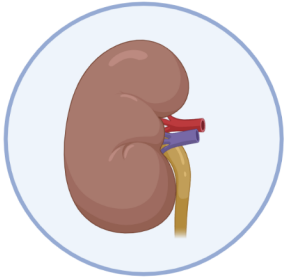
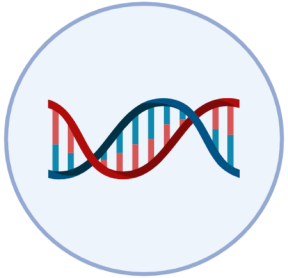
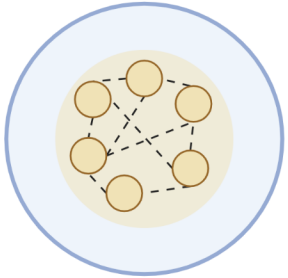
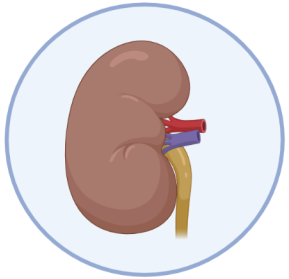
Data-driven Diagnosis: Using R to Advance Kidney Disease Research

Claire Hill
Research Fellow at Queen's University Belfast

A bit about me

2011 - 2016	Undergraduate MSci Biochemistry *Queen's University Belfast*	*No real coding experience* *Beginner bioinformatics*
2016 - 2021	PhD Interdisciplinary Bioscience *University of Oxford*	*Python* *Pandas* *Jupyter notebook* *Bioinformatics*
2021 - present	Molecular Epidemiology Research Fellow *Queen's University Belfast*	*R / R studio* Big data analysis eCarlton - R for Biologists - Free course



Why focus on kidney disease research?

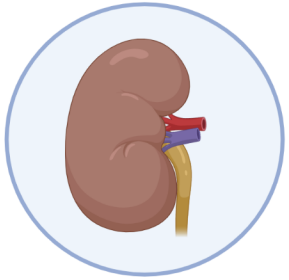
	2017	2040
- Leading cause of death:	12th	5th

- End-stage kidney disease, requiring **dialysis / kidney transplant.**

- UN Goal 3 - Decrease non-communicable disease 1/3 by 2030

SUSTAINABLE DEVELOPMENT GOALS

United Nations, 2015
Carney, 2020
Bikbov et al., 2020
Foreman et al., 2019



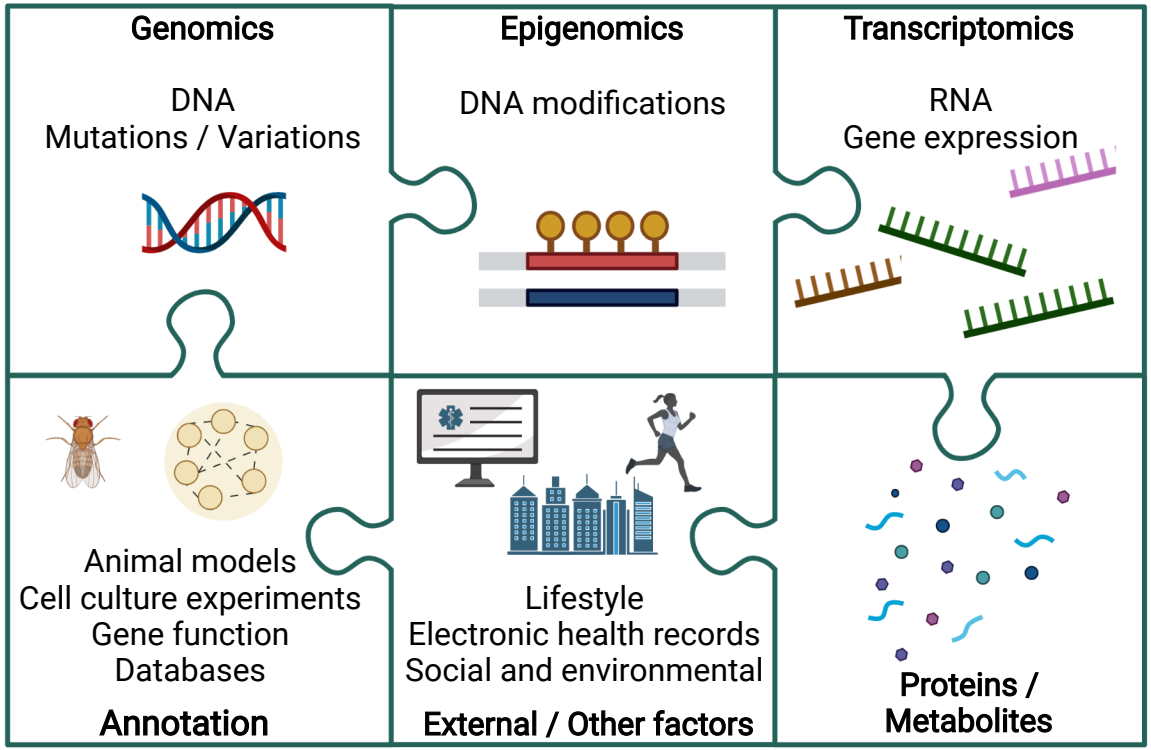
Methods to study kidney disease

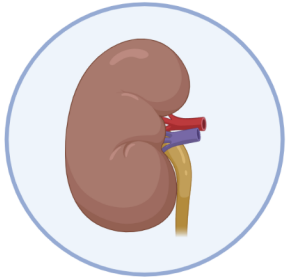
- Study **multiple factors** to understand complexity.

- Data reliability and quality control.

- **Belfast Renal Transplant** cohort:

50 years follow-up





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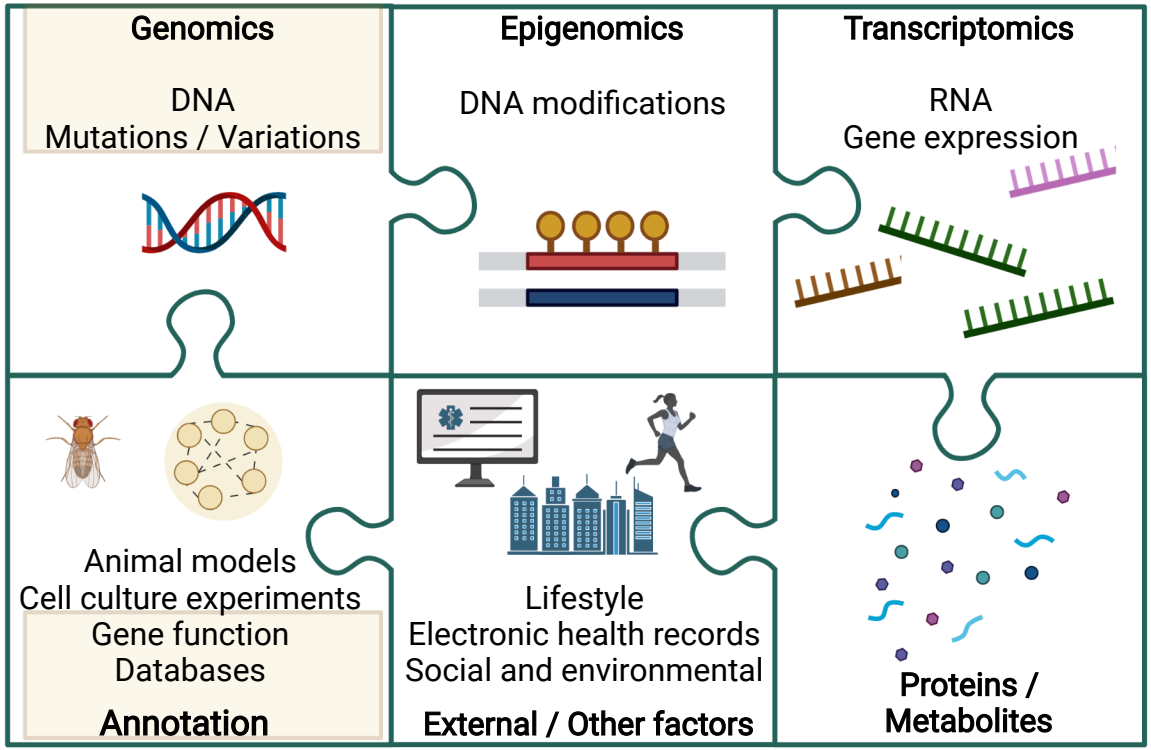
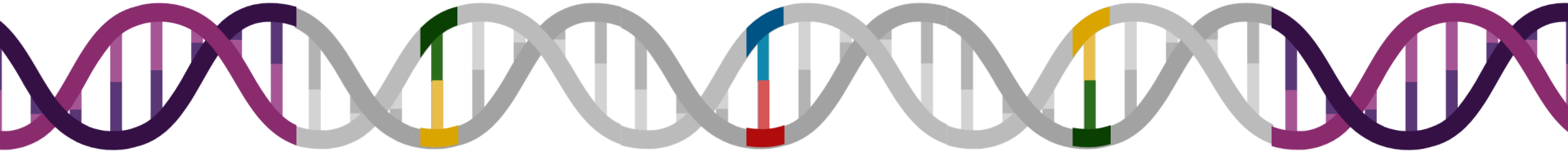


Genomic analysis of kidney disease

- Genetic differences influence **onset** and **progression**.

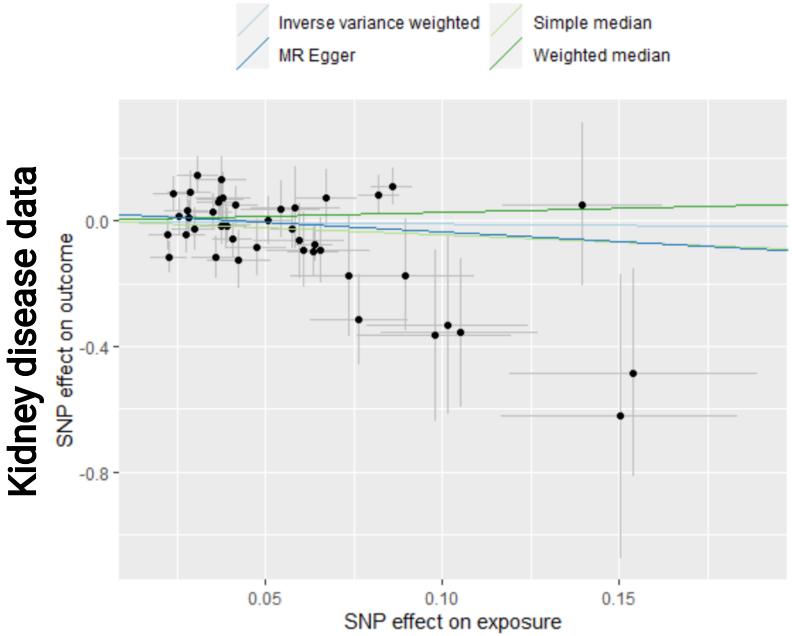
- Identifying **genetic variants** to **aid diagnosis**.

- **Overlap** with variants associated with modifiable risks to identify **causal associations**, e.g **Telomere** shortening.



Genomic analysis of kidney disease

```
library(MendelianRandomization)
library(TwoSampleMR)
#read in outcome data (e.g. kidney disease)
outcome_data <- read_outcome_data(....)
#read in exposure data (e.g. telomere associated variants)
exposure_df <- read_csv("~/MyDocuments/exp.csv")
#format your data for MR
exposure_data <- format_data(exposure_df, type="exposure")
#harmonise datasets
harmonised_data <- harmonise_data(
  exposure_dat = exposure_data ,
  outcome_dat = outcome_data)
#run multiple MR analyses at once
harmonised_res <- mr(harmonised_data,
method_list=c("mr_ivw", "mr_simple_median",
"mr_weighted_median","mr_egger_regression"))
#generate a range of plots e.g.
harmonised_p1 <- mr_scatter_plot(harmonised_res, harmonised_data)
```



Park at al., 2021

Hill and Duffy et al., in preparation

Genomic analysis of kidney disease

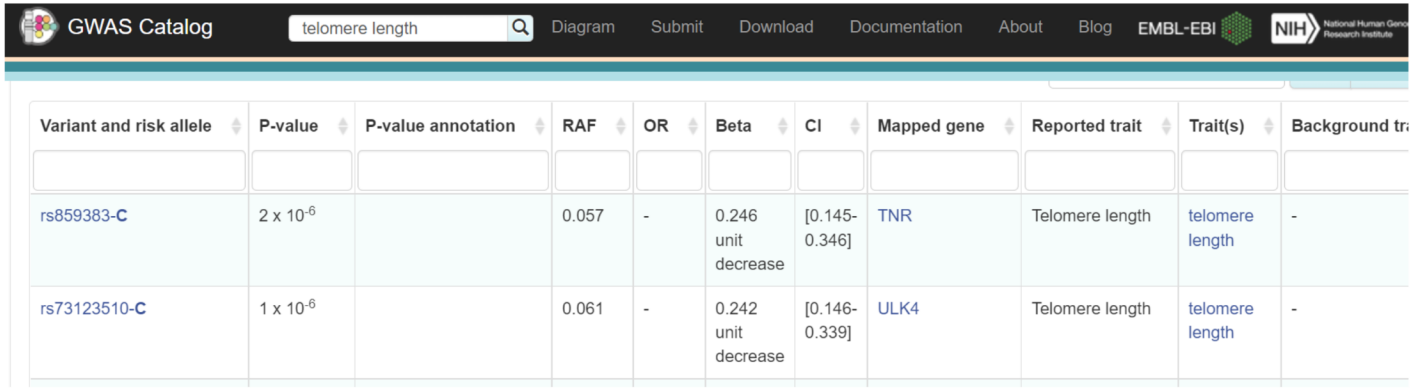
- Online version: MRBASE University of BRISTOL MRC Integrative Epidemiology Unit CANCER RESEARCH UK

- Previously published summary statisitcs:

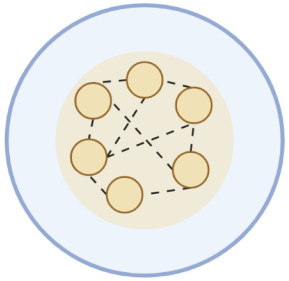
No identifiable data relased - **data protection**.

Sharing resources saves research time, effort and money.

R library **MRInstruments** (*MRC Integrative Epidemiology Unit*) pulls this data into R.



Variant and risk allele	P-value	P-value annotation	RAF	OR	Beta	CI	Mapped gene	Reported trait	Trait(s)	Background tra
rs859383-C	2 x 10^{-6}		0.057	-	0.246 unit decrease	[0.145-0.346]	TNR	Telomere length	telomere length	-
rs73123510-C	1 x 10^{-6}		0.061	-	0.242 unit decrease	[0.146-0.339]	ULK4	Telomere length	telomere length	-

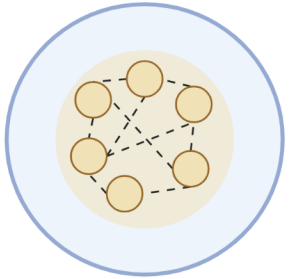


Gene ontology analysis of kidney disease

- Discover novel genetic variants associated with kidney disease, but…
  what do these genes, or the proteins they encode, do?

- Annotation via gene ontology databases:
  Molecular function,
  Biological processes,
  Cellular compartments.

- R package for analysis and **data visualisation** to aid interpretation:
  ViSEAGO (*Brionne et al., 2019*)



Gene ontology analysis of kidney disease

ViSEAGO

Background genes

Genes of interest Kidney disease genes

Enriched gene ontologies

Clustering of similar gene ontologies

Highlight hits for future study

```
##################
# load genes background
background<-scan(
    "background.txt",
    quiet=TRUE,
    what=""
)

##################
# load gene selection
selection<-scan(
    "selection.txt",
    quiet=TRUE,
    what=""
)
```

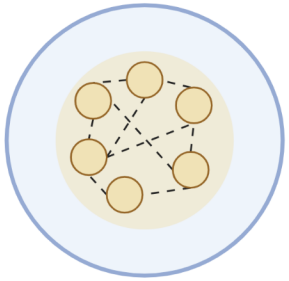
```
##################
# connect to EntrezGene
EntrezGene<-ViSEAGO::EntrezGene2GO()

 ##################
 # load GO annotations from EntrezGene
 myGENE2GO<-ViSEAGO::annotate(
     "EntrezGene_id",
     EntrezGene
 )

 ##################
 # create topGOdata for BP
 BP<-ViSEAGO::create_topGOdata(
     geneSel=selection,
     allGenes=background,
     gene2GO=myGENE2GO,
     ont="BP",
     nodeSize=5
 )
```

```
##################
# initialyse
myGOs<-ViSEAGO::build_GO_SS(
    gene2GO=myGENE2GO,
    enrich_GO_terms=BP_sResults
)
```

```
##################
# compute all available Semantic Similarity (SS) measures
myGOs<-ViSEAGO::compute_SS_distances(
    myGOs,
    distance="Wang"
)
```

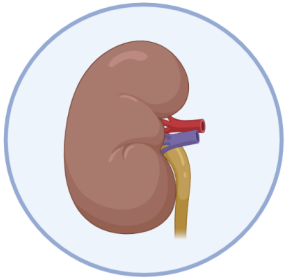
```
Wang_clusters_wardD2<-ViSEAGO::GOterms_heatmap(
    myGOs,
    showIC=TRUE,
    showGOlabels=TRUE,
    GO.tree=list(
        tree=list(
            distance="Wang",
            aggreg.method="ward.D2"
        ),
        cut=list(
            dynamic=list(
                pamStage=TRUE,
                pamRespectsDendro=TRUE,
                deepSplit=2,
                minClusterSize =2
            )
        )
    ),
    samples.tree=NULL
)
```

Brionne et al., 2019



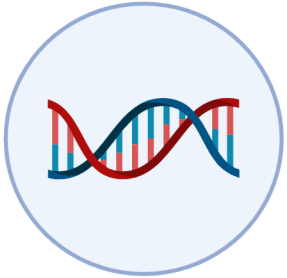
Hill and Duffy et al., in preparation

Key points



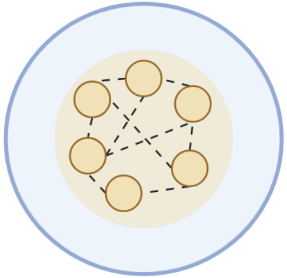
- Kidney disease is a **leading cause of death**, globally.

- **Integrating multiple approaches** advances diagnosis and treatment.



- **Genetic variants** associated with kidney disease used to aid diagnosis.

- **Mendelian Randomisation** used to identify new causal risk exposures.



- **Gene annotation** translates this knowledge into **functional insights**.

- Guiding experimental analysis for **diagnostic and therapeutic development**.

Awknowledgements

- Centre for Public Health
Molecular Epidemiology group:
Prof. AJ McKnight
Dr. Laura Smyth
Dr. Katie Kerr
Dr. Seamus Duffy
Jill Kilner
all staff and students.

- Jordan Jones

- Women Techmakers Belfast Team

- Slides created via **Biorender.com**

Fondúireacht Eolaíochta Éireann
Dá bhfuil romhainn
Science Foundation Ireland
For what's next

DfE
Department for the Economy
www.economy-ni.gov.uk

Key Tools / Links

R for Biologists (eCarlton) - Free online course

Mendelian Randomisation (Yavorska and Staley)

Two sample MR (MRC Integrative Epidemiology Unit)

MRBase (MRC Integrative Epidemiology Unit)

MRInstruments (MRC Integrative Epidemiology Unit)

ViSEAGO (Brionne et al., 2019)

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Brionne A, Juanchich A, and Hennequet-Antier C. **An overview of ViSEAGO: Visualisation, Semantic similarity, Enrichment Analysis of Gene Ontology.** [Internet]. 2020 Available from: https://bioconductor.org/packages/devel/bioc/vignettes/ViSEAGO/inst/doc/ViSEAGO.html

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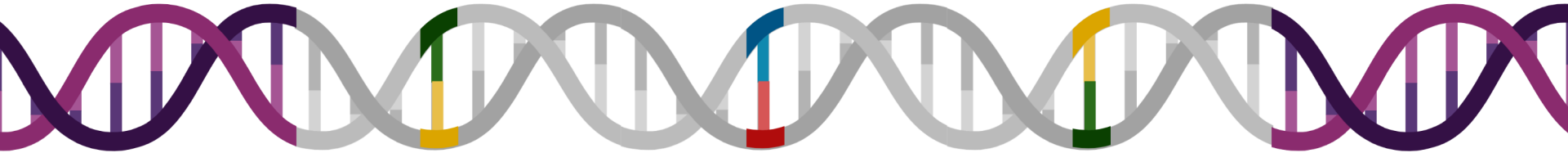
Extra slides

Genomic analysis of kidney disease

- **Telomere shortening:**
  - Premature aging
  - Age related diseases
  - Diabetes mellitus
  - Cardiovascular disease
  - Hypertension

Is this associated with onset / progression of kidney disease?

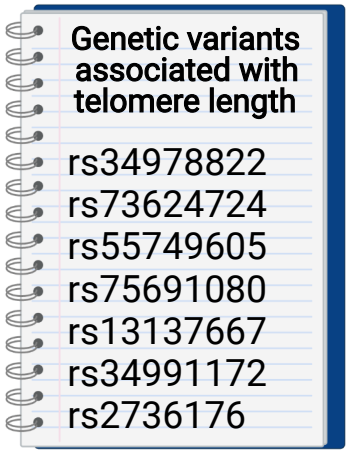


Genomic analysis of kidney disease

- Mendelian Randomisation (MR):

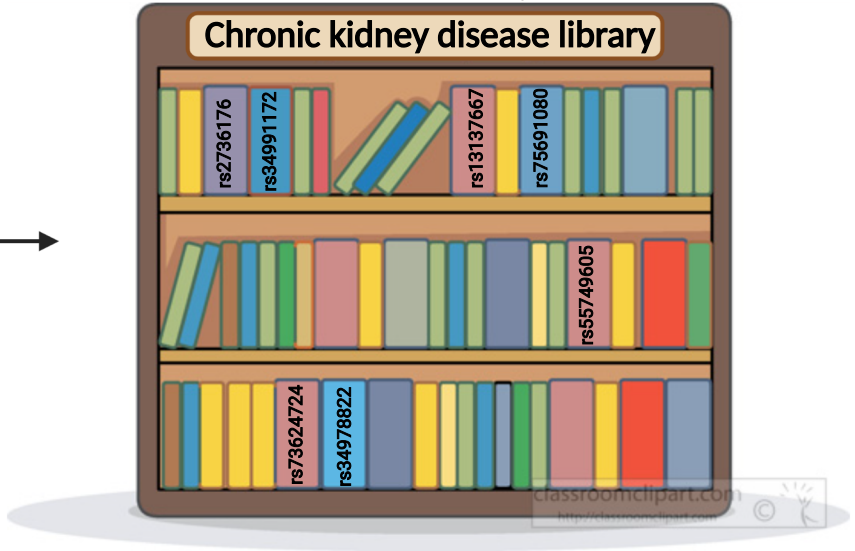
Step 1

*Genetic variants known to be associated with **proposed risk exposure***



Step 2

*Build evidence that this risk exposure is **causally associated** with kidney disease*



Genetic analysis of kidney disease

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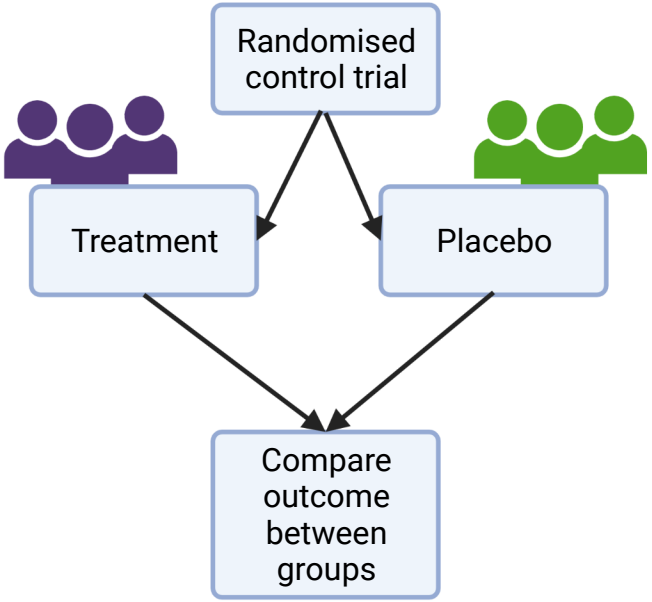
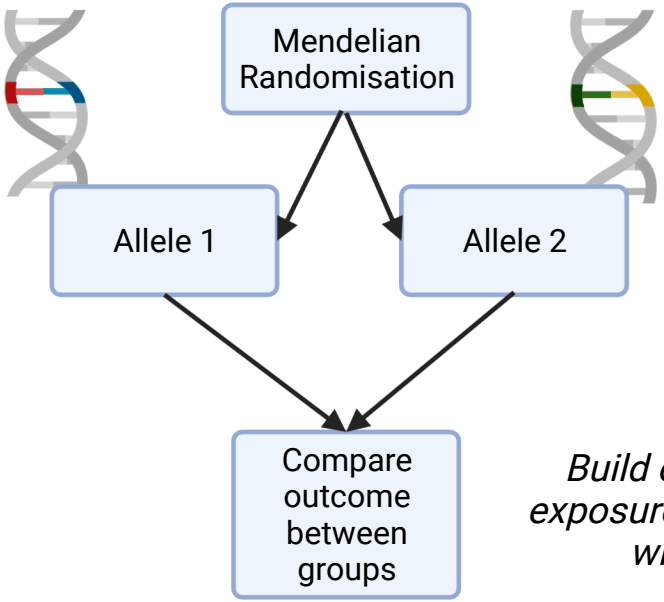




Step 1

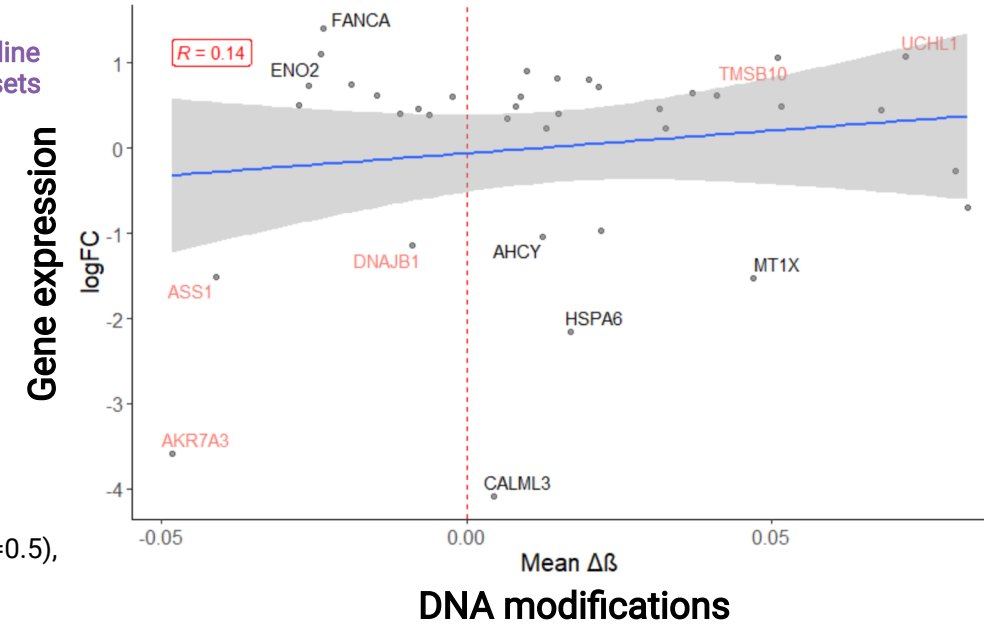
Genetic variants known to be associated with ***proposed risk exposure***

Step 2

Build evidence that this risk exposure is ***causally associated*** *with kidney disease*

Genomic analysis of kidney disease

```
ggplot(dataframe, aes(x=m, y=logFC)) +   #pull in your data
 geom_point(alpha = 0.4)+    #make points slightly transparent
 geom_vline(xintercept=0, linetype="dashed", color = "red")+  #add vertical line
 geom_text_repel(data=subset(dataframe, m < 0 & logFC < -1),   #label subsets
           aes(label=Gene, colour='red'))+
 geom_text_repel(data=subset(dataframe, m > 0 & logFC > 1),
           aes(label=Gene, colour='red'))+
 geom_text_repel(data=subset(dataframe, m > 0 & logFC < -1),
           aes(label=Gene))+
 geom_text_repel(data=subset(dataframe, m < 0 & logFC > 1),
           aes(label=Gene))+
 scale_y_continuous(breaks=seq(-4,2,1))+  #axis limits and increments
 theme(panel.grid.major = element_blank(),   #how graph looks
      panel.grid.minor = element_blank(),
      panel.background = element_blank(),
      axis.text.x = element_text(size=12),
      axis.text.y = element_text(size=12),
      legend.position = 'none',
      plot.title = element_text(color="black", size=12, face="bold.italic", hjust=0.5),
      axis.title.x = element_text(size = 15, colour = 'black'),
      axis.title.y = element_text(size = 15, colour = 'black'),
      axis.line = element_line(colour = "black") )+
 labs(x='Mean Δ β',y='logFC')+     #label axis
 geom_smooth(method='lm')+     #linear model
 stat_cor(aes(label = ..r.label..), color = "red", size=4, geom = "label")    #add correltion label
```



DNA modifications

Levin et al, 2020

Hill and Duffy et al., in preparation