lee HJ, Bae EJ, et al.: Inclusion formation and neuronal cell death through neuron-to-neuron transmission of alpha-synuclein. Proc Natl Acad Sci U S A. 2009; 106(31): 13010–5. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
111. Luk KC, Song C, O’Brien P, et al.: Exogenous alpha-synuclein fibrils seed the formation of Lewy body-like intracellular inclusions in cultured cells. Proc Natl Acad Sci U S A. 2009; 106(47): 20001–6. Published Abstract | Publisher Full Text | Free Full Text
112. Volpesez-Daley LA, Luk KC, Patel TP, et al.: Exogenous α-synuclein fibrils induce Lewy body pathology leading to synaptic dysfunction and neuron death. Neuron. 2011; 72(1): 57–71. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
113. Luk KC, Kehm V, Carroll J, et al.: Pathological α-synuclein transmission initiates Parkinson-like neurodegeneration in nontransgenic mice. Science. 2012; 338(6109): 949–53. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
114. Rey NL, Steiner JA, Marod N, et al.: Widespread transneuronal propagation of α-synucleinoopathy triggered in olfactory bulb mimics prodromal Parkinson’s disease. J Exp Med. 2016; 213(9): 1759–78. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
115. Brundin P, Ma J, Kordower JH: How strong is the evidence that Parkinson’s disease is a prion disorder? Curr Opin Neurol. 2016; 29(4): 459–66. Published Abstract | Publisher Full Text | Free Full Text
116. Peeleaerts W, Boussert L, van der Perren A, et al.: α-Synuclein strains cause distinct synucleinopathies after local and systemic administration. Nature. 2015; 522(756): 340–4. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
117. Guo JL, Covell DJ, Daniels JP, et al.: Distinct α-synuclein strains differentially promote tau inclusions in neurons. Cell. 2013; 154(1): 103–17. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
118. Ma X, Ou MT, Karuppagounder SS, et al.: Pathological α-synuclein transmission initiated by binding lymphocyte-activation gene 3. Science. 2016; 353(6307): pii: aah3374. Published Abstract | Publisher Full Text | F1000 Recommendation
119. McCann H, Cartwright H, Halliday GM: Neuropathology of α-synuclein propagation and braak hypothesis. Mov Disord. 2016; 31(2): 152–60. Published Abstract | Publisher Full Text
120. Hansen C, Li JY: Beyond α-synuclein transfer: pathology propagation in Parkinson’s disease. Trends Mol Med. 2012; 18(5): 248–55. Published Abstract | Publisher Full Text
121. Hallett PJ, Cooper O, Sadi O, et al.: Long-term health of dopaminergic neuron transplants in Parkinson’s disease patients. Cell Rep. 2014; 7(8): 1755–61. Published Abstract | Publisher Full Text | Free Full Text
122. Postuma RB, Berg D: Advances in markers of prodromal Parkinson disease. Nat Rev Neurol. 2016; 12(11): 622–34. Published Abstract | Publisher Full Text | Free Full Text
123. Rabinowicz I, Cogswell M, De-young K, et al.: Evaluation of potential infectivity of Alzheimer and Parkinson disease proteins in recipients of cadaver-derived human growth hormone. JAMA Neurol. 2013; 70(4): 462–6. Published Abstract | Publisher Full Text | Free Full Text
124. A Gandy R, Barlow C, et al.: A practical review of gastrointestinal manifestations in Parkinson’s disease. Parkinsonism Relat Disord. 2017; 39: 17–26. Published Abstract | Publisher Full Text
125. Postuma RB, Koller W, et al.: Idiopathic Parkinson’s disease: possible routes by which vulnerable neuronal types may be subject to neuroinvasion by an unknown pathogen. J Neural Transm (Vienna). 2003; 110(5): 517–36. Published Abstract | Publisher Full Text
126. Hils-Burns EM, Debelius JW, Morton JT, et al.: Parkinson’s disease and Parkinson’s disease medications have distinct signatures of the gut microbiome. Mov Disord. 2017; 32(5): 739–49. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
127. Marco-H: Parkinson disease: could gut microbiota influence severity of Parkinson disease? Nat Rev Neurol. 2017; 13(2): 66–7. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
128. Keshavarzian A, Green SJ, Engen PA, et al.: Colonic bacterial composition in Parkinson’s disease. Mov Disord. 2015; 30(10): 1351–60. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
129. Wood H: Parkinson disease. Gut reactions–can changes in the intestinal microbiome provide new insights into Parkinson disease? Nat Rev Neuro. 2015; 11(2): 66. Published Abstract | Publisher Full Text
130. Sampson TR, Debelius JW, Tron T, et al.: Gut microbiota regulate motor deficits and neuroinflammation in a model of Parkinson’s disease. Cell. 2016; 167(6): 1469–1480 e12. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
131. Parashar A, Udayababu M: Gut microbiota: implications in Parkinson’s disease. Parkinsonism Relat Disord. 2017; 38: 1–7. Published Abstract | Publisher Full Text | Free Full Text
132. Braak H, Del Tredici K: Neuropathological staging of brain pathology in sporadic Parkinson’s disease: separating the wheat from the chaff. J Parkinsons Dis. 2017; 7(4): S73–S87. Published Abstract | Publisher Full Text | Free Full Text
133. Liu B, Fang F, Pedersen NL, et al.: Vogtomy and Parkinson disease: a Swedish register-based matched-cohort study. Neurology. 2017; 88(21): 1996–2002. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
134. Svensson E, Horváth-Puhi E, Thomsen RW, et al.: Vogtomy and subsequent risk of Parkinson’s disease. Ann Neurol. 2016; 88(4): 522–9. Published Abstract | Publisher Full Text | F1000 Recommendation
135. Terakad A, Jankovic J: Diagnosis and management of Parkinson’s disease. Semin Neurol. 2017; 37(2): 118–26. Published Abstract | Publisher Full Text
Open Peer Review

Current Peer Review Status: ✅ ✅

Editorial Note on the Review Process

Faculty Reviews are review articles written by the prestigious Members of Faculty Opinions. The articles are commissioned and peer reviewed before publication to ensure that the final, published version is comprehensive and accessible. The reviewers who approved the final version are listed with their names and affiliations.

The reviewers who approved this article are:

Version 1

1. David G Standaert
   Department of Neurology, The University of Alabama at Birmingham, Birmingham, Alabama, USA
   Competing Interests: No competing interests were disclosed.

2. Roger A Barker
   University of Cambridge, Cambridge, UK
   Competing Interests: No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com