Regional brain iron and gene expression provide insights into neurodegeneration in Parkinson’s disease

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ABSTRACT

The mechanisms which are responsible for the selective vulnerability of specific neuronal populations in Parkinson’s disease are poorly understood. Oxidative stress secondary to brain iron accumulation is one postulated mechanism. We measured iron deposition in 180 cortical regions in 96 patients with Parkinson’s disease and 35 controls using quantitative susceptibility mapping. We estimated the expression of 15 745 genes in the same regions using transcriptomic data from the Allen Human Brain Atlas. Using partial least squares regression, we then identified the profile of gene transcription in the healthy brain that underlies increased cortical iron in patients with Parkinson’s disease relative to controls. With gene ontological tools, we investigated the biological processes and cell types associated with this transcriptomic profile. We identified the sets of genes whose spatial expression profiles in control brains correlated significantly with the spatial pattern of cortical iron deposition in Parkinson’s disease. Gene ontological analyses revealed that these genes were enriched for biological processes relating to heavy metal detoxification, synaptic function and nervous system development and were predominantly expressed in astrocytes and glutamatergic neurons. We also show that genes found to be differentially expressed in Parkinson’s disease play a role in explaining the pattern of cortical expression we identified. Our findings provide mechanistic insights into regional selective vulnerabilities in Parkinson’s disease, particularly into processes involving iron accumulation.

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Introduction

In Parkinson’s disease, bradykinesia and rigidity severity correlate approximately with the degree of nigrostriatal dopamine denervation (Hornykiewicz, 2002) and the presence of alpha synuclein rich cell inclusions (Spillantini et al., 1997), called Lewy bodies, are the pathological signature associated with the disease. However, alpha synuclein inclusions do not correlate with symptom severity (Jellinger, 2009) and the pathophysiological processes that are responsible for the associated dementia seen in many elderly patients is not known. As such, there is an increasing need to understand why some brain regions display selective vulnerability to neurodegeneration (Surmeier et al., 2017) as well as the mechanisms which trigger the degenerative cascade (Johnson et al., 2019).
For more than 25 years, it has been known that iron accumulates in the basal ganglia and substantia nigra in Parkinson’s disease (Sofic et al., 1988; Dexter et al., 1989, 1991) with resultant oxidative stress of interest as an important potential driver of neurodegeneration (Fahn and Cohen, 1992; Halliwell, 1992; Gerlach et al., 1994), an observation which has been corroborated by more recent advances (Ward et al., 2014; Ndayisaba et al., 2019). Iron accumulates in the brain during aging, especially within the basal ganglia (Betts et al., 2016), partly due to increased blood brain barrier permeability (Farrall and Wardlaw, 2009). High levels of tissue iron cause build-up of toxic reactive oxygen species that interfere with mitochondrial function (Horowitz and Greenamyre, 2010), damage DNA (Melis et al., 2013), catalyse dopamine oxidation reactions to produce toxic quinones (Hare and Double, 2016) and irreversibly modify proteins via highly reactive aldehydes (Dalle-Donne et al., 2003). All these causes of cell stress ultimately lead to iron-mediated cell death (Cozzi et al., 2019). Intriguingly, lipofuscin formation, previously characterised in Parkinson’s disease (Braak et al., 2001), has recently been shown to be driven by disruption of lysosomal lipid metabolism in neurons (Tian et al., 2020). This in turn leads to iron accumulation, oxidative stress and ferroptosis (Tian et al., 2020), providing a direct link between the pathological inclusions in Parkinson’s disease and excess brain iron. Excess iron also interacts directly and indirectly via free radical species with key pathological proteins in Parkinson’s by promoting aggregation of α-synuclein (Ostrerova-Golts et al., 2000), stimulating production of amyloid-beta via downregulation of furin (Silvestri and Camaschella, 2008) and increasing the toxicity of amyloid-beta either directly (Huang et al., 1999), or via increased tau phosphorylation (Lovell et al., 2005).

Until recently, whole brain neuroimaging using conventional structural MRI has had only a limited role in uncovering mechanisms for neurodegeneration in Parkinson’s disease as cortical atrophy is not prominent, particularly early in the disease process (Lanskey et al., 2018). However, quantitative susceptibility mapping (QSM) (Shmueli et al., 2009) has emerged as a powerful new technique, sensitive to local sources of magnetic susceptibility and in particular to variation in brain iron content (Langkammer et al., 2012; Sun et al., 2015). We recently used QSM to show that brain iron is associated with disease severity in Parkinson’s (Thomas et al., 2020), with higher levels of hippocampal iron linked to poorer cognition, and higher putaminal iron linked with poorer motor performance. Whether brain iron deposition is a cause of...
neuronal damage, or a surrogate marker is still unknown, but QSM offers a new way to explore regional effects of neurodegeneration.

We have used the spatial information about iron accumulation provided by QSM and combined it with regional gene expression profiles from the Allen Human Brain Atlas transcriptomic data (Hawrylycz et al., 2012) to identify possible mechanisms for regional selective vulnerability in Parkinson’s disease. This approach, of combining neuroimaging with transcriptomic data has previously been used to investigate autism (Romero-Garcia et al., 2019), schizophrenia (Morgan et al., 2019) and Huntington’s disease (McColgan et al., 2018). Here, we used partial least squares (PLS) regression to test whether cortical brain iron accumulation in Parkinson’s disease measured using QSM correlates with specific patterns of gene expression in order to shed light on the gene expression profiles that render specific cortical regions vulnerable to higher levels of brain iron accumulation and subsequent neurodegeneration. We also performed an enrichment analysis on the identified gene expression patterns linked to higher brain iron to determine the biological and cell processes linked with this degenerative process of aberrant iron accumulation and neurodegeneration.

Materials and Methods

Participants

96 patients with Parkinson’s seen with a disease duration of less than 10 years (age 49-80 years, mean=66.4, SD=7.7, 48 female) volunteered to participate from October 2017 to December 2018. Inclusion criteria were clinically diagnosed, early to mid-stage Parkinson’s disease (Queen Square Brain Bank Criteria (Gibb and Lees, 1988)) aged 49-80 years. Exclusion criteria were confounding neurological or psychiatric disorders, dementia and metallic implants considered unsafe for MRI. Participants continued their usual therapy (including l-DOPA) for all assessments. No patients were taking cholinesterase inhibitors. In addition, we recruited 35 age-matched controls (50-80 years, mean=66.1, SD=9.4, 21 female) that included some unaffected patient spouses. All participants gave written informed consent, and the study was approved by the Queen Square Research Ethics Committee. All participants underwent clinical assessments of motor function, cognition, vision, mood and sleep, as previously described (Thomas et al., 2020). Motor assessment was performed using the MDS-UPDRS (Goetz et al.,
2008), general cognition was assessed using the Mini-Mental State Examination (MMSE) (Folstein et al., 1975) and the Montreal Cognitive Assessment (MOCA) (Nasreddine et al., 2005) (see Table 1 for clinical and demographic information).

MRI acquisition

MRI acquisition is as previously described (Thomas et al., 2020). In brief, MRI measurements consisting of susceptibility- and T1-weighted MRI scans were performed on a Siemens Prisma-fit 3T MRI system using a 64-channel receive array coil (Siemens Healthcare, Erlangen, Germany). Susceptibility-weighted MRI signals were obtained from a 2×1-accelerated, 3D flow-compensated spoiled-gradient-recalled echo sequence with flip angle 12°; echo time, 18ms; repetition time, 25ms; receiver bandwidth, 110Hz/pixel; matrix dimensions 204×224×160 with 1×1×1 mm voxel resolution (scan time 5 minutes 41 seconds). T1-weighted magnetization-prepared 3D rapid gradient-echo (MPRAGE) anatomical images were acquired using a 2×1-accelerated sequence with inversion time, 1100ms; flip angle, 7°; first echo time, 3.34ms; echo spacing, 7.4ms; repetition time, 2530ms; receiver bandwidth, 200Hz/pixel; matrix dimensions 256×256×176 with 1×1×1 mm voxel size (scan time 6 minutes 3 seconds).

QSM pre-processing and spatial standardization

QSM pre-processing is as previously described (Thomas et al., 2020). 3D phase images were unwrapped with a discrete Laplacian method (Abdul-Rahman et al., 2007). Brain masks were calculated using the BET2 algorithm (Smith, 2002) in the FMRIB software library (FSL version 5.0, https://fsl.fmrib.ox.ac.uk/fsl/fslwiki). Background field removal was completed using Laplacian boundary value extraction (Zhou et al., 2014) and variable spherical mean-value filtering (Schweser et al., 2011), and susceptibility maps were estimated using Multi-Scale Dipole Inversion (Acosta-Cabronero et al., 2018) in MATLAB R2014b (The MathWorks, Inc., Natick, MA, USA). A study-wise template was created using advanced normalisation tools (ANTs) (Avants et al., 2011) via normalisation of participant MPRAGE volumes (Acosta-Cabronero et al., 2017; Thomas et al., 2020). QSM images were coregistered to normalised space using a warp composition of the above transformation and an affine GRE-magnitude-to-MPRAGE transformation using ANTs.
Regional QSM analysis

In our previous whole-brain analysis, absolute QSM was used to improve statistical conditioning in the cortex (Betts et al., 2016). However, as the current study involves multiple individual regions of interest (ROIs), here, we used signed QSM as it enables discrimination between paramagnetic and diamagnetic susceptibility sources. To generate ROIs, we used a version of the Glasser atlas surface parcellation (Glasser et al., 2016) transformed into a set of volumetric labels in MNI space (https://neurovault.org/collections/1549/). The 180 cortical ROIs from the left hemisphere were brought into the study-wise template space using an ANTs-based deformable b-spline co-registration routine and nearest-neighbour interpolation. To reduce partial-volume contamination, each cortical region of interest was intersected with a study-wise average grey matter mask binarized at a grey matter density cut-off of 0.25 using FSL. Mean, signed QSM values in each ROI were extracted from all participants and age- and sex-adjusted using MATLAB. A linear model was fitted to each ROI in all the control subjects such that: $\hat{Y}_i = \alpha_i + \beta_i A$. Where $\hat{Y}_i$ is the estimated mean susceptibility value in region $i$, $A$ is the subject’s age and $\alpha_i$ and $\beta_i$ are the fitted model parameters. The QSM susceptibility values in the control and PD patients were then age adjusted according to: $\chi_{ij} = Y_{ij} + \beta_i (\mu - A_j)$. Where, for region $i$ in subject $j$, $Y_{ij}$ is the unadjusted susceptibility value, $\mu$ is the mean age of the control subjects, $A_j$ is subject’s age and $\chi_{ij}$ is the age-adjusted susceptibility value. We used the fitted parameters from the control group to age-correct the susceptibility values measured in the Parkinson’s disease patients to avoid removing effects of disease progression with age. For the sex adjustment, as sex may affect magnetic susceptibility values differently in controls and Parkinson’s disease patients, a linear model was fitted to each ROI for each participant group separately: $\hat{\chi}_{ik} = \alpha_{ik} + \beta_{ik} S_k$. Where, for region $i$ in participant group $k$, $\hat{\chi}_{ik}$ is estimated age-adjusted susceptibility, $S_k$ is the sex of the subject and $\alpha_{ik}$ and $\beta_{ik}$ are the fitted parameters. We then adjusted for the effect of sex in each group using: $\chi'_{ijk} = \chi_{ijk} + \beta_{ik}(\mu_k - S_{jk})$. Where, for region $i$ in subject $j$ in participant group $k$, $\chi_{ijk}$ is age-adjusted susceptibility, $\mu_k$ is the group’s ‘mean’ sex, $S_{jk}$ is subject’s sex and $\chi'_{ijk}$ is the age- and sex-adjusted susceptibility. To generate a continuous measure of magnetic susceptibility difference in Parkinson’s disease relative to controls, Parkinson’s age- and sex-adjusted regional means were normalised to the control mean for that region by a Z-score transformation using MATLAB (Arnatkevičiūtė et al., 2019), giving a 180x1 age-adjusted QSM score vector, $Y$ (see Fig 1 for
an overview of QSM and gene expression processing). Statistically significant differences between ROIs were probed using t-tests and are reported at $p<0.05$ and $q<0.05$ (Yekutieli and Benjamini, 1999).

**Estimation of regional gene expression**

We used Allen Human Brain Atlas microarray transcriptomic data for five male and one female donors with no history of neurological or psychiatric disease (mean age 42.5 years), available from the Allen Institute for Brain Science (Hawrylycz et al., 2012). As data from six donors are available for the left hemisphere but from only two donors for the right hemisphere, we assessed left hemisphere samples only, as these are considered more robust (Romme et al., 2017; McColgan et al., 2018). Using MATLAB, each tissue sample was assigned to one of the 180 left cortical regions of the Glasser atlas (Glasser et al., 2016), using the AHBA MRI data for each donor, in a process that has been described in detail (Arnatkevičiūtė et al., 2019). Genes with expression levels above a background threshold of 50% were selected and gene expression data was normalised across the left cortex, as in previous literature (Arnatkevičiūtė et al., 2019). Regional expression levels for each gene were compiled to form a $180 \times 15745$ regional transcription matrix, $X$ (see Fig 1).

**Partial least squares regression**

We used partial least squares regression (PLSR) to examine the association between the healthy brain transcriptome and cortical QSM in Parkinson’s disease as PLSR is well suited to the high collinearity of gene expression data (Abdi, 2010). PLSR is a multivariate analysis technique, similar to Principal Component Analysis, which combines dimension reduction and linear regression, producing components from $X$ (the $180 \times 15475$ predictor matrix of 180 regional mRNA measurements for 15475 genes) that have maximum covariance with $Y$ (the $180 \times 1$ regional QSM score vector). The second PLS component (PLS2) was used to weight and rank gene predictor variables. 10000 permutations based on sphere-projection-rotations (Váša et al., 2018) of the QSM-score cortical map were examined to test the null hypothesis that PLS2 explained no more variance in $Y$ than chance. Bootstrapping was used to estimate the variability of each gene’s positive or negative weight on PLS2 and the ratio of the weight of each gene to its bootstrapped standard error was used to rank its contribution to PLS2. All PLS
and bootstrapping analyses were conducted in MATLAB. We tested the null hypothesis of zero weight for each gene using a false discovery rate inverse quantile transformation correction to account for winner's curse bias using R version 3.6.1 (Bigdeli et al., 2016). Only genes that survived this correction at q<0.05 were included in the enrichment analyses, and upweighted and downweighed genes were assessed separately.

**Gene ontological analysis**

We used the g:Profiler (Raudvere et al., 2019) toolset, implemented in R, to perform a gene ontological (GO) enrichment analysis of the significant positively and negatively weighted genes defined by PLS2. We filtered the resulting list of GO terms by retaining only those that were significantly enriched at P<0.05 (corrected for multiple comparisons using the g:SCS algorithm (Raudvere et al., 2019)) and discarded terms associated with over 2500 genes as being too general. To reduce and visualize gene ontology terms, we used the REViGO web page tool, which is based on semantic similarity (Supek et al., 2011).

We performed additional GO enrichment analyses using R to mitigate against the possibility of false-positive bias for GO terms in the enrichment analyses of brain-wide transcriptomic data due to null models not accounting for gene-gene co-expression and spatial autocorrelation present within such data (Fulcher et al., 2020). Specifically, we ran GO enrichment analyses for both a random and a spatial-spin permutation of our QSM-score data (generated in MATLAB) and compared the associated GO terms to the GO terms arising from our main analysis.

**Cell-type analysis**

As gene expression is often driven by the underlying cell-type distribution we used expression weighted cell-type enrichment analysis (EWCE), implemented in R, to investigate whether the most strongly weighted genes were more significantly expressed in particular cell types (Skene and Grant, 2016). We took the top 20% of the most significantly upweighted and downweighted genes identified by PLS2 and assessed their relative expression in the cell types defined in the Allen Institute for Brain Science single-cell transcription dataset ([https://portal.brain-map.org/atlas-and-data/rnaseq](https://portal.brain-map.org/atlas-and-data/rnaseq)) (Hawrylycz et al., 2015) against a
background set of all 15745 genes included in our initial analysis. We replicated our analysis using a separate human derived dataset (Habib et al., 2017), and performed replication analyses with the top 10, 30, 40 and 50% of PLS2 genes to ensure that results were not driven by threshold selection.

**Comparison with external Parkinson’s disease post mortem-derived gene expression data**

To test whether differentially expressed genes in Parkinson’s disease and associated disease states helped explain the pattern of PLS2 expression, we matched sets of genes identified in cortical (Stamper et al., 2008; Riley et al., 2014; Dumitriu et al., 2016) and subcortical (Bossers et al., 2009; Riley et al., 2014; Dijkstra et al., 2015) brain regions in Parkinson’s disease, as well as cortical regions in Parkinson’s disease dementia (Stamper et al., 2008) and dementia with Lewy bodies (Riley et al., 2014) to our weighted PLS2 gene list. This included re-analysis of one dataset (Dumitriu et al., 2016) using available clinical data to differentiate between Parkinson’s disease and Parkinson’s disease dementia, with RNA-sequencing data in each group analysed using the R package DESeq2 version 1.30.0. We used age at death and RNA integrity number (RIN) as covariates and generated lists of genes that were differentially expressed: in Parkinson’s disease relative to controls; in Parkinson’s disease dementia relative to controls and in Parkinson’s disease dementia relative to Parkinson’s disease (significant at \( p<0.05 \)). We tested whether genes in each dataset were significantly more positively or negatively weighted than due to chance by 10,000 random permutations of the same sample size. To maintain consistency across datasets, we only included genes with an absolute fold change of greater than 1.5. Significant results are reported at Bonferroni corrected \( p<0.0023 \) (22 comparisons across 5 studies).

**Results**

**Brain iron content is increased in Parkinson’s disease compared with controls**
Regional cortical signed QSM score showed increases in magnetic susceptibility in Parkinson’s disease relative to controls in frontal, posterior parietal and insular cortices, and slightly decreased susceptibility values in Parkinson’s disease relative to controls in the occipital cortex (Fig 2A, 3B) indicating differences in brain iron content in these brain regions. These differences were statistically significant (p<0.05) in 17 Glasser ROIs, with increased magnetic susceptibility in Parkinson’s disease in 16 ROIs and increased magnetic susceptibility in controls compared with Parkinson’s disease in one ROI (Fig 2B and Table 2). The anterior agranular insular complex survived correction for multiple comparisons (FDR-adjusted q<0.05) (Yekutieli and Benjamini, 1999). These results are qualitatively similar to the whole-brain results we obtained using absolute QSM in our previous study, where we found increased absolute QSM susceptibility values in Parkinson’s in frontal and posterior parietal cortices (Thomas et al., 2020).

**Relating cortical brain iron in Parkinson’s disease to variation in gene expression patterns**

We used partial least squares regression to identify the pattern of gene expression that correlated with the anatomical distribution of brain iron content, as measured using QSM. The second partial least squares component (PLS2) explained the most (20%) variance in QSM score (p<0.01) and PLS2 gene expression weights showed a strong positive correlation with QSM score (r=0.44, p=4.46e-10, Fig 3), meaning that genes that were positively weighted on PLS2 were also more highly expressed in cortical brain regions with higher magnetic susceptibilities. Similarly, genes that were negatively weighted on PLS2 showed relatively low expression in cortical brain regions with high QSM scores. The spatial profile of PLS2 weightings matched that of QSM score, particularly in frontal, posterior cingulate and insular cortices. We therefore used PLS2 to rank and select significantly weighted genes, giving a set of 1622 significantly upweighted and 1068 significantly downweighed genes (q<0.05). We assessed these lists separately (see Fig 3). The complete set of PLS2 gene weights and associated statistics are provided in supplementary Table 1.

Using gene ontological (GO) analysis, we found sets of biological processes associated with upweighted genes (Fig 4A). Genes more highly expressed in regions with higher QSM values in Parkinson’s disease were enriched for GO terms relating to nervous system development,
synaptic transmission and signalling, and detoxification of and stress response to metal ions (Table 3). We also found these genes to be enriched for the REACTOME pathways “Metallothioneins bind metals” and “Response to metal ions”. In contrast, we did not find any GO biological processes to be enriched in the downweighted gene set. Full tables of GO terms associated with up- and downweighted genes are provided in supplementary Tables 2&3.

Our additional control analyses confirmed that these findings were specific to the QSM data, rather than spuriously arising from gene-gene co-expression. Specifically, the control analysis using a spatial spin of the cortical data revealed upweighted genes that were not enriched for any GO biological processes, and downweighted genes enriched for biological processes relating to immune cell proliferation and viral transcription. See supplementary Tables 4&5 for the full list of GO terms associated with the up- and downweighted genes from the control spatial-spin null analyses. The second control analysis was performed using a random-null model and generated an upweighted gene list that was not significantly enriched for any GO biological processes, and a downweighted list that was enriched only for the term “response to lipopolysaccharide”. These control analyses provide additional support that gene lists arising from our experimental analysis relating spatial variation in QSM with gene expression are specific to this relationship and have not arisen spuriously due to gene-gene co-expression or spatial autocorrelation.

**Cell types linked with variation in brain cortical iron content**

We assessed whether the most significantly upweighted and downweighted genes associated with brain iron, as measured using QSM, were more strongly expressed in specific cell types. Cell-type expression was defined using the human-derived single-nucleus data from the Allen Institute for Brain Science (Hawrylycz et al., 2015) and increased expression assessed using EWCE (Skene and Grant, 2016). We found that the top 20% of upweighted genes (324 genes) were significantly enriched in astrocytes, glutamatergic and GABAergic neurons, and oligodendrocyte precursor cells, implying a relatively higher proportion of these cell types in healthy brains in regions with a higher iron content in Parkinson’s disease (Fig 4B). We found that the top 20% of downweighted genes (214 genes) showed significantly greater expression in GABAergic and glutamatergic neurons, implying a higher proportion of these cells in healthy brains in regions with lower iron content in Parkinson’s disease. Adjusting the EWCE
threshold to include the top 10, 30, 40 or 50% of upweighted or downweighted genes did not alter the cell transcription profile seen in either case, nor did defining cell-type expression using a different human-derived single-cell nucleus dataset (Habib et al., 2017) (see Supplementary Fig 1).

**Association with Parkinson’s disease post mortem-derived gene expression data**

We found that genes upregulated in Parkinson’s disease cortex relative to controls as well as genes upregulated in cortex in Parkinson’s disease dementia relative to controls (Dumitriu et al., 2016) were more negatively weighted than expected by chance in our analysis ($p=0.0002$, $p<0.0001$ respectively). Similarly, genes identified as being upregulated in cortex amongst individuals with dementia with Lewy bodies relative to controls (Riley et al., 2014) were significantly more downweighted than expected by chance in our analysis ($p<0.0001$), whereas genes found to be downregulated were significantly more upweighted ($p<0.0001$). Additionally, we found that downregulated genes identified in Parkinson’s disease substantia nigra (Riley et al., 2014) were more positively weighted than due to chance in our analysis ($p<0.0001$). Full results from the above weighting analysis can be found in supplementary Table 8. Together these finding suggests that the gene expression profiles we identified based on susceptibility to cortical iron deposition and using baseline gene expression (as determined by the Allen Atlas dataset) are more perturbed in Parkinson’s disease than expected by chance, and this finding is replicable across independent sample cohorts and experimental approaches. Furthermore, these findings hold at later disease stages linked with Parkinson’s disease dementia and dementia with Lewy bodies.

**Discussion**

We have demonstrated that regional increases in magnetic susceptibility that are most likely due to brain iron accumulation, are associated with the expression of genes involved in metal detoxification and synaptic function. Regional differences in the probable proportion of astrocytes, glutamatergic and GABAAergic neurons and oligodendrocyte precursor cells were also present.
Increased QSM susceptibility values were found in prefrontal and posterior parietal cortices, consistent with our previous findings (Thomas et al., 2020) and were also present in insular and cingulate cortices. The use of signed rather than absolute QSM values allowed us to separate the effects of paramagnetic from diamagnetic substances, allowing for a more detailed analysis of the gene expression profiles. The cortical pattern of increased QSM values implicates similar cortical regions to those where Lewy pathology predominates in advanced Parkinson’s, namely the insular, cingulate and prefrontal cortices (Braak et al., 2003). High levels of intracellular iron promote α-synuclein fibril aggregation (Ostrerova-Golts et al., 2000; Li et al., 2011), and produce free radical species causing cellular damage (Dalle-Donne et al., 2003; Horowitz and Greenamyre, 2010; Melis et al., 2013; Hare and Double, 2016). We believe therefore that, high QSM susceptibility in these regions is likely to reflect early tissue changes leading to neurodegeneration in Parkinson’s disease.

To connect the regional pattern of iron accumulation to underlying biological vulnerabilities, we used partial least squares regression to identify the pattern of gene expression that best mapped to the spatial distribution of the quantitative susceptibility mapping signal. Those genes with higher expression in regions with more brain iron deposition in Parkinson’s disease as measured by QSM (upweighted on PLS2) were significantly enriched for GO processes relating to heavy metal detoxification and synaptic signalling. The specific link between brain iron and cellular vulnerability in Parkinson’s disease was recently shown using a CRISPR interference platform. Mutations in genes related to lysosomal pathways, which are highly implicated in Parkinson’s disease, strongly sensitized neurons to oxidative stress and ferroptosis (Tian et al., 2020).

Our finding of gene expression relating to detoxification of other heavy metals is intriguing. This is likely to be explained by the fact that iron-copper homeostasis is controlled by proteins involved in both the metabolism of iron and copper (Kozlowski et al., 2012; Zheng and Monnot, 2012). As well as iron, altered levels of copper also occur in Parkinson’s disease. Reduced copper is seen alongside increased iron in the substantia nigra in Parkinson’s disease (Dexter et al., 1991), whereas increased copper has been reported in the CSF (Boll et al., 1999). Free copper can catalyse reactions generating reactive oxygen species in a manner similar to iron (Wang et al., 2012) and has similar potential to cause cell damage. However, some copper binding and transport proteins, notably metallothioneins, may have a neuroprotective role (Michael et al., 2011). The altered binding of copper by cuproproteins in Parkinson’s could...
Contribute to the inability of cells to deal with increased oxidative load (Montes et al., 2014), which is in turn aggravated by a large pool of labile iron (Genoud et al., 2017). Copper has a high binding affinity with α-synuclein and is more potent than iron in aggregating α-synuclein (Binolfi et al., 2012), as well as being implicated in the formation of beta-amyloid plaques and neurofibrillary tangles (Sayre et al., 2000). When bound to α-synuclein it also has the capacity to reduce ferric to ferrous iron, facilitating further reactive oxygen species (Davies et al., 2011).

Disturbances in synaptic function are known to play a role in vulnerability and progression in Parkinson’s disease. A number genes mutated in familial forms of Parkinson’s are involved in synaptic function and autophagy (Kumaran and Cookson, 2015), and genes implicated in sporadic Parkinson’s disease also relate to mechanisms of synaptic homeostasis (Soukup et al., 2018). Aberrant synaptic autophagy and excess synaptic pruning may lead to downstream synaptic loss, which is expected to precede neurodegeneration (Day et al., 2006; Plowey and Chu, 2011; Soukup et al., 2018).

The distribution of gene expression could be partly explained by variation in the distribution of particular cell types, leading us to perform an expression-weighted cell-type enrichment (EWCE) analysis. We found that upweighted genes showed significantly greater expression in astrocytes and glutamatergic and GABAergic neurons, and oligodendrocyte precursor cells. The finding of relative enrichment of astrocytes in regions with high levels of brain iron is intriguing as astrocytes play an important role in brain iron uptake and metabolism (Abbott et al., 2006; Dringen et al., 2007) and in distributing iron to other neuronal cells (Jeong and David, 2003; Ward et al., 2014).

Enrichment in glutamatergic neurons is also of note. Higher levels of brain iron have been reported to correlate with higher N-methyl-D-aspartate (NMDA) receptor overactivity (Xu et al., 2017), and increased NMDA receptor activity may also stimulate release of iron from the lysosome (White et al., 2016). Glutamate-mediated excitotoxicity has been linked with neurodegeneration in Parkinson’s (Ambrosi et al., 2014). Glutamate induces Parkin accumulation in mitochondria in a calcium and NMDA-receptor dependent manner (Van Laar et al., 2015) and α-synuclein may perturb intracellular calcium levels via α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors (Hüls et al., 2011).
We compared the pattern of cortical gene expression from our analyses against differentially expressed gene lists identified in a number of external post-mortem datasets in Parkinson’s disease, Parkinson’s disease dementia and dementia with Lewy bodies (Stamper et al., 2008; Bossers et al., 2009; Riley et al., 2014; Dijkstra et al., 2015; Dumitriu et al., 2016). We found that genes altered in these conditions were linked with the profile of healthy brain gene expression underlying higher magnetic susceptibility in Parkinson’s disease (Riley et al., 2014; Dumitriu et al., 2016). This provides further evidence for processes involving brain iron in the selective vulnerabilities driving degeneration in Parkinson’s disease and Lewy body dementia.

Our findings demonstrate that regions showing increased iron deposition in Parkinson’s disease have a functional abundance of cells and proteins involved in the homeostasis and detoxification of metals in the healthy brain as well as in synaptic function. Any disturbance of such pathways, as has been reported in Parkinson’s disease and models of Parkinson’s disease, could potentially render these regions vulnerable to excess iron accumulation, aberrant iron processing and subsequent downstream oxidative stress, proteinopathy and cell death.

**Limitations**

Our main analysis used gene expression data derived from donors unaffected by neurological or psychiatric disease. Spatial expression profiles are therefore based on unaffected individuals and conclusions only apply to this intrinsic variability, not to changes in gene expression that occur in Parkinson’s disease.

Due to the availability of data in the Allen Human Brain Atlas, we used only left hemisphere data, as data from the right hemisphere is only available from two brains (Hawrylycz et al., 2012; Arnatkevičiūtė et al., 2019). There is a lateralisation effect for the distribution of QSM susceptibility, with higher magnetic susceptibilities in the right compared with the left hemisphere. Differences between hemispheres could be examined in future work when gene expression data are available for both hemispheres from a larger number of donors. When Parkinson’s disease gene expression data with widespread cortical coverage become available, it will be of interest to relate gene expression in Parkinson’s disease directly to brain iron levels. We were unable to validate our PLS analysis with any additional healthy gene expression
databases as those currently available (Kang et al., 2011; Ramasamy et al., 2014) are derived from only a small number of cortical regions.

We used QSM to measure increased iron deposition in Parkinson’s and investigated the genetic makeup of implicated regions as there is strong evidence implicating neurodegeneration as a process downstream of iron-induced oxidative stress (Fahn and Cohen, 1992; Halliwell, 1992; Gerlach et al., 1994; Ward et al., 2014; Ndayisaba et al., 2019). However, QSM will be unable to capture all drivers of neurodegeneration, especially those that occur independently of iron accumulation, or do not exhibit positive correlations with it. This may explain, for example, why we did not see any significant GO terms relating to autophagy or mitochondrial function in our analysis, despite reported associations between these two processes and Parkinson’s disease neurodegeneration (Menzies et al., 2017; Tan et al., 2020). There may also be other drivers of QSM changes in magnetic susceptibility apart from iron. For example, it is possible that demyelination could lead to increases in cortical paramagnetic susceptibility (Stüber et al., 2014) and diamagnetic metals such as calcium and magnesium have been reported at comparable levels to iron in several brain regions post mortem (Krebs et al., 2014).

Iron deposits have been shown to colocalise with amyloid (Van Bergen et al., 2016) and tau pathology (Spotorno et al., 2020). How these contribute to one another and lead to neurodegeneration is not known. Future studies of this kind could incorporate contemporaneous data on brain amyloid and tau, e.g. using amyloid or tau PET, with QSM and transcriptomic data to try and shed light on the biological processes which may contribute to this.

Our cell-type analysis was performed using the Allen Institute for Brain Science single nucleus RNA-sequencing dataset, which is based on the profiling of cortical brain samples. Future analyses could specifically examine the role of cells in other brain regions, for example midbrain dopaminergic neurons.

Participants in our study were scanned whilst taking their usual dopaminergic medications. There is no evidence that L-DOPA causally affects brain tissue iron content or magnetic susceptibility and L-DOPA does not alter cortical iron levels in a mouse model of Parkinson’s disease (Billings et al., 2019). In humans, magnetic susceptibility in subcortical regions of interest analyses do correlate with L-DOPA dose (Langkammer et al., 2016), but whether this
relates specifically to the effects of L-DOPA, or is a function of disease severity is not yet known. The effects of being on versus off L-DOPA on cortical QSM magnetic susceptibility could be specifically examined in future work. Finally, our study is cross sectional. Longitudinal studies of brain iron accumulation in patients with Parkinson’s progressing to dementia will provide further insights into the temporal order of brain iron accumulation in specific brain regions and the associated biological processes.

Conclusions

Regional increases in magnetic susceptibility in Parkinson’s disease, most likely due to brain iron accumulation in frontal, cingulate and insular cortices are associated with a distinct gene expression profile. In these regions, we found higher intrinsic levels of gene expression relating to heavy metal detoxification and synaptic function as well as a probable relative abundance of astrocytes and glutamatergic neurons. These findings shed light onto the processes driving neurodegeneration in Parkinson’s disease and the selective vulnerabilities of brain regions that are most affected, providing potential insights into future therapeutic targets to slow the progression of neurodegeneration in Parkinson’s disease.

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Competing interests
RSW has received personal fees from GE healthcare. JA-C has equity and a full-time appointment at Tenoke Limited.

Data availability

Regional QSM score and gene expression matrices, along with code to carry out the PLSR, gene ontological, and weighting of differentially expressed genes analyses can be found at (https://github.com/gecthomas/QSM_and_AHBA_transcription_in_PD). Code for cortical parcellation of AHBA data into Glasser regions (Arnatkevičiūtė et al., 2019): (https://github.com/BMHLab/AHBAprocessing). Code for creating spatial spin permutations of MRI data was adapted from (Váša et al., 2018): (https://github.com/frantisekvasa/rotate_parcellation). Code for PLS analyses and bootstrapping was adapted from (Whitaker et al., 2016): (https://github.com/KirstieJane/NSPN_WhitakerVertes_PNAS2016/tree/master/SCRIPTS). GO analyses were conducted using the gprofiler2 R package (https://CRAN.R-project.org/package=gprofiler2). Cell-type analyses were conducted using the EWCE R package (Skene and Grant, 2016) (https://github.com/NathanSkene/EWCE). BrainNet Viewer was used to visualise data on cortical surfaces: (https://www.nitrc.org/projects/bnv/) (Xia et al., 2013).
Figure Legends

Figure 1 – An overview of the methodology used for regional QSM extraction, estimation of regional gene expression, PLS regression, gene ontological and cell type analyses.

(A) Mean, signed QSM values were extracted from 180 left-cortical regions for 96 Parkinson’s disease patients and 35 controls. (B) A QSM score, $Y_{PD}$, was calculated for each region by a Z-score transformation. (C,D) Allen Human Brain Atlas samples of gene expression data were mapped to the 180 left-cortical regions according to the anatomical parcellation and were used to create a matrix containing the average expression of 15745 genes in those regions. (E) A bootstrapped PLS regression was performed using gene expression (X) as the predictor variable and QSM score (Y) as the response variable. The second component of X explained maximum variance in Y and bootstrapped z-scores were used to rank each gene’s contribution to this component. (F) Genes that were significant at $q_{(corrected\,P)}<0.05$ underwent gene ontological analyses for biological processes and expression-weighted cell-type enrichment analyses.

Figure 2 – Regional cortical differences in magnetic susceptibility between Parkinson’s disease and controls.

(A) Cortical plot of QSM score, calculated by Z-score transforming to the Parkinson’s disease to the control mean for each of the 180 Glasser regions of interest. (B) Glasser regions of interest where a significant difference was observed (blue, controls greater than Parkinson’s disease at $P<0.05$; red, Parkinson’s disease greater than controls at $P<0.05$; orange, Parkinson’s disease greater than controls at $q_{(FDR-adjusted\,P)}<0.05$).

Figure 3 – Spatial profiles of gene expression and significantly weighted genes associated with cortical iron deposition in Parkinson’s disease.

(A) The cortical map of QSM score had a similar spatial pattern to the regional linearly weighted sum of gene expression scores defined by the second PLS component (PLS2). (B) Scatterplot of regional PLS2 scores versus QSM score demonstrating a positive correlation, each data point represents one of 180 cortical regions. (C) Distribution of bootstrapped gene weights on PLS2 - an FDR inverse quantile transform (FIQT) was used to correct for multiple...
comparisons, giving a set of 1622 upweighted (red) and 1068 downlighted (blue) genes significant at q(FIQT-corrected $P<0.05$) that were used in gene ontological and cell-type analyses.

**Figure 4 – Enrichment analyses for genes associated with cortical iron deposition in Parkinson’s disease.**

(A) Gene ontological (GO) terms for biological processes that are significantly enriched in significantly upweighted genes defined by PLS2. Terms are plotted in semantic space with more similar terms clustered together. Non-redundant GO terms significant at g:SCS corrected $P<1\times10^{-5}$ have been labelled in each case. Larger, darker circles indicate greater significance (see colour bar). (B) Expression-weighted cell-type enrichment (EWCE) analyses using the Allen Institute for Brain Science single-cell transcription dataset. Data are presented as standard deviations of the mean expression of upweighted target gene lists from the mean expression of the bootstrap replicates. Cell types in which the target gene lists are significantly enriched are marked with an asterisk (FDR corrected results). ASC = astrocytes, GABA = GABAergic neurons, GLU = glutamatergic neurons, MG = microglia, OPC = oligodendrocyte precursor cells, ODC = oligodendrocytes.
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| Measure                                           | Control (n = 35) | Parkinson’s disease (n = 96) | P-value    |
|--------------------------------------------------|-----------------|------------------------------|------------|
| Gender, male:female                              | 15:20           | 51:45                        | 0.329      |
| Age, years                                       | 66.26 (9.16)    | 64.52 (7.79)                 | 0.284      |
| Years of education                               | 17.71 (2.38)    | 17.02 (2.83)                 | 0.196      |
| MoCA (out of 30)                                 | 28.71 (1.34)    | 27.97 (2.06)                 | 0.049*     |
| UPDRS-III                                        | 5.20 (4.21)     | 22.25 (11.46)                | 2.66 × 10⁻¹⁴*** |
| Binoocular LogMAR visual acuity                  | −0.08 (0.23)    | −0.09 (0.13)                 | 0.802      |
| HADS depression score                            | 1.66 (1.89)     | 3.81 (2.83)                  | 5.37 × 10⁻⁵*** |
| HADS anxiety score                               | 3.91 (3.43)     | 5.92 (4.07)                  | 0.011**    |
| RBDSQ score                                      | 1.94 (1.39)     | 4.13 (2.46)                  | 2.17 × 10⁻⁶*** |
| Smell test (Sniffin’ Sticks)                     | 12.49 (2.43)    | 7.63 (3.13)                  | 1.08 × 10⁻¹³*** |
| Disease duration, years                          | N/A             | 4.20 (2.52)                  | N/A        |
| Levodopa equivalent dose, mg                      | N/A             | 459 (256)                    | N/A        |
| Motor deficit dominance, left:right:both         | N/A             | 37:54:5                      | N/A        |

Table 1: Participant demographics

Means (SDs) reported. Binocular LogMAR: lower score is better visual acuity; HADS = Hospital Anxiety and Depression Scale; MoCA = Montreal Cognitive Assessment; RBDSQ = REM (Rapid Eye Movement) Sleep Behaviour Disorder Screening Questionnaire; UPDRS = Unified Parkinson’s Disease Rating Scale.

***P < 0.001; **P < 0.01; *P < 0.05; ns = not significant.
| ROI number | ROI name                                | HC, ppm   | PD, ppm   | P-value | Q-value |
|------------|-----------------------------------------|-----------|-----------|---------|---------|
| 18         | Fusiform face complex                    | $9.44 \times 10^{-4}$ | $3.02 \times 10^{-3}$ | 0.025*  | 0.366   |
| 27         | Precuneus visual area                    | $-4.41 \times 10^{-4}$ | $-1.43 \times 10^{-3}$ | 0.016*  | 0.290   |
| 34         | Area dorsal 23a+b                        | $-5.90 \times 10^{-4}$ | $-1.27 \times 10^{-3}$ | 8.76 $\times 10^{-4}$*** | 0.079   |
| 37         | Area 5mm ventral                         | $2.14 \times 10^{-3}$ | $4.42 \times 10^{-3}$ | 0.042*  | 0.447   |
| 38         | Area 23c                                | $-5.25 \times 10^{-3}$ | $-3.01 \times 10^{-3}$ | 0.028*  | 0.366   |
| 57         | Area posterior 24 prime                  | $-1.12 \times 10^{-3}$ | $-8.39 \times 10^{-3}$ | 0.014*  | 0.290   |
| 59         | Anterior 24 prime                        | $-9.01 \times 10^{-3}$ | $-6.37 \times 10^{-3}$ | 0.037*  | 0.443   |
| 73         | Area 8C                                 | $2.79 \times 10^{-3}$ | $4.46 \times 10^{-3}$ | 3.81 $\times 10^{-3}$*** | 0.229   |
| 83         | Area posterior 9-46v                      | $-2.01 \times 10^{-4}$ | $-9.25 \times 10^{-4}$ | 0.041*  | 0.447   |
| 85         | Area anterior 9-46v                       | $-6.73 \times 10^{-4}$ | $6.27 \times 10^{-4}$ | 9.71 $\times 10^{-3}$*** | 0.265   |
| 86         | Area 9-46d                               | $-3.48 \times 10^{-4}$ | $7.59 \times 10^{-4}$ | 0.028*  | 0.366   |
| 91         | Area 11I                                | $-4.68 \times 10^{-4}$ | $1.21 \times 10^{-3}$ | 7.63 $\times 10^{-3}$*** | 0.265   |
| 112        | Anterior agranular insula complex        | $-6.48 \times 10^{-4}$ | $-2.32 \times 10^{-3}$ | 3.80 $\times 10^{-4}$*** | 6.85 $\times 10^{-4}$*   |
| 130        | Area STSv posterior                      | $-3.40 \times 10^{-3}$ | $-1.23 \times 10^{-3}$ | 0.015*  | 0.290   |
| 153        | Ventromedial visual area 1               | $2.76 \times 10^{-3}$ | $1.00 \times 10^{-3}$ | 9.58 $\times 10^{-3}$*** | 0.265   |
| 167        | Area posterior insular 1                 | $-1.13 \times 10^{-3}$ | $-8.34 \times 10^{-3}$ | 0.018*  | 0.298   |
| 176        | Area STSv anterior                       | $5.67 \times 10^{-3}$ | $2.83 \times 10^{-3}$ | 0.010*  | 0.265   |

Table 1 Cortical regions with significant differences in QSM signal between PD and controls
ROI numbers and names are according to Glasser atlas denomination. Age adjusted mean signed QSM values are reported. HC = healthy controls; PD = Parkinson’s disease. Q is FDR-adjusted P. 
***(P or Q) < 0.001; **(P or Q) < 0.01; *(P or Q) < 0.05; ns = not significant.
### Table 3 Most significantly enriched GO biological processes in upweighted gene list

GO = gene ontology. Term ID is a unique GO identifier; term name gives a brief description; and term size is the number of unique genes associated with a given term. Reported P-values are corrected for multiple comparisons using the g:SCS algorithm in g:profiler.

| Term ID   | Term name                                | Term size | p-value     |
|-----------|------------------------------------------|-----------|-------------|
| GO:0007399| nervous system development               | 2483      | 1.53 × 10^{-10} |
| GO:0099537| trans-synaptic signaling                 | 702       | 1.25 × 10^{-7}  |
| GO:0007268| chemical synaptic transmission           | 694       | 1.32 × 10^{-7}  |
| GO:0098916| anterograde trans-synaptic signaling     | 694       | 1.32 × 10^{-7}  |
| GO:0007267| cell-cell signaling                      | 1806      | 3.70 × 10^{-7}  |
| GO:0099536| synaptic signaling                        | 724       | 4.04 × 10^{-7}  |
| GO:0099177| regulation of trans-synaptic signaling   | 421       | 1.06 × 10^{-6}  |
| GO:010273 | detoxification of copper ion             | 14        | 1.57 × 10^{-6}  |
| GO:1990169| stress response to copper ion            | 14        | 1.57 × 10^{-6}  |
| GO:0000902| cell morphogenesis                       | 1058      | 2.26 × 10^{-6}  |
| GO:0050804| modulation of chemical synaptic transmission | 420       | 2.60 × 10^{-6}  |
| GO:0097501| stress response to metal ion             | 16        | 6.40 × 10^{-6}  |
| GO:0061687| detoxification of inorganic compound     | 16        | 6.40 × 10^{-6}  |
| GO:0071294| cellular response to zinc ion            | 23        | 6.73 × 10^{-6}  |
| GO:1902930| regulation of alcohol biosynthetic process | 83        | 1.38 × 10^{-5}  |
| GO:0022008| neurogenesis                             | 1710      | 2.57 × 10^{-5}  |
| GO:0048666| neuron development                       | 1167      | 2.90 × 10^{-5}  |
| GO:0071280| cellular response to copper ion          | 27        | 4.78 × 10^{-5}  |
| GO:0031175| neuron projection development            | 1030      | 5.28 × 10^{-5}  |
| GO:0006882| cellular zinc ion homeostasis            | 37        | 7.43 × 10^{-5}  |
Figure 1 – An overview of the methodology used for regional QSM extraction, estimation of regional gene expression, PLS regression, gene ontological and cell type analyses. (A) Mean, signed QSM values were extracted from 180 left-cortical regions for 97 Parkinson’s disease patients and 35 controls. (B) A QSM score, YPD, was calculated for each region by a Z-score transformation. (C,D) Allen Human Brain Atlas samples of gene expression data were mapped to the 180 left-cortical regions according to the anatomical parcellation and were used to create a matrix containing the average expression of 15745 genes in those regions. (E) A bootstrapped PLS regression was performed using gene expression (X) as the predictor variable and QSM score (Y) as the response variable. The second component of X explained maximum variance in Y and bootstrapped z-scores (z) were used to rank each gene’s contribution to this component (q = FDR corrected p-value). (F) Genes that were significant at q<0.05 underwent gene ontological analyses for biological processes and expression-weighted cell-type enrichment analyses.
Figure 2 – Regional cortical differences in magnetic susceptibility between Parkinson’s disease and controls. (A) Cortical plot of QSM score, calculated by Z-score transforming to the Parkinson’s disease to the control mean for each of the 180 Glasser regions of interest. (B) Glasser regions of interest where a significant difference was observed (blue, controls greater than Parkinson’s disease at $P<0.05$; red, Parkinson’s disease greater than controls at $P<0.05$; orange, Parkinson’s disease greater than controls at $q$(FDR-adjusted $P)<0.05$).
Figure 3 – Spatial profiles of gene expression and significantly weighted genes associated with cortical iron deposition in Parkinson’s disease. (A) The cortical map of QSM score had a similar spatial pattern to the regional linearly weighted sum of gene expression scores defined by the second PLS component (PLS2). (B) Scatterplot of regional PLS2 scores versus QSM score demonstrating a positive correlation, each data point represents one of 180 cortical regions. (C) The top five most upweighted (red) and downweighted (blue) genes on PLS2 with accompanying bootstrapped Z-scores, p-values and FDR-corrected q-values. (D) Distribution of bootstrapped gene weights on PLS2 - an FDR inverse quantile transform was used to correct for multiple comparisons, giving a set of 1622 significantly upweighted (red) and 1068 significantly downlighted (blue) genes that were used in gene ontological and cell-type analyses.
Figure 4 – Enrichment analyses for genes associated with cortical iron deposition in Parkinson’s disease. (A) Gene ontological (GO) terms for biological processes that are significantly enriched in significantly upweighted genes defined by PLS2. Terms are plotted in semantic space with more similar terms clustered together. Non-redundant GO terms significant at q:SCS corrected \( p < 1 \times 10^{-5} \) have been labelled in each case. Larger, darker circles indicate greater significance (see colour bar). (B) Expression-weighted cell-type enrichment (EWCE) analyses using the Allen Institute for Brain Science single-cell transcription dataset. Data are presented as standard deviations of the mean expression of upweighted target gene lists from the mean expression of the bootstrap replicates. Cell types in which the target gene lists are significantly enriched are marked with an asterisk (FDR corrected results). ASC = astrocytes, GABA = GABAergic neurons, GLU = glutamatergic neurons, MG = microglia, OPC = oligodendrocyte precursor cells, ODC = oligodendrocytes.
Investigating gene expression underlying cortical iron deposition in Parkinson’s disease.
### STROBE Statement—Checklist of items that should be included in reports of cross-sectional studies

| Item No | Recommendation | Page No |
|---------|----------------|---------|
| **Title and abstract** | 1. (a) Indicate the study’s design with a commonly used term in the title or the abstract  <br> (b) Provide in the abstract an informative and balanced summary of what was done and what was found | 2 |
| **Introduction** | 2. Explain the scientific background and rationale for the investigation being reported | 3-4 |
| **Objectives** | 3. State specific objectives, including any prespecified hypotheses | 4 |
| **Methods** | 4. Present key elements of study design early in the paper | 5 |
| **Study design** | 5. Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection | 5,6 |
| **Setting** | 6. (a) Give the eligibility criteria, and the sources and methods of selection of participants | 5 |
| **Participants** | 7. Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable | 5-9, Table 1 |
| **Variables** | 8*. For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group | 5,7,9-10 |
| **Data sources/measurement** | 9. Describe any efforts to address potential sources of bias | 7,9-10 |
| **Bias** | 10. Explain how the study size was arrived at | 5 |
| **Study size** | 11. Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why | 6-10 |
| **Quantitative variables** | 12. (a) Describe all statistical methods, including those used to control for confounding  <br> (b) Describe any methods used to examine subgroups and interactions  <br> (c) Explain how missing data were addressed  <br> (d) If applicable, describe analytical methods taking account of sampling strategy  <br> (e) Describe any sensitivity analyses | 6-10, N/A, 7,8, 7,9-10, 9-10 |
| **Statistical methods** | 13*. (a) Report numbers of individuals at each stage of study—e.g. numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed  <br> (b) Give reasons for non-participation at each stage  <br> (c) Consider use of a flow diagram | 5, N/A, 5 |
| **Participants** | 14*. (a) Give characteristics of study participants (e.g. demographic, clinical, social) and information on exposures and potential confounders  <br> (b) Indicate number of participants with missing data for each variable of interest | Table 1, N/A |
| **Descriptive data** | 15*. Report numbers of outcome events or summary measures | 10-13 |
| **Outcome data** | 16. (a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (e.g., 95% confidence interval). Make clear which confounders were adjusted for and why they were included | 10-13, Tables 1-3 |
(6) Report category boundaries when continuous variables were categorized  

(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period N/A

| Other analyses | 17 | Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses | 11-13 |

### Discussion

| Key results | 18 | Summarise key results with reference to study objectives | 13-14 |
| Limitations | 19 | Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias | 18-19 |
| Interpretation | 20 | Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence | 20 |
| Generalisability | 21 | Discuss the generalisability (external validity) of the study results | 14-19 |

### Other information

| Funding | 22 | Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based | 20 |

*Give information separately for exposed and unexposed groups.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.
Oxidative stress secondary to brain iron accumulation has been implicated in extrastriatal neurodegeneration in Parkinson’s disease. Thomas et al. show that cortical iron deposition in Parkinson’s disease is associated with differences in the expression of genes involved in metal detoxification and synaptic function.