Reanalysis of 06-Wile

Simon Schwab

13/05/2020

Reference

Wile et al. (2017). Serotonin and dopamine transporter PET changes in the premotor phase of LRRK2 parkinsonism: cross-sectional studies. *Lancet Neurology*, 16(5), 351–359. https://doi.org/10.1016/S1474-4422(17)30056-X

Notes from reading methods section

- Dependant variable: PET radiotracer called 11C-DASB in cortex (first of many ANCOVAs reported)
- Independant variable: Group
  - Healty controls (n=9)
  - LRRK2 w/o PD (n=9)
  - LRRK2 w PD (n=7)
  - sporadic PD (n=13)
- Covariate: age
- age was not estimable for LRRK2 without manifest Parkinson's disease
- Design: 4-way ANCOVA with group as IV and age as covariate

Reading data

Data is loaded, reshaped if necessary, and factors are specified.

```
PATH = file.path(path.expand("~"), "Data", "ancova") # ancova project folder
data = read_excel(file.path(PATH, "dataPrimaryStudies", "06-Wile", "06-Wile.xlsx"))

# group variable is dummy coded, key for AncovaVariable file
# 0 = healthy control, 1 = LRRK2 premanifest, 2 = LRRK2 affected, 3 = sporadic PD
data$Group.factor = NA
data$Group.factor[data$Group == 0] = "healthy control"
data$Group.factor[data$Group == 1] = "LRRK2 premanifest"
data$Group.factor[data$Group == 2] = "LRRK2 affected"
data$Group.factor[data$Group == 3] = "sporadic PD"
data$Group.factor = factor(data$Group.factor,
                           levels = c("healthy control", "LRRK2 premanifest",
                                      "LRRK2 affected", "sporadic PD"))
```

Descriptives

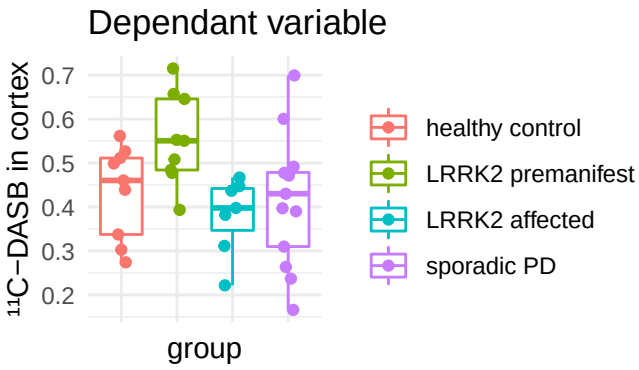
Dependant variable

Number of samples and mean (SD) in levels of the independant variables. We reproduce Table 3 and Figure 2A of the study.

```
idx = c(1, 3, 2, 4) # sorting as in publication
tab.dv = array(NA, dim=c(4,3))
tab.dv[,1] = levels(data$Group.factor)
tab.dv[,2] = summary(data$Group.factor)
tab.dv[,3] = tapply(data$Cortex, data$Group.factor,
                    function (x) sprintf("%0.2f (%0.2f)", mean(x), sd(x)))
colnames(tab.dv) = c("group", "n", "mean (SD)")
print(tab.dv)
```

```
##      group               n    mean (SD)
## [1,] "healthy control"   "9"  "0.43 (0.10)"
## [2,] "LRRK2 premanifest" "9"  "0.55 (0.10)"
## [3,] "LRRK2 affected"    "7"  "0.38 (0.09)"
## [4,] "sporadic PD"       "13" "0.42 (0.15)"
```

```
ggplot(data, aes(y=Cortex, x=Group.factor, color=Group.factor)) +
  geom_boxplot() +
  geom_point(position = position_jitter(width = 0.15, height = 0)) +
  theme_minimal() +
  theme(axis.text.x = element_blank(), legend.title = element_blank()) +
  xlab("group") + ylab("[11]C-DASB in cortex") + ggtitle("Dependant variable")
```



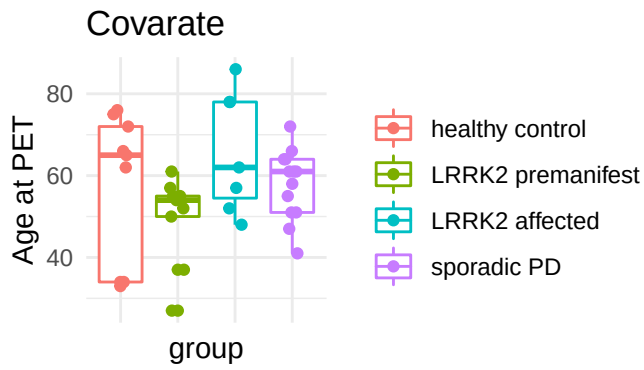
Covariate

```
idx = c(1, 3, 2, 4) # sorting as in publication
tab.cv = array(NA, dim=c(4,3))
tab.cv[,1] = levels(data$Group.factor)
tab.cv[,2] = summary(data$Group.factor)
tab.cv[,3] = tapply(data$`Age at PET`, data$Group.factor,
                    function (x) sprintf("%0.1f (%0.1f)", mean(x), sd(x)))
colnames(tab.cv) = c("group", "n", "mean (SD)")
print(tab.cv)
```

```
##      group               n    mean (SD)
```

```
## [1,] "healthy control"   "9"  "57.4 (18.4)"
## [2,] "LRRK2 premanifest" "9"  "49.8 (10.8)"
## [3,] "LRRK2 affected"    "7"  "65.9 (14.7)"
## [4,] "sporadic PD"       "13" "57.8 (8.5)"
```

```
ggplot(data, aes(y=`Age at PET`, x=Group.factor, color=Group.factor)) +
  geom_boxplot() +
  geom_point(position = position_jitter(width = 0.15, height = 0)) +
  theme_minimal() +
  theme(axis.text.x = element_blank(), legend.title = element_blank()) +
  xlab("group") + ylab("Age at PET") + ggtitle("Covarate")
```



ANCOVA

We will use Type I sum of squares for balanced designs (equal n per group), and Type III for unbalanced designs and used in SAS and SPSS.

```
contrasts(data$Group.factor) = contr.helmert(4) # when is this necessary?

fit = aov(Cortex ~ `Age at PET` + Group.factor, data = data)
# result = summary(fit) # Type I
result = Anova(fit, type=3) # Type III
print(result)
```

```
## Anova Table (Type III tests)
##
## Response: Cortex
##               Sum Sq Df F value    Pr(>F)
## (Intercept)  0.36361  1 25.1466 1.767e-05 ***
## `Age at PET` 0.00073  1  0.0502    0.8241
## Group.factor 0.12125  3  2.7951    0.0555 .
## Residuals    0.47717 33
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
# For Type I SS
# stats.reanalysis = data.frame(Fvalue = sprintf("%.2f",result[[1]]$`F value`[1]),
#                               df1 = result[[1]]$Df[1],
#                               df2 = result[[1]]$Df[2],
#                               pvalue = formatPval(result[[1]]$`Pr(>F)`[1]))
```

```r
# For Type III SS
stats.rep.IV = data.frame(Fvalue = sprintf("%.2f",result$`F value`[3]),
                          df1 = result$Df[3],
                          df2 = result$Df[4],
                          pvalue = formatPval(result$`Pr(>F)`[3]))

stats.rep.CV = data.frame(Fvalue = sprintf("%.2f",result$`F value`[2]),
                          df1 = result$Df[2],
                          df2 = result$Df[4],
                          pvalue = formatPval(result$`Pr(>F)`[2]))
```

Comparing ANCOVA in original study with reanalysis

Independant variable

```r
tab.IV = rbind(stats.orig.IV, stats.rep.IV)
rownames(tab.IV) = c("original Study", "reanalysis")
print(t(tab.IV))
```

```
##        original Study reanalysis
## Fvalue NA             "2.80"
## df1    NA             " 3"
## df2    NA             "33"
## pvalue "0.026"        "0.056"
```

Covariate

```r
tab.CV = rbind(stats.orig.CV, stats.rep.CV)
rownames(tab.CV) = c("original Study", "reanalysis")
print(t(tab.CV))
```

```
##        original Study reanalysis
## Fvalue NA             "0.05"
## df1    NA             " 1"
## df2    NA             "33"
## pvalue NA             "0.82"
```

Assumptions

1. Homogeneity of variance

- ANOVA/ANCOVA is fairly robust in terms of the error rate when sample sizes are equal.
- When groups with larger sample sizes have larger variances than the groups with smaller sample sizes, the resulting F-ratio tends to be conservative. That is, it's more likely to produce a non-significant result when a genuine difference does exist in the population.
- Conversely, when the groups with larger sample sizes have smaller variances than the groups with smaller samples sizes, the resulting F-ratio tends to be liberal and can inflate the false positive rate.

```
tapply(data$Cortex, data$Group.factor, sd)
```

```
##   healthy control LRRK2 premanifest    LRRK2 affected       sporadic PD
##        0.10480960        0.10221440        0.08699411        0.14748558
```

```
leveneTest(Cortex ~ Group.factor, data = data)
```

```
## Levene's Test for Homogeneity of Variance (center = median)
##       Df F value Pr(>F)
## group  3   0.821 0.4914
##       34
```

2. Independence between covariate and IV

When the covariate and the experimental effect (independent variable) are not independent the treatment effect is obscured, spurious treatment effects can arise and the interpretation of the ANCOVA is seriously compromised.

We test whether our groups differ on the CV. If the groups do not significantly differ then is appropriate to use the covariate.

```
fit.cv = aov(`Age at PET` ~ Group.factor, data = data)
summary(fit.cv)
```

```
##              Df Sum Sq Mean Sq F value Pr(>F)
## Group.factor  3   1026   342.0   1.994  0.133
## Residuals    34   5830   171.5
```

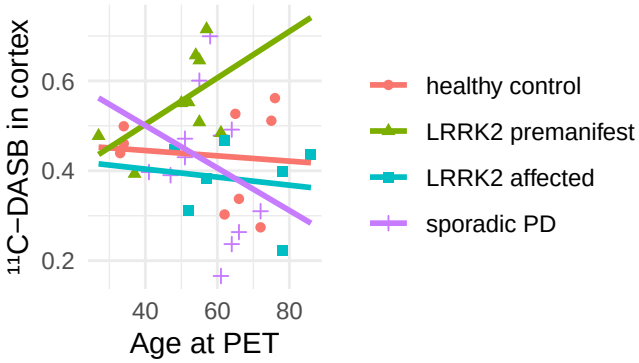
3. Homogeneity of regression slopes

```
fit.hrs = aov(Cortex ~ `Age at PET`*Group.factor, data = data)
Anova(fit.hrs, type=3)
```

```
## Anova Table (Type III tests)
##
## Response: Cortex
##                             Sum Sq Df F value    Pr(>F)
## (Intercept)                0.31357  1 21.8078 5.908e-05 ***
## `Age at PET`               0.00032  1  0.0226    0.8816
## Group.factor               0.02337  3  0.5417    0.6574
## `Age at PET`:Group.factor 0.04580  3  1.0617    0.3800
## Residuals                  0.43137 30
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
ggplot(data, aes(y=Cortex, x=`Age at PET`, color=Group.factor, shape=Group.factor)) +
  geom_point() +
  geom_smooth(method=lm, se=FALSE, fullrange=TRUE) +
  theme_minimal() +
  theme(legend.title = element_blank()) +
  xlab("Age at PET") + ylab("[11]C-DASB in cortex")
```

```
## `geom_smooth()` using formula 'y ~ x'
```



Additional analyses: MANCOVA

The paper performed four ANCOVAs for cortex, striatum, brainstem, and hypothalamus but did not correct for multiple testing. Therefore, we also perform a MANCOVA.

```
fit = manova(cbind(Cortex, Striatum, Brainstem, Hypothalamus) ~ Group.factor + `Age at PET`, data=data)
summary(fit)
```

```
##              Df  Pillai approx F num Df den Df    Pr(>F)
## Group.factor  3 0.95385   3.7293     12     96 0.0001241 ***
## `Age at PET`  1 0.36886   4.3832      4     30 0.0065618 **
## Residuals    33
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

Data was analyzed according to recommendations by Field, Miles, & Field (2012).