Supplementary Material 3 : Simulate SNV data for affected individuals in pedigrees

Nirodha Epasinghege Dona, Jinko Graham

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This document discusses the gene-dropping step in our work-flow (the orange box labelled 3). Gene-dropping in the ascertained pedigrees is the third and final step required to simulate the exome-sequencing data of affected individuals and their relatives connecting them along a line of descent in the ascertained pedigrees.

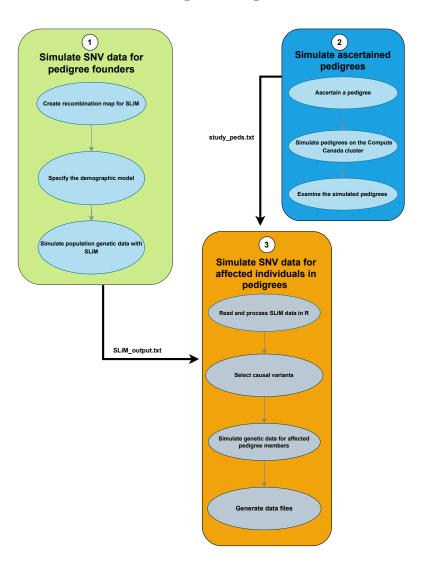


Figure 1: Work-flow for simulating the exome-sequencing data for ascertained pedigrees.

1 Read and process SLiM data in R

Our final goal is to simulate exome sequences for disease-affected members of the ascertained pedigrees. In the first supplementary materials document we obtained exome sequences for an American-admixed population. In the second supplementary materials document we obtained the ascertained pedigrees. Now, we have only to select sequences for the pedigree founders from the population, and then "drop" them through the pedigrees to descendants. We use the gene-dropping functions available in the SimRVSequences (Nieuwoudt,

Brooks-Wilson, and Graham 2020) R package, which require sparse matrices of SNV sequences. Unfortunately, the large population size and number of single-nucleotide variants (SNVs) exceeds R's memory capacity for a single sparse matrix. Therefore, we read in the population sequences and create the sparse matrices chromosome-by-chromosome, as described next.

We start by reading the SLiM simulation output, SLiM_output.txt, into R. This text file is of size approximately 6 GB and contains all the exome sequences in the American-admixed population. The file takes approximately 1 minute to read on a Windows OS with an i7-8550U @ 1.8GHz,16GB of RAM.

```
library(Matrix) #this package is required throughout this document
# Read the text file to R.
# Note: Change the path for the file as necessary.
exData <- readLines("D:/SFU_Vault/SLiM_Output/SLiM_output.txt")
```

Next, we select rare SNVs based on their population derived (mutated) allele frequencies, as described in the next subsection.

1.1 Extract the rare variants

First, we find the line numbers of the mutation and genome header sections in the SLiM output. The formatting of the SLiM output is described in first supplementary materials document.

```
# Find heading location (i.e. file line number) for mutations.
MutHead <- which(exData == "Mutations:")

# Find heading location (i.e. file line number) for genomes.
GenHead <- which(exData == "Genomes:")</pre>
```

We create a data frame to store all the SNVs as follows.

```
##
     tempID type position selCoef count
                                 0 60626
## 1
       7229
              m2 51287555
## 2
     13218
              m2 39812003
                                 0 9536
## 3
       5202
              m2 36490340
                                 0 64099
     25103
                                 0 3732
              m2 41991968
      14264
                                 0 46668
## 5
              m2 54793604
       4333
              m2 30267920
                                 0 79908
```

The MutData data frame contains all the SNVs in the simulated American-admixed population. The rows of this data frame correspond to SNVs and the columns to the following SNV characteristics of interest:

- 1. tempID- specifies the SLiM-generated identifier number which helps to identify the SNV.
- 2. type- represents the type of the SNV."m_1" and "m_2" catalog the non-synonymous and synonymous SNVs, respectively.

- 3. position- indicates the base-pair position of the SNV on the chromosome.
- 4. selCoef- represents the selection coefficient of the SNV.
- 5. count- specifies the number of copies of the SNV in the population.

Next we change the default behavior of the starting positions in SLiM. The starting position is zero in SLiM, but R starts its indexing at position one. To accommodate R indexing, we add one to the tempID and position columns in the MutData data frame.

```
# Add 1 to temp ID so that we can easily associate mutations to columns.
# By default SLiM's first tempID is 0, not 1.
MutData$tempID <- MutData$tempID + 1

# First position in slim is 0, not 1
MutData$position <- MutData$position + 1</pre>
```

Then we calculate the population derived-allele frequencies of the SNVs. We divide the number of copies of the SNV in the population (the count column in the MutData) by the total number of sequences in the population.

After calculating the population derived-allele frequencies, we keep the SNVs which are rare in the population. To select only the rare variants (RVs), we assign a colID to each based on a threshold value for its minor-allele frequency (MAF). RVs with MAFs below the threshold are assigned non-zero colIDs that increase according to their physical order on the exome. Common SNVs are assigned a colID of zero, as they will be discarded.

```
# Create a threshold value
maf <- 0.01
keep_SNVs <- (MutData$afreq <= maf | MutData$afreq >= (1 - maf))

# Variants with MAF below the threshold are assigned non-zero
# colIDs according their physical order on the exome.

MutData$colID <- cumsum(keep_SNVs)*(keep_SNVs)</pre>
```

We create a new data frame, RareMutData, of RVs in the American-admixed population.

```
# Using the identified colID, create data frame of rare mutations only. RareMutData <- MutData[MutData$colID > 0, ]
```

We identify the chromosome of each RV with the reMap_mutations() internal function in the SimRVSequences package. This function requires a recombination map identifying the exon positions on chromosomes. The recombination map is obtained by calling the create_slimMap() function in the SimRVSequences R package.

```
# Create recombination map for exon-only data using
# the hg_exons dataset.(From SimRVSequences.)
recomb_map <- SimRVSequences:::create_slimMap(exon_df = hg_exons)</pre>
```

The call to reMap_mutation() adds a new column, chr, to RareMutData. We are now in a position to extract RV sequences from the SLiM output, as discussed in the next subsection.

1.2 Extract sequences of RVs

First we get the line number of the genome-header section in the SLiM output:

```
# Find heading location (i.e. file line number) for genomes.
GenHead <- which(exData == "Genomes:")</pre>
```

In the genomes section of the SLiM output, rows and columns represent, respectively, exome sequences and SLiM-generated identifier numbers for SNVs. We extract RV sequences with the extract_tempIDs() internal function of the SimRVSequences package.

For the American-admixed population, we now have a database of the RVs (RareMutdata) as well as a catalog of the sequences containing them (RareGenomes). These sequences will be separated by chromosome in the next subsection.

1.3 Prepare chromosome-specific population data

The code chunk below separates the large number of RVs and sequences in the American-admixed population by chromosome. As the code chunk takes approximately 16 hours to run on a Windows OS with an i7-8550U @ 1.8GHz,16GB, we recommend against running it to knit the document. Instead, load the Chromwide.Rdata file which can be found in the Zenodo repository.

We use the foreach() function to parallelize the looping over the 22 chromosomes. To start, we create a "haplotypes" matrix for the corresponding chromosome. The haplotypes matrix is a sparse matrix of class dgCMatrix defined in the SimRVSequences package. The rows of the matrix correspond to sequences in the population and the columns to RVs that lie on the targeted chromosome. For any chromosome, the number of rows in the haplotypes matrix is the number of sequences in the population. We get the IDs for RVs that lie on a particular chromosome from the RareMutData R object and match them to the column IDs of the haplotypes in the RareGenome R object. The RVs are ordered according to their base-pair position along the chromosome. The RVs in the columns of the chromosome-specific haplotypes matrix GenoData and in the rows of the chromosome-specific mutation data-frame RareMutData_new are named according to their chromosome and base-pair position. The following code chunk implements these steps.

```
#-----#

# Get the results by chromosome

#------#

# Load required libraries to parallel the code

library(foreach)

library(doParallel)

# Get unique chromosome IDs.

chrID <- unique(RareMutData$chrom)
```

```
# Create empty lists to save the results
chrby_haplotype <- list()</pre>
chrby_SNVs <- list()</pre>
output <- list()</pre>
# Since we have a large number of SNVs in each chromosome,
# we parallelize the function to speed up the simulation time.
# Make the clusters.
cl <- makeCluster(detectCores() - 1)</pre>
# Register the clusters.
registerDoParallel(cl)
# Create a foreach loop.
out <- foreach(k= 1:length(chrID),</pre>
               .packages = c("Matrix", "tidyverse", "data.table",
                               "SimRVSequences"),
               .multicombine = TRUE)%dopar%{
    #----#
    # Genotypes #
    #----#
    # Get the column positions of the sparse matrix for the kth chromosome.
    # We use the jpos output that contains the
    # data from the genome section of the SLiM output.
    jpos_chr <- lapply(RareGenomes, function(x){</pre>
                      x[RareMutData[RareMutData$colID]
                                    %in% x, ]$chrom == chrID[k]]})
    # Get the rows of the sparse matrix for the kth chromosome.
    ipos_chr <- lapply(1:length(jpos_chr), function(x){</pre>
                        rep(x, length(jpos_chr[[x]]))})
    # Create sparse matrix containing SNVs (columns)
    # for each genome (row).
    GenoData <- sparseMatrix(i = unlist(ipos_chr),</pre>
                              j = unlist(jpos_chr),
                              x = rep(1, length(unlist(jpos_chr))))
    GenoData <- GenoData[, -which(colSums(GenoData) == 0)]</pre>
    #----#
    # SNVs #
    #----#
    # Identify the SNV matrix for each chromosome.
    Mute_uni <- unlist(jpos_chr)</pre>
    RareSNVs <- RareMutData[RareMutData$colID %in% Mute_uni, ]</pre>
    # Order by genomic position of rare SNV.
    GenoData <- GenoData[, order(RareSNVs$position)]</pre>
    RareSNVs <- RareSNVs[order(RareSNVs$position), ]</pre>
    RareSNVs$colID <- 1:nrow(RareSNVs)</pre>
    # Remove the old tempID.
```

```
RareSNVs <- RareSNVs[, -1]</pre>
    # Change the row names and column names of the mutation data frame.
    RareMutData new <- RareSNVs
    row.names(RareMutData_new) = NULL
    # Create unique SNV names.
    RareMutData new$SNV <- make.unique(paste0(RareMutData new$chrom,</pre>
                                                   sep = " ",
                                                   RareMutData new$position))
    # Reduce RareMutData, to the columns we actually need.
    RareMutData new <- RareMutData new[, c("colID", "chrom", "position",</pre>
                                             "afreq", "SNV", "type",
                                             "selCoef")]
    # Store the SNVs and haplotypes by chromosome.
    output[[k]] <- list(Haplotypes = GenoData,</pre>
                         Mutations = RareMutData_new)
               }
stopCluster(cl)
# Save the result
save(out, file = "Chromwide.Rdata")
```

1.4 Identify pathway RVs

To identify the RVs that lie on the pathway of interest, we use the identify_pathwaySNVs() function in the SimRVSequences package. We supply the apoptosis sub-pathway centered about the TNFSF10 gene in the UCSC Genome Browser's Gene Interaction Tool as discussed in Nieuwoudt, Brooks-Wilson, and Graham (2020). The data in this sub-pathway are contained in the hg_apopPath data set in SimRVSequences R package.

```
# Load the output generated from the previous code chunk.
# Note: Change the path for the file as necessary.

load("Chromwide.Rdata")

#-----#
# Identify Pathway SNVs #
#-------#
pathway_out <- lapply(out, function(x){
   RareMutData_pathway = SimRVSequences:::identify_pathwaySNVs(markerDF = x$Mutations, pathwayDF = hg_apopPath )})</pre>
```

The call to identify_pathwaySNVs() adds an additional column to the mutation data frame labelled pathwaySNV. This column identifies RVs that lie on the pathway as TRUE.

We combine the chromosome-specific haplotypes matrices with the chromosome-specific mutation data frames to get list elements for chromosomes. We then combine the chromosome-specific list elements into a list of chromosomes as follows.

```
# Create a list of 22 elements representing chromosomes.
# Each element is itself a list which contains the haplotypes
```

The format of slim_out is discussed in the next subsection.

1.5 Discuss format of chromosome-specific population data

The structure of the first element of the slim_out, for chromosome 1, is shown below.

```
# Get the structure of each list elements of output.
str(slim_out[[1]])
```

```
## List of 2
    $ Haplotypes:Formal class 'dgCMatrix' [package "Matrix"] with 6 slots
##
                    : int [1:8343173] 86549 579 1814 3424 4089 4909 5912 6565 7663 8422 ...
##
     .. ..@ p
##
                    : int [1:84665] 0 1 144 146 189 295 349 362 366 367 ...
##
     .. ..@ Dim
                    : int [1:2] 107752 84664
##
     .. .. @ Dimnames:List of 2
##
     .. .. ..$ : NULL
##
     .. .. ..$ : NULL
##
     .. ..@ x
                    : num [1:8343173] 1 1 1 1 1 1 1 1 1 1 ...
##
     .. .. @ factors : list()
##
   $ Mutations :'data.frame': 84664 obs. of 8 variables:
##
     ..$ colID
                   : int [1:84664] 1 2 3 4 5 6 7 8 9 10 ...
                   : int [1:84664] 1 1 1 1 1 1 1 1 1 1 ...
##
     ..$ chrom
     ..$ position : num [1:84664] 11951 11975 12224 12626 13231 ...
##
##
     ..$ afreq
                   : num [1:84664] 9.28e-06 1.33e-03 1.86e-05 3.99e-04 9.84e-04 ...
                   : chr [1:84664] "1_11951" "1_11975" "1_12224" "1_12626" ...
##
     ..$ SNV
                   : Factor w/ 2 levels "m1", "m2": 1 1 1 1 2 2 1 1 1 1 ...
##
     ..$ type
                   : num [1:84664] -3.64e-03 -1.33e-06 -2.45e-02 -4.53e-03 0.00 ...
##
     ..$ selCoef
     ...$ pathwaySNV: logi [1:84664] FALSE FALSE FALSE FALSE FALSE FALSE ...
```

The object slim_out is a list of 22 elements. Each element corresponds to a chromosome and is itself a list with two elements, a haplotypes matrix and a mutation data frame. The haplotypes matrix contains the chromosome-specific exome sequences of all 107,752 individuals in the simulated American-admixed population. Exome sequences for pedigree founders are sampled from the haplotypes matrix. The mutation data frame contains information on the SNVs that reside on the chromosome. For chromosome 1, the dimensions of these elements are as follows.

```
# dimensions of the list element 1
dim(slim_out[[1]]$Haplotypes)
## [1] 107752 84664
```

```
## [1] 84664 8
```

dim(slim out[[1]]\$Mutations)

The number of columns in haplotypes matrix is equal to the number of rows in the mutation data frame. The first chromosome has 84,664 SNVs. Let's print the first four rows and 30 columns of its haplotypes matrix.

```
slim_out[[1]]$Haplotypes[1:6, 1:30]
```

##	[2,]						•									•	
	[3,]																
##	[4,]																
##	[5,]																
##	[6,]																

The haplotypes matrix is a sparse matrix of class dgCMatrix from the Matrix package. Rows correspond to individuals and columns correspond to RVs on chromosome 1. Entries with "1" and "." indicate the derived (mutated) allele and the ancestral allele, respectively.

Let's print the first six rows of the mutation data frame for chromosome 1.

head(slim_out[[1]]\$Mutations)

##		${\tt colID}$	${\tt chrom}$	position	afreq	SNV	type	selCoef	${\tt pathwaySNV}$
##	1	1	1	11951	9.280570e-06	1_11951	m1	-3.638366e-03	FALSE
##	2	2	1	11975	1.327122e-03	1_11975	m1	-1.328507e-06	FALSE
##	3	3	1	12224	1.856114e-05	1_12224	m1	-2.454038e-02	FALSE
##	4	4	1	12626	3.990645e-04	1_12626	m1	-4.526557e-03	FALSE
##	5	5	1	13231	9.837404e-04	1_13231	m2	0.000000e+00	FALSE
##	6	6	1	13411	5.011508e-04	1_13411	m2	0.000000e+00	FALSE

The rows and columns of the mutation data frame represent the RVs and their characteristics, respectively. The column variable colID links the rows in the mutation data frame to the columns of haplotypes matrix, chrom is the chromosome of the RV, position is the position of the RV along the chromosome in base pairs, afreq is the RV's population derived allele frequency, SNV is an unique character identifier for the RV, and pathwaySNV identifies whether or not RVs are located within the apoptosis sub-pathway of interest.

The next task is to select the causal rare variants (cRVs).

2 Select causal variants

We create a function, select_cRV(), to select cRVs. The functions considers RVs in genes on an apoptosis sub-pathway as candidates for cRVs. Among these, cRVs are selected from population singletons, on the basis of their absolute selection coefficients, until the cumulative probability of a sequence carrying a cRV in the population is 0.001. Note that selection coefficients are less than or equal to zero because mutations are deleterious or selectively neutral in the SLiM simulation. The function has two required arguments:

- 1. chrm_by_out represents the chromosome-by-chromosome results in the slim_out R object.
- 2. cumAF specifies the cumulative probability of a sequence carrying a risk variant in the population.

select_cRV() selects the relevant mutation data frames and haplotypes matrices from the slim_out R object. Singleton SNVs which lie on the specified pathway (apoptosis sub-pathway) are determined from the haplotypes matrices of the cumAF argument. A weight is assigned to each singleton in the pathway according to the value of its selection coefficient (which is ≤ 0 because mutations are set to be deleterious or selectively neutral in the SLiM simulation). The weights are calculated as:

$$w_i = \frac{|S_i|}{|\sum_i^N S_i|},$$

where w_i is weight of i^{th} SNV; S_i is the selection coefficient of the i^{th} SNV and N is the total number of singletons in the specified pathway. These weights are used as the sampling probabilities for drawing causal rare variants (cRVs) from the pool of singleton SNVs. The cRVs are sampled randomly with these weights until their cumulative, derived-allele frequency in the population is 0.001.

The select_cRV() function is:

```
select_cRV <- function(chrm_by_out, cumAF){</pre>
  # Select all the mutation data frames in the slim_out object.
  SNV_df <- lapply( chrm_by_out, `[[`, 'Mutations')</pre>
  # Select all haplotypes matrices in the slim_out object.
  haplo <- lapply( chrm_by_out, `[[`, 'Haplotypes')</pre>
  # Get the ColIDs of singletons under each chromosome.
  sing_colID <- lapply(lapply(haplo, function(x){</pre>
    which(colSums(x) == 1)}), unlist)
  # Select only singletons in the mutation data frames.
  singletons <- lapply(1:22, function(x){</pre>
    SNV_df[[x]][SNV_df[[x]]$colID %in% sing_colID[[x]], ]})
  # Combine all the 22 data frames (contains only singletons)
  # into one data frame.
  SNV_singletons <- do.call(rbind, singletons)</pre>
  # Assign weights to each marker based on their selection coefficient values.
  SNV_singletons$weight <-</pre>
    abs(SNV_singletons$selCoef)/abs(sum(SNV_singletons$selCoef))
  # Select SNVs(singletons) which lie on our pathway of interest.
  SNV_pathway <- SNV_singletons[SNV_singletons$pathwaySNV == TRUE, ]</pre>
  # Initialize vectors to store the cumulative sum of allele frequencies and
  # cRVs.
  cum <- 0
  cSNVs <- c()
  # The loop runs while the cumulative sum of the allele frequency
  # is less than or equal to CumAF (0.001).
  while (cum <= cumAF) {</pre>
    # Select a SNV proportional to its weights.
    selected_cRVs <- sample(SNV_pathway$SNV, 1,</pre>
                             prob = c(SNV_pathway$weight))
    # Get the allele frequency of the selected SNV.
    af <- SNV_pathway[SNV_pathway$SNV == selected_cRVs, 4]</pre>
    \# Update the cumulative sum of the allele frequencies of causal SNVs.
    cum <- cum + af
    # Remove the selected SNV from the mutation data frame.
    # If not we may get this same SNV again.
    SNV_pathway <- SNV_pathway[-(which(SNV_pathway$SNV == selected_cRVs)),</pre>
    # Save the selected cRVs in a vector
    cSNVs <- c(cSNVs, selected_cRVs)
  }
  # Create a new variable in our mutation data frame to represent
  # whether a SNV is a cRV or not.
  SNV_combine <- do.call("rbind", SNV_df)</pre>
  SNV_combine$is_CRV <- SNV_combine$SNV %in% cSNVs</pre>
```

```
return(SNV_combine)
}
```

Below is an example call to select_cRV().

```
# Set a seed value.
set.seed(1987)

# Run the function.
cRV_data <- select_cRV(chrm_by_out = slim_out , cumAF = 0.001)

# Display the function output.
head(cRV_data)</pre>
```

```
##
     colID chrom position
                                  afreq
                                            SNV type
                                                            selCoef pathwaySNV
## 1
                    11951 9.280570e-06 1_11951
                                                  m1 -3.638366e-03
         1
               1
                                                                         FALSE
## 2
         2
                    11975 1.327122e-03 1_11975
                                                  m1 -1.328507e-06
                                                                         FALSE
               1
                    12224 1.856114e-05 1_12224
## 3
         3
                                                  m1 -2.454038e-02
                                                                         FALSE
               1
         4
## 4
               1
                    12626 3.990645e-04 1_12626
                                                  m1 -4.526557e-03
                                                                         FALSE
## 5
         5
               1
                    13231 9.837404e-04 1_13231
                                                  m2 0.00000e+00
                                                                         FALSE
## 6
         6
                    13411 5.011508e-04 1_13411
                                                  m2 0.000000e+00
                                                                         FALSE
##
     is_CRV
## 1 FALSE
## 2
     FALSE
## 3 FALSE
## 4
     FALSE
## 5 FALSE
## 6 FALSE
```

The output of the function is a mutation data frame with an additional column, is_CRV. This column gives the RVs selected as causal variants. We may then print the number of cRVs in the population, their cumulative allele frequencies in the population and their chromosomes, as follows.

```
# Display the number of selected cRVs in the population.
length(which(cRV_data$is_CRV == TRUE))

## [1] 108

# Print the cumulative allele frequency in the population.
round(sum(cRV_data$afreq[which(cRV_data$is_CRV==TRUE)]), 5)

## [1] 0.001

# Display the number of cRVs that are selected from each chromosome.
table(cRV_data[cRV_data$is_CRV == TRUE, ]$chrom)

##

## 1 2 3 4 5 6 7 8 10 11 16 17 18 19 21 22

## 1 21 5 3 8 8 5 11 8 4 4 4 13 1 7 5
```

According to the above outputs, 108 RVs are sampled as cRVs. Their cumulative derived-allele frequency in the population is 0.001. The table summarizes the number of cRVs on each chromosome. For example, only one cRV is on chromosome 1; 21 cRVs reside in chromosome 2 and so forth.

Then we add the is_CRV column to all 22 mutation data frames in the slim_out object as follows.

```
# Add is_CRV column to all 22 mutation data frames
slim_out <- lapply(1:22, function(x){
```

We are now ready to simulate exome sequences for the affected individuals in the 150 ascertained pedigrees, as described in the next section.

3 Simulate genetic data for affected pedigree members

The sim_RVstudy() function of the SimRVSequences R package simulates genetic sequence data in pedigrees, but expects only a single population database of sequences as an argument in the form of a sparse matrix of SNV haplotypes and an associated mutation data frame. Unfortunately, we cannot use sim_RVstudy() without modification because the number of individuals and RVs in our American-admixed population far exceeds R's memory. The fundamental problem is that the population sequences of RVs cannot be contained in a single sparse matrix. We therefore modify sim_RVstudy() and various supporting functions in the SimRVSequences package to handle chromosome-specific databases, as described in the next subsections.

3.1 Modify sim_RVstudy() to simulate data by chromosome

We add a new argument, fam_RVs, to sim_RVstudy() that identifies the familial cRVs. This argument is used to check whether or not the familial cRV lies on the targeted chromosome. If the familial cRV is on the targeted chromosome, the chromosome is segregated through the pedigree with conditional gene-dropping (Nieuwoudt, Brooks-Wilson, and Graham 2020). Otherwise, the chromosome is segregated through the pedigree according to Mendelian law. Below, the updated sim_RVstudy_new() function has these changes as marked in the comments.

```
sim_RVstudy_new <- function(ped_files, SNV_data, fam_RVs,</pre>
                             affected_only = TRUE,
                             remove_wild = TRUE,
                             pos_in_bp = TRUE,
                             gamma_params = c(2.63, 2.63/0.5),
                             burn_in = 1000,
                             SNV_map = NULL, haplos = NULL){
  if (!(is.null(SNV_map)) | !is.null(haplos)) {
    stop("Arguments 'SNV_map' and 'haplos' have been deprecated.
         \n Instead, please supply to argument 'SNV_data' an object of class SNVdata.
         Execute help(SNVdata) for more information." )
  }
  if (!(SimRVSequences:::is.SNVdata(SNV_data))) {
    stop("Expecting SNV_data to be an object of class SNVdata")
  }
  #check to see if DA1 and DA2 are both missing, if so
  #assume fully sporadic and issue warning
  if (is.null(ped_files$DA1) & is.null(ped_files$DA2)) {
    ped_files$DA1 <- 0</pre>
    ped_files$DA2 <- 0</pre>
    warning("\n The variables DA1 and DA2 are missing from ped_files.
            \n Assuming fully sporadic ...
            \n...setting DA1 = DA2 = 0 for all pedigrees.")
  }
  #check ped_files for possible issues
```

```
SimRVSequences:::check_peds(ped_files)
#assign generation number if not included in ped_file
if(!"Gen" %in% colnames(ped_files)){
 ped_files$Gen <- unlist(lapply(unique(ped_files$FamID),</pre>
                            function(x){
                              SimRVSequences:::assign_gen(ped_files[
                                 ped files$FamID == x, ])}))
}
# save mutations and haplotypes in SNV_map and haplos objects
SNV_map = SNV_data$Mutations
haplos = SNV_data$Haplotypes
#check to see that the sample contains affected relatives when the
#affected_only setting is used
if (affected_only & all(ped_files$affected == FALSE)) {
 stop("\n There are no disease-affected relatives in this sample of pedigrees.
       \n To simulate data for pedigrees without disease-affected
       relatives use affected_only = FALSE.")
}
#collect list of FamIDs
FamIDs <- unique(ped_files$FamID)</pre>
#check for pedigree formatting issues
for (i in FamIDs){
  SimRVSequences:::check_ped(ped_files[ped_files$FamID == i, ])
#Reduce to affected-only pedigrees
if (affected_only) {
  #reduce pedigrees to contain only disease-affected relative and
  #the individuals who connect them along a line of descent.
 Afams <- lapply(FamIDs, function(x){
 SimRVSequences:::affected_onlyPed(ped_file = ped_files[which(ped_files$FamID == x),])
 })
  #combine the reduced pedigrees
 ped_files <- do.call("rbind", Afams)</pre>
 pedfiles <- ped_files</pre>
 #check to see if any pedigrees were removed due to lack of
  #disease affected relatives and issue warning for removed pedigrees
 removed_peds <- setdiff(FamIDs, unique(ped_files$FamID))</pre>
 if (length(removed_peds) > 0){
   FamIDs <- unique(ped_files$FamID)</pre>
    warning("\n There are no disease-affected relatives in the pedigrees with FamID: ",
            paste0(removed_peds, collapse = ", "),
            "\n These pedigrees have been removed from ped_files.")
 }
}
```

```
# Add is_CRV column again if it is not present
if (is.null(SNV_map$is_CRV)) {
  SNV map$is CRV = FALSE
   # warning("The variable is CRV is missing from SNV map.",
             "\n ... randomly sampling one SNV to be the cRV for all pedigrees.")
}
# Check whether any candidates for the familial cRV lie on the chromosome.
# This next block of code is changed from the original.
for(k in 1:length(FamIDs)){
  if(any(SNV_map$SNV == fam_RVs[k])){
     ped_files[ped_files$FamID == k, ]$DA1 <- ped_files[ped_files$FamID ==</pre>
                                                            k, ]$DA1
    ped_files[ped_files$FamID == k, ]$DA2 <- ped_files[ped_files$FamID ==</pre>
                                                            k, ]$DA2
  } else {
     ped_files[ped_files$FamID == k, ]$DA1 <- 0</pre>
    ped_files[ped_files$FamID == k, ]$DA2 <- 0</pre>
  }
}
#Given the location of familial risk variants, sample familial founder
#haplotypes from conditional haplotype distribution
 f_genos <- lapply(c(1:length(FamIDs)), function(x){</pre>
 sim_FGenos(founder_ids = ped_files$ID[which(ped_files$FamID == FamIDs[x]
                                                & is.na(ped files$dadID))],
              RV_founder = ped_files$ID[which(ped_files$FamID == FamIDs[x]
                                               & is.na(ped_files$dadID)
                                               & (ped_files$DA1 + ped_files$DA2) != 0)],
              founder_pat_allele = ped_files$DA1[which(ped_files$FamID == FamIDs[x]
                                                         & is.na(ped_files$dadID))],
              founder_mat_allele = ped_files$DA2[which(ped_files$FamID == FamIDs[x]
                                                         & is.na(ped_files$dadID))],
              haplos, RV_col_loc = which(SNV_map$SNV == fam_RVs[x]),
              RV_pool_loc = SNV_map$colID[SNV_map$is_CRV])
})
#If desired by user, reduce the size of the data by removing
#markers not carried by any member of the study.
if (remove wild) {
  reduced_dat <- SimRVSequences:::remove_allWild(f_haps = f_genos, SNV_map)</pre>
  f_genos <- reduced_dat[[1]]</pre>
  SNV_map <- reduced_dat[[2]]</pre>
}
#create chrom_map, this is used to determine the segments over
#which we will simulate genetic recombination
chrom_map <- SimRVSequences:::create_chrom_map(SNV_map)</pre>
#convert from base pairs to centiMorgan
if (pos_in_bp) {
  options(digits = 9)
  chrom_map$start_pos <- SimRVSequences:::convert_BP_to_cM(chrom_map$start_pos)</pre>
```

```
chrom_map$end_pos <- SimRVSequences:::convert_BP_to_cM(chrom_map$end_pos)</pre>
    SNV_map$position <- SimRVSequences:::convert_BP_to_cM(SNV_map$position)</pre>
  }
  #simulate non-founder haploypes via conditional gene drop
  ped_seqs <- lapply(c(1:length(FamIDs)), function(x){</pre>
    sim_seq(ped_file = ped_files[ped_files$FamID == FamIDs[x], ],
            founder genos = f genos[[x]],
            SNV_map, chrom_map,
            RV_marker = fam_RVs[x],
            burn_in, gamma_params)
  })
  ped_haplos <- do.call("rbind", lapply(ped_seqs, function(x){x$ped_genos}))</pre>
  haplo_map <- do.call("rbind", lapply(ped_seqs, function(x){x$geno_map}))
  #convert back to base pairs if we converted to CM
  if (pos_in_bp) {
    options(digits = 9)
    SNV_map$position <- SimRVSequences:::convert_CM_to_BP(SNV_map$position)</pre>
  return(SimRVSequences:::famStudy(list(ped_files = pedfiles, ped_haplos = ped_haplos,
                        haplo_map = haplo_map, SNV_map = SNV_map)))
}
```

sim_RVstudy() requires the argument SNV_data, an object of class SNVdata as defined by SimRVSequences package. The SNVdata() function in the SimRVSequences R package converts haplotypes matrices and mutation data frames into an object of class SNVdata. We modified the SNVdata() and the check_SNV_map() functions of the package to align with our changes in sim_RVstudy_new(). The SNVdata() and check_SNV_map() functions remain the same except that the mutation data frame provided as an argument is now expected to have a column named SNV rather than marker. When the targeted chromosome contains no cRV, the original check_SNV_map() function exits with an error. The original function exits because it inappropriately checks whether the is_CRV column is FALSE for all the SNVs in the mutation data frame. To avoid the inappropriate exit, we remove this check. The modified versions of SNVdata() and check_SNV_map() are renamed as SNVdata_new() and check_SNV_map_new() and defined in the next code chunk.

```
# Define the SNVdata_new() and check_SNV_map_new() functions

# Constructor function for an object of class SNVdata
SNVdata_new <- function(Haplotypes, Mutations, Samples = NULL) {

#check SNV_map for possible issues
check_SNV_map_new(Mutations)

if (!"SNV" %in% colnames(Mutations)) {

   Mutations$SNV <- make.unique(pasteO(Mutations$chrom, sep = "_", Mutations$position))
}

if (nrow(Mutations) != ncol(Haplotypes)) {
   stop("\n nrow(Mutations) != ncol(Haplotypes).
        \n Mutations must catalog every SNV in Haplotypes.")
}</pre>
```

```
#create list containing all relevant of SNVdata information
  SNV_data = list(Haplotypes = Haplotypes,
                  Mutations = Mutations,
                  Samples = Samples)
  class(SNV_data) <- c("SNVdata", class(SNV_data))</pre>
  return(SNV_data)
}
# Check SNV_map for possible issues: modified version
check_SNV_map_new <- function(SNV_map){</pre>
  #check to see if SNV_map contains the column information we expect
  # and check to see if we have any missing values.
  ## Check colID variable
  if (!"colID" %in% colnames(SNV_map)) {
   stop('The variable "colID" is missing from SNV_map.')
  if (any(is.na(SNV_map$colID))) {
   stop('Error SNV_map: The variable "colID" contains missing values.')
  }
  if (any(duplicated(SNV map$colID))) {
   stop('Error SNV_map: The variable "colID" contains duplicate values.')
  }
  ## Check chrom variable
  if (!"chrom" %in% colnames(SNV_map)) {
    stop('The variable "chrom" is missing from SNV_map.')
  if (any(is.na(SNV_map$chrom))) {
    stop('Error SNV_map: The variable "chrom" contains missing values.')
  ## Check position variable
  if (!"position" %in% colnames(SNV_map)) {
    stop('The variable "position" is missing from SNV_map.')
  if (any(is.na(SNV_map$position))) {
    stop('Error SNV_map: The variable "position" contains missing values.')
  # Check to see if marker variable exists, and if so do all SNVs have a unique name
  if ("SNV" %in% colnames(SNV_map)) {
   if (length(unique(SNV_map$SNV)) != nrow(SNV_map)) {
      stop('Expecting each SNV to have a unique SNV name in SNV_map.')
   }
   if (any(is.na(SNV_map$SNV))) {
      stop('Error SNV_map: The variable "marker" contains missing values.')
   }
 }
```

The next subsection discusses how we set the arguments of sim_RVstudy_new().

3.2 Set arguments to sim_RVstudy_new()

sim_RVstudy_new() requires three arguments: fam_RVs giving the cRV for each ascertained pedigree, ped_files giving the ascertained pedigrees and SNV_data giving the chromosome-specific exome sequences and associated mutation data frames for everyone in the American admixed population. We prepare these three arguments as follows.

(1). fam_RVs

Familial cRVs are sampled on the basis of their population derived-allele frequencies as follows.

```
# Load all 150 pedigrees.
# Note: Change the path for the file as necessary.
study_peds <- read.table("study_peds.txt", header=TRUE, sep= " ")</pre>
# Collect list of FamIDs.
FamIDs <- unique(study peds$FamID)</pre>
# Set the sampling probabilities for causal RVs.
# When the derived-allele frequencies are provided, we sample cRVs
# according to their derived-allele frequency.
sample prob <- cRV data$afreq[cRV data$is CRV]/</pre>
    sum(cRV_data$afreq[cRV_data$is_CRV])
set.seed(1987)
# Sample the familial cRV from the pool of potential cRVs with replacement.
familial_RVs <- sample(x = cRV_data$SNV[cRV_data$is_CRV],</pre>
                  size = length(FamIDs),
                  prob = sample_prob,
                  replace = TRUE)
# Display first five candidates for familial cRVs.
familial_RVs[1:5]
```

```
## [1] "2_201170321" "18_63125128" "10_89015222" "6_108683999" "1_155738619"
```

The final output, familial_RVs is a vector of length 150 that contains the familial cRVs for each of the ascertained pedigrees.

- (2). ped_files is a data frame that represents the ascertained pedigrees. We have loaded this data frame previously, in the object study_peds.
- (3). SNV_data gives a database of exome sequences for a single chromosome, for everyone in the American-admixed population. This argument is an object of class SNVdata. Objects of class SNVdata are comprised of a sparse matrix of exome sequences together with an associated data frame of mutation information.

We first make a list, by chromosome, of objects of class SNVdata by applying the SNVdata_new() function:

The SNV_data argument of sim_RVstudy_new() will be extracted from the appropriate list element of the chrom_data object.

The remaining arguments to sim_RVstudy_new() are optional and set with the defaults of the original sim_RVstudy() function. For example, we use the default value affected_only = TRUE to simulate sequence data for disease-affected members in the pedigree, as is typical for exome-sequencing studies of families ascertained for multiple affected relatives. Affected relatives from such families are more likely to carry a

cRV. We also set the default value of remove_wild = TRUE, to shrink the sequence data for the study by removing monomorphic SNVs.

To obtain the genetic sequences for disease-affected family members, we loop over chromosomes and apply the simRV_study_new() function to each. First, however, we load functions required by simRV_study_new(). These functions are slightly modified versions of sim_FGenos() and sim_seq(), two non-exported functions from the SimRVSequences R package. The modified versions are the same as their counterparts in SimRVsequences, except they use the Matrix package's which() function instead of base R's.

```
# Draw founder genotypes from haplotype distribution given familial RV
sim_FGenos <- function(founder_ids, RV_founder,</pre>
                       founder_pat_allele, founder_mat_allele,
                       haplos, RV_col_loc, RV_pool_loc) {
  #Determine which haplotypes carry the familial RV and which do not
  #Determine which haplotypes carry the familial cRV
  RV_hap_loc <- which(haplos[, RV_col_loc] == 1)</pre>
  #Determine which haplotypes do not carry ANY cRV in the pool
  no_CRVrows <- SimRVSequences:::find_no_cSNV_rows(haplos, RV_pool_loc)
  #here we handle the fully sporadic families
  #i.e. families that do not segregate any cSNVs
  #In this case, the haplotypes for ALL founders
  #is sampled from no CRVhaps
  if(length(RV_founder) == 0){
    #sample all founder data from this pool
    founder_genos <- haplos[sample(x = no_CRVrows,</pre>
                                    size = 2*length(founder_ids),
                                    replace = TRUE), ]
  } else {
    #sample the paternally inherited founder haplotypes
    pat_inherited_haps <- sapply(founder_pat_allele, function(x){</pre>
      if(x == 0){
        SimRVSequences:::resample(x = no_CRVrows, size = 1)
        SimRVSequences:::resample(x = RV_hap_loc, size = 1)
      }})
    #sample the maternally inherited founder haplotypes
    mat_inherited_haps <- sapply(founder_mat_allele, function(x){</pre>
      if(x == 0){
        SimRVSequences:::resample(x = no_CRVrows, size = 1)
        SimRVSequences:::resample(x = RV_hap_loc, size = 1)
      }})
    #pull the sampled haplotypes from the haplos matrix
    founder_genos <- haplos[c(pat_inherited_haps, mat_inherited_haps), ]</pre>
  #create IDs to associate founders to rows in founder_qenos
  founder_genos_ID <- rep(founder_ids, 2)</pre>
  #re-order so that founder haplotypes appear in order
```

```
founder_genos <- founder_genos[order(founder_genos_ID), ]</pre>
  founder_genos_ID <- founder_genos_ID[order(founder_genos_ID)]</pre>
 return(list(founder_genos, founder_genos_ID))
}
#Now the modified version of the sim_seq() function.
sim seq <- function(ped file, founder genos,
                    SNV_map, chrom_map, RV_marker,
                    burn_in = 1000, gamma_params = c(2.63, 2.63/0.5)){
  #Get parent/offspring information
  #i.e. for each offspring find RV status,
  #parent IDs, and parent alleles at RV locus
  PO_info <- SimRVSequences:::get_parOffInfo(ped_file)
  PO_info <- PO_info[order(PO_info$Gen, PO_info$offspring_ID),]
  ped_genos <- founder_genos[[1]]</pre>
  ped_geno_IDs <- founder_genos[[2]]</pre>
  #determine the chromosome number and location of the familial RV locus
  #then store as a data frame with chrom in the first column
  RVL <- SNV_map[which(SNV_map$SNV == RV_marker),</pre>
                 which(colnames(SNV_map) %in% c("chrom", "position"))]
  if(colnames(RVL[1]) != "chrom"){
    RVL \leftarrow RVL[, c(2, 1)]
  }
  #for each offspring simulate transmission of parental data
  for (i in 1:nrow(PO_info)) {
    #simulate recombination events for this parent offspring pair
    loop_gams <- SimRVSequences:::sim_gameteInheritance(RV_locus = RVL,</pre>
                                        parent_RValleles = PO_info[i, c(6, 7)],
                                        offspring_RVstatus = PO_info[i, 5],
                                        chrom_map,
                                        allele_IDs = c(1, 2),
                                        burn_in, gamma_params)
    #construct offspring's inherited material from this parent
    loop_seq <- lapply(c(1:nrow(chrom_map)),</pre>
                        function(x){
                          SimRVSequences:::reconstruct_fromHaplotype(
                            parental_genotypes = ped_genos[which(ped_geno_IDs == PO_info[i, 4]),
                                                            which(SNV_map$chrom == chrom_map$chrom[x])],
                            CSNV_map = SNV_map[which(SNV_map$chrom == chrom_map$chrom[x]),],
                            inherited_haplotype = loop_gams$haplotypes[[x]],
                            chiasmata_locations = loop_gams$cross_locations[[x]],
                            REDchrom_map = chrom_map(x, ])
                        })
    #append ID for this haplotype to the list of IDs
    ped_geno_IDs <- c(ped_geno_IDs, PO_info[i, 1])</pre>
```

```
ped_genos <- rbind(ped_genos, unlist(loop_seq))</pre>
  }
  #Determine if this is a sporadic pedigree
  printed_FamRV <- ifelse(all(ped_file[, c("DA1", "DA2")] == 0), "no_CRV", RV_marker)</pre>
  #create a data.frame to store identifying info
  geno_map <- data.frame(FamID = rep(ped_file$FamID[1], length(ped_geno_IDs)),</pre>
                          ID = ped_geno_IDs,
                          affected = rep(FALSE, length(ped_geno_IDs)),
                          FamCRV = rep(printed_FamRV, length(ped_geno_IDs)),
                          stringsAsFactors = FALSE)
  #identify affected individuals
  geno_map$affected[geno_map$ID %in% ped_file$ID[ped_file$affected]] <- TRUE</pre>
  #Return the genomes matrix and a data.frame containing identifying
  #information for the of IDs to identify the
  #family member to whom
  return(list(ped_genos = ped_genos, geno_map = geno_map))
}
```

We are now ready to call sim_RVstudy_new() on each chromosome.

Simulating exome-wide sequences for disease-affected members in the study families takes about 6 minutes on a Windows OS with an i7-8550U @ 1.8GHz,16GB of RAM. The times to simulate chromosomes 1, 2, 8 and 9 are shown in Table 1.

Tab	ole	1:	Simu	lation	time	for	selected	chrom	osomes.

Chromosome	No. of RVs	No. of cRVs	Time (s)
1	84664	1	36.23
2	60995	21	53.63
8	31396	11	22.35
9	34248	0	19.57

From the table, we see that chromosome 1 takes less time to simulate than chromosome 2, despite having more rare variants. We attribute this to chromosome 1 having fewer cRVs than chromosome 2. By contrast, chromosome 8 has more cRVs than chromosome 1 yet takes less time to simulate because it has fewer RVs overall. Simulation time therefore depends on both the overall number of RVs and the number of cRVs on chromosome.

The sim_RVstudy_new() function returns the same set of outputs as the sim_RVstudy() function, as discussed in the next subsection.

3.3 Discuss the sim_RVstudy_new() output

The output study_seq from the call to sim_RVstudy_new() is a list containing 22 elements, one for each chromosome. As the output format of each chromosome is the same, we focus on the first chromosome. Each element of the list study_seq is itself a list containing four elements as follows.

(1). The ped_files data frame gives details about the individuals in the pedigrees. When we set affected_only = TRUE, the results contain only the affected individuals and the individuals who connect them along a line of descent within a pedigree. Note that the ped_files data frame is exactly the same for all 22 chromosomes; though wasteful of space, this unnecessary repetition is convenient for looping.

```
# View the first 4 individuals in the ped_files data frame (the same regardless of chromosome). head(study_seq[[1]]ped_files, n = 4)
```

```
##
      FamID ID sex dadID momID affected DA1 DA2 birthYr onsetYr deathYr available
## 1
           1
              1
                   1
                        NA
                                NA
                                        TRUE
                                               0
                                                    1
                                                          1881
                                                                   1952
                                                                            1955
                                                                                       TRUE
                          2
## 3
           1
              3
                   1
                                1
                                       TRUE
                                               0
                                                    1
                                                          1901
                                                                   1970
                                                                            1981
                                                                                       TRUE
## 5
           1
              4
                   0
                          2
                                1
                                       TRUE
                                               0
                                                    1
                                                          1910
                                                                   2000
                                                                            2002
                                                                                       TRUE
           1 20
                   0
                         19
                                8
                                       TRUE
                                                          1957
                                                                   1997
                                                                            2016
                                                                                       TRUE
## 25
                                               0
                                                    1
##
      Gen proband
## 1
         1
             FALSE
## 3
         2
             FALSE
## 5
         2
              TRUE
## 25
             FALSE
```

(2). The sparse matrix ped_haplos contains simulated SNVs on the exome sequences of the disease-affected individuals and the individuals connecting them in the ascertained pedigrees.

```
# View the first 30 SNVs of the first 6 exome sequences on the first chromosome. study_seq[[1]]$ped_haplos[1:6, 1:30]
```

Rows of this sparse matrix correspond to exomes and columns to RVs on the first chromosome. The entry "1" represents the derived (mutated) allele and "." the ancestral allele.

(3). The SNV_map data frame contains information about RVs in the study. Since the remove_wild argument of sim_RVstudy_new() is set to its default value of TRUE, this data frame contains only RVs carried by at least one study individual.

```
# View the first 4 rows of SNV_map
head(study_seq[[1]]$SNV_map, n = 4)
```

```
SNV type
##
     colID chrom position
                                    afreq
                                                                selCoef pathwaySNV
## 1
         1
               1
                    12626 0.000399064519 1 12626
                                                     m1 -0.00452655694
                                                                             FALSE
## 2
         2
                    13411 0.000501150791 1_13411
                                                         0.0000000000
                                                                             FALSE
               1
                                                     m2
## 3
         3
                    14230 0.001568416364 1 14230
                                                         0.0000000000
                                                                             FALSE
               1
                                                     m2
## 4
         4
               1
                    14231 0.000631078773 1 14231
                                                     m1 -0.00146789732
                                                                             FALSE
##
     is CRV
## 1
      FALSE
## 2
      FALSE
## 3 FALSE
```

4 FALSE

The rows of the data frame represent the RVs carried by at least one individual in the study. The columns are characteristics of the RVs explained in subsection 1.5 of this document.

(4). The haplo_map data frame maps the exome sequences in ped_haplos to the individuals in ped_files. The rows of haplo_map correspond to sequences and the columns to characteristics of individuals to which these sequences belong. Let's look at the first family's information on chromosome 1.

```
# View family 1's entries of haplo_map
fam1<-(study_seq[[1]] $haplo_map[,"FamID"] == 1)
study_seq[[1]] $haplo_map[fam1,]</pre>
```

```
##
      FamID ID affected FamCRV
## 1
          1
             1
                    TRUE no CRV
## 2
          1
                    TRUE no_CRV
              1
## 3
          1
              2
                   FALSE no_CRV
          1
             2
                   FALSE no_CRV
## 4
## 5
          1
             6
                   FALSE no_CRV
## 6
          1
             6
                   FALSE no_CRV
## 7
          1 19
                   FALSE no_CRV
## 8
          1 19
                   FALSE no_CRV
                    TRUE no_CRV
## 9
             3
          1
## 10
          1
              3
                    TRUE no CRV
## 11
          1
              4
                    TRUE no CRV
## 12
          1
             4
                    TRUE no CRV
                   FALSE no_CRV
## 13
          1
             8
                   FALSE no CRV
## 14
          1
             8
                    TRUE no CRV
## 15
          1 20
## 16
          1 20
                    TRUE no CRV
```

We can see that the two sequences of an individual are stored in consecutive rows of the data frame. The FamCRV column of the data frame gives the identifier of the familial cRV and is the same for all family members. If a family does not have a cRV on the selected chromosome, the entry of FamCRV is no_CRV. For example, family ID 1 does not carry a cRV on chromosome 1.

With the complete data now available in the list study_seq, our final task is to deliver it in human-readable flat-file formats, as described next.

4 Generate data files

Throughout this section, we will refer to the list study_seq generated in the previous subsection. The list element for chromosome 21 has the following structure.

```
# The study_seq object is a list of length 22 elements.
# We print the 21st element of study_seq, for chromosome 21.
str(study_seq[[1]])
```

```
## List of 4
   $ ped files :'data.frame': 1247 obs. of 14 variables:
##
     ..$ FamID
                  : int [1:1247] 1 1 1 1 1 1 1 1 2 2 ...
                  : int [1:1247] 1 3 4 20 2 8 19 6 1 3 ...
     ..$ ID
##
                  : int [1:1247] 1 1 0 0 0 1 0 0 1 0 ...
     ..$ sex
                  : int [1:1247] NA 2 2 19 NA 6 NA NA NA 2 ...
##
     ..$ dadID
##
                  : int [1:1247] NA 1 1 8 NA 3 NA NA NA 1 ...
     ..$ momID
     ...$ affected : logi [1:1247] TRUE TRUE TRUE TRUE FALSE FALSE ...
                  : int [1:1247] 0 0 0 0 0 0 0 0 0 0 ...
##
     ..$ DA1
```

```
: int [1:1247] 1 1 1 1 0 1 0 0 1 1 ...
##
##
                 : int [1:1247] 1881 1901 1910 1957 NA 1924 NA NA 1914 1931 ...
     ..$ birthYr
##
     ..$ onsetYr : int [1:1247] 1952 1970 2000 1997 NA NA NA NA 1987 1979 ...
     ..$ deathYr : int [1:1247] 1955 1981 2002 2016 NA 1957 NA NA 1990 2014 ...
##
##
     ...$ available: logi [1:1247] TRUE TRUE TRUE TRUE FALSE TRUE ...
                  : int [1:1247] 1 2 2 4 1 3 3 2 1 2 ...
##
     ..$ Gen
     ...$ proband : logi [1:1247] FALSE FALSE TRUE FALSE FALSE FALSE ...
##
    $ ped_haplos:Formal class 'dgCMatrix' [package "Matrix"] with 6 slots
##
##
     .. ..@ i
                    : int [1:193252] 1689 1700 1706 983 986 990 918 924 2246 2256 ...
                    : int [1:19394] 0 3 6 10 14 15 19 25 29 38 ...
##
     .. ..@ р
##
     .. ..@ Dim
                    : int [1:2] 2494 19393
##
     .. .. @ Dimnames:List of 2
##
     .. .. ..$ : NULL
     .. .. ..$ : NULL
##
##
     .. ..0 x
                    : num [1:193252] 1 1 1 1 1 1 1 1 1 1 ...
##
     .. .. @ factors : list()
    $ haplo_map :'data.frame': 2494 obs. of 4 variables:
##
##
                : int [1:2494] 1 1 1 1 1 1 1 1 1 1 ...
##
                 : int [1:2494] 1 1 2 2 6 6 19 19 3 3 ...
     ...$ affected: logi [1:2494] TRUE TRUE FALSE FALSE FALSE FALSE ...
##
##
     ..$ FamCRV : chr [1:2494] "no_CRV" "no_CRV" "no_CRV" "no_CRV" ...
                :'data.frame': 19393 obs. of 9 variables:
##
   $ SNV map
##
     ..$ colID
                   : int [1:19393] 1 2 3 4 5 6 7 8 9 10 ...
                   : int [1:19393] 1 1 1 1 1 1 1 1 1 1 ...
##
     ..$ chrom
     ..$ position : num [1:19393] 12626 13411 14230 14231 15836 ...
##
##
     ..$ afreq
                   : num [1:19393] 0.000399 0.000501 0.001568 0.000631 0.000343 ...
##
                   : chr [1:19393] "1_12626" "1_13411" "1_14230" "1_14231" ...
     ..$ SNV
                   : Factor w/ 2 levels "m1", "m2": 1 2 2 1 2 1 1 2 2 2 ...
##
     ..$ type
##
     ..$ selCoef
                   : num [1:19393] -0.00453 0 0 -0.00147 0 ...
     ...$ pathwaySNV: logi [1:19393] FALSE FALSE FALSE FALSE FALSE FALSE ...
##
                   : logi [1:19393] FALSE FALSE FALSE FALSE FALSE FALSE ...
   - attr(*, "class")= chr [1:2] "famStudy" "list"
```

We use study_seq to create a .sam file containing information about genotyped individuals in the ascertained pedigrees, chromosome-specific .geno files containing RV genotypes and chromosome-specific .var files containing information about RVs. As described next, the data files are in flat-file format similar to PLINK files (Purcell et al. 2007).

4.1 .sam file

The .sam file contains pedigree information about the disease-affected individuals and the individuals connecting them along a line of descent in their pedigrees. These individuals are prioritized for exome sequencing in our family study. The function plink_format_samp() generates the .sam file using the argument, peds. The argument peds is a data frame giving information on the study pedigrees. The function selects specific columns of the peds data frame and aligns them in a format similar to the .psam PLINK file.

```
# Get the information for .sam file
plink_format_samp <- function(peds){
    # Convert sex. In PLINK 1 is male 2 is female.
    # We have 0 s to represent male and 1 for female.
    peds$sex[peds$sex == 1] <- c(2)
    peds$sex[peds$sex == 0] <- c(1)

# Affected variable consists logical values.
# Need to change it as character to assign values to</pre>
```

```
# represent the phenotype.
  peds$affected <- as.character(peds$affected)</pre>
  # If affected is NA consider it as missing.
  # In PLINK missing is denoted as 0 or -9.
  peds$affected[is.na(peds$affected)] <- c(0)</pre>
  # Non-affected is represented as 1 in PLINK.
  peds$affected[peds$affected == "FALSE"] <- c(1)</pre>
  # Affected is represented as 2 in PLINK.
  peds$affected[peds$affected == "TRUE"] <- c(2)</pre>
  peds$FamID <- as.numeric(peds$FamID)</pre>
  peds$affected <- as.numeric(peds$affected)</pre>
  # Create the data frame with required columns.
  psam_file <- data.frame(peds$FamID, peds$ID, peds$dadID, peds$momID,
                           peds$sex, peds$affected,
                           peds$birthYr, peds$deathYr, peds$proband)
  colnames(psam_file) <- c("FID", "IID", "PAT", "MAT", "SEX", "PHENO1",
                            "BIRTHYr", "DEATHYr", "PROBAND")
  return(psam_file)
}
```

To get the .sam file, we apply the plink_format_samp() function to chromosome 21. Note that the .sam file is the same regardless of which chromosome is used.

```
# Call the function for chromosome 21
sample_data <- plink_format_samp(study_seq[[21]]$ped_files)

# How many individuals.
nrow(sample_data)

## [1] 1247</pre>
```

```
# Print the first 6 individuals
head(sample_data, n= 6)
```

```
FID IID PAT MAT SEX PHENO1 BIRTHYR DEATHYR PROBAND
##
## 1
       1
           1 NA NA
                        2
                               2
                                    1881
                                            1955
                                                    FALSE
## 2
       1
           3
               2
                        2
                               2
                                    1901
                                             1981
                                                    FALSE
               2
## 3
       1
           4
                   1
                       1
                               2
                                    1910
                                            2002
                                                     TRUE
          20
             19
                   8
                        1
                               2
                                    1957
                                                   FALSE
       1
                                             2016
           2 NA
                                                    FALSE
## 5
       1
                  NA
                        1
                               1
                                      NA
                                              NA
                   3
                                    1924
                                            1957
                                                    FALSE
```

The rows of sample_data are the 1247 genotyped individuals in the study pedigrees. The individuals are either disease-affected or connect disease-affected individuals along a line of descent in a study pedigree. The columns of sample_data contain information about the individuals as follows:

- 1. FID- the identification number of the family that the individual belongs to.
- 2. IID- the individual identification number.
- 3. PAT- the father's identification number.
- 4. MAT- the mother's identification number.

- 5. SEX- the individual's sex, with 1 and 2 corresponding to male and female, respectively.
- 6. PHEN01- the disease-affected status, with 1 and 2 corresponding to unaffected and affected, respectively.
- 7. BIRTHYr- the individual's birth year.
- 8. DEATHYr- the death year of the individual, with NA indicating that the individual is still alive at the end of the study.
- 9. PROBAND- a logical value indicating whether or not the individual is the proband for their pedigree.

We save the .sam file as a text file, sample_info.txt, as follows. The text file can be found in our Zenodo repository.

```
# Write the sample information to a single text file
write.table(sample_data, "sample_info.txt", row.names=FALSE, quote = FALSE)
```

4.2 .geno files

A .geno file gives the RV genotypes in gene-dosage format. An individual's dosage of the derived allele is the number of copies they inherited from their parents (i.e. 0, 1 or 2). The gene_data() function below converts [pair of RV RV-haplotype pairs into genotypes in gene-dosage format.

```
# Convert haplotype pairs into genotypes in gene-dosage format.
gene_data <- function(geno){
   gene_dosage <- list()
   IDs <- seq(from = 1, to = nrow(geno), by = 2)

# Get the column sums.
for(i in 1: length(IDs)){
    gene_dosage[[i]] <- colSums(geno[IDs[i]:(IDs[i] + 1), ])
    genotypes <- do.call(rbind, gene_dosage)
}
genotypes <- do.call(rbind, gene_dosage)
return(genotypes)
}</pre>
```

Let's call gene_data() on chromosome 21 as an example. The function's argument, geno, is filled with the sparse matrix ped_haplos from the study_seq output.

```
# Apply the function to 21st chromosome
genotype_data <- gene_data(study_seq[[21]]$ped_haplos)</pre>
```

To convert chromosome 21 haplotypes to individual genotypes in gene-dosage format, gene_data() takes approximately 15 seconds on a Windows OS with an i7-8550U @ 1.8GHz,16GB of RAM. Let's view the first few rows and columns of the data frame returned by gene_data().

```
# View the first four rows and 12 columns
genotype_data[1:4, 1:12]
```

```
##
               [,2] [,3] [,4] [,5] [,6]
                                              [,7] [,8] [,9] [,10] [,11] [,12]
          [,1]
## [1,]
                               0
                                     0
                                           0
                                                 0
                                                       0
                                                             0
                                                                     0
## [2,]
             0
                               0
                                           0
                                                             0
                                                                                   0
                   0
                         0
                                     0
                                                 0
                                                       0
                                                                     0
                                                                            0
## [3,]
             0
                         0
                               0
                                           0
                                                       0
                                                             0
                                                                     0
                                                                            0
                                                                                   0
## [4,]
             0
                   0
                         0
                               0
                                     0
                                           0
                                                 0
                                                       0
                                                             0
                                                                     0
                                                                            0
                                                                                   0
```

The rows of the data frame represent the 1247 genotyped individuals in our study. The columns represent RVs that reside on the exome of chromosome 21. Each entry of the data frame gives the dosage of the derived allele of an RV (i.e. 0, 1 or 2). Most of the entries are 0, as would be expected for RVs.

The gene_data() function is applied to all the chromosomes as follows.

```
# Apply function to all chromosomes
genotype_data <- lapply(study_seq, function(x){
  result <- gene_data(x$ped_haplos)
  colnames(result) <- x$SNV_map$SNV
  result
})</pre>
```

Below, the resulting chromosome-specific .geno files are written to text files named genotypes_chr_i.txt, where "i" indicates the chromosome number. These text files can be found in the Zenodo repository.

4.3 .var files

A .var file contains information about the RVs in the columns of the associated .geno file. The variant_data() function below selects the relevant characteristics of the RVs and stores them in a data frame.

```
# Get the variant information to create the .var file
variant_data <- function(variant){</pre>
  # Chromosome number.
  CHROM <- variant$chrom
  # Position.
  POS <- variant$position
  # Reference allele.
  REF <- rep("A", length(CHROM))</pre>
  # Alternate allele.
  ALT <- rep("T", length(CHROM))
  # Selection coefficient.
  sel_coef <- variant$selCoef</pre>
  # Population allele frequency.
  pop_afreq <- variant$afreq</pre>
  # Pathway SNV or not.
  pathwaySNV <- variant$pathwaySNV</pre>
  # Causal SNV or not.
  C_SNV <- variant$is_CRV</pre>
  # label the type as NS and S where NS- non-synonymous and
  # S- Synonymous.
  levels(variant$type) <- c("NS", "S")</pre>
  # Type of the SNV
  Type <- variant$type
  # Create the data frame.
  SNV <- data.frame(CHROM, POS, REF, ALT,
                     pop_afreq, sel_coef, pathwaySNV, C_SNV, Type)
  return(SNV)
}
```

Let's call variant_data() on chromosome 21 as an example. The function's argument, variant, is filled with the SNV_map data frame from the study_seq output.

```
# Run the function on chromosome 21 SNV_map data
variant_info <- variant_data(study_seq[[21]]$SNV_map)</pre>
```

The function returns the data frame variant_info. Let's view information about the first four RVs in variant info.

```
# View the first 4 rows of the resulting data frame
head(variant_info, n = 4)
     CHROM
##
               POS REF ALT
                                pop_afreq
                                                  sel_coef pathwaySNV C_SNV Type
## 1
        21 5591572
                         T 0.000371222808 0.00000000e+00
                                                                FALSE FALSE
                                                                                S
                     Α
## 2
        21 5591931
                         T 0.000575395352 -8.08714731e-08
                                                                FALSE FALSE
                                                                               NS
                     Α
        21 6066994
## 3
                     Α
                         T 0.000092805702 0.00000000e+00
                                                                FALSE FALSE
                                                                                S
## 4
        21 6112121
                     Α
                         T 0.000566114782 0.00000000e+00
                                                                FALSE FALSE
                                                                                S
```

The rows of variant_info contain exomic RVs on chromosome 21 that are carried by at least one study participant. The columns give the following information about these RVs:

- 1. CHROM- the chromosome number of the RV.
- 2. POS- the RV position, in base pairs, on the chromosome.
- 3. REF- the reference allele for the RV
- 4. ALT- the alternate allele for the RV
- 5. pop_afreq- the population alternate allele frequency for the RV.
- 6. sel_coef- the selection coefficient for the RV.
- 7. pathwaySNV- whether or not the RV comes from a gene in our disease pathway.
- 8. C_SNV- whether or not the RV is causal.
- 9. Type- whether the RV is a synonymous (S) or non-synonymous (NS) mutation.

The variant_data() function is applied to all the chromosomes as follows.

```
# Apply function to all 22 chromosomes
SNV_map <- lapply(study_seq, function(x){
  variant_data(x$SNV_map)
})</pre>
```

Below, the resulting chromosome-specific .var files are written to text files named SNV_map_chr_i.txt, where "i" indicates the chromosome number. These text files can be found in the Zenodo repository.

The next section provides a data frame listing the cRVs for each ascertained family.

4.4 List the familial cRVs

First, we obtain a list of family-specific cRVs by chromosome. Each list item corresponds to a chromosome and is a data frame with the family identifiers and the familial cRVs on that chromosome. We print the first two chromosomes in this list for illustration.

```
# Get the FamIDs and their familial cRVs, by chromosome
famcRV_bychrom <- lapply(study_seq, function(x){
   unique(x$haplo_map[, c("FamID", "FamCRV")])})

str(famcRV_bychrom[1:2])

## List of 2

## $ :'data.frame': 150 obs. of 2 variables:

## ..$ FamID : int [1:150] 1 2 3 4 5 6 7 8 9 10 ...

## ..$ FamCRV: chr [1:150] "no_CRV" "no_CRV" "no_CRV" "no_CRV" ...

## $ :'data.frame': 150 obs. of 2 variables:

## ..$ FamID : int [1:150] 1 2 3 4 5 6 7 8 9 10 ...

## ..$ FamCRV: chr [1:150] 1 2 3 4 5 6 7 8 9 10 ...

## ..$ FamCRV: chr [1:150] 1 2 3 4 5 6 7 8 9 10 ...</pre>
```

From the output of str(), we see that each chromosome in the list famcRV_bychrom has a data frame containing the family identifiers and the family's cRV, if any, on that particular chromosome.

Next, we create the function familial_cRV() to collapse famRV_bychrom into a single data frame containing the familial identifier and cRV for each family across all chromosomes.

```
# Get the familial_cRVs
familial cRV <- function(cRV bychrom){</pre>
  # From haplomap data frame get the familial
  # cRVs from the last column FamCRV.
  f_CRV <- lapply(cRV_bychrom, function(x){</pre>
    unique(x[which(x$FamCRV != "no_CRV"), ])
  })
  # Combine all of them into a single data frame.
  family_CRV <- do.call("rbind", f_CRV)</pre>
  # Order them the data frame according to the family ID.
  family_CRV <- family_CRV[order(as.numeric(family_CRV$FamID)), ]</pre>
  # Get the family IDS that are not carrying a cRV,
  # by comparing two data frames.
  no_CRV <- as.numeric(setdiff(cRV_bychrom[[1]]$FamID, family_CRV$FamID))</pre>
  no_CRVfam <- rep(c("no_CRV"), length(no_CRV))</pre>
  # Create a data frame with families without a cRV.
  df <- data.frame(no_CRV, no_CRVfam)</pre>
  # Give the same column names as in families with a cRV.
  colnames(df) <- colnames(family_CRV)</pre>
  # Combine both of the data frames.
  family_CRV <- rbind(family_CRV, df)</pre>
  # Order the final data frame based on the family ID.
  family_CRV <- family_CRV[order(as.numeric(family_CRV$FamID)), ]</pre>
  return(family_CRV)
```

We apply the function as follows and get the familial cRV for each family.

```
# Apply the function.
cRVS <- familial_cRV(famcRV_bychrom)

# View the output
head(cRVS, n=5)</pre>
```

FamID FamCRV

The output gives the cRVs for each family. As an example, family ID 1 has a cRV labeled "2_201170321" indicating that it is on chromosome 2 in base-pair position 20117032. Although not shown in the output, there are a few families without a cRV:

```
# Select families which do not carry cRVs
cRVS[cRVS$FamCRV == "no_CRV", ]

## FamID FamCRV
## 11   72 no_CRV
## 2   95 no_CRV
## 3   103 no_CRV
```

Three families with IDs 72, 95 and 103 do not carry a cRV. These families have affected individuals with sporadically occurring disease.

We save the results in a text file, familial_cRV.txt, which can be found in the Zenodo repository.

4.5 Create PLINK files

- We convert .sam, .var and .geno files to PLINK file formats, .fam, .bim and .bed file formats, respectively.
- For this task we use the R package, snpStats. The function write.plink() in the snpStats package builds the three PLINK file formats, .fam, .bim and .bed.
- Let's discuss about the write.plink() function and how we arrange the arguments accordingly.
- To this function, we need to supply our sample information, variant information and genotype information. For this, we can use the functions that we created in the previous section to generate .sam, .var and .geno files. As an example, let's write the chromosome 21's .bim, .fam and .bed files using the write.plink() function.
- First we get the sample information. We use plink_format_samp() function for this. (We discussed about this function in sub-section 4.2.)

```
# Call the plink_format_samp function for chromosome 21
sample_data <- plink_format_samp(study_seq[[21]]$ped_files)</pre>
```

- The format of the sample data object is discussed in sub-section 4.2.
- Then we get the variant information for chromosome 21 by using the variant_data() function as follows. (We discussed about this function in sub-section 4.3.)

```
# Run the function on chromosome 21 SNV_map data
variant_info <- variant_data(study_seq[[21]]$SNV_map)
# Add row names to the variant data data frame
row.names(variant_info) <- study_seq[[21]]$SNV_map$SNV</pre>
```

• The format of the variant_info object is discussed in the sub-section 4.3.

• Finally, we need to get the genotypes data for chromosome 21. We use the gene_data() function, with a tiny modification. The reason for this modification is that when we use the write.plink() function, we need to supply our genotype matrix as an object of class SnpMatrix. In this class, the genotypes are coded as 1, 2 and 3 and 0 indicates the missing values. Therefore, we changed our genotype format as 1, 2 and 3 instead of 0, 1 and 2. The following is the modified gene_data_new() function.

```
# Convert haplotype pairs into genotypes in gene-dosage format.

gene_data_new <- function(geno){
    gene_dosage <- list()
    IDs <- seq(from = 1, to = nrow(geno), by = 2)

# Get the column sums.
for(i in 1: length(IDs)){
    gene_dosage[[i]] <- colSums(geno[IDs[i]:(IDs[i] + 1), ]) + 1
    genotypes <- do.call(rbind, gene_dosage)
}
genotypes <- do.call(rbind, gene_dosage)
return(genotypes)
}</pre>
```

• We apply the gene_data_new() function to the chromosome 21 genotypes.

```
# Apply the function to 21st chromosome
genotype_data <- gene_data_new(study_seq[[21]]$ped_haplos)</pre>
```

• Let's view the first few rows and columns of the data frame returned by gene_data().

```
# View the first four rows and 12 columns
genotype_data[1:4, 1:12]
         [,1] [,2] [,3] [,4] [,5] [,6] [,7] [,8] [,9] [,10] [,11] [,12]
##
## [1,]
                                         1
                             1
                                   1
                                              1
                                                          1
                                                                 1
                        1
                                                    1
## [2,]
            1
                        1
                             1
                                   1
                                         1
                                              1
                                                    1
                                                          1
                                                                 1
                                                                              1
## [3,]
            1
                  1
                       1
                             1
                                   1
                                         1
                                              1
                                                    1
                                                          1
                                                                 1
                                                                        1
                                                                              1
## [4,]
            1
                             1
                                         1
                                              1
                                                                 1
                                                                              1
```

- The above output shows that the dosage of the derived allele format is now converted to 1, 2 and 3. (Majority is 1 corresponding to no copies of the variant because the SNVs are rare.)
- Then we convert the genotype_data object into a SnpMatrix object as follows.

```
# Add row and column names
rownames(genotype_data) <- 1:nrow(genotype_data)
colnames(genotype_data) <- study_seq[[21]]$SNV_map$SNV

# The mode of the genotype_data change as raw
mode(genotype_data) <- "raw"

# Change the class of the object ad SnpMatrix
genotypes <- new("SnpMatrix", genotype_data)</pre>
```

• Then, we apply the write.plink() function to get the .fam, .bim and .bed files of chromosome 21 as follows.

```
mother = as.numeric(MAT), sex = as.numeric(SEX),
phenotype = as.numeric(PHENO1),
snp.data = variant_info, chromosome = as.numeric(CHROM),
position = as.numeric(POS), allele.1 = REF,
allele.2 = ALT,
na.code = 0, human.genome=TRUE)
```

```
## Writing FAM file to chr_21.fam
## Writing extended MAP file to chr_21.bim
## Writing BED file to chr_21.bed (SNP-major mode)
## NULL
```

- The function output the .fam, .bim and .bed file corresponding to chromosome 21.
- The above steps are then apply to all the chromosomes as follows.
- Apply the variant_data() function to all the chromosomes.

```
# Apply function to all 22 chromosomes
SNV_map <- lapply(study_seq, function(x){
  variant_data(x$SNV_map)
})</pre>
```

• Apply the gene_data_new() function to all the chromosomes.

```
# Apply function to all chromosomes
genotype_all <- lapply(study_seq, function(x){
  result <- gene_data_new(x$ped_haplos)
  colnames(result) <- x$SNV_map$SNV
  rownames(result) <- 1:nrow(result)
  result
})</pre>
```

• Convert all the genotype_data as the object of class SnpMatrix.

```
# Convert objects as the class of SnpMatrix
genotypes <- lapply(genotype_all, function(x){
  mode(x) <- "raw"
  new("SnpMatrix", x)})</pre>
```

• Apply the write.plink() function to all chromosomes.

As a final step, for future reference, we provide the R version and the version of the SimRVSequences R package that was used to generate this document.

```
library(SimRVSequences)
sessionInfo()
## R version 4.1.1 (2021-08-10)
## Platform: x86_64-w64-mingw32/x64 (64-bit)
## Running under: Windows 10 x64 (build 22000)
## Matrix products: default
##
## locale:
## [1] LC COLLATE=English Canada.1252 LC CTYPE=English Canada.1252
## [3] LC_MONETARY=English_Canada.1252 LC_NUMERIC=C
## [5] LC_TIME=English_Canada.1252
##
## attached base packages:
## [1] parallel stats
                           graphics grDevices utils
                                                          datasets methods
## [8] base
##
## other attached packages:
  [1] snpStats_1.44.0
                             survival_3.2-11
                                                   reshape2_1.4.4
## [4] doRNG_1.8.2
                             rngtools_1.5.2
                                                   doParallel_1.0.16
                             foreach_1.5.1
## [7] iterators_1.0.13
                                                   data.table_1.14.0
## [10] Matrix_1.2-12
                             forcats_0.5.1
                                                   stringr_1.4.0
## [13] dplyr_1.0.7
                             purrr_0.3.4
                                                   readr_2.0.1
## [16] tidyr_1.1.3
                             tibble_3.1.4
                                                   ggplot2_3.3.5
                             SimRVSequences_0.2.7
## [19] tidyverse_1.3.1
## loaded via a namespace (and not attached):
## [1] httr_1.4.2
                            jsonlite_1.7.2
                                                 splines_4.1.1
   [4] modelr_0.1.8
                            assertthat_0.2.1
                                                 cellranger 1.1.0
##
## [7] yaml_2.2.1
                            pillar_1.7.0
                                                 backports_1.2.1
## [10] lattice_0.20-44
                            glue_1.4.2
                                                 quadprog_1.5-8
                            rvest_1.0.2
## [13] digest_0.6.27
                                                 colorspace_2.0-2
## [16] htmltools_0.5.2
                            plyr_1.8.6
                                                 pkgconfig_2.0.3
                            SimRVPedigree_0.4.4 haven_2.4.3
## [19] broom_0.8.0
## [22] zlibbioc_1.40.0
                            scales_1.2.0
                                                 intervals_0.15.2
## [25] tzdb_0.1.2
                            generics_0.1.2
                                                 ellipsis_0.3.2
## [28] withr_2.5.0
                            BiocGenerics_0.40.0 cli_3.1.0
## [31] magrittr_2.0.1
                            crayon_1.5.1
                                                 readxl_1.3.1
## [34] evaluate_0.15
                            kinship2_1.8.5
                                                 fs_{1.5.0}
## [37] fansi_0.5.0
                            xm12_1.3.3
                                                 tools_4.1.1
## [40] hms_1.1.1
                            lifecycle_1.0.1
                                                 munsell_0.5.0
## [43] reprex_2.0.1
                            compiler_4.1.1
                                                 rlang_1.0.2
## [46] grid_4.1.1
                            rstudioapi_0.13
                                                 rmarkdown_2.13
## [49] gtable 0.3.0
                            codetools 0.2-18
                                                 DBI 1.1.2
## [52] R6_2.5.1
                            lubridate_1.7.10
                                                 knitr 1.38
## [55] fastmap 1.1.0
                            utf8 1.2.2
                                                 stringi 1.7.4
## [58] Rcpp_1.0.7
                            vctrs_0.3.8
                                                 dbplyr_2.1.1
## [61] tidyselect_1.1.2
                            xfun_0.30
```

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