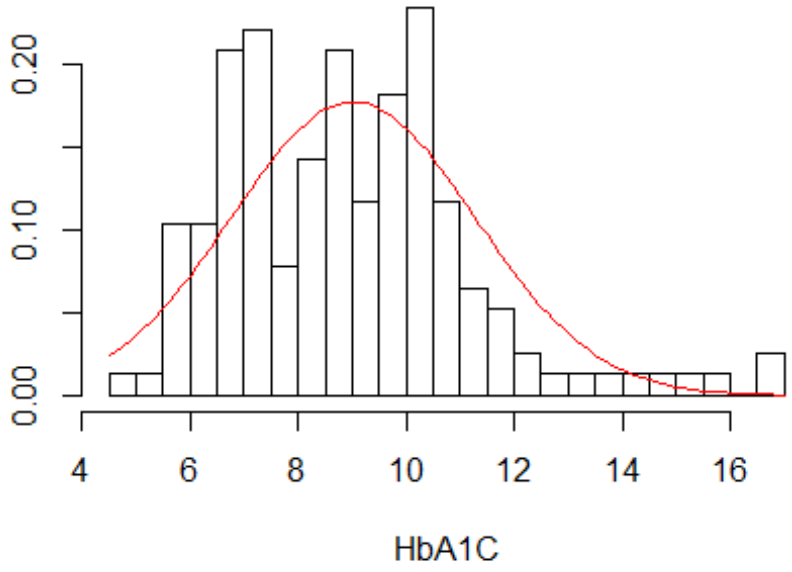
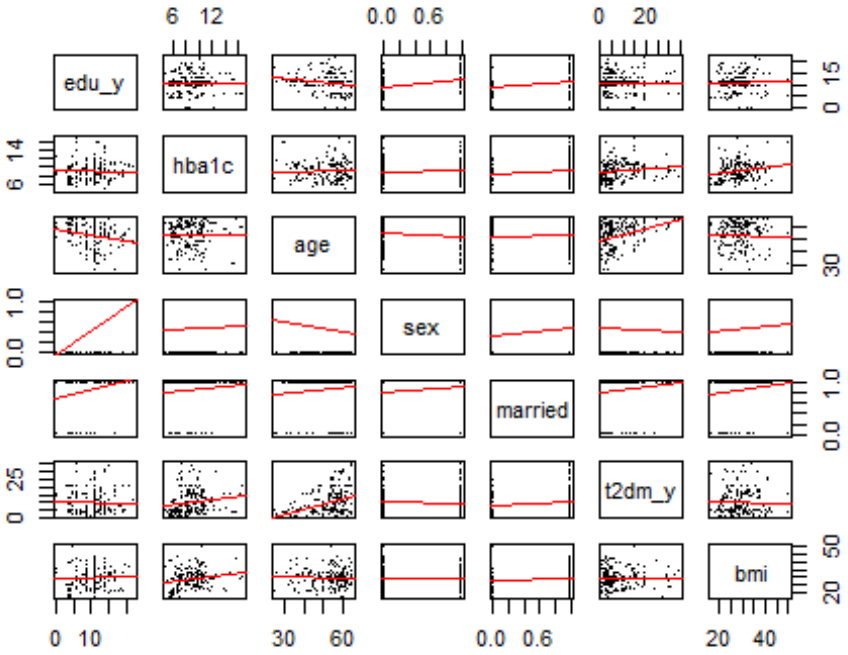
AppendixSyntax.R

```
# Using R-version 3.3.0
rm(list = ls())

################################################################################
############################## Data preparation ################################
################################################################################

######################
##### comment 1 #####
######################

# Using data from PS Shu, Chan YM, and Huang SL "Higher body mass index
# and lower intake of dairy products predict poor glycaemic control
# among Type 2 Diabetes patients in Malaysia" Plos One 2017;
# a cross-sectional study.

dt1 <- read.csv("ShuPlosOne.csv")[-72,] # removing the 72th patient which has
a very long history with T2DM (100 years!)

# preparing data
dt2 <- dt1[,c("AGE", "SEX", "MARITAL.STATUS", "EDUC_YEARS", "DURATION._.yeAR"
, "bmi", "HBA1c_average" )] # using a subset of the predictors
colnames(dt2) <- c("age", "sex", "married", "edu_y", "t2dm_y", "bmi", "hba1c"
) #renaming
dt2 <- dt2[,c(4,7, 1:3,5:6)] # reordering columns

# Recoding
dt2$sex <- as.numeric(dt2$sex) -1 # zero = female
dt2$married <- ifelse(dt2$married == "Married", 1, 0)

dt1 <- dt2
rm(dt2)

################################################################################
############################## Data exploration ################################
################################################################################


dt1[1:6,] # first 6 observations

##   edu_y hba1c age sex married t2dm_y   bmi
## 1    11   7.0  60   1       1     28 17.13
## 2    14  11.4  55   1       1      5 25.70
## 3    16   6.5  57   0       1      5 36.78
## 4     5  12.3  52   0       1      7 28.39
```

```
## 5    11   6.8  50   1       1      1 23.23
## 6    14   9.7  37   0       1      5 42.35

# exploring the central locations and min/max
summary(dt1)

##      edu_y           hba1c            age             sex        
##  Min.   : 0.00   Min.   : 4.800   Min.   :25.00   Min.   :0.0000  
##  1st Qu.: 8.25   1st Qu.: 7.225   1st Qu.:46.25   1st Qu.:0.0000  
##  Median :11.00   Median : 8.900   Median :56.00   Median :0.0000  
##  Mean   :10.53   Mean   : 9.023   Mean   :52.88   Mean   :0.4675  
##  3rd Qu.:13.00   3rd Qu.:10.375   3rd Qu.:60.00   3rd Qu.:1.0000  
##  Max.   :22.00   Max.   :16.800   Max.   :65.00   Max.   :1.0000  
##     married           t2dm_y           bmi        
##  Min.   :0.0000   Min.   : 1.00   Min.   :16.88  
##  1st Qu.:1.0000   1st Qu.: 4.00   1st Qu.:25.07  
##  Median :1.0000   Median : 7.00   Median :28.95  
##  Mean   :0.8636   Mean   : 9.81   Mean   :29.38  
##  3rd Qu.:1.0000   3rd Qu.:15.00   3rd Qu.:32.51  
##  Max.   :1.0000   Max.   :35.00   Max.   :50.59  

# Note extreme maxima for t2dm_y and bmi.
# Sadly we don't have access to primary data source
# so we can't double check these values. For now we will keep them.

par(mfrow = c(1,1))
breaks1 <- 20
xlab1 = "HbA1C"
hist(dt1$hba1c, freq = FALSE, breaks = breaks1,
     xlab = xlab1, main = "", ylab = "")
curve(dnorm(x,mean(dt1$hba1c),sd(dt1$hba1c)),
      col = 2, add = TRUE) # expected normal density
```




```
# There is a tail towards larger HbA1c values.

#################################################
#exploring pairwise distributions and relations#
#################################################
pairs(dt1,
      panel = function(x,y,...){
        points(x,y,...)
        abline(lm(y ~ x), col = "red", lwd = 1.2)
      }, pch = ".", cex = 1.7
      )
```



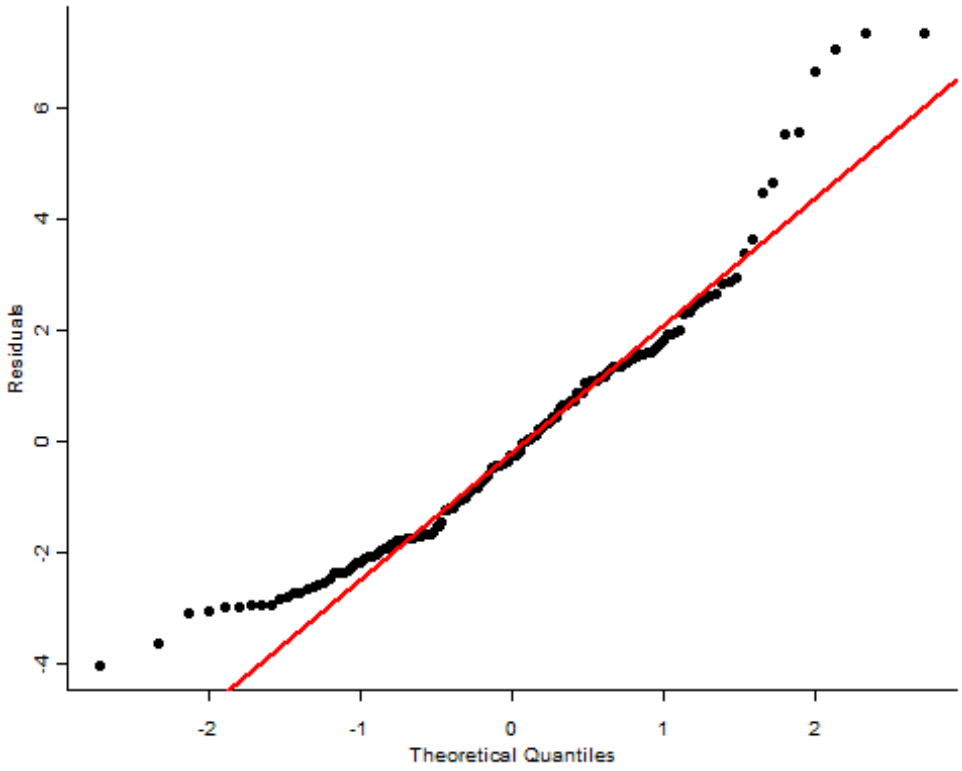

```
######################
##### comment 2 #####
######################

# Time since T2DM diagnosis and BMI seem related to HbA1c,
# while most other variables show a flat relation
# Time since T2DM diagnosis itself is strongly related to age.
# BMI seems to be independent of most other variables
# except, perhaps, marital status.

#############################################################################
# Pairwise linear model regressing HbA1c on times since T2DM diagnosis #
#############################################################################



######################
##### comment 3 #####
######################

# For illustrative purposes we first focus on a simple pairwise linear model

fit.0 <- lm(hba1c ~ t2dm_y, data=dt1)

summary(fit.0) # Model estimates
```

```
##
## Call:
## lm(formula = hba1c ~ t2dm_y, data = dt1)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -4.0175 -1.7462 -0.2527  1.3364  7.3545
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  8.60346    0.28754  29.921   <2e-16 ***
## t2dm_y       0.04280    0.02284   1.874   0.0629 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 2.236 on 152 degrees of freedom
## Multiple R-squared:  0.02258,    Adjusted R-squared:  0.01615
## F-statistic: 3.512 on 1 and 152 DF,  p-value: 0.06285
```

```
cbind(fit.0$coef,confint(fit.0))
```

```
##                               2.5 %     97.5 %
## (Intercept) 8.6034615  8.035372873 9.17155021
## t2dm_y      0.0428048 -0.002323315 0.08793291
```

```
###################################
# distribution of the residuals #
###################################

par(bg = 'white')
par(mfrow = c(1,1))
par(cex.axis = 0.6, cex.lab  = 0.6, cex.main = 0.7)
par(mgp = c(1.0, 0.35, 0),  oma = c(0,0,0,0), mar = c(2.2, 1.7, 0.2, 0.4)) #
mar (b,l, t,r)
par(tcl = -0.25)

xlab1 <- "Fitted values"
ylab1 <- "Residuals"
lwd1 <- 2
cex2 <- 0.7

res <- summary(fit.0)$res # extracting residuals
qqnorm(res, main = "", ylab = "Residuals", pch =16, bty = "l", cex = cex2)
qqline(res, col = "red", lwd =lwd1) # line indicating perfect fit
```




```
######################
##### comment 3 #####
######################

# The residual plot indicates tail-area deviations from the normal distributi
on

################################################################################
######
############################## Exploration of the      ######################
######
############################## parametric relationship ######################
######
################################################################################
######

# figure resolutions and dimensions
res1 <- 600
h <- 6 * 0.393701
w <- 6 * 0.393701
#
############################
# exploring non-linearity #
############################

# tiff("Figure1.tiff", width = w, height = h, res = res1, units = "in", compr
```

```r
ession = "lzw", type = "cairo")
par(bg = 'white')
par(mfrow = c(1,1))
par(cex.axis = 0.6, cex.lab  = 0.6, cex.main = 0.7)
par(mgp = c(1.0, 0.35, 0),  oma = c(0,0,0,0), mar = c(2.2, 1.7, 0.2, 0.4)) #
mar (b,l, t,r)
par(tcl = -0.25)

mat <- matrix(cbind(predict(fit.0), res), ncol = 2) # predict(fit.0) extracts
the fitted values
plot(x = mat[,1], y = mat[,2], ylab = ylab1, xlab = xlab1, bty = "l", pch = 1
6, cex = cex2)

# loess curve
loess.fit <- loess(mat[,2]~ mat[,1])
xloess <-  seq(min(mat[,1]), max(mat[,1]), length.out = 100)
yloess <- predict(loess.fit, newdata = xloess)

lines(x = xloess, yloess, col = "red", lwd = lwd1) # add the loess curve to t
he graph

# line of perfect fit
abline(h = 0, lwd = lwd1, col = "grey", lty = 2) # if there is a perfect line
ar relation the residuals should cluster allong the grey line

# dev.off()

######################
##### comment 4 #####
######################

# The figure indicates that time since T2DM diagnosis is
# initially related with an increased HbA1c, which switches to a negative
# relation as subjects report a longer experience with T2DM.
# Potentially this is a true effect related to increased disease
# managment skills.

###################################
# Modeling the non-linear effect #
###################################

######################
##### comment 5 #####
######################

# Non-linear trends can be modelled in many different ways here we use restri
cted cubic splines.
# Loading the rms package for the restricted cubic spline function
```

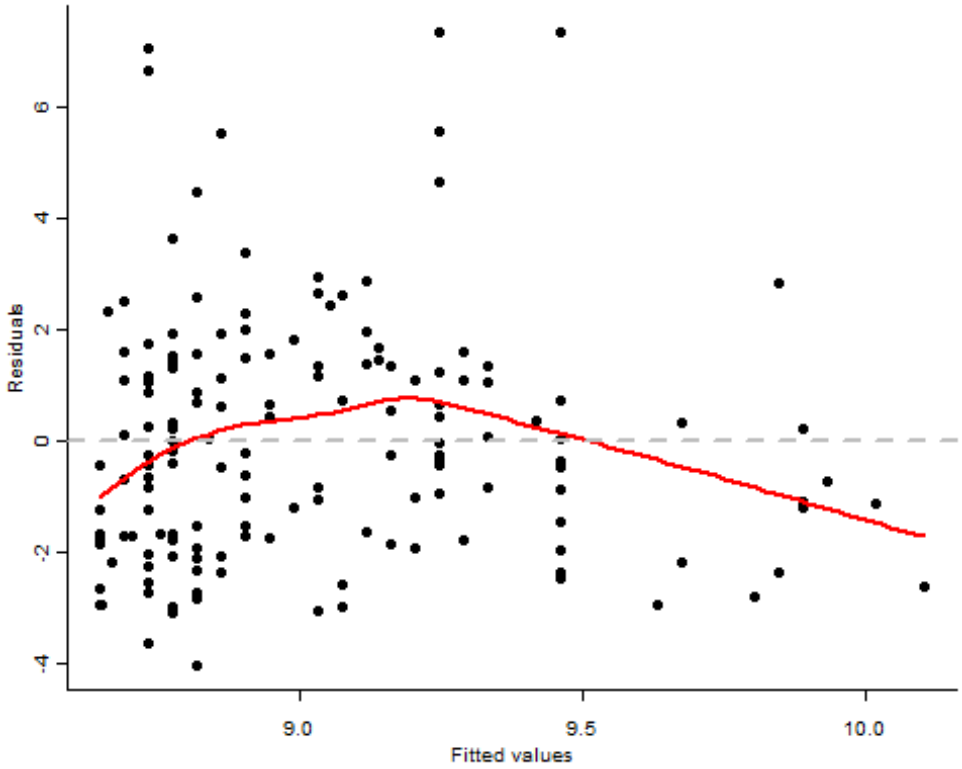
```
ev <- try(require("rms", quietly =T), silent = T)
```

```
##
## Attaching package: 'Hmisc'
```

```
## The following objects are masked from 'package:base':
##
##     format.pval, round.POSIXt, trunc.POSIXt, units
```

```
##
## Attaching package: 'SparseM'
```

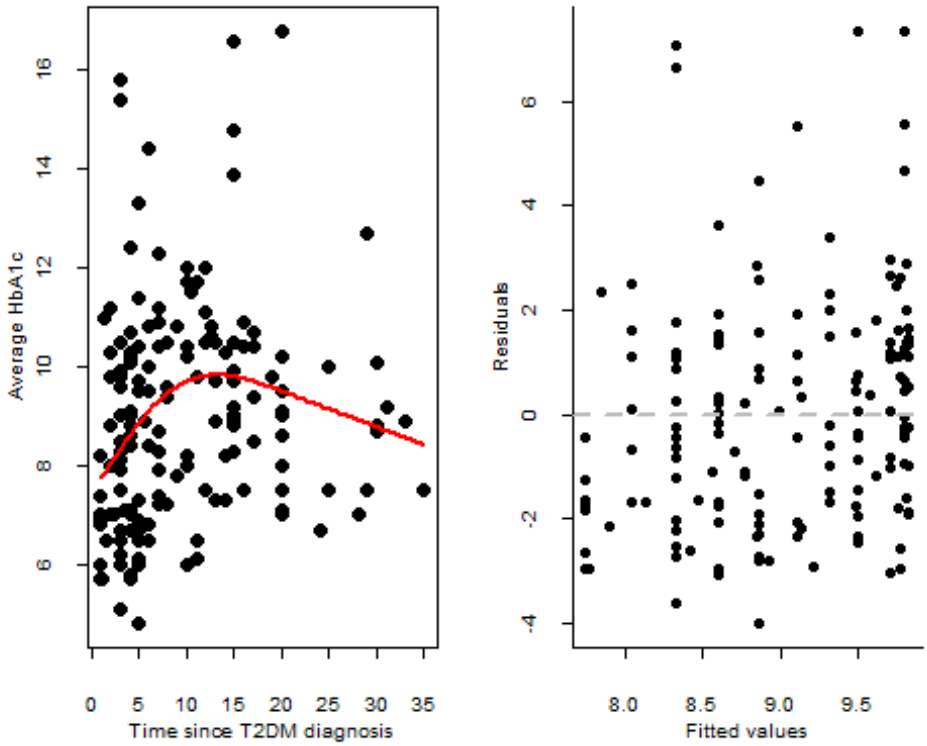
```
## The following object is masked from 'package:base':
##
##     backsolve
```




```
if(ev == FALSE){
  options(repos=c(CRAN ="http://cran-mirror.cs.uu.nl/"))
  install.packages('rms', dependencies = TRUE)
  library(rms)
}

fit.1 <- lm(hba1c~ rcs(t2dm_y,3), data=dt1)
anova(fit.0, fit.1) # The non-linear model fits the data better
```

```
## Analysis of Variance Table
##
```

```
## Model 1: hba1c ~ t2dm_y
## Model 2: hba1c ~ rcs(t2dm_y, 3)
##   Res.Df    RSS Df Sum of Sq      F   Pr(>F)
## 1    152 760.02
## 2    151 711.53  1    48.485 10.289 0.001634 **
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

# Plotting the non-linear association
X <- seq(min(dt1$t2dm_y), max(dt1$t2dm_y), length.out =100)

kn1 <- rcspline.eval(dt1$t2dm_y, nk =3,
           knots.only = T) # extracting the cubic spline transformation of th
e independent variable
designX <- cbind(1,rcspline.eval(X,
           knots = kn1, inclx = T)) # design matrix storing the predictor val
ues
cf <- coef(fit.1)
pred1 <- matrix(cf, ncol = 3) %*% t(designX)

par(mfrow = c(1,2))
par(mgp = c(1.5, 0.8, 0),  oma = c(0,0,0,0), mar = c(3, 3, 0.5, 0.5))

plot(y = dt1$hba1c, x = dt1$t2dm_y, pch = 16,
     xlab = "Time since T2DM diagnosis",
     ylab = "Average HbA1c")
lines(x = X, pred1, col = "red", lwd = lwd1)

# Plotting the new residuals ~ fitted values plot
mat <- matrix(cbind(predict(fit.1), res), ncol = 2)
plot(x = mat[,1], y = mat[,2], ylab = ylab1, xlab = xlab1,
     bty = "l", pch = 16, cex = cex2)

abline(h = 0, lwd = lwd1, col = "grey", lty = 2) # expected line
```




```
######################
##### comment 6 #####
######################
# The left graph shows that the restricted cubic splines adequately model
# the non-linear trend observed.The right graph shows an absence of
# trend between the residuals and fitted value confirming the improved model
fit.


#############################################################################
###############
############################## Corrections for  heteroscedastic ##############
###############
############################### or correlated errors             ##############
###############
#############################################################################
###############

######################
##### comment 7 #####
######################

# It is likely this non-linear trend is a true reflection of the
# population association (i.e., T2DM management skills improve with
#  time). However, an alternative explanation of the trend
# between the residuals and fitted values (of the linear model)
```

```r
# could be heteroscedasticity or correlation between errors.
# As an illustration of how to correct for these issues,
# we will replace the naive standard errors
# by heteroscedastic robust standard errors.

# loading the sandwich package for the "robust" standard error function
ev <- try(require("sandwich", quietly = T), silent = T)
if(ev == FALSE){
  options(repos=c(CRAN ="http://cran-mirror.cs.uu.nl/"))
  install.packages('sandwich', dependencies = TRUE)
  library(sandwich)
}

# original model
summary(fit.0)

##
## Call:
## lm(formula = hba1c ~ t2dm_y, data = dt1)
##
## Residuals:
##     Min      1Q  Median      3Q     Max
## -4.0175 -1.7462 -0.2527  1.3364  7.3545
##
## Coefficients:
##             Estimate Std. Error t value Pr(>|t|)
## (Intercept)  8.60346    0.28754  29.921   <2e-16 ***
## t2dm_y       0.04280    0.02284   1.874   0.0629 .
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
##
## Residual standard error: 2.236 on 152 degrees of freedom
## Multiple R-squared:  0.02258,    Adjusted R-squared:  0.01615
## F-statistic: 3.512 on 1 and 152 DF,  p-value: 0.06285

# new robust standard errors compared to the naive standard errors.
(se.r <- sqrt(diag(vcovHC(fit.0))))

## (Intercept)      t2dm_y
##  0.28648195  0.02249715

summary(fit.0)$coef[,"Std. Error"]

## (Intercept)      t2dm_y
##  0.28753880  0.02284165

se.r/summary(fit.0)$coef[,"Std. Error"] # ratio between new and old standard
errors

## (Intercept)      t2dm_y
##   0.9963245   0.9849177
```

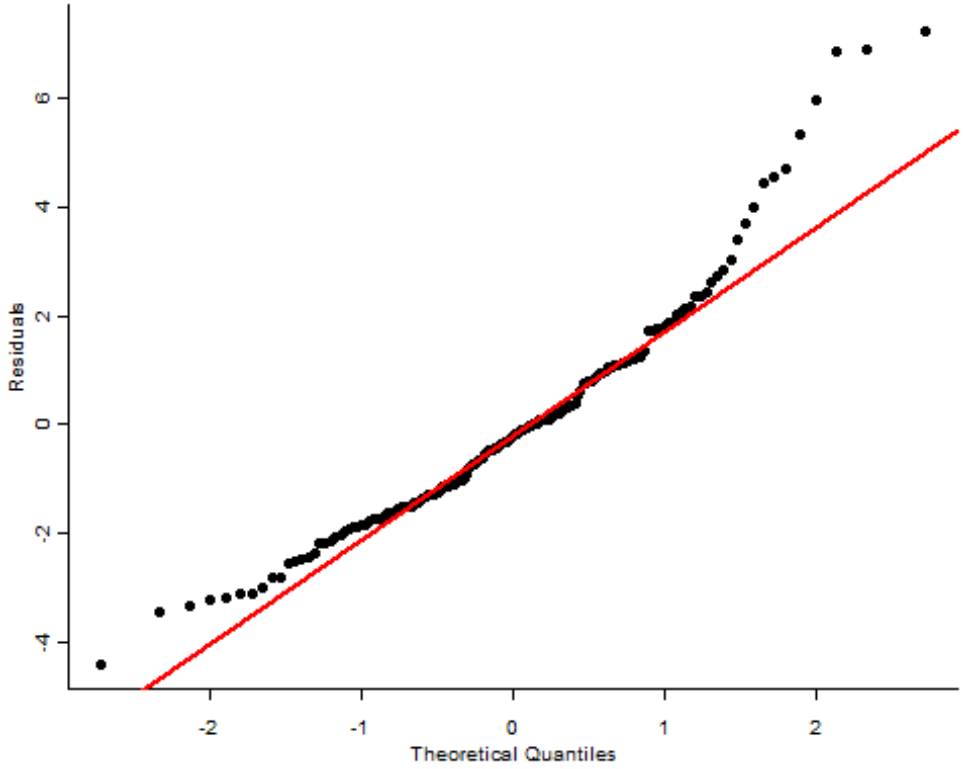
```r
# new robust confidence intervals compared to the naive CI's
df1 <- dim(dt1)[1] -1 # degrees of freedom
a1 <- 0.05 # alpha, the significant cut-off value

(ci.r <- summary(fit.0)$coef[,"Estimate"] + matrix(c(-1,1), 2, 2, byrow = T)
* qt(1-a1/2, df = df1) * se.r)

##              [,1]       [,2]
## [1,]  8.037490579 9.16943250
## [2,] -0.001640349 0.08724995

confint(fit.0)

##                    2.5 %     97.5 %
## (Intercept)  8.035372873 9.17155021
## t2dm_y      -0.002323315 0.08793291

# new robust p-values compared to the naive p-values
t.r <- summary(fit.0)$coef[,"Estimate"]/se.r # t-values

(pval.r <- pt(abs(t.r), df = df1, lower.tail = F) * 2)

##  (Intercept)       t2dm_y
## 4.966966e-66 5.896134e-02

summary(fit.0)$coef[,"Pr(>|t|)"]

##  (Intercept)       t2dm_y
## 1.379007e-65 6.285256e-02

######################
##### comment 8 #####
######################

# Comparing the robust standard errors to the naive standard errors
# showed little difference (the quotient was close to 1),
# confirming what we already suspected ~ the observed trend is
# most likely explained by the non-linear association of
# time since diagnosis. More generally it is often difficult to determine if
# the errors are correlated or not, especially in the case of non-time series
# data. As such external, prior knowledge is key here.
# When the rows in the data can be ordered in an informative way (e.g, as in
time-series),
# a possible data driven grafic to check dependency between errors is to simp
ly
# plot the residuals against the ordered observations (e.g., time).

##############################################################################
##
############################## Multivariable model ############################
##
```

```
##############################################################################
##

#####################
##### comment 9 #####
#####################

# As we saw before the relation between HbA1c seemed
# to be related to duration of T2DM, and BMI.
# Furthermore, these variables themselves seemed related
# to age and marital status. To further explore the relation between HbA1c
# and duration of T2DM we will next include these variables
# in a multivariable linear model.


fit.full <- lm(hba1c~ rcs(t2dm_y,3) + bmi + age + married, data=dt1)
anova(fit.full)
```

```
## Analysis of Variance Table
##
## Response: hba1c
##                 Df Sum Sq Mean Sq F value  Pr(>F)
## rcs(t2dm_y, 3)   2  66.04  33.022  7.1977 0.00104 **
## bmi              1  30.18  30.180  6.5782 0.01132 *
## age              1   2.07   2.069  0.4511 0.50287
## married          1   0.28   0.276  0.0602 0.80648
## Residuals      148 679.01   4.588
## ---
## Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1
```

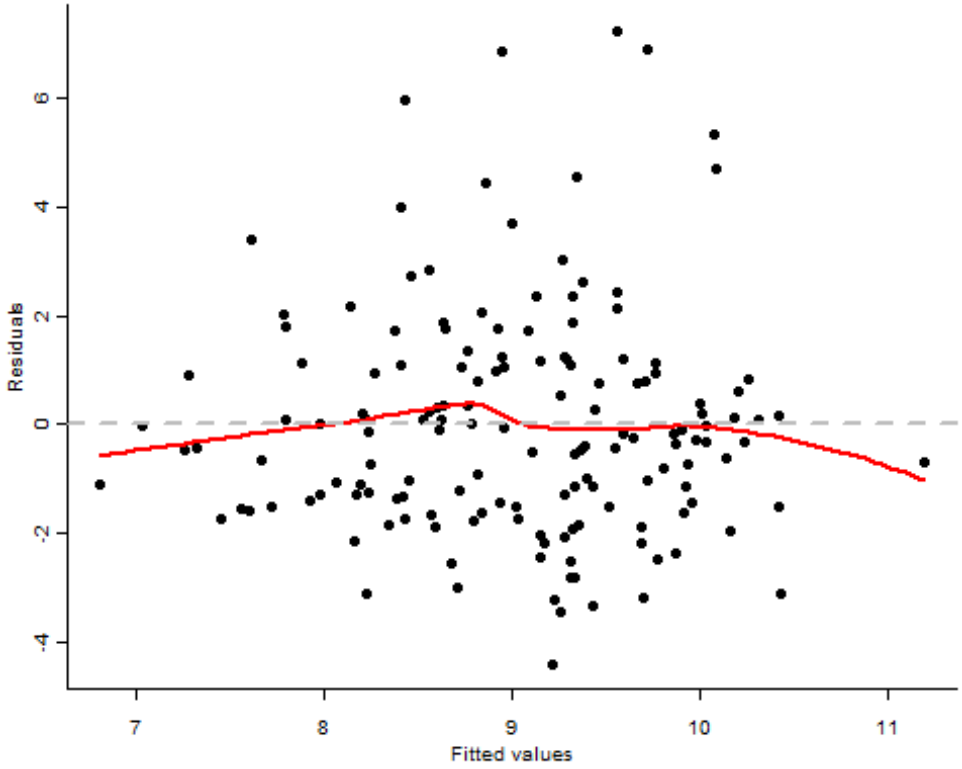
```
######################
##### comment 10 #####
######################

# conditional on covariables, both time since diagnosis
# and bmi are significantly associated with HbA1c.
# Let's check how well this "full" model,
# fits the collected data and if
# there are potential model assumption violations.

##############################################
# distribution of the residuals, full model #
##############################################

par(bg = 'white')
par(mfrow = c(1,1))
par(cex.axis = 0.6, cex.lab  = 0.6, cex.main = 0.7)
par(mgp = c(1.0, 0.35, 0),  oma = c(0,0,0,0), mar = c(2.2, 1.7, 0.2, 0.4)) #
mar (b,l, t,r)
par(tcl = -0.25)
```

```r
xlab1 <- "Fitted values"
ylab1 <- "Residuals"
lwd1 <- 2
cex2 <- 0.7

res <- summary(fit.full)$res # extracting residuals
qqnorm(res, main = "", ylab = "Residuals", pch =16, bty = "l", cex = cex2)
qqline(res, col = "red", lwd =lwd1) # line indicating perfect fit
```



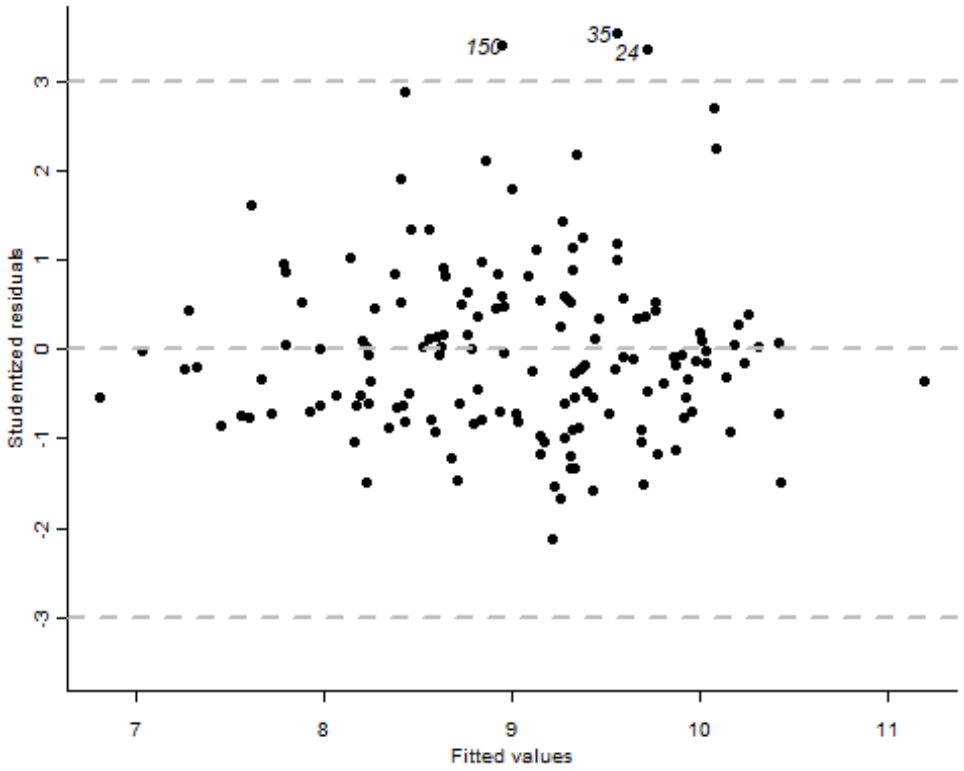

```r
######################
##### comment 11 #####
######################

# as before the more outlying residuals
# deviate from the normal distribution

#########################################################
# Comparing the fitted values and residuals to detect #
# issue with heteroscedasticity or correlated errors  #
#########################################################

mat <- matrix(cbind(predict(fit.full), res), ncol = 2) # predict(fit.0) extra
cts the fitted values
plot(x = mat[,1], y = mat[,2], ylab = ylab1, xlab = xlab1, bty = "l", pch = 1
6, cex = cex2)
```

```r
# loess curve
loess.fit <- loess(mat[,2]~ mat[,1])
xloess <-  seq(min(mat[,1]), max(mat[,1]), length.out = 100)
yloess <- predict(loess.fit, newdata = xloess)

lines(x = xloess, yloess, col = "red", lwd = lwd1) # add the loess curve to t
he graph
abline(h = 0, lwd = lwd1, col = "grey", lty = 2) # if there is a perfect line
ar relation the
```



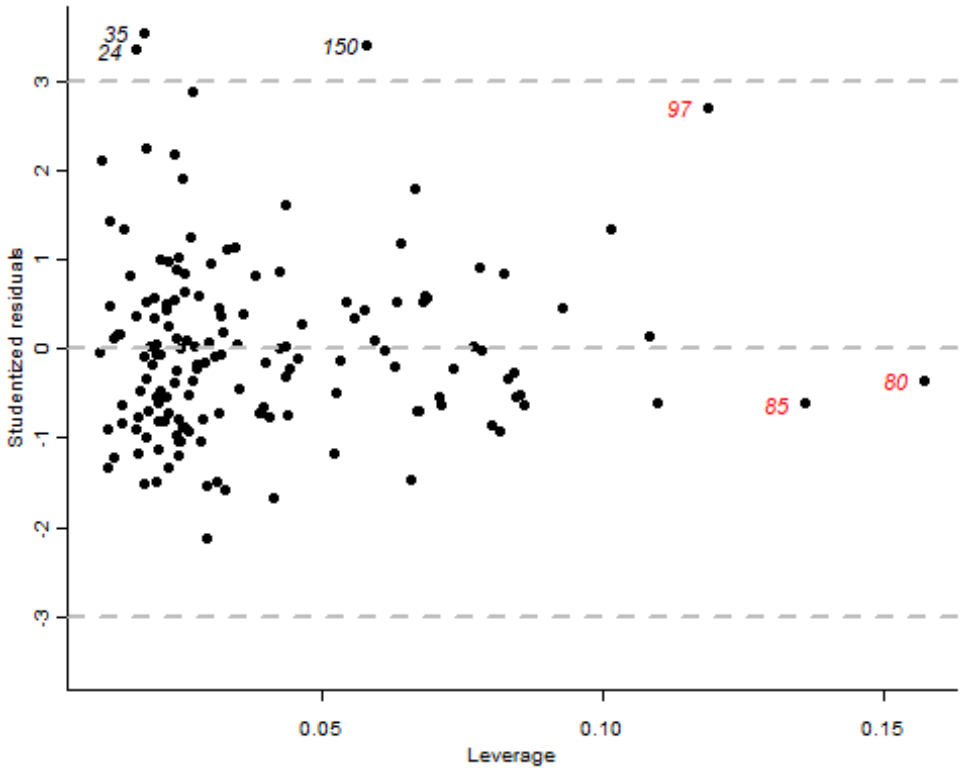

```r
#residuals should cluster along the grey line

#######################
##### comment 12 #####
#######################

# No clear trend can be observed so we don't have to worry
# about heteroscedasticity or correlated errors

############################
# Multivariable outliers #
############################

rm(res, mat ) # removing unstandardized residuals
sres <- rstudent(fit.full) # extracting Studentized residuals
```

```
mat <- matrix(cbind(predict(fit.full), sres), ncol = 2) # predict(fit.0) extr
acts the fitted values
plot(x = mat[,1], y = mat[,2], ylab = "Studentized residuals", xlab = xlab1,
bty = "l", pch = 16, cex = cex2,
     ylim = c(-max(abs(sres)),max(abs(sres)) ))
abline(h = c(-3,0,3), lwd = lwd1, col = "grey", lty = 2)

# extracting outlying observations
out1 <- as.numeric(as.character(rownames(dt1)))[abs(sres) > 3] # outlying obs
ervations
yout1 <- sres[abs(sres) > 3]
xout1 <- predict(fit.full)[abs(sres) > 3]
text(y = yout1, xout1-0.1, cex = 0.7, labels = as.character(out1),  font = 3)
```



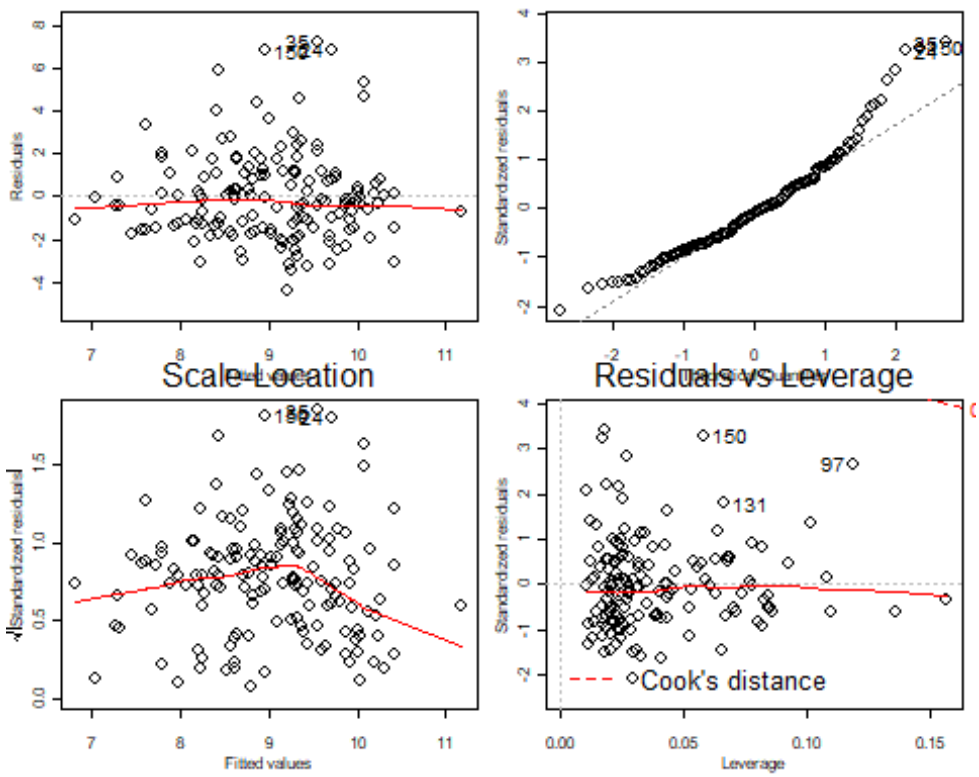

```
######################
##### comment 13 #####
######################

# by using Studentized residuals we can more easily identify
# outlying HbA1c values. For example we would not expect many
# Studentized residuals larger or smaller than 3. In this graph
# we find 3 outlying values which deserve further consideration.

############
# Leverage #
```

```
#############

rm(mat )
sres <- rstudent(fit.full) # extracting Studentized residuals
leverage <- hatvalues(fit.full)



mat <- matrix(cbind(leverage, sres), ncol = 2)
plot(x = mat[,1], y = mat[,2], ylab = "Studentized residuals", xlab = "Levera
ge", bty = "l", pch = 16, cex = cex2, ylim =
       c(-max(abs(sres)),max(abs(sres)) ))
abline(h = c(-3,0,3), lwd = lwd1, col = "grey", lty = 2)

# adding points with outlying HbA1c values
xout1 <- leverage[abs(sres) > 3]
text(y = yout1, xout1 - 0.005, cex = 0.7, labels = as.character(out1),  font
= 3)

# adding points with high leverage only
cut <- 3*mean(leverage)   # heuristic rule to define 3 times the mean as  ext
reme
lev1 <- as.numeric(as.character(rownames(dt1)))[leverage > cut] # outlying ob
servations
ylev1 <- sres[leverage > cut]
xlev1 <- leverage[leverage > cut]
text(y = ylev1, xlev1-0.005, cex = 0.7, labels = as.character(lev1),  font =
3, col = "red")
```




```
########################
##### comment 14 #####
########################

# It turns out that from the  the previously defined outlying values 150 also
has a slightly high  leverage.
# Furthermore, we identified 3 observations with high leverage without outlyi
ng HbA1c values.

########################################
# Exploring these extreme observations #
########################################

dt1[rownames(dt1) %in% c(lev1,out1),]

##     edu_y hba1c age sex married t2dm_y   bmi
## 24      6  16.6  59   0       1     15 28.22
## 35      6  16.8  59   0       1     20 30.08
## 80      5  10.5  54   0       0     15 49.98
## 85      6   7.5  65   1       1     35 34.41
## 97     12  15.4  30   1       1      3 50.59
## 150     6  15.8  64   1       1      3 41.19

########################
##### comment 15 #####
########################
```

```r
# It turns out that the observations with high leverage (80,85,97)
# are related to a long history with T2DM and/or a high BMI value.
# The outlying observations are (not surprisingly) related to a
# relatively high HbA1c values (which are naturally difficult to model)

# In itself this having extreme outcome or predictor values
# does not mandate removal. In fact every analyes is expected to have a few
# unusual observations. Clustering of such observations would be more worriso
me,
# especially if these unusual observations would be outliers and have high le
verage.
# Where possible it is however important to check whether these observations
are due to errors
# in data collection.



################################
#Short-cut for diagnostic plots#
################################

######################
##### comment 16 #####
######################

# Manually creating these plot is great fun and a useful skill
# if one would want to include these in a publication. However,
# to quickly check the modelling assumptions one can simply use
# the plot() function

# diagnostic plots
par(mfrow = c(2,2))
plot(fit.full)
```




```
# note that the bottom left most plot compares the Studentized residuals
# against leverage (as done above as well), and additionally provides
# Cook's distance which indicates oberservations with both high
# leverage and high residual (outliers). The Cook's distance
# indicates that there is little reason to worry about the
# observations previously highlighted as "unusual".
```