WHICH ARE THE VARIANTS BEHIND COLORECTAL CANCER?

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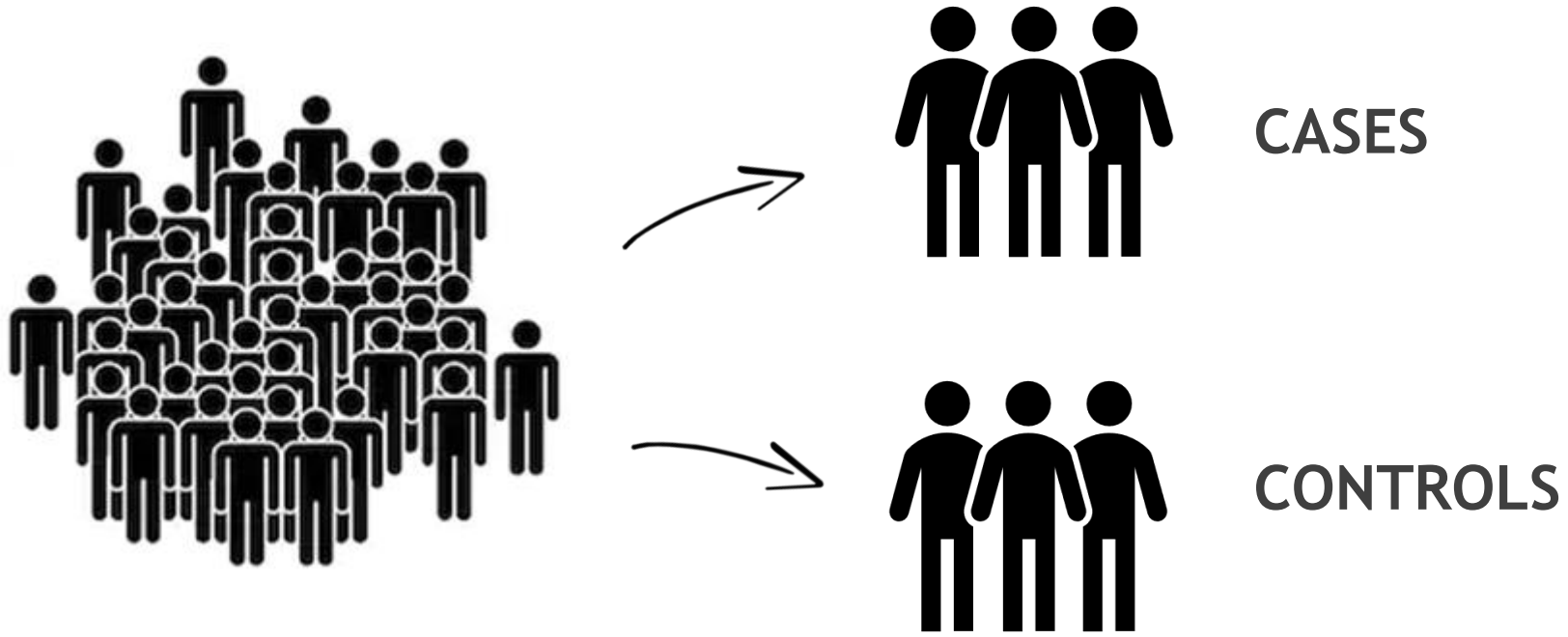
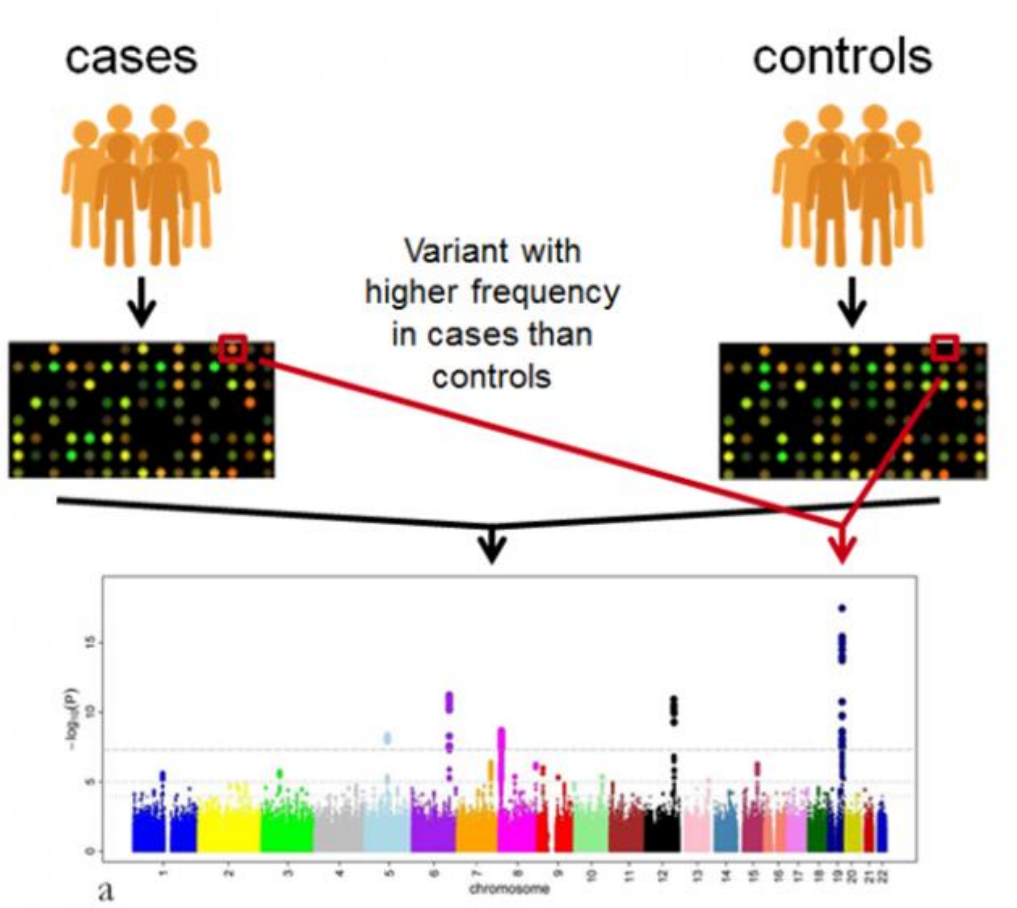
INTRODUCTION

GWAS = Genome-Wide Association Studies



INTRODUCTION



EMBL-EBI Train Online, 2020, https://www.ebi.ac.uk/training/online/course/gwas-catalog-exploring-snp-trait-associations-2019/what-gwas-catalog/what-are-genome-wide

Requires:

- Statistical tests
- Large number of subjects

In our analysis:

COLORECTAL CANCER

METHODS

PACKAGES AND TOOLS:

- ggplot2
- dplyr
- ggrepel
- devtools
- isglobal-brge/SNPassoc
- BiocManager
- snpStats
- SNPRelate

METHODS

DATA DESCRIPTION:

PLINK colorectal cancer dataset

- Colorectal.bed → Genomic SNP data
- Colorectal.bim → SNP annotation
- Colorectal.fam → Individual's family information

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Upload to R

Colorectal.plink

- Colorectal.genotype
- Annotation
- Individuals

METHODS

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Upload to R

Colorectal.plink

- Colorectal.genotype
- Annotation
- Individuals

Full phenotype information → Additional txt files

Upload to R

- Colorectal.phenotype

METHODS

DATA DESCRIPTION:

```
```{r read-plink-data}
# Read PLINK data of the obestiy dataset
colorectal.plink <-  read.plink(bed = "Project3_cancer/data/colorectal_cancer/colorectal.bed",
                                bim = "Project3_cancer/data/colorectal_cancer/colorectal.bim",
                                fam = "Project3_cancer/data/colorectal_cancer/colorectal.fam")
```
```

METHODS

DATA DESCRIPTION:

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                                fam = "Project3_cancer/data/colorectal_cancer/colorectal.fam")
```
```

```
```{r genotypes}
# Get genotypes information
colorectal.genotype <- colorectal.plink$genotypes
colorectal.genotype
```
```

METHODS

DATA DESCRIPTION:

```
```{r read-plink-data}
# Read PLINK data of the obestiy dataset
colorectal.plink <-  read.plink(bed = "Project3_cancer/data/colorectal_cancer/colorectal.bed",
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```
```

```
```{r genotypes}
# Get genotypes information
colorectal.genotype <- colorectal.plink$genotypes
colorectal.genotype
```
```

```
```{r individuals}
# Get individuals information
individuals <- colorectal.plink$fam
head(individuals)
```
```

METHODS

DATA DESCRIPTION:

```
```{r read-plink-data}
# Read PLINK data of the obestiy dataset
colorectal.plink <-  read.plink(bed = "Project3_cancer/data/colorectal_cancer/colorectal.bed",
                                bim = "Project3_cancer/data/colorectal_cancer/colorectal.bim",
                                fam = "Project3_cancer/data/colorectal_cancer/colorectal.fam")
```
```

```
```{r genotypes}
# Get genotypes information
colorectal.genotype <- colorectal.plink$genotypes
colorectal.genotype
```
```

```
```{r annotation}
# Get annotation information
annotation <- colorectal.plink$map
head(annotation)
```
```

```
```{r individuals}
# Get individuals information
individuals <- colorectal.plink$fam
head(individuals)
```
```

METHODS

```{r obesity}
colorectal.phenotype <- read.delim("Project3_cancer/data/colorectal_cancer/colorectal.txt")
head(colorectal.phenotype)
```

DATA DESCRIPTION:

```{r read-plink-data}
# Read PLINK data of the obestiy dataset
colorectal.plink <-  read.plink(bed = "Project3_cancer/data/colorectal_cancer/colorectal.bed",
                                bim = "Project3_cancer/data/colorectal_cancer/colorectal.bim",
                                fam = "Project3_cancer/data/colorectal_cancer/colorectal.fam")
```

```{r genotypes}
# Get genotypes information
colorectal.genotype <- colorectal.plink$genotypes
colorectal.genotype
```

```{r annotation}
# Get annotation information
annotation <- colorectal.plink$map
head(annotation)
```

```{r individuals}
# Get individuals information
individuals <- colorectal.plink$fam
head(individuals)
```

METHODS

DATA DESCRIPTION:

```
```{r rename-rownames}
# Rename the rownames with the id
rownames(colorectal.phenotype) <- colorectal.phenotype$id
head(colorectal.phenotype)
```
```

```
```{r check-order}
# We check if the rownames of the two objects are identical
identical(rownames(colorectal.phenotype), rownames(colorectal.genotype))
```
```

```
[1] TRUE
```

METHODS

DATA DESCRIPTION:

```
```{r fix-individuals}

ids <- intersect(rownames(colorectal.phenotype), rownames(colorectal.genotype))
genotype <- colorectal.genotype[ids, ]
phenotype <- colorectal.phenotype[ids, ]
identical(rownames(phenotype), rownames(genotype))
individuals <- individuals[ids, ]
```
```

METHODS

Control individuals → subjects that do not have colorectal cancer

Cascon == 0

```
```{r controls}
# Controls are not subjects with colorectal cancer
controls <- phenotype$cascon == 0 & !is.na(phenotype$cascon)
genotype.controls <- genotype[controls, ]
info.controls <- col.summary(genotype.controls)
nrow(genotype.controls)
```
```

METHODS

QUALITY CONTROL:

Before GWAS analysis to make sure that the data is good enough for the analysis.

Two levels:

1. Quality control of SNPs
2. Quality control of individuals

METHODS

QUALITY CONTROL: 1. QUALITY CONTROL OF SNPs

Measures:

- SNPs with high rate of missing → SNPs with a call rate less than 95% are removed
- Rare SNPs (MAF) → SNPs with less than 5% minor allele frequency are deleted
- SNPs that do not pass the HWE test → controls with a Z-value bigger than 3.3 are removed

METHODS

QUALITY CONTROL: 1. QUALITY CONTROL OF SNPs

```
```{r quality2}
# Filter QC
use <- info.snps$call.rate > 0.95 &
       info.snps$MAF > 0.05 &
       abs(info.controls$z.HWE < 3.3)
mask.snps <- use & !is.na(use)

# We keep those SNPs that pass the QC
genotype.qc.snps <- genotype[, mask.snps]
genotype.qc.snps
annotation <- annotation[mask.snps, ]

# Original SNPs
genotype
# Filtered SNPs
genotype.qc.snps
```
```

METHODS

QUALITY CONTROL: 1. QUALITY CONTROL OF SNPs

Number of deleted individuals:

```
```{r snp-quality-report}
# Number of SNPs removed for a bad call rate
sum(info.snps$call.rate < 0.95, na.rm = TRUE)

# Number of SNPs removed for low MAF
sum(info.snps$MAF < 0.05, na.rm = TRUE)

# Number of SNPs removed that do not pass HWE
sum(abs(info.controls$z.HWE > 3.3), na.rm = TRUE)

# The total number of SNPs removed for any reason
sum(!mask.snps)
```
```

```
[1] 875

[1] 10669

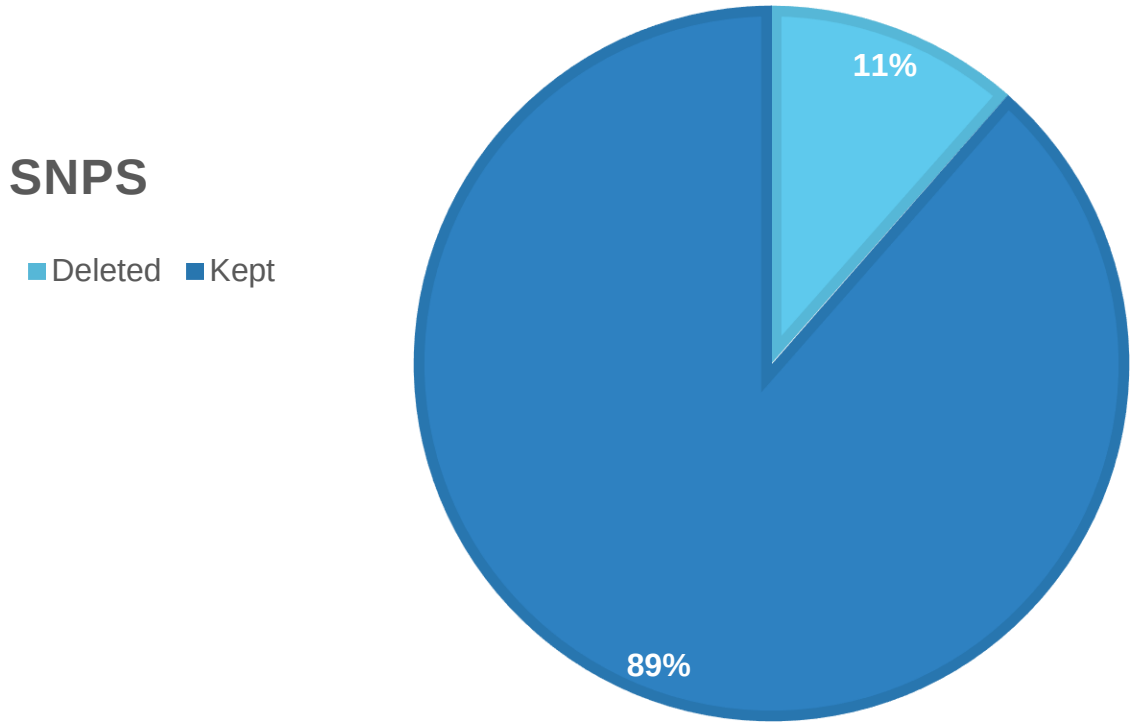
[1] 72

[1] 11479
```

METHODS

QUALITY CONTROL: 1. QUALITY CONTROL OF SNPs

From 100,000 SNPs, we keep 88,521



METHODS

QUALITY CONTROL: 2. QUALITY CONTROL OF INDIVIDUALS

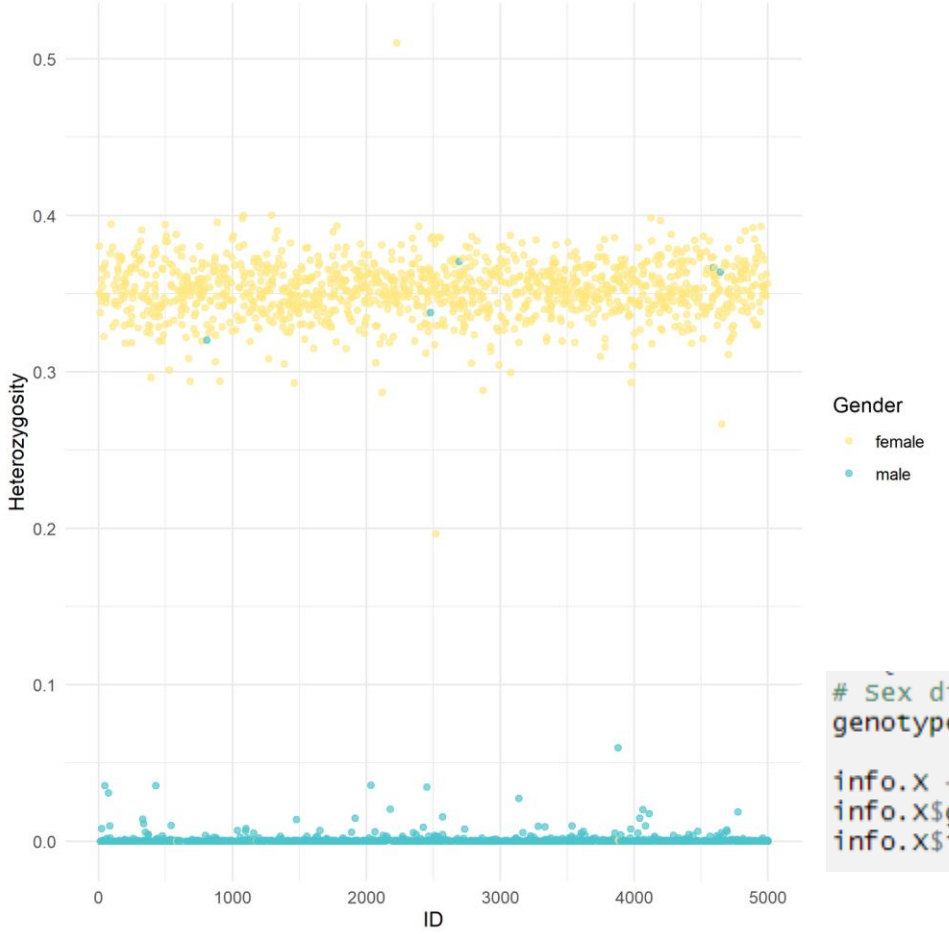
Information at individuals' level

Steps

- Sex discrepancies → Heterozygosity of chromosome X
- Individuals with outlying heterozygosity from the overall genomic heterozygosity
- Delete close familiar relatedness between individuals
- Remove individuals with more than 5% missing genotypes

METHODS

QUALITY CONTROL: 2. QUALITY CONTROL OF INDIVIDUALS



SEX DISCREPANCIES:

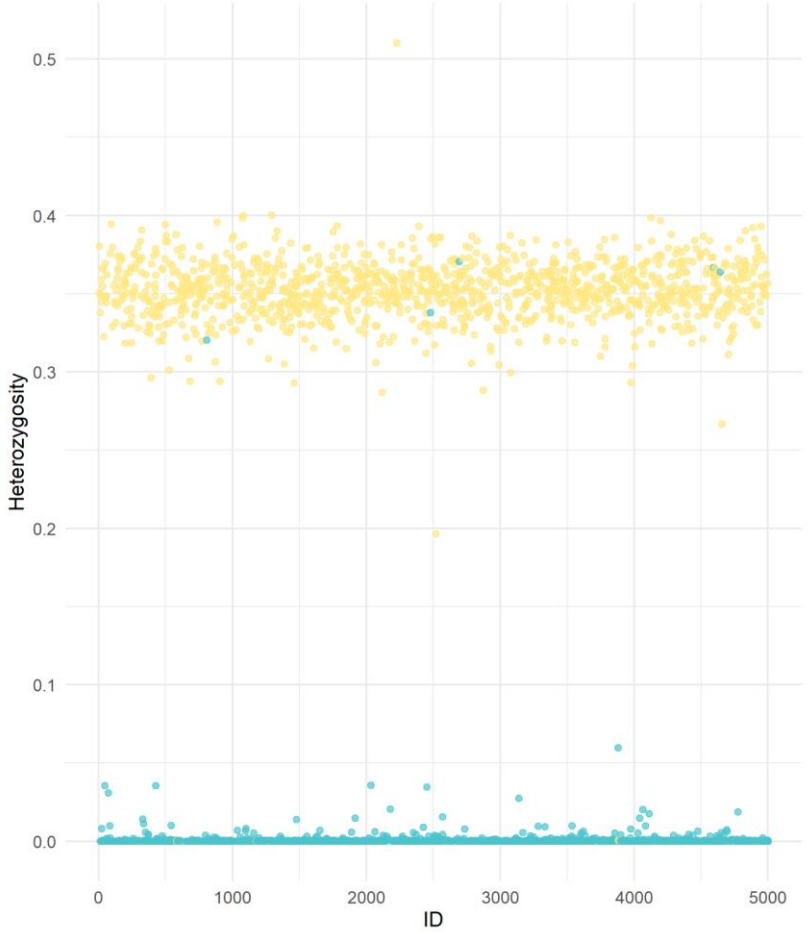
- Males → expected heterozygosity = 0
- Females → expected heterozygosity = 0.30

```
# Sex discrepancies
genotype.X <- genotype.qc.snps[,annotation$chromosome=="23" & !is.na(annotation$chromosome)]

info.X <- row.summary(genotype.X)
info.X$gender <- phenotype$sex
info.X$id <- phenotype$id
```

METHODS

QUALITY CONTROL: 2. QUALITY CONTROL OF INDIVIDUALS



SEX DISCREPANCIES:

- Males → expected heterozygosity = 0
- Females → expected heterozygosity = 0.30

```
# Plot with ggplot2
ggplot(info.X, aes(y = Heterozygosity, x = id)) +
  geom_point(aes(color=gender), alpha = 0.7) +
  labs(y = "Heterozygosity", x = "ID", color = "Gender") +
  theme_minimal() + scale_color_manual(values = c("#FFE882", "#4DC4CC"))
```

```
# Sex discrepancies
genotype.X <- genotype.qc.snps[,annotation$chromosome=="23" & !is.na(annotation$chromosome)]

info.X <- row.summary(genotype.X)
info.X$gender <- phenotype$sex
info.X$id <- phenotype$id
```

METHODS

QUALITY CONTROL: 2. QUALITY CONTROL OF INDIVIDUALS

SEX DISCREPANCIES:

- Males → expected heterozygosity = 0
- Females → expected heterozygosity = 0.30

```
```{r sex-discr2}
sex.discrep <- (info.x$gender == "Male" &
                 info.x$Heterozygosity > 0.2) |
                 (info.x$gender=="Female" &
                  info.x$Heterozygosity < 0.2)
```
```

METHODS

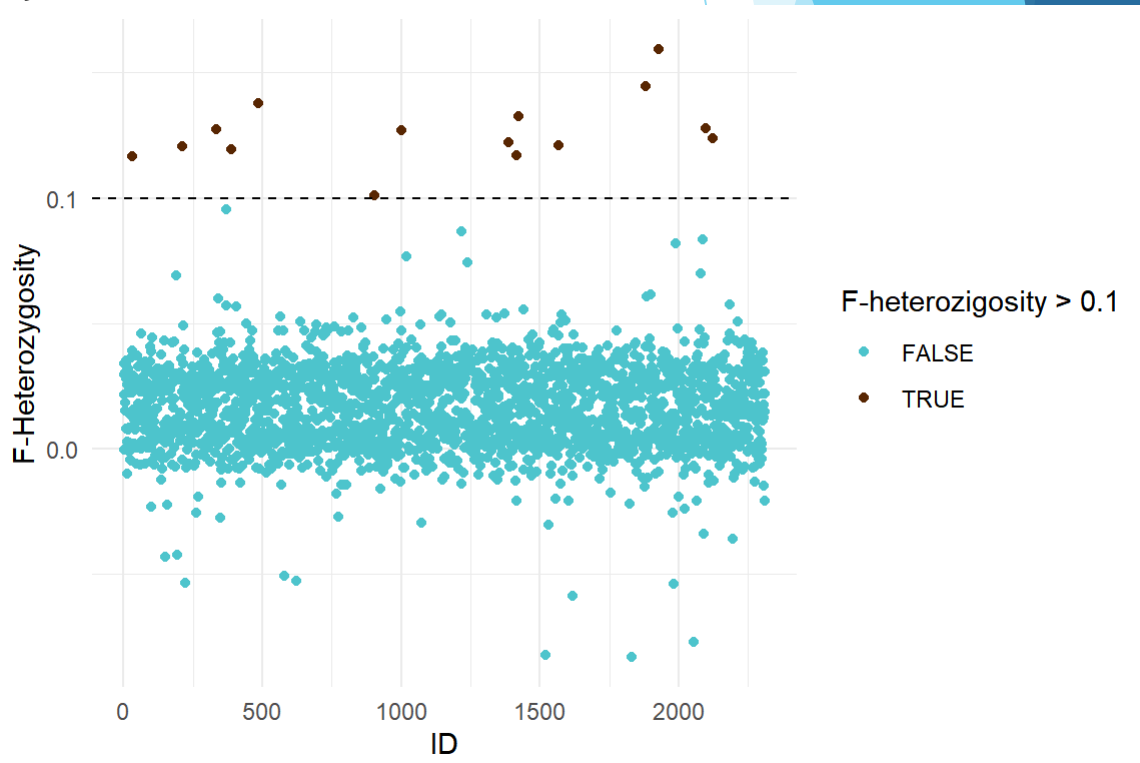
QUALITY CONTROL: 2. QUALITY CONTROL OF INDIVIDUALS

OUTLYING HETEROZYGOSITY FROM THE OVERALL GENOMIC HETEROZYGOSITY

- Heterozygosity rate lower than 0.32 or F-heterozigosity > 0.1 → outliers that need to be removed

```
MAF <- col.summary(genotype.qc.snps)$MAF
callmatrix <- !is.na(genotype.qc.snps)
hetExp <- callmatrix %*% (2*MAF*(1-MAF))
hetObs <- with(info.indv,
               Heterozygosity*(ncol(genotype.qc.snps))*Call.rate)
info.indv$hetF <- 1 - (hetObs/hetExp)
head(info.indv)
```

```
ggplot(info.indv, aes(x = 1:nrow(info.indv), y = hetF))
  geom_point(aes(color = hetF > 0.1)) +
  geom_hline(yintercept = 0.1, linetype = "dashed") +
  labs(y = "F-Heterozygosity", x = "ID", color = "F-heterozigosity > 0.1") +
  theme_minimal() + scale_color_manual(values = c("#4DC4CC", "#582602"))
```



METHODS

QUALITY CONTROL: 2. QUALITY CONTROL OF INDIVIDUALS

RELATEDNESS

```
# Open the file
genofile <- snpgdsOpen("colorectalGDS")
# Using a seed allows to reproduce the analysis
set.seed(12345)
snps.qc <- colnames(genotype.qc.snps)
snp.prune <- snpgdsLDpruning(genofile,ld.threshold = 0.2, snp.id=snps.qc)
```

```
snps.ibd <- unlist(snp.prune, use.names=FALSE)
ibd <- snpgdsIBDMoM(genofile, kinship = TRUE,
                    snp.id = snps.ibd,
                    num.thread = 1)
ibd.kin <- snpgdsIBDSelection(ibd)
head(ibd.kin)
```

Individuals with higher kindship than 0.1 are removed

```
ibd.kin.thres <- subset(ibd.kin, kinship > 0.1)
head(ibd.kin.thres)
```

```
##          ID1  ID2        k0        k1   kinship
## 46484   1049  188 0.2933008 0.5060649 0.2268334
## 232848  1202 1330 0.0000000 0.0000000 0.5000000
## 281069  1237  872 0.2903871 0.4285650 0.2476652
## 640474   155 1682 0.2608786 0.4202985 0.2644860
## 806337   170 2015 0.2593817 0.5409794 0.2350643
## 1158509 2055  825 0.0000000 0.0000000 0.5000000
```

We removed them by using their Id

```
ids.rel <- related(ibd.kin.thres)
ids.rel
```

METHODS

QUALITY CONTROL: 2. QUALITY CONTROL OF INDIVIDUALS

SUMMARY

```
 use <- info.indv$Call.rate > 0.95 &
  abs(info.indv$hetF) < 0.1 &     # or info.inv$Heterozygosity < 0.32
  !sex.discrep &
  !rownames(info.indv)%in%ids.rel
mask.indiv <- use & !is.na(use)
genotype.qc <- genotype.qc.snps[mask.indiv, ]

phenotype.qc <- colorectal.phenotype[mask.indiv, ]
identical(rownames(phenotype.qc), rownames(genotype.qc))

dim(phenotype)
dim(phenotype.qc)
```

METHODS

QUALITY CONTROL: 2. QUALITY CONTROL OF INDIVIDUALS

Number of deleted individuals:

```
# Number of individuals removed to bad call rate
sum(info.indv$Call.rate < 0.95)
## [1] 32

# Number of individuals removed for heterozygosity problems
sum(abs(info.indv$hetF)>0.1)
## [1] 15

# Number of individuals removed for sex discrepancies
sum(sex.discrep)
## [1] 9

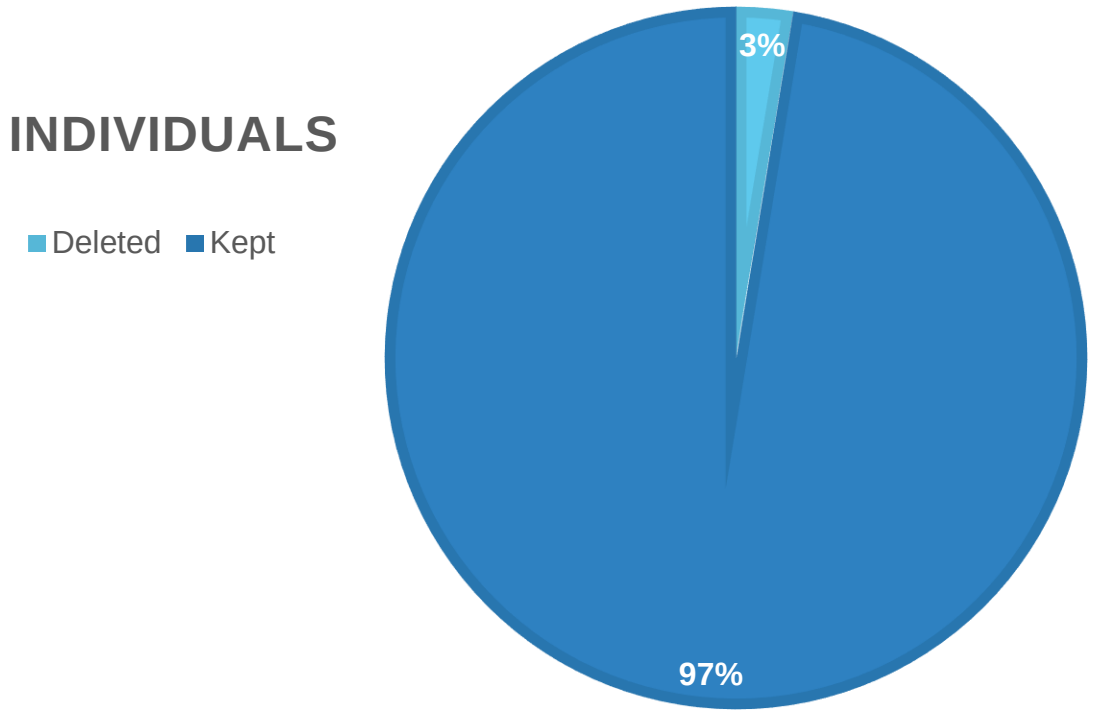
# Number of individuals removed to be related with others
length(ids.rel)
## [1] 15

# The total number of individuals that do not pass QC
sum(!mask.indiv)
## [1] 69
```

METHODS

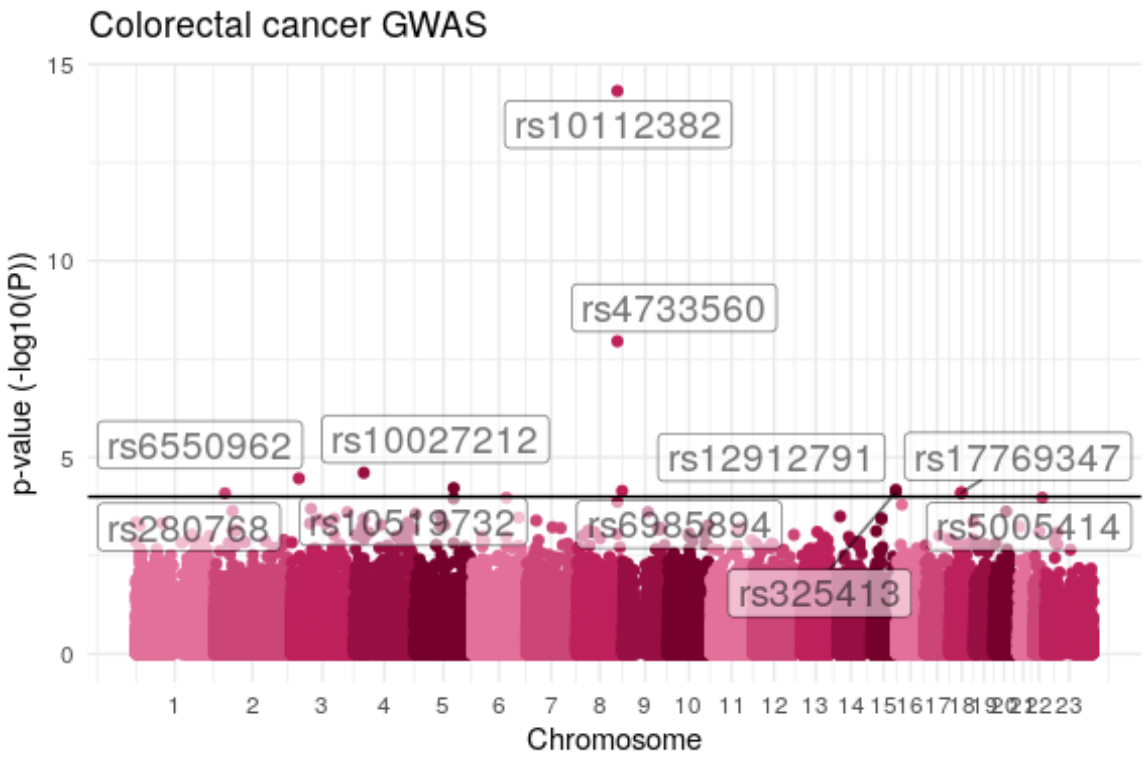
QUALITY CONTROL: 2. QUALITY CONTROL OF INDIVIDUALS

From 2312 individuals, we kept 2243



RESULTS

GWAS ⟶ Manhattan plot



One point for every SNP.

Using the Bonferroni-corrected threshold:

$$\frac{0{,}05}{\text{Number of SNPs}}$$

DISCUSSION

SNPs associated to colorectal cancer

SNP	CHROMOSOME
rs280768	2
rs6550962	3
rs10027212	4
rs10519732	5
rs4733560	8
rs10112382	8
rs6985894	8
rs12912791	15
rs325413	15
rs5005414	18
rs17769347	18

DISCUSSION

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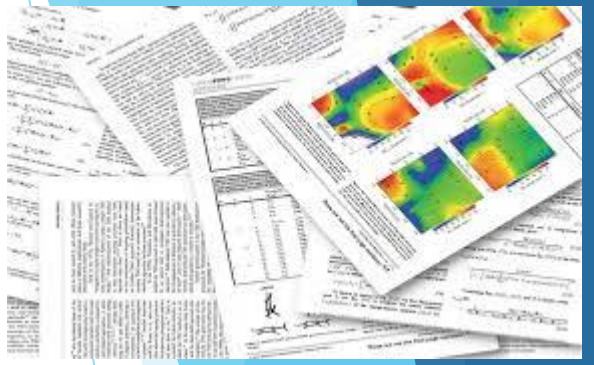
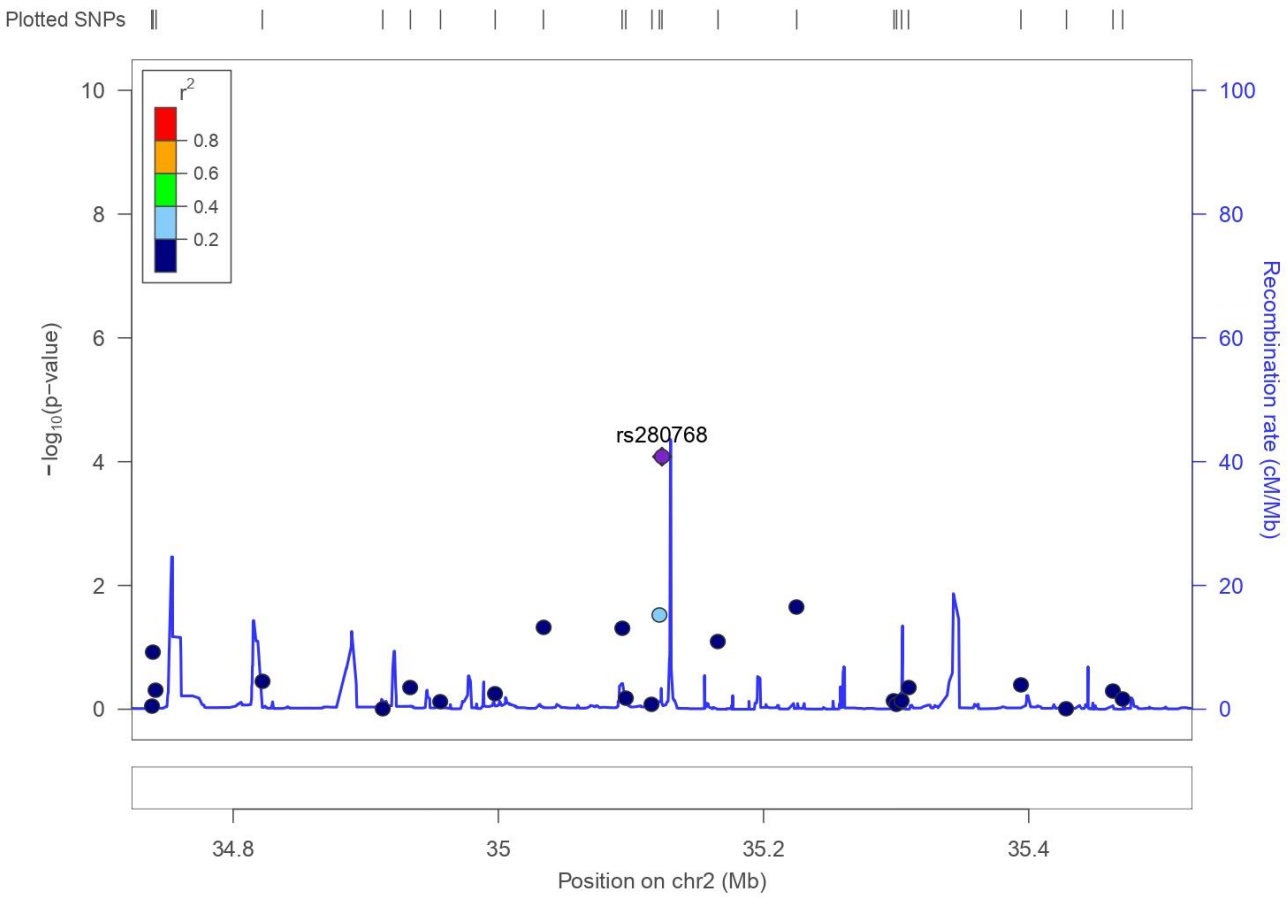


DISCUSSION

rs280768



C > T

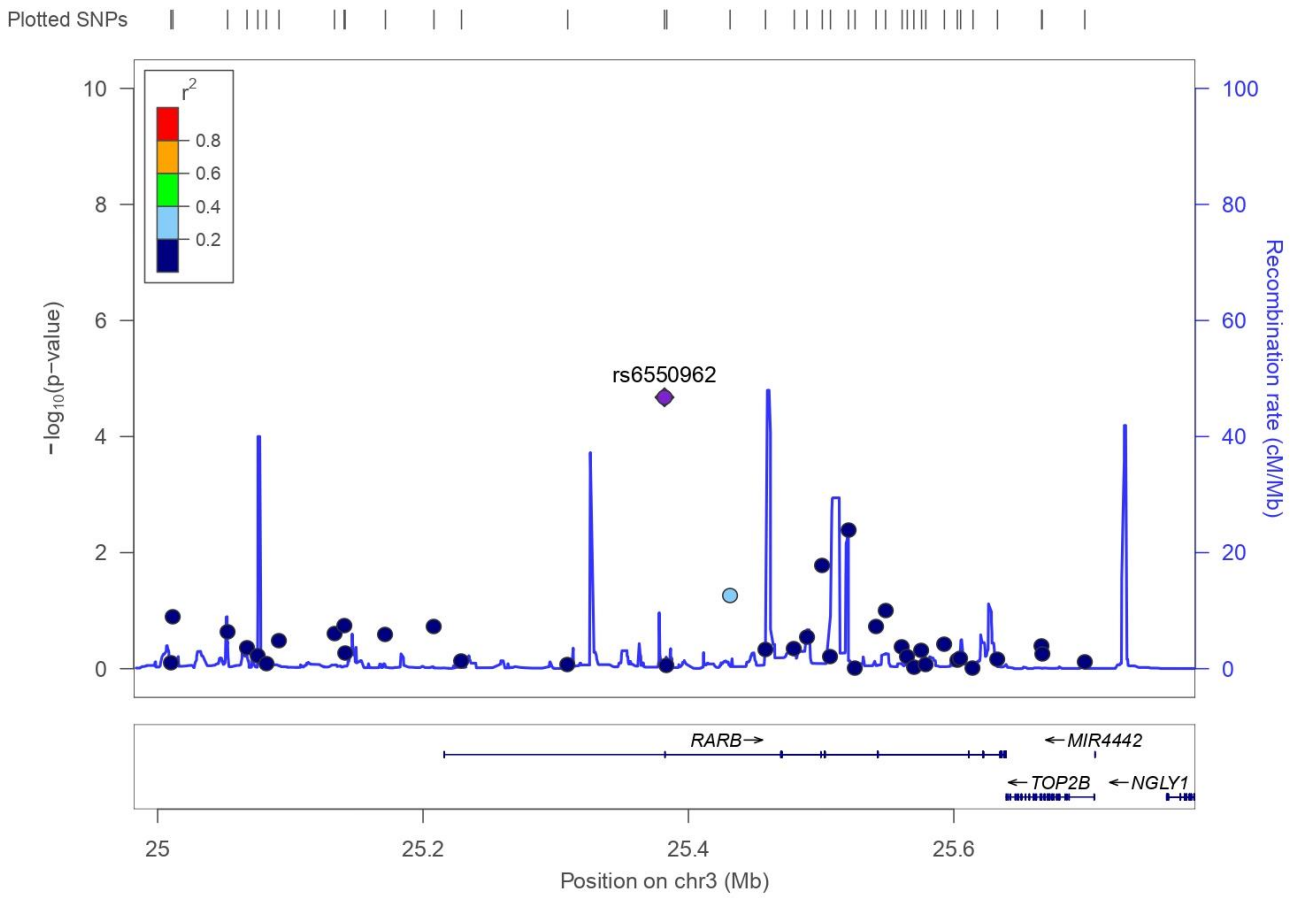
CHROMOSOME 2

FREQUENCY:

- C → 0,529
- T → 0,471

DISCUSSION

rs6550962



A > G

CHROMOSOME 3

- *RARB* gene

FREQUENCY:

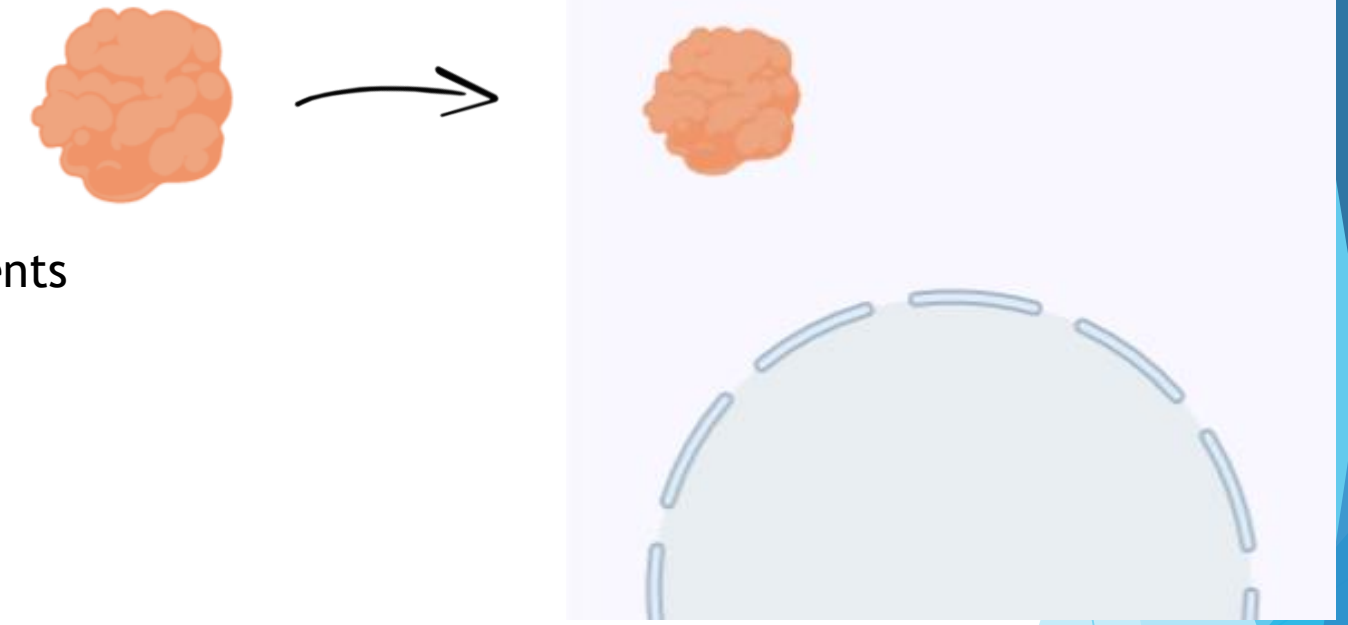
- A → 0,868
- G → 0,132

SO Term

- Genic Upstream Transcription Variant
- Intron variant

DISCUSSION

rs6550962 – *RARB* gene

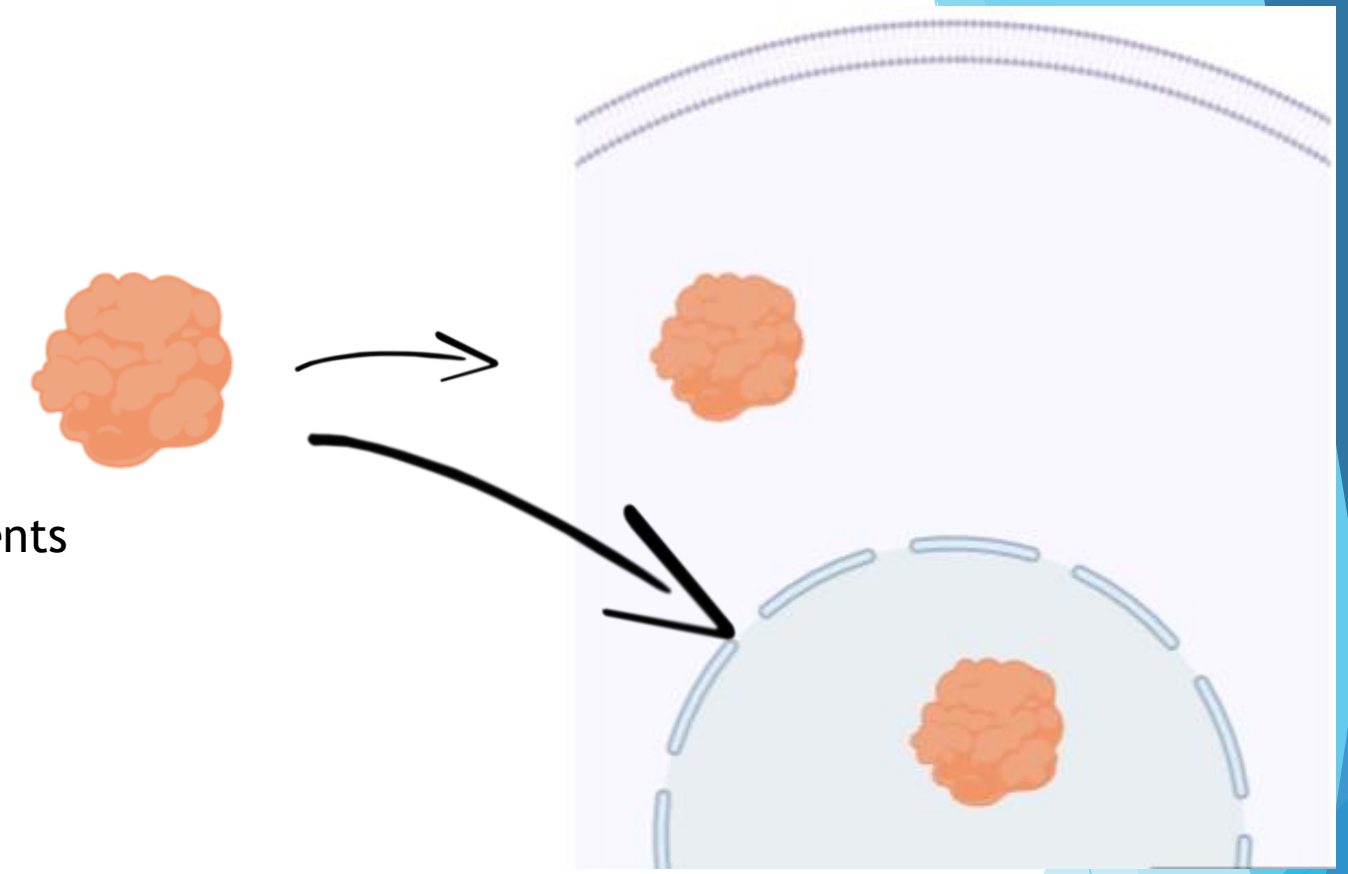


- Nuclear transcriptional regulator
- Cytoplasm and subnuclear compartments

DISCUSSION

rs6550962 – *RARB* gene

- Nuclear transcriptional regulator
- Cytoplasm and subnuclear compartments



DISCUSSION

rs6550962 – *RARB* gene

- Nuclear transcriptional regulator
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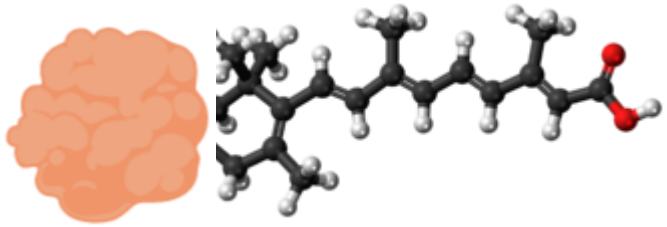

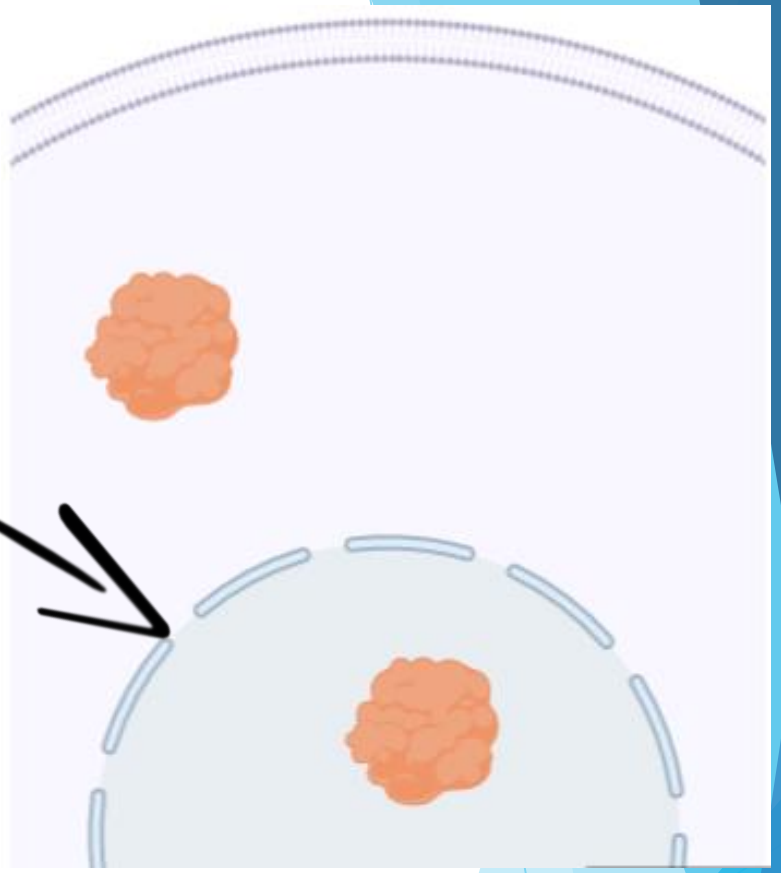

Cellular signalling in embryonic morphogenesis

Cell growth

Differentiation

¿ Cancer ?

DISCUSSION

rs6550962 – *RARB* gene

2019

Research Article

High Expression of RARβ Is a Favorable Factor in Colorectal Cancer

Wei Wang,[1] Shuang Liu,[2] Chunyi Jiang,[1] Yan Wang,[1] Huijun Zhu,[1] and XuDong Wang[3,4]

RARB expression was strongly correlated with several clinicopathological factors of colorectal cancer and may represent a favourable prognostic marker in patients with this cancer

DISCUSSION

rs6550962 – *RARB* gene

2019

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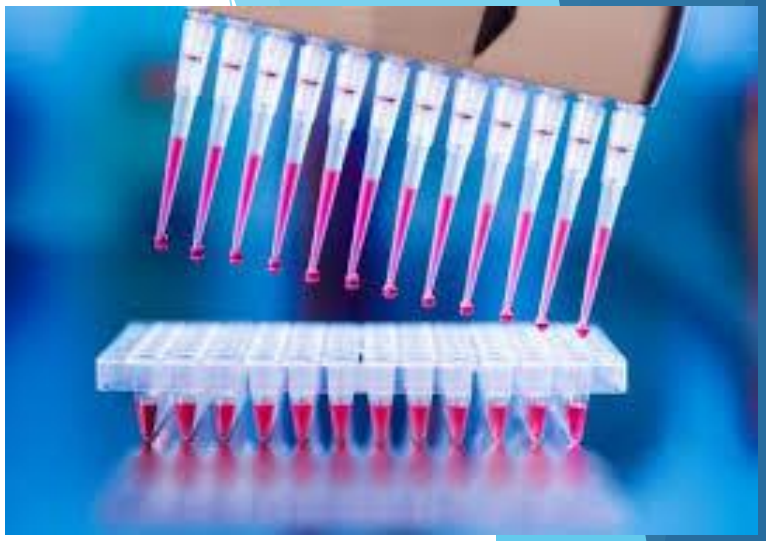
COSMIC
Catalogue Of Somatic Mutations In Cancer

67 samples with mutation

2340 samples tested

DISCUSSION



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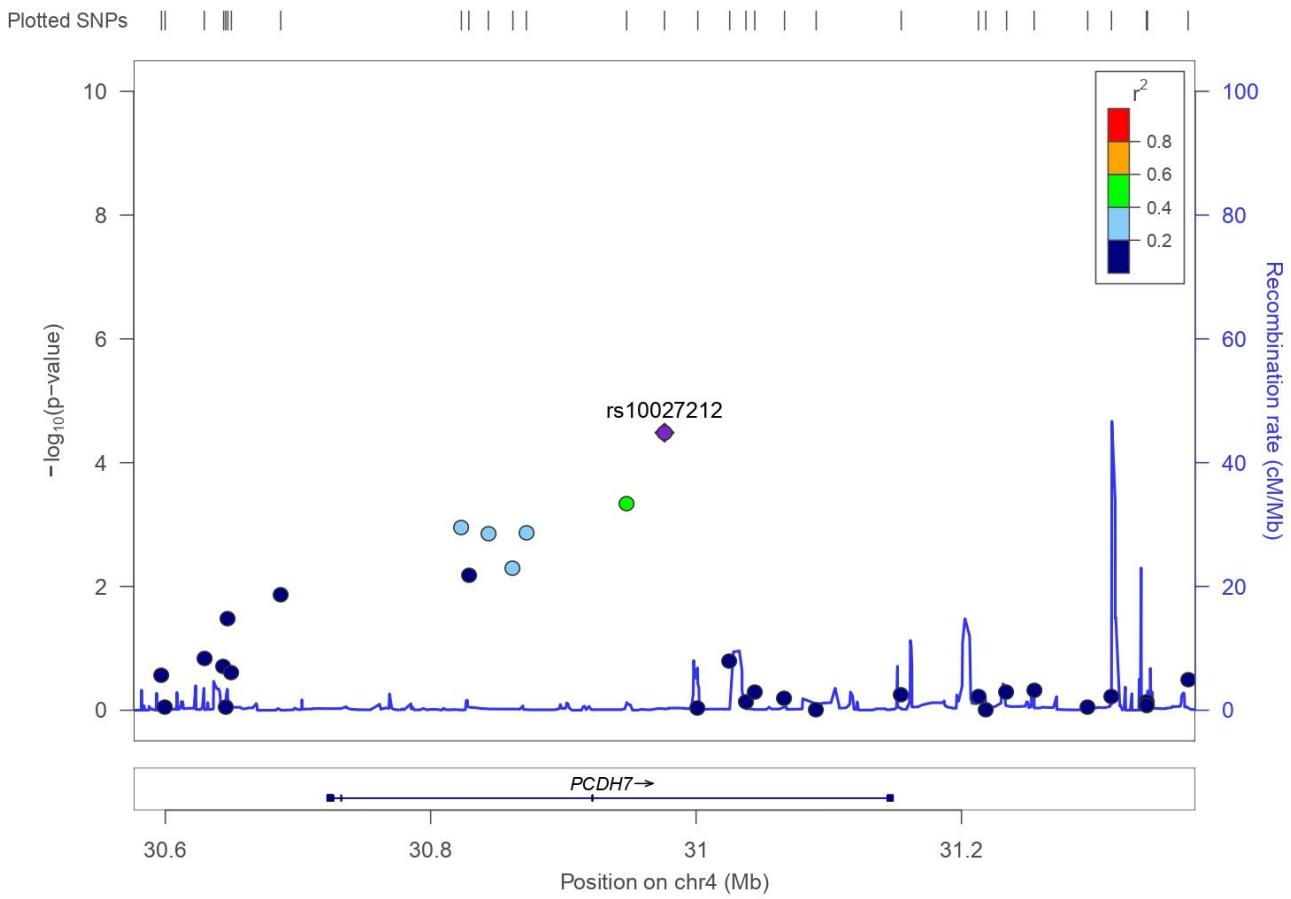
DISCUSSION

rs10027212



T > G

CHROMOSOME 4

- *PCDH7* gene

FREQUENCY:

- T → 0,544
- G → 0,456

SO Term

- Genic Downstream Transcription Variant
- Intron variant

DISCUSSION

rs10027212 – *PCDH7* gene

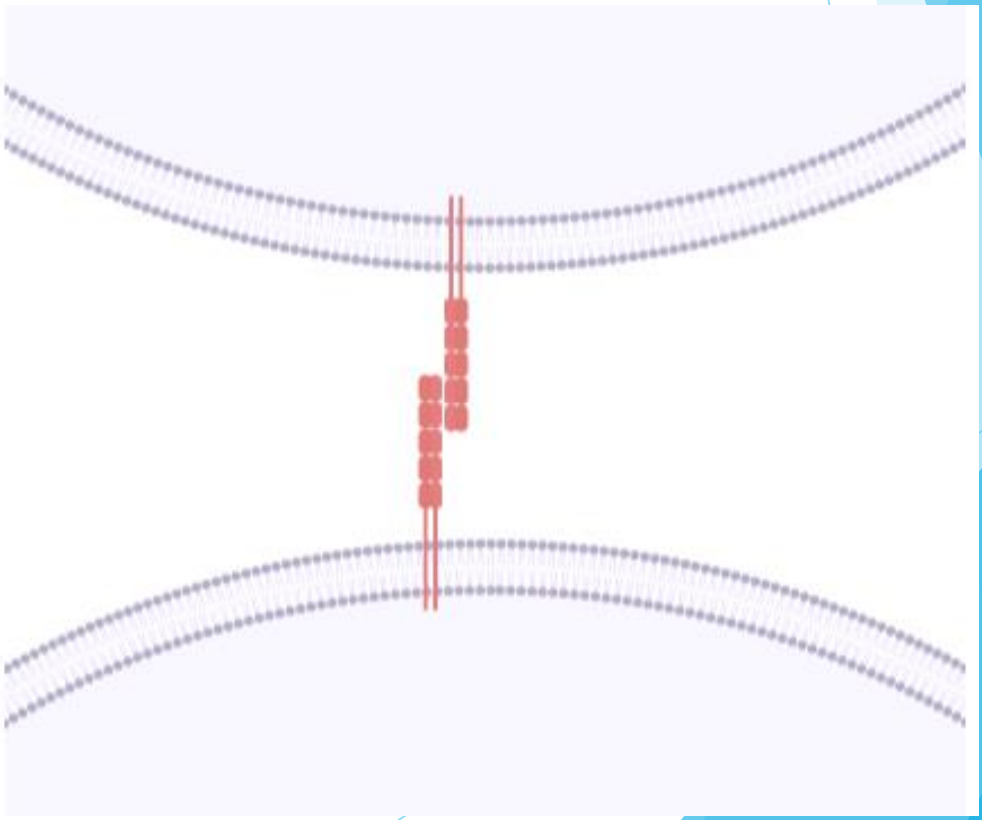
- Integral membrane protein

DISCUSSION

rs10027212 – *PCDH7* gene

- Integral membrane protein

- Cell-Cell recognition and adhesion



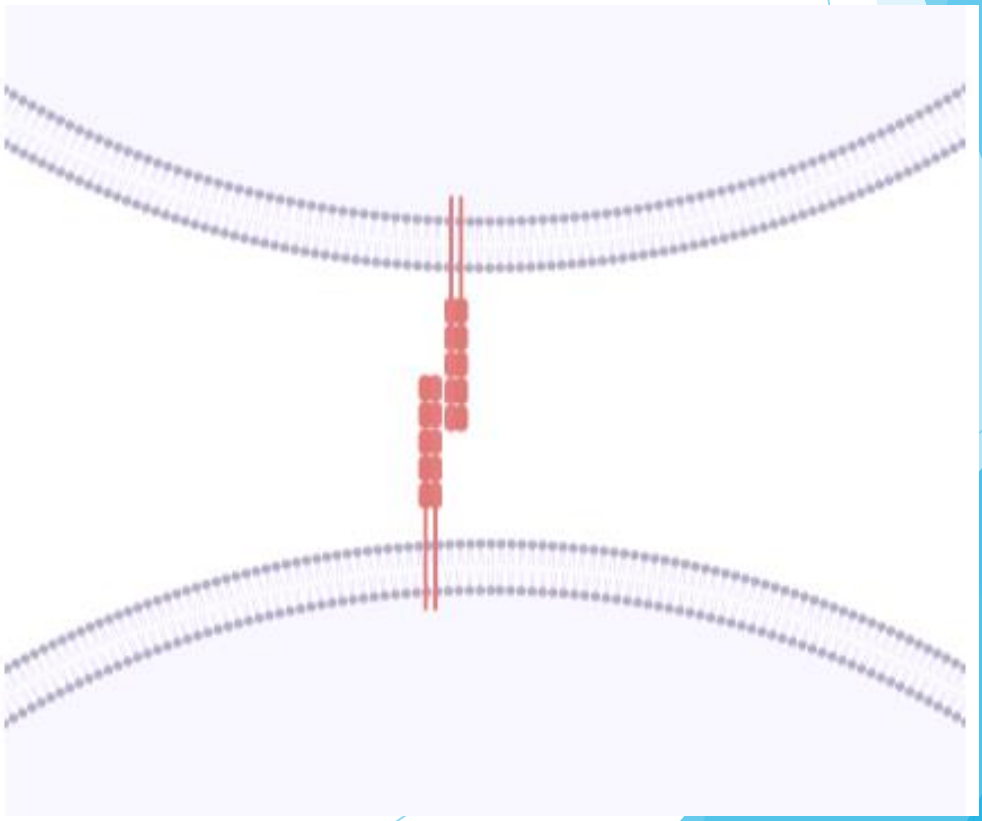
DISCUSSION

rs10027212 – *PCDH7* gene

- Integral membrane protein

¿ Cancer ?

- Cell-Cell recognition and adhesion



DISCUSSION

rs10027212 – *PCDH7* gene

2018

AQP8 inhibits colorectal cancer growth and metastasis by down-regulating PI3K/AKT signaling and PCDH7 expression

De Qing Wu[1,2], Zi Feng Yang[2], Ke Jian Wang[3], Xing Yu Feng[2], Ze Jian Lv[2], Yong Li[2], Zhi Xiang Jian[1,2]

They found molecular evidences which support the vital role of a novel AQP8-PCDH7 signalling axis in growth and metastasis of colorectal carcinoma.

It was already known that PCDH7 is overexpressed in several malignancies and are correlated with cancer cells metastasis.

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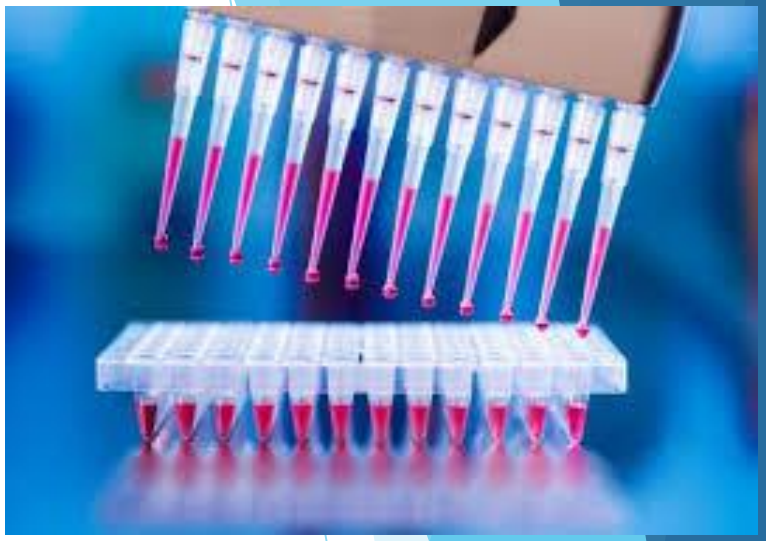
COSMIC
Catalogue Of Somatic Mutations In Cancer

125 samples with mutation

2314 samples tested

DISCUSSION



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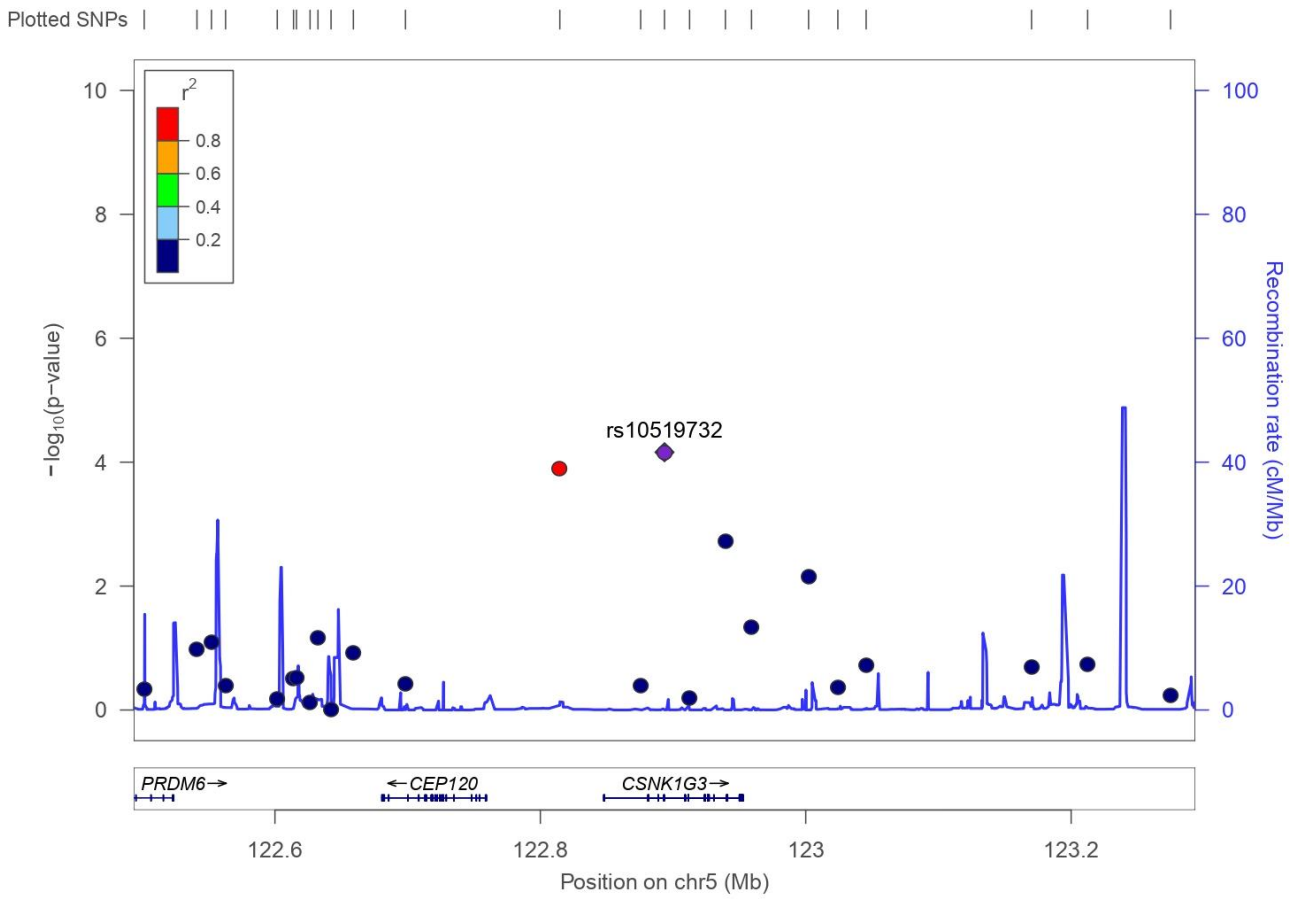
rs10519732



T > C

CHROMOSOME 5

- *CSNK163* gene

FREQUENCY:

- T → 0,893
- C → 0,107

SO Term

- Intron variant

DISCUSSION

rs10519732 – *CSNK163* gene

- Serine/Threonine protein kinases

DISCUSSION

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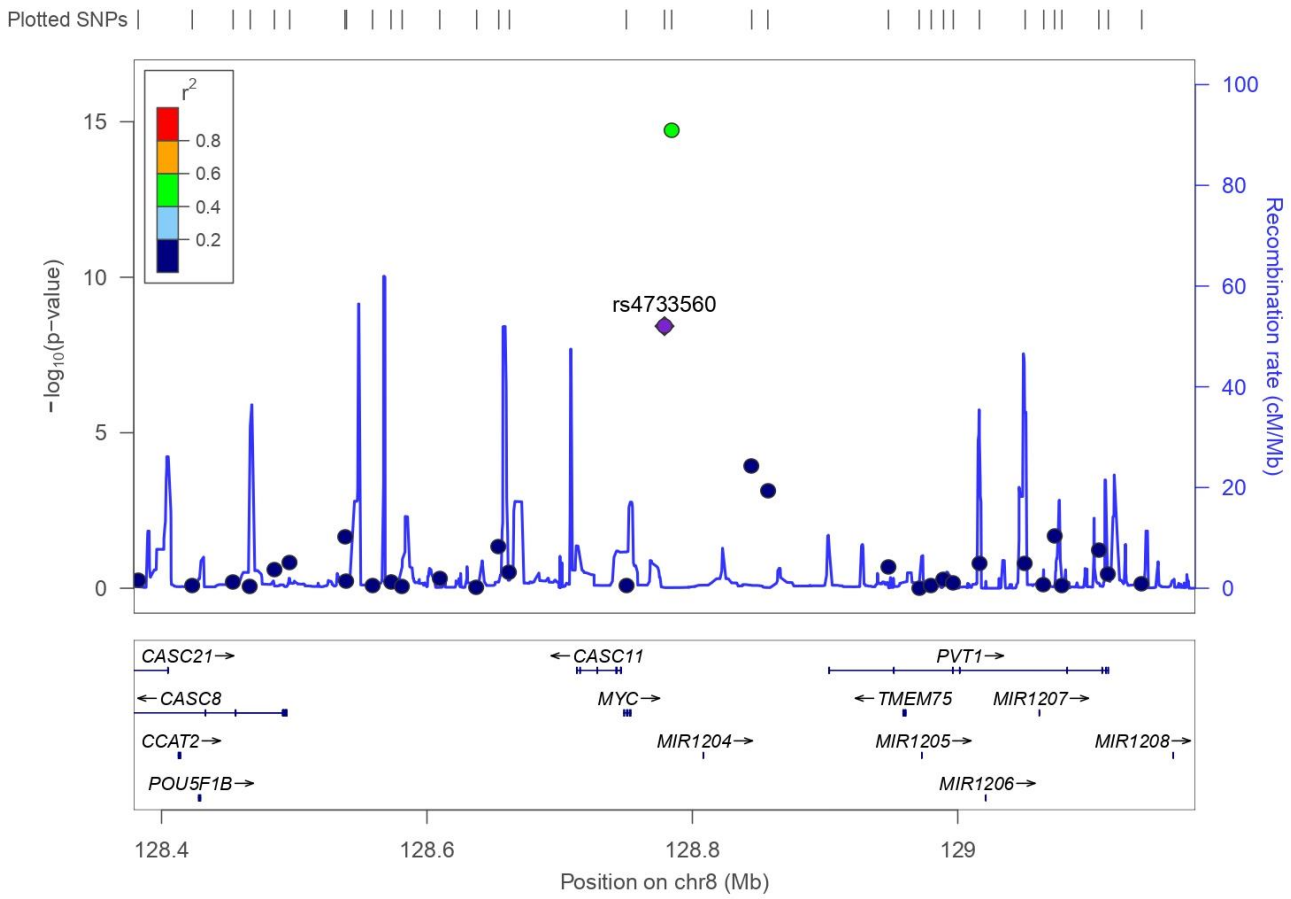
DISCUSSION

rs4733560



G > A

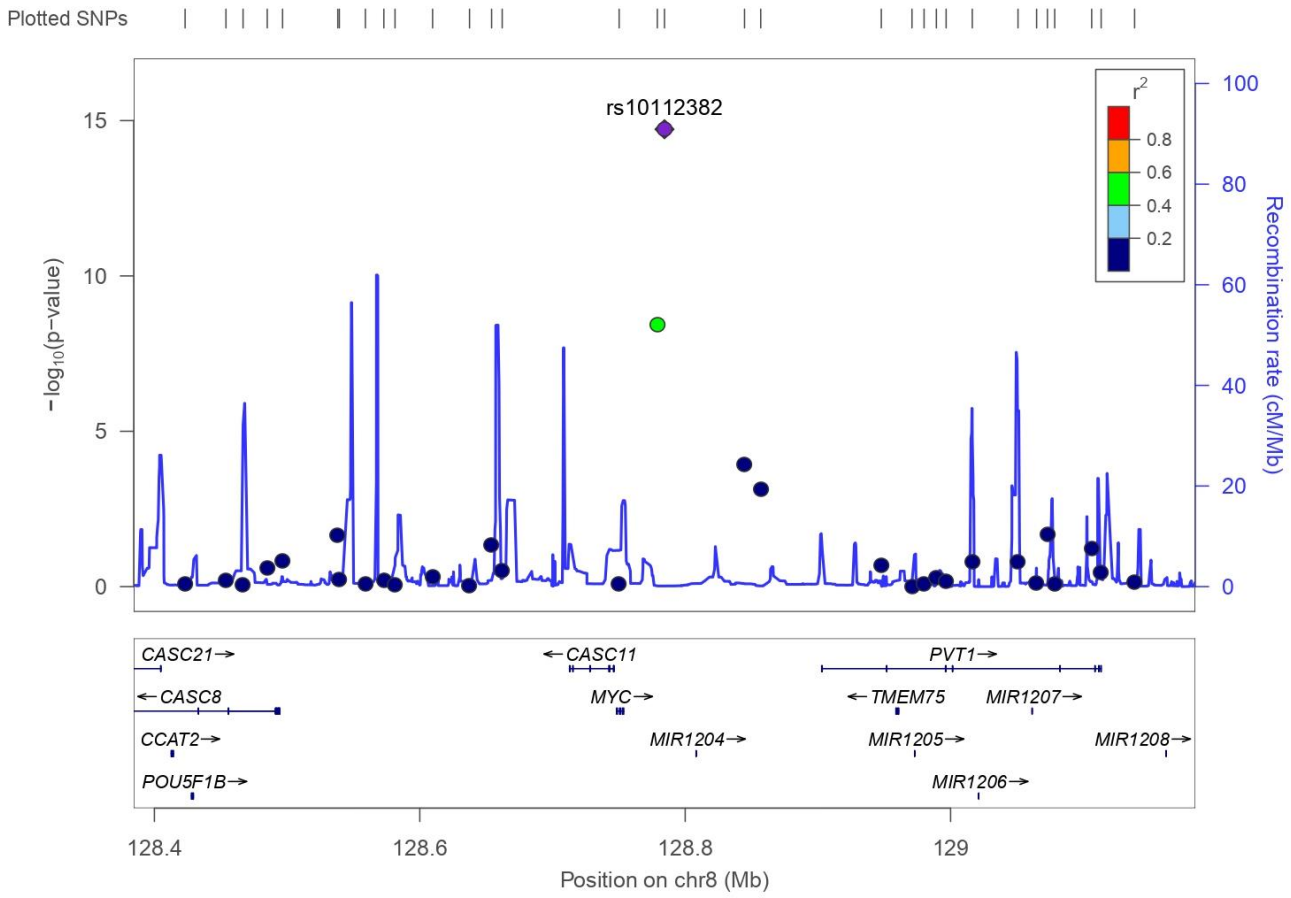
CHROMOSOME 8

FREQUENCY:

- G → 0,740
- A → 0,260

DISCUSSION

rs10112382



T > C

CHROMOSOME 8

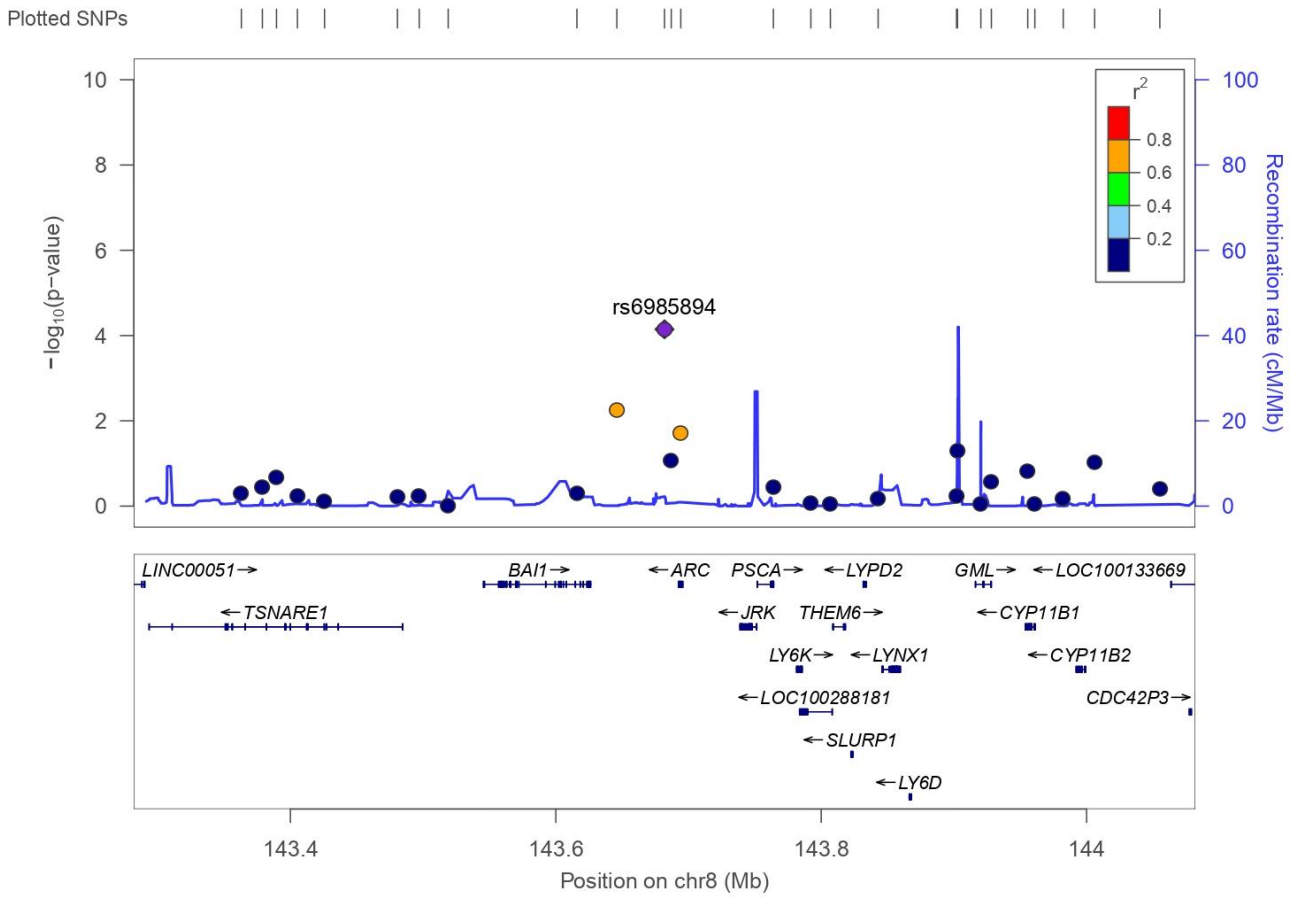
FREQUENCY:

- T → 0,339
- C → 0,661

DISCUSSION

A > G

rs6985894



CHROMOSOME 8

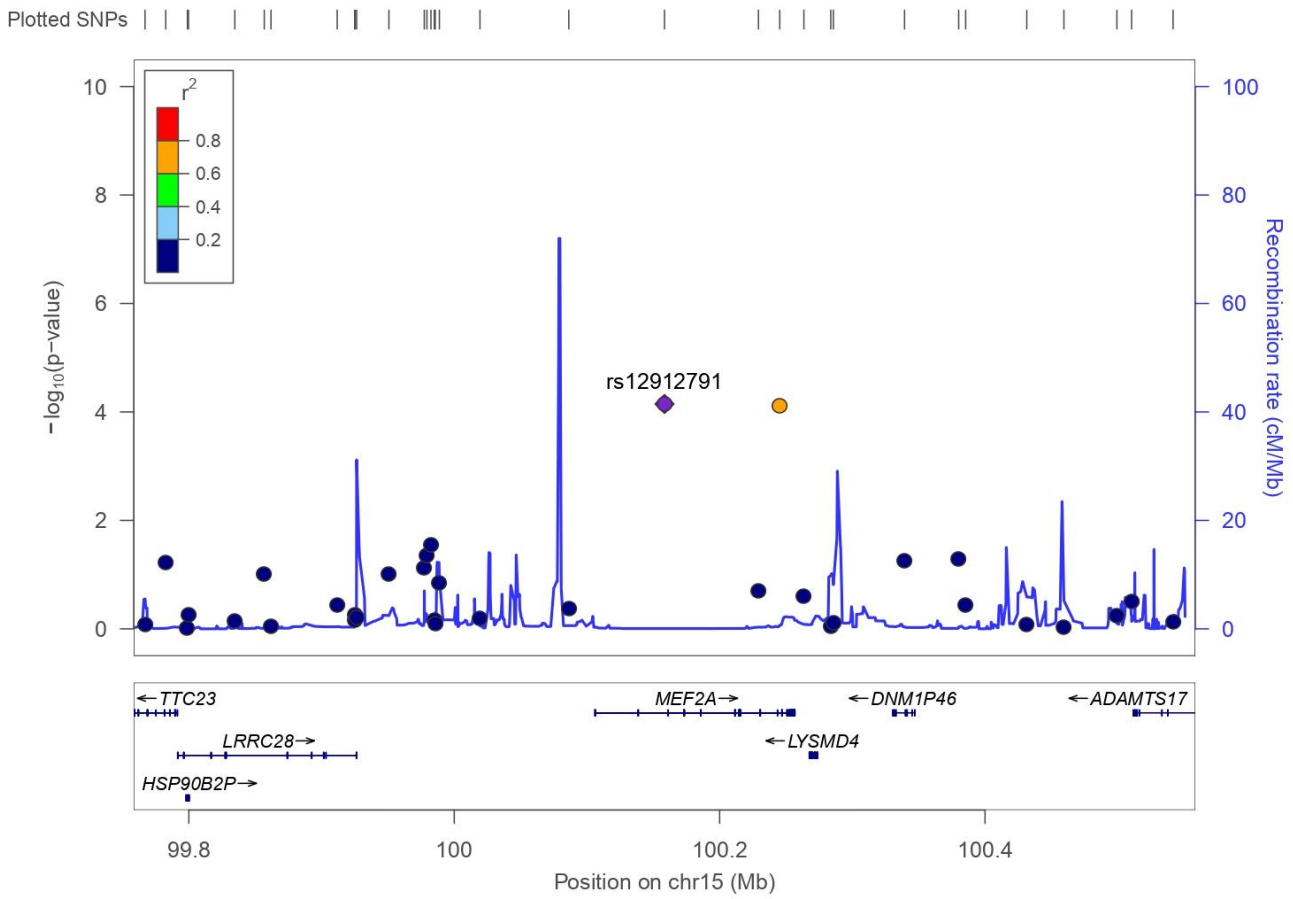
FREQUENCY:

- A → 0,218
- G → 0,782

DISCUSSION

T > C

rs12912791



CHROMOSOME 15

- *MEF2A* gene

FREQUENCY:

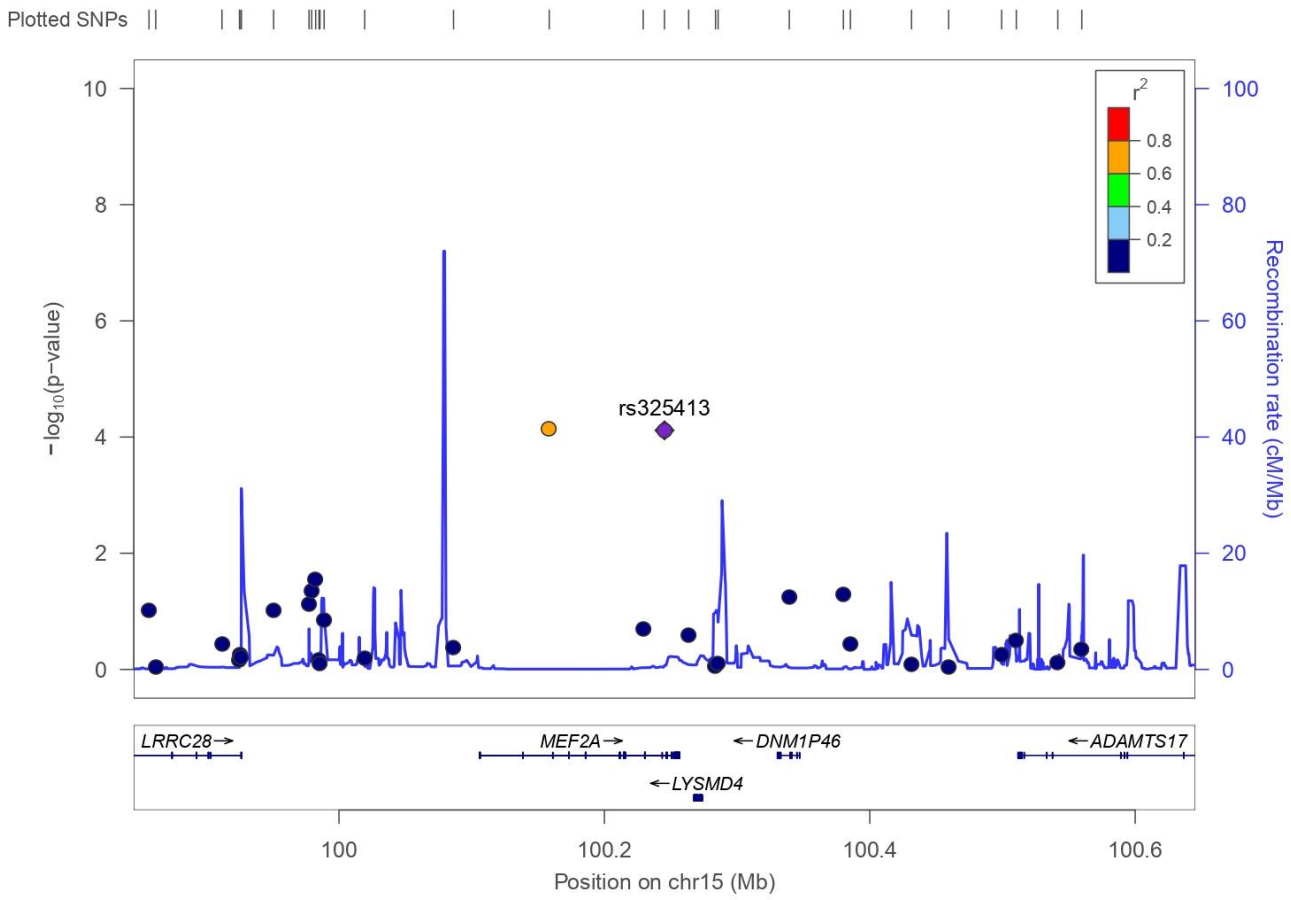
- T → 0,732
- C → 0,268

SO Term

- Intron variant
- Genic Upstream Transcript Variant

DISCUSSION

rs325413



G > A

CHROMOSOME 15

- *MEF2A* gene

FREQUENCY:

- G → 0,379
- A → 0,621

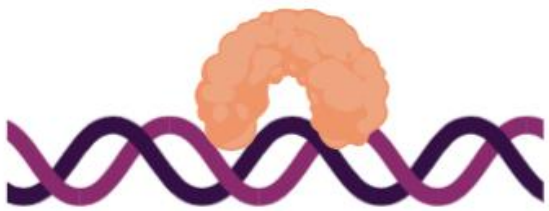
SO Term

- Intron variant
- Genic Downstream Transcript Variant

DISCUSSION

rs12912791 and rs325413 – *MEF2A* gene

- DNA-binding transcription factor



Muscle-specific genes

Growth factor-induced genes

Stress-induced genes

DISCUSSION

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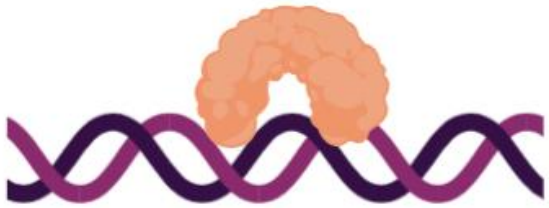

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¿ Cancer ?

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DISCUSSION

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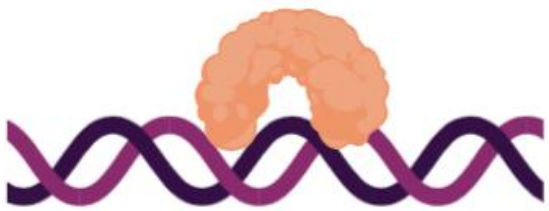

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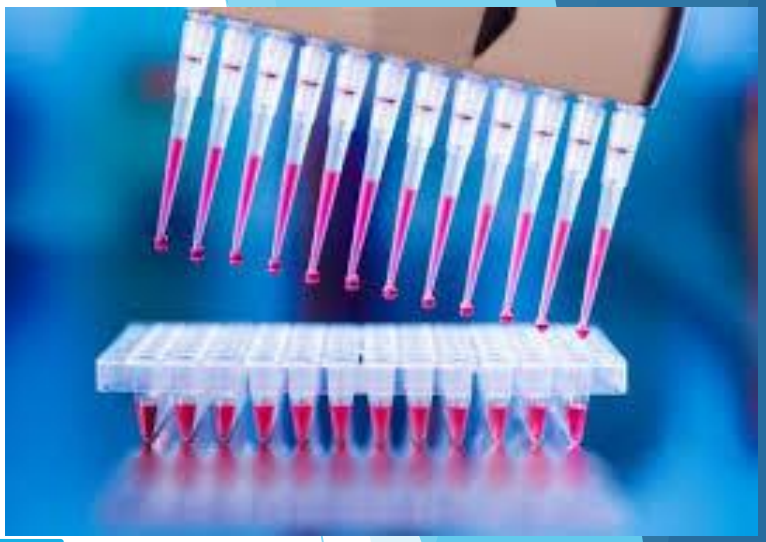
54 samples with mutation

2303 samples tested

DISCUSSION



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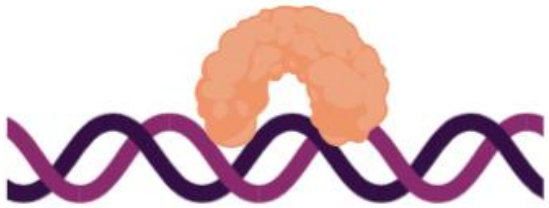

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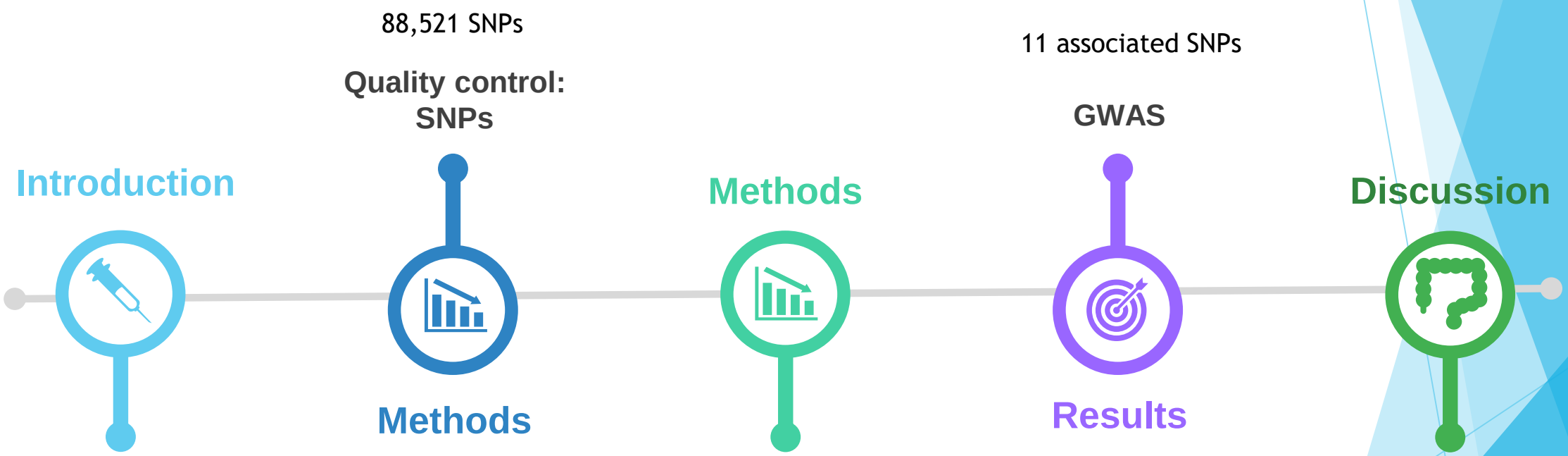
SUMMARY


Introduction
DNA samples collection
2,312 individuals
100,000 SNPs
88,521 SNPs
Quality control: SNPs
Methods
Methods
Quality control: individuals
2,243 individuals
11 associated SNPs
GWAS
Results
Discussion
SNPs analysis
4 SNPs considered for molecular studies