

Review

Simvastatin as a Potential Disease-Modifying Therapy for Patients with Parkinson's Disease: Rationale for Clinical Trial, and Current Progress

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Abstract. Many now believe the holy grail for the next stage of therapeutic advance surrounds the development of disease-modifying approaches aimed at intercepting the year-on-year neurodegenerative decline experienced by most patients with Parkinson's disease (PD). Based on recommendations of an international committee of experts who are currently bringing multiple, potentially disease-modifying, PD therapeutics into long-term neuroprotective PD trials, a clinical trial involving 198 patients is underway to determine whether *Simvastatin* provides protection against chronic neurodegeneration. Statins are widely used to reduce cardiovascular risk, and act as competitive inhibitors of HMG-CoA reductase. It is also known that statins serve as ligands for PPAR α , a known arbiter for mitochondrial size and number. Statins possess multiple cholesterol-independent biochemical mechanisms of action, many of which offer neuroprotective potential (suppression of proinflammatory molecules & microglial activation, stimulation of endothelial nitric oxide synthase, inhibition of oxidative stress, attenuation of α -synuclein aggregation, modulation of adaptive immunity, and increased expression of neurotrophic factors). We describe the biochemical, physiological and pharmaceutical credentials that continue to underpin the rationale for taking *Simvastatin* into a disease-modifying trial in PD patients. While unrelated to the *Simvastatin* trial (because this conducted in patients who *already* have PD), we discuss conflicting epidemiological studies which variously suggest that statin use for cardiovascular prophylaxis may increase or decrease risk of developing PD. Finally, since so few disease-modifying PD trials have ever been launched (compared to those of symptomatic therapies), we discuss the rationale of the trial structure we have adopted, decisions made, and lessons learnt so far.

Keywords: Parkinson's disease, Simvastatin, disease modification, clinical trial

INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative condition with age being the main risk factor for its development [1]. With longevity

having increased in most Western countries, a conservative estimation in 2007 predicted that the global number of PD patients will increase to approximately 10 million by 2030 [2]. By 2010, there were approximately 630,000 PD patients in the USA, a figure that was thought set to double by 2040 [3]. However, recent figures suggest these striking predictions may themselves be substantial underestimates since the incidence rates for PD now appear to be

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39 increasing each decade [4]. While these figures are
40 alarming in themselves, especially given the burden
41 to patients and to their families, these demographics
42 also demonstrate the massive impact on each
43 country's healthcare services that PD brings.

44 For example, the costs in the USA of managing
45 PD were estimated in 2013 at \$23 billion, which is
46 \$38,000 per patient per year [5], a figure to
47 which must be added the additional \$10,000 per
48 patient/family of indirect costs that their PD incurs
49 them. Furthermore, PD patients get progressively
50 more expensive to manage as their condition deteriorates
51 over time. Accordingly, increasing annual healthcare
52 costs per PD patient are associated with more advanced
53 stages of the disease, with greater burden resulting
54 from cognitive decline, increased non-motor symptoms
55 and development of balance impairment and falls. Therefore
56 there is a compelling need, shared by patients, families
57 and healthcare systems alike, to identify a cost-effective
58 approach to intercept disease progression, to slow, stop
59 or even reverse neurodegeneration in a rapidly expanding
60 global population of PD patients. It is projected that
61 if PD disease progression could be slowed by just
62 20% it would overall save approximately \$76,000 per
63 patient, rising to a saving of approximately \$440,000
64 per patient if PD progression could be stopped altogether
65 [5]. Both these scenarios would translate to far better
66 long-term quality of life for PD patients, as well as
67 saving billions of healthcare dollars every year by all
68 major Western countries. Currently, only symptomatic
69 treatments are available to PD patients since no
70 disease-modifying therapy has yet been demonstrated
71 to be effective in slowing PD progression, which
72 highlights what is currently a huge unmet need for
73 the identification of effective neuroprotective PD
74 therapeutics.

75 For this reason, the International PD Linked Clinical
76 Trials initiative was established in 2012 with the
77 specific aim of identifying disease-modifying
78 treatments for PD that would slow, stop or reverse
79 the neurodegenerative aspects of this condition. The
80 International PD Linked Clinical Trials is run by a
81 committee of 15 global PD experts who, under the
82 stewardship of the Cure Parkinson's Trust, are
83 tasked with selecting, and sending into appropriately-
84 designed clinical trials, compelling new and repurposed
85 therapeutics to evaluate their disease-modifying
86 potential in various different populations of patients
87 with PD. At their first ever committee meeting in
88 2012, 26 potential disease-modifying candidate drug
89 approaches for slowing

91 PD progression were evaluated. At that meeting,
92 several of these therapeutics were prioritized to
93 enter PD disease-modifying trials, and they have
94 since entered, or have now recently completed
95 (Bydureon), these clinical evaluations. On the basis
96 of compelling biochemical, physiological and pharmaceutical
97 arguments, coupled with a strong safety record,
98 *Simvastatin* was one of the drugs prioritized at that
99 meeting [6]. Accordingly, funds were subsequently
100 raised and this *Simvastatin* clinical trial in PD
101 patients was commenced in September 2015 [7]. This
102 *Simvastatin* study is co-funded by the Cure Parkinson's
103 Trust and the JP Moulton Foundation. This on-going
104 2 year trial involves 198 patients with mid-stage
105 idiopathic PD and is currently being carried out in
106 movement disorder units in 23 hospitals across the
107 UK. Projected completion of this trial is in early
108 2020.

109 The current paper discusses the original biochemical,
110 physiological and pharmaceutical rationale that led
111 the committee in 2012 to agree that this trial was
112 strongly merited to explore the disease-modifying
113 potential of *Simvastatin* for treating PD. It also
114 updates to October 2017 the rationale for conducting
115 this trial in terms of our current understanding of
116 the relevant mechanisms of action and biological
117 targets of *Simvastatin* that continues to maintain
118 our enthusiasm about the use of this therapeutic as a
119 disease-modifying approach for patients with PD.

120 This paper also strives to achieve a balanced view
121 of a range of conflicting epidemiological studies
122 surrounding the use of statins for cardiovascular
123 protection, and whether statin use for this purpose
124 may increase or decrease PD risk.

125 Finally, this paper describes details about our ongoing
126 *Simvastatin* trial and outlines the decisions made
127 about its design, as well as aspects about patient
128 selection, patient recruitment, the dose of *Simvastatin*
129 chosen, investigator site selection, rationale on how
130 the duration of the trial was chosen, and the choices
131 of which patient outcomes are being measured.

132 **WHY DOES SIMVASTATIN REPRESENT A** 133 **STRONG CANDIDATE TO BE A** 134 **DISEASE-MODIFYING THERAPEUTIC** 135 **FOR PATIENTS WITH PARKINSON'S** 136 **DISEASE?**

137 What is the biochemical, physiological & pharmaceutical
138 rationale for testing *Simvastatin* in PD patients
139 as a long-term disease-modifying therapy?

140 Although statins have been widely adopted in mil- 191
141 lions of patients worldwide as cholesterol lowering 192
142 drugs to reduce cardiovascular risk, a very wide 193
143 range of laboratory studies (described below) coa- 194
144 lesce to suggest that statins also modulate some of 195
145 the important biochemical processes involved with 196
146 driving neurodegenerative changes, and may there- 197
147 fore offer a beneficial long-term disease-modifying 198
148 therapeutic approach to reduce neurological decline 199
149 in PD patients. 200

150 Several laboratory studies have demonstrated mul- 201
151 tiple biochemical neuroprotective effects of statins 202
152 in models of PD; these will be reviewed and dis- 203
153 cussed below. *Simvastatin*, like all statins, is a specific 204
154 inhibitor of the rate-limiting enzyme in cholesterol 205
155 biosynthesis, and it is one of the most effective of the 206
156 statins in terms of crossing the blood-brain barrier, 207
157 while *Pravastatin* shows almost no penetration [8]. 208
158 In fact the permeability of different statins into the 209
159 brain directly relates to the level of their individual 210
160 lipophilicity [9, 10].

161 In addition to their original pharmaceutical use 211
162 in lowering cholesterol, statins display multiple 212
163 neuroprotective effects. For example, Selley [11] 213
164 reported that *Simvastatin* prevents methyl-4-phenyl- 214
165 1,2,3,6-tetrahydropyridine (MPTP)-induced striatal 215
166 dopamine depletion and protein tyrosine nitration 216
167 in mice. Ghosh et al. [12] then found, at a dose 217
168 of 1 mg/kg body weight/day (which is equivalent to 218
169 the FDA-approved dose in adults), that *Simvastatin* 219
170 enters the substantia nigra, inhibits the activation of 220
171 p21(ras), suppresses the activation of NF- κ B, atten- 221
172 uates the expression of proinflammatory molecules, 222
173 protects dopaminergic neurons, restores striatal fibers 223
174 and dopamine levels, and improves locomotor func- 224
175 tion in an acute MPTP model of PD. They concluded 225
176 by suggesting that statins are capable of slowing down 226
177 the progression of neuronal loss in the MPTP mouse 227
178 model. 228

179 In an excellent and extensive review, Roy and 229
180 Pahan in 2011 [13] outlined the evidence for five sep- 230
181 arate pathways, each thought to be of relevance in 231
182 PD neurodegenerative aetiopathogenesis, by which 232
183 *Simvastatin* may improve dopaminergic neuronal 233
184 survival :- 234

- 185 ● suppression of proinflammatory molecules and 235
186 microglial activation 236
- 187 ● stimulation of endothelial nitric oxide synthase 237
- 188 ● inhibition of oxidative stress 238
- 189 ● attenuation of α -synuclein aggregation 239
- 190 ● modulation of adaptive immunity 240

One of the objectives of the current review is to 191
update these biochemical and pharmaceutical find- 192
ings to the present day to help give a perspective 193
on the rationale of why a clinical trial testing *Sim-* 194
vastatin as a potential disease-modifying therapeutic 195
for patients with PD is currently underway. Below is 196
our current interpretation (updated to October 2017) 197
of the multiple cholesterol-independent biochemical 198
mechanisms of action of *Simvastatin* as originally 199
cited by Roy and Pahan in 2011 [13] that we believe 200
specifically support the biochemical, physiological 201
and pharmaceutical reasons underpinning this inno- 202
vative clinical trial. We add to this 2011 list, the topic 203
of the stimulation of increased expression of neu- 204
rotrophic factors by statins which was not covered 205
by Roy & Pahan in 2011 but since then, in the con- 206
text of neurodegenerative diseases, has shown also to 207
be of considerable relevance to the other pleiotropic 208
effects of statins mentioned above. 209

210 *Suppression of proinflammatory molecules and* 211 *microglial activation*

212 In 2011, Roy & Pahan [13] collated evidence that 213
214 inflammation and oxidative stress represent impor- 215
216 tant components in nigrostriatal degeneration in PD 217
218 [14–20]. At that time it was already well established 219
220 that cytokines were central to the inflammatory pro- 221
222 cesses that accompany various forms of acute and 223
224 chronic brain injury, and many research laboratories 225
226 around the world had begun to focus with therapeutic 227
228 intent on PD. Ghosh et al. [19] also notably found 229
230 that NF- κ B was activated within the substantia 231
232 nigra pars compacta of PD patients and in MPTP- 233
234 intoxicated mice. Roy and Pahan [13] then discussed 235
236 how statins might be harnessed to reduce neuroin- 237
238 flammation in a Parkinsonian context. 239

240 At that time, the evidence for this potentially 241
242 important property of statins was that Pahan et al. 243
244 [21] had already shown *Lovastatin* inhibits NF- κ B, 245
246 iNos expression and the proinflammatory cytokines, 247
248 TNF- α , IL-1 β and IL-6 in lipolysaccharide (LPS)- 249
250 stimulated rat primary astrocytes. Adding to earlier 251
252 work by Stanislaus et al. [22], Neuhaus et al. [23] 253
254 using in cells taken from multiple sclerosis patients 255
256 demonstrated that *Simvastatin* is more potent as 257
258 an effective immunomodulatory agent than either 259
260 *Lovastatin* and *Mevastatin*. 261

262 To add to this, Clarke et al. [24], building 263
264 on the fact that they knew statins generate pow- 265
266 erful anti-inflammatory effects in brain, reported 267
268 that *Atorvastatin* exerts these effects via IL-4, and 269
270

241 completely independent of its cholesterol-lowering
242 actions. These results were supported by other find-
243 ings [12, 25] which showed how p21(ras) inhibits the
244 expression of iNOS by inhibiting the activation of
245 NF-kappaB, while Pahan et al. [21, 26] demonstrated
246 how farnesylation can impact on these biochemi-
247 cal processes, and how the sphingomyelin-ceramide
248 signaling pathway is involved with stimulating the
249 expression of iNOS via LPS- or cytokine-mediated-
250 activation of NF-kappaB in astrocytes. The current
251 state of knowledge at that time on these aspects had
252 been well described and summarized by van der Most
253 et al. [27], after which Santiago et al. [28] then showed
254 that *Simvastatin* protected striatal dopaminergic ter-
255 minals against the neurotoxic damage caused by LPS,
256 but not in an MPP+ toxic model. Liu et al. [29] have
257 recently explored how the inflammatory responses in
258 microglia may be controlled in PD-related models
259 and postulating that Nur77 may be a modulator of
260 microglia-mediated dopaminergic neurotoxicity.

261 Building on earlier work which showed that statins
262 protect neurons in models of long-lasting status
263 epilepticus and seizures, Gouveia et al. [30] found
264 that *Lovastatin* protectively decreased mRNA expres-
265 sion levels of the proinflammatory cytokines, IL-1 β ,
266 IL-6, and TNF α in hippocampal neurons during
267 experimental status epilepticus. Using a mouse model
268 of Alzheimer's disease (AD), Kurata et al. [31] found
269 that, after 15–20 months of treatment, both *Atorvas-*
270 *tatin* and *Pitavastatin* were protective of senile plaque
271 formation, and that this protection was preceded by a
272 reduction of proinflammatory events including levels
273 both of activated microglia and TNF- α . This sup-
274 ported earlier work by Tong et al. [32] who had
275 found in amyloid precursor protein transgenic mice
276 that *Simvastatin* attenuated inflammation, oxidative
277 stress and reduced amyloid beta levels and the num-
278 ber of affected neurites. *Simvastatin* had also been
279 shown to protect against tissue injury in the context
280 of ischemia-reperfusion injury [33], and in a model
281 of cardiopulmonary bypass to protect against cere-
282 bral and systemic inflammation, neuronal loss and
283 memory impairment [34].

284 Using a 6-hydroxydopamine model of PD and
285 a 3 week administration of *Simvastatin*, Yan et al.
286 [35] presented evidence that NMDA receptor mod-
287 ulation, MMP9 (matrix metalloproteinase-9) and
288 TNF- α by *Simvastatin* could partially explain its
289 anti-inflammatory, neuroprotective effects. Using a
290 similar model of PD, Kumar et al. [36] then found in
291 a mixed behavioral and biochemical study that *Ator-*
292 *vastatin* (20 mg/kg) and *Simvastatin* (30 mg/kg) were

293 both protective of weight loss, locomotor activity, and
294 also decreased levels of the inflammatory cytokines,
295 TNF- α and IL-6 that are characteristic of this model.
296 They also found that these statins restored the deficits
297 in mitochondrial enzyme complex activity that are
298 also generated in their 6-hydroxydopamine model.

299 The notion that mitochondrial function might be
300 involved with the anti-inflammatory action of statins
301 was also highlighted by Esposito et al. [37] in a com-
302 pletely different model, that of spinal cord injury
303 (which also displays inflammation, neutrophil infil-
304 tration, nitrotyrosine formation, pro-inflammatory
305 cytokine expression, and nuclear factor (NF)- κ B
306 activation). They showed that PPAR- α (a major
307 arbiter of mitochondrial size and number) *contributes*
308 to the anti-inflammatory activity of *Simvastatin*.
309 Specifically, and describing their findings as the
310 demonstration of a new mechanism for the action of
311 statins, they showed that the anti-inflammatory prop-
312 erties of *Simvastatin* were substantially reduced in
313 a PPAR- α knockout model. Since, as well as over-
314 all mitochondrial function, the PPAR-alpha nuclear
315 receptor also regulates genes involved with inflam-
316 mation and oxidative stress, it is of particular
317 interest that they found PPAR- α mediates the anti-
318 inflammatory effects of *Simvastatin in vivo* models of
319 acute neuroinflammation. This built on their earlier
320 observation [38] that *Simvastatin* similarly worked
321 in synergy with PPAR- α to protect cellular damage
322 caused by systemic inflammation in a model of mul-
323 tiple organ failure. A recent report by Zhou et al.
324 [39] expands Esposito's findings in that they showed
325 *Simvastatin* is both neuroprotective and inhibits
326 secondary inflammatory damage by markedly down-
327 regulating the expression of the proteins (NF)- κ B,
328 TLR4 and IL-1 β .

329 Xu et al. [40] studied how *Simvastatin* affects
330 6-hydroxydopamine-lesioned PC12 through regula-
331 tion of PI3K/AKT/caspase 3 and by modulating
332 inflammatory mediators, and how it might be used
333 therapeutically to treat patients with PD. In a cel-
334 lular RNA study involving 6-OHDA administration,
335 Yan et al. [41] explored the involvement of N-methyl-
336 D-aspartic acid receptor 1 (NMDAR1) finding that
337 *Simvastatin* inhibits the expression of NMDAR1,
338 and of the cytokines, TNF- α , IL-1 β , and IL-6, in
339 a manner just as potent as using siRNA for the
340 receptor. In a retinal cell model, Zhang et al. [42]
341 reported that that *Simvastatin* inhibits apoptosis fol-
342 lowing IR-induced retinal injury by inhibition of
343 the TNF- α /NF- κ B pathway. With a PD therapeu-
344 tic perspective in mind, Zhang's findings should be

345 seen in the context that Malu Tansey's group had
346 previously described [43] how the TNF- α /NF- κ B
347 pathway mediates chronic inflammation which, in
348 turn may generate a reduction in Parkin levels, and
349 thereby increasing the vulnerability for degeneration
350 of the nigrostriatal pathway. They argued that chronic
351 inflammation offers a clear biochemical mechanism
352 which can promote the development of PD. Huang
353 et al. [44] recently showed in multiple models that
354 Simvastatin ameliorated memory deficits in patients
355 with Alzheimer's disease as well as in laboratory
356 models of AD, and that it achieved this through
357 reduction of mRNA expression of inflammatory
358 cytokines and mediators as well as by improv-
359 ing neuronal survival, supporting earlier work by
360 Wang et al. [45].

361 In summary, by directly inhibiting key inflamma-
362 tory processes, *Simvastatin* may therefore represent
363 a therapeutically beneficial disease modifying agent
364 with considerable potential to reduce the rate of PD
365 progression.

366 *Stimulation of endothelial nitric oxide synthase*

367 Roy & Pahan [13] also collated robust evidence
368 [14-15, 17, 21, 24-25] in 2011 which supported the
369 view that the upregulation of endothelial nitric oxide
370 synthase (eNOS) is generated by statins via suppres-
371 sion of mevalonate and concomitant activation of the
372 PI-3 kinase-Akt pathway. This built on Flint Beal's
373 supposition [46] that modulating eNOS might offer
374 a valuable neuroprotective therapeutic approach for
375 the treatment of PD. Statins inhibit iNOS expression,
376 while in contrast, they stimulate eNOS-derived nitric
377 oxide production, and this property appears biochemi-
378 cally unrelated to their ability to reduce cholesterol
379 [47]. Statin-induced upregulation of eNOS can be
380 reversed by geranylgeranyl pyrophosphate (but not
381 by farnesyl pyrophosphate) which intimates [13]
382 that Rac/Rho (rather than Ras) may be involved
383 in the regulation of eNOS. Fulton et al. [48] and
384 Skaletz-Rorowski et al. [49] demonstrated that Akt
385 phosphorylates eNOS, while mevalonate inhibits
386 phosphatidylinositol-3 kinase and thereby reduces
387 Akt activation. As statins lower mevalonate levels
388 (via inhibition of HMG-CoA reductase) it therefore
389 seems likely that reduction of mevalonate may trigger
390 increased eNOS production, and thereby increasing
391 NO levels. Atorvastatin has been shown [50, 51]
392 to promote NOS-derived nitric oxide production by
393 reducing expression of caveolin-1, and the therapeu-
394 tic implications of these HMG-CoA reductase effects

395 of statins are still being actively clarified in cardio-
396 vascular medicine [52, 53].

397 A recent review by Saeedi Saravi et al. [54] focuses
398 more specifically on the potential relevance of the
399 mevalonate pathway to the potential therapeutic ben-
400 efit that statins may offer in protecting against long
401 term neurodegeneration in PD patients. Bezar's
402 group [55] now consider downstream modulation
403 of the sterol regulatory element-binding protein 1
404 (SREBP-1) pathway to be important in inducing
405 phenotypic changes in dopaminergic cells, includ-
406 ing increases in cell growth, synaptic connections
407 and protein expression. They have recently presented
408 additional data on this that supports a potential pro-
409 tective role of statins in PD [56]. Since SREBP-1 (and
410 SREBP-2) regulates promoter activity of PCSK9 [57]
411 there is therefore a clear link, with therapeutic impli-
412 cations, between SREBP-1 and PCSK9, and it was
413 recently shown that *Simvastatin* increases PCSK9
414 expression [58, 59] which may be therapeutically
415 relevant [60-63].

416 Sun et al. [64] showed in a cardiovascular context
417 that eNOS is a direct target of miR-155. Inflam-
418 matory cytokines such as TNF- α increase miR-155
419 expression and inhibition of miR-155 reverses TNF-
420 α -induced downregulation of eNOS expression.
421 They found that *Simvastatin* decreased TNF- α -
422 induced upregulation of miR-155 and ameliorated
423 the effects of tumor necrosis factor- α on eNOS
424 via the mevalonate-geranylgeranyl-pyrophosphate-
425 RhoA signaling pathway.

426 Pierucci et al. [65] reviewed in 2011 the promise
427 and opportunities of harnessing the NOS system to
428 treat PD, essentially building on the work by Hoang
429 et al. [66] who assessed the aspects and extent of the
430 nitrate damage, including in nuclear and mitochon-
431 drial DNA, that is caused in an MPTP model of PD,
432 and in a NOS knockout model, and from which they
433 concluded DNA damage may contribute to the over-
434 all neurodegenerative process in PD. Peter Jenner's
435 group [67] found evidence of a major role for i-NOS-
436 mediated nitrate stress in microglia in their MPP+
437 model of PD, which they concluded had important
438 implications for developing neuroprotective strate-
439 gies for PD, an argument which was further supported
440 by Tripathy et al. [68], and also recently reviewed by
441 Jiménez-Jiménez et al. [69] from the perspective of
442 studies both in PD patients, and in various PD models.

443 Li et al. [70] reported in 2015 how *Simvastatin*
444 is therapeutically beneficial following LPS-induced
445 experimental lung injury, showing it had a protec-
446 tive effect by alleviating lung injury via decreasing

447 iNOS levels. This ties in with the earlier findings
448 by Pahan et al. [21] who, as described above, had
449 already demonstrated that *Lovastatin* inhibits NF- κ B,
450 iNOS expression and the proinflammatory cytokines,
451 TNF- α , IL-1 β and IL-6 in LPS-stimulated rat primary
452 astrocytes.

453 Therefore, as well as its beneficial effects through
454 suppression of proinflammatory molecules and
455 reduction of microglial activation (as outlined in the
456 previous section), *Simvastatin* also appears to offer
457 substantial long-term disease-modifying benefits for
458 PD patients on the basis of decreasing microglia
459 iNOS levels and reducing chronic nitrative stress.

460 Along with the continued research into how NOS
461 may contribute to the neurodegenerative process in
462 PD, and may thus offer a therapeutic opportunity,
463 such as using *Simvastatin*, to delay PD progression,
464 a parallel line of research has explored how NOS
465 might be modulated for therapeutic benefit in treat-
466 ing a widespread clinical complication experienced
467 by many PD patients on long-term dopaminergic
468 support, that of L-DOPA-induced dyskinesias. How
469 NOS inhibitors might be employed in the treatment
470 of dyskinesias has been explored in experimental
471 studies using various PD models [71–74], and is
472 clearly showing promise for clinical translation for
473 the treatment of dyskinesias in PD patients. The
474 basis for this [75, 76] is that when inhibitors of the
475 MAPK signaling cascade impede the inappropriate
476 dyskinesia-inducing response of striatal neurons this
477 offers considerable evidence that MAPK inhibitors
478 may offer therapeutic efficacy in reducing incidence
479 and/or severity of dyskinesias experienced by PD
480 patients.

481 The isoprenylation of Ras is inhibited by statins
482 which underpins their ability to curb the stimulation
483 of ERK 1/2 MAP kinases, and Schuster et al. [77]
484 found that *Lovastatin* reduces the number and sever-
485 ity of dyskinesias in their 6-OHDA model of PD. In
486 particular, Tison et al. [78] found that *Simvastatin* was
487 indeed effective in reducing dyskinesias in a monkey
488 model of PD, but only at high doses that would be
489 incompatible with their long-term administration in
490 man, and which were 3-6 times higher than is being
491 used in the current clinical trial of *Simvastatin* in PD
492 patients (see below).

493 *Inhibition of oxidative stress*

494 Roy & Pahan [13] reviewed the evidence for
495 the involvement of statins in inhibiting the pro-
496 cess by which oxidative stress contributes to

497 neurodegeneration in PD, particularly focusing on
498 the roles of nicotinamide adenosine dinucleotide and
499 Rac, collating evidence that NADPH oxidase is vital
500 in terms of attrition of dopaminergic neurons. In fact
501 it was already known that nigral NADPH oxidase
502 is upregulated in MPTP mice, but that, conversely,
503 this toxin had no effect on dopaminergic neurons
504 in gp91phox (–/–) mice [79, 80]. Building on the
505 review by van der Most et al. [27], Roy & Pahan
506 [13] provided evidence that the inhibition by statins
507 of the geranylgeranylation of Rac leads to reduced
508 NADPH oxidase-mediated generation of superoxide,
509 which they interpreted as evidence statins may atten-
510 uate oxidative stress by diminishing the production of
511 reactive oxygen species both in the substantial nigra
512 of MPTP mice, and in PD patients via this biochem-
513 ical process.

514 Since then, much research has focused on the
515 role of Nrf2 (nuclear factor erythroid 2-related fac-
516 tor 2) in oxidative stress [81, 82], and how, in several
517 therapeutic areas, including PSP and PD [83], age-
518 related macular degeneration [84], oncology [85, 86],
519 cardiovascular disease [87, 88], arterial calcification
520 [89], spinal cord injury [90] and radiation dermati-
521 tis [91], this emerging biochemical insight might
522 be manipulated to therapeutic advantage. Nrf2 is
523 a cytoprotective master regulator of the transcrip-
524 tional response to oxidative stress; it has a rapid
525 turnover, and its role in neurodegenerative diseases
526 has been well described by Gan and Johnson [92],
527 and its diversity of actions and control with respect
528 to mitochondrial function were recently well reviewed
529 by Holmstrom et al. [93], and also by Dinkova-
530 Kostova et al. [94]. When reactive oxidative species
531 are at low levels, nuclear Nrf2 is suppressed by the
532 inhibitory protein, KEAP1, which sequesters Nrf2 in
533 the cytoplasm to prepare it for proteasomal degrada-
534 tion [95, 96], and which maintains Nrf2 at a relatively
535 low steady state level. However, increasing levels of
536 reactive oxidative species influence KEAP1 in a way
537 that progressively impairs its ability to target Nrf2 for
538 degradation. A link between Nrf2, MAPT expression
539 and the risk of PD has recently been postulated by
540 Wang et al. [97], and may possibly offer a mechanis-
541 tic glimpse of why tau/MAPT repeatedly appears in
542 large-scale GWAS studies of PD patients [98, 99] yet
543 its role in the generalized risk of developing PD, and
544 its specific role in neuroinflammation with regards to
545 PD, are both poorly understood [100].

546 Several agents (particularly Nrf2 activators), which
547 act on these biochemical pathways (by upregu-
548 lating antioxidant, anti-inflammatory, mitochondrial

549 biosynthetic, apoptotic mediator and cytoprotective
550 genes) have promising potential for the long-term
551 protection from neurodegeneration in PD patients.
552 These include monomethylfumarate [101], dimethyl-
553 fumarate [102], gliptins [103] and the triterpenoid,
554 RTA-408 [85], each of which have already been prior-
555 itized by the International PD Linked Clinical Trials
556 committee to enter clinical trials in PD patients to
557 determine their disease-modifying potential. As a
558 practical therapeutic approach in neurology, much
559 of the new understanding of the protective poten-
560 tial of activating Nrf2 resides in these emerging
561 publications and it is being rapidly translated into
562 disease-modifying agendas in PD, as well as in other
563 therapeutic areas. Urate probably also acts via the
564 Nrf2 antioxidant response pathway [104] and is cur-
565 rently being tested (using oral inosine) in a Phase
566 III trial in 270 PD patients to assess its disease-
567 modifying potential over a treatment duration of 2
568 years [105]. To add to all the other biochemical
569 actions of statins outlined in this review we can add
570 another LCT-prioritized drug, *Simvastatin*, to this
571 important list of Nrf2 activators that may all have
572 the potential to be used clinically to slow neurode-
573 generation in PD patients. In 2014 Abdanipour et al.
574 [106], studying PGC-1 α and Nrf2 expression on cell
575 survival and apoptosis demonstrated that *Lovastatin*
576 protects bone marrow stromal cell-derived neural
577 stem cells against oxidative stress-induced cell death,
578 and suggested it as a candidate for the treatment of
579 neurological diseases that involve oxidative stress.
580 Since then, several papers have added further sup-
581 port to the view that statins act as Nrf2 activators. Wu
582 et al. [107] recently reported that *Atorvastatin* reduces
583 damage in liver injury when exposed to inflammatory
584 stress, citing loss of the adaptive antioxidant response
585 mediated by Nrf2 as the basis for the biochemical
586 mechanism involved. *Simvastatin* was found by Jang
587 et al. [108] to induce heme oxygenase-1 via direct
588 activation of Nrf2 in human colon cancer cell lines.
589 Ferraro et al. [109] studied the effects of *Simvastatin*
590 in both lung inflammation and in a human neuroblas-
591 toma cell line and concluded that *Simvastatin* may
592 provide neuroprotection against neurotoxicity via
593 Nrf2 independently of its ability to inhibit cholesterol
594 synthesis. Furthermore, Yeh et al. [110] demonstrated
595 that the well known effect of statins to protect against
596 atrial fibrillation was generated by the activation of
597 Akt/Nrf2/heme oxygenase-1 signaling.

598 Hsieh et al. [111] was the first to show that iron
599 production from Heme oxygenase-1 activity may
600 play an important role in the increased apoptosis

601 in response to glucose deprivation in neuronal cells
602 pretreated with *Simvastatin* which acted by inducing
603 of Heme oxygenase-1, a process which was medi-
604 ated by Nrf2. They found in neuronal cells that the
605 iron chelator, desferrioxamine, blocked apoptosis,
606 which suggested that iron production from Heme
607 oxygenase-1 activity might drive increased apoptosis
608 in situations of glucose deprivation in neuronal cells
609 that had been pretreated with *Simvastatin*. Two PD
610 trials also prioritized by the international PD Linked
611 Clinical Trials committee in 2012 are underway to test
612 iron chelator therapy as a potential disease-modifying
613 treatment for patients with PD. One trial involves 338
614 early-stage PD patients who are not currently taking
615 antiparkinsonian medication (disease duration less
616 than 18 months) and who are taking the iron chela-
617 tor, Deferiprone [112], and the second dose-finding
618 trial [113], also using Deferiprone, is comprised of
619 140 PD patients who have been diagnosed with PD
620 within the last 3 years and who are currently taking
621 antiparkinsonian medication.

622 *Attenuation of α -synuclein aggregation*

623 In their 2011 review of the potential for using
624 statins to treat PD, Roy and Pahan [13] summarized
625 the knowledge at that time relating to how alpha-
626 synuclein impacts on dopaminergic toxicity and cell
627 loss, motor deficits, the synthesis of cholesterol, and
628 the deposition of alpha-synuclein-rich Lewy bod-
629 ies in the substantia nigra. They concluded that,
630 since statins suppress the release of proinflammatory
631 molecules from activated glial cells (see above), it
632 is likely they should also subdue malformed alpha-
633 synuclein-mediated glial cell activation in a manner
634 that is completely independent of cholesterol. As with
635 all the other sections in this review, much has moved
636 on over the past 6 years. A current view, held by many
637 (but not in 2011) is that malformed alpha-synuclein is
638 capable of cell-to-cell transmission and that this may
639 underpin the development of PD throughout the body,
640 but particularly involving spread from the enteric
641 nerves, and/or olfactory bulb, to the substantia nigra,
642 raphe, locus coeruleus, the cortex and several other
643 important anatomical sites which each contribute in
644 their own way to the range of PD symptoms we see
645 clinically [114–118].

646 Roy and Pahan [13] reflected on how *Lovas-*
647 *tatin*, *Simvastatin* and *Pravastatin* each generate
648 large reductions in alpha-synuclein accumulation
649 both in a transfected neuronal cell line, and in
650 primary human neurons [119], and that *oxidized*

651 cholesterol even promotes increased alpha-synuclein
652 aggregation [120]. This observation has recently been
653 somewhat supported by Eriksson et al. [121] who
654 interpreted their findings in a neuroblastoma cell line
655 exposed to MPP⁺ to reason that high cholesterol in
656 PD stimulates the accumulation of alpha-synuclein.

657 Once *Lovastatin* was also shown [122] to reduce
658 alpha-synuclein accumulation and aggregation in
659 transgenic models, it was logical to contemplate that,
660 since statins lower cholesterol levels, then they may
661 therefore directly reduce the aggregation of alpha-
662 synuclein in PD patients. Koob et al. concluded
663 that, while it was known as early as 2006 [123]
664 that, once mutated, alpha-synuclein causes a much
665 stronger glial cell inflammatory response, but that
666 since statins reduce the expression of these proinflam-
667 matory molecules they may well be found beneficial
668 as a long-term treatment for patients with PD. Given
669 the knowledge available at that time, Roy and Pahan
670 [13] therefore posed the logical question; do statins
671 suppress mutated alpha-synuclein-mediated glial cell
672 activation in a manner that is completely independ-
673 ent of cholesterol? Several papers, mostly published
674 since then, have gone some way to answering that
675 question [24, 124–131] – the widely-held consensus
676 answer is definitely yes, although there is still slightly
677 less clarity on the mechanistic details than we might
678 have wished for.

679 *Modulation of adaptive immunity*

680 Statins have been repurposed into several diseases
681 where innate and adaptive immunity and endothelial
682 damage play an important role [132]. To date, statins
683 have been specifically tested and used in atheroscle-
684 rosis [133], multiple sclerosis [134, 135], rheumatoid
685 arthritis [136], Behcet's disease [137], and Kawasaki
686 disease [138, 139] in many cases with very promis-
687 ing results. It was pointed out in the review by Roy
688 & Pahan [13] that effector T cells may exacerbate
689 disease progression (which can be demonstrated in
690 post mortem PD brains), while regulatory T cells
691 (Tregs) tend to occupy a protective role. It has been
692 found that T-cell responses in an MPTP model of
693 PD add to the rate of neurodegeneration [140–142]
694 while conversely, Tregs have been shown to be protec-
695 tive in an MPTP model of PD [143], and the reasons
696 behind this duality have previously been discussed
697 by Mosley et al. [144] and, since Tregs can be mod-
698 ulated *in vivo*, this gives strong support to the use
699 of an immunomodulatory approach to treat PD. In
700 2010, Reynolds et al. [145] demonstrated that natural

701 Tregs reversed the T cell nigrostriatal degeneration
702 caused by malformed alpha-synuclein, proposing that
703 this observation forms a sound rationale for future
704 PD immunization strategies. This approach has been
705 reviewed and expanded upon by several with consid-
706 erable clarity and insight [124, 130, 146, 147], and
707 most recently by Gendelman and Mosley [148] who
708 discussed approaching this topic in therapeutic terms
709 by postulating simultaneously to seek enhancing the
710 suppressive function of Tregs, while downregulating
711 proinflammatory cytokine production. They balanced
712 and tempered this by recognizing that immune system
713 activation is also necessary in order to clear debris
714 to help sustain and restore damaged neurons. They
715 offered 'mounting and strong evidence' that immune
716 transformation can affect the pathogenesis of neu-
717 rodegenerative diseases, and that modulation of the
718 inflammatory response, while restoring a homeostatic
719 immune system via immunopharmacological strate-
720 gies, may lead to new therapeutic opportunities for
721 PD and other neurodegenerative disorders.

722 Acting as a cytokine and neuropeptide which
723 impacts on immune responses, Vasoactive Intesti-
724 nal Peptide (VIP) induces Tregs. The neuroprotective
725 capability of Tregs is mediated through TH17, and It
726 has been suggested that shifting the balance between
727 effector and regulatory T cell activity by adaptive
728 immune regulation of glial homeostasis could be used
729 to attenuate neurotoxic inflammatory events [149].
730 By peptide modifications similar to those for GLP-
731 1 agonists that have given them greater potency and
732 much longer metabolically stable half-life in blood
733 than the native hormone, Olsen et al. [150] developed
734 an analogue of VIP and showed that to be an effective
735 immunomodulatory agent in an MPTP model of PD.
736 They concluded by stating they had provided "strong
737 evidence" that VIP receptor agonism has the potential
738 to slow the pathogenesis of PD through modulation
739 of the inflammatory response. This builds on the ear-
740 lier observation by Brachmachari and Pahan [151]
741 who, and citing Foxp3 as a master regulator in Treg
742 formation and function, discovered that *Simvastatin*
743 upregulates Foxp3 by inhibiting nitric oxide produc-
744 tion, going on to suggest that Treg enrichment by
745 *Simvastatin* may help to protect dopaminergic neu-
746 rons in the substantia nigra.

747 *Increased expression of neurotrophic factors*

748 This potential biochemical effect of statins was not
749 covered in the earlier review by Roy & Pahan [13].
750 Hernandez-Romero et al. [152], as well as demon-

751 strating the potency of *Simvastatin* in markedly
752 reducing inflammatory responses in LPS-induced PD
753 rats, including interleukin-1, TGF- α and iNOS (as
754 have several other groups, as described above), also
755 found that *Simvastatin* stimulated the activation of the
756 neurotrophic factor, BDNF. This suggests there may
757 be a profoundly neurogenic aspect to the mechanism
758 of action of *Simvastatin* in dopaminergic neurons.
759 This was followed up by Wu et al. [153] who showed
760 that *Simvastatin* increases the hippocampal expres-
761 sion of BDNF and VEGF in a model of brain injury.

762 They concluded that the neurorestorative effect of
763 *Simvastatin* they observed was probably be medi-
764 ated via the Akt-mediated signaling pathway, which
765 thereby upregulated expression of growth factors,
766 thus stimulating via neurogenesis the restoration of
767 cognitive function they observed with *Simvastatin*.
768 They employed a similar dose to that used in our
769 current clinical trial of *Simvastatin* in PD patients
770 (see below). It was then reported [154] in a model
771 of spinal cord injury that *Simvastatin* generated sig-
772 nificantly improved locomotor recovery, and this
773 improvement was ascribed to the higher levels of
774 expression of BDNF and GDNF which observed
775 in this study after administration of *Simvastatin*.
776 This contention was further supported when it was
777 reported [155] that both *Simvastatin* and *Atorvastatin*
778 increased the expression of BDNF, VEGF and NGF,
779 as well as activation of the Akt-mediated signaling
780 pathway, in an experimental model of intracerebral
781 hemorrhage. Furthermore, it was shown [156] that
782 *Simvastatin* modulates the profile of the release of
783 cytokines and trophic factors from microglia, (partic-
784 ularly interleukin-1 β , TNF- α , and BDNF) through a
785 mechanism that is cholesterol-dependent, and which
786 went some way to explain previously confusing con-
787 tradictions in laboratory results. Rana et al. [157]
788 then reported that *Simvastatin* increased expression
789 of BDNF exon-IIC transcripts in stressed mice. Wang
790 et al. [158] then reported that, in A β 25-35-mice,
791 *Simvastatin* is protective of neurogenesis through
792 reduction of farnesyl pyrophosphate level which
793 then generates α 7nAChR-cascading PI3K-Akt and
794 increased levels of BDNF. Next, Gao et al. [159]
795 demonstrated that *Simvastatin* significantly increases
796 the levels both of BDNF and GDNF in a model of
797 spinal cord injury, and then went on to show that
798 *Simvastatin* also reduces neuronal apoptosis while
799 promoting locomotor recovery in this model [160]. It
800 was recently shown [161] that *Simvastatin* promoted
801 the neurogenesis and migration of neural stem cells
802 with a mode of action involving the ROCK/CGTase

803 pathway. *Simvastatin* has also recently been demon-
804 strated to improve peripheral nerve regeneration and
805 functional recovery in an experimental model of sci-
806 atic damage that involves elevation of levels of GDNF
807 and several other growth factors [162]. Earlier this
808 year, it was reported [163] that *Atorvastatin* increased
809 serum BDNF levels and improved functional recov-
810 ery (modified Rankin and Barthel scales) in patients
811 following atherothrombotic stroke.

812 It had previously highlighted [20] that the
813 Nurr1/CoREST pathway in microglia and astrocytes
814 protects dopaminergic neurons from inflammatory
815 damage, and this is thought to be particularly relevant
816 here because Nurr1 activity is known to be closely
817 related to GDNF activity [164]. Wang et al. [158] also
818 demonstrated that *Simvastatin* induces autophagy by
819 inhibiting the mTOR signaling pathway. In 2015, Roy
820 et al. [165] then took a step forward to help tie the
821 various threads together in reporting that statins serve
822 as ligands for PPAR α and ascribed the neurotrophic
823 action of statins to be via the PPAR α -CREB path-
824 way. Until then there had been no receptor protein
825 identified for statins (they exert their *lipid-lowering*
826 actions quite differently, more structurally, as com-
827 petitive inhibitors of HMG-CoA reductase).

828 Finally, and here focusing on PD, unlike most of
829 the other neurological models described in this sec-
830 tion from which we are definitely able to draw useful
831 parallels, Castro et al. [166] reported on how *Ator-*
832 *vastatin* in an intranasal PD rat model caused in a
833 significant increase in striatal and hippocampal lev-
834 els of nerve growth factor, adding that their findings
835 ‘extend the notion of the neuroprotective potential
836 of *Atorvastatin* and suggest that it may represent a
837 new therapeutic tool for the management of motor
838 and non-motor symptoms of PD’, while in 2016,
839 Tan et al. [167] showed in an LPS model of PD
840 that *Simvastatin* restored the expression of BDNF, as
841 well as replicating earlier findings by many groups
842 that it reduces oxidative stress and improves nigral
843 function.

844 EPIDEMIOLOGICAL STUDIES ON THE 845 USE OF STATINS AND THE RISK OF PD

846 The purpose of this section is not intended as a
847 critical appraisal of epidemiological research in this
848 area, nor to generate data synthesis (in fact others
849 have previously attempted to do this - see below),
850 but rather to provide a catalogue, and a context, of
851 published studies.

Valid interpretation of published studies has been consistently confounded by the core reason why statins are taken, i.e., to reduce high levels of cholesterol, which in turn means there is inevitably a high correlation between the two explanatory variables, statin use and blood cholesterol levels. Partly because of this confounding inter-relationship, there is currently no clarity about whether statin use is protective of an individual developing PD, has no effect, or makes it more likely that an individual may develop the disease.

Most would agree that the hypothetical risk of a healthy individual acquiring PD through taking a particular medication, represents a very different scenario to using that same medication to treat the disease once it has already developed. Nevertheless, it is appropriate to discuss here, and bring a balance to, the various studies that have either linked the taking of statins to protecting healthy individuals against developing PD, or the converse.

We re-emphasize this ongoing epidemiological debate is actually of questionable relevance to patients who *already* have PD but it is appropriate in terms of our on-going trial of Simvastatin to use this opportunity to give a balanced scientific review of the viewpoints, the available evidence, and to highlight strengths and weaknesses in published papers in this area of research.

First, it is important to make the point that, since the initial isolation of statins from microorganisms in the 1970s, there has been a huge growth in their specific use in primary and secondary prevention of various forms of cardiovascular disease. In 2016, the US Preventative Services Task Force advised the use of statins for people between 40 and 75 years old who carry at least one risk factor for heart disease, and who have more than a 10% risk of heart disease [168]. Similarly, the UK National Institute for Health and Clinical Excellence has endorsed the use of statins in those with an estimated 10% risk of developing cardiovascular disease over the next decade [169]. The nature, interactions and pharmacokinetic relationships between cholesterol, apolipoproteins and statins were well described from a neurological perspective in a Cochrane review [170, 171] as a part of an original, then updated, analysis to consider the possible use of statins in the context of dementia prevention or treatment. They found 'insufficient evidence to recommend statins for the treatment of dementia'. Many PD patients develop cognitive impairment, but while none of those in that meta-analysis were PD patients, a recent paper

by Deck et al. [172] found that PD patients taking statins performed better on tests of global cognition, semantic fluency and phonemic fluency. Furthermore, although it is known that statins increase HDL and apolipoprotein A1 levels [173, 174], and that lower apolipoprotein A1 levels are associated with later stages of PD progression, Deck did not find that baseline apolipoprotein A1 levels correlated with any baseline neuropsychological measures.

Turning now to the question of whether the use of statins may positively or negatively influence the risk of developing PD, in 2006 and citing that epidemiologic investigations had revealed an association between low LDL-C levels and the risk of PD, with several studies previously having suggested a role of lipid and cholesterol metabolism in the pathogenesis of PD, de Lau et al. [175], studying >6000 patients, felt there might well be a role involving lipids in the pathogenesis of PD, and suggested that this provided support for the notion of an important role of oxidative stress in the pathogenesis of the disease.

An extensive review of patients in the Veterans Affairs healthcare system then found *Simvastatin* (but not *Atorvastatin* or *Lovastatin*) use was associated with a strong reduction in incidence of dementia and PD [176]. Wahner et al. [177] then found that all statins are inversely associated with PD (except for *Pravastatin*). They observed a higher frequency of statin use among controls versus PD cases. The strongest protective association between statin use and PD was observed in long-term (>5 yr) statin users. Huang et al. [178] then reported the results of a small epidemiological study of 124 PD patients and 110 controls which inferred that low LDL-C may be associated with a higher occurrence of PD, and that statin use for prophylactic cardiovascular protection may lower PD occurrence. What Huang did not report is whether their patients had low LDL levels prior to their diagnosis of PD, nor whether their LDL levels decreased after this diagnosis. Therefore, since statins are effective at lowering LDL cholesterol levels, it may well be that their study design intrinsically confused cause with effect. What is more is that this research was vastly underpowered in the sense that fewer than 20 of the 124 PD patients in this study were actually taking statins so the results cannot be viewed as reliable. Becker et al then reported in a case-control analysis involving 3637 PD patients and 3637 controls that the long-term use of statins or fibrates was not associated with a substantially altered relative risk of developing PD [179].

956 The same year, a study of approximately 50,000
957 Finnish citizens, with their baseline serum total
958 cholesterol stratified into five groups, reported that
959 those individuals with the highest levels of chole-
960 sterol were almost 90% more likely to develop PD
961 than those with the lowest levels of cholesterol [180],
962 concluding that, in subjects under 55 years of age,
963 our ‘large prospective study suggests that high total
964 cholesterol at baseline is associated with an increased
965 risk of Parkinson’s disease’.

966 A retrospective study involving a cohort of 419 PD
967 patients, showed that in PD patients who received
968 either a statin or a fibrate, their mean age of disease
969 onset was delayed by nearly 9 years when com-
970 pared with PD patients who were not taking any
971 lipid-lowering treatment [181]. They also found the
972 increase in the levodopa-equivalent daily dose over
973 2 years was significantly smaller in the group tak-
974 ing a statin (+24 mg) than in the matched control
975 group (+212 mg) ($p=0.004$), whereas the UPDRS
976 motor score progression was similar. Their conclu-
977 sion was that lipid-lowering drugs may have a disease
978 modifying effect.

979 The 2011 DATATOP study [182] then provided
980 evidence that higher total serum cholesterol con-
981 centrations may be associated with a modest slower
982 clinical progression of PD. The same team at Harvard,
983 using 12 years of patient follow-up, and following
984 644 documented incident cases of PD, then reported
985 [183] that regular use of statins was associated with
986 a modest reduction in PD risk. They suggested that
987 “the possibility that some statins may reduce PD risk
988 deserves further consideration”.

989 The following year Undela et al. [184] conducted
990 a robust meta-analysis of published healthy subjects
991 and found, across five separate case-control studies
992 ($n=43,526$) and three cohort studies ($n=1.4$ million),
993 that statin use reduced an individual’s risk of getting
994 PD by 23% ($p=0.005$), but no such effect was found
995 for *long-term* statin use. They substantiated their find-
996 ings by conducting further sensitivity analysis and
997 concluded that their results ‘suggest a decreased rel-
998 ative risk of PD in statin users as identified by a
999 combined meta-analysis of eight observational stud-
1000 ies’. Friedman et al. [185] then reported their findings
1001 following 94,308 (initially) non-statin users who did
1002 not have PD. By the end of their study, there had
1003 been 1035 incident cases of PD. Furthermore, 29,714
1004 participants (31.5%) had started using statins for a
1005 minimum of 6 months during the study period. This
1006 statin use was associated with a significant decrease in
1007 the incidence of PD ($p=0.001$), while no association

1008 was found between baseline LDL-C levels and PD
1009 risk. Friedman felt their results provided evidence
1010 relating to a lower incidence of PD among statin
1011 users.

1012 This contention was further supported by a report
1013 from Taiwan [186] following for several years 43,810
1014 individuals who had started taking a statin, and
1015 backed up by an excellent commentary by Tan and
1016 Tan, [187]. It was found that continuation of tak-
1017 ing lipophilic statins was associated with a decreased
1018 incidence of PD, whereas taking hydrophilic statins
1019 appeared not to generate this benefit.

1020 Then, Huang et al. [188] reported results of a
1021 prospective study involving 15,291 individuals with-
1022 out PD and mostly who were not statin users at study
1023 commencement. Over approximately a decade statin
1024 usage had increased to 11.2% of the study popula-
1025 tion, and there were 56 incident cases of PD. As
1026 in their 2011 paper [182] they reported that higher
1027 total cholesterol was associated with a lower risk of
1028 developing PD, even after adjustment for statin use.
1029 Unlike their earlier studies they calculated that statin
1030 use may be associated with a higher risk of acquiring
1031 PD which added further uncertainty to this topic, and
1032 also attracted considerable journalistic interest.

1033 To try to gain some clarity on whether statins were
1034 protective or not in terms of initially developing PD,
1035 Bai et al. [189] and Sheng et al. [190] both pub-
1036 lished extensive meta analyses of relevant results to
1037 date. Bai’s meta-analysis involved 3,513,209 indi-
1038 viduals and included 21,011 incident cases of PD.
1039 Sheng’s meta-analysis involved 2,787,249 individ-
1040 uals. The results of both studies were in complete
1041 agreement that statin use was associated with a much
1042 lower risk of PD ($p=0.001$) and that sensitivity anal-
1043 yses confirmed the robustness of these results. They
1044 found that statin use was less protective of PD in
1045 North America than in other geographies, which is
1046 something that may account for some of the con-
1047 jecture and mixed results that had been published
1048 previously. Furthermore, and adding to this complex-
1049 ity in terms of confounding interpretation of statin
1050 use in the context of PD epidemiology, Clark et al.
1051 [191] found that the frequency of treatment success
1052 in dyslipidemia management was significantly lower
1053 in African American patients in the USA than in
1054 non-Hispanic white patients, while Yood et al [192]
1055 found that African American patients initiating statin
1056 therapy are less likely to achieve LDL goals, even
1057 after controlling for adherence differences and other
1058 factors, and suggested that this group may require
1059 different pharmacologic management.

Huang's group then reported in 2016 [193] that higher levels of LDL-cholesterol were associated with improved executive set shifting and fine motor scores in PD patients, but not in healthy controls. This small study (64 PD cases) did not contain many statin users to be meaningful on interpreting this aspect but interestingly, they hypothesized from their results that there may possibly be an association between cholesterol and cognition that is nigrostriatal-based while very fairly pointing out that they could not currently ascertain whether this relationship was causative, reverse-causative or a parallel process.

Earlier this year Huang's group [194] used a large US claims database of people who had chosen to enroll in private healthcare insurance schemes in order to interrogate this team's earlier 2015 contention [188] that statin use may be linked to a higher risk of PD. This time they included 21,599 individuals who, during the period of their analysis generated 2322 incident cases of PD who, for statistical analysis, were then matched with an identical number of healthy controls. Consistent with several earlier studies, they found that higher levels of cholesterol was associated with a lower risk of PD. They also reported that the use of statins (especially lipophilic statins) was associated with higher risk of PD.

Rozani et al. [195] recently published a 232,877 population-based cohort study of new statin users in whom 2,550 developed PD during a mean follow-up of 7.6 years. The study was unusual in that throughout this time the researchers comprehensively made multiple repeated measurements both of statin exposure and LDL-levels. Contrary to Huang's findings [188, 194], and agreeing with the results of many other studies [176–179, 181, 183–187, 189, 190] they found no association between annual statin adherence and PD risk regardless of age, or type of statin taken.

Understandably, those taking statins, or consider taking a statin, to reduce their cardiovascular risk want to know whether this choice would also bring them an increased likelihood of developing PD? This is a very different question to whether a statin might represent a disease-modifying therapeutic for use in patients who have *already* developed PD, and our ongoing 2 year clinical trial involving approximately 200 PD patients seeks specifically to determine whether *Simvastatin* slows PD progression.

As can be seen above, there have been several epidemiological studies investigating whether there may be an association, protective or otherwise, of statin use in relation to subsequent development of PD. These have recently been evaluated in a systematic

review and meta-analysis by Bykov et al. [196] that helpfully discusses the methodological strengths and weaknesses of each these earlier epidemiological studies. It is fair to say that the methodologies utilized in the epidemiological papers cited above all have limitations. Association does not imply causation. Bykov found that overall there seems to be a protective effect of statins against development of PD, but that if cholesterol levels are adjusted for, then this protective effect disappears, and there is no association one way or the other with PD development. The authors also describe some of the limitations of epidemiological study design, including the 'healthy user' and 'immortal time' biases among others [197].

A brief description of the key findings from many of the various types of epidemiological studies that have attempted in recent years to determine whether the use of statins is positively or negatively associated with PD risk are summarized in Table 1.

With regard to Huang's most recent publication suggesting that *Simvastatin* may facilitate development of PD [194], there are some key limitations which merit highlighting: large sectors of the population were not represented in the database that was analyzed (particularly the elderly, i.e., over 65 s, were excluded); and only those with private health insurance were included. It is possible that some patients in this study were misdiagnosed because the clinical details of the participants, and diagnostic confirmation, was not available to the researchers. The authors found that PD was more likely to be diagnosed within the first year or so of starting *Simvastatin*. However, it is well acknowledged that PD starts at least 5 years, if not 10 years before diagnosis; it has also been demonstrated by others that healthcare contacts increase in the year or two prior to diagnosis, which could be what led to their being prescribed a statin. It is well known that vascular risk factors increase risk of dementia, and it would not be unreasonable to suppose that the same might hold true for other neurodegenerative diseases. It is likely that most people taking a statin were started on it because of their vascular risk, and that this might have been the contributory factor that was identified in the study. Indeed, a UK cohort study has demonstrated worse PD severity with increased cardiovascular risk, and underutilization of statins in the PD population [198]. The association of cholesterol and statins as risk factors for PD development has recently been discussed in a *Lancet Neurology* review by Ascherio & Schwarzschild [104], which concluded that any possible association remains uncertain.

Table 1

Key findings from various epidemiological studies that have attempted to determine whether the use of statins is positively or negatively associated with PD risk

| Reference and Research team | Study Type & Size | Results | Statistics and additional information |
|-----------------------------|---|--|--|
| Wolozin et al. [176] | Retrospective analysis of VA database of 4.5 million subjects, which included 700,000 SV users | Protective. SV 'strongly protective' of PD risk | SV use reduced the Hazard Ratio (0.51) $p < 0.001$ |
| Huang et al. [178] | Case control study involving 124 PD cases and 112 controls | Protective. Use of cholesterol-lowering drugs (primarily statins) in this study group was associated with a lower occurrence of PD | Odds Ratio = 0.36–0.41 |
| Wahner et al. [177] | 312 PD cases versus 343 controls. Population-based case control study of incident PD | Protective. 3 of 5 different statins were associated with approximately a 55% reduction in PD risk | Statistically significant Risk Reduction for SV, AV, LV, but not for PV. Odds Ratios ($p < 0.01$) were 0.38, 0.39 and 0.27, respectively |
| Becker et al. [179] | Case-control observational analysis involving 3,637 PD patients (378 of whom had or were taking statins), and 3,637 controls | No difference found between PD patients and controls | Odds Ratio = 1.06 |
| Mutez et al. [181] | Retrospective analysis of a cohort of 419 PD patients | Protective. Mean age of onset of PD delayed 9 years by statin use | Levodopa-equivalent daily dose also reduced in the group taking a statin |
| Gao et al. [183] | Prospective study of 129,066 healthy subjects, 644 of whom developed PD over 12 years of follow-up | Protective. Regular use of statins was associated with a modest reduction in PD risk | Relative Risk was 0.74, $p < 0.05$, and for subjects under 60 years, Relative Risk was 0.31, $p = 0.02$ |
| Undela et al. [184] | Meta-analysis, combining 5 case control studies and 3 cohort studies which studied 1.4 million subjects including 15,102 PD cases | Protective. Statin use over a 2–14 year follow-up reduced risk of PD by 23% | Relative Risk = 0.77, $p = 0.005$ |
| Friedman et al. [185] | 94,308 subjects without PD or statin use at baseline. Over 7 years, 1035 developed PD, and 29,714 took statins for at least 6 months | Protective. Statin use up to 2.5 years of follow-up in 15,394 patients was associated with a significantly reduced risk of PD | Odds Ratio for reduced risk of PD = 0.69, $p < 0.001$ No association noted between baseline LDL-C levels and PD risk |
| Lee et al. [186] | Study followed 43,810 statin users without PD, 1,985 of whom went on to develop PD | Protective. Use of lipophilic statins, either SV or AV, each reduced the incidence of emergent PD | SV and AV significantly reduced the Hazard Ratio (0.23–0.42) for PD risk depending on age and gender. The incidence rate for PD was 1.68 and 3.52 per 1,000,000 person-days for lipophilic and hydrophilic statins, respectively |
| Huang X et al. [188] | Prospective study of 15,792 subjects over 9 years, 106 of whom developed PD while another 187 subjects may have developed PD but this could not be clinically confirmed | Disadvantageous. Statin use was associated with a higher risk for PD when adjusted for cholesterol levels | Odds Ratio = 2.30, $p < 0.05$ In addition, higher total cholesterol was associated with lower risk for PD after adjustment for statin use |
| Liu et al. [194] | Retrospective case control analysis involving 2,322 probable incident PD cases and 2,322 controls. Same research team as 188 | Disadvantageous. Statin usage was significantly associated with PD risk | Strongest associations with PD risk was for lipophilic statins (Odds Ratio = 1.58, $p < 0.0001$), whereas for hydrophilic statins (Odds Ratio = 1.19, $p = 0.25$) |

(Continued)

Table 1
(Continued)

| Reference and Research team | Study Type & Size | Results | Statistics and additional information |
|-----------------------------|---|--|--|
| Rozani et al. [195] | Population-based cohort of statin initiators. 232,877 new statin users were followed for 7+ years, and in whom there were 2,550 emergent cases of PD | Statin adherence over time did not affect PD risk | All-risk estimates were close to unity (except for a slightly reduced PD risk: Hazard Ratio = 0.77 among women aged 40–45 with LDL-C level 160 mg/dl at baseline) Results unaffected by whether statins were lipophilic or hydrophilic |
| Bai et al. [189] | Meta-analysis, combining 5 case control studies and 6 cohort studies which studied 3.5 million subjects (1.1 million statin users), and including 21,011 incident cases of PD | Protective. Statin use was associated with a reduced risk of PD | Because of the low incidence of PD, the authors felt distinctions among RR, HR, and OR could be ignored, allowing combined case-control and cohort studies, and calculated the summary Relative Risks and 95% CIs. Relative Risk for reduced PD risk was 0.81, $p = 0.002$ |
| Bykov et al. [196] | Meta-analysis, of ten eligible epidemiological studies | Protective, but only in the six studies analyzed that did not adjust for cholesterol. Protective effect of statins against PD risk, Relative Risk = 0.75 95%CI: 0.60 to 0.92 | No protective effect was observed among the four studies that adjusted for either cholesterol or hyperlipidemia Relative Risk = 0.91; 95%CI 0.68 to 1.22 |
| Sheng et al. [190] | Meta-analysis, of 11 studies (2,787,249 subjects) including 5 case-control and 6 cohort studies | Protective. Use of statins was associated with a significant reduction in risk of developing PD | Adjusted Relative Risk = 0.74, 95% CI 0.62 to 0.90, $P < 0.001$ |

Results, and outcomes measures used, are as described by the respective authors. SV, Simvastatin; AV, Atorvastatin; LV, Lovastatin; PV, Pravastatin.

In conclusion, we reiterate, whether PD risk is increased or decreased by taking a statin to lower cardiovascular risk (and a clear future demonstration of the reality of this would be valuable and welcome), that the testing of a statin to treat PD neurodegeneration in patients who already have established PD is a completely separate and unrelated question. It therefore remains highly reasonable to pursue Simvastatin in a randomized clinical trial to test its disease-modifying potential in a population of PD patients (see biochemical/pharmaceutical rationale described earlier). In this Simvastatin trial [7] we are measuring PD severity and so will pick up whether or not the rate of PD progression is affected by Simvastatin, hopefully in a positive direction as that is the point of the trial. We are carefully monitoring for adverse events and at the end of the study in 2020 we will finally be able to evaluate the unblinded data.

DESCRIPTION OF CURRENT LCT CLINICAL TRIAL OF SIMVASTATIN

No drug has yet been shown to slow or reverse the neurodegenerative process of PD. All currently

licensed therapies act as symptom-relieving agents but have a limited lifespan of effectiveness because of continued neuronal loss. The purpose of *this* study, as mandated by the International PD Linked Clinical Trials Committee [6], is to determine whether *Simvastatin*, a widely used cholesterol-lowering drug (statin) with an excellent safety profile, is capable of reducing the rate of neurodegenerative decline in patients with PD. Details of this clinical trial are described on the US clinical trials website [7]: <https://clinicaltrials.gov/show/NCT02787590>.

Briefly, after considerable discussion about how best to configure an appropriate long-term *disease-modifying* trial in PD patients (rather than a symptomatic study), a randomized, double-blind, placebo-controlled, two year study was chosen, to be conducted in idiopathic PD patients who, at study commencement, had been characterized as Modified Hoehn and Yahr stage ≤ 3.0 in the ON medication state. It was made a requirement for entry into the trial that they were on dopaminergic treatment with wearing-off phenomenon.

Participants are randomly allocated to one of two treatment groups. In one group, participants are given capsules of *Simvastatin* to take orally (by mouth) for 24 months.

The other group receives placebo capsules to take orally for 24 months. At the start of the study, when they receive their medication, participants complete a number of questionnaires and motor (movement) tests (a walking test and a finger tapping test). Participants in both groups also attend a further 6 clinic visits after 1, 6, 12, 18 and 24 and 26 months, where they are asked about their health and any medication they are taking, as well as repeating the questionnaires and motor tests. For 4 of the clinic visits, having omitted their usual PD medication that day, the participants are asked to attend in the 'OFF medication' state so that the researchers can get a true picture of their disease without it being masked by their normal medication.

The *Simvastatin* trial is a 198 patient, 23 Centre, Phase II, randomized, placebo-controlled, double-blinded study, officially titled '*Simvastatin* as a Neuroprotective Treatment for Parkinson's Disease: a Double-blind, Randomised, Placebo Controlled Futility Study in Patients of Moderate Severity'. The primary outcome measure is Change in MDS-UPDRS part III (OFF) score, and the duration of treatment is 24 Months. The Secondary Outcome Measures include MDS-UPDRS total score in the practically defined ON state, MDS-UPDRS part II subscale score in the practically defined ON state, Timed motor tests - finger tapping and timed walk test, Timed Motor Tests include evaluating the number of hand taps that an individual can perform within 30 seconds and a timed walk test. In addition, the following rating scales are used to evaluate the study participants: Montgomery and Asberg Depression Rating Scale (MADRS), The Addenbrooke's Cognitive Assessment-III (ACE-III), Non-Motor Symptom assessment scale (NMSS), Parkinson's disease Questionnaire (PDQ-39), Changes in PD medication as measured by levodopa-equivalent dose (LED), Cholesterol levels (total, HDL, total/HDL ratio), King's PD pain scale (KPPS), EuroQoL 5D-5L health status questionnaire (EQ-5D-5L), Safety and tolerability of trial medication by adverse events (AEs) review, and Incidence of diabetes mellitus.

Active comparator: A one month low dose phase of 40 mg oral *Simvastatin* daily is followed by a 23-month high dose phase of 80 mg oral *Simvastatin* daily and a final two month phase off trial medication.

Matched Placebo Comparator: A one month low dose phase of 40 mg matched placebo daily is followed by a 23 month high dose phase of 80 mg matched placebo daily and a final two month phase off trial medication.

Outline of choices made about the Simvastatin trial

Patient population

We elected to recruit patients in mid-stage disease, H&Y < or = 3 in the ON state, but who had developed motor fluctuations. The reason for this was two-fold. First, we felt that the trial findings would be of relevance to people living with PD today. Second, the presence of wearing off reduces the degree of heterogeneity in the study population, particularly for ensuring, as much as possible, consistency in the 'practically-defined off' state used for the primary outcome measure.

Study sites

We selected a multi-center design as it would not be possible to recruit the required number of participants from a single center. Within the UK we have an established Clinical Research Network that facilitates study delivery within centers experienced in PD clinical study delivery. The multi-center nature of the study does introduce issues relating to quality control, particularly with regard to rater experience and training. We therefore carried out feasibility assessments with sites expressing interest, stipulating the study requirements in terms of rater uniformity for the study duration, the need for an independent rater (separate from the rest of study delivery), rater experience and training (kindly provided by the MDS for this study). In addition, the study co-ordinating center has robust data management and site monitoring processes to ensure quality data collection across all sites.

Dose of Simvastatin chosen

We chose to use a dose of 80 mg of *Simvastatin* for this study, as this was the dose shown to have an excellent protective effect in the MS STAT trial involving treating Multiple Sclerosis patients [135, 199]. There are safety concerns regarding the use of high dose *Simvastatin*, particularly in the elderly. The overall risk of statin-induced myopathy is approximately 1%. We have introduced robust guidance and safety procedures into the protocol to mitigate this risk.

Choice of selected duration of clinical trial

Study duration was chosen as 24 months to maximize the potential for differences in progression between placebo and active treatment group. It is known that the placebo effect in PD studies is large and sustained. In addition, with a relatively small sample size and a clinically heterogeneous condition, it is important to allow sufficient time for measurable disease progression across the study population.

Choice of primary patient outcome

Choice of primary outcome measure was the OFF state MDS-UPDRS part III as this is the most likely to correlate with underlying disease severity and therefore be indicative of disease progression. This does not reflect clinical meaningfulness for patients whose OFF state UPDRS score will be improved by symptomatic medication; however, demonstrating clinical utility is not the purpose of this preliminary study. If this study suggests that *Simvastatin* does have potential as a neuroprotective agent, then a further Phase III study can evaluate impact on clinically meaningful outcomes, such as patient reported measures, quality of life measures, and cognitive decline.

Other design aspects

In order to distinguish a protective effect from a potential symptomatic effect a washout design was chosen with a 2-month washout period after the end of the 24-month treatment period. The half-life of *Simvastatin* is less than 5 hours, and so this period provides sufficient time for drug elimination.

DISCUSSION

The international PD linked clinical trials committee, based on a range of evidence compiled into a detailed dossier, and followed by extensive committee discussions, agreed in 2012 to prioritize *Simvastatin* to enter a trial in PD patients to assess its potential as a disease-modifying therapy [6]. Clinical trials of potential neuroprotective agents in PD are difficult to design. This is partially because of the variability in disease phenotype and rate of progression, and also, the potential confounding factor of a symptomatic response. In addition, there is no reliable biomarker for disease progression. The International PD Linked Clinical Trial committee is tasked to analyze potential new target therapies for PD and for which the biochemical evidence indicates the likelihood that they may have benefit to slow, halt

or reverse disease progression in patients with PD. This large global committee of PD experts, many of whom have extensive experience in PD trials and their design, is coordinated by The Cure Parkinson's Trust. The detailed biochemical, physiological, and pharmaceutical evidence available to the committee in 2012 which led them to choose to prioritize *Simvastatin* to enter a disease-modifying clinical trial is summarized in the first section of the current paper, with very substantial updating to October 2017. It is interesting to observe that the rationale, then in 2012, for taking *Simvastatin* into a PD trial to assess its disease-modifying potential (in fact, there are several separate mechanistic rationales as outlined above) has continued to strengthen over those 5 years on all biochemical and physiological fronts. Another recent review by Saeedi Saravi et al. [200] also summarizes, but more generally across several neurodegenerative diseases, the biochemical and pharmaceutical mechanisms of action of how statins may be beneficial in the management of these conditions. In their discussion of the treatment of multiple sclerosis they explore research into how immunomodulatory and anti-inflammatory properties of statins may helpfully unite for therapeutic benefit [201–203] and this may also be of direct relevance to the long-term management of PD. This is especially poignant since *Simvastatin* has already shown encouraging long-term clinical results in patients with multiple sclerosis [135, 199, 204], and a major Phase III study involving almost 1200 patients being given high dose *Simvastatin* (80 mg daily) will commence in the coming months. We have long established strong lines of communication between those involved with the multiple sclerosis (MS-STAT) and *Simvastatin* (PD STAT) trials.

Taking the example, also conceptually relevant to PD, of the costs of some of the newer multiple sclerosis (patented) therapeutics that are pursuing disease-modification objectives, several of these currently exceed \$75,000 per patient per year [205]. By contrast, the annual cost per patient of 80 mg *Simvastatin* (now unpatented) is \$37 per year [206], although that is not to say that the cost effectiveness of those high value therapies render them financially unusable [207–209], as this can vary across patient subgroups which in turn means that direct therapeutic, or even financial, comparisons with *Simvastatin* cannot always readily be made. However, there may be situations in the future where low cost unpatented drugs like *Simvastatin* may look a very attractive therapeutic alternative for healthcare providers, while

the patient perspective can be somewhat different and must also be taken in to account [210] because health economic algorithms currently used often miss substantial additional social value.

The first, biochemical, section of this paper demonstrates that *Simvastatin* acts on a number of separate, distinct intracellular processes, each of which appear of relevance to the long-term management of PD. These remain highly active research topics and we eagerly anticipate developments into this growing insight. When a repurposed therapeutic such as *Simvastatin*, has multiple pleiotropic effects, all or any of which may be clinically beneficial, there sometimes comes a point when one may just acknowledge we cannot identify a clear therapeutic target (because there are so many positively-orientated candidate modes of action), and go ahead and test it in the clinic. To quote John Overington from BenevolentAI, ‘although the concept of a single drug target is a natural one for researchers in the field, there are substantial operational difficulties in consistently mapping this target concept to specific genes and gene products’ [211]. Clearly, having a strong and extensive safety record has helped us move *Simvastatin* into a long-term trial in PD patients to explore its disease-modifying potential. We anticipate our current *Simvastatin* trial in PD patients will finish in 2020.

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CONFLICTS OF INTEREST

RW is the Director of Research and Development at the Cure Parkinson’s Trust which is an international grant-giving charity focused on delivering fundamentally innovative disease-modifying treatments that slow, stop or reverse Parkinson’s disease. He declares no conflicts of interest relevant to this publication.

CC is the chief investigator of PD STAT, a clinical trial exploring *Simvastatin* as a neuroprotective treatment for patients with Parkinson’s disease. She has no other conflicts of interest relevant to this publication.

REFERENCES

- [1] Hirsch L, Jette N, Frolkis A, Steeves T, & Pringsheim T (2016) The incidence of Parkinson’s disease: A sys-

tematic review and meta-analysis. *Neuroepidemiology*, **46**, 292-300.

- [2] Dorsey ER, Constantinescu R, Thompson JP, Biglan KM, Holloway RG, Kieburtz K, Marshall FJ, Ravina BM, Schifitto G, Siderowf A, & Tanner CM (2007) Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology*, **30**, 384-386.
- [3] Kowal SL, Dall TM, Chakrabarti R, Storm MV, & Jain A (2013) The current and projected economic burden of Parkinson’s disease in the United States. *Mov Disord*, **28**, 311-318.
- [4] Savica R, Grossardt BR, Bower JH, Ahlskog JE, & Rocca WA (2016) Time Trends in the Incidence of Parkinson Disease. *JAMA Neurol*, **73**, 981-989.
- [5] Johnson SJ, Diener MD, Kaltenboeck A, Birnbaum HG, & Siderowf AD (2013) An economic model of Parkinson’s disease: Implications for slowing progression in the United States. *Mov Disord*, **28**, 319-326.
- [6] Brundin P, Barker RA, Conn PJ, Dawson TM, Kieburtz K, Lees AJ, Schwarzschild MA, Tanner CM, Isaacs T, Duffen J, Matthews H, & Wyse RK (2013) Linked clinical trials—the development of new clinical learning studies in Parkinson’s disease using screening of multiple prospective new treatments. *J Parkinsons Dis*, **3**, 231-239.
- [7] *Simvastatin as a Neuroprotective Treatment for Moderate Parkinson’s Disease (PD STAT)* <https://clinicaltrials.gov/ct2/show/NCT02787590> Last verified April 2017. Accessed on October, 1, 2017.
- [8] Vuletic S, Riekse RG, Marcovina SM, Peskind ER, Hazzard WR, & Albers JJ (2006) Statins of different brain penetrability differentially affect CSF PLTP activity. *Dement Geriatr Cogn Disord* **22**, 392-398.
- [9] Wood WG, Eckert GP, Igbavboa U, & Müller WE (2010) Statins and neuroprotection: A prescription to move the field forward. *Ann N Y Acad Sci*, **1199**, 69-76.
- [10] Wood WG, Müller WE, & Eckert GP (2014) Statins and neuroprotection: Basic pharmacology needed. *Mol Neurobiol*, **50**, 214-220.
- [11] Selley ML (2005) *Simvastatin* prevents 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced striatal dopamine depletion and protein tyrosine nitration in mice. *Brain Res*, **1037**, 1-6.
- [12] Ghosh A, Roy A, Matras J, Brahmachari S, Gendelman HE, & Pahan K (2009) *Simvastatin* inhibits the activation of p21ras and prevents the loss of dopaminergic neurons in a mouse model of Parkinson’s disease. *J Neurosci*, **43**, 13543-13556.
- [13] Roy A, & Pahan K (2011) Prospects of statins in Parkinson disease. *Neuroscientist*, **17**, 244-255.
- [14] Hunot S, Boissière F, Faucheux B, Brugg B, Mouatt-Prigent A, Agid Y, & Hirsch EC (1996) Nitric oxide synthase and neuronal vulnerability in Parkinson’s disease. *Neuroscience*, **72**, 355-363.
- [15] Qureshi GA, Baig S, Bednar I, Södersten P, Forsberg G, & Siden A (1995) Increased cerebrospinal fluid concentration of nitrite in Parkinson’s disease. *Neuroreport*, **6**, 1642-1644.
- [16] Bessler H, Djaldetti R, Salman H, Bergman M, & Djaldetti M (1999) IL-1 beta, IL-2, IL-6 and TNF-alpha production by peripheral blood mononuclear cells from patients with Parkinson’s disease. *Biomed Pharmacother*, **53**, 141-145.
- [17] Dehmer T, Lindenau J, Haid S, Dichgans J, & Schulz JB (2000) Deficiency of inducible nitric oxide synthase protects against MPTP toxicity in vivo. *J Neurochem*, **74**, 2213-2216.

- 1518 [18] Sriram K, Matheson JM, Benkovic SA, Miller DB, Luster
1519 MI, O'Callaghan JP (2006) Deficiency of TNF receptors
1520 suppresses microglial activation and alters the susceptibility
1521 of brain regions to MPTP-induced neurotoxicity: Role
1522 of TNF-alpha. *FASEB J*, **20**, 670-682.
- 1523 [19] Ghosh A, Roy A, Liu X, Kordower JH, Mufson EJ, Hart-
1524 ley DM, Ghosh S, Mosley RL, Gendelman HE, & Pahan K
1525 (2007) Selective inhibition of NF-kappaB activation pre-
1526 vents dopaminergic neuronal loss in a mouse model of
1527 Parkinson's disease. *Proc Natl Acad Sci USA*, **104**, 18754-
1528 18759.
- 1529 [20] Saijo K, Winner B, Carson CT, Collier JG, Boyer L, Rosen-
1530 feld MG, Gage FH, & Glass CK (2009) A Nurr1/CoREST
1531 pathway in microglia and astrocytes protects dopaminergic
1532 neurons from inflammation-induced death. *Cell*, **137**,
1533 47-59.
- 1534 [21] Pahan K, Sheikh FG, Namboodiri AM, & Singh I (1997)
1535 Lovastatin and phenylacetate inhibit the induction of nitric
1536 oxide synthase and cytokines in rat primary astrocytes,
1537 microglia, and macrophages. *J Clin Invest*, **100**, 2671-
1538 2679.
- 1539 [22] Stanislaus R, Singh AK, & Singh I (2001) Lovastatin treat-
1540 ment decreases mononuclear cell infiltration into the CNS
1541 of Lewis rats with experimental allergic encephalomyelitis.
1542 *J Neurosci Res*, **66**, 155-162.
- 1543 [23] Neuhaus O, Strasser-Fuchs S, Fazekas F, Kieseier BC,
1544 Niederwieser G, Hartung HP, & Archelos JJ (2002) Statins
1545 as immunomodulators: Comparison with interferon-beta
1546 1b in MS. *Neurology*, **59**, 990-997.
- 1547 [24] Clarke RM, Lyons A, O'Connell F, Deighan BF, Barry
1548 CE, Anyakoha NG, Nicolaou A, & Lynch MA (2008)
1549 A pivotal role for interleukin-4 in atorvastatin-associated
1550 neuroprotection in rat brain. *J Biol Chem*, **283**, 1808-1817.
- 1551 [25] Pahan K, Liu X, McKinney MJ, Wood C, Sheikh FG, &
1552 Raymond JR (2000) Expression of a dominant-negative
1553 mutant of p21(ras) inhibits induction of nitric oxide syn-
1554 thase and activation of nuclear factor-kappaB in primary
1555 astrocytes. *J Neurochem*, **74**, 2288-2295.
- 1556 [26] Pahan K, Sheikh FG, Khan M, Namboodiri AM, & Singh
1557 I (1998) Sphingomyelinase and ceramide stimulate the
1558 expression of inducible nitric-oxide synthase in rat pri-
1559 mary astrocytes. *J Biol Chem*, **273**, 2591-2600.
- 1560 [27] van der Most PJ, Dolga AM, Nijholt IM, Luiten PG, &
1561 Eisel UL (2009) Statins: Mechanisms of neuroprotection.
1562 *Prog Neurobiol*, **88**, 64-75.
- 1563 [28] Santiago M, Hernández-Romero MC, Machado A, &
1564 Cano J (2009) Zocor Forte (simvastatin) has a neuropro-
1565 tective effect against LPS striatal dopaminergic terminals
1566 injury, whereas against MPP+ does not. *Eur J Pharmacol*,
1567 **609**, 58-64.
- 1568 [29] Liu TY, Yang XY, Zheng LT, Wang GH, & Zhen XC (2017)
1569 Activation of Nur77 in microglia attenuates proinflamma-
1570 tory mediators production and protects dopaminergic
1571 neurons from inflammation-induced cell death. *J Neuro-
1572 chem*, **140**, 589-604.
- 1573 [30] Gouveia TL, Scorza FA, Silva MJ, Bandeira Tde A, Perosa
1574 SR, Argañaraz GA, Silva Mde P, Araujo TR, Frangiotti
1575 MI, Amado D, Cavalheiro EA, Silva JA Jr, & Naffah-
1576 Mazzacoratti Mda G (2011) Lovastatin decreases the
1577 synthesis of inflammatory mediators in the hippocampus
1578 and blocks the hyperthermia of rats submitted to long-
1579 lasting status epilepticus. *Epilepsy Behav*, **20**, 1-5.
- 1580 [31] Kurata T, Miyazaki K, Kozuki M, Morimoto N, Ohta
1581 Y, Ikeda Y, & Abe K (2012) Atorvastatin and pitavas-
1582 tatin reduce senile plaques and inflammatory responses in
a mouse model of Alzheimer's disease. *Neurol Res*, **34**,
601-610.
- [32] Tong XK, Nicolakakis N, Fernandes P, Ongali B, Brouil-
lette J, Quirion R, & Hamel E (2009) Simvastatin improves
cerebrovascular function and counters soluble amyloid-
beta, inflammation and oxidative stress in aged APP mice.
Neurobiol Dis, **35**, 406-414.
- [33] Zhao Y, Feng Q, Huang Z, Li W, Chen B, Jiang L, Wu B,
Ding W, Xu G, Pan H, Wei W, Luo W, & Luo Q (2014)
Simvastatin inhibits inflammation in ischemia-reperfusion
injury. *Inflammation*, **37**, 1865-1875.
- [34] Ouk T, Amr G, Azzaoui R, Delassus L, Fossaert E, Tailleux
A, Bordet R, & Modine T (2016) Lipid-lowering drugs
prevent neurovascular and cognitive consequences of car-
diopulmonary bypass. *Vascul Pharmacol*, **80**, 59-66.
- [35] Yan J, Xu Y, Zhu C, Zhang L, Wu A, Yang Y, Xiong Z,
Deng C, Huang XF, Yenari MA, Yang YG, Ying W, &
Wang Q (2011) Simvastatin prevents dopaminergic neu-
rodegeneration in experimental parkinsonian models: The
association with anti-inflammatory responses. *PLoS One*,
6, e20945.
- [36] Kumar A, Sharma N, Gupta A, Kalonia H, & Mishra
J (2012) Neuroprotective potential of atorvastatin and
simvastatin (HMG-CoA reductase inhibitors) against
6-hydroxydopamine (6-OHDA) induced Parkinson-like
symptoms. *Brain Res*, **1471**, 13-22.
- [37] Esposito E, Rinaldi B, Mazzon E, Donniacuo M, Impel-
lizzeri D, Paterniti I, Capuano A, Bramanti P, & Cuzzocrea
S (2012) Anti-inflammatory effect of simvastatin in an
experimental model of spinal cord trauma: Involvement
of PPAR- α . *J Neuroinflammation*, **9**, 81.
- [38] Rinaldi B, Donniacuo M, Esposito E, Capuano A, Sodano
L, Mazzon E, Di Palma D, Paterniti I, Cuzzocrea S, &
Rossi F (2011) PPAR α mediates the anti-inflammatory
effect of simvastatin in an experimental model of
zymosan-induced multiple organ failure. *Br J Pharmacol*,
163, 609-623.
- [39] Zhou HX, Gao LH, Meng LL, Zhang YX, Wei ZF, &
Si DW (2017) Preventive and therapeutic effect of sim-
vastatin on secondary inflammatory damage of rats with
cerebral hemorrhage. *Asian Pac J Trop Med*, **10**, 152-156.
- [40] Xu YQ, Long L, Yan JQ, Wei L, Pan MQ, Gao HM, Zhou P,
Liu M, Zhu CS, Tang BS, & Wang Q (2013) Simvastatin
induces neuroprotection in 6-OHDA-lesioned PC12 via
the PI3K/AKT/caspase 3 pathway and anti-inflammatory
responses. *CNS Neurosci Ther*, **19**, 170-177.
- [41] Yan J, Sun J, Huang L, Fu Q, & Du G (2014) Simvastatin
prevents neuroinflammation by inhibiting N-methyl-D-
aspartic acid receptor 1 in 6-hydroxydopamine-treated
PC12 cells. *J Neurosci Res*, **92**, 634-640.
- [42] Zhang Y, Zhang Z, & Yan H (2015) Simvastatin inhibits
ischemia/reperfusion injury-induced apoptosis of retinal
cells via downregulation of the tumor necrosis factor- α /
nuclear factor- κ B pathway. *Int J Mol Med*, **36**, 399-405.
- [43] Tran TA, Nguyen AD, Chang J, Goldberg MS, Lee JK,
& Tansey MG (2011) Lipopolysaccharide and tumor
necrosis factor regulate Parkin expression via nuclear
factor-kappa B. *PLoS One*, **6**, e23660.
- [44] Huang W, Li Z, Zhao L, & Zhao W (2017) Simvastatin
ameliorate memory deficits and inflammation in clinical
and mouse model of Alzheimer's disease via modulating
the expression of miR-106b. *Biomed Pharmacother*, **92**,
46-57.
- [45] Wang Q, Wei X, Gao H, Li J, Liao J, Liu X, Qin B,
Yu Y, Deng C, Tang B, & Huang XF (2014) Simvas-

- tatin reverses the downregulation of M1/4 receptor binding in 6-hydroxydopamine-induced parkinsonian rats: The association with improvements in long-term memory. *Neuroscience*, **267**, 57-66.
- [46] Beal MF (1998) Excitotoxicity and nitric oxide in Parkinson's disease pathogenesis. *Ann Neurol* **44**(3 Suppl 1), S110-S114.
- [47] Hernández-Perera O, Pérez-Sala D, Navarro-Antolín J, Sánchez-Pascuala R, Hernández G, Díaz C, & Lamas S (1998) Effects of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors, atorvastatin and simvastatin, on the expression of endothelin-1 and endothelial nitric oxide synthase in vascular endothelial cells. *J Clin Invest*, **101**, 2711-2719.
- [48] Fulton D, Gratton JP, McCabe TJ, Fontana J, Fujio Y, Walsh K, Franke TF, Papapetropoulos A, & Sessa WC (1999) Regulation of endothelium-derived nitric oxide production by the protein kinase Akt. *Nature*, **399**, 597-601.
- [49] Skaletz-Rorowski A, Lutchman M, Kureishi Y, Lefer DJ, Faust JR, & Walsh K (2003) HMG-CoA reductase inhibitors promote cholesterol-dependent Akt/PKB translocation to membrane domains in endothelial cells. *Cardiovasc Res*, **57**, 253-264.
- [50] Feron O, Dessy C, Desager JP, & Balligand JL (2001) Hydroxy-methylglutaryl-coenzyme A reductase inhibition promotes endothelial nitric oxide synthase activation through a decrease in caveolin abundance. *Circulation*, **103**, 113-118.
- [51] Arora R, Hare DL, & Zulli A (2012) Simvastatin reduces endothelial NOS: Caveolin-1 ratio but not the phosphorylation status of eNOS in vivo. *J Atheroscler Thromb*, **19**, 705-711.
- [52] Pugh SD, MacDougall DA, Agarwal SR, Harvey RD, Porter KE, & Calaghan S (2014) Caveolin contributes to the modulation of basal and β -adrenoceptor stimulated function of the adult rat ventricular myocyte by simvastatin: A novel pleiotropic effect. *PLoS One*, **9**, e106905.
- [53] Chang CC, Hsu YH, Chou HC, Lee YG, & Juan SH (2017) 3-methylcholanthrene/aryl-hydrocarbon receptor-mediated hypertension through eNOS inactivation. *J Cell Physiol*, **232**, 1020-1029.
- [54] Saeedi Saravi SS, Saeedi Saravi SS, Khoshbin K, & Dehpour AR (2017) Current insights into pathogenesis of Parkinson's disease: Approach to mevalonate pathway and protective role of statins. *Biomed Pharmacother*, **90**, 724-730.
- [55] Schmitt M, Dehay B, Bezaud E, & Garcia-Ladona FJ (2016) Harnessing the trophic and modulatory potential of statins in a dopaminergic cell line. *Synapse*, **70**, 71-86.
- [56] Schmitt M, Dehay B, Bezaud E, & Garcia-Ladona FJ (2017) U18666A, an activator of sterol regulatory element binding protein pathway, modulates presynaptic dopaminergic phenotype of SH-SY5Y neuroblastoma cells. *Synapse* 71. doi: 10.1002/syn.21980
- [57] Miyosawa K, Watanabe Y, Murakami K, Murakami T, Shibata H, Iwashita M, Yamazaki H, Yamazaki K, Ohgiya T, Shibuya K, Mizuno K, Tanabe S, Singh SA, & Aikawa M (2015) New CETP inhibitor K-312 reduces PCSK9 expression: A potential effect on LDL cholesterol metabolism. *Am J Physiol Endocrinol Metab*, **309**, E177-E190.
- [58] Ferri N, Marchianò S, Lupo MG, Trenti A, Biondo G, Castaldello P, & Corsini A (2017) Geranylgeraniol prevents the simvastatin-induced PCSK9 expression: Role of the small G protein Rac1. *Pharmacol Res*, **122**, 96-104.
- [59] Benn M, Nordestgaard BG, Frikke-Schmidt R, & Tybjaerg-Hansen A (2017) Low LDL cholesterol, PCSK9 and HMGCR genetic variation, and risk of Alzheimer's disease and Parkinson's disease: Mendelian randomisation study. *BMJ*, **357**, j3170.
- [60] Chaudhary R, Garg J, Shah N, & Sumner A (2017) PCSK9 inhibitors: A new era of lipid lowering therapy. *World J Cardiol*, **9**, 76-91.
- [61] Scherer DJ, Nelson A, Psaltis PJ, & Nicholls SJ (2017) Targeting LDL cholesterol with PCSK9 inhibitors. *Intern Med J*, **47**, 856-865.
- [62] Jaworski K, Jankowski P, & Kosior DA (2017) PCSK9 inhibitors - from discovery of a single mutation to a groundbreaking therapy of lipid disorders in one decade. *Arch Med Sci*, **13**, 914-929.
- [63] Berthold HK, Seidah NG, Benjannet S, Gouni-Berthold I (2013) Evidence from a randomized trial that simvastatin, but not ezetimibe, upregulates circulating PCSK9 levels. *PLoS One*, **8**, e60095.
- [64] Sun HX, Zeng DY, Li RT, Pang RP, Yang H, Hu YL, Zhang Q, Jiang Y, Huang LY, Tang YB, Yan GJ, & Zhou JG (2012) Essential role of microRNA-155 in targeting endothelium-dependent vasorelaxation by targeting endothelial nitric oxide synthase. *Hypertension*, **60**, 1407-1414.
- [65] Pierucci M, Galati S, Valentino M, Di Matteo V, Benigno A, Pitruzzella A, Muscat R, & Di Giovanni G (2011) Nitric oxide modulation of the basal ganglia circuitry: Therapeutic implication for Parkinson's disease and other motor disorders. *CNS Neurol Disord Drug Targets*, **10**, 777-791.
- [66] Hoang T, Choi DK, Nagai M, Wu DC, Nagata T, Prou D, Wilson GL, Vila M, Jackson-Lewis V, Dawson VL, Dawson TM, Chesselet MF, & Przedborski S (2009) Neuronal NOS and cyclooxygenase-2 contribute to DNA damage in a mouse model of Parkinson disease. *Free Radic Biol Med*, **47**, 1049-1056.
- [67] Brzozowski MJ, Jenner P, & Rose S (2015) Inhibition of i-NOS but not n-NOS protects rat primary cell cultures against MPP(+)-induced neuronal toxicity. *J Neural Transm (Vienna)*, **122**, 779-788.
- [68] Tripathy D, Chakraborty J, & Mohanakumar KP (2015) Antagonistic pleiotropic effects of nitric oxide in the pathophysiology of Parkinson's disease. *Free Radic Res*, **49**, 1129-1139.
- [69] Jiménez-Jiménez FJ, Alonso-Navarro H, Herrero MT, García-Martín E, & Agúndez JA (2016) An Update on the Role of Nitric Oxide in the Neurodegenerative Processes of Parkinson's Disease. *Curr Med Chem*, **23**, 2666-2679.
- [70] Li WC, Zou ZJ, Zhou MG, Chen L, Zhou L, Zheng YK, & He ZJ (2015) Effects of simvastatin on the expression of inducible NOS in acute lung injury in septic rats. *Int J Clin Exp Pathol*, **8**, 15106-15111.
- [71] Padovan-Neto FE, Echeverry MB, Chiavegatto S, & Del-Bel E (2011) Nitric Oxide Synthase Inhibitor Improves De Novo and Long-Term L-DOPA-Induced Dyskinesia in Hemiparkinsonian Rats. *Front Syst Neurosci*, **5**, 40.
- [72] Padovan-Neto FE, Cavalcanti-Kiwiatkovski R, Carolino RO, Anselmo-Franci J, & Del Bel E (2015) Effects of prolonged neuronal nitric oxide synthase inhibition on the development and expression of L-DOPA-induced dyskinesia in 6-OHDA-lesioned rats. *Neuropharmacology*, **89**, 87-99.

- 1778 [73] Solís O, Espadas I, Del-Bel EA, & Moratalla R (2015) Nitric oxide synthase inhibition decreases L-DOPA-
1779 induced dyskinesia and the expression of striatal molecular
1780 markers in Pitx3(-/-) aphakia mice. *Neurobiol Dis*, **73**,
1781 49-59.
- 1782 [74] Bortolanza M, Cavalcanti-Kwiatkoski R, Padovan-Neto
1783 FE, da-Silva CA, Mitkovski M, Raisman-Vozari R, & Del-
1784 Bel E (2015) Glial activation is associated with L-DOPA
1785 induced dyskinesia and blocked by a nitric oxide synthase
1786 inhibitor in a rat model of Parkinson's disease. *Neurobiol*
1787 *Dis*, **73**, 377-387.
- 1788 [75] Gerfen CR, Miyachi S, Paletzki R, & Brown P (2002)
1789 D1 dopamine receptor supersensitivity in the dopamine-
1790 depleted striatum results from a switch in the regulation
1791 of ERK1/2/MAP kinase. *J Neurosci*, **22**, 5042-5054.
- 1792 [76] Westin JE, Vercammen L, Strome EM, Konradi C, & Cenci
1793 MA (2007) Spatiotemporal pattern of striatal ERK1/2
1794 phosphorylation in a rat model of L-DOPA-induced dyski-
1795 nesia and the role of dopamine D1 receptors. *Biol*
1796 *Psychiatry*, **62**, 800-810.
- 1797 [77] Schuster S, Nadjar A, Guo JT, Li Q, Ittrich C, Hengerer B,
1798 & Bezdard E (2008) The 3-hydroxy-3-methylglutaryl-CoA
1799 reductase inhibitor lovastatin reduces severity of L-DOPA-
1800 induced abnormal involuntary movements in experimental
1801 Parkinson's disease. *J Neurosci*, **28**, 4311-4316.
- 1802 [78] Tison F, Nègre-Pagès L, Meissner WG, Dupouy S, Li
1803 Q, Thiolat ML, Thiollier T, Galitzky M, Ory-Magne F,
1804 Milhet A, Marquine L, Spampinato U, Rascol O, &
1805 Bezdard E (2013) Simvastatin decreases levodopa-induced
1806 dyskinesia in monkeys, but not in a randomized, placebo-
1807 controlled, multiple cross-over ("n-of-1") exploratory trial
1808 of simvastatin against levodopa-induced dyskinesia in
1809 Parkinson's disease patients. *Parkinsonism Relat Disord*,
1810 **19**, 416-421.
- 1811 [79] Wu DC, Teismann P, Tieu K, Vila M, Jackson-Lewis
1812 V, Ischiropoulos H, & Przedborski S (2003) NADPH
1813 oxidase mediates oxidative stress in the 1-methyl-4-
1814 phenyl-1,2,3,6-tetrahydropyridine model of Parkinson's
1815 disease. *Proc Natl Acad Sci USA*, **100**, 6145-6150.
- 1816 [80] Gao HM, Liu B, Zhang W, & Hong JS (2003) Critical role
1817 of microglial NADPH oxidase-derived free radicals in the
1818 in vitro MPTP model of Parkinson's disease. *FASEB J*, **17**,
1819 1954-1956.
- 1820 [81] Sykietis GP, & Bohmann D (2010) Stress-activated
1821 cap'n'collar transcription factors in aging and human dis-
1822 ease. *Sci Signal*, **3**, re3.
- 1823 [82] Lacher SE, Lee JS, Wang X, Campbell MR, Bell DA, &
1824 Slattery M (2015) Beyond antioxidant genes in the ancient
1825 Nrf2 regulatory network. *Free Radic Biol Med* **88**(Pt B),
1826 452-465.
- 1827 [83] Lacher SE, & Slattery M (2016) Gene regulatory effects
1828 of disease-associated variation in the NRF2 network. *Curr*
1829 *Opin Toxicol*, **1**, 71-79.
- 1830 [84] Liu X, Ward K, Xavier C, Jann J, Clark AF, Pang IH, & Wu
1831 H (2016) The novel triterpenoid RTA 408 protects human
1832 retinal pigment epithelial cells against H₂O₂-induced cell
1833 injury via NF-E2-related factor 2 (Nrf2) activation. *Redox*
1834 *Biol*, **8**, 98-109.
- 1835 [85] Probst BL, Trevino I, McCauley L, Bumeister R, Dulubova
1836 I, Wigley WC, & Ferguson DA (2015) RTA 408, A Novel
1837 Synthetic Triterpenoid with Broad Anticancer and Anti-
1838 Inflammatory Activity. *PLoS One*, **10**, e0122942.
- 1839 [86] Cho HY, Kim K, Kim YB, Kim H, & No JH
1840 (2017) Expression patterns of Nrf2 and Keap1 in ovar-
1841 ian cancer cells and their prognostic role in disease
1842 recurrence and patient survival. *Int J Gynecol Cancer*, **27**,
1843 412-419.
- 1844 [87] Lu J, Guo S, Xue X, Chen Q1 Ge J, Zhuo Y, Zhong H,
1845 Chen B, Zhao M, Han W, Suzuki T, Zhu M, Xia L, Schnei-
1846 der C, Blackwell TS, Porter NA, Zheng L, Tsimikas S,
1847 & Yin H (2017) Identification of a novel series of anti-
1848 inflammatory and anti-oxidative phospholipid oxidation
1849 products containing cyclopentenone moiety in vitro and
1850 in vivo: Implication in atherosclerosis. *J Biol Chem*, **292**,
1851 5378-5391.
- 1852 [88] Sharma A, Rizky L, Stefanovic N, Tate M, Ritchie RH,
1853 Ward KW, & de Haan JB (2017) The nuclear factor
1854 (erythroid-derived 2)-like 2 (Nrf2) activator dh404 pro-
1855 tects against diabetes-induced endothelial dysfunction.
1856 *Cardiovasc Diabetol*, **16**, 33.
- 1857 [89] Lin CP, Huang PH, Lai CF, Chen JW, Lin SJ, & Chen
1858 JS (2015) Simvastatin attenuates oxidative stress, NF- κ B
1859 activation, and artery calcification in LDLR^{-/-} mice fed
1860 with high fat diet via down-regulation of tumor necrosis
1861 factor- α and TNF receptor 1. *PLoS One*, **10**, e0143686.
- 1862 [90] Sohn HM, Hwang JY, Ryu JH, Kim J, Park S, Park
1863 JW, & Han SH (2017) Simvastatin protects ischemic
1864 spinal cord injury from cell death and cytotoxicity through
1865 decreasing oxidative stress: In vitro primary cultured rat
1866 spinal cord model under oxygen and glucose deprivation-
1867 reoxygenation conditions. *J Orthop Surg Res*, **12**, 36.
- 1868 [91] Nakagami Y, & Masuda K (2016) A novel Nrf2 activator
1869 from microbial transformation inhibits radiation-induced
1870 dermatitis in mice. *J Radiat Res*, **57**, 567-571.
- 1871 [92] Gan L, & Johnson JA (2014) Oxidative damage and
1872 the Nrf2-ARE pathway in neurodegenerative diseases.
1873 *Biochim Biophys Acta*, **1842**, 1208-1218.
- 1874 [93] Holmström KM, Kostov RV, & Dinkova-Kostova AT
1875 (2016) The multifaceted role of Nrf2 in mitochondrial
1876 function. *Curr Opin Toxicol*, **1**, 80-91.
- 1877 [94] Dinkova-Kostova AT, Baird L, Holmström KM, Meyer
1878 CJ, & Abramov AY (2015) The spatiotemporal regulation
1879 of the Keap1-Nrf2 pathway and its importance in cellular
1880 bioenergetics. *Biochem Soc Trans*, **43**, 602-610.
- 1881 [95] Dinkova-Kostova AT, Holtzclaw WD, Cole RN, Itoh K,
1882 Wakabayashi N, Katoh Y, Yamamoto M, & Talalay P
1883 (2002) Direct evidence that sulfhydryl groups of Keap1
1884 are the sensors regulating induction of phase 2 enzymes
1885 that protect against carcinogens and oxidants. *Proc Natl*
1886 *Acad Sci USA*, **99**, 11908-11913.
- 1887 [96] McMahon M, Itoh K, Yamamoto M, & Hayes JD (2003)
1888 Keap1-dependent proteasomal degradation of transcrip-
1889 tion factor Nrf2 contributes to the negative regulation
1890 of antioxidant response element-driven gene expression.
1891 *J Biol Chem*, **278**, 21592-21600.
- 1892 [97] Wang X, Campbell MR, Lacher SE, Cho HY, Wan M,
1893 Crowl CL, Chorley BN, Bond GL, Kleeberger SR, Slattery
1894 M, & Bell DA (2016) A polymorphic antioxidant response
1895 element links NRF2/sMAF binding to enhanced MAPT
1896 expression and reduced risk of parkinsonian disorders.
1897 *Cell Rep*. pii: S2211-1247(16)30357-6.
- 1898 [98] Vandrovcova J, Pittman AM, Malzer E, Abou-Sleiman
1899 PM, Lees AJ, Wood NW, & de Silva R. (2009) Asso-
1900 ciation of MAPT haplotype-tagging SNPs with sporadic
1901 Parkinson's disease. *Neurobiol Aging*, **30**, 1477-1482.
- 1902 [99] Edwards TL, Scott WK, Almonte C, Burt A, Powell EH,
1903 Beecham GW, Wang L, Züchner S, Konidari I, Wang G,
1904 Singer C, Nahab F, Scott B, Stajich JM, Pericak-Vance M,
1905 Haines J, Vance JM, & Martin ER (2010) Genome-wide
1906 association study confirms SNPs in SNCA and the MAPT
1907

- 1908 region as common risk factors for Parkinson disease. *Ann*
 1909 *Hum Genet*, **74**, 97-109.
- 1910 [100] Kumaran R, & Cookson MR (2015) Pathways to Parkin-
 1911 sonism Redux: Convergent pathobiological mechanisms
 1912 in genetics of Parkinson's disease. *Hum Mol Genet* **24**(R1),
 1913 R32-44.
- 1914 [101] Ahuja M, Ammal Kaidery N, Yang L, Calingasan N,
 1915 Smirnova N, Gaisin A, Gaisina IN, Gazaryan I, Hush-
 1916 pulian DM, Kaddour-Djebbar I, Bollag WB, Morgan JC,
 1917 Ratan RR, Starkov AA, Beal MF, & Thomas B (2016) Dis-
 1918 tinct Nrf2 signaling mechanisms of fumaric acid esters
 1919 and their role in neuroprotection against 1-methyl-4-
 1920 phenyl-1,2,3,6-tetrahydropyridine-induced experimental
 1921 Parkinson's-like disease. *Neuroscience*, **36**, 6332-6351.
- 1922 [102] Campolo M, Casili G, Biundo F, Crupi R, Cordaro M,
 1923 Cuzzocrea S1, & Esposito E (2017) The neuroprotective
 1924 effect of dimethyl fumarate in an MPTP-mouse model
 1925 of Parkinson's disease: Involvement of reactive oxy-
 1926 gen species/nuclear factor- κ B/nuclear transcription factor
 1927 related to NF-E2. *Antioxid Redox Signal*, **27**, 453-471.
- 1928 [103] Abdelsalam RM, & Safar MM (2015) Neuroprotective
 1929 effects of vildagliptin in rat rotenone Parkinson's disease
 1930 model: Role of RAGE-NF κ B and Nrf2-antioxidant signal-
 1931 ing pathways. *J Neurochem*, **133**, 700-707.
- 1932 [104] Ascherio A, & Schwarzschild MA (2016) The epidemiol-
 1933 ogy of Parkinson's disease: Risk factors and prevention.
 1934 *Lancet Neurol*, **15**, 1257-1272.
- 1935 [105] Study of Urate Elevation in Parkinson's Disease,
 1936 Phase 3 (SURE-PD3) [https://clinicaltrials.gov/show/
 1937 NCT02642393](https://clinicaltrials.gov/show/NCT02642393) Verified June 28 2017. Accessed October
 1938 1 2017.
- 1939 [106] Abdanipour A, Tiraihi T, Noori-Zadeh A, Majdi A, &
 1940 Gosaili R (2014) Evaluation of lovastatin effects on
 1941 expression of anti-apoptotic Nrf2 and PGC-1 α genes in
 1942 neural stem cells treated with hydrogen peroxide. *Mol*
 1943 *Neurobiol*, **49**, 1364-1372.
- 1944 [107] Wu W, Zhao L, Yang P, Zhou W, Li B, Moorhead JF,
 1945 Varghese Z, Ruan XZ, & Chen Y (2016) Inflammatory
 1946 stress sensitizes the liver to atorvastatin-induced injury in
 1947 ApoE $^{-/-}$ mice. *PLoS One*, **11**, e0159512.
- 1948 [108] Jang HJ, Hong EM, Kim M, Kim JH, Jang J, Park SW,
 1949 Byun HW, Koh DH, Choi MH, Kae SH, & Lee J (2016)
 1950 Simvastatin induces heme oxygenase-1 via NF-E2-related
 1951 factor 2 (Nrf2) activation through ERK and PI3K/Akt
 1952 pathway in colon cancer. *Oncotarget*, **7**, 46219-46229.
- 1953 [109] Ferraro SA, Astort F, Yakisich JS, & Tasat DR (2016)
 1954 Particulate matter cytotoxicity in cultured SH-SY5Y cells
 1955 is modulated by simvastatin: Toxicological assessment for
 1956 oxidative damage. *Neurotoxicology*, **53**, 108-114.
- 1957 [110] Yeh YH, Kuo CT, Chang GJ, Chen YH, Lai YJ,
 1958 Cheng ML, & Chen WJ (2015) Rosuvastatin sup-
 1959 presses atrial tachycardia-induced cellular remodeling via
 1960 Akt/Nrf2/heme oxygenase-1 pathway. *J Mol Cell Cardiol*,
 1961 **82**, 84-92.
- 1962 [111] Hsieh CH, Rau CS, Hsieh MW, Chen YC, Jeng SF, Lu TH,
 1963 & Chen SS (2008) Simvastatin-induced heme oxygenase-
 1964 1 increases apoptosis of Neuro 2A cells in response to
 1965 glucose deprivation. *Toxicol Sci*, **101**, 112-121.
- 1966 [112] Conservative Iron Chelation as a Disease-modifying
 1967 Strategy in Parkinson's Disease (FAIRPARKII),
 1968 <https://clinicaltrials.gov/show/NCT02655315> Verified
 1969 May 19 2017. Accessed October 1, 2017.
- 1970 [113] Study of Parkinson's Early Stage With Deferiprone
 1971 (SKY), <https://clinicaltrials.gov/show/NCT02728843>
 1972 Verified September 26 2017. Accessed October 1, 2017.
- [114] Rey NL, Steiner JA, Maroof N, Luk KC, Madaj Z, Tro-
 1973 janowski JQ, Lee VM, & Brundin P (2016) Widespread
 1974 transneuronal propagation of α -synucleinopathy triggered
 1975 in olfactory bulb mimics prodromal Parkinson's disease.
 1976 *J Exp Med*, **213**, 1759-1778.
- [115] Luk KC, Kehm VM, Zhang B, O'Brien P, Trojanowski
 1978 JQ, & Lee VM (2012) Intracerebral inoculation of
 1979 pathological α -synuclein initiates a rapidly progressive
 1980 neurodegenerative α -synucleinopathy in mice. *J Exp Med*,
 1981 **209**, 975-986.
- [116] George S, Rey NL, Reichenbach N, Steiner JA, & Brundin
 1983 P (2013) α -Synuclein: The long distance runner. *Brain*
 1984 *Pathol*, **23**, 350-357.
- [117] Brundin P, Ma J, & Kordower JH (2016) How strong is
 1986 the evidence that Parkinson's disease is a prion disorder?
 1987 *Curr Opin Neurol* **29**, 459-466.
- [118] Braak H, Del Tredici K (2017) Neuropathological stag-
 1989 ing of brain pathology in sporadic Parkinson's disease:
 1990 Separating the wheat from the chaff. *J Parkinsons Dis*, **7**,
 1991 S73-S87.
- [119] Bar-On P, Crews L, Koob AO, Mizuno H, Adame A,
 1993 Spencer B, & Masliah E (2008) Statins reduce neuronal
 1994 alpha-synuclein aggregation in in vitro models of Parkin-
 1995 son's disease. *J Neurochem*, **105**, 1656-1667.
- [120] Bosco DA, Fowler DM, Zhang Q, Nieva J, Powers ET,
 1997 Wentworth P Jr, Lerner RA, & Kelly JW (2006) Elevated
 1998 levels of oxidized cholesterol metabolites in Lewy body
 1999 disease brains accelerate alpha-synuclein fibrilization. *Nat*
 2000 *Chem Biol*, **2**, 249-253.
- [121] Eriksson I, Nath S, Bornefall P, Giraldo AM, & Öllinger K
 2002 (2017) Impact of high cholesterol in a Parkinson's disease
 2003 model: Prevention of lysosomal leakage versus stimu-
 2004 lation of α -synuclein aggregation. *Eur J Cell Biol*, **96**,
 2005 99-109.
- [122] Koob AO, Ubhi K, Paulsson JF, Kelly J, Rockenstein E,
 2007 Mante M, Adame A, & Masliah E (2010) Lovastatin ame-
 2008 liorates alpha-synuclein accumulation and oxidation in
 2009 transgenic mouse models of alpha-synucleinopathies. *Exp*
 2010 *Neurol*, **221**, 267-274.
- [123] Klegeris A, Giasson BI, Zhang H, Maguire J, Pelech S,
 2012 & McGeer PL (2006) Alpha-synuclein and its disease-
 2013 causing mutants induce ICAM-1 and IL-6 in human
 2014 astrocytes and astrocytoma cells. *FASEB J*, **20**, 2000-2008.
- [124] Sanchez-Guajardo V, Febraro F, Kirik D, & Romero-
 2016 Ramos M (2010) Microglia acquire distinct activation
 2017 profiles depending on the degree of alpha-synuclein neu-
 2018 ropathology in a rAAV based model of Parkinson's
 2019 disease. *PLoS One*, **5**, e8784.
- [125] Gan L, Vargas MR, Johnson DA, & Johnson JA (2012)
 2021 Astrocyte-specific overexpression of Nrf2 delays motor
 2022 pathology and synuclein aggregation throughout the CNS
 2023 in the alpha-synuclein mutant (A53T) mouse model.
 2024 *J Neurosci*, **32**, 17775-17787.
- [126] Chen J, Costa LG, & Guizzetti M (2011) Retinoic acid
 2026 isomers up-regulate ATP binding cassette A1 and G1 and
 2027 cholesterol efflux in rat astrocytes: Implications for their
 2028 therapeutic and teratogenic effects. *J Pharmacol Exp Ther*,
 2029 **338**, 870-878.
- [127] Kang SY, Lee SB, Kim HJ, Kim HT, Yang HO, & Jang W
 2031 (2017) Autophagic modulation by rosuvastatin prevents
 2032 rotenone-induced neurotoxicity in an in vitro model of
 2033 Parkinson's disease. *Neurosci Lett*, **642**, 20-26.
- [128] Paul R, Choudhury A, & Borah A (2015) Cholesterol -
 2035 A putative endogenous contributor towards Parkinson's
 2036 disease. *Neurochem Int*, **90**, 125-133.
- 2037

- 2038 [129] Saito T, Nito C, Ueda M, Inaba T, Kamiya F, Muraga K, Katsura K, & Katayama Y (2014) Continuous oral administration of atorvastatin ameliorates brain damage after transient focal ischemia in rats. *Life Sci*, **94**, 106-114. 2103
- 2039 2104
- 2040 [130] Allen Reish HE, & Standaert DG (2015) Role of α -synuclein in inducing innate and adaptive immunity in Parkinson disease. *J Parkinsons Dis*, **5**, 1-19. 2105
- 2041 2106
- 2042 [131] Davies JT, Delfino SF, Feinberg CE, Johnson MF, Nappi VL, Olinger JT, Schwab AP, & Swanson HI (2016) Current and emerging uses of statins in clinical therapeutics: A review. *Lipid Insights*, **9**, 13-29. 2107
- 2043 2108
- 2044 [132] Bedi O, Dhawan V, Sharma PL, & Kumar P (2016) Pleiotropic effects of statins: New therapeutic targets in drug design. *Naunyn Schmiedebergs Arch Pharmacol*, **389**, 695-712. 2109
- 2045 2110
- 2046 [133] Tousoulis D, Psarros C, Demosthenous M, Patel R, Antoniadis C, & Stefanadis C (2014) Innate and adaptive inflammation as a therapeutic target in vascular disease: The emerging role of statins. *J Am Coll Cardiol*, **63**, 2491-2502. 2111
- 2047 2112
- 2048 [134] Zhang X, Tao Y, Wang J, Garcia-Mata R, & Markovic-Plese S (2013) Simvastatin inhibits secretion of Th17-polarizing cytokines and antigen presentation by DCs in patients with relapsing remitting multiple sclerosis. *Eur J Immunol*, **43**, 281-289. 2113
- 2049 2114
- 2050 [135] Chataway J, Schuerer N, Alsanousi A, Chan D, MacManus D, Hunter K, Anderson V, Bangham CR, Clegg S, Nielsen C, Fox NC, Wilkie D, Nicholas JM, Calder VL, Greenwood J, Frost C, & Nicholas R (2014) Effect of high-dose simvastatin on brain atrophy and disability in secondary progressive multiple sclerosis (MS-STAT): A randomised, placebo-controlled, phase 2 trial. *Lancet*, **383**, 2213-2221. 2115
- 2051 2116
- 2052 [136] Soubrier M, Pei J, Durand F, Gullestad L, & John A (2017) Concomitant use of statins in tocilizumab-treated patients with rheumatoid arthritis: A post hoc analysis. *Rheumatol Ther*, **4**, 133-149. 2117
- 2053 2118
- 2054 [137] Inanc MT, Kalay N, Heyit T, Ozdogru I, Kaya MG, Dogan A, Duran M, Kasapkara HA, Gunebakmaz O, Borlu M, Yarlioglu M, & Oguzhan A (2010) Effects of atorvastatin and lisinopril on endothelial dysfunction in patients with Behçet's disease. *Echocardiography*, **27**, 997-1003. 2119
- 2055 2120
- 2056 [138] Duan C, Du ZD, Wang Y, & Jia LQ (2014) Effect of pravastatin on endothelial dysfunction in children with medium to giant coronary aneurysms due to Kawasaki disease. *World J Pediatr*, **10**, 232-237. 2121
- 2057 2122
- 2058 [139] Suda K, Tahara N, Honda A, Yoshimoto H, Kishimoto S, Kudo Y, Kaida H, Abe T, Ueno T, & Fukumoto Y (2015) Statin reduces persistent coronary arterial inflammation evaluated by serial ¹⁸Ffluorodeoxyglucose positron emission tomography imaging long after Kawasaki disease. *Int J Cardiol*, **179**, 61-62. 2123
- 2059 2124
- 2060 [140] Benner EJ, Mosley RL, Destache CJ, Lewis TB, Jackson-Lewis V, Gorantla S, Nemachek C, Green SR, Przedborski S, & Gendelman HE (2004) Therapeutic immunization protects dopaminergic neurons in a mouse model of Parkinson's disease. *Proc Natl Acad Sci USA*, **101**, 9435-9440. 2125
- 2061 2126
- 2062 [141] Benner EJ, Banerjee R, Reynolds AD, Sherman S, Pisarev VM, Tsiperson V, Nemachek C, Ciborowski P, Przedborski S, Mosley RL, & Gendelman HE (2008) Nitrated alpha-synuclein immunity accelerates degeneration of nigral dopaminergic neurons. *PLoS One*, **3**, e1376. 2127
- 2063 2128
- 2064 [142] Martin HL, Santoro M, Mustafa S, Riedel G, Forrester JV, & Teismann P (2016) Evidence for a role of adaptive immune response in the disease pathogenesis of the MPTP mouse model of Parkinson's disease. *Glia*, **64**, 386-395. 2129
- 2065 2130
- 2066 [143] Reynolds AD, Banerjee R, Liu J, Gendelman HE, & Mosley RL (2007) Neuroprotective activities of CD4+CD25+ regulatory T cells in an animal model of Parkinson's disease. *J Leukoc Biol*, **82**, 1083-1094. 2131
- 2067 2132
- 2068 [144] Mosley RL, Benner EJ, Kadiu I, Thomas M, Boska MD, Hasan K, Laurie C, & Gendelman HE (2006) Neuroinflammation, oxidative stress and the pathogenesis of Parkinson's disease. *Clin Neurosci Res*, **6**, 261-281. 2133
- 2069 2134
- 2070 [145] Reynolds AD, Stone DK, Hutter JA, Benner EJ, Mosley RL, & Gendelman HE (2010) Regulatory T cells attenuate Th17 cell-mediated nigrostriatal dopaminergic neurodegeneration in a model of Parkinson's disease. *J Immunol*, **184**, 2261-2271. 2135
- 2071 2136
- 2072 [146] Hutter-Saunders JA, Mosley RL, & Gendelman HE (2011) Pathways towards an effective immunotherapy for Parkinson's disease. *Expert Rev Neurother*, **11**, 1703-1715. 2137
- 2073 2138
- 2074 [147] Kannarkat GT, Boss JM, & Tansey MG (2013) The role of innate and adaptive immunity in Parkinson's disease. *J Parkinsons Dis*, **3**, 493-514. 2139
- 2075 2140
- 2076 [148] Gendelman HE, & Mosley RL (2015) A perspective on roles played by innate and adaptive immunity in the pathobiology of neurodegenerative disorders. *J Neuroimmune Pharmacol*, **10**, 645-650. 2141
- 2077 2142
- 2078 [149] Kosloski LM, Ha DM, Hutter JA, Stone DK, Pichler MR, Reynolds AD, Gendelman HE, & Mosley RL (2010) Adaptive immune regulation of glial homeostasis as an immunization strategy for neurodegenerative diseases. *J Neurochem*, **114**, 1261-1276. 2143
- 2079 2144
- 2080 [150] Olson KE, Kosloski-Bilek LM, Anderson KM, Diggs BJ, Clark BE, Gledhill JM Jr, Shandler SJ, Mosley RL, & Gendelman HE (2015) Selective VIP receptor agonists facilitate immune transformation for dopaminergic neuroprotection in MPTP-intoxicated mice. *J Neurosci*, **35**, 16463-16478. 2145
- 2081 2146
- 2082 [151] Brahmachari S, & Pahan K (2010) Myelin basic protein priming reduces the expression of Foxp3 in T cells via nitric oxide. *J Immunol*, **184**, 1799-1809. 2147
- 2083 2148
- 2084 [152] Hernández-Romero MC, Argüelles S, Villarán RF, de Pablos RM, Delgado-Cortés MJ, Santiago M, Herrera AJ, Cano J, & Machado A (2008) Simvastatin prevents the inflammatory process and the dopaminergic degeneration induced by the intranigral injection of lipopolysaccharide. *J Neurochem*, **105**, 445-459. 2149
- 2085 2150
- 2086 [153] Wu H, Lu D, Jiang H, Xiong Y, Qu C, Li B, Mahmood A, Zhou D, & Chopp M (2008) Simvastatin-mediated upregulation of VEGF and BDNF, activation of the PI3K/Akt pathway, and increase of neurogenesis are associated with therapeutic improvement after traumatic brain injury. *J Neurotrauma*, **25**, 130-139. 2151
- 2087 2152
- 2088 [154] Han X, Yang N, Xu Y, Zhu J, Chen Z, Liu Z, Dang G, & Song C (2011) Simvastatin treatment improves functional recovery after experimental spinal cord injury by upregulating the expression of BDNF and GDNF. *Neurosci Lett*, **487**, 255-259. 2153
- 2089 2154
- 2090 [155] Yang D, Han Y, Zhang J, Chopp M, & Seyfried DM (2012) statins enhance expression of growth factors and activate the PI3K/Akt-mediated signaling pathway after experimental intracerebral hemorrhage. *World J Neurosci*, **2**, 74-80. 2155
- 2091 2156
- 2092 [156] Churchward MA, & Todd KG (2014) Statin treatment affects cytokine release and phagocytic activity in primary cultured microglia through two separable mechanisms. *Mol Brain*, **7**, 85. 2157
- 2093 2158
- 2094 2159
- 2095 2160
- 2096 2161
- 2097 2162
- 2098 2163
- 2099 2164
- 2100 2165
- 2101 2166
- 2102 2167

- [157] Rana DG, Patel AK, Joshi CG, Jhala MK, & Goyal RK (2014) Alteration in the expression of exon IIC transcripts of brain-derived neurotrophic factor gene by simvastatin [correction of simvastain] in chronic mild stress in mice: A possible link with dopaminergic pathway. *Can J Physiol Pharmacol*, **92**, 985-992.
- [158] Wang C, Chen T, Li G, Zhou L, Sha S, & Chen L (2015) Simvastatin prevents β -amyloid(25-35)-impaired neurogenesis in hippocampal dentate gyrus through α 7nAChR-dependent cascading PI3K-Akt and increasing BDNF via reduction of farnesyl pyrophosphate. *Neuropharmacology*, **97**, 122-132.
- [159] Gao K, Wang G, Wang Y, Han D, Bi J, Yuan Y, Yao T, Wan Z, Li H, & Mei X (2015) Neuroprotective effect of simvastatin via inducing the autophagy on spinal cord injury in the rat model. *Biomed Res Int*, **2015**, 260161.
- [160] Gao K, Shen Z, Yuan Y, Han D, Song C, Guo Y, & Mei X (2016) Simvastatin inhibits neural cell apoptosis and promotes locomotor recovery via activation of Wnt/ β -catenin signaling pathway after spinal cord injury. *J Neurochem*, **138**, 139-149.
- [161] Zhang C, Wu JM, Liao M, Wang JL, & Xu CJ (2016) The ROCK/GGTase pathway are essential to the proliferation and differentiation of neural stem cells mediated by simvastatin. *J Mol Neurosci*, **60**, 474-485.
- [162] Guo Q, Liu C, Hai B, Ma T, Zhang W, Tan J, Fu X, Wang H, Xu Y, & Song C (2017) Chitosan conduits filled with simvastatin/Pluronic F-127 hydrogel promote peripheral nerve regeneration in rats. *J Biomed Mater Res B Appl Biomater*. doi: 10.1002/jbm.b.33890
- [163] Zhang J, Mu X, Breker DA, Li Y, Gao Z, & Huang Y (2017) Atorvastatin treatment is associated with increased BDNF level and improved functional recovery after atherothrombotic stroke. *Int J Neurosci*, **127**, 92-97.
- [164] Decressac M, Kadkhodaei B, Mattsson B, Laguna A, Perlmann T, & Björklund A (2012) α -Synuclein-induced down-regulation of Nurr1 disrupts GDNF signaling in nigral dopamine neurons. *Sci Transl Med*, **4**, 163ra156.
- [165] Roy A, Jana M, Kundu M, Corbett GT, Rangaswamy SB, Mishra RK, Luan CH, Gonzalez FJ, & Pahan K (2015) HMG-CoA reductase inhibitors bind to PPAR α to upregulate neurotrophin expression in the brain and improve memory in mice. *Cell Metab*, **22**, 253-265.
- [166] Castro AA, Wiemes BP, Matheus FC, Lapa FR, Viola GG, Santos AR, Tasca CI, & Prediger RD (2013) Atorvastatin improves cognitive, emotional and motor impairments induced by intranasal 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration in rats, an experimental model of Parkinson's disease. *Brain Res*, **1513**, 103-116.
- [167] Tan W, Xue-bin C, Tian Z, Xiao-wu C, Pei-pei H, Zhi-bin C, & Bei-sha T (2016) Effects of simvastatin on the expression of inducible nitric oxide synthase and brain-derived neurotrophic factor in a lipopolysaccharide-induced rat model of Parkinson disease. *Int J Neurosci*, **126**, 278-286.
- [168] US Preventive Services Task Force, Bibbins-Domingo K, Grossman DC, Curry SJ, Davidson KW, Epling JW Jr, Garcia FA, Gillman MW, Kemper AR, Krist AH, Kurth AE, Landefeld CS, LeFevre ML, Mangione CM, Phillips WR, Owens DK, Phipps MG, & Pignone MP (2016) Statin use for the primary prevention of cardiovascular disease in adults: US Preventive Services Task Force Recommendation Statement. *JAMA*, **316**, 1997-2007.
- [169] "Cardiovascular disease: Risk assessment and reduction, including lipid modification at www.nice.org.uk". Last updated, September 2016. Assessed 1 October 2017.
- [170] McGuinness B, O'Hare J, Craig D, Bullock R, Malouf R, & Passmore P (2013) Cochrane review on 'Statins for the treatment of dementia'. *Int J Geriatr Psychiatry*, **28**, 119-26.
- [171] McGuinness B, Craig D, Bullock R, Malouf R, & Passmore P (2014) Statins for the treatment of dementia. *Cochrane Database Syst Rev*, CD007514.
- [172] Deck BL, Rick J, Xie SX, Chen-Plotkin A, Duda JE, Morley JF, Chahine LM, Dahodwala N, Trojanowski JQ, & Weintraub D (2017) Statins and cognition in Parkinson's disease. *J Parkinsons Dis*. doi: 10.3233/JPD-171113
- [173] Swanson CR, Li K, Unger TL, Gallagher MD, Van Deerlin VM, Agarwal P, Leverenz J, Roberts J, Samii A, Gross RG, Hurtig H, Rick J, Weintraub D, Trojanowski JQ, Zabetian C, Chen-Plotkin AS (2015) Lower plasma apolipoprotein A1 levels are found in Parkinson's disease and associate with apolipoprotein A1 genotype. *Mov Disord*, **30**, 805-812.
- [174] Swanson CR, Berlyand Y, Xie SX, Alcalay RN, Chahine LM, Chen-Plotkin AS (2015) Plasma apolipoprotein A1 associates with age at onset and motor severity in early Parkinson's disease patients. *Mov Disord*, **30**, 1648-1656.
- [175] de Lau LM, Koudstaal PJ, Hofman A, & Breteler MM (2006) Serum cholesterol levels and the risk of Parkinson's disease. *Am J Epidemiol*, **164**, 998-1002.
- [176] Wolozin B, Wang SW, Li NC, Lee A, Lee TA, & Kazis LE (2007) Simvastatin is associated with a reduced incidence of dementia and Parkinson's disease. *BMC Med*, **5**, 20.
- [177] Wahner AD, Bronstein JM, Bordelon YM, & Ritz B (2008) Statin use and the risk of Parkinson disease. *Neurology* **70**(16 Pt 2), 1418-1422.
- [178] Huang X, Chen H, Miller WC, Mailman RB, Woodard JL, Chen PC, Xiang D, Murrow RW, Wang YZ, & Poole C (2007) Lower low-density lipoprotein cholesterol levels are associated with Parkinson's disease. *Mov Disord*, **22**, 377-381.
- [179] Becker C, Jick SS, & Meier CR (2008) Use of statins and the risk of Parkinson's disease: A retrospective case-control study in the UK. *Drug Safety*, **31**, 399-407.
- [180] Hu G, Antikainen R, Jousilahti P, Kivipelto M, & Tuomilehto J (2008) Total cholesterol and the risk of Parkinson disease. *Neurology*, **70**, 1972-1979.
- [181] Mutez E, Duhamel A, Defebvre L, Bordet R, Destée A, & Kreisler A (2009) Lipid-lowering drugs are associated with delayed onset and slower course of PD. *Pharmacol Res*, **60**, 41-45.
- [182] Huang X, Auinger P, Eberly S, Oakes D, Schwarzschild M, Ascherio A, Mailman R, Chen H, & Parkinson Study Group DATATOP Investigators (2011) Serum cholesterol and the progression of Parkinson's disease: Results from DATATOP. *PLoS One*, **6**, e22854.
- [183] Gao X, Simon KC, Schwarzschild MA, & Ascherio A (2012) Prospective study of statin use and risk of Parkinson disease. *Arch Neurol*, **69**, 380-384.
- [184] Undela K, Gudala K, Malla S, & Bansal D (2013) Statin use and risk of Parkinson's disease: A meta-analysis of observational studies. *J Neurol*, **260**, 158-165.
- [185] Friedman B, Lahad A, Dresner Y, & Vinker S (2013) Long-term statin use and the risk of Parkinson's disease. *Am J Manag Care*, **19**, 626-632.

- 2298 [186] Lee Y-C, Lin C-H, Wu R-M, Lin M-S, Lin J-W, Chang
2299 C-H, & Lai M-S (2013) Discontinuation of statin ther-
2300 apy associates with Parkinson disease: A population-based
2301 study. *Neurology*, **81**, 410-416. 2355
- 2302 [187] Tan EK, & Tan LC (2013) Holding on to statins in Parkin- 2356
2303 son disease. *Neurology*, **81**, 406-407. 2357
- 2304 [188] Huang X, Alonso A, Guo X, Umbach DM, Lichtenstein 2358
2305 ML, Ballantyne CM, Mailman RB, Mosley TH, & Chen H 2359
2306 (2015) Statins, plasma cholesterol, and risk of Parkinson's 2360
2307 disease: A prospective study. *Mov Disord*, **30**, 552-559. 2361
- 2308 [189] Bai S, Song Y, Huang X, Peng L, Jia J, Liu Y, & Lu H 2362
2309 (2016) Statin use and the risk of Parkinson's disease: An 2363
2310 updated meta-analysis. *PLoS One*, **11**, e0152564. 2364
- 2311 [190] Sheng Z, Jia X, & Kang M (2016) Statin use and risk of 2365
2312 Parkinson's disease: A meta-analysis. *Behav Brain Res*, 2366
2313 **309**, 29-34. 2367
- 2314 [191] Clark LT, Maki KC, Galant R, Maron DJ, Pearson TA, & 2368
2315 Davidson MH (2006) Ethnic differences in achievement 2369
2316 of cholesterol treatment goals. Results from the National 2370
2317 Cholesterol Education Program Evaluation Project Util- 2371
2318 izing Novel E-Technology II. *J Gen Intern Med*, **21**, 2372
2319 320-326. 2373
- 2320 [192] Yood MU, McCarthy BD, Kempf J, Kucera GP, Wells K, 2374
2321 Oliveria S, & Stang P (2006) Racial differences in reach- 2375
2322 ing target low-density lipoprotein goal among individuals 2376
2323 treated with prescription statin therapy. *Am Heart J*, **152**, 2377
2324 777-784. 2378
- 2325 [193] Sterling NW, Lichtenstein M, Lee EY, Lewis MM, Evans 2379
2326 A, Eslinger PJ, Du G, Gao X, Chen H, Kong L, & Huang X 2380
2327 (2016) Higher plasma LDL-cholesterol is associated with 2381
2328 preserved executive and fine motor functions in Parkin- 2382
2329 son's disease. *Aging Dis*, **7**, 237-245. 2383
- 2330 [194] Liu G, Sterling NW, Kong L, Lewis MM, Mailman RB, 2384
2331 Chen H, Leslie D, & Huang X (2017) Statins may facilitate 2385
2332 Parkinson's disease: Insight gained from a large, national 2386
2333 claims database. *Mov Disord*, **32**, 913-917. 2387
- 2334 [195] Rozani V, Giladi N, El-Ad B, Gurevich T, Tsamir J, 2388
2335 Hemo B, & Peretz C (2017) Statin adherence and the risk 2389
2336 of Parkinson's disease: A population-based cohort study. 2390
2337 *PLoS One*, **12**, e0175054. 2391
- 2338 [196] Bykov K, Yoshida K, Weisskopf MG, & Gagne JJ 2392
2339 (2017) Confounding of the association between statins and 2393
2340 Parkinson disease: Systematic review and meta-analysis. 2394
2341 *Pharmacoepidemiol Drug Saf*, **26**, 294-300. 2395
- 2342 [197] Shrank WH, Patrick AR, & Brookhart MA (2011) Healthy 2396
2343 user and related biases in observational studies of preven- 2397
2344 tive interventions: A primer for physicians. *J Gen Intern 2398
2345 Med*, **26**, 546-550. 2399
- 2346 [198] Swallow DM, Lawton MA, Grosset KA, Malek N, Klein J, 2400
2347 Baig F, Ruffmann C, Bajaj NP, Barker RA, Ben-Shlomo 2401
2348 Y, Burn DJ, Foltynic T, Morris HR, Williams N, Wood 2402
2349 NW, Hu MT, & Grosset DG (2016) Statins are under- 2403
2350 used in recent-onset Parkinson's disease with increased 2404
2351 vascular risk: Findings from the UK Tracking Parkinson's 2405
2352 and Oxford Parkinson's Disease Centre (OPDC) discovery 2406
2353 cohorts. *J Neurol Neurosurg Psychiatry*, **87**, 1183-1190. 2407
- 2354 [199] Chan D, Binks S, Nicholas JM, Frost C, Cardoso MJ, 2408
2355 Ourselin S, Wilkie D, Nicholas R, & Chataway J (2017) 2409
2356 Effect of high-dose simvastatin on cognitive, neuropsy- 2410
2357 chiatric, and health-related quality-of-life measures in 2411
2358 secondary progressive multiple sclerosis: Secondary anal- 2412
2359 yses from the MS-STAT randomised, placebo-controlled 2413
2360 trial. *Lancet Neurol*, **16**, 591-600. 2414
- [200] Saeedi Saravi SS, Saeedi Saravi SS, Arefidoust A, & 2415
2361 Dehpour AR (2017) The beneficial effects of HMG-CoA 2416
2362 reductase inhibitors in the processes of neurodegeneration. 2417
2363 *Metab Brain Dis*, **32**, 949-965. 2418
- [201] Ciurleo R, Bramanti P, & Marino S (2014) Role of statins 2419
2364 in the treatment of multiple sclerosis. *Pharmacol Res*, **87**, 2420
2365 133-143. 2421
- [202] Wong B, Lumma WC, Smith AM, Sisko JT, Wright SD, 2422
2366 & Cai TQ (2001) Statins suppress THP-1 cell migration 2423
2367 and secretion of matrix metalloproteinase 9 by inhibiting 2424
2368 geranylgeranylation. *J Leukoc Biol*, **69**, 959-962. 2425
- [203] Ganné F, Vasse M, Beaudoux JL, Peynet J, François 2426
2369 A, Mishal Z, Chartier A, Tobelem G, Vannier JP, 2427
2370 Soria J, & Soria C (2000) Cerivastatin, an inhibitor 2428
2371 of HMG-CoA reductase, inhibits urokinase/urokinase- 2429
2372 receptor expression and MMP-9 secretion by peripheral 2430
2373 blood monocytes—a possible protective mechanism against 2431
2374 atherothrombosis. *Thromb Haemost*, **84**, 680-688. 2432
- [204] Sena A, Pedrosa R, & Morais MG (2007) Beneficial 2433
2375 effect of statins in multiple sclerosis: Is it dose-dependent? 2434
2376 *Atherosclerosis* **191**, 462. 2435
- [205] MS Drug Treatment Costs Start the Year Headed Up, 2436
2377 [https://multiplesclerosisnewstoday.com/2017/01/13/ms-](https://multiplesclerosisnewstoday.com/2017/01/13/ms-drug-treatment-costs-start-year-headed-up/) 2437
2378 [drug-treatment-costs-start-year-headed-up/](https://multiplesclerosisnewstoday.com/2017/01/13/ms-drug-treatment-costs-start-year-headed-up/)Published 2438
2379 January 13, 2017. Accessed October 1, 2017. 2439
- [206] £6m statin trial raises hope drug can be used to 2440
2380 treat multiple sclerosis, [https://www.theguardian.com/](https://www.theguardian.com/society/2017/may/09/statin-trial-raises-hope-drug-can-be-used-to-treat-multiple-sclerosis-disability-progressive-) 2441
2381 [society/2017/may/09/statin-trial-raises-hope-drug-can-be-](https://www.theguardian.com/society/2017/may/09/statin-trial-raises-hope-drug-can-be-used-to-treat-multiple-sclerosis-disability-progressive-) 2442
2382 [used-to-treat-multiple-sclerosis-disability-progressive-](https://www.theguardian.com/society/2017/may/09/statin-trial-raises-hope-drug-can-be-used-to-treat-multiple-sclerosis-disability-progressive-) 2443
2383 [ms](https://www.theguardian.com/society/2017/may/09/statin-trial-raises-hope-drug-can-be-used-to-treat-multiple-sclerosis-disability-progressive-), Published May 9, 2017. Accessed October 1, 2444
2384 2017. 2445
- [207] Su W, Kansal A, Vicente C, Deniz B, & Sarda S (2016) The 2446
2385 cost-effectiveness of delayed-release dimethyl fumarate 2447
2386 for the treatment of relapsing-remitting multiple sclerosis 2448
2387 in Canada. *J Med Econ*, **19**, 718-727. 2449
- [208] Bozkaya D, Livingston T, Migliaccio-Walle K, & Odom T 2450
2388 (2017) The cost-effectiveness of disease-modifying ther- 2451
2389 apies for the treatment of relapsing-remitting multiple 2452
2390 sclerosis. *J Med Econ*, **20**, 297-302. 2453
- [209] Soini E, Joutseno J, & Sumelahti ML (2017) Cost-utility 2454
2391 of First-line Disease-modifying Treatments for Relapsing- 2455
2392 Remitting Multiple Sclerosis. *Clin Ther*, **39**, 537-557.e10. 2456
- [210] Shih T, Wakeford C, Meletiche D, Sussell J, Chung A, 2457
2393 Liu Y, Shim JJ, & Lakdawalla D (2016) Reconsidering 2458
2394 the economic value of multiple sclerosis therapies. *Am J 2459
2395 Manag Care*, **22**, e368-e374. 2460
- [211] Santos R, Ursu O, Gaulton A, Bento AP, Donadi RS, 2461
2396 Bologna CG, Karlsson A, Al-Lazikani B, Hersey A, Oprea 2462
2397 TI, & Overington JP (2017) A comprehensive map of 2463
2398 molecular drug targets. *Nat Rev Drug Discov*, **16**, 19-34. 2464