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Meta-analysis of copper and iron in Parkinson's disease brain and biofluids

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ABSTRACT

Background: Variations in study quality and design complicate interpretation of the clinical significance of consistently reported changes in copper and iron levels in human Parkinson's disease brain and biofluids.

Methods and findings: We systematically searched literature databases for quantitative reports of biometal levels in the degenerating substantia nigra (SN), CSF, serum and plasma in Parkinson's disease compared with healthy age-matched controls and assessed the quality of these publications. The primary outcomes of our analysis confirmed SN copper and iron levels are decreased and increased, respectively, in the Parkinson's disease brain. We applied a novel Quality Assessment Scale for Human Tissue to categorize the quality of individual studies and investigated the effects of study quality on our outcomes. We undertook a random-effects meta-analysis and meta-regression subgroup analysis. In the 18 eligible studies identified (211 Parkinson's disease, 215 control) SN copper levels were significantly lower (d , -2.00; 95% CI, -2.81 to -1.19; $P < 0.001$) and iron levels significantly higher (d , 1.31; CI 0.38 – 2.24; $P < 0.01$) in Parkinson's disease. No changes were detected in CSF, serum or plasma for any metals (29 studies; 2443 Parkinson's disease and 2183 control cases) except serum iron which was lower in Parkinson's disease (14 studies; 1177 Parkinson's disease and 1447 controls).

Conclusion: Reductions in copper levels and elevations in iron are confirmed as characteristic of the degenerating SN of Parkinson's disease. Iron in serum is also changed, but in the opposite direction to that in the SN and to a lesser extent.

INTRODUCTION

Motor dysfunction in Parkinson's disease (PD) is caused by the progressive loss of dopaminergic neurons, particularly in the ventral substantia nigra (SN), and the consequent diminution of dopaminergic transmission by the nigrostriatal pathway. As available antiparkinsonian therapies do not modify this disease process,¹ identifying pathophysiological processes that may be effective targets for neuroprotective therapies is a major research focus. Over the past decade, dysfunction of metal-catalyzed pathways in neurodegenerative disorders has attracted substantial interest². Genetic disorders involving dysregulation of pathways that require copper and iron, such as Wilson's disease,³ Menkes disease,⁴ and neurodegeneration with brain iron accumulation⁵ illustrate the essential roles played by copper and iron in neuronal health and the pathologic consequences of major shifts in their levels.

Increased local iron levels in the brains of people with PD were first noted during the 1920s,⁶ and abnormal iron accumulation in sites of neurodegeneration has since been regularly reported. More recently, reduced copper concentrations, together with increased iron levels, have been reported in degenerating brain regions in PD.⁷ However, the quality of such studies has been heterogeneous and affected by problems including small and diverse sample cohorts, the possibility of post mortem artefacts caused by metal chelation or contamination, and differences in preparation procedures and analytic techniques. Altered metal levels in

biofluids, such as CSF, sera and plasma, have also been described,⁸ but the relationship between these changes and those of brain metals is unclear.

Pharmacological modulation of brain metal levels in PD is being investigated in several clinical trials, including the administration of the orally bioavailable, blood brain barrier-permeable copper complex Cu(II)ATSM for restoring normal copper levels in the SN⁹ (phase 1; ClinicalTrials.gov Identifier: NCT03204929), and of the chelator deferiprone to reduce iron levels¹⁰ (phase 2; ClinicalTrials.gov Identifier: NCT02728843). Confirming copper and iron changes in regions that degenerate in PD would support these therapeutic strategies.

To overcome limitations of individual observation studies and to increase the statistical power of analysis, we undertook a systematic review and meta-analysis of all English language publications on SN and biofluid copper and iron levels in PD. Meta-analysis is useful for estimating the degree to which variation in the outcomes of different studies are related to limited sample sizes (sampling variance), and methodology (true heterogeneity). Further, we evaluated study bias and the validity of methodological design and reporting in individual post mortem and biofluid studies with a scale we specifically developed for assessing the quality of biochemical studies of human samples. Study quality provides clinometric information that enables statistical adjustment of individual datasets in subsequent subgroup meta-analyses.

METHODS AND MATERIALS

Search Strategy and Study Selection

Author SG searched Web of Science, EMBASE and MEDLINE for the terms “*copper*”/“*Cu*” or “*iron*”/“*Fe*” or “*metal**” AND “*Parkinson**” on 13 September 2017. All articles in English published before the search date were initially included in the analysis. No search restrictions regarding analytic methods or experimental approach (*i.e.*, *in vitro* or *in vivo*) were applied. We undertook this systematic review and meta-analysis according to the guidelines of the Preferred Reporting Items for Systematic Reviews and Metal-analyses (PRISMA) statement (Fig. 1).¹¹

Data extraction

The initial search identified 14,487 articles. After duplicates were excluded, 9,026 unique articles were pre-screened by two authors (SG, KLD) according to pre-defined exclusion criteria (quantitative measurements of copper, iron, zinc or manganese in human post-mortem SN tissue, CSF, sera or blood in PD patients); 8929 records were excluded (Figure 1), and 97 articles further assessed for eligibility:

- Forty-one articles reporting metal concentrations in post mortem SN tissue: 23 articles were excluded as ineligible according to pre-defined criteria (Supplementary Table 2); 18 articles describing data for 211 PD and 215 control brains were included in our meta-analysis of post mortem SN reports (Supplementary Table 3).

- Fifty-six articles reporting metal concentrations in CSF, serum, or plasma samples: 27 were excluded as ineligible according to pre-defined criteria (Supplementary Table 4); 29 articles describing data from 2443 PD and 2183 control cases were included in our meta-analysis of biofluid metal levels (Supplementary Tables 5 to 7).

The characteristics of each study (numbers of samples; the mean metal levels, with standard deviations) were extracted for analysis. The primary outcome was difference in metal concentrations (iron and copper) between PD and control tissues. Differences in zinc and manganese levels were also examined as secondary outcomes, as associations between these biometals and PD pathology have been discussed.^{12, 13}

The included articles described samples from tissue donors from a range of ethnic backgrounds and continents (Europe, Asia, Australia, the Americas). No donors contributed tissue to both the brain and biofluid metal datasets. Studies reporting metal concentrations in SN tissue often also reported data for other brain regions (frontal, occipital, temporal cortices; cerebellum; striatum). However, given the small sample sizes for these other regions and the significance of the SN for PD etiology, we restricted our analysis of brain tissue levels to the SN.

The Quality Assessment Scale for Biochemical Analysis of Human Samples

There is no gold standard technique for assessing metal levels in human tissues. Despite our rigorous exclusion criteria, the included studies differed in design; differences in techniques

for measuring minor and trace metals may have affected data quality.¹⁴ The sensitivity of the various analytic options differs markedly; for example, the sensitivity of Mössbauer spectroscopy is relatively low,¹⁵ while synchrotron-microprobe x-ray fluorescence is the most sensitive technique, although it is usually applied to single cell, rather than regional, analyses. As biometals are extremely sensitive to local environmental changes,¹⁶ we also included studies that assessed both fresh frozen and formalin-fixed tissues to compare the effect of tissue preparation.

Protocols for assessing the quality of quantitative data from human tissue analyses have not been published. We therefore developed the Quality Assessment Scale for Biochemical Analysis of Human Samples (see Supplementary Materials for detail and *R* code), based on the Cochrane Collaboration-approved Newcastle–Ottawa scale for assessing the quality of nonrandomised studies in meta-analyses.¹⁷ Our scale scores aspects of study design, sample selection, tissue quality, detection method, and statistical analysis. A maximum 15 points can be allotted: a study with 11–15 points is deemed high quality, 6–10 points moderate quality, and 0–5 points low quality (Table 1). We applied our scale to both SN tissue and biofluid studies. Test-retest reliability was assessed by one author (SG) who applied the scale to both the SN and biofluids datasets at two separate time-points on one-third of studies. Inter-rater reliability was assessed by two authors (SG, KLD) by independently applying the scale to the same studies.

Data analysis

All analyses were performed with the *metafor* package for R 3.4 (R Core Team). The differences between the means of the two groups in each study (in units of pooled SD), expressed as effect size, were assessed with Hedges' d statistic,¹⁸ calculated with the 'escalc' function in *metafor*.¹⁹ Effect sizes were calculated so that a negative value indicated that metal concentration was lower in the PD than the control tissue group. Standard interpretive benchmarks for Hedges' d were applied (0.2 = small, 0.5 = medium, 0.8 = large).

Data for metal concentrations in post mortem SN tissue, CSF, serum, and plasma were analyzed separately. For each dataset, a random-effects meta-analysis (REMA) model was applied using the 'rma' function in *metafor* to estimate the overall magnitude and statistical significance of an effect. The REMA model was preferred to a fixed effects model because the included studies included potential sources of heterogeneity arising from differences in donor cohorts and analytic methods. A REMA model weights individual studies according to both the inverse of within-study variance (square root of the SE for sampling variances), and between-study variance, which also depends on sample size. Variation in effect sizes that could not be attributed to sampling variance, or 'heterogeneity', was also estimated by each REMA model. Heterogeneity statistics quantify the degree to which the results of different studies differ on factors other than sampling (e.g., analytic methods). Each REMA estimates whether effect size heterogeneity was statistically significant with the Q statistic. We also report the proportion of variance in reported effects not attributable to sampling variance

alone (and therefore ascribed to differences in analytic approach) as I^2 ;^{20, 21} values below 25% were deemed low, 25–75% medium, and more than 75% high heterogeneity.

Random effects meta-regression (REMR) and subgroup analyses were conducted on datasets categorized by study quality according to our scale (Supplementary Table 8), patient age, sex, and post-mortem interval (SN tissue only to assess observed data heterogeneity). Subgroup analyses also assessed overall effect sizes as a function of analytic technique, tissue preparation method, and the significance of differences between subgroups in REMA.

Assessment of Publication Bias

Publication bias was investigated when ten or more datasets reported data on the same variable²² by Egger's regression ('regtest' function in *metafor*)²³ and visualized as funnel plots (Supplementary Fig. 1-3). Where Egger's regression detected statistically significant evidence of bias ($P < 0.05$), a trim and fill analysis was applied (*metafor* 'trimfill' function) to estimate the number, magnitude, and precision of potentially missing effect sizes, and to calculate a revised overall effect.^{21, 24} The statistical methodology is described in greater detail in the Supplementary Materials.

RESULTS

Demographic characteristics of tissue donors: age

The mean age of SN tissue donors was 76.1 years (SD, 3.7 years) for donors with PD, 74.8 years (SD, 5.7 years) for control donors; the difference in mean age between the two groups was 1.6 years (SD, 5.0 years). The mean age of CSF donors was 65.0 years (SD, 3.5 years) for donors with PD, 61.0 years (SD, 5.3 years) for control donors; the difference in mean age was 5.9 years (SD, 11.9 years). The mean age of serum donors was 64.3 years (SD, 4.4 years) for donors with PD, 60.0 years (SD, 5.3 years) for control donors; the difference in mean age was 3.7 years (SD, 4.0 years). The mean age of plasma donors was 61.5 years (SD, 5.6 years) for donors with PD, 58.4 years (SD, 13.9 years) for control donors; the difference in mean age between the two groups was 3.1 years (SD, 0.8 years). Further demographic data are reported in Supplementary Tables 4-7.

Quality Assessment Scale for Biochemical Analysis of Human Samples

Test-retest reliability of the scale was high (correlation, 0.99; $P < 0.0001$) with 91% agreement ($n, 11$; $\kappa, 0.889$; $-P < 0.0001$). Inter-rater reliability for two independent raters was also high (correlation, 0.99; $P < 0.0001$), with 82% agreement ($n, 11$; $\kappa, 0.778$; $P < 0.0001$). Seven of 18 human post mortem tissue studies were deemed high quality, nine moderate quality and two limited quality studies. Five of the 12 CSF studies were categorized as high quality studies, seven as moderate quality. Seven of 17 serum studies were deemed high quality, seven moderate quality and one low quality studies. It should be acknowledged that one factor the Quality Assessment Scale cannot account for is the analytical validity of the method used. Blank concentrations, limits of analysis, and potential contamination sources

are not typically reported. This may have the effect of masking changes in studies that did not report changes due to the sensitivity of the method used.

Copper levels in the substantia nigra are reduced in PD

Nine of 18 reports on post mortem SN tissue included quantitative data on copper levels (PD, 127; control, 134 samples); six reported that copper levels were significantly lower in PD than control samples, three studies found no significant difference (Figure 2A). Overall, copper levels were significantly lower in PD samples (d , -2.00 [large effect]; 95% CI, -2.81 to -1.19 ; $P = 0.0051$; Fig. 2A), but study heterogeneity was substantial (I^2 , 83.9%; Q , 59.9; $P < 0.0001$). The effect size was similar in the six high quality studies that measured copper (d , -2.05 ; 95% CI, -2.95 to -1.16 ; $P < 0.0001$) and the three moderate quality studies (d , -1.84 ; 95% CI, -4.04 to 0.36 ; $P = 0.10$). The four studies that employed inductively coupled plasma–mass spectrometry (ICP-MS) (d , -2.42 ; 95% CI, -3.92 to -0.92 ; $P = 0.0016$) and the three atomic absorption spectroscopy studies (AAS; d , -2.25 ; 95% CI, -4.02 to -0.47 ; $P = 0.013$) reported the largest effect sizes; the effect sizes of the three microprobe X-ray fluorescence/emission and one Mössbauer spectroscopy study were not statistically significant (Supplementary Table 10). The effect sizes in the eight studies using fresh tissue were significant (d , -2.06 ; 95% CI, -3.01 to -1.12 ; $P < 0.0001$), in contrast to the two studies using formalin-fixed tissue (d , -1.76 ; 95% CI, -3.72 to 0.21 ; $P = 0.07$). Effect sizes were not influenced by age, sex, or post mortem interval (Supplementary Table 10), consistent with

within-study matching for these variables. No publication bias was identified (Supplementary Figure 1A).

Iron levels are elevated in the in the substantia nigra in PD

All but one of the 18 post mortem SN tissue articles reported quantitative iron levels (PD, 203; control, 221 samples); 13 studies reported that iron levels were significantly higher in PD than control samples, four studies found no significant difference (Figure 2B). Overall, iron levels were significantly higher in PD samples (d , 1.22; 95% CI, 0.36–2.09; $P = 0.0055$, but study heterogeneity was substantial (I^2 , 92.8%; Q , 148; $P < 0.0001$; Fig. 2B). The effect size was slightly larger if two low quality studies (PD, 22; control, 30 samples) were excluded (d , 1.35; 95% CI, 0.37–2.33; $P < 0.007$), and was also greater for studies using unfixed, fresh tissue (14 studies: d , 1.48; 95% CI, 0.50–2.46; $P < 0.003$). Within analytical technique subgroups, no technique demonstrated a significant difference in effect size (Supplementary Table 10). The between-group difference in post mortem interval significantly influenced effect size, with increasing difference in interval associated with larger effects (slope for d , 0.09; 95% CI, 0.04–0.15; $P < 0.0008$), while disease duration, donor age, and sex did not influence effect size (Supplementary Table 10). Evidence of publication bias was statistically significant ($P < 0.0001$; Supplementary Fig 1B), but trim and fill analyses did not alter the overall effect.

Zinc levels in the substantia nigra are unchanged in PD

Three of seven studies that assessed zinc levels in the SN reported that levels were significantly higher in PD than control samples (Figure 2C). Meta-analysis of all seven studies indicated that zinc levels were similar in PD and healthy aged control samples (d , 0.64, 95% CI -0.28 to 1.56, $P = 0.18$; Fig. 2C). Analysis of the four high quality studies found no difference (Fig. 2C), but the effect size for the three moderate quality studies was significant (d , 1.83, 95% CI, 0.34–3.32, $P=0.016$). Age, post mortem interval, sex, and disease duration did not significantly influence the effect (Supplementary Table 9). Publication bias was statistically significant ($P = 0.0072$); Supplementary Fig 1C), but trim and fill analyses estimated no missing studies.

Biometal levels in CSF, serum, and plasma in people with PD

Eight of the ten studies that assessed copper levels in CSF found no difference in levels between samples from donors with or without PD, while one study each found them to be significantly lower or higher in PD donors (Figure 3A). Assessments of CSF iron levels in CSF were also mixed, with four reporting they were lower in PD, two that they were higher, and three that they were similar to those of control donors. Of seven studies of CSF zinc levels three found that they were lower in PD, one that they were higher, and three that they were similar to those of control donors (Figure 3C). Of seven studies assessing CSF manganese levels, one reported that they were higher in PD, six that levels were similar in PD and control samples (Figure 3D). Overall (11 CSF studies; PD, 576; control, 293

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samples) there copper, iron, zinc, and manganese levels in PD were not significantly different to those of control donors (Fig. 3). The only statistically significant differences in effect size by study quality were that iron (d , -0.53 ; 95% CI, -0.79 to -0.26 ; $P < 0.0001$; $I^2=23.0\%$) and zinc levels in four high quality studies (d , -0.52 ; 95% CI, -0.82 to -0.23 ; $P = 0.0004$; $I^2= 0$) were lower in PD than control samples. There was no evidence of publication bias (Supplementary Figure 3).

Of the 15 studies that assessed serum copper, nine found that levels were lower in PD, one that they were higher, and five that they were similar in PD and control samples. Overall, serum copper levels were similar in PD and control samples (14 studies: PD, 1235; control, 1132 samples); study heterogeneity was high (I^2 , 98.8%; Q , 603; $P < 0.0001$) but Figure 4A).

Five serum iron studies reported that they were lower in PD, one that they were higher, and eight that they were similar in PD and control samples (Figure 4B). Overall, serum iron levels were significantly lower in PD samples (14 studies [six high, seven moderate, one low quality]: PD, 1177; control 1447 samples; d , -0.77 ; CI, -1.43 to -0.10 ; $P = 0.024$ Fig. 4B); excluding the low quality study (35 PD, 33 control samples) rendered the effect non-significant (d , -0.63 ; CI, -1.28 to 0.03 ; $P = 0.06$; Fig 4C), as did including only the six high quality studies (PD, 701; control, 761 samples: d , -0.97 ; CI, -2.31 to 0.57 ; $P = 0.24$; Fig. 4C). Meta-regression analyses indicated that serum iron level increased with age (slope, 0.18, 95% CI, 0.06–0.22; $P < 0.005$); but the ages of donors in the PD and control cohorts did not

differ, nor between these cohorts when high, moderate and low quality studies were individually tested.

Four of 12 studies that assessed serum zinc found that levels were lower in PD, and eight that they were similar in PD and control samples (Figure 4D). Of the seven studies that assessed serum manganese, one found that levels were lower in PD and one that they were reduced (Figure 4E). Overall, serum zinc and manganese levels were similar in PD and control samples (Fig. 4D,E). There was no evidence of publication bias for serum metal studies (Supplementary Figure 3).

Meta-analysis of plasma metal data was limited by the low number of studies that met our selection criteria (five) and data heterogeneity for all three metals assessed (copper: d , 0.0, 95% CI -1.82 to 1.81, $P = 0.996$, $I^2 = 98.6\%$; iron: d , 0.35, 95% CI, -1.07 to 1.77, $P = 0.63$, $I^2 = 96.4\%$; zinc, d , -0.40, 95% CI -0.78 to -0.01, $P = 0.043$, $I^2 = 0\%$). One copper and one zinc study were deemed low quality, but their omission did not change our findings. (Supplementary Table 9). There was no evidence of publication bias for plasma studies.

No association between copper and iron levels in the substantia nigra and in biofluids

Meta-regression indicated that the lower SN copper levels in PD samples were not correlated with lower biofluid copper levels in age-matched individuals. Similarly, higher SN iron

levels in PD samples were not mirrored by higher biofluid iron levels, before or after adjusting for age (Supplementary Figure 4).

DISCUSSION

In the first meta-analysis of SN copper and zinc levels in people with PD, and in accordance with that a previous meta-analysis of iron³², we found evidence that copper levels are consistently lower than in healthy controls, and confirmed that iron levels are significantly higher. The magnitudes of these differences were similar, but in opposite directions. The studies we analyzed included donors of different ethnic backgrounds from multiple continents, suggesting our findings can be generalized to people with PD worldwide.

Although studies of copper and iron levels in PD consistently report changes restricted to degenerating brain regions, particularly the SN,^{25, 26} the available data did not permit meaningful analysis of metal level changes in other brain regions. The measured differences in SN iron and copper levels in PD may reflect redistribution of metals within the SN or between the SN and other tissues.²⁷ Intranigral redistribution would be consistent with the significantly higher levels of intraneuronal iron and lower levels of intraneuronal copper in in PD we have described.⁷

Despite the high degree of study heterogeneity, the magnitudes of the overall changes our meta-analysis identified are consistent with those we and others have found in studies of

within-dopaminergic neuron metal concentrations in PD.^{26, 28} We have found these changes in cases of probable pre-clinical disease, which suggests that iron and copper levels change at the cellular level early in the disease process.²⁹ These findings, however, do not provide unequivocal evidence for their etiological significance. Nevertheless, like the discovery of markedly reduced dopamine levels that motivated the first trials of dopamine replacement therapy in the early 1960s, our findings provide strong empirical evidence of specific changes in the primary region of neurodegeneration in PD, supporting the rationale of current clinical trials investigating these changes as targets for disease modification in PD.^{9,10}

Our data support the hypothesis that increased SN iron levels are at least a biomarker of the disease process, including in pre-clinical PD. Elevated SN iron levels detected by functional ultrasound imaging detection have been proposed a risk marker for pre-clinical PD by the International Parkinson and Movement Disorders Society Task Force,³⁰ and quantifying SN iron by MRI imaging is being investigated both as a biomarker of PD and of disease progress.³¹ We did not include functional imaging of brain iron in PD in our analysis, as this was recently the subject of meta-analysis.³² Copper and zinc cannot currently be assessed in vivo with functional imaging.

We also examined whether the effect sizes for changes in copper and iron levels in the post mortem SN were matched by changes in intra vitam CSF, serum, and plasma levels in people with PD. Given the close physical contacts between the brain, CSF, and capillary network, changes in biometal levels in the more accessible biofluids could potentially be useful

biomarkers of central disease processes.³³ Our analysis indicated that biofluid copper levels are within normal limits in PD, but serum iron levels are lower than in age-matched healthy control people. One major limitation of our analysis, however, was that brain and biofluid tissue samples were not sourced from the same donors; a study that compared both sample types within individuals would be invaluable, but investigations of this type have not been reported. Biofluid metal levels are derived from various sources however, and therefore circulating metal levels may therefore not be useful for estimating brain metal levels.

We initially weighted individual studies according to donor group size and variance, but other design aspects, such as analytic technique and tissue preparation, significantly influenced effect size, suggesting such factors should be carefully considered during experimental design. In contrast, other variables reported to influence biometal levels, including sex³⁴ and post-mortem interval³⁵ did not have significant effects. No robust evidence that medications influence biometal levels in the human brain or biofluids has been reported. Monoamine oxidase inhibitors prescribed for patients with PD may have iron-chelating properties³⁶ that may be relevant to reports of reduced serum iron levels in PD.³⁷ Little is known, however, about the effects on brain biometal levels of other dopamine replacement therapies or of other medications frequently taken by older people.

Conclusion

Copper levels are significantly lower and iron levels significantly higher in post mortem SN tissue from PD patients than from healthy controls. Serum iron levels, however, are lower in PD than in samples from healthy age-matched controls. Our findings provide evidence for marked changes in iron and copper levels in the major site of neurodegeneration in PD. These findings warrant further investigation to define the significance of SN biometal levels for the pathophysiology of PD, and to determine whether therapies targeting these metals would be both feasible and clinically useful.

Author contributions

S.G., D.J.H. and K.L.D. designed the study. S.G. performed the literature search, did the statistical analysis with the help of A.M.S., and wrote the first version of the report with the help of D.J.H. and K.L.D.

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no other financial interests to declare.

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Table 1: Scoring rubric for the Quality Assessment Scale for Biochemical Analysis of Human Samples.

SELECTION		
1. <i>Is the case definition adequate?</i>		
a.	Yes, both clinical and pathologically defined	2
b.	Either clinical or pathologically defined	1
c.	No description	0
2. <i>Representation of population?</i>		
a.	Cases from multiple tissue banks or live cohorts	2
b.	Tissue bank or live cohort stated	1
c.	Potential for selection biases or not stated	0
3. <i>Selection of controls</i>		
a.	Controls selected from same tissue banks or live population	1
b.	Controls from different source or no description	0
4. <i>Definition of controls</i>		
a.	No history of disease or confounding condition	1
b.	Diagnosed conditions provided but effect on variable of interest unknown	0
c.	No description	0
COMPARABILITY		
1. <i>Comparability of cases and controls on the basis of the design or analysis</i>		
a.	Study controls for the most significant potential moderating variables x and y (where x is the most important factor) <i>e.g.</i> age, sex, genetic background; 2 points	2
a.	Study controls for only one (most important) factor	1
b.	Not controlled or not stated	0
2. <i>Quality of tissue</i>		
a.	Post-mortem delay is <24hrs for post-mortem tissues; or biofluids were stored at -20C <1hr after collection (1 point).	1
b.	>24hrs for post-mortem or >1hr for biofluids or not defined.	0
3. <i>Method detail</i>		
a.	An appropriate method applied and described	2
b.	Methods and/or description limited	1
c.	Inappropriate method and/or missing crucial information.	0
ANALYSIS		
1. <i>Ascertainment of exposure</i>		
a.	Blinded to case/control status	1
b.	Unblinded.	0
c.	No description	0
2. <i>Appropriate and detailed statistical analysis</i>		
a.	Detailed and appropriate analysis	2
b.	Statistical test stated	1
c.	No description.	0
3. <i>Elimination of outliers</i>		
a.	All participants included	1
b.	Similar proportion excluded for both groups	1
c.	Different exclusion or not stated	0
Maximum score: SELECTION + COMPARABILITY + ANALYSIS =		15

FIGURE LEGENDS

Figure 1: PRISMA flowchart of literature search, screening, and eligibility assessment. CSF = cerebrospinal fluid; PD = Parkinson's disease; SN = substantia nigra.

Figure 2: Forest plots of analysis of (A) copper, (B) iron, and (C) zinc in human post-mortem SN tissue. Square marker indicates effect size and statistical weight of study; horizontal lines indicate 95% CIs. The diamond data markers represent the subtotal and overall effect size and 95% CI. The vertical dashed line shows the summary effect estimate; the dotted line shows the line of no effect ($d = 0$). Asterisk (*) represents studies identifying a reduction and ^ indicates studies reporting a significant increase. df: degrees of freedom. Q : Cochran's Q .

Figure 3: Forest plots of analysis of (A) copper, (B) iron, (C) zinc, and (D) manganese in human CSF.

Figure 4: Forest plots of analysis of (A) copper, (B) iron, (C) zinc, and (D) manganese in human serum.

```
# Script to do all analyses in Genoud et al.
# Written by Sian Genoud and Alistair Senior at the University of Sydney, 2018-2019
# Chunks of Code are broadly split up by Figure and associated analyses, each chunk is
independent of the others and can be run on its own

# Files required in the working directory are
# 1) Cu SN quality order.csv - Copper levels in PD and Ct substantia nigra brain tissue -
subgroup of study quality
# 2) Fe SN quality order.csv - Iron levels in PD and Ct substantia nigra brain tissue - subgroup
of study quality
# 3) Zn SN quality order.csv - Zinc levels in PD and Ct substantia nigra brain tissue - subgroup
of study quality
# 4) Serum Cu quality order.csv - Copper levels in PD and Ct serum - subgroup of study
quality
# 5) Serum Fe quality order.csv - Iron levels in PD and Ct serum - subgroup of study quality
# 6) Serum Zn quality order.csv - Zinc levels in PD and Ct serum - subgroup of study quality
# 7) Serum Mn quality order.csv - Manganese levels in PD and Ct serum - subgroup of study
quality
# 8) CSF Cu quality data.csv - Copper levels in PD and Ct CSF - subgroup of study quality
# 9) CSF Fe quality order.csv - Iron levels in PD and Ct CSF - subgroup of study quality
# 10) CSF Zn quality order.csv - Zinc levels in PD and Ct CSF - subgroup of study quality
# 11) Plasma Cu quality order.csv - Copper levels in PD and Ct plasma - subgroup of study
quality
# 12) Plasma Fe quality order.csv - Iron levels in PD and Ct plasma - subgroup of study
quality
# 13) Plasma Zn quality order.csv - Zinc levels in PD and Ct plasma - subgroup of study
quality
# 14) Combined Cu data 18.2.19.csv - Copper levels in PD and Ct of all tissues - subgroup of
tissue type
# 15) Fe combined data 16.2.19.csv - Iron levels in PD and Ct of all tissues - subgroup of
tissue type

#####
##### Meta-analyses For SN CU data #####
#####

# Deletes any potentially old info hanging around
rm(list=ls())

# Set the working directory
setwd("/Users/sianguenoud/Desktop/Forests")

# Install if needs be on the first time - intall.packages("metafor") and intall.packages("plyr")

# Load packages we need
library("metafor")
library("plyr")
```

```
# Load dataset
data<-read.csv("Cu SN quality order.csv")

# Check the data looks good
head(data)

# Calculate the effect size ("SMD" - Hedges d) using escalc function
#slab function labels studies
data.ES<-escalc(measure = "SMD", n1i = PD.cases, n2i = Ct.cases, m1i = PD.Cu, m2i = Ct.Cu,
sd1i = PD.Cu.SD, sd2i = Ct.Cu.SD, data = data, slab=paste(Author, Year, sep=" "))

# Fit the meta analysis for all data
MA1<-rma(yi = yi, vi = vi, data = data.ES)
summary(MA1)

# Fit subsets for high and moderate quality studies
res.h <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="High"), method="REML")
res.m <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Moderate"), method="REML")
summary(res.h)
summary(res.m)

#Publication bias test
regtest(MA1)
#Check for missing studies
trimfill(MA1)
#Funnel plot
funnel(MA1)

##### Meta-regressions #####

####Meta-regression analysis for effect of technique
# This first line tells you the order - by default R will put the first one of these categories as
the intercept group
sort(unique(data.ES$Technique))

# Here you change the intercept, by changing base=x at the end - here I changed it to the
second in the list above
contrasts(data.ES$Technique)<-contr.treatment(levels(data.ES$Technique), base=2)
# Overall effect of analytical technique

MRTechnique<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Technique-1)
summary(MRTechnique)

# Overall effect of tissue prep
MRPrep<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Prep-1)
summary(MRPrep)
```

```
# Mean centered age
data.ESS$Age.PD<-data.ESS$Age.PD - mean(data.ESS$Age.PD, na.rm=T)
MRPDage<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.PD)
summary(MRPDage)

data.ESS$Age.Ct<-data.ESS$Age.Ct - mean(data.ESS$Age.Ct, na.rm=T)
MRCTage<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.Ct)
summary(MRCTage)

# Difference in age
MRDiffAge<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffAge)
summary(MRDiffAge)

# Difference in PMI
MRDiffPMI<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffPMI)
summary(MRDiffPMI)

# Difference in SR
MRDiffPercMale<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffPercMale)
summary(MRDiffPercMale)

# Mean centered disease duration
data.ESS$Duration<-data.ESS$Duration - mean(data.ESS$Duration, na.rm=T)
MRDuration<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Duration)
summary(MRDuration)

#####
##### Meta-analyses For SN Fe data #####
#####

# Deletes any potentially old info hanging around
rm(list=ls())

# Set the working directory
setwd("/Users/siangenoud/Desktop/Forests")

# Install if needs be on the first time - intall.packages("metafor") and intall.packages("plyr")

# Load packages we need
library("metafor")
library("plyr")

# Load dataset
data<-read.csv("Fe SN quality order.csv")

# Check the data looks good
```

```

head(data)

# Calculate the effect size ("SMD" - Hedges d) using escalc function
#slab function labels studies
data.ES<-escalc(measure = "SMD", n1i = PD.cases, n2i = Ct.cases, m1i = PD.Fe, m2i = Ct.Fe,
sd1i = PD.Fe.SD, sd2i = Ct.Fe.SD, data = data, slab=paste(Author, Year, sep=", "))

# Fit the meta analysis for all data
MA1<-rma(yi = yi, vi = vi, data = data.ES)
summary(MA1)

# Fit subsets for high and moderate quality studies
res.nL <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="High" | Quality == "Moderate"),
method="REML")
summary(res.nL)
res.h <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="High"), method="REML")
summary(res.h)
res.m <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Moderate"), method="REML")
summary(res.m)
res.l <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Limited"), method="REML")
summary(res.l)

#Publication bias test
regtest(MA1)
#Check for missing studies
trimfill(MA1)
#Funnel plot
funnel(MA1)

##### Meta-regressions #####

####Meta-regression analysis for effect of technique
# This first line tells you the order - by default R will put the first one of these categories as
the intercept group
sort(unique(data.ES$Technique))

# Here you change the intercept, by changing base=x at the end - here I changed it to the
second in the list above

# Overall effect of analytical technique
contrasts(data.ES$Technique)<-contr.treatment(levels(data.ES$Technique), base=2)
MRTechnique<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Technique-1)
summary(MRTechnique)

# Overall effect of tissue prep
MRPrep<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Prep-1)

```

```
summary(MRPrep)

# Mean centered age
data.ES$Age.PD<-data.ES$Age.PD - mean(data.ES$Age.PD, na.rm=T)
MRPDage<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.PD)
summary(MRPDage)

data.ES$Age.Ct<-data.ES$Age.Ct - mean(data.ES$Age.Ct, na.rm=T)
MRCTage<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.Ct)
summary(MRCTage)

# Difference in age
MRDiffAge<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffAge)
summary(MRDiffAge)

# Difference in PMI
MRDiffPMI<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffPMI)
summary(MRDiffPMI)

# Difference in SR
MRDiffPercMale<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffPercMale)
summary(MRDiffPercMale)

# Mean centered disease duration
data.ES$Duration<-data.ES$Duration - mean(data.ES$Duration, na.rm=T)
MRDuration<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Duration)
summary(MRDuration)

#####
##### Meta-analyses For SN Zn data #####
#####

# Deletes any potentially old info hanging around
rm(list=ls())

# Set the working directory
setwd("/Users/siangenoud/Desktop/Forests")

# Install if needs be on the first time - intall.packages("metafor") and intall.packages("plyr")

# Load packages we need
library("metafor")
library("plyr")

# Load dataset
data<-read.csv("Zn SN quality order.csv")
```

```

# Check the data looks good
head(data)

# Calculate the effect size ("SMD" - Hedges d) using escalc function
#slab function labels studies
data.ES<-escalc(measure = "SMD", n1i = PD.cases, n2i = Ct.cases, m1i = PD.Zn, m2i = Ct.Zn,
sd1i = PD.Zn.SD, sd2i = Ct.Zn.SD, data = data, slab=paste(Author, Year, sep=" , "))

# Fit the meta analysis for all data
MA1<-rma(yi = yi, vi = vi, data = data.ES)
summary(MA1)

# Fit subsets for high and moderate quality studies
res.nL <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="High" | Quality == "Moderate"),
method="REML")
summary(res.nL)
res.h <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="High"), method="REML")
summary(res.h)
res.m <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Moderate"), method="REML")
summary(res.m)

#Publication bias test
regtest(MA1)
#Check for missing studies
trimfill(MA1)
#Funnel plot
funnel(MA1)

##### Meta-regressions #####

####Meta-regression analysis for effect of technique
# This first line tells you the order - by default R will put the first one of these categories as
the intercept group
sort(unique(data.ES$Technique))

# Here you change the intercept, by changing base=x at the end - here I changed it to the
second in the list above

# Overall effect of analytical technique
contrasts(data.ES$Technique)<-contr.treatment(levels(data.ES$Technique), base=2)
MRTechnique<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Technique-1)
summary(MRTechnique)

# Overall effect of tissue prep
MRPrep<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Prep-1)
summary(MRPrep)

```

```
# Mean centered age
data.ES$Age.PD<-data.ES$Age.PD - mean(data.ES$Age.PD, na.rm=T)
MRPDage<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.PD)
summary(MRPDage)

data.ES$Age.Ct<-data.ES$Age.Ct - mean(data.ES$Age.Ct, na.rm=T)
MRCTage<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.Ct)
summary(MRCTage)

# Difference in age
MRDiffAge<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffAge)
summary(MRDiffAge)

# Difference in PMI
MRDiffPMI<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffPMI)
summary(MRDiffPMI)

# Difference in SR
MRDiffPercMale<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffPercMale)
summary(MRDiffPercMale)

# Mean centered disease duration
data.ES$Duration<-data.ES$Duration - mean(data.ES$Duration, na.rm=T)
MRDuration<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Duration)
summary(MRDuration)

#####
##### Meta-analyses For CSF Cu data #####
#####

# Deletes any potentially old info hanging around
rm(list=ls())

# Set the working directory
setwd("/Users/siangenoud/Desktop/Forests")

# Install if needs be on the first time - intall.packages("metafor") and intall.packages("plyr")

# Load packages we need
library("metafor")
library("plyr")

# Load dataset
data<-read.csv("CSF Cu quality data.csv")

# Check the data looks good
```

```
head(data)

# Calculate the effect size ("SMD" - Hedges d) using escalc function
#slab function labels studies
data.ES<-escalc(measure = "SMD", n1i = PD.cases, n2i = Ct.cases, m1i = PD.Cu, m2i = Ct.Cu,
sd1i = PD.Cu.SD, sd2i = Ct.Cu.SD, data = data, slab=paste(Author, Year, sep=", "))

# Fit the meta analysis for all data
MA1<-rma(yi = yi, vi = vi, data = data.ES)
summary(MA1)

# Fit subsets by quality
res.h <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="High"), method="REML")
summary(res.h)
res.m <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Moderate"), method="REML")
summary(res.m)

#Publication bias test
regtest(MA1)
#Check for missing studies
trimfill(MA1)
#Funnel plot
funnel(MA1)

##### Meta-regressions #####

####Meta-regression analysis for effect of technique
# This first line tells you the order - by default R will put the first one of these categories as
the intercept group
sort(unique(data.ES$Technique))

# Here you change the intercept, by changing base=x at the end - here I changed it to the
second in the list above

# Overall effect of analytical technique
contrasts(data.ES$Technique)<-contr.treatment(levels(data.ES$Technique), base=2)
MRTechnique<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Technique-1)
summary(MRTechnique)

# Mean centered age
data.ES$Age.PD<-data.ES$Age.PD - mean(data.ES$Age.PD, na.rm=T)
MRPDage<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.PD)
summary(MRPDage)

data.ES$Age.Ct<-data.ES$Age.Ct - mean(data.ES$Age.Ct, na.rm=T)
```

```
MRCTAge<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.Ct)
summary(MRCTAge)

# Difference in age
MRDiffAge<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffAge)
summary(MRDiffAge)

# Difference in SR
MRDiffPercMale<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffPercMale)
summary(MRDiffPercMale)

# Mean centered disease duration
data.ES$Duration<-data.ES$Duration - mean(data.ES$Duration, na.rm=T)
MRDuration<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Duration)
summary(MRDuration)

#####
##### Meta-analyses For CSF Fe data #####
#####

# Deletes any potentially old info hanging around
rm(list=ls())

# Set the working directory
setwd("/Users/siangenoud/Desktop/Forests")

# Install if needs be on the first time - intall.packages("metafor") and intall.packages("plyr")

# Load packages we need
library("metafor")
library("plyr")

# Load dataset
data<-read.csv("CSF Fe quality order.csv")

# Check the data looks good
head(data)

# Calculate the effect size ("SMD" - Hedges d) using escalc function
#slab function labels studies
data.ES<-escalc(measure = "SMD", n1i = PD.cases, n2i = Ct.cases, m1i = PD.Fe, m2i = Ct.Fe,
sd1i = PD.Fe.SD, sd2i = Ct.Fe.SD, data = data, slab=paste(Author, Year, sep=", "))

# Fit the meta analysis for all data
MA1<-rma(yi = yi, vi = vi, data = data.ES)
summary(MA1)
```

```
# Fit subsets by quality
res.h <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="High"), method="REML")
summary(res.h)
res.m <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Moderate"), method="REML")
summary(res.m)

# No publication bias as  $k < 10$ 

##### Meta-regressions #####

####Meta-regression analysis for effect of technique
# This first line tells you the order - by default R will put the first one of these categories as
the intercept group
sort(unique(data.ES$Technique))

# Here you change the intercept, by changing base=x at the end - here I changed it to the
second in the list above

# Overall effect of analytical technique
contrasts(data.ES$Technique)<-contr.treatment(levels(data.ES$Technique), base=2)
MRTechnique<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Technique-1)
summary(MRTechnique)

# Mean centered age
data.ES$Age.PD<-data.ES$Age.PD - mean(data.ES$Age.PD, na.rm=T)
MRPDage<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.PD)
summary(MRPDage)

data.ES$Age.Ct<-data.ES$Age.Ct - mean(data.ES$Age.Ct, na.rm=T)
MRCTage<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.Ct)
summary(MRCTage)

# Difference in age
MRDiffAge<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffAge)
summary(MRDiffAge)

# Difference in SR
MRDiffPercMale<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffPercMale)
summary(MRDiffPercMale)

# Mean centered disease duration
data.ES$Duration<-data.ES$Duration - mean(data.ES$Duration, na.rm=T)
MRDuration<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Duration)
summary(MRDuration)
```

```
#####
##### Meta-analyses For CSF Zn data #####
#####

# Deletes any potentially old info hanging around
rm(list=ls())

# Set the working directory
setwd("/Users/siangenoud/Desktop/Forests")

# Install if needs be on the first time - intall.packages("metafor") and intall.packages("plyr")

# Load packages we need
library("metafor")
library("plyr")

# Load dataset
data<-read.csv("CSF Zn quality order.csv")

# Check the data looks good
head(data)

# Calculate the effect size ("SMD" - Hedges d) using escalc function
#slab function labels studies
data.ES<-escalc(measure = "SMD", n1i = PD.cases, n2i = Ct.cases, m1i = PD.Zn, m2i = Ct.Zn,
sd1i = PD.Zn.SD, sd2i = Ct.Zn.SD, data = data, slab=paste(Author, Year, sep=", "))

# Fit the meta analysis for all data
MA1<-rma(yi = yi, vi = vi, data = data.ES)
summary(MA1)

# Fit subsets by quality
res.h <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="High"), method="REML")
summary(res.h)
res.m <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Moderate"), method="REML")
summary(res.m)

# No publicaiton bias as k < 10

##### Meta-regressions #####

####Meta-regression analysis for effect of technique
# This first line tells you the order - by default R will put the first one of these categories as
the intercept group
sort(unique(data.ES$Technique))
```

```
# Here you change the intercept, by changing base=x at the end - here I changed it to the
second in the list above
```

```
# Overall effect of analytical technique
contrasts(data.ES$Technique)<-contr.treatment(levels(data.ES$Technique), base=2)
MRTechnique<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Technique-1)
summary(MRTechnique)
```

```
# Mean centered age
data.ES$Age.PD<-data.ES$Age.PD - mean(data.ES$Age.PD, na.rm=T)
MRPDage<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.PD)
summary(MRPDage)
```

```
data.ES$Age.Ct<-data.ES$Age.Ct - mean(data.ES$Age.Ct, na.rm=T)
MRCTage<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.Ct)
summary(MRCTage)
```

```
# Difference in age
MRDiffAge<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffAge)
summary(MRDiffAge)
```

```
# Difference in SR
MRDiffPercMale<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffPercMale)
summary(MRDiffPercMale)
```

```
# Mean centered disease duration
data.ES$Duration<-data.ES$Duration - mean(data.ES$Duration, na.rm=T)
MRDuration<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Duration)
summary(MRDuration)
```

```
#####
##### Meta-analyses For CSF Mn data #####
#####
```

```
# Deletes any potentially old info hanging around
rm(list=ls())
```

```
# Set the working directory
setwd("/Users/siangenoud/Desktop/Forests")
```

```
# Install if needs be on the first time - intall.packages("metafor") and intall.packages("plyr")
```

```
# Load packages we need
library("metafor")
library("plyr")
```

```

# Load dataset
data<-read.csv("CSF Mn quality order.csv")

# Check the data looks good
head(data)

# Calculate the effect size ("SMD" - Hedges d) using escalc function
#slab function labels studies
data.ES<-escalc(measure = "SMD", n1i = PD.cases, n2i = Ct.cases, m1i = PD.Mn, m2i = Ct.Mn,
sd1i = PD.Mn.SD, sd2i = Ct.Mn.SD, data = data, slab=paste(Author, Year, sep=", "))

# Fit the meta analysis for all data
MA1<-rma(yi = yi, vi = vi, data = data.ES)
summary(MA1)

# Fit subsets by quality
res.h <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="High"), method="REML")
summary(res.h)
res.m <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Moderate"), method="REML")
summary(res.m)

# No publicaition bias as k < 10

##### Meta-regressions #####

####Meta-regression analysis for effect of technique
# This first line tells you the order - by default R will put the first one of these categories as
the intercept group
sort(unique(data.ES$Technique))

# Here you change the intercept, by changing base=x at the end - here I changed it to the
second in the list above

# Overall effect of analytical technique
contrasts(data.ES$Technique)<-contr.treatment(levels(data.ES$Technique), base=2)
MRTechnique<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Technique-1)
summary(MRTechnique)

# Mean centered age
data.ES$Age.PD<-data.ES$Age.PD - mean(data.ES$Age.PD, na.rm=T)
MRPDAGE<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.PD)
summary(MRPDAGE)

data.ES$Age.Ct<-data.ES$Age.Ct - mean(data.ES$Age.Ct, na.rm=T)
MRCtAge<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.Ct)
summary(MRCtAge)

```

```
# Difference in age
MRDiffAge<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffAge)
summary(MRDiffAge)

# Difference in SR
MRDiffPercMale<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffPercMale)
summary(MRDiffPercMale)

# Mean centered disease duration
data.ES$Duration<-data.ES$Duration - mean(data.ES$Duration, na.rm=T)
MRDuration<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Duration)
summary(MRDuration)

#####
##### Meta-analyses For Serum Cu data #####
#####

# Deletes any potentially old info hanging around
rm(list=ls())

# Set the working directory
setwd("/Users/siangenoud/Desktop/Forests")

# Install if needs be on the first time - intall.packages("metafor") and intall.packages("plyr")

# Load packages we need
library("metafor")
library("plyr")

# Load dataset
data<-read.csv("Serum Cu quality order.csv")

# Check the data looks good
head(data)

# Calculate the effect size ("SMD" - Hedges d) using escalc function
#slab function labels studies
data.ES<-escalc(measure = "SMD", n1i = PD.cases, n2i = Ct.cases, m1i = PD.Cu, m2i = Ct.Cu,
sd1i = PD.Cu.SD, sd2i = Ct.Cu.SD, data = data, slab=paste(Author, Year, sep=" "))

# Fit the meta analysis for all data
MA1<-rma(yi = yi, vi = vi, data = data.ES)
summary(MA1)

# Fit subsets by quality
res.h <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="High"), method="REML")
```

```
summary(res.h)
res.m <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Moderate"), method="REML")
summary(res.m)
res.l <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Limited"), method="REML")
summary(res.l)
```

```
#Publication bias test
regtest(MA1)
#Check for missing studies
trimfill(MA1)
#Funnel plot
funnel(MA1)
```

```
##### Meta-regressions #####
```

```
####Meta-regression analysis for effect of technique
# This first line tells you the order - by default R will put the first one of these categories as
the intercept group
sort(unique(data.ES$Technique))
```

```
# Here you change the intercept, by changing base=x at the end - here I changed it to the
second in the list above
```

```
# Overall effect of analytical technique
contrasts(data.ES$Technique)<-contr.treatment(levels(data.ES$Technique), base=2)
MRTechnique<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Technique-1)
summary(MRTechnique)
```

```
# Mean centered age
data.ES$Age.PD<-data.ES$Age.PD - mean(data.ES$Age.PD, na.rm=T)
MRPDAGE<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.PD)
summary(MRPDAGE)
```

```
data.ES$Age.Ct<-data.ES$Age.Ct - mean(data.ES$Age.Ct, na.rm=T)
MRCTAGE<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.Ct)
summary(MRCTAGE)
```

```
# Difference in age
MRDiffAGE<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffAGE)
summary(MRDiffAGE)
```

```
# Difference in PMI
MRDiffPMI<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffPMI)
summary(MRDiffPMI)
```

```
# Difference in SR
MRDiffPercMale<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffPercMale)
```

```
summary(MRDiffPercMale)

# Mean centered disease duration
data.ES$Duration<-data.ES$Duration - mean(data.ES$Duration, na.rm=T)
MRDuration<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Duration)
summary(MRDuration)

#####
##### Meta-analyses For serum Fe data #####
#####

# Deletes any potentially old info hanging around
rm(list=ls())

# Set the working directory
setwd("/Users/siangenoud/Desktop/Forests")

# Install if needs be on the first time - intall.packages("metafor") and intall.packages("plyr")

# Load packages we need
library("metafor")
library("plyr")

# Load dataset
data<-read.csv("Serum Fe quality order.csv")

# Check the data looks good
head(data)

# Calculate the effect size ("SMD" - Hedges d) using escalc function
#slab function labels studies
data.ES<-escalc(measure = "SMD", n1i = PD.cases, n2i = Ct.cases, m1i = PD.Fe, m2i = Ct.Fe,
sd1i = PD.Fe.SD, sd2i = Ct.Fe.SD, data = data, slab=paste(Author, Year, sep=", "))

# Fit the meta analysis for all data
MA1<-rma(yi = yi, vi = vi, data = data.ES)
summary(MA1)

# Fit subsets by quality
res.nL <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="High" | Quality == "Moderate"),
method="REML")
summary(res.nL)
res.h <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="High"), method="REML")
summary(res.h)
res.m <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Moderate"), method="REML")
summary(res.m)
res.l <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Limited"), method="REML")
```

```
summary(res.l)

#Publication bias test
regtest(MA1)
#Check for missing studies
trimfill(MA1)
#Funnel plot
funnel(MA1)

##### Meta-regressions #####

####Meta-regression analysis for effect of technique
# This first line tells you the order - by default R will put the first one of these categories as
the intercept group
sort(unique(data.ES$Technique))

# Here you change the intercept, by changing base=x at the end - here I changed it to the
second in the list above

# Overall effect of analytical technique
contrasts(data.ES$Technique)<-contr.treatment(levels(data.ES$Technique), base=2)
MRTechnique<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Technique-1)
summary(MRTechnique)

# Mean centered age
data.ES$Age.PD<-data.ES$Age.PD - mean(data.ES$Age.PD, na.rm=T)
MRPDage<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.PD)
summary(MRPDage)

data.ES$Age.Ct<-data.ES$Age.Ct - mean(data.ES$Age.Ct, na.rm=T)
MRCTage<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.Ct)
summary(MRCTage)

# Difference in age
MRDiffAge<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffAge)
summary(MRDiffAge)

# Difference in SR
MRDiffPercMale<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffPercMale)
summary(MRDiffPercMale)

# Mean centered disease duration
data.ES$Duration<-data.ES$Duration - mean(data.ES$Duration, na.rm=T)
MRDuration<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Duration)
summary(MRDuration)

#####
```

```
##### Meta-analyses For serum Zn data #####
#####

# Deletes any potentially old info hanging around
rm(list=ls())

# Set the working directory
setwd("/Users/siangenoud/Desktop/Forests")

# Install if needs be on the first time - intall.packages("metafor") and intall.packages("plyr")

# Load packages we need
library("metafor")
library("plyr")

# Load dataset
data<-read.csv("Serum Zn quality order.csv")

# Check the data looks good
head(data)

# Calculate the effect size ("SMD" - Hedges d) using escalc function
#slab function labels studies
data.ES<-escalc(measure = "SMD", n1i = PD.cases, n2i = Ct.cases, m1i = PD.Zn, m2i = Ct.Zn,
sd1i = PD.Zn.SD, sd2i = Ct.Zn.SD, data = data, slab=paste(Author, Year, sep=" ", ))

# Fit the meta analysis for all data
MA1<-rma(yi = yi, vi = vi, data = data.ES)
summary(MA1)

# Fit subsets by quality
res.nL <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="High" | Quality == "Moderate"),
method="REML")
summary(res.nL)
res.h <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="High"), method="REML")
summary(res.h)
res.m <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Moderate"), method="REML")
summary(res.m)

#Publication bias test
regtest(MA1)
#Check for missing studies
trimfill(MA1)
#Funnel plot
funnel(MA1)

##### Meta-regressions #####
```

```

#####Meta-regression analysis for effect of technique
# This first line tells you the order - by default R will put the first one of these categories as
the intercept group
sort(unique(data.ES$Technique))

# Here you change the intercept, by changing base=x at the end - here I changed it to the
second in the list above

# Overall effect of analytical technique
contrasts(data.ES$Technique)<-contr.treatment(levels(data.ES$Technique), base=2)
MRTechnique<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Technique-1)
summary(MRTechnique)

# Mean centered age
data.ES$Age.PD<-data.ES$Age.PD - mean(data.ES$Age.PD, na.rm=T)
MRPDage<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.PD)
summary(MRPDage)

data.ES$Age.Ct<-data.ES$Age.Ct - mean(data.ES$Age.Ct, na.rm=T)
MRCTage<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.Ct)
summary(MRCTage)

# Difference in age
MRDiffAge<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffAge)
summary(MRDiffAge)

# Difference in SR
MRDiffPercMale<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffPercMale)
summary(MRDiffPercMale)

# Mean centered disease duration
data.ES$Duration<-data.ES$Duration - mean(data.ES$Duration, na.rm=T)
MRDuration<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Duration)
summary(MRDuration)

#####
##### Meta-analyses For serum Mn data #####
#####

# Deletes any potentially old info hanging around
rm(list=ls())

# Set the working directory
setwd("/Users/siangenoud/Desktop/Forests")

# Install if needs be on the first time - intall.packages("metafor") and intall.packages("plyr")

```

```

# Load packages we need
library("metafor")
library("plyr")

# Load dataset
data<-read.csv("Serum Mn quality order.csv")

# Check the data looks good
head(data)

# Calculate the effect size ("SMD" - Hedges d) using escalc function
#slab function labels studies
data.ES<-escalc(measure = "SMD", n1i = PD.cases, n2i = Ct.cases, m1i = PD.Mn, m2i = Ct.Mn,
sd1i = PD.Mn.SD, sd2i = Ct.Mn.SD, data = data, slab=paste(Author, Year, sep=", "))

# Fit the meta analysis for all data
MA1<-rma(yi = yi, vi = vi, data = data.ES)
summary(MA1)

# Fit subsets by quality
res.nL <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="High" | Quality == "Moderate"),
method="REML")
summary(res.nL)
res.h <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="High"), method="REML")
summary(res.h)
res.m <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Moderate"), method="REML")
summary(res.m)

#Publication bias test
regtest(MA1)
#Check for missing studies
trimfill(MA1)
#Funnel plot
funnel(MA1)

##### Meta-regressions #####

####Meta-regression analysis for effect of technique
# This first line tells you the order - by default R will put the first one of these categories as
the intercept group
sort(unique(data.ES$Technique))

# Here you change the intercept, by changing base=x at the end - here I changed it to the
second in the list above

# Overall effect of analytical technique

```

```
contrasts(data.ESS$Technique)<-contr.treatment(levels(data.ESS$Technique), base=2)
MRTechnique<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Technique-1)
summary(MRTechnique)

# Mean centered age
data.ESS$Age.PD<-data.ESS$Age.PD - mean(data.ESS$Age.PD, na.rm=T)
MRPDAGE<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.PD)
summary(MRPDAGE)

data.ESS$Age.Ct<-data.ESS$Age.Ct - mean(data.ESS$Age.Ct, na.rm=T)
MRCTAGE<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.Ct)
summary(MRCTAGE)

# Difference in age
MRDiffAGE<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffAGE)
summary(MRDiffAGE)

# Difference in SR
MRDiffPercMale<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffPercMale)
summary(MRDiffPercMale)

# Mean centered disease duration
data.ESS$Duration<-data.ESS$Duration - mean(data.ESS$Duration, na.rm=T)
MRDuration<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Duration)
summary(MRDuration)

#####
##### Meta-analyses For Plasma Cu data #####
#####

# Deletes any potentially old info hanging around
rm(list=ls())

# Set the working directory
setwd("/Users/siangenoud/Desktop/Forests")

# Install if needs be on the first time - intall.packages("metafor") and intall.packages("plyr")

# Load packages we need
library("metafor")
library("plyr")

# Load dataset
data<-read.csv("Plasma Cu quality order.csv")

# Check the data looks good
head(data)
```

```
# Calculate the effect size ("SMD" - Hedges d) using escalc function
#slab function labels studies
data.ES<-escalc(measure = "SMD", n1i = PD.cases, n2i = Ct.cases, m1i = PD.Cu, m2i = Ct.Cu,
sd1i = PD.Cu.SD, sd2i = Ct.Cu.SD, data = data, slab=paste(Author, Year, sep=", "))

# Fit the meta analysis for all data
MA1<-rma(yi = yi, vi = vi, data = data.ES)
summary(MA1)

# Fit subsets by quality
res.nL <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality == "Moderate" |
Quality=="Limited"), method="REML")
summary(res.nL)
res.m <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Moderate"), method="REML")
summary(res.m)
res.l <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Limited"), method="REML")
summary(res.l)

#Publication bias test
regtest(MA1)
#Check for missing studies
trimfill(MA1)
#Funnel plot
funnel(MA1)

##### Meta-regressions #####

####Meta-regression analysis for effect of technique
# This first line tells you the order - by default R will put the first one of these categories as
the intercept group
sort(unique(data.ES$Technique))

# Here you change the intercept, by changing base=x at the end - here I changed it to the
second in the list above

# Overall effect of analytical technique
contrasts(data.ES$Technique)<-contr.treatment(levels(data.ES$Technique), base=2)
MRTechnique<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Technique-1)
summary(MRTechnique)

# Mean centered age
data.ES$Age.PD<-data.ES$Age.PD - mean(data.ES$Age.PD, na.rm=T)
MRPDAGE<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.PD)
summary(MRPDAGE)

data.ES$Age.Ct<-data.ES$Age.Ct - mean(data.ES$Age.Ct, na.rm=T)
```

```
MRCTAge<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Age.Ct)
summary(MRCTAge)

# Difference in age
MRDiffAge<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffAge)
summary(MRDiffAge)

# Difference in SR
MRDiffPercMale<-rma(yi = yi, vi = vi, data = data.ES, mods= ~DiffPercMale)
summary(MRDiffPercMale)

# Not enough studies to mean centered disease duration

#####
##### Meta-analyses For Plasma Fe data #####
#####

# Deletes any potentially old info hanging around
rm(list=ls())

# Set the working directory
setwd("/Users/siangenoud/Desktop/Forests")

# Install if needs be on the first time - intall.packages("metafor") and intall.packages("plyr")

# Load packages we need
library("metafor")
library("plyr")

# Load dataset
data<-read.csv("Plasma Fe quality order.csv")

# Check the data looks good
head(data)

# Calculate the effect size ("SMD" - Hedges d) using escalc function
#slab function labels studies
data.ES<-escalc(measure = "SMD", n1i = PD.cases, n2i = Ct.cases, m1i = PD.Fe, m2i = Ct.Fe,
sd1i = PD.Fe.SD, sd2i = Ct.Fe.SD, data = data, slab=paste(Author, Year, sep=" ", ""))

# Fit the meta analysis for all data
MA1<-rma(yi = yi, vi = vi, data = data.ES)
summary(MA1)

# Fit subsets by quality
res.m <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Moderate"), method="REML")
summary(res.m)
```

```
#Publication bias test
regtest(MA1)
#Check for missing studies
trimfill(MA1)
#Funnel plot
funnel(MA1)

##### Meta-regressions #####

####Meta-regression analysis for effect of technique
# This first line tells you the order - by default R will put the first one of these categories as
the intercept group
sort(unique(data.ES$Technique))

# Not enough techniques to analyse overall effect of analytical technique

# Not enough studies to analyse effect of Age, Sex ratio or disease duration

#####
##### Meta-analyses For Plasma Zn data #####
#####

# Deletes any potentially old info hanging around
rm(list=ls())

# Set the working directory
setwd("/Users/siangenoud/Desktop/Forests")

# Install if needs be on the first time - intall.packages("metafor") and intall.packages("plyr")

# Load packages we need
library("metafor")
library("plyr")

# Load dataset
data<-read.csv("Plasma Zn quality order.csv")

# Check the data looks good
head(data)

# Calculate the effect size ("SMD" - Hedges d) using escalc function
#slab function labels studies
data.ES<-escalc(measure = "SMD", n1i = PD.cases, n2i = Ct.cases, m1i = PD.Zn, m2i = Ct.Zn,
sd1i = PD.Zn.SD, sd2i = Ct.Zn.SD, data = data, slab=paste(Author, Year, sep=" ", ""))
```

```
# Fit the meta analysis for all data
MA1<-rma(yi = yi, vi = vi, data = data.ES)
summary(MA1)

# Fit subsets by quality
res.m <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Moderate"), method="REML")
summary(res.m)
res.l <- rma(yi=yi, vi=vi, data=data.ES, subset=(Quality=="Limited"), method="REML")
summary(res.l)

#Publication bias test
regtest(MA1)
#Check for missing studies
trimfill(MA1)
#Funnel plot
funnel(MA1)

##### Meta-regressions #####

####Meta-regression analysis for effect of technique
# This first line tells you the order - by default R will put the first one of these categories as
the intercept group
sort(unique(data.ES$Technique))

# Not enough techniques to analyse overall effect of analytical technique

# Not enough studies to analyse effect of Age, Sex ratio or disease duration

#####
##### REMA Regression of Brain vs Biofluids ###
#####

# Deletes any potentially old info hanging around
rm(list=ls())

# Set the working directory
setwd("/Users/siangenoud/Desktop/Forests")

# Intall if needs be on the first time - intall.packages("metafor")

# Load packages we need
library("metafor")

# Load dataset
data<-read.csv("Combined Cu data 16.2.19.csv")
```

```

# Check the data looks good
head(data)

data.ES<-escalc(measure = "SMD", n1i = noPD, n2i = noCt, m1i = Cu.PD, m2i = Cu.Ct, sd1i =
SD.PD, sd2i = SD.Ct, data = data, slab=paste(Author, Year, sep=", "))

# Again aded na.rm
data.ES$Age.PD<-data.ES$Age.PD - mean(data.ES$Age.PD, na.rm=T)

# Here you change the intercept, by changing base=x at the end - here I changed it to the
second in the list above
sort(unique(data.ES$Tissue))
contrasts(data.ES$Tissue)<-contr.treatment(levels(data.ES$Tissue), base=4)

# OK effect of PD on CU levels is significantly different in SN than all bio fluids
# Effect of tissue
MRTissue<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Tissue)
summary(MRTissue)

# Agaein there are differences in age, so could this affect the results
mean(data.ES$Age.PD[which(data.ES$Tissue == "SN")], na.rm=T)
mean(data.ES$Age.PD[which(data.ES$Tissue == "Serum")], na.rm=T)

MRTissueAge<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Tissue+Age.PD)
summary(MRTissueAge)
# No the differences remain - no real effect of age

##### Repeat for the Fe data #####

# Deletes any potentially old info hanging around
rm(list=ls())

# Set the working directory
setwd("/Users/siangenoud/Desktop/Forests")

# Intall if needs be on the first time - intall.packages("metafor")

# Load packages we need
library("metafor")

# Load dataset
data<-read.csv("Fe combined tissue data 16.2.19.csv")

# Check the data looks good
head(data)

```

```
data.ES<-escalc(measure = "SMD", n1i = noPD, n2i = noCt, m1i = Fe.PD, m2i = Fe.Ct, sd1i = SD.PD, sd2i = SD.Ct, data = data, slab=paste(Author, Year, sep=", "))
```

```
# This was causing a problem because of the NAs in the dataset - I added na.rm=T at the end and htis fixed the issue
```

```
data.ES$Age.PD<-data.ES$Age.PD - mean(data.ES$Age.PD, na.rm=T)
```

```
# Here you change the intercept, by changing base=x at the end - here I changed it to the second in the list above
```

```
sort(unique(data.ES$Tissue))
```

```
contrasts(data.ES$Tissue)<-contr.treatment(levels(data.ES$Tissue), base=4)
```

```
# Effect of tissue
```

```
MRTissue<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Tissue)
```

```
summary(MRTissue)
```

```
# Could results be affected by age - after all by the time brain tissue is collected PD is more advanced
```

```
mean(data.ES$Age.PD[which(data.ES$Tissue == "SN")], na.rm=T)
```

```
mean(data.ES$Age.PD[which(data.ES$Tissue == "Serum")], na.rm=T)
```

```
MRTissueAge<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Tissue+Age.PD)
```

```
summary(MRTissueAge)
```

```
##### Repeat for the Zn data #####
```

```
# Deletes any potentially old info hanging around
```

```
rm(list=ls())
```

```
# Set the working directory
```

```
setwd("/Users/siangenoud/Desktop/Forests")
```

```
# Intall if needs be on the first time - intall.packages("metafor")
```

```
# Load packages we need
```

```
library("metafor")
```

```
# Load dataset
```

```
data<-read.csv("Combined Zn data 18.2.19.csv")
```

```
# Check the data looks good
```

```
head(data)
```

```
data.ES<-escalc(measure = "SMD", n1i = noPD, n2i = noCt, m1i = Zn.PD, m2i = Zn.Ct, sd1i = SD.PD, sd2i = SD.Ct, data = data, slab=paste(Author, Year, sep=", "))
```

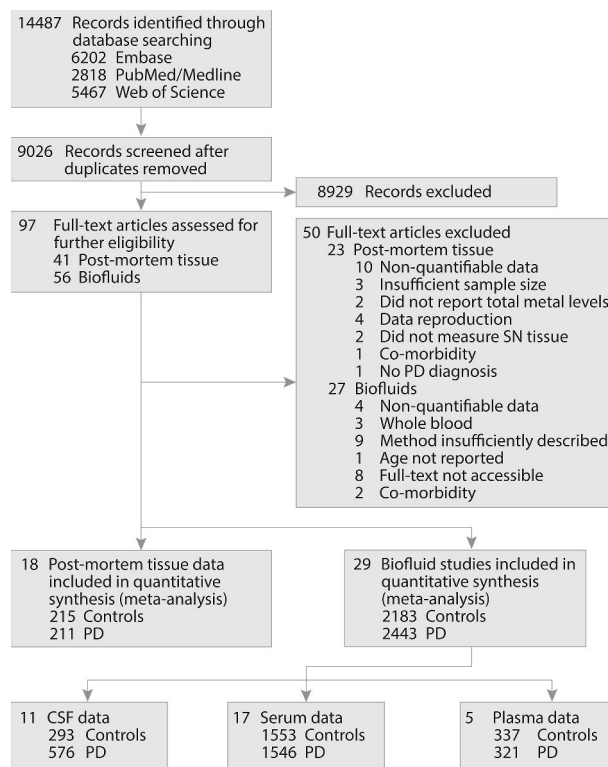
```
# This was causing a problem because of the NAs in the dataset - I added na.rm=T at the end  
and htis fixed the issue  
data.ESS$Age.PD<-data.ESS$Age.PD - mean(data.ESS$Age.PD, na.rm=T)
```

```
# Here you change the intercept, by changing base=x at the end - here I changed it to the  
second in the list above  
sort(unique(data.ESS$Tissue))  
contrasts(data.ESS$Tissue)<-contr.treatment(levels(data.ESS$Tissue), base=4)
```

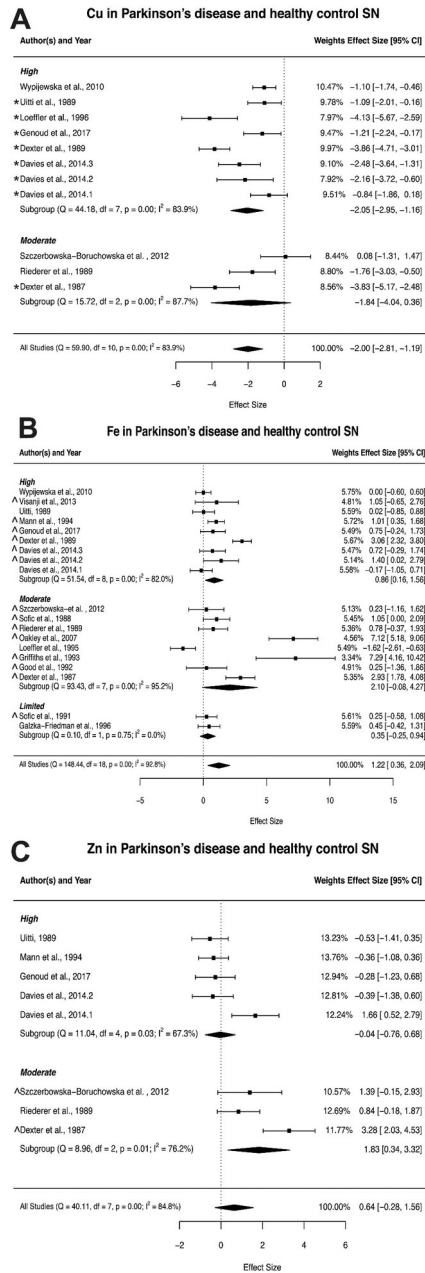
```
# Effect of tissue  
MRTissue<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Tissue)  
summary(MRTissue)
```

```
# Could results be affected by age - after all by the time brain tissue is collected PD is more  
advanced  
mean(data.ESS$Age.PD[which(data.ESS$Tissue == "SN")], na.rm=T)  
mean(data.ESS$Age.PD[which(data.ESS$Tissue == "Serum")], na.rm=T)
```

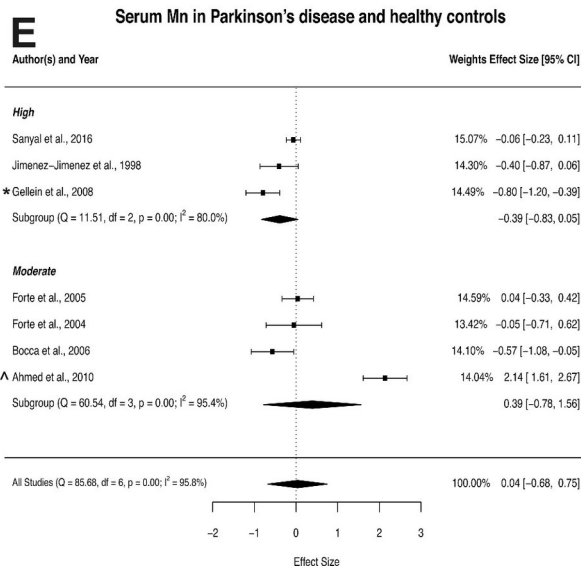
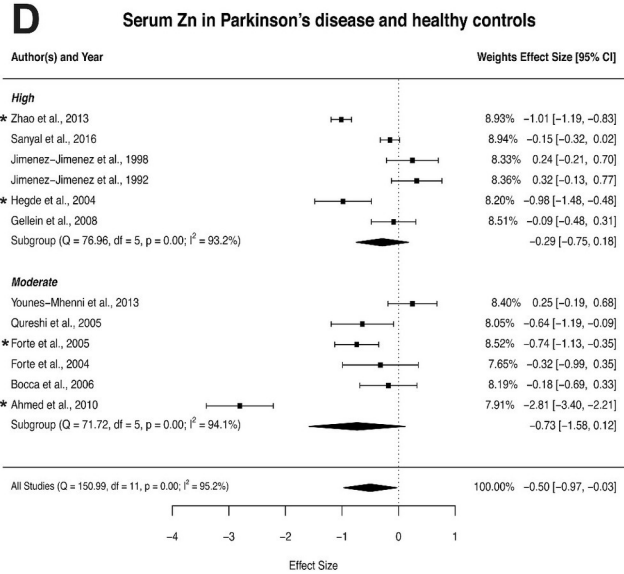
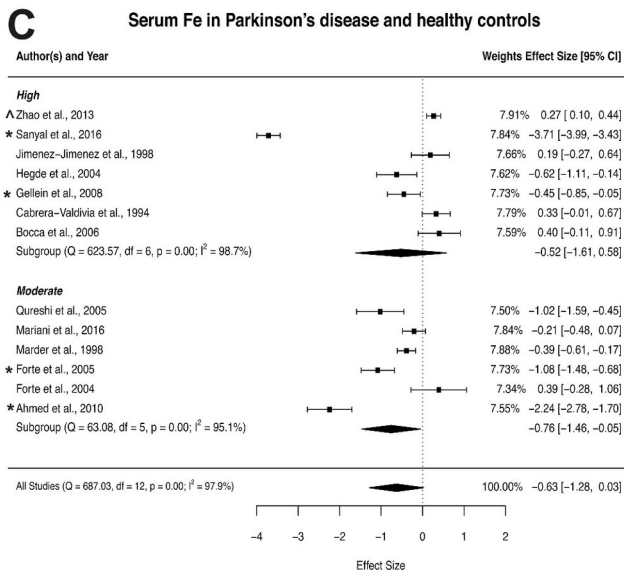
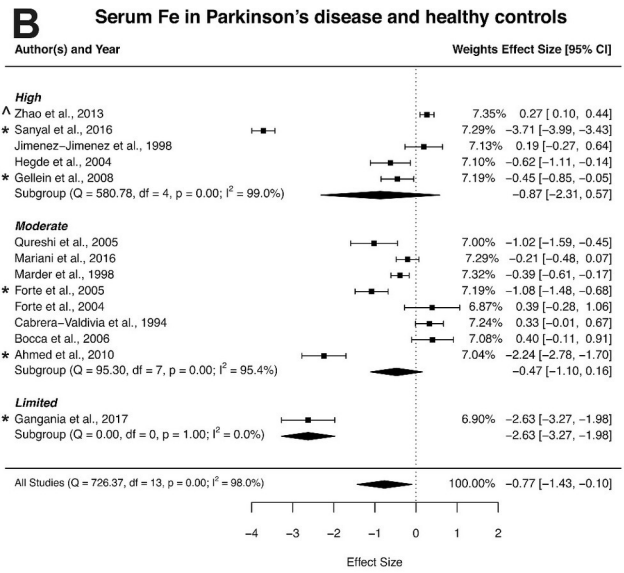
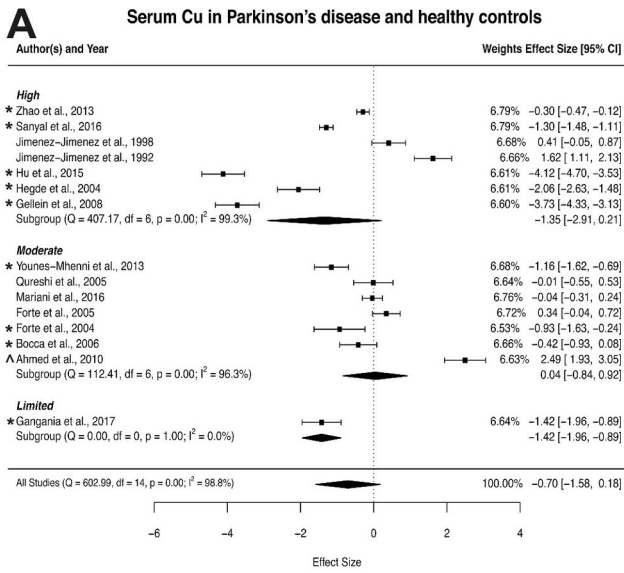
```
MRTissueAge<-rma(yi = yi, vi = vi, data = data.ES, mods= ~Tissue+Age.PD)  
summary(MRTissueAge)
```



mds_27947_figure_1.eps

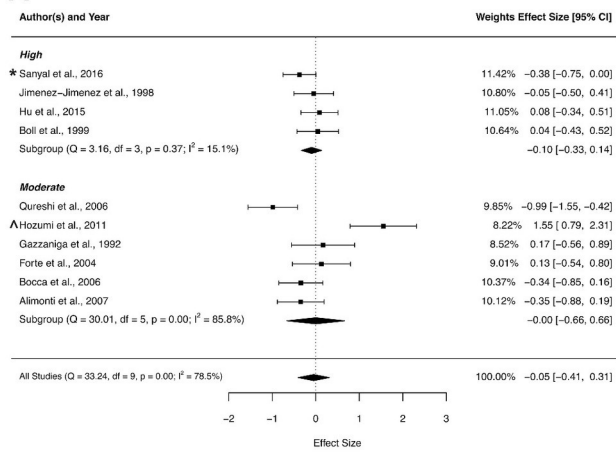


mds_27947_figure_2.eps

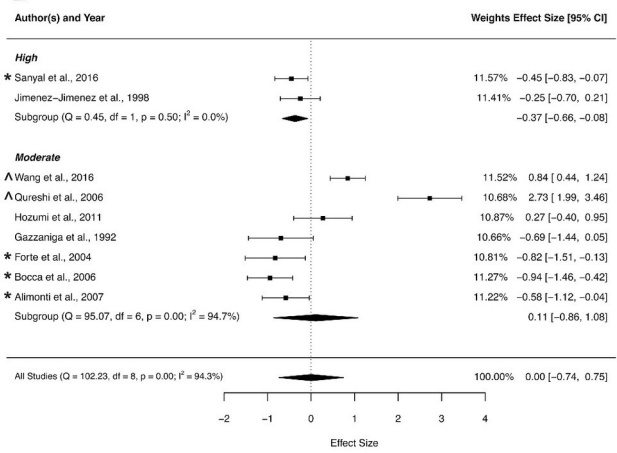


mds_27947_figure_3.eps

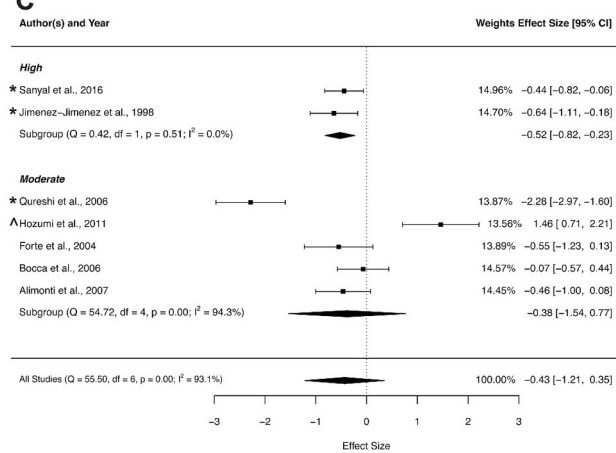
A CSF Cu in Parkinson's disease and healthy controls



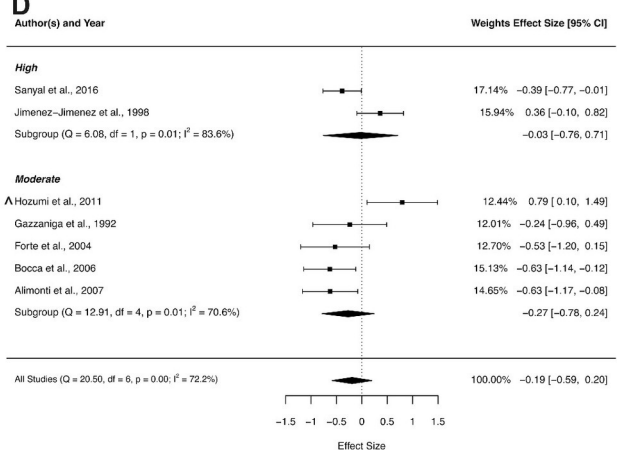
B CSF Fe in Parkinson's disease and healthy controls



C CSF Zn in Parkinson's disease and healthy controls



D CSF Mn in Parkinson's disease and healthy controls



mds_27947_figure_4.eps

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Contributor address: 94-100 Mallet St, Camperdown, Sydney, NSW, Australia

Manuscript number: MDS-19-0841

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