DFNA9 genotype ~ phenotype analysis

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Introduction

This R Markdown notebook is used to document the various aspects of the genotype-phenotype analysis in many subjects with hereditary hearing loss based on mutation in the DFNA9 gene. We have data collected [. . .].

Load R-packages

```r
library(ggplot2)
library(drc)
```

```
## Loading required package: MASS

##
## 'drc' has been loaded.

## Please cite R and 'drc' if used for a publication,

## for references type 'citation()' and 'citation('drc')'.

##
## Attaching package: 'drc'

## The following objects are masked from 'package:stats':
##
##     gaussian, getInitial
```

```r
library(sjPlot)
```

```
## Registered S3 methods overwritten by 'lme4':
##   method                          from
##   cooks.distance.influence.merMod car
##   influence.merMod                car
##   dfbeta.influence.merMod         car
##   dfbetas.influence.merMod        car

## Learn more about sjPlot with 'browseVignettes("sjPlot")'.
```

```r
library("readxl")
library("nlme")
library(lme4)
```

```
## Loading required package: Matrix

##
## Attaching package: 'lme4'
## The following object is masked from 'package:nlme':
##
##     lmList
```

```r
library(knitr)
library(forcats)
library(tidyr)
```

```
##
## Attaching package: 'tidyr'

## The following objects are masked from 'package:Matrix':
##
##     expand, pack, unpack
```

```r
library(dplyr)
```

```
##
## Attaching package: 'dplyr'

## The following object is masked from 'package:nlme':
##
##     collapse

## The following object is masked from 'package:MASS':
##
##     select

## The following objects are masked from 'package:stats':
##
##     filter, lag

## The following objects are masked from 'package:base':
##
##     intersect, setdiff, setequal, union
```

Load data and clean data-frames

Load data from Excel file and select only relevant columns/rows. The first analyses will be based on pure-tone average (PTA). The selected subset dataframe consists of the columns patient id (pid), group, age (Leeftijd), and the PTA (PTA54ADS).

```r
data_raw <- read_excel("../data/raw_data/database_20-04-2020.xlsx")
data_raw$group = factor(data_raw$Domain)
#leave out data with only n=1 dataset per domain/certain unpublished data.
data_subset <- subset(data_raw, Smits=='no' & Domainrec!=1 & group!="Ivd1")
data <- subset(data_subset, select=c('pid', 'group', 'Leeftijd', 'PTA54ADS'))
data <- droplevels(data)
#data_raw %>% group_by(group) %>% summarize(count=n())
#head(data)
#check for NaNs in PTA and Leeftijd, should be 0.
nrow(data[is.na(data$PTA54ADS) | is.na(data$Leeftijd), ])
```

```
## [1] 0
```

```r
#number (n) of counts (i.e. audiograms) per subject (pid)
summarytable <- data %>% count(group, pid)
```

Group description

In the group of DFNA9 patients we have some for which there is longitudinal data, i.e. multiple audiograms over time/age (Leeftijd). First, create a table and histogram of number of measurements for each subject id (pid) across the groups

```
num_meas_per_id <- aggregate(PTA54ADS ~ pid , data, function(x) length(unique(x)))
t1 <- table(num_meas_per_id$PTA54ADS)
```

In total there are 283 subjects with 716 measurements; 159 patients with only 1 measurement and 123 patients with 2 or more measurements, see e.g. table 1 or the histogram.

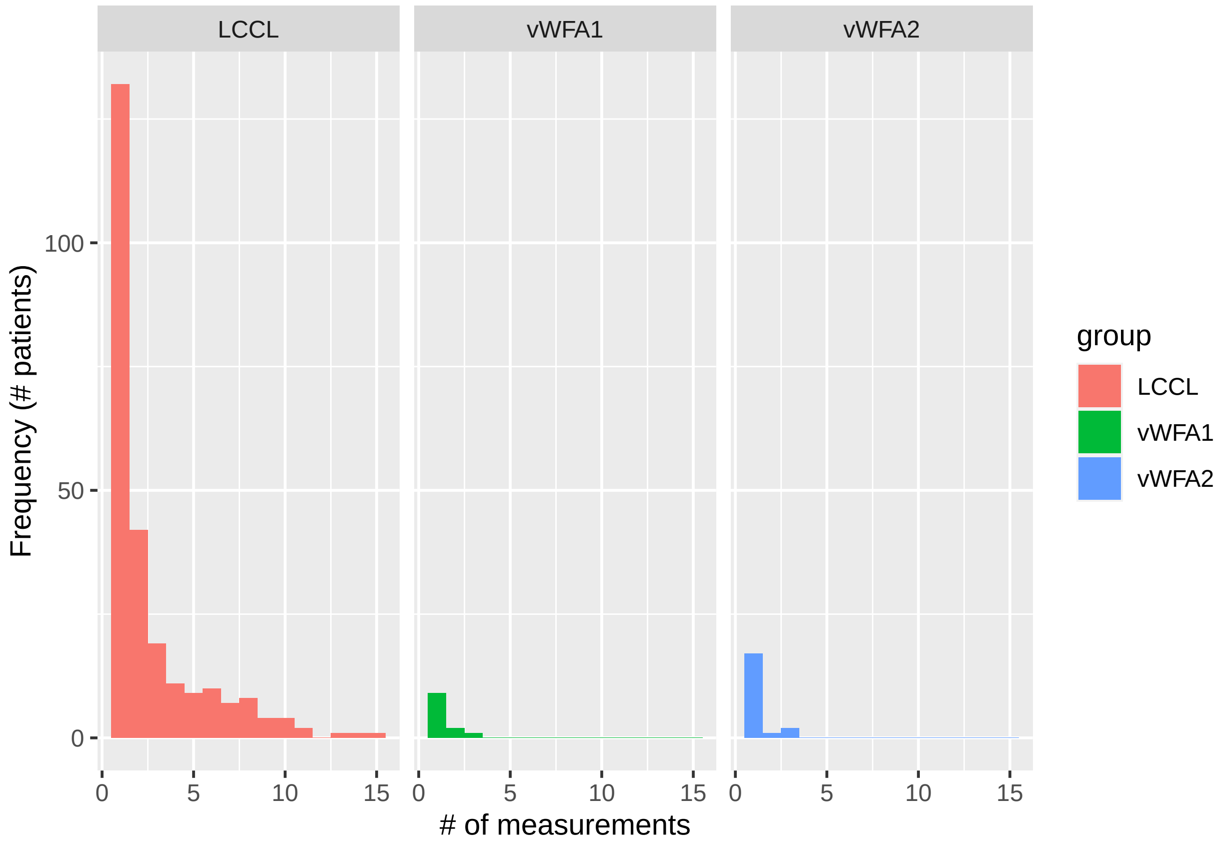
```
kable(t1, caption = "Table 1. The number of subjects that each have n audiograms", col.names = c("# audi
```

Table 1: Table 1. The number of subjects that each have n audiograms

# audiograms	# subjects
1	159
2	47
3	21
4	10
5	13
6	11
7	4
8	7
9	3
10	3
11	3
12	1
15	1

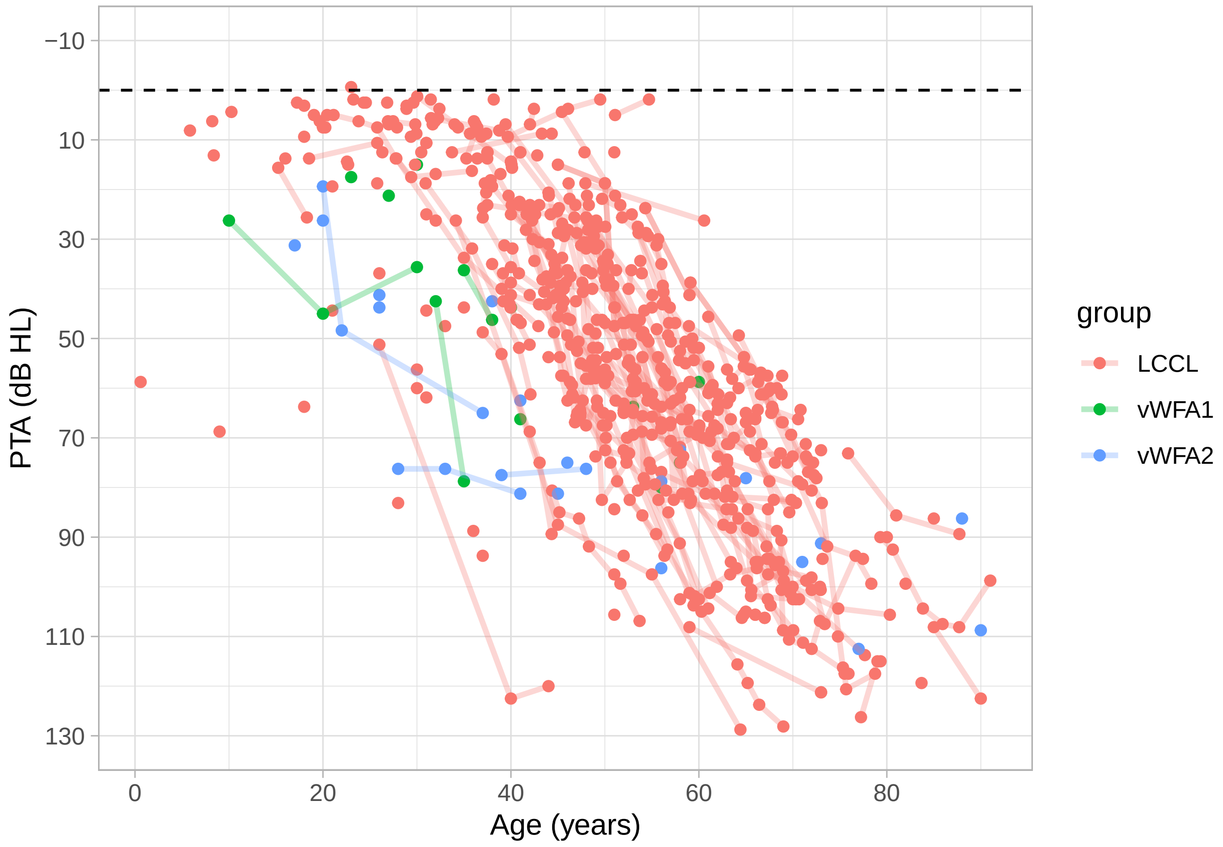
Now make a histogram of the number of audiograms across patients in each of the groups

```
ggplot(data=summarytable, aes(x=n,fill=group)) +
  geom_histogram(binwidth=1) +
  facet_wrap(~group) +
  xlab("# of measurements") +
  ylab("Frequency (# patients)")
```



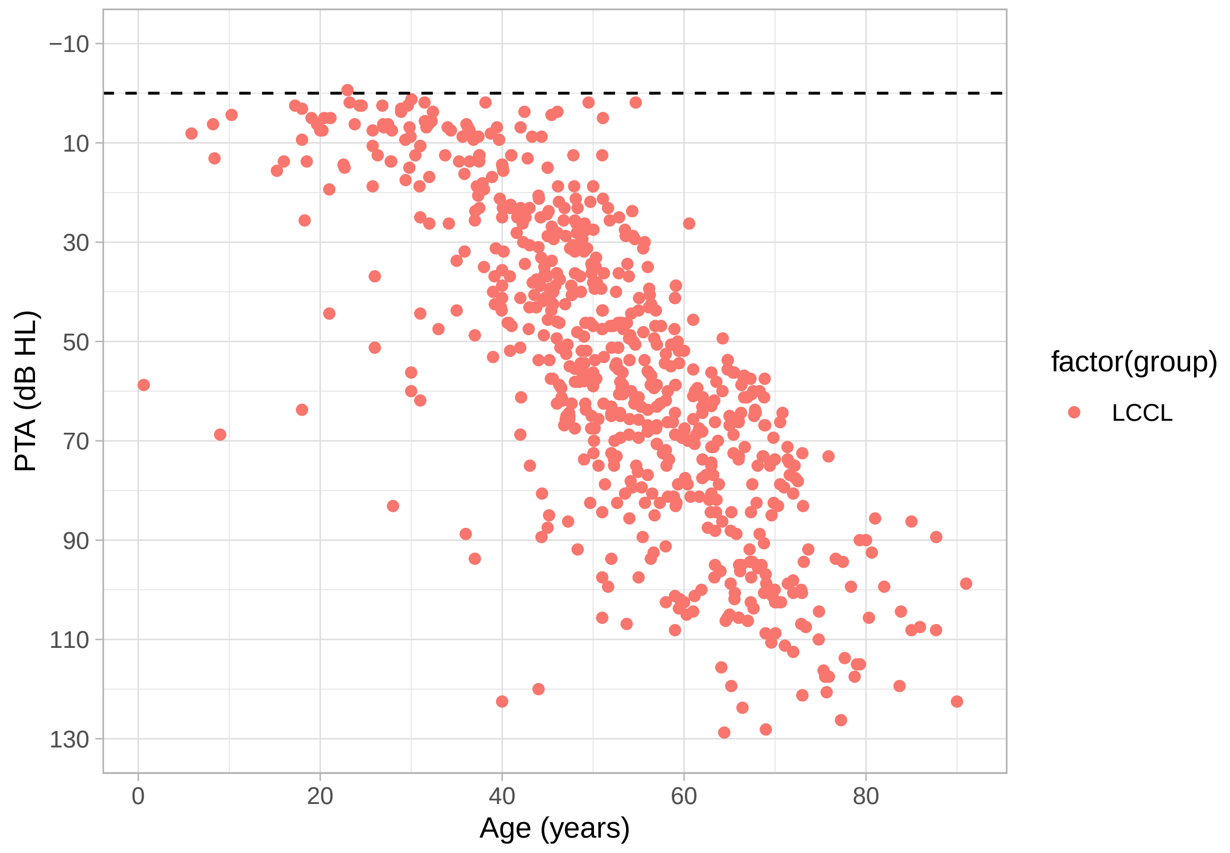
Relation of PTA with age for the different groups; connecting lines show longitudinal data of patients' PTA over time

```
ggplot(data, aes(x = Leeftijd, y = PTA54ADS, group = pid, color = group)) +
  #xlab("Age (years)") +
  #ylab("PTA (dB HL)") +
  geom_point(aes(colour = factor(group))) +
  geom_line(data=data, size=1, alpha = .3) +
  geom_hline(yintercept=0,linetype="dashed") +
  scale_x_continuous(breaks=seq(0,100,20)) +
  scale_y_reverse(breaks=seq(-10,130,20), limits=c(130,-10)) +
  #scale_y_reverse(limits=c(130,-10)) +
  xlab("Age (years)") +
  ylab("PTA (dB HL)") +
  theme_light()
```



## Logistic fit of PTA with age Perform non-linear fit; first focus on LCCL domain.

```
lccl = subset(data, group=="LCCL")
ggplot(lccl, aes(x=Leeftijd, y=PTA54ADS), group=pid, color=group) +
  xlab("Age (years)") +
  ylab("PTA (dB HL)") +
  geom_point(aes(colour = factor(group))) +
  geom_hline(yintercept=0,linetype="dashed") +
  scale_x_continuous(breaks=seq(0,100,20)) +
  scale_y_reverse(breaks=seq(-10,130,20), limits=c(130,-10)) +
  #scale_y_reverse(limits=c(130,-10)) +
  theme_light()
```



```r
lin_fit <- nls(PTA54ADS ~ a*Leeftijd + b, lccl)
```

```
## Warning in nls(PTA54ADS ~ a * Leeftijd + b, lccl): No starting values specified for some parameters.
## Initializing 'a', 'b' to '1.'.
## Consider specifying 'start' or using a selfStart model
```

```r
summary(lin_fit)
```

```
##
## Formula: PTA54ADS ~ a * Leeftijd + b
##
## Parameters:
##    Estimate Std. Error t value Pr(>|t|)
## a   1.60851    0.05331  30.170   <2e-16 ***
## b -28.43929    2.89132  -9.836   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 20.01 on 673 degrees of freedom
##
## Number of iterations to convergence: 1
## Achieved convergence tolerance: 9.99e-10
```

```r
nls_fit <- nls(PTA54ADS ~ a*Leeftijd^b, lccl, start = list(a = 0.05, b = 1.5))
summary(nls_fit)
```

```
##
## Formula: PTA54ADS ~ a * Leeftijd^b
##
```

```
## Parameters:
##   Estimate Std. Error t value Pr(>|t|)
## a  0.07298    0.01859   3.926 9.51e-05 ***
## b  1.66646    0.06175  26.988  < 2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 19.54 on 673 degrees of freedom
##
## Number of iterations to convergence: 5
## Achieved convergence tolerance: 2.93e-06
```

```
startvec <- c(Asym = 120, xmid = 50, scal = 15)
nls_logis<- nls(PTA54ADS ~ SSlogis(Leeftijd, Asym, xmid, scal),
                 data=lccl,
                 start = startvec)
summary(nls_logis)
```

```
##
## Formula: PTA54ADS ~ SSlogis(Leeftijd, Asym, xmid, scal)
##
## Parameters:
##      Estimate Std. Error t value Pr(>|t|)
## Asym  126.351      9.477   13.33   <2e-16 ***
## xmid   56.974      2.619   21.76   <2e-16 ***
## scal   15.410      1.371   11.24   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 19.29 on 672 degrees of freedom
##
## Number of iterations to convergence: 4
## Achieved convergence tolerance: 3.603e-06
```

Compare power-law fit and the logistic functione and display the results

```
anova(nls_fit,nls_logis)
```

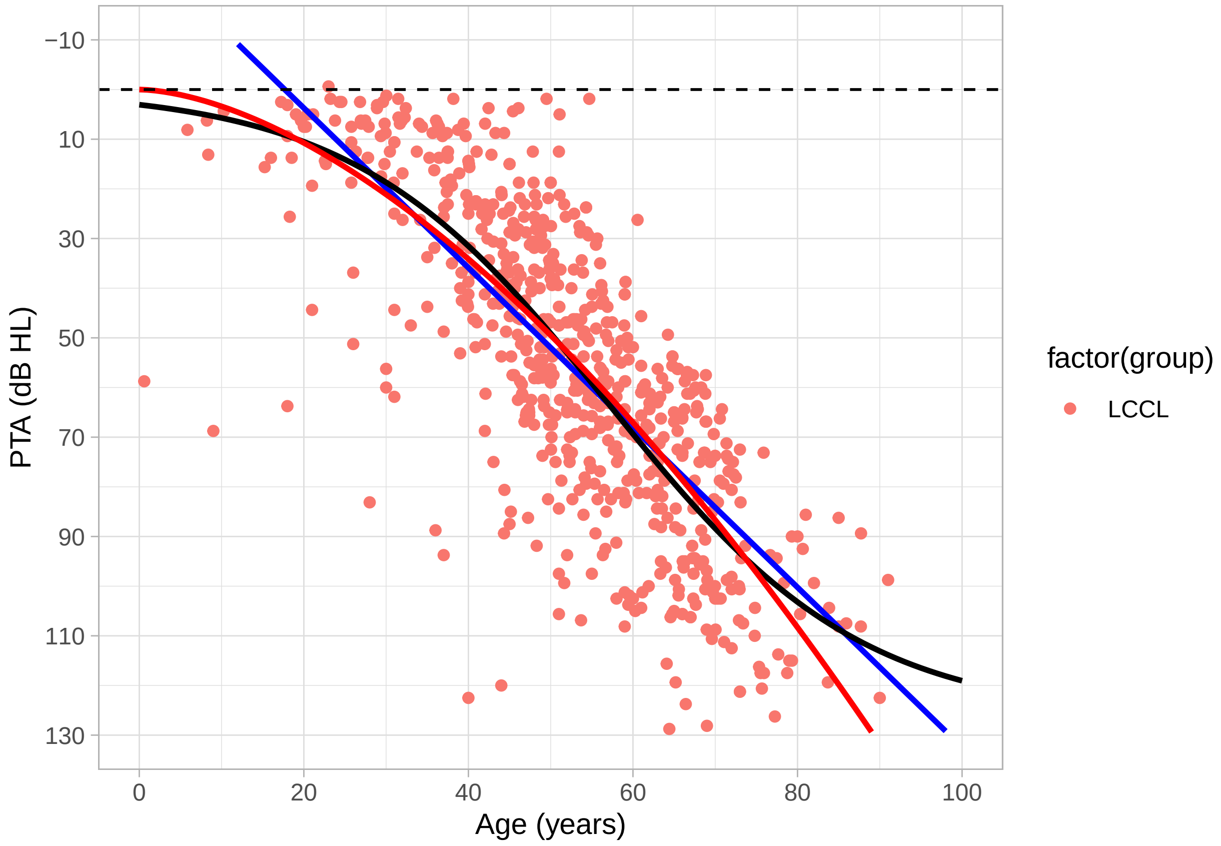
```
## Analysis of Variance Table
##
## Model 1: PTA54ADS ~ a * Leeftijd^b
## Model 2: PTA54ADS ~ SSlogis(Leeftijd, Asym, xmid, scal)
##   Res.Df Res.Sum Sq Df Sum Sq F value    Pr(>F)
## 1    673     257033
## 2    672     250020  1 7013.2   18.85 1.632e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

```
newdat = expand.grid(Leeftijd = seq(0, 100, by = 1))
newdat$lin <-predict(lin_fit, newdata = newdat)
newdat$pta_logistic <- predict(nls_logis,newdata = newdat)
newdat$pta_power <- predict(nls_fit,newdata = newdat)
#newdat
ggplot(lccl, aes(x=Leeftijd, y=PTA54ADS)) +
  geom_point(aes(colour = factor(group))) +
  geom_line(data = newdat, aes(y = lin), size = 1, col='blue') +
  geom_line(data = newdat, aes(y = pta_logistic), size = 1) +
  geom_line(data = newdat, aes(y = pta_power), size = 1, col='red') +

  geom_hline(yintercept=0,linetype="dashed") +
  scale_x_continuous(breaks=seq(0,100,20)) +
  scale_y_reverse(breaks=seq(-10,130,20), limits=c(130,-10)) +
  #scale_y_reverse(limits=c(130,-10)) +
  xlab("Age (years)") +
  ylab("PTA (dB HL)") +
  theme_light()
```

```
## Warning: Removed 14 row(s) containing missing values (geom_path).

## Warning: Removed 11 row(s) containing missing values (geom_path).
```



As we can see, the logistic function (SSlogis) describes the data better than the power-law function (F = 18,9; p = 1.6 e-5) This function has also been used in desribing the (frequency-specific) thresholds in Pauw et al., 2011 and will used in the subsequent sections.

Group comparison

The main questions is whether the function that describes the PTA (dB HL) as a function of age (years) differs between the groups @ref(fig:plot__pta__age__groups).

Start with a group-fit; discarding grouping information

```
fit0 <- nls(PTA54ADS ~ SSlogis(Leeftijd, Asym, xmid, scal), data=data)
summary(fit0)
```

```
##
## Formula: PTA54ADS ~ SSlogis(Leeftijd, Asym, xmid, scal)
##
## Parameters:
##      Estimate Std. Error t value Pr(>|t|)
## Asym  135.557     12.188   11.12   <2e-16 ***
## xmid   59.258      3.517   16.85   <2e-16 ***
## scal   17.807      1.639   10.86   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 19.78 on 713 degrees of freedom
##
## Number of iterations to convergence: 4
## Achieved convergence tolerance: 2.948e-06
```

```
coef(fit0)
```

```
##      Asym      xmid      scal
## 135.55740  59.25776  17.80669
```

Now, add a grouping-variable with the mid-point (xmid)

```
# https://stats.stackexchange.com/questions/27273/how-do-i-fit-a-nonlinear-mixed-effects-model-for-repe
# https://stats.stackexchange.com/questions/316801/how-to-compare-logistic-regression-curves
fit1 <- nls(PTA54ADS ~ SSlogis(Leeftijd, Asym, xmid[group], scal),
            data=data,
            start=list(Asym=rep(120,1), xmid=rep(50,3), scal=rep(15,1)))
summary(fit1)
```

```
##
## Formula: PTA54ADS ~ SSlogis(Leeftijd, Asym, xmid[group], scal)
##
## Parameters:
##       Estimate Std. Error t value Pr(>|t|)
## Asym   116.114      6.242   18.60   <2e-16 ***
## xmid1   54.188      1.813   29.89   <2e-16 ***
## xmid2   43.644      3.462   12.61   <2e-16 ***
## xmid3   34.631      3.185   10.87   <2e-16 ***
## scal    14.394      1.127   12.77   <2e-16 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 19.13 on 711 degrees of freedom
##
## Number of iterations to convergence: 7
## Achieved convergence tolerance: 4.654e-06
```

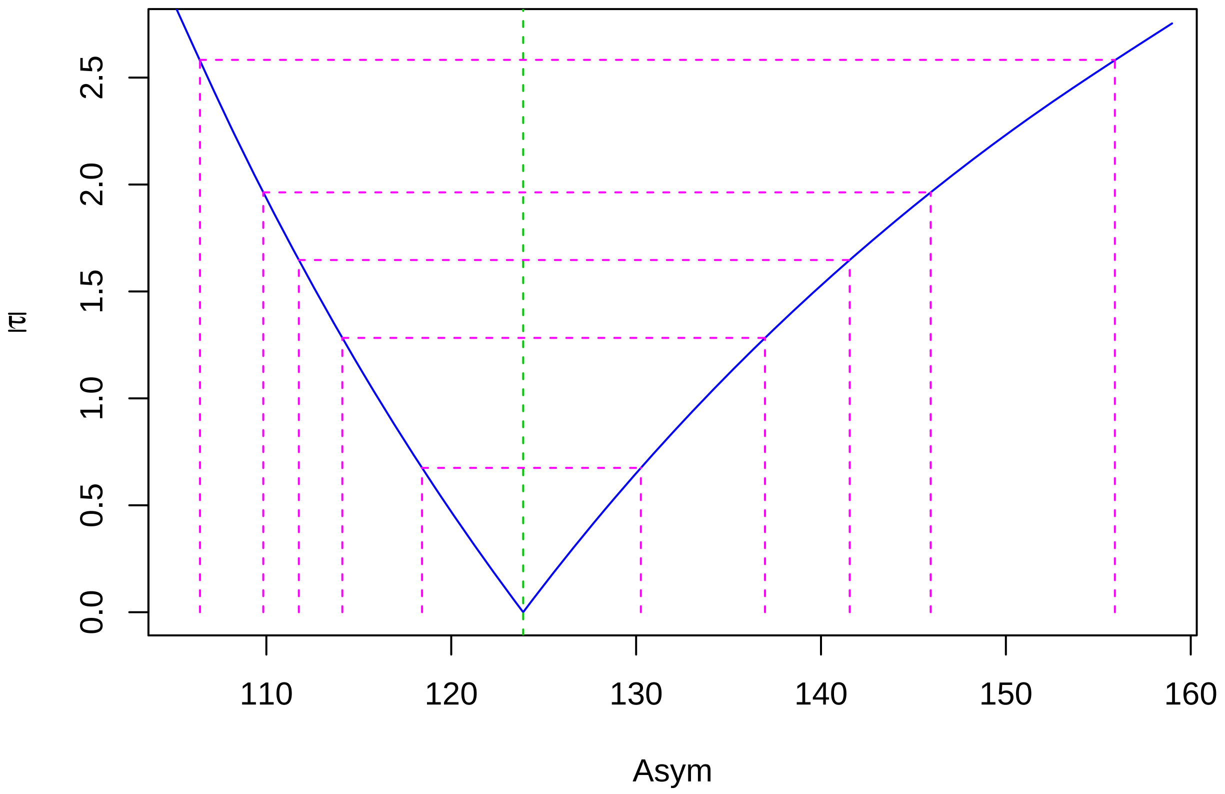
And add the scaling [scal] as a grouping variable; does it futher explain differences between groups?

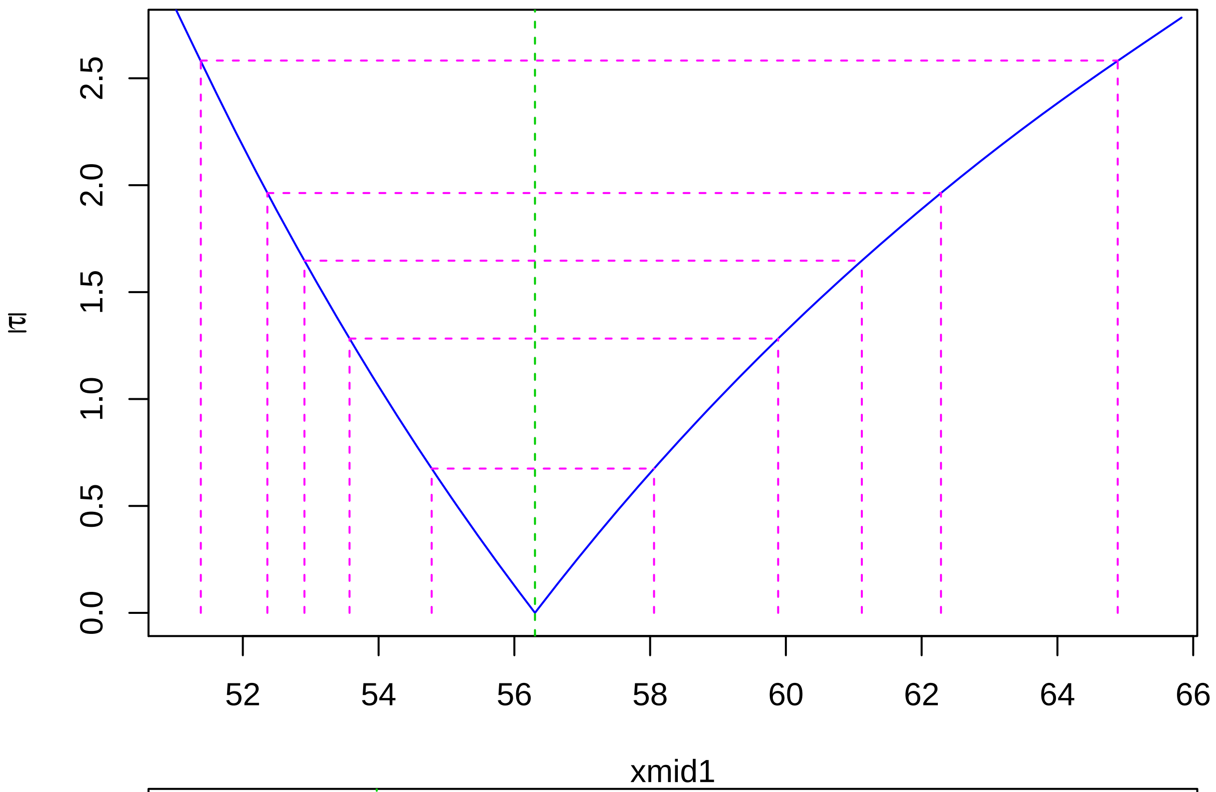
```
fit2 <- nls(PTA54ADS ~ SSlogis(Leeftijd, Asym, xmid[group], scal[group]),
            data=data,
            start=list(Asym=rep(120,1), xmid=rep(50,3), scal=rep(15,3)))
summary(fit2)
```

```
##
## Formula: PTA54ADS ~ SSlogis(Leeftijd, Asym, xmid[group], scal[group])
```

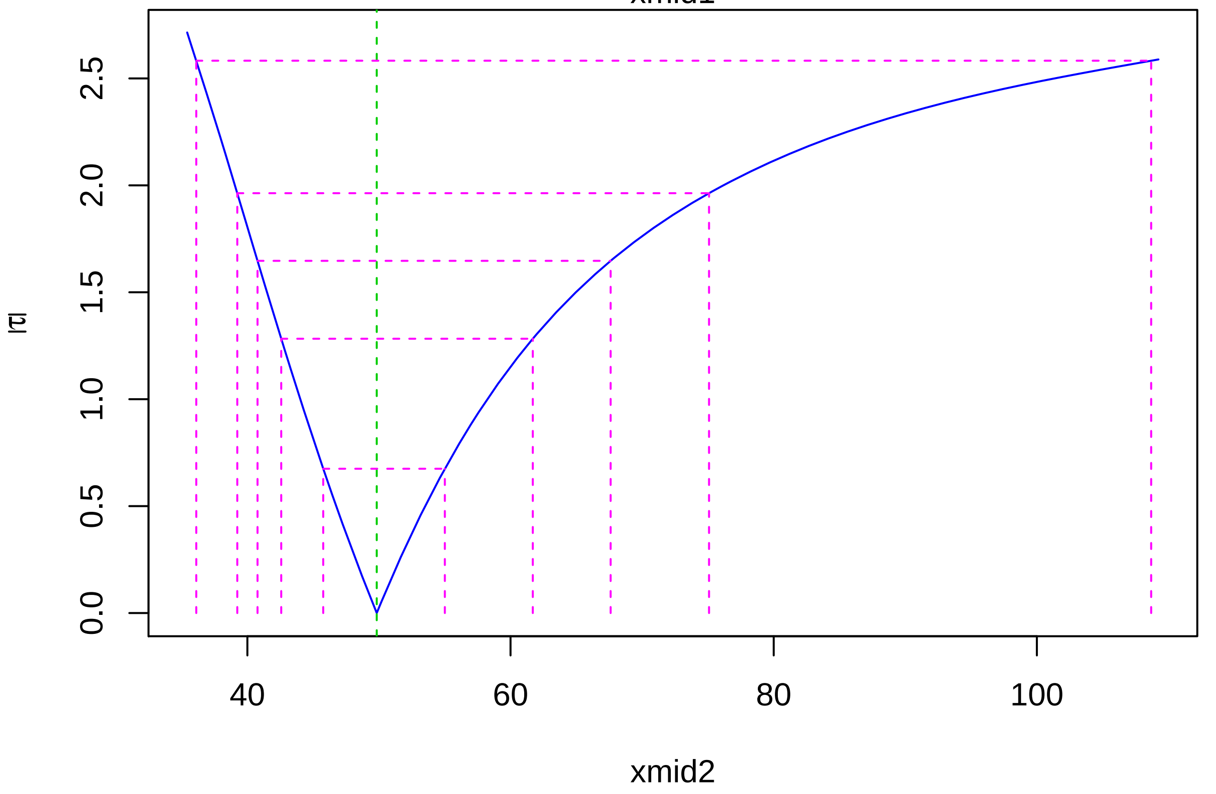
```
##
## Parameters:
##       Estimate Std. Error t value Pr(>|t|)
## Asym   123.891      8.563  14.468  < 2e-16 ***
## xmid1   56.304      2.381  23.652  < 2e-16 ***
## xmid2   49.833      6.541   7.618 8.24e-14 ***
## xmid3   37.568      5.595   6.715 3.86e-11 ***
## scal1   15.084      1.291  11.682  < 2e-16 ***
## scal2   24.884      8.419   2.956  0.00322 **
## scal3   26.860      6.824   3.936 9.09e-05 ***
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 19.05 on 709 degrees of freedom
##
## Number of iterations to convergence: 6
## Achieved convergence tolerance: 2.195e-06
```

```r
plot(profile(fit2))
```

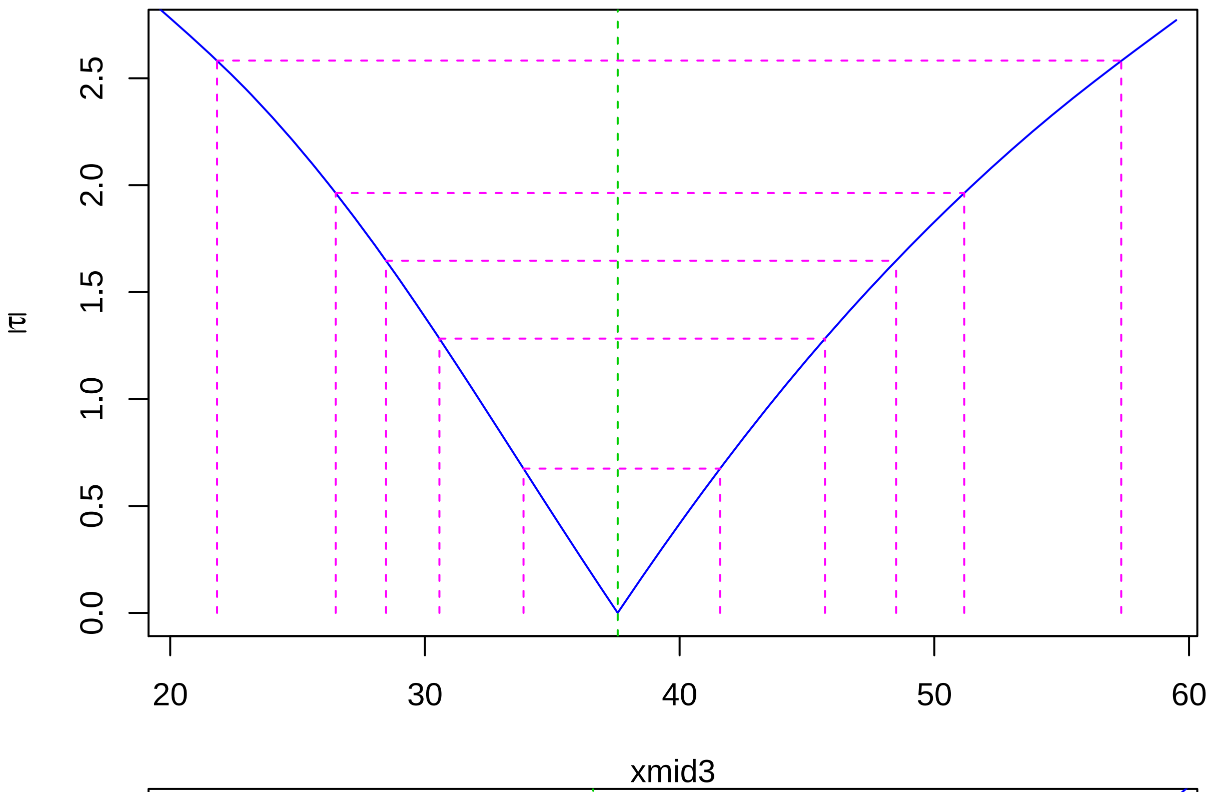




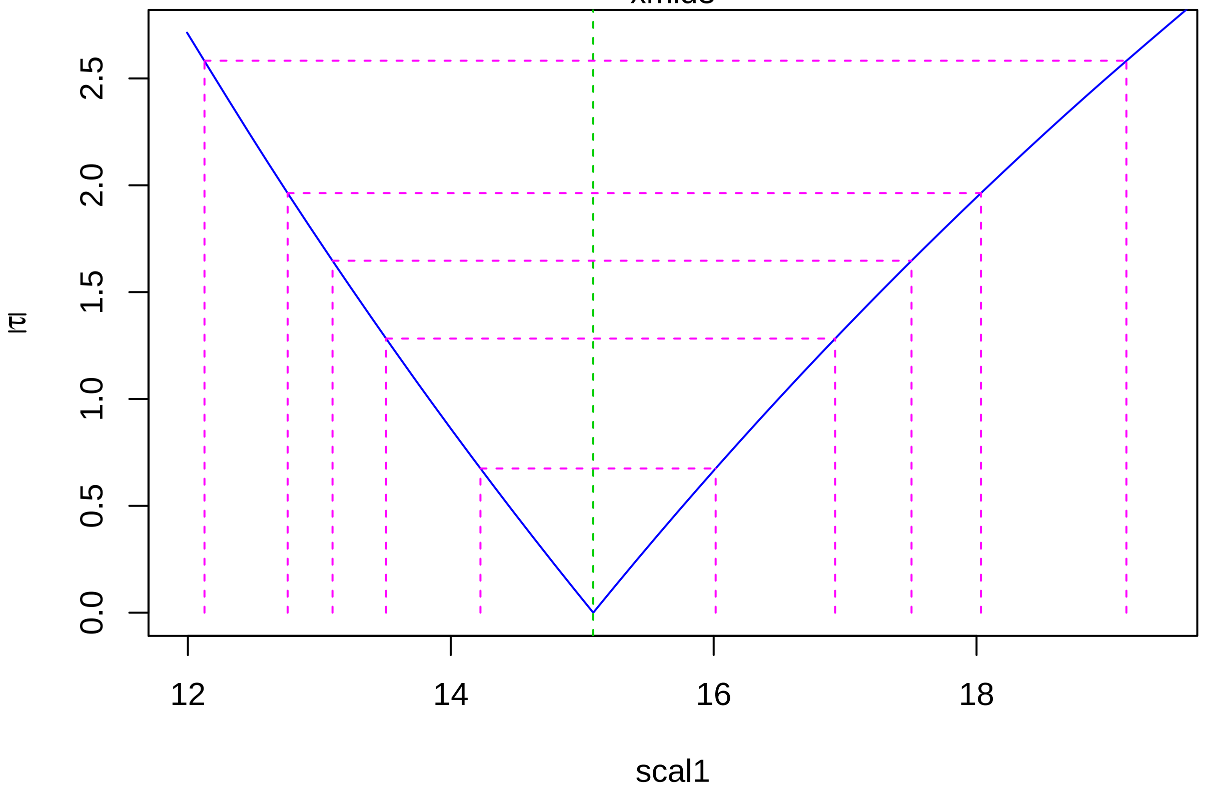
$|\tau|$
0.0
0.5
1.0
1.5
2.0
2.5
52
54
56
58
60
62
64
66
xmid1



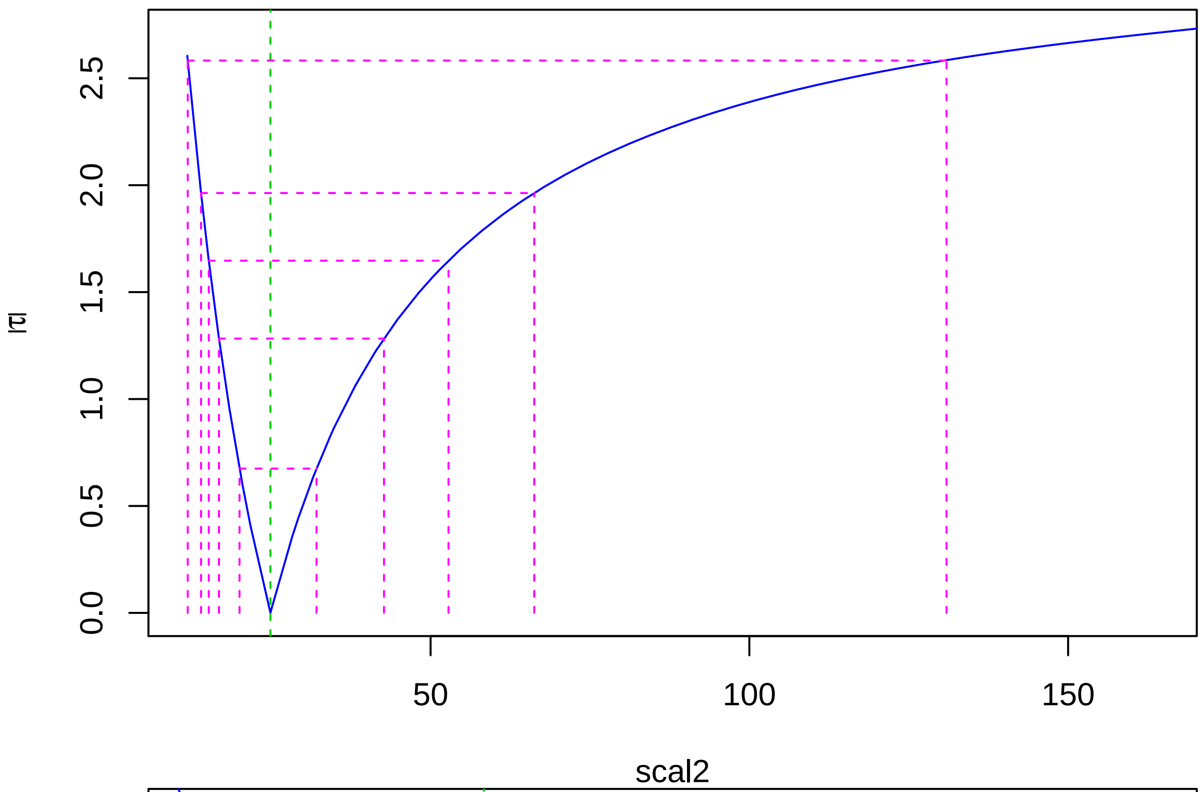
$|\tau|$
0.0
0.5
1.0
1.5
2.0
2.5
40
60
80
100
xmid2

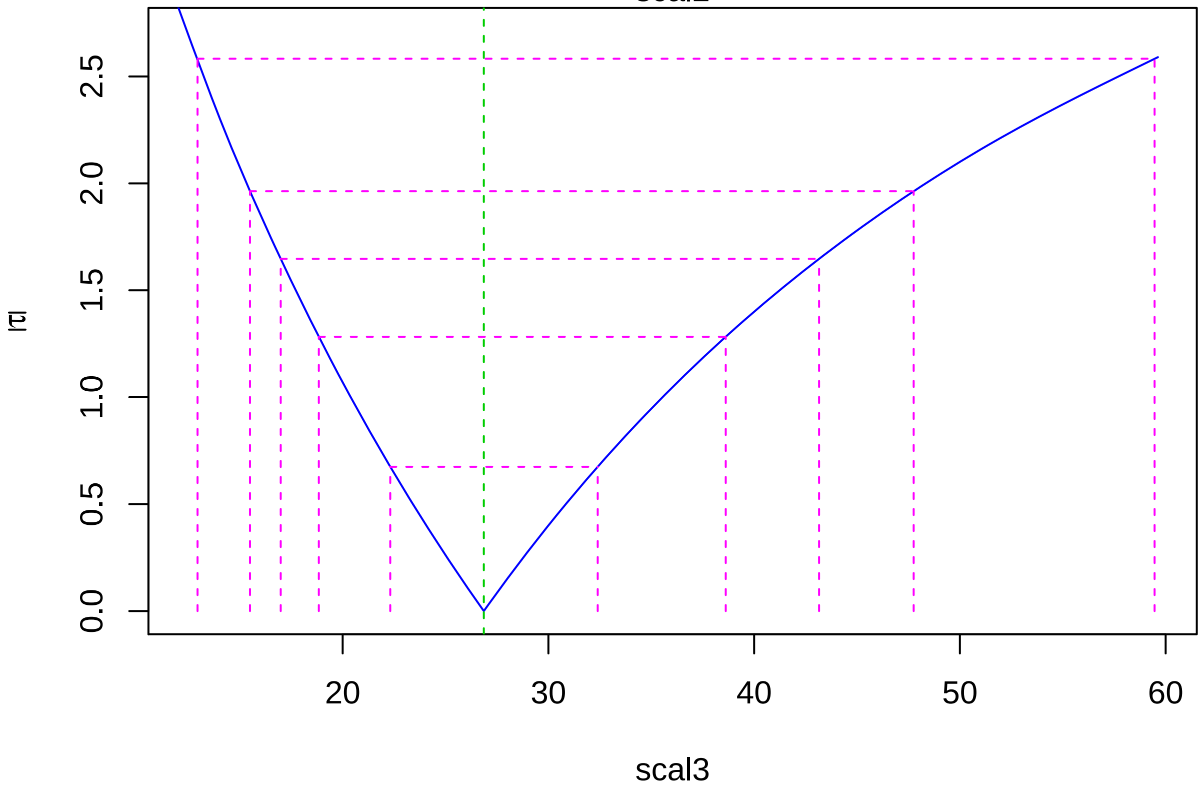


2.5
2.0
1.5
1.0
0.5
0.0
|τ|
20
30
40
50
60
xmid3



2.5
2.0
1.5
1.0
0.5
0.0
|τ|
12
14
16
18
scal1





And now add the asymptotic value of the fit (Leeftijd -> infinity) (Asym):

```
fit3 <- nls(PTA54ADS ~ SSlogis(Leeftijd, Asym[group], xmid[group], scal[group]),
            data=data,
            start=list(Asym=rep(120,3), xmid=rep(50,3), scal=rep(15,3)))
```

```r
summary(fit3)
```

```
##
## Formula: PTA54ADS ~ SSlogis(Leeftijd, Asym[group], xmid[group], scal[group])
##
## Parameters:
##       Estimate Std. Error t value Pr(>|t|)
## Asym1  126.350      9.361  13.497  < 2e-16 ***
## Asym2   89.933     60.449   1.488   0.1373
## Asym3   97.280     10.751   9.049  < 2e-16 ***
## xmid1   56.974      2.587  22.025  < 2e-16 ***
## xmid2   34.989     26.673   1.312   0.1900
## xmid3   27.148      4.666   5.819 8.99e-09 ***
## scal1   15.410      1.354  11.383  < 2e-16 ***
## scal2   17.424     17.929   0.972   0.3315
## scal3   13.988      5.807   2.409   0.0163 *
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 19.05 on 707 degrees of freedom
##
## Number of iterations to convergence: 8
## Achieved convergence tolerance: 3.258e-06
```

Now test the various models. Which of the parameters explain the data best?

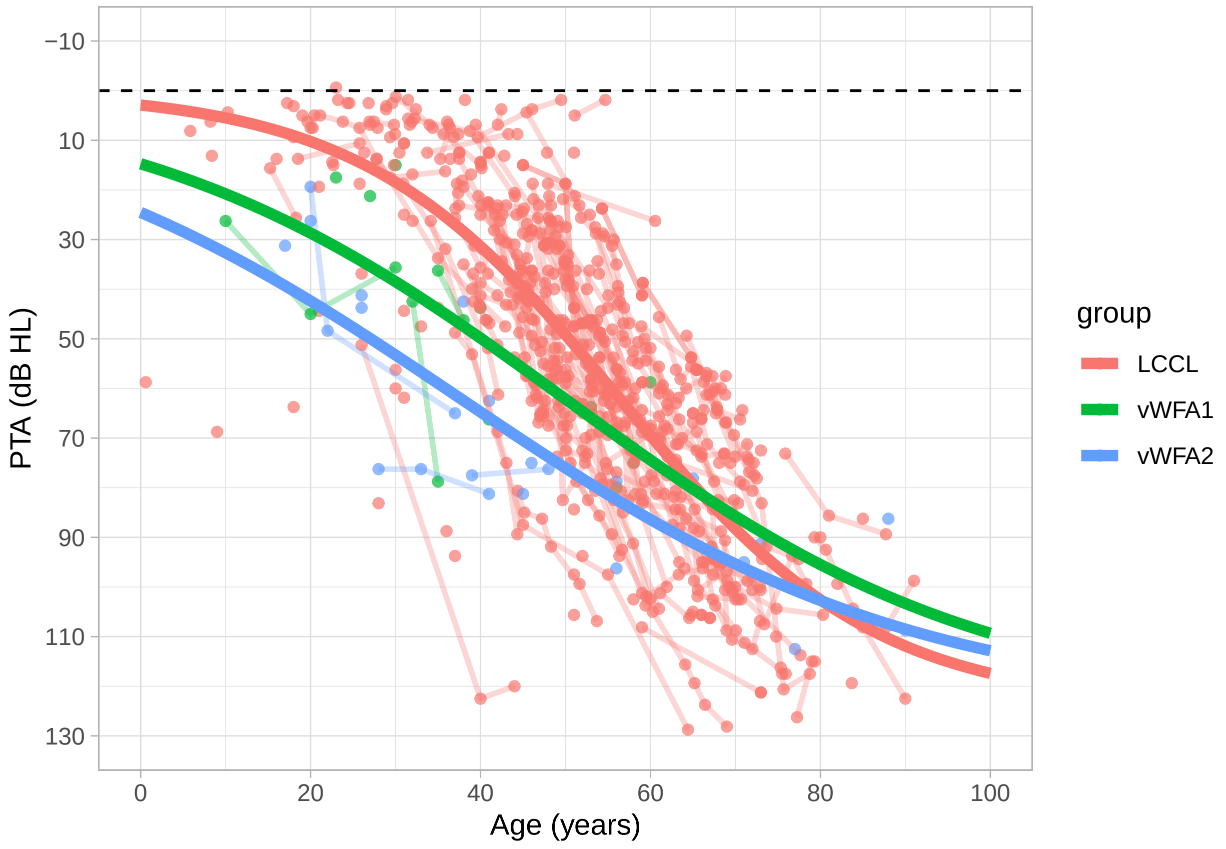
```r
anova(fit0,fit1,fit2,fit3)
```

```
## Analysis of Variance Table
##
## Model 1: PTA54ADS ~ SSlogis(Leeftijd, Asym, xmid, scal)
## Model 2: PTA54ADS ~ SSlogis(Leeftijd, Asym, xmid[group], scal)
## Model 3: PTA54ADS ~ SSlogis(Leeftijd, Asym, xmid[group], scal[group])
## Model 4: PTA54ADS ~ SSlogis(Leeftijd, Asym[group], xmid[group], scal[group])
##   Res.Df Res.Sum Sq Df  Sum Sq F value    Pr(>F)
## 1    713     279067
## 2    711     260324  2 18743.2 25.5958 1.844e-11 ***
## 3    709     257309  2  3014.8  4.1535   0.01609 *
## 4    707     256670  2   639.6  0.8809   0.41487
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

It turns out the both the variables [xmid] and [scale], i.e. the midpoint and slope at the midpoint significantly differ between the three groups, but that adding the asymptotic value does not describe the data signigicantly better.

```r
newdat = expand.grid(Leeftijd = seq(0, 100, by = 1), group = c("LCCL","vWFA1","vWFA2"))
newdat$fit <- predict(fit2, newdata = newdat)
```

```r
ggplot(data, aes(x=Leeftijd, y=PTA54ADS, group = pid, color = group)) +
  geom_point(aes(colour = factor(group)),alpha = .7) +
  geom_line(data=data, size=1, alpha = .3) +
  geom_line(data = newdat, aes(y = fit, group = group, colour = factor(group)), size = 2) +
  geom_hline(yintercept=0,linetype="dashed") +
  scale_x_continuous(breaks=seq(0,100,20)) +
  scale_y_reverse(breaks=seq(-10,130,20), limits=c(130,-10)) +
  #scale_y_reverse(limits=c(130,-10)) +
  xlab("Age (years)") +
  ylab("PTA (dB HL)") +
  theme_light()
```



Now perform fit on individual data by subsetting the data to keep individuals with more that 4 longitudinal datapoints. Is it the case that using a non-linear mixed-model approach may helpt us?

```
#https://stackoverflow.com/questions/14439770/filter-rows-in-dataframe-by-number-of-rows-per-level-of-a
pidlengths <- ave(as.numeric(data$pid),
                   data$pid, FUN = length)
#df2 <- lccl[pidlengths > 5, ]
df2 <- data[pidlengths > 2, ]
with(df2, table(group))
```

```
## group
##  LCCL vWFA1 vWFA2
##   459     3     6
```

```
models <- nlsList(PTA54ADS ~ SSlogis(Leeftijd, Asym, xmid, scal) | pid, data = df2)
```

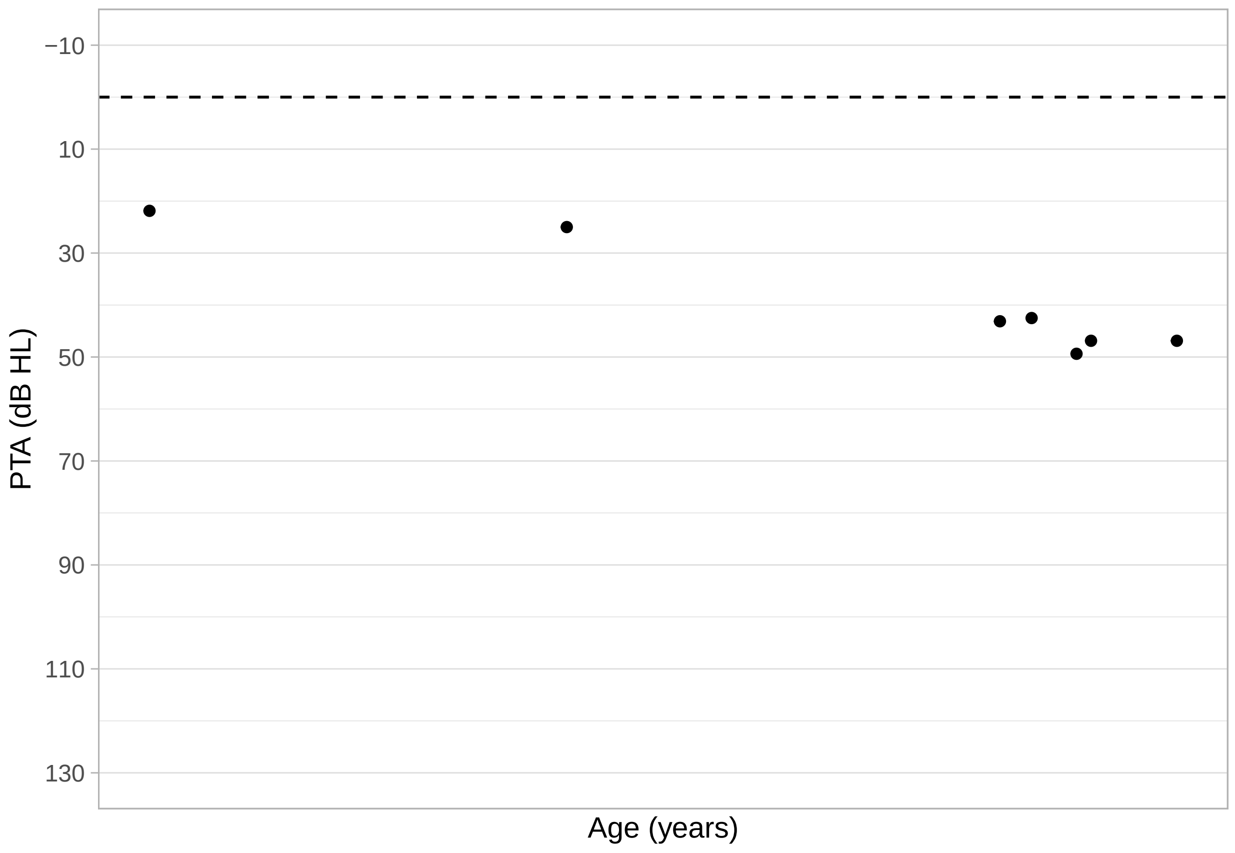
```
## Warning: 37 errors caught in (attr(object, "initial"))(mCall = mCall, data = data, LHS = LHS).  The
##
##                                        singular matrix 'a' in solve
##                                                                   1
##                                                    singular gradient
##                                                                   2
## step factor 0.000488281 reduced below 'minFactor' of 0.000976562
##                                                               12
##             too few distinct input values to fit a logistic model
##                                                               22
```

As we can see, some model-predictions failed; they end up with NaNs in the model fit list (nlslist); see e.g. pid 147

```
data_id <- subset(df2, pid=="147")
data_id
```

```
## # A tibble: 7 x 4
##     pid group Leeftijd PTA54ADS
##   <dbl> <fct>    <dbl>    <dbl>
## 1   147 LCCL      49.7     21.9
## 2   147 LCCL      52.9     25
## 3   147 LCCL      56.4     42.5
## 4   147 LCCL      56.1     43.1
## 5   147 LCCL      56.8     46.9
## 6   147 LCCL      57.5     46.9
## 7   147 LCCL      56.7     49.4
```

```
ggplot(data=data_id,  aes(x=Leeftijd, y=PTA54ADS)) +
  geom_point() +
  geom_hline(yintercept=0,linetype="dashed") +
  scale_x_continuous(breaks=seq(0,100,20)) +
  scale_y_reverse(breaks=seq(-10,130,20), limits=c(130,-10)) +
  #scale_y_reverse(limits=c(130,-10)) +
  xlab("Age (years)") +
  ylab("PTA (dB HL)") +
  theme_light()
```



Predict for all pid's the fit to the model and remove the pid's that give NaNs. Check how many subjects per group we end up with.

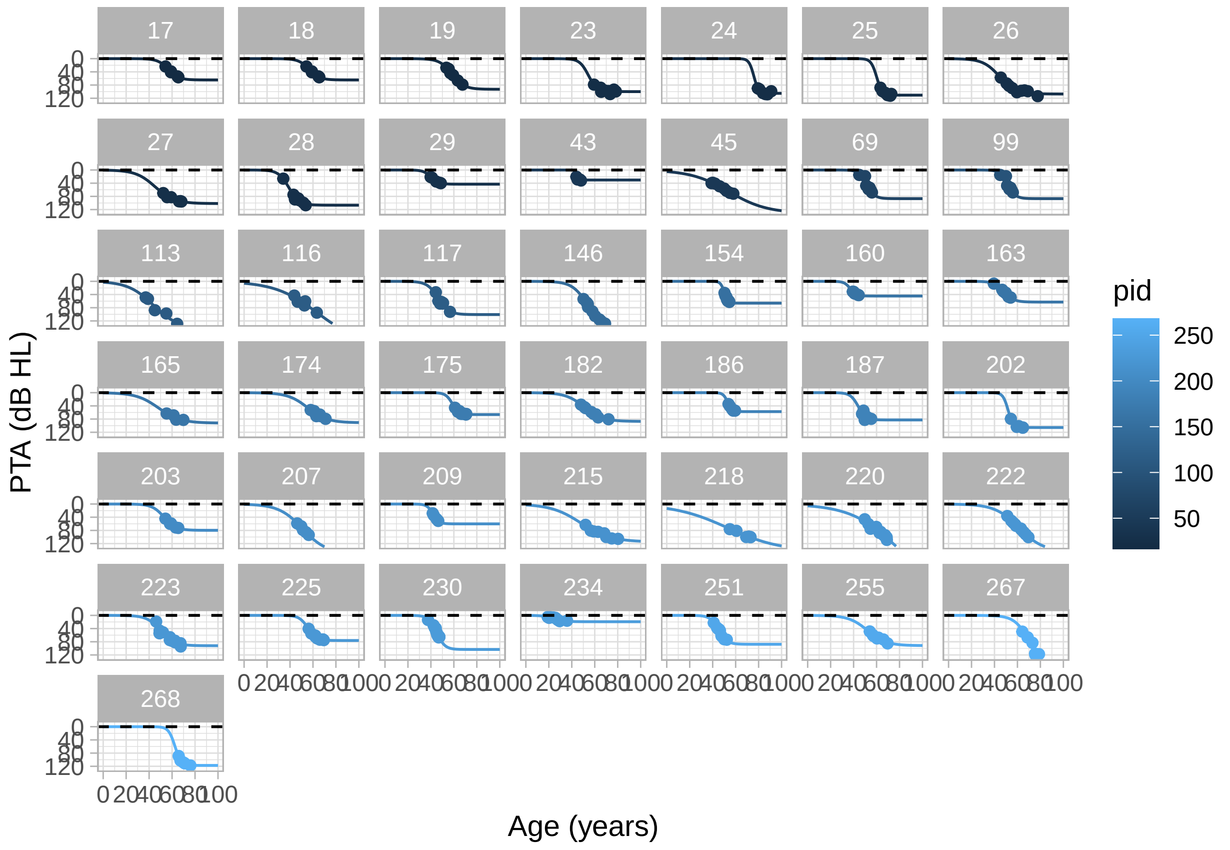
```
df2$Pred <-predict(models)
df2_na <- na.omit(df2)
with(df2_na, table(group, pid))
```

```
##        pid
## group   17  18  19  23  24  25  26  27  28  29  43  45  69  99  113  116  117  146  154  160  163
##   LCCL   6   6   6   8   6   6   9   6   9   5   4   8   7   7    5    5    7   10   10    5    8
##   vWFA1  0   0   0   0   0   0   0   0   0   0   0   0   0   0    0    0    0    0    0    0    0
##   vWFA2  0   0   0   0   0   0   0   0   0   0   0   0   0   0    0    0    0    0    0    0    0
##        pid
## group   165  174  175  182  186  187  202  203  207  209  215  218  220  222  223  225  230  234
##   LCCL    4    7    8    8    5    6    5    5    5    4    8    6   11   10   11    9   14   10
##   vWFA1   0    0    0    0    0    0    0    0    0    0    0    0    0    0    0    0    0    0
##   vWFA2   0    0    0    0    0    0    0    0    0    0    0    0    0    0    0    0    0    0
##        pid
## group   251  255  267  268
##   LCCL    8    7    8    5
##   vWFA1   0    0    0    0
##   vWFA2   0    0    0    0
```

We only keep the pid's from the LCCL group. The pid's in the other groups are not properly fitted.

```
le <- unique(df2_na$pid)
#le
newdat = expand.grid(Leeftijd = seq(0, 100, by = 1), pid=le)
newdat$prednlm <-predict(models, newdata=newdat)
```

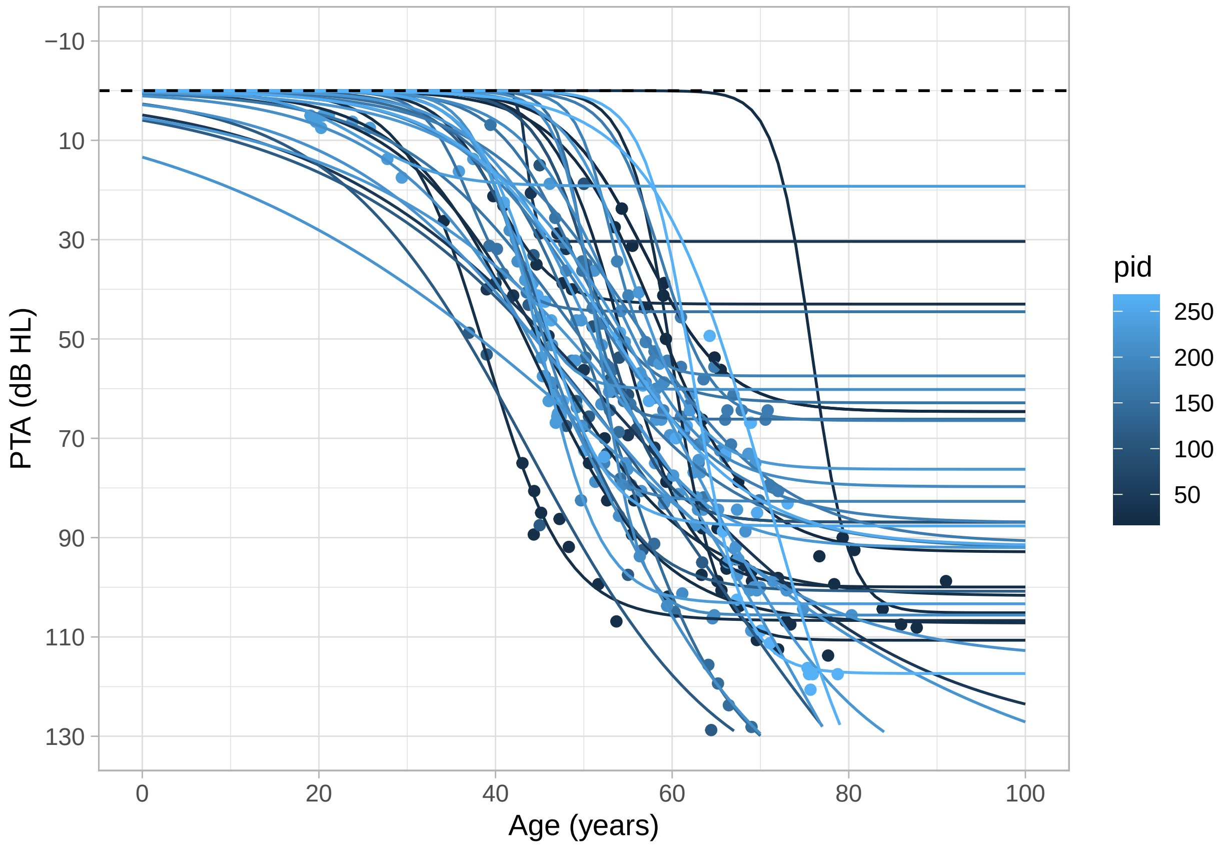
```r
#https://stackoverflow.com/questions/37122994/plotting-a-list-of-non-linear-regressions-with-ggplot
#https://aosmith.rbind.io/2018/11/16/plot-fitted-lines/
ip <- ggplot(data=df2_na,  aes(x=Leeftijd, y=PTA54ADS, colour = pid)) +
  geom_point() +
  geom_line(data=newdat,aes(y=prednlm)) +
  facet_wrap(~pid) +
  geom_hline(yintercept=0,linetype="dashed") +
  scale_x_continuous(breaks=seq(0,100,20)) +
  scale_y_reverse(breaks=seq(0,120,40), limits=c(130,-10)) +
  #scale_y_reverse(limits=c(130,-10)) +
  xlab("Age (years)") +
  ylab("PTA (dB HL)") +
  theme_light()
ip
```



So, it seeems we can fit the data for individual subjects by some extent. It often ‘fails’ by over- or underestimating the tail (coef.lmlist Asym column). We can also plot it all in one figure.

```r
ggplot(data=df2_na,  aes(x=Leeftijd, y=PTA54ADS, colour = pid)) +
  geom_point() +
  geom_line(data=newdat,aes(y=prednlm, group = pid)) +
  geom_hline(yintercept=0,linetype="dashed") +
  scale_x_continuous(breaks=seq(0,100,20)) +
  scale_y_reverse(breaks=seq(-10,130,20), limits=c(130,-10)) +
  #scale_y_reverse(limits=c(130,-10)) +
  xlab("Age (years)") +
  ylab("PTA (dB HL)") +
  theme_light()
```

```
## Warning: Removed 176 row(s) containing missing values (geom_path).
```



Feed the remaining data into the non-linear mixed-models with the parameters Asym, xmid, and scal as random factors.

```
nm1 <- nlmer(PTA54ADS ~ SSlogis(Leeftijd, Asym, xmid, scal) ~ Asym + xmid + scal | pid, df2_na, start =
summary(nm1)
```

```
## Warning in vcov.merMod(object, use.hessian = use.hessian): variance-covariance matrix computed from 
## not positive definite or contains NA values: falling back to var-cov estimated from RX

## Warning in vcov.merMod(object, correlation = correlation, sigm = sig): variance-covariance matrix co
## not positive definite or contains NA values: falling back to var-cov estimated from RX

## Nonlinear mixed model fit by maximum likelihood  ['nlmerMod']
## Formula: PTA54ADS ~ SSlogis(Leeftijd, Asym, xmid, scal) ~ Asym + xmid +
##     scal | pid
##    Data: df2_na
##
##      AIC      BIC   logLik deviance df.resid
##   2152.6   2189.9  -1066.3   2132.6      297
##
## Scaled residuals:
##      Min       1Q   Median       3Q      Max
## -3.14923 -0.50051 -0.03471  0.44682  2.94717
##
```

```
## Random effects:
##  Groups   Name Variance Std.Dev. Corr
##  pid      Asym 166.742  12.913
##           xmid  44.562   6.675    0.23
##           scal   4.541   2.131   -0.53  0.11
##  Residual       24.231   4.922
## Number of obs: 307, groups:  pid, 43
##
## Fixed effects:
##      Estimate Std. Error t value
## Asym 113.0219     3.6536   30.93
## xmid  52.5762     1.1214   46.88
## scal   8.4703     0.5342   15.86
##
## Correlation of Fixed Effects:
##      Asym  xmid
## xmid 0.409
## scal 0.343 0.265
```

```
coef(nm1)
```

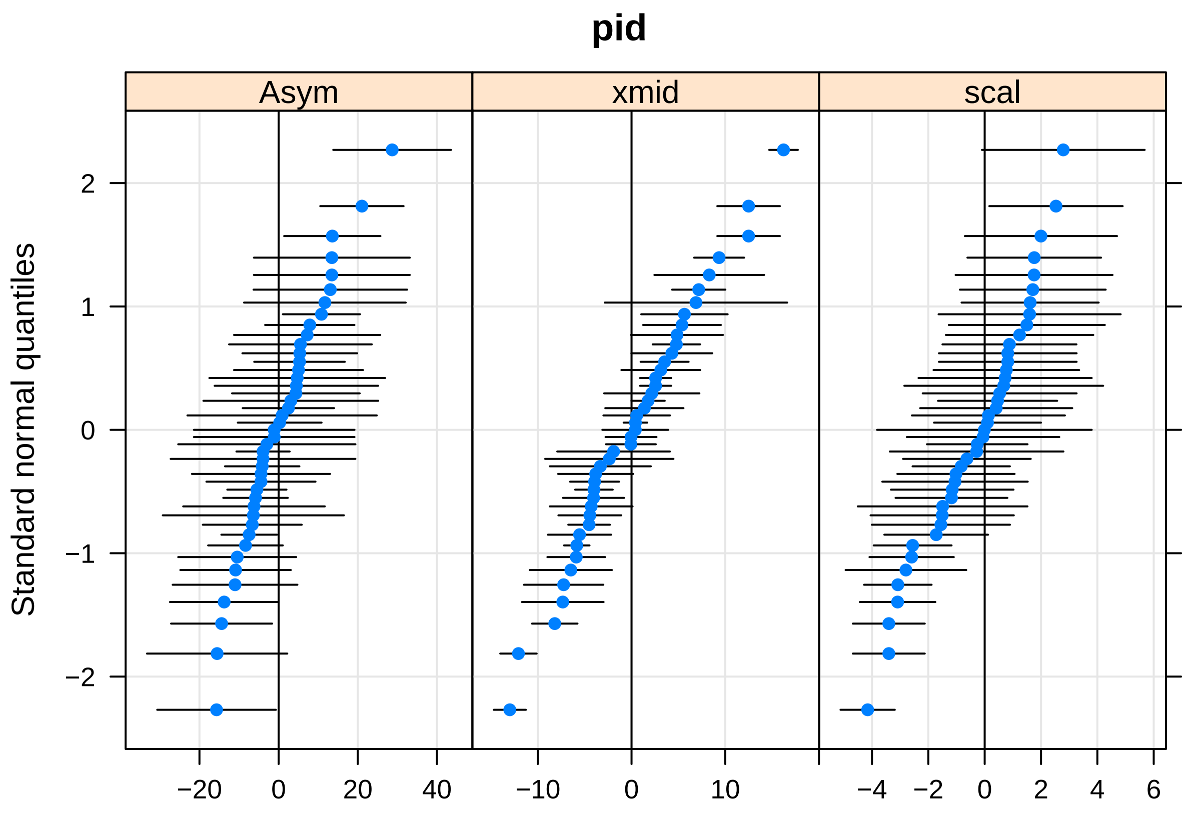
```
## $pid
##          Asym     xmid      scal
## 17  111.91187 65.06896  9.290262
## 18  111.91187 65.06896  9.290262
## 19  118.54557 61.92052  7.638449
## 23  105.58459 49.24825  8.413491
## 24  108.87821 59.45191  9.966515
## 25  120.90609 54.73558  7.417888
## 26  109.08970 44.38473  7.839455
## 27  104.65608 46.09939  8.880052
## 28  113.29662 39.58856  5.379525
## 29  110.04858 52.98010  9.196106
## 43  113.90947 55.69913  8.459887
## 45   97.34802 45.33117 11.001626
## 69  126.47538 55.14536  5.067873
## 99  126.47538 55.14536  5.067873
## 113 123.85066 40.51042  7.292063
## 116 108.56522 47.02774  9.350318
## 117 118.05750 48.05015  6.749991
## 146 134.07677 52.99453  5.877904
## 154 124.71754 54.34416  5.675409
## 160 109.08630 48.12987  9.147920
## 163 117.51108 56.10667  7.451837
## 165  99.26396 50.66147 10.224177
## 174 102.01522 57.42787 10.467515
## 175  97.48613 60.86962 11.260595
## 182  98.61739 53.12833 10.230050
## 186 116.11258 59.74771  8.184632
## 187 108.57918 45.24103  8.603256
## 202 118.30537 48.75103  6.916644
## 203 106.80804 56.87895  9.709881
## 207 117.38917 46.67863  7.318511
## 209 117.73015 48.63513  6.977685
## 215 107.53529 48.52569  9.239854
## 218 107.19360 48.27288  9.002671
## 220 115.49471 52.50751  8.928806
## 222 118.37714 57.36307  8.206486
## 223 106.36951 52.52182  8.571337
## 225 102.56742 57.96347 10.083785
## 230 126.08938 46.73509  4.317173
## 234 106.64376 50.20636 10.065763
## 251 120.23932 48.56520  5.913720
## 255 102.15156 53.94762 10.178729
## 267 141.71632 68.79567  5.386864
## 268 126.58949 58.22265  6.962851
##
## attr(,"class")
## [1] "coef.mer"
```

```
require(lattice)
```

```
## Loading required package: lattice
```

```
qqmath(ranef(nm1, condVar=TRUE))
```

```
## $pid
```



Now subset the data to contain individual frequencies

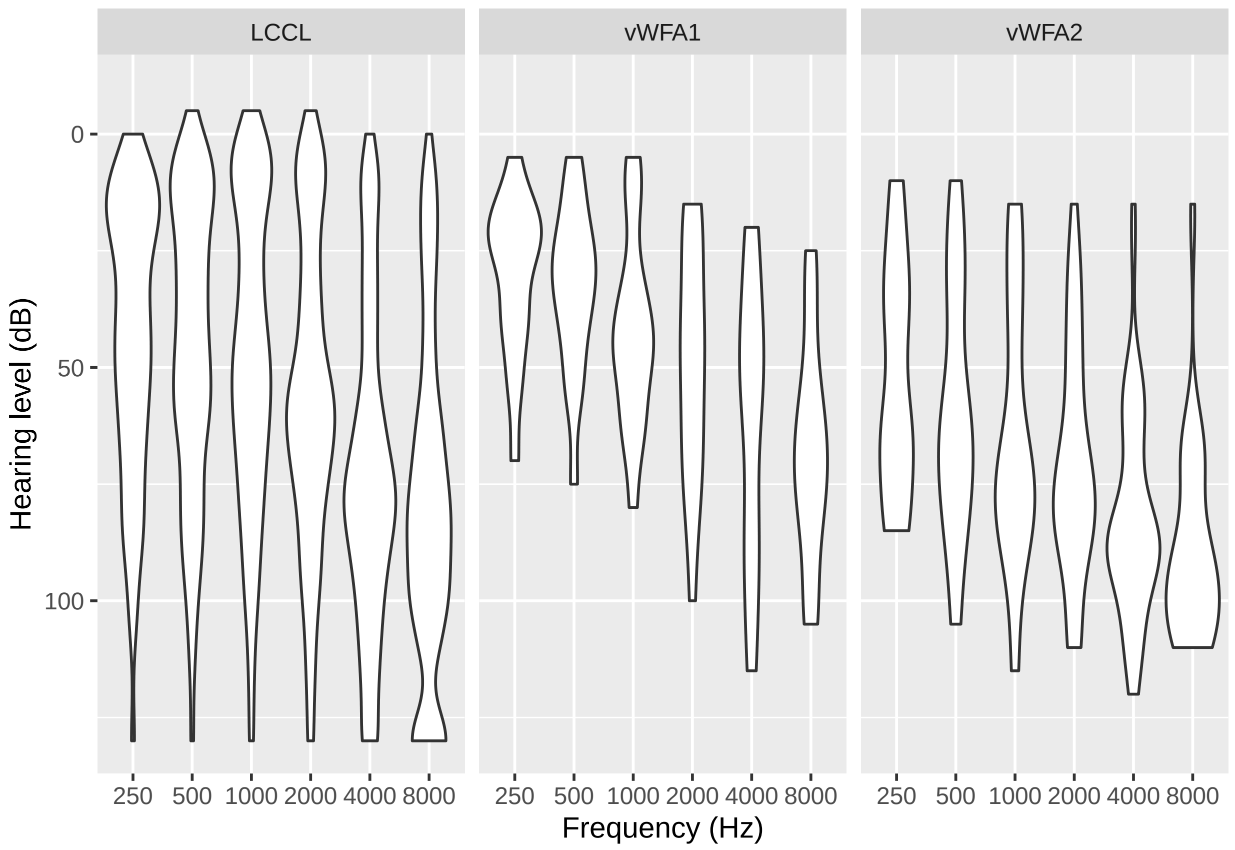
```
data_all <- subset(data_subset, select=c('pid', 'group', 'Leeftijd', '250.AD','500.AD', '1000.AD','2000
head(data_all)
```

```
## # A tibble: 6 x 15
##     pid group Leeftijd `250.AD` `500.AD` `1000.AD` `2000.AD` `4000.AD` `8000.AD`
##   <dbl> <fct>    <dbl>    <dbl>    <dbl>     <dbl>     <dbl>     <dbl>     <dbl>
## 1      1 LCCL         60        NA        45        50        60        80        85
## 2      2 LCCL         82        70        75       100       130       130        NA
## 3      3 LCCL         68        80        80        80        90       130       130
## 4      4 LCCL         48        10         5        10        15        65        60
## 5      4 LCCL         55        20        20        15        40        80        85
## 6      5 LCCL         48         5         5         0         0        45        60
## # ... with 6 more variables: `250.AS` <dbl>, `500.AS` <dbl>, `1000.AS` <dbl>,
## #   `2000.AS` <dbl>, `4000.AS` <dbl>, `8000.AS` <dbl>
```

Convert 'wide' dataset into 'long' format using tidyr and remove NaNs

```
tidier <- data_all %>%
  gather(f, dB, -pid, -group, -Leeftijd)
data_all_l <- tidier %>%
  separate(f, into = c("frequency", "ear"), sep = "\\.")
#head(data_all_l)
data_all_l$frequency = factor(data_all_l$frequency)
data_all_l$ear= factor(data_all_l$ear)
data_all_l <- na.omit(data_all_l)
#head(data_all_l)
```

```
p <- data_all_l%>%
  mutate(frequency = fct_relevel(frequency,"250", "500", "1000", "2000", "4000", "8000")) %>%
  ggplot(aes(x = frequency, y = dB)) +
    #geom_bar(stat="identity") +
    #geom_histogram() +
    geom_violin() +
    facet_wrap(~ group, ncol = 3) +
    #geom_point()
    xlab("Frequency (Hz)") +
    ylab("Hearing level (dB)") +
    scale_y_reverse(limits=c(130,-10))
    #theme_classic()
p
```



simple linear model: PTA is a function of the affected domain; here are two levels in this mixed model; 1: timepoints for each patient; 2: genetic domain, with domain the fixed effect and patient the random effect allowing the intercept to vary across patient (~1|pid).

```
random_intercept <-lme(dB ~ frequency ,
            random = ~1|pid,    #p. 896
            method = "ML",
            na.action = na.exclude,
            control = list(opt="optim"),
            correlation = corAR1(),  #see p.897; timepoints are not equally spaced;use corCAR1
            data = data_all_l)
summary(random_intercept)
```

```
## Linear mixed-effects model fit by maximum likelihood
##  Data: data_all_l
##      AIC      BIC logLik
##    70816 70879.26 -35399
##
## Random effects:
##  Formula: ~1 | pid
##         (Intercept) Residual
## StdDev:    30.66465 17.63885
##
## Correlation Structure: AR(1)
##  Formula: ~1 | pid
##  Parameter estimate(s):
##       Phi
## 0.4384654
## Fixed effects: dB ~ frequency
##                   Value Std.Error   DF  t-value p-value
## (Intercept)   43.50693 1.9538723 8068 22.26703  0.0000
## frequency2000  6.76735 0.6766851 8068 10.00074  0.0000
## frequency250  -2.45973 0.7709764 8068 -3.19041  0.0014
## frequency4000 22.58725 0.7647918 8068 29.53386  0.0000
## frequency500  -1.96178 0.6766216 8068 -2.89937  0.0037
## frequency8000 30.51271 0.7951280 8068 38.37459  0.0000
##  Correlation:
##               (Intr) fr2000 frq250 fr4000 frq500
## frequency2000 -0.173
## frequency250  -0.197  0.410
## frequency4000 -0.196  0.565  0.479
## frequency500  -0.174  0.362  0.561  0.414
## frequency8000 -0.201  0.454  0.540  0.612  0.436
##
## Standardized Within-Group Residuals:
##        Min         Q1        Med         Q3        Max
## -4.2543260 -0.5913403  0.0373855  0.6194437  3.9226279
##
## Number of Observations: 8347
## Number of Groups: 274
```

```r
anova(random_intercept)
```

```
##             numDF denDF  F-value p-value
## (Intercept)     1  8068 783.1713  <.0001
## frequency       5  8068 538.4169  <.0001
```

now add Leeftijd as fixed effect; PTA ~ Domain + Leeftijd‘ (see. e.g. p.897)

```r
timeRI <- update(random_intercept, .~. + Leeftijd)
summary(timeRI)
```

```
## Linear mixed-effects model fit by maximum likelihood
##  Data: data_all_l
##        AIC      BIC    logLik
##   67842.65 67912.94 -33911.32
##
## Random effects:
##  Formula: ~1 | pid
##         (Intercept) Residual
## StdDev:   0.1104064 24.88384
##
## Correlation Structure: AR(1)
##  Formula: ~1 | pid
##  Parameter estimate(s):
##       Phi
## 0.8322339
## Fixed effects: dB ~ frequency + Leeftijd
##                    Value Std.Error   DF   t-value p-value
## (Intercept)   -50.31584 1.6018876 8067 -31.41034  0.0000
## frequency2000   5.13859 0.5814116 8067   8.83812  0.0000
## frequency250   -1.30225 0.7394264 8067  -1.76116  0.0782
## frequency4000  20.24899 0.7377572 8067  27.44668  0.0000
## frequency500   -0.94485 0.5808559 8067  -1.62665  0.1038
## frequency8000  29.31740 0.8033935 8067  36.49196  0.0000
## Leeftijd        1.93967 0.0258142 8067  75.13958  0.0000
##  Correlation:
##               (Intr) fr2000 frq250 fr4000 frq500 fr8000
## frequency2000 -0.196
## frequency250  -0.228  0.283
## frequency4000 -0.262  0.635  0.419
## frequency500  -0.176  0.195  0.631  0.284
## frequency8000 -0.304  0.464  0.551  0.713  0.369
## Leeftijd      -0.819  0.018 -0.023  0.032 -0.015  0.049
##
## Standardized Within-Group Residuals:
##         Min          Q1         Med          Q3         Max
## -3.07541243 -0.70824164 -0.07201688  0.58548467  5.01653320
##
## Number of Observations: 8347
## Number of Groups: 274
```

```
sessionInfo()
```

```
## R version 3.6.0 (2019-04-26)
## Platform: x86_64-apple-darwin15.6.0 (64-bit)
## Running under: macOS  10.15.4
##
## Matrix products: default
## BLAS:   /Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRblas.0.dylib
## LAPACK: /Library/Frameworks/R.framework/Versions/3.6/Resources/lib/libRlapack.dylib
##
## locale:
## [1] en_US.UTF-8/en_US.UTF-8/en_US.UTF-8/C/en_US.UTF-8/en_US.UTF-8
##
## attached base packages:
## [1] stats     graphics  grDevices utils     datasets  methods   base
##
## other attached packages:
##  [1] lattice_0.20-40 dplyr_0.8.4     tidyr_1.0.2     forcats_0.5.0
##  [5] knitr_1.28      lme4_1.1-21     Matrix_1.2-18   nlme_3.1-145
##  [9] readxl_1.3.1    sjPlot_2.8.2    drc_3.0-1       MASS_7.3-51.5
## [13] ggplot2_3.3.0
##
## loaded via a namespace (and not attached):
##  [1] splines_3.6.0     carData_3.0-3     modelr_0.1.6      gtools_3.8.1
##  [5] assertthat_0.2.1  highr_0.8         cellranger_1.1.0  yaml_2.2.1
##  [9] bayestestR_0.5.2  pillar_1.4.3      backports_1.1.5   glue_1.4.0
## [13] digest_0.6.25     minqa_1.2.4       colorspace_1.4-1  sandwich_2.5-1
## [17] htmltools_0.4.0   pkgconfig_2.0.3   broom_0.5.5       haven_2.2.0
## [21] purrr_0.3.3       xtable_1.8-4      mvtnorm_1.1-0     scales_1.1.0
## [25] openxlsx_4.1.4    rio_0.5.16        emmeans_1.4.5     tibble_2.1.3
## [29] generics_0.0.2    farver_2.0.3      car_3.0-6         ellipsis_0.3.0
## [33] sjlabelled_1.1.3  TH.data_1.0-10    withr_2.1.2       cli_2.0.2
## [37] survival_3.1-8    magrittr_1.5      crayon_1.3.4      effectsize_0.2.0
## [41] estimability_1.3  evaluate_0.14     fansi_0.4.1       foreign_0.8-76
## [45] tools_3.6.0       data.table_1.12.8 hms_0.5.3         lifecycle_0.2.0
## [49] multcomp_1.4-12   stringr_1.4.0     munsell_0.5.0     plotrix_3.7-7
## [53] zip_2.0.4         ggeffects_0.14.1  compiler_3.6.0    rlang_0.4.5
## [57] grid_3.6.0        nloptr_1.2.1      parameters_0.5.0  labeling_0.3
## [61] rmarkdown_2.1     boot_1.3-24       gtable_0.3.0      codetools_0.2-16
## [65] abind_1.4-5       sjstats_0.17.9    curl_4.3          sjmisc_2.8.3
## [69] R6_2.4.1          zoo_1.8-7         performance_0.4.4 utf8_1.1.4
## [73] insight_0.8.1     stringi_1.4.6     Rcpp_1.0.4.6      vctrs_0.2.4
## [77] tidyselect_1.0.0  xfun_0.12
```