Example data analysis

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Contents

1 Create the example dataset

- We start by loading the necessary R packages and functions.

```
library(SimRVSequences)
library(RVMethods)
library(Matrix)
# Load the cd_new() function from its R source file
source("cd_new.R")
# Load worker functions we will call from a source file
source("workerfuncs.R")
```

- Next we read into our R environment the population sequences that we will sample from to create the data.

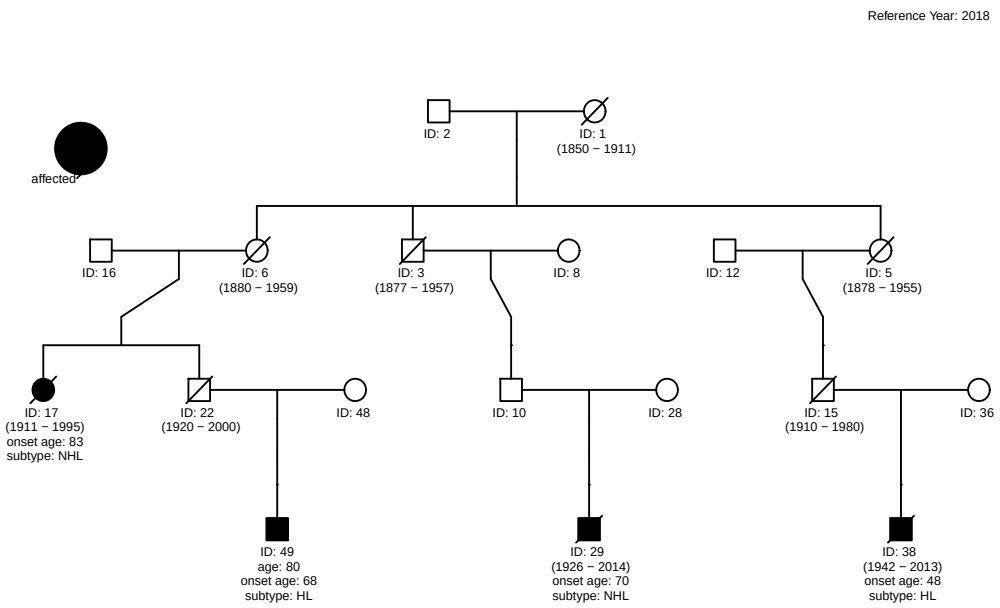
```
infileDir <- "FJdata"
load(paste0(infileDir,"/chr8.RData"))
```

- We also create the vector of carrier probabilities to consider for calculating global likelihood-ratio statistics and configuration probabilities under the null hypothesis (for p-values in the global approaches).
  - The true value of the carrier probability used to simulate the data is 0.00032. We will consider this value plus misspecified values of 1/100,1/10, 1/2, 2 times, 10 times and 100 times the true value.

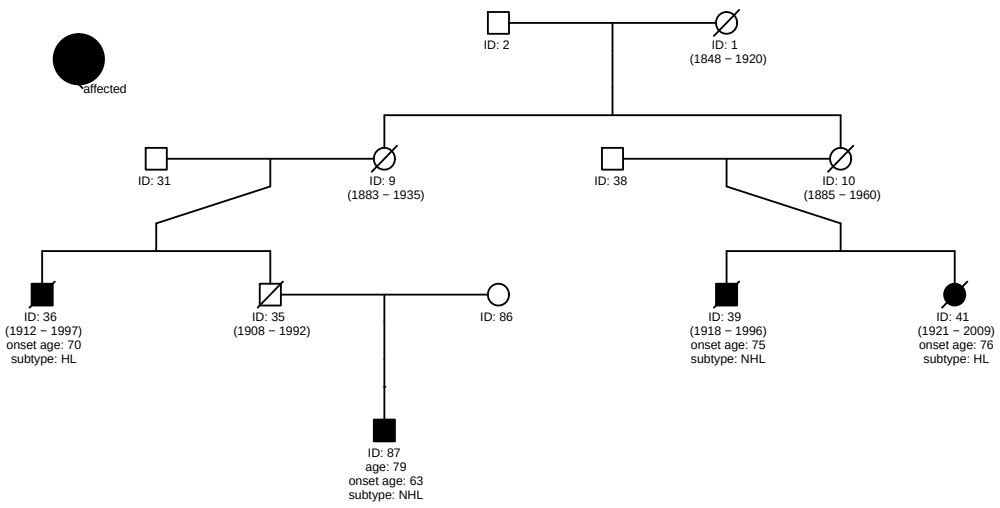
```
true_carrier_prob <- 0.00032
carrier_probs <- true_carrier_prob*c(1/100,1/10,1/2,1,2,10,100)
```

- Next, we re-create the pedigrees from Figure 4.4 of Christina's thesis.
- We don't have the random seeds Christina used but we can re-create the pedigree data structures in a CSV file and read that into an R data frame called `expeds`. Pedigrees are distinguished by a `FamID` variable in the data frame.
- To plot a pedigree, we select it from `expeds` and coerce it to class `ped`. We hide the RV status in the pedigree plots by setting the RV status variables `DA1` and `DA2` in the pedigree data structure to `NULL`.

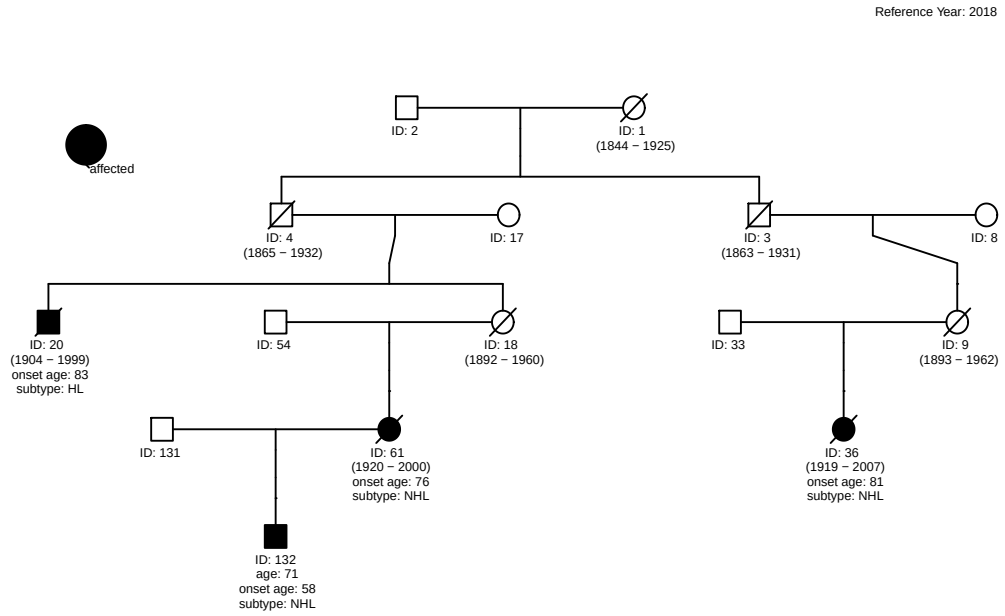
```
expeds <- read.csv("exdatpeds.csv")
class(expeds) <- c("ped","data.frame")
ped1 <- expeds[expeds$FamID==1,]
ped1$DA1 <- ped1$DA2 <- NULL
plot(ped1,ref_year=2018,cex=.4)
```




```
ped2 <- expeds[expeds$FamID==2,]
ped2$DA1 <- ped2$DA2 <- NULL
plot(ped2,ref_year=2018,cex=.4)
```




```
ped3 <- expeds[expeds$FamID==3,]
ped3$DA1 <- ped3$DA2 <- NULL
plot(ped3,ref_year=2018,cex=.4)
```



- Lastly, we simulate sequence data for the pedigrees.
- We use `sim_RVstudy()` from Christina's `SimRVSequences` package. `sim_RVstudy()` takes the study data-frame as input, but operates on the individual pedigrees separately.
- For each pedigree it (i) samples a cSNV from the pool of cSNVs, (ii) samples founder sequences from Nirodha's American Admixed population, and (iii) conditionally gene drops these sequences through the pedigree.
- After simulating sequences, we filter RVs to those that are observed in the affected individuals.
- Note: The next code chunk is the first use of the random number generator so we set the seed here.

```
set.seed(1)
#  Generate sequence data with sim_RVstudy() and then filter RVs further
# to those observed in affected individuals.
s_seqs <- sim_RVstudy(expeds,chr8) # *Christina's*
a_seqs <- filter2aff(s_seqs) # *worker*
```

2 Summarize sequence data of the study

- I use a modified version of Christina's summary method for objects of class "famStudy" (output of `sim_RVstudy`) to get counts for each RV observed in each family, *split by the subtype* of the affected individuals.

```
allele_summary <- mysummary.famStudy(a_seqs) # *worker*
```

- On chromosome 8, 74 RVs are observed in the affected individuals. Fifty seven of the RVs appear only once in the study, 14 appear twice and three appear three times:

```
total_counts <- rowSums(allele_summary[,c("tot1","tot2","tot3")])
table(total_counts)
```

```
## total_counts
##  1  2  3
## 57 14  3
```

- To reduce the output, we focus on the RVs that appear more than once in the study and construct a LaTeX table to show their counts in the paper. From the summary data in `allele_summary` we omit the columns of total counts within each pedigree.

```
exclude_cols <- c(5,8,11)
red_allele_sum <- allele_summary[total_counts > 1,-exclude_cols]
```

- A template for the header and footer of the LaTeX table is shown below, followed by the output of `xtable()`, which includes the body of the LaTeX table. Finally, I manually cut-and-paste the template and table body together to make the table.

```
\begin{table}[ht]
\centering
\begin{tabular}{lrrrrrrrr}
& & & \multicolumn{2}{c}{ped 1} & \multicolumn{2}{c}{ped 2} & \multicolumn{2}{c}{ped 3} \\ \cline{4-9}
\multicolumn{3}{l}{} & NHL & HL & NHL & HL & NHL & HL  \\
  \hline
\multicolumn{3}{l}{Number of affected individuals:} & 2 & 2 & 2 & 2 & 3 & 1 \\ \hline
RV  & chrom & position & & & & & &  \\
  \hline
% Table body starts here

% Table body ends here
\end{tabular}
\caption{Simulated RVs that appear in more than one affected individual, with counts by disease subtype within each pedigree.  Only one RV, 8\_6708389, appears in multiple families.}
\end{table}
```

```
library(xtable)
tem <- xtable(red_allele_sum,digits=0) #print integers
print(tem,type="latex")
```

```
## % latex table generated in R 4.0.2 by xtable 1.8-4 package
## % Fri May 19 23:00:34 2023
## \begin{table}[ht]
## \centering
## \begin{tabular}{rrrrrrrrr}
##   \hline
##  & chrom & position & NHL1 & HL1 & NHL2 & HL2 & NHL3 & HL3 \\
##   \hline
## 8\_6708389 & 8 & 6708389 & 1 & 0 & 0 & 1 & 0 & 0 \\
##   8\_7972232 & 8 & 7972232 & 0 & 0 & 0 & 2 & 0 & 0 \\
##   8\_8376739 & 8 & 8376739 & 1 & 1 & 0 & 0 & 0 & 0 \\
##   8\_12116292 & 8 & 12116292 & 0 & 0 & 0 & 2 & 0 & 0 \\
##   8\_23020454 & 8 & 23020454 & 0 & 2 & 0 & 0 & 0 & 0 \\
##   8\_23068413 & 8 & 23068413 & 0 & 0 & 0 & 0 & 2 & 0 \\
##   8\_28452476 & 8 & 28452476 & 0 & 0 & 0 & 0 & 0 & 2 \\
##   8\_30846116 & 8 & 30846116 & 0 & 0 & 2 & 0 & 0 & 0 \\
##   8\_39680176 & 8 & 39680176 & 1 & 2 & 0 & 0 & 0 & 0 \\
##   8\_41261959 & 8 & 41261959 & 0 & 0 & 2 & 0 & 0 & 0 \\
##   8\_54047456 & 8 & 54047456 & 0 & 0 & 1 & 1 & 0 & 0 \\
##   8\_55454701 & 8 & 55454701 & 0 & 0 & 0 & 0 & 2 & 0 \\
##   8\_60866956 & 8 & 60866956 & 1 & 1 & 0 & 0 & 0 & 0 \\
##   8\_65161224 & 8 & 65161224 & 0 & 0 & 0 & 0 & 2 & 1 \\
##   8\_103382957 & 8 & 103382957 & 1 & 1 & 0 & 0 & 0 & 0 \\
##   8\_107249922 & 8 & 107249922 & 0 & 0 & 1 & 1 & 0 & 0 \\
##   8\_142271244 & 8 & 142271244 & 0 & 0 & 1 & 1 & 0 & 0 \\
##    \hline
## \end{tabular}
## \end{table}
```

- Table 1 below shows the resulting table of RVs appearing more than once in the affected individuals of the study.

3 Analyze the sequence data of the study

- We want to prioritize the RVs in our dataset for further investigation. To prioritize, we use the value of the RV test statistics to rank them with each method. We first call `cd_new()` to get lookup tables of the statistic values.

```
lookupTabs <- cd_new(peds = expeds, subtypes = c("HL", "NHL"),
                     carrier_probs = carrier_probs)
```

- All of the statistical methods assume that cRVs enter pedigrees through at most one founder. We therefore filter out "invalid" RVs that are not consistent with this assumption.

```
a_seqs <- remove_invalid_configs(a_seqs,lookupTabs) # *worker*
```

3.1 Summary of rank results

- We rank each RV in our filtered dataset using the value of the test statistics for its global configuration.

			ped 1		ped 2		ped 3	
			NHL	HL	NHL	HL	NHL	HL
Number of affected individuals:			2	2	2	2	3	1
RV	chrom	position						
8_6708389	8	6708389	1	0	0	1	0	0
8_7972232	8	7972232	0	0	0	2	0	0
8_8376739	8	8376739	1	1	0	0	0	0
8_12116292	8	12116292	0	0	2	0	0	0
8_23020454	8	23020454	0	2	0	0	0	0
8_23068413	8	23068413	0	0	0	2	0	0
8_28452476	8	28452476	0	0	0	0	2	1
8_30846116	8	30846116	0	0	2	0	0	0
8_39680176	8	39680176	1	2	0	0	0	0
8_41261959	8	41261959	0	0	2	0	0	0
8_54047456	8	54047456	0	0	1	1	0	0
8_55454701	8	55454701	0	0	0	0	2	0
8_60866956	8	60866956	1	1	0	0	0	0
8_65161224	8	65161224	0	0	0	0	2	1
8_103382957	8	103382957	1	1	0	0	0	0
8_107249922	8	107249922	0	0	1	1	0	0
8_142271244	8	142271244	0	0	1	1	0	0

Table 1: Counts of RVs within each pedigree by disease subtype, for RVs that appear in more than one affected individual in the study. Only one RV, 8_6708389, appears in multiple families.

- The ranks from the first six global LR methods, with assumed carrier probabilities of 1/100, 1/10, 1/2, 1, 2 and 10 times the true value, are all identical and are represented by the variable `globalLR1to6` in the following code.

```
RVs <- a_seqs$SNV_map$marker
is_CRV <- a_seqs$SNV_map$is_CRV
allranks <- get_ranks(RVs,lookupTabs,a_seqs) # *worker*
rankcols <- c(paste0("globalLR",1:length(carrier_probs)),
              "globaltrans","localLR","RVS","modRVS")
colnames(allranks) <- rankcols
rownames(allranks) <- RVs
# Rankings of first 6 LR methods are the same; e.g.,
all.equal(allranks[,"globalLR1"],allranks[,"globalLR6"])
```

```
## [1] TRUE
```

```
# Use globalLR1 as a representative of the first 6; i.e., remove
# globalLR2, ..., globalLR6 and relabel globalLR1 as globalLR1to6
allranks <- allranks[,-c(2:6)]
colnames(allranks)[1] <- "globalLR1to6"
```

- We print out the names of top-10-ranked RVs and mark cRVs with asterisks.

```
RVs <- a_seqs$SNV_map$marker
is_CRV <- a_seqs$SNV_map$is_CRV
# add one asterisk to name of the first cRV, two asterisks to the
# name of the second cRV and three to the third cRV
RVs[is_CRV] <- paste0(RVs[is_CRV],c("*","**","***"))
top10RVs <-  matrix(nrow=10,ncol=ncol(allranks))
colnames(top10RVs) <- colnames(allranks)
```

```
for(i in 1:ncol(top10RVs)){
  ord <- order(allranks[,i])
  top10RVs[,i] <- RVs[ord][1:10]
}
```

```
options(width=100)
noquote(top10RVs)
```

```
##       globalLR1to6 globalLR7    globaltrans  localLR      RVS         modRVS
##  [1,] 8_39680176   8_39680176   8_39680176   8_39680176   8_39680176  8_39680176
##  [2,] 8_7972232    8_7972232    8_7972232    8_7972232    8_65161224  8_65161224
##  [3,] 8_23068413** 8_23068413** 8_23068413** 8_23068413** 8_8376739   8_8376739
##  [4,] 8_65161224   8_65161224   8_65161224   8_65161224   8_23020454* 8_23020454*
##  [5,] 8_23020454*  8_23020454*  8_23020454*  8_23020454*  8_12116292  8_12116292
##  [6,] 8_8376739    8_60866956   8_54047456   8_54047456   8_30846116  8_30846116
##  [7,] 8_60866956   8_103382957  8_107249922  8_107249922  8_41261959  8_41261959
##  [8,] 8_103382957  8_8376739    8_8376739    8_8376739    8_60866956  8_60866956
##  [9,] 8_54047456   8_54047456   8_60866956   8_60866956   8_103382957 8_103382957
## [10,] 8_107249922  8_107249922  8_103382957  8_103382957  8_7972232   8_7972232
```

- The raw ranks of the three cRVs sampled in the study are shown below.

```
cRVs <- unique(a_seqs$haplo_map$FamCRV) # in peds 1, 2 and 3, respectively
allranks[rownames(allranks)%in%cRVs,]*nrow(allranks)
```

```
##             globalLR1to6 globalLR7 globaltrans localLR RVS modRVS
## 8_23020454             5         5           5       5   4      4
## 8_23068413             3         3           3       3  11     11
## 8_118923860           32        32          38      15  54     32
```

- We can also look at the summaries of the three cRVs.

```
allele_summary[rownames(allele_summary) %in% cRVs,]
```

```
##             chrom  position NHL1 HL1 tot1 NHL2 HL2 tot2 NHL3 HL3 tot3
## 8_23020454      8  23020454    0   2    2    0   0    0    0   0    0
## 8_23068413      8  23068413    0   0    0    0   2    2    0   0    0
## 8_118923860     8 118923860    0   0    0    0   0    0    0   1    1
```

- We see three cRVs, each of which is unique to a pedigree.
- The cRV 8_23020454 from pedigree 1 had a top-10 rank from all methods. From the pedigree diagram we see that this RV is in two HL-affected individuals who are second cousins.
- The cRV 8_23068413 from pedigree 2 had a top-10 rank from the LR methods only. From the pedigree diagram we see that this RV is in two HL-affected individuals who are first cousins.
- The cRV 8_118923860 from pedigree 3 did not have a top-10 ranking from any of the methods. This RV is only observed in one HL-affected individual.

3.2 P-values for sampled cRVS

- For each familial cRV, we find its p-value from the lookup tables.

```
cRVs <- unique(a_seqs$haplo_map$FamCRV)
pvals <- get_pvals(cRVs,lookupTabs,a_seqs) # *worker*
pvalcols <- c(paste0("globalLR",1:length(carrier_probs)),
              paste0("globaltrans",1:length(carrier_probs)),
              "localLR_1","RVS_1","modRVS_1")
colnames(pvals) <- pvalcols
rownames(pvals) <- cRVs
pvals
```

```
##                globalLR1    globalLR2    globalLR3    globalLR4    globalLR5    globalLR6
## 8_23020454  0.0426263041 0.0426328640 0.0426620377 0.0426985474 0.0427717090 0.0433688318
## 8_23068413  0.0174106483 0.0174124672 0.0174205551 0.0174306745 0.0174509448 0.0176147233
## 8_118923860 0.3532146103 0.3532387598 0.3533461167 0.3534803727 0.3537490840 0.3559088472
##                globalLR7 globaltrans1 globaltrans2 globaltrans3 globaltrans4 globaltrans5
## 8_23020454  0.0456594785 0.0426263790 0.0426336125 0.0426657782 0.0427060239 0.0427866438
## 8_23068413  0.0201133004 0.0174107042 0.0174130258 0.0174233496 0.0174362672 0.0174621449
## 8_118923860 0.3883242776 0.6143686747 0.6143691567 0.6143713052 0.6143740055 0.6143794550
##             globaltrans6 globaltrans7    localLR_1        RVS_1     modRVS_1
## 8_23020454  0.0434378160 0.0515801992 0.0202296337 0.0240568617 0.0240568617
## 8_23068413  0.0176712257 0.0202943367 0.0414507772 0.0777202073 0.0414507772
## 8_118923860 0.6144254180 0.6152624632 0.3177339901 0.7093596059 0.3152709360
```

- The cRVs 8_23020454 (from ped 1) and 8_23068413 (from ped 2) were observed in two HL-affected individuals and have reasonably small p-values (e.g., 0.043 and 0.017, respectively by the global methods).
- The cRV 8_118923860 was observed in only one affected individual in pedigree 3 and so, not surprisingly, has large p-values (about 0.35 by the global methods) and ranks (about 0.44 by the LR methods).

4 Appendix: Worker functions

- Here we document the worker functions written specifically for this example data analysis. The functions are sourced into the above analysis from the source file `workerfuncs.R`.
- `workerfuncs.R` also includes functions from R scripts in the simulation study workflow. These other functions are documented in their respective .Rmd files.
- The first worker function written for this analysis removes RVs whose configurations are incompatible with the assumption that RVs enter a pedigree through at most one founder.
- Christina calls such configurations "invalid", and they have been removed from the rows of the lookup tables of p-values and statistics output by `cd_new()`.
- For each RV, we see if its configuration is in the lookup tables, and if not we remove it from the dataset.

```
remove_invalid_configs <- function(a_seqs,lookupTabs) {
  # configs are identified in the lookup table by their base-10
  # representation, or "binID"
  valid_binIDs <- lookupTabs$statvals[,"binID"]
  # initialize an empty vector to hold info on which RV configs are valid
  include <- rep(NA,nrow(a_seqs$SNV_map))
  # loop over RVs
  for(i in 1:length(include)) {
    RV_binID <- find_binID(a_seqs$SNV_map$marker[i],a_seqs)
    include[i] <- (RV_binID %in% valid_binIDs)
  }
  # Use the include vector to subset the input a_seqs and return
  out <- list(ped_files=a_seqs$ped_files,
              ped_haplos=a_seqs$ped_haplos[,include],
              haplo_map=a_seqs$haplo_map,
              SNV_map=a_seqs$SNV_map[include,],
              ped_genos=a_seqs$ped_genos[,include])
  return(out)
}
```

- The second function written for this analysis is a modified version of Christina's `summary.famStudy()` function.

- `famStudy` objects are the output of `sim_RVstudy()` and `summary.famStudy()` is a summary function that returns the counts of each variant in the affected members of each pedigree.
- My modification is to split the counts of each variant by disease subtype.
  - Note: This is a *very* quick-and-dirty function that assumes that there are three families in the study and that there are 12 affected individuals in total.

```
mysummary.famStudy <- function (object, ...)
{
  Fids <- sort(unique(object$ped_files$FamID))
  # The following 6 lines are from Christina's summary function.
  # They get the total allele count in affected individuals
  aff_allele_counts <- lapply(Fids, function(x) {
    SimRVSequences:::affected_allele_count(
      ped_haps = object$ped_haplos[object$haplo_map$FamID == x, ],
      hap_map = object$haplo_map[object$haplo_map$FamID == x, ],
      ped_file = object$ped_files[object$ped_files$FamID == x, ])
  })
  tot_allele_count <- do.call(rbind, aff_allele_counts)
  totals <- colSums(tot_allele_count)
  # I now do the analogous thing to ge the alelle counts in those
  # affected with NHL
  aff_allele_counts <- lapply(Fids, function(x) {
    affected_allele_count_NHL(
      ped_haps = object$ped_haplos[object$haplo_map$FamID == x, ],
      hap_map = object$haplo_map[object$haplo_map$FamID == x, ],
      ped_file = object$ped_files[object$ped_files$FamID == x, ])
  })
  NHL_allele_count <- do.call(rbind, aff_allele_counts)
  # Finally, get the alelle counts in those affected with HL
  aff_allele_counts <- lapply(Fids, function(x) {
    affected_allele_count_HL(
      ped_haps = object$ped_haplos[object$haplo_map$FamID == x, ],
      hap_map = object$haplo_map[object$haplo_map$FamID == x, ],
      ped_file = object$ped_files[object$ped_files$FamID == x, ])
  })
  HL_allele_count <- do.call(rbind, aff_allele_counts)
  # We can now put together our counts and return the results
  fam_allele_count <- cbind(
    NHL_allele_count[ 1,],HL_allele_count[1,],tot_allele_count[1,],
    NHL_allele_count[2,],HL_allele_count[2,],tot_allele_count[2,],
    NHL_allele_count[3,],HL_allele_count[3,],tot_allele_count[3,]
  )
  colnames(fam_allele_count) <-
    c("NHL1","HL1","tot1","NHL2","HL2","tot2","NHL3","HL3","tot3")
  rownames(fam_allele_count) <- object$SNV_map$marker
  out <- data.frame(
    chrom = object$SNV_map$chrom,
    position = object$SNV_map$position,
    fam_allele_count)
  # only return RVs present in at least one affected individual
  return(out[totals>0,])
}

# Worker functions to count variants in NHL and HL subtypes. These are
```

```r
# based on Christina's affected_allele_count() function.
affected_allele_count_NHL <- function (ped_haps, hap_map, ped_file)
{
  aff_IDs <- ped_file$ID[ped_file$affected & ped_file$subtype=="NHL"]
  aff_rows <- which(hap_map$ID %in% aff_IDs)
  total_count <- colSums(ped_haps[aff_rows, ])
  return(total_count)
}
affected_allele_count_HL <- function (ped_haps, hap_map, ped_file)
{
  aff_IDs <- ped_file$ID[ped_file$affected & ped_file$subtype=="HL"]
  aff_rows <- which(hap_map$ID %in% aff_IDs)
  total_count <- colSums(ped_haps[aff_rows, ])
  return(total_count)
}
```