# Cystic Fibrosis and Pulmonary Function

Cystic fibrosis is a genetic disease that leads to pulmonary complications and ultimately death.

These data represent a subsample of measurements available in a registry database.

Several specific aspects are of interest:

- What is the rate of decline in FEV1?
- Is the time course different for males and females?
- Is the time course different for F508 homozygous subjects?

Davis P.B. (1997) *Journal of Pediatrics*

(Borrowed from Patrick)

# Data

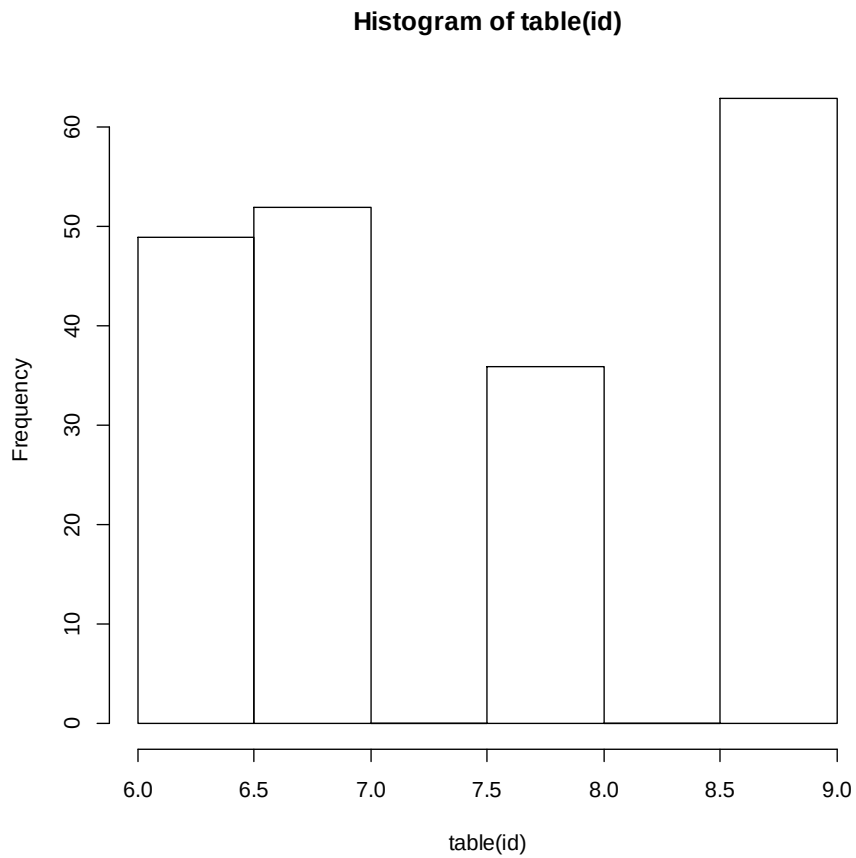
```
> data <- read.table( "NewCFkids.dat", header=F )

> cfkids <- data.frame(
                      id = data[,1],
                      fev1 = data[,2],
                      age = data[,3],
                      female = as.integer( data[,4]==2 ),
                      pseudoA = as.integer( data[,5]==3 ),
                      f508 = 3-data[,6],
                      pancreat = as.integer( data[,7]==2 ))

> cfkids[1:10,]
       id   fev1    age female pseudoA f508 pancreat
1  100073 113.80  8.452      1       1    2        1
2  100073  98.18  8.783      1       1    2        1
3  100073  98.73  9.785      1       1    2        1
4  100073 101.79 10.538      1       1    2        1
5  100073  98.04 12.329      1       1    2        1
6  100073  94.32 13.306      1       1    2        1
7  100073  95.48 14.418      1       1    2        1
8  100111  96.85 12.515      1       0    0        0
9  100111 101.05 13.103      1       0    0        1
10 100111 100.33 15.105      1       0    0        1
```

- ID = patient id
- FEV1 = percent-predicted forced expiratory volume in 1 second
- AGE = age (years)
- GENDER = sex (1=male, 2=female)
- PSEUDOA = infection with Pseudo Aeruginosa (0=no, 3=yes)
- F508 = genotype (1=homozygous, 2=heterozygous, 3=none)
- PANCREAT = pancreatic enzyme supplmentation (0,1=no, 2=yes)

# Data

```
> attach(cfkids)

> length(id)
[1] 1513

> length(table(id))
[1] 200

> hist(table(id))

> table(female)
female
  0   1
773 740

> summary(age)
   Min. 1st Qu.  Median    Mean 3rd Qu.    Max.
  5.002  11.617  15.255  15.776  19.677  29.906



> table(f508)
f508
  0   1   2
165 660 688
```

What's wrong with these?

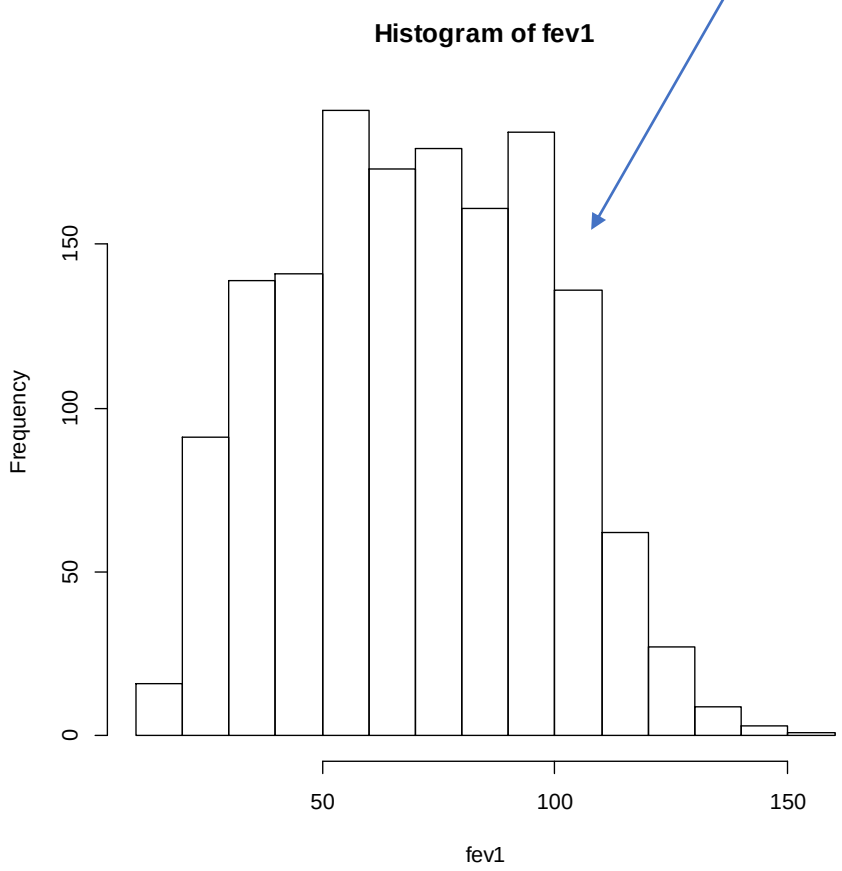


# Age at Entry

```
> age_entry = (cfkids %>% group_by(id) %>% top_n(-1, age))$age  ## this uses the dplyr package
> cfkids$age0 <- age0 <- rep(age_entry, times = table(id)) ## age at entry
> cfkids$ageL <- ageL <- cfkids$age - cfkids$age0  ## age since entry

> stem(age_entry)

  The decimal point is at the |

   5 | 002355666788889
   6 | 0111222334555567789999
   7 | 0001234446778889
   8 | 011223345566899
   9 | 00011244788
  10 | 0111113349
  11 | 2223446678
  12 | 00111222344455577888888999
  13 | 01234456
  14 | 111245555779
  15 | 001223357
  16 | 0012347899
  17 | 1223567779
  18 | 4899
  19 | 4
  20 | 0123778
  21 | 15577
  22 | 2459
  23 | 001128
```

# FEV1

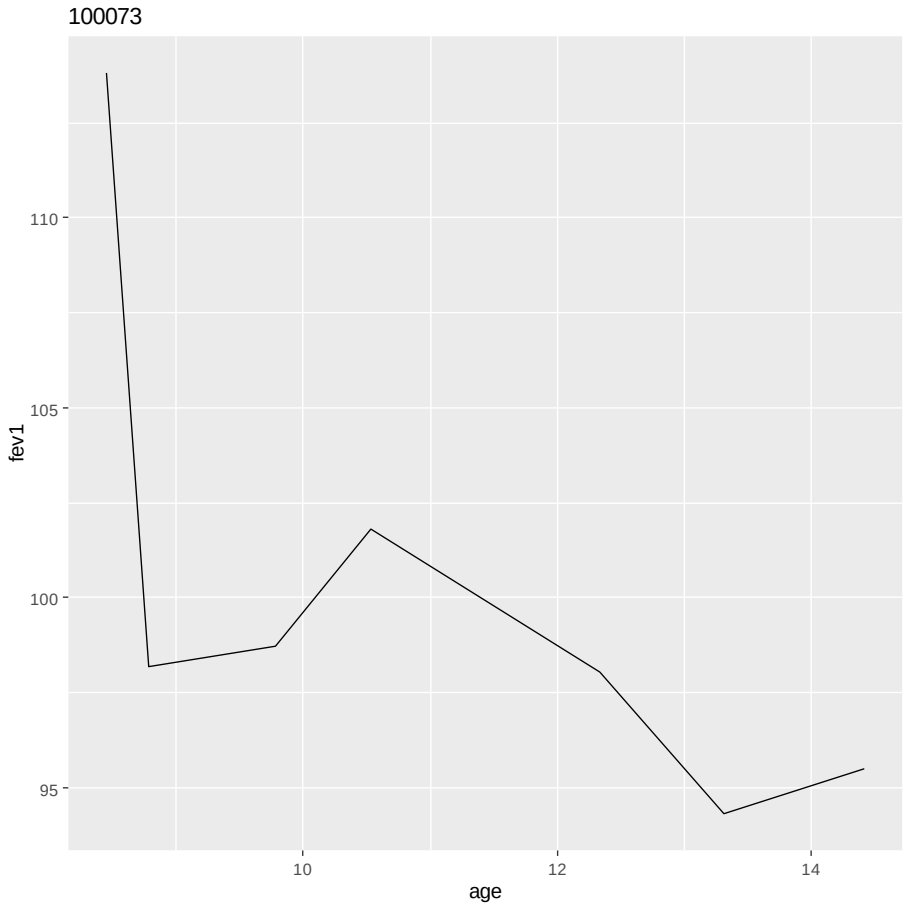
Consider transformation *IF* ugly distribution: log, sqrt, square, arctan;
here, it looks ok



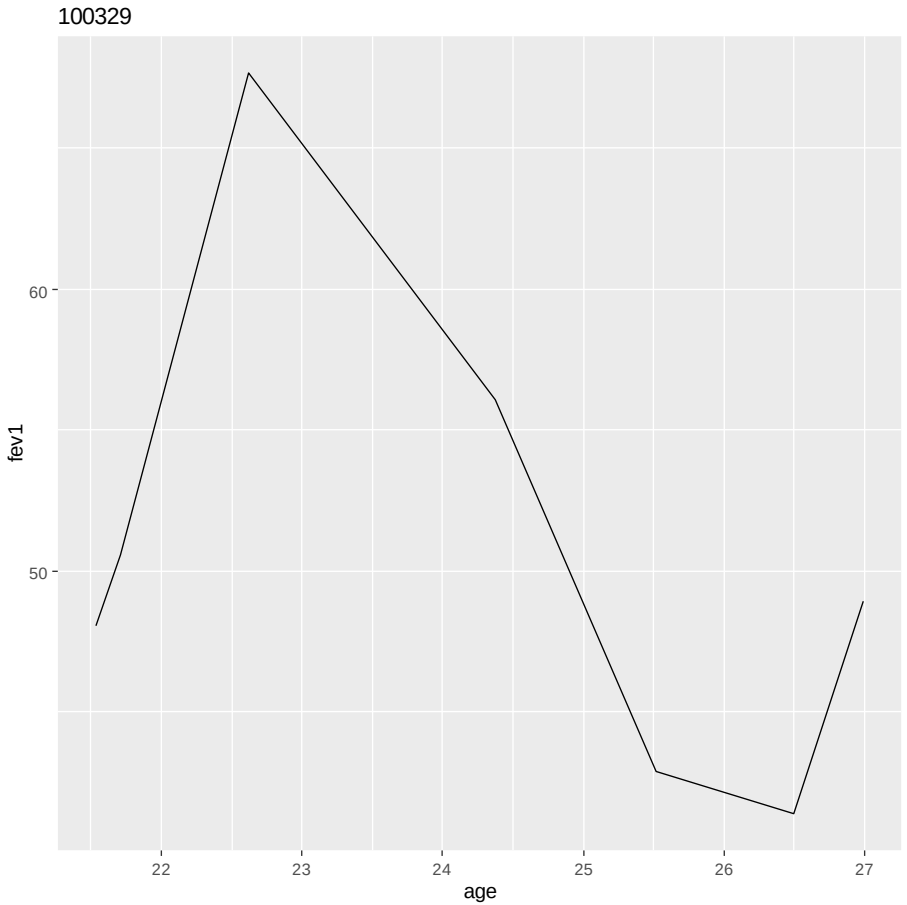

- ID = patient id
- FEV1 = percent-predicted forced expiratory volume in 1 second
- AGE = age (years)
- GENDER = sex (1=male, 2=female)
- PSEUDOA = infection with Pseudo Aeruginosa (0=no, 3=yes)
- F508 = genotype (1=homozygous, 2=heterozygous, 3=none)
- PANCREAT = pancreatic enzyme supplmentation (0,1=no, 2=yes)

- What is the rate of decline in FEV1?
- Is the time course different for males and females?
- Is the time course different for F508 homozygous subjects?

# FEV1 over Time

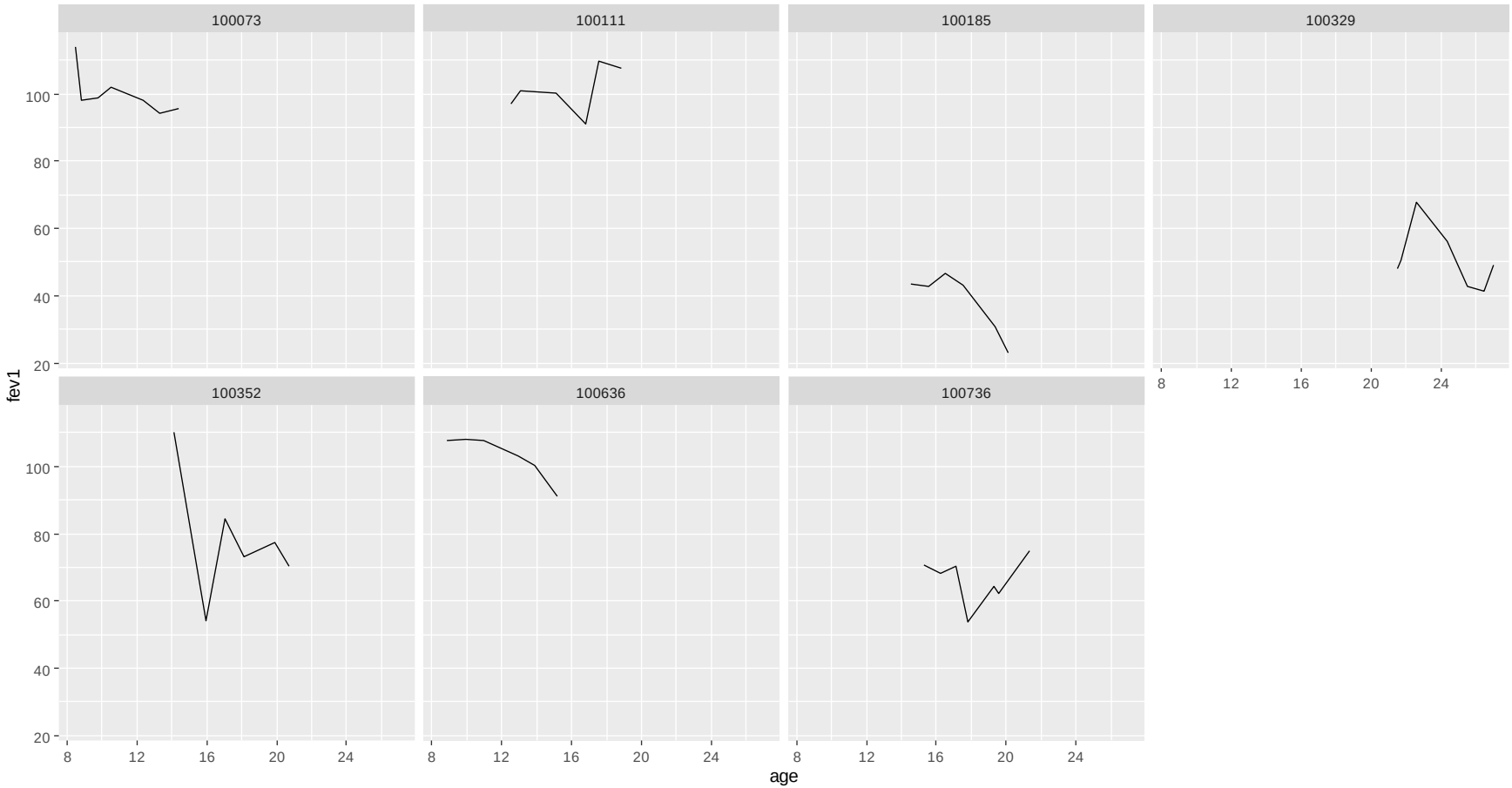



```
> ggplot(data= cfkids %>%filter(id== 100073), aes(x=age,
y=fev1)) + geom_line()+ggtitle("100073")
```



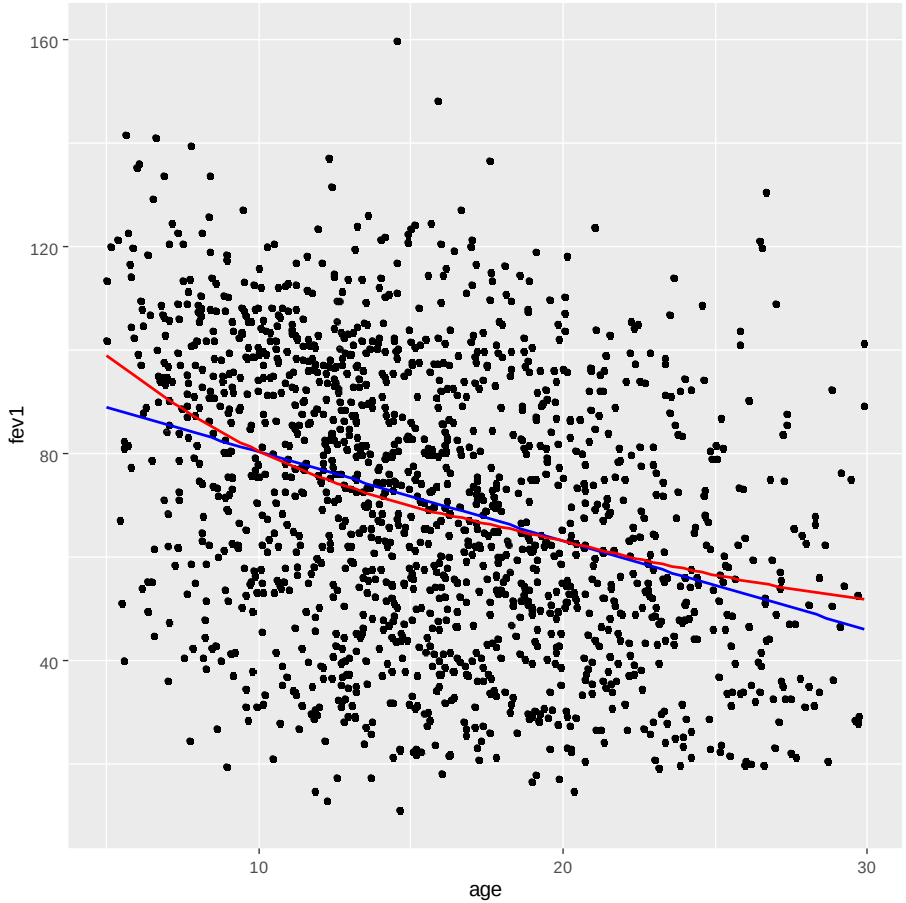

```
ggplot(data= cfkids %>%filter(id== 100329), aes(x=age, y=fev1)) +
geom_line()+ggtitle("100329")
```

# More individual plots

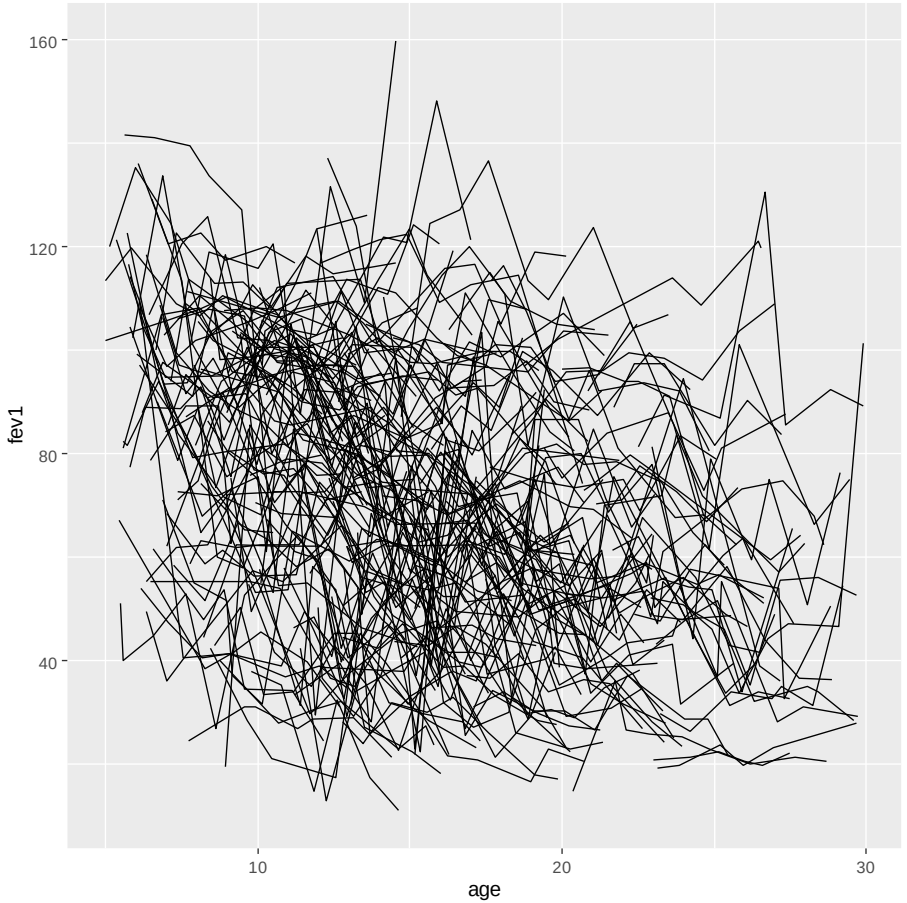



```
> ggplot(data= cfkids[1:45,] , aes(x=age, y=fev1))+geom_line()+facet_wrap(~id, ncol=4)
```

# FEV1 over Time

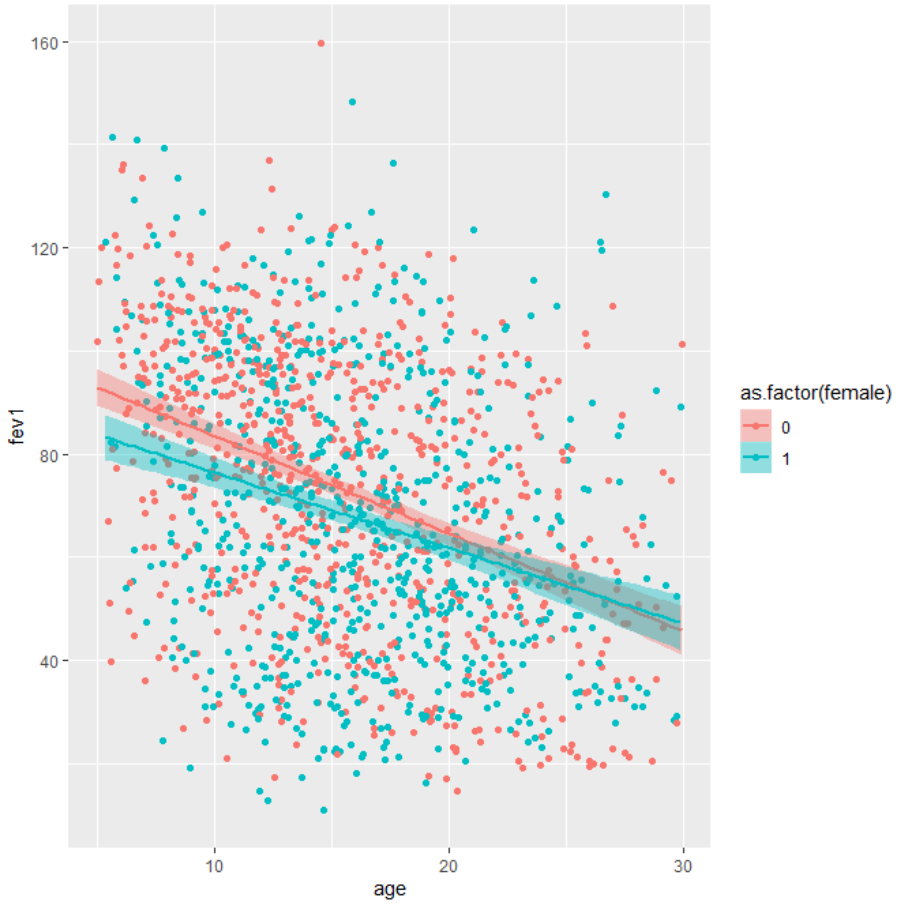



```
ggplot(data=cfkids, aes(x=ageggplot(data=cfkids, aes(x=age,
y=fev1)) + geom_point()+ geom_smooth(method = lm, color =
"blue")+geom_smooth(method = loess, color = "red")
```

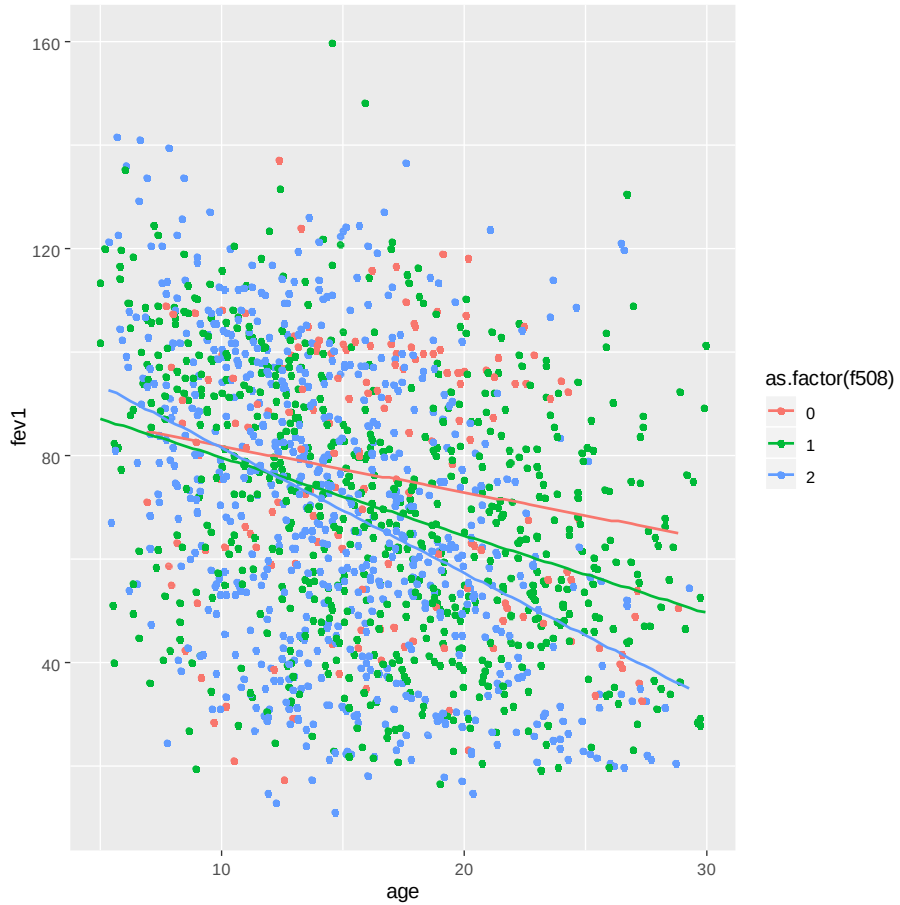



```
ggplot(data=cfkids, aes(x=age, y=fev1, group=id)) + geom_line()
```

# Sex and Mutations




```
ggplot(data= cfkids, aes(x=age, y=fev1, color =
as.factor(female)))+geom_point()+geom_smooth(method=lm,
aes(fill=as.factor(female)))
```




```
ggplot(data= cfkids, aes(x=age, y=fev1, color =
as.factor(f508)))+geom_point()+geom_smooth(method=lm,
aes(fill=as.factor(f508)))
```

# General Observations

- Systematic trends in the date:
  - Time
  - Sex
  - F508
- Two different time scales
- How to estimate effects and test?
  - What is the rate of decline in FEV1? (slope of time)
  - Is the time course different for males and females?
  - Is the time course different for F508 homozygous subjects?

# Fitting LMMs in R

- Many different software packages for fitting LMMs
  - lmm ← avoid
  - nlme (lme function)
  - lme4 (lmer function)
  - Others…

- SAS is probably better overall

# Random Intercept – lme4

```
> summary(lmer(fev1~ age0+ageL+female+(f508==1) + (f508==2)+female*ageL + (f508==1)*ageL + (f508==2)*ageL+ (1|id), REML = T))
Linear mixed model fit by REML ['lmerMod']
Formula: fev1 ~ age0 + ageL + female + (f508 == 1) + (f508 == 2) + female *      ageL + (f508 == 1) * ageL + (f508 == 2) * ageL + (1 | id)

REML criterion at convergence: 12498.1

Scaled residuals:
    Min      1Q  Median      3Q     Max
-4.9430 -0.5282 -0.0037  0.5168  4.2899

Random effects:
 Groups   Name        Variance Std.Dev.
 id       (Intercept) 510.7    22.6
 Residual             148.8    12.2
Number of obs: 1513, groups:  id, 200

Fixed effects:
                   Estimate Std. Error t value
(Intercept)        103.8063     6.7060  15.480
age0                -1.8553     0.3343  -5.550
ageL                -0.5878     0.3931  -1.496
female              -1.1620     3.3979  -0.342
f508 == 1TRUE       -4.2810     5.6110  -0.763
f508 == 2TRUE       -6.7404     5.6426  -1.195
ageL:female         -0.8257     0.2494  -3.311
ageL:f508 == 1TRUE  -0.4877     0.4225  -1.154
ageL:f508 == 2TRUE  -0.6575     0.4214  -1.560

Correlation of Fixed Effects:
            (Intr) age0   ageL   female f508=1 f508=2 agL:fm aL:5=1
age0        -0.630
ageL        -0.212  0.006
female      -0.164 -0.088  0.073
f508==1TRUE -0.657 -0.002  0.229 -0.021
f508==2TRUE -0.725  0.123  0.226 -0.062  0.788
ageL:female  0.062 -0.005 -0.272 -0.262  0.004  0.011
aL:508==1TR  0.181 -0.004 -0.856  0.005 -0.267 -0.214 -0.022
aL:508==2TR  0.179 -0.003 -0.853  0.011 -0.215 -0.266 -0.041  0.805
```

# Random Intercept - nlme

```
> summary(lme(fev1~ age0+ageL+female+(f508==1) + (f508==2)+female*ageL + (f508==1)*ageL + (f508==2)*ageL, random = ~1|id))
Linear mixed-effects model fit by REML
 Data: NULL
       AIC      BIC    logLik
  12520.08 12578.56 -6249.041

Random effects:
 Formula: ~1 | id
        (Intercept) Residual
StdDev:    22.59935 12.19734

Fixed effects: fev1 ~ age0 + ageL + female + (f508 == 1) + (f508 == 2) + female *      ageL + (f508 == 1) * ageL + (f508 == 2) * ageL
                       Value Std.Error   DF   t-value p-value
(Intercept)        103.80627  6.706026 1309 15.479550  0.0000
age0                -1.85532  0.334312  195 -5.549663  0.0000
ageL                -0.58782  0.393060 1309 -1.495504  0.1350
female              -1.16203  3.397872  195 -0.341987  0.7327
f508 == 1TRUE       -4.28096  5.611017  195 -0.762956  0.4464
f508 == 2TRUE       -6.74044  5.642572  195 -1.194569  0.2337
ageL:female         -0.82574  0.249427 1309 -3.310541  0.0010
ageL:f508 == 1TRUE  -0.48769  0.422469 1309 -1.154391  0.2486
ageL:f508 == 2TRUE  -0.65745  0.421359 1309 -1.560310  0.1189
 Correlation:
                   (Intr) age0   ageL   female f508=1 f508=2 agL:fm aL:5=1
age0               -0.630
ageL               -0.212  0.006
female             -0.164 -0.088  0.073
f508 == 1TRUE      -0.657 -0.002  0.229 -0.021
f508 == 2TRUE      -0.725  0.123  0.226 -0.062  0.788
ageL:female         0.062 -0.005 -0.272 -0.262  0.004  0.011
ageL:f508 == 1TRUE  0.181 -0.004 -0.856  0.005 -0.267 -0.214 -0.022
ageL:f508 == 2TRUE  0.179 -0.003 -0.853  0.011 -0.215 -0.266 -0.041  0.805

Standardized Within-Group Residuals:
         Min           Q1          Med           Q3          Max
-4.942954121 -0.528230691 -0.003676248  0.516817787  4.289855016

Number of Observations: 1513
Number of Groups: 200
```

# Random Intercept/Slope – lme4

```
> summary(lmer(fev1~ age0+ageL+female+(f508==1) + (f508==2)+female*ageL + (f508==1)*ageL + (f508==2)*ageL+(1+ageL|id), REML = T))
Linear mixed model fit by REML ['lmerMod']
Formula: fev1 ~ age0 + ageL + female + (f508 == 1) + (f508 == 2) + female *      ageL + (f508 == 1) * ageL + (f508 == 2) * ageL + (1 + ageL |      id)

REML criterion at convergence: 12389.2

Scaled residuals:
    Min      1Q  Median      3Q     Max
-5.3289 -0.4618  0.0052  0.4784  4.3196

Random effects:
 Groups   Name        Variance Std.Dev. Corr
 id       (Intercept) 512.406  22.636
          ageL          4.513   2.124   -0.16
 Residual             118.023  10.864
Number of obs: 1513, groups:  id, 200

Fixed effects:
                   Estimate Std. Error t value
(Intercept)        104.5192     6.6443  15.731
age0                -1.9105     0.3310  -5.771
ageL                -0.6028     0.5903  -1.021
female              -1.3005     3.3701  -0.386
f508 == 1TRUE       -4.2381     5.5636  -0.762
f508 == 2TRUE       -6.6523     5.5944  -1.189
ageL:female         -0.7624     0.3812  -2.000
ageL:f508 == 1TRUE  -0.5001     0.6357  -0.787
ageL:f508 == 2TRUE  -0.7459     0.6345  -1.176

Correlation of Fixed Effects:
            (Intr) age0   ageL   female f508=1 f508=2 agL:fm aL:5=1
age0        -0.630
ageL        -0.211  0.002
female      -0.164 -0.088  0.075
f508==1TRUE -0.657 -0.002  0.230 -0.021
f508==2TRUE -0.725  0.122  0.227 -0.062  0.788
ageL:female  0.060 -0.003 -0.279 -0.265  0.005  0.012
aL:508==1TR  0.179 -0.001 -0.850  0.005 -0.269 -0.214 -0.023
aL:508==2TR  0.178  0.000 -0.845  0.012 -0.215 -0.268 -0.046  0.797
```

# Random Intercept/Slope - nlme

```
> summary(lme(fev1~ age0+ageL+female+(f508==1) + (f508==2)+female*ageL + (f508==1)*ageL + (f508==2)*ageL, random = ~1+ageL|id))
Linear mixed-effects model fit by REML
 Data: NULL
       AIC      BIC    logLik
  12415.17 12484.28 -6194.586

Random effects:
 Formula: ~1 + ageL | id
 Structure: General positive-definite, Log-Cholesky parametrization
            StdDev    Corr
(Intercept) 22.636839 (Intr)
ageL         2.124421 -0.158
Residual    10.863789

Fixed effects: fev1 ~ age0 + ageL + female + (f508 == 1) + (f508 == 2) + female *      ageL + (f508 == 1) * ageL + (f508 == 2) * ageL
                      Value Std.Error   DF   t-value p-value
(Intercept)        104.51929  6.644377 1309 15.730489  0.0000
age0                -1.91054  0.331049  195 -5.771174  0.0000
ageL                -0.60278  0.590297 1309 -1.021148  0.3074
female              -1.30048  3.370124  195 -0.385886  0.7000
f508 == 1TRUE       -4.23809  5.563682  195 -0.761742  0.4471
f508 == 2TRUE       -6.65233  5.594495  195 -1.189085  0.2359
ageL:female         -0.76242  0.381214 1309 -1.999991  0.0457
ageL:f508 == 1TRUE  -0.50011  0.635753 1309 -0.786642  0.4316
ageL:f508 == 2TRUE  -0.74591  0.634493 1309 -1.175599  0.2400
 Correlation:
                   (Intr) age0   ageL   female f508=1 f508=2 agL:fm aL:5=1
age0               -0.630
ageL               -0.211  0.002
female             -0.164 -0.088  0.075
f508 == 1TRUE      -0.657 -0.002  0.230 -0.021
f508 == 2TRUE      -0.725  0.122  0.227 -0.062  0.788
ageL:female         0.060 -0.003 -0.279 -0.265  0.005  0.012
ageL:f508 == 1TRUE  0.179 -0.001 -0.850  0.005 -0.269 -0.214 -0.023
ageL:f508 == 2TRUE  0.178  0.000 -0.845  0.012 -0.215 -0.268 -0.046  0.797

Standardized Within-Group Residuals:
        Min          Q1         Med          Q3         Max
-5.32898196 -0.46179233  0.00517294  0.47838439  4.31967651

Number of Observations: 1513
Number of Groups: 200
```

# Key Aspects of Output

- Random Effects (each has mean zero, so only variance)
- Fixed effects: lmer does not give p-values
- (Standardized) Residuals
- AIC/BIC
- lmer does not by default output p-values: lots of caveats with p-values in mixed models
  - Can use anova function to compare models (LRT)
  - lmerTest also will spit out p-values

# anova with lmer

```
> mod0 = lmer(fev1~ age0+ageL+female+(f508==1) + (f508==2)+ (f508==1)*ageL + (f508==2)*ageL+(1+ageL|id), REML = T)
> mod1 = lmer(fev1~ age0+ageL+female+(f508==1) + (f508==2)+female*ageL + (f508==1)*ageL + (f508==2)*ageL+(1+ageL|id), REML = T)
> anova(mod1,mod0)

refitting model(s) with ML (instead of REML)
Data: NULL
Models:
mod0: fev1 ~ age0 + ageL + female + (f508 == 1) + (f508 == 2) + (f508 ==
mod0:     1) * ageL + (f508 == 2) * ageL + (1 + ageL | id)
mod1: fev1 ~ age0 + ageL + female + (f508 == 1) + (f508 == 2) + female *
mod1:     ageL + (f508 == 1) * ageL + (f508 == 2) * ageL + (1 + ageL |
mod1:     id)
     Df   AIC   BIC  logLik deviance  Chisq Chi Df Pr(>Chisq)
mod0 12 12432 12496 -6204.2    12408
mod1 13 12430 12500 -6202.2    12404 4.0441      1    0.04433 *
---
Signif. codes:  0 ‘***’ 0.001 ‘**’ 0.01 ‘*’ 0.05 ‘.’ 0.1 ‘ ’ 1
```

# Choosing a Model

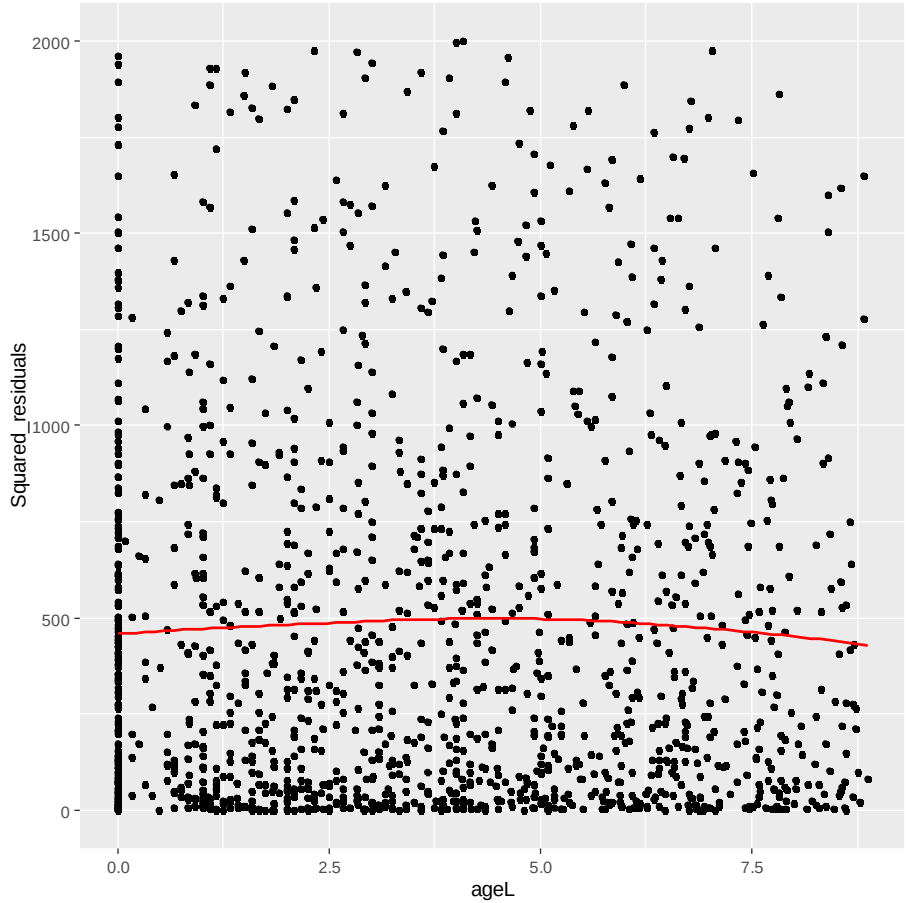
- For LMM, important to get appropriate mean AND covariance structure
- Overly simplistic models lead to invalid inference
- Overly complicated models lead to poor efficiency
- General guidelines:
  - get fixed effects
  - get random effects
  - get covariance structure
  - Reduce the model

# Choosing Initial Mean Model

- Start with bigger model than necessary
- Saturated model: kitchen sink
- Not seeing much need for quadratic effects over time
- Possibly some heterogeneity by sex and F508

# Choosing Random Effects

- Usually only random effects for variables that change
  - For non time dependent variables, subject specific changes are absorbed into random intercept
- Add variables that are included as fixed effects (require **b** to have mean zero)
- Prefer hierarchical models
- (squared) OLS residuals are helpful
  - Constant variability over time implies stationarity
- Err on side of bigger models
  - But may require estimation of lots of effects which can be unstable



# Selection of Residual Covariance Structure

- Generally challenging problem

- Basic idea:
  - Fit range of models
  - Compare models via AIC/BIC (sometimes LRT is possible)

- For unbalanced data with lots of measurements, typically select fairly simplistic models which leads to simply models for **V**

# Model Reduction

- Backward selection
- Beware of LRT for random effects (not the usual null distributions)
- Can sequentially test for fixed effects
- Inference post model selection?