# DEVELOPMENT OF A NEONATAL EPIGENETIC SCORE TO CAPTURE PARENT-CHILD TRANSMISSION LOAD FOR PSYCHIATRIC RISK

## ANALYSIS PLAN

### (WP4 – TASK 4.2)

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19-06-2024

Elena Isaevska

## Little bit about me…

- Started working at ERASMUS MC in December 2024 as a postdoctoral researcher with Charlotte and Alex
- FAMILY project – intergenerational transmission of mental health risk (WP4)

- MD (University of Skopje, Macedonia)
- PhD (University of Turin, Italy)

- Main research interests are on prenatal and early childhood exposures, their associations with long term mental health outcomes

- Interested in statistical methods applied in population based birth cohorts

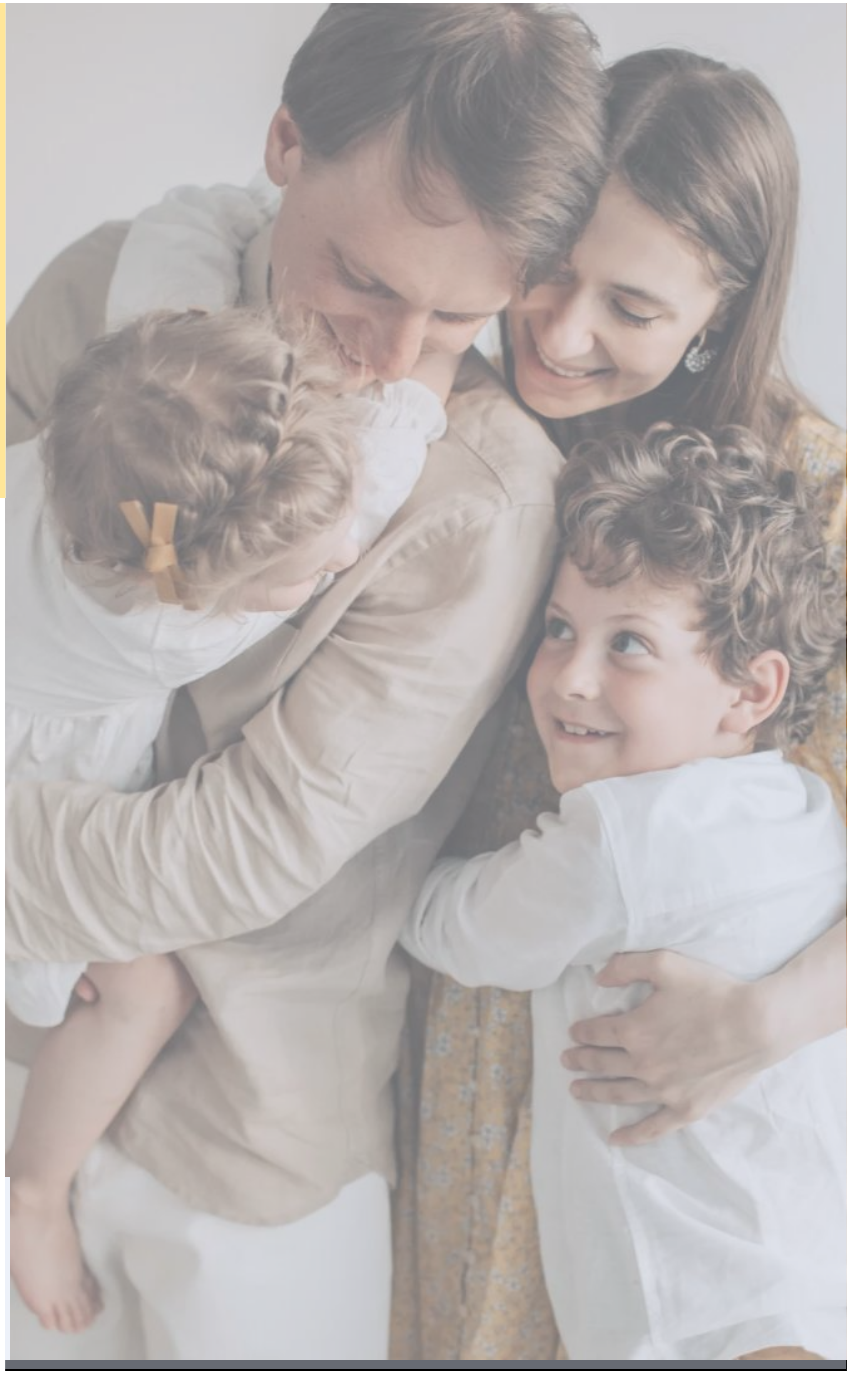
# Introduction

## Intergenerational Transmission of Mental Illness

Half of children born to parents with mental illness might develop one themselves

Risk varies significantly & affected children may not necessarily develop the same illness as their parents

Utilizing all available & relevant information is important for early risk prediction within families



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# Introduction

## Intergenerational transmission: a complex interplay of factors

| | | | |
|---|---|---|---|
| Genetics |  | Family history | • High heritability according to twin and family studies |
| |  | Genetic liability | • SNP-based heritability estimates notably lower |
| Prenatal factors |  | Maternal health and pregnancy complications | • Maternal BMI, smoking<br>• Maternal diabetes<br>• (Pre)eclampsia<br>• Maternal infection and/or fever<br>• Maternal auto-immune diseases |
| |  | Birth complications | • Preterm birth<br>• Cesarean section<br>• Low APGAR<br>• Low birth weight<br>• Breach/transversal presentation<br>• Incubator |

**EARLY RISK PREDICTION**

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# Introduction

## Previous efforts to integrate exposures into a “score” for mental illnesses


- Difficult to measure lot of determinants
- Quality of measurement? (ex. genetic predisposition)
- Biologically relevant/measurable exposure?

- Mostly focused on postnatal exposures
- Mostly focused on single diagnosis

Received: 14 September 2022 | Accepted: 10 February 2023
DOI: 10.1002/jcv2.12152

ORIGINAL ARTICLE

JCPP Advances

Joint polygenic and environmental risks for childhood attention-deficit/hyperactivity disorder (ADHD) and ADHD symptom dimensions

**Predictive Performance of Exposome Score for Schizophrenia in the General Population**

Lotta-Katrin Pries[1,8], Gamze Erzin[2,8], Jim van Os[1,3,4], Margreet ten Have[5], Ron de Graaf[5], Saskia van Dorsselaer[5], Maarten Bak[1,6], Bart P. F. Rutten[1], and Sinan Guloksuz[*,1,7]

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# Introduction

## Assessing the "transmission load" can offer valuable insights

?

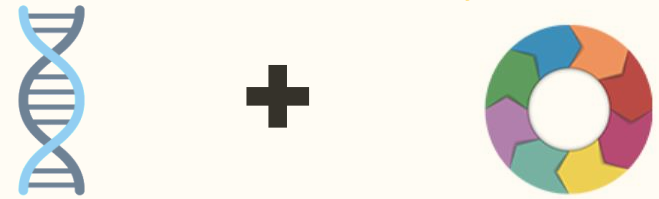
1. “Transmission load" – a collection of genetic and prenatal factors linked to the transfer of psychiatric risk from parents to offspring

2. Aid in early risk prediction and support the development of preventive strategies.

3. Measure surrogate markers of genetic and prenatal exposures in biological samples

4. Presence of exposures and their combined effect on the child + continuous and objective measurements at individual level

*Genetics* + *Early environment*

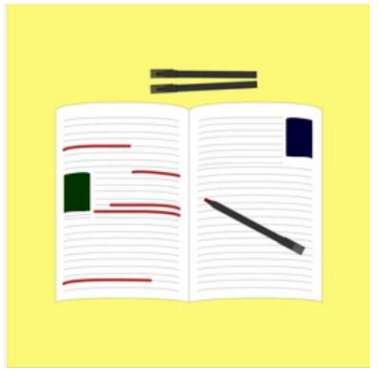


*...recent advancements in epigenetics, hold significant promise in this area*

# Introduction

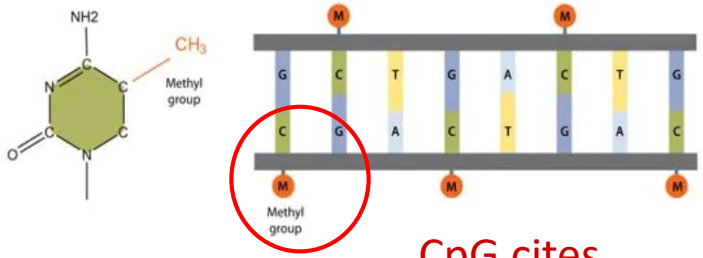
## EPIGENETICS

The study of heritable changes in gene function without altering the underlying DNA sequence.



**DNA methylation – the most well studied epigenetic mechanism**

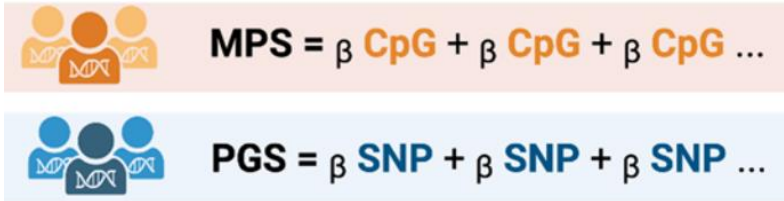
- Influenced by genetics and the environment
- Pregnancy is a sensitive window
- Time and tissue sensitive




CpG cites

**Methylation Profile Scores (MPS) – summarize specific patterns**

- Surrogate measure for exposures (biomarker of exposure)
- Stratify disease risk - predict future health outcomes




*Nabais et al. Genome Biology (2023)*

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# Main objectives

**1**

Create a transmission load methylation profile score (TL-MPS) by integrating genetic and prenatal factors implicated in parent-child transmission of psychiatric risk

**2**

Does the TL-MPS at birth predict offspring mental health problems in childhood, and does it add predictive utility over the individual genetic and environmental risk factors used to create it?

***Such approach could help us better understand the role of DNA methylation in the intergenerational transmission of mental health risk.***

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# Secondary objectives

1. Is the TL-MPS temporally stable?
2. Does the TL-MPS associate to other ASD/ADHD/SCZ phenotypes-related assessed at different time points?
3. Does sex influence the TL-MPS's predictive ability?

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# Our focus will be on:

## Genetic and prenatal risk factors for ADHD, ASD and SCZ

- high heritability
- common prenatal risk factors
- linked to disruptions in fetal neurodevelopment
- likely epigenetic involvement

## Psychopathological problems in childhood as the main outcome:

- Total Problems score from Child Behavior Checklist (CBCL) at age 6
- Total Difficulties Score from the Strengths and Difficulties Questionnaire (SDQ) at age 7

*The age of assessment (6/7 years) is coincides with the peak age of onset for ADHD and is close to the mean age when ASD is diagnosed.*

CBCL and SDQ
are validated questionnaires used in clinical practice and research to identify a wide range of behavioral and emotional problems in children.

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# Methods – study population

## Generation R – discovery

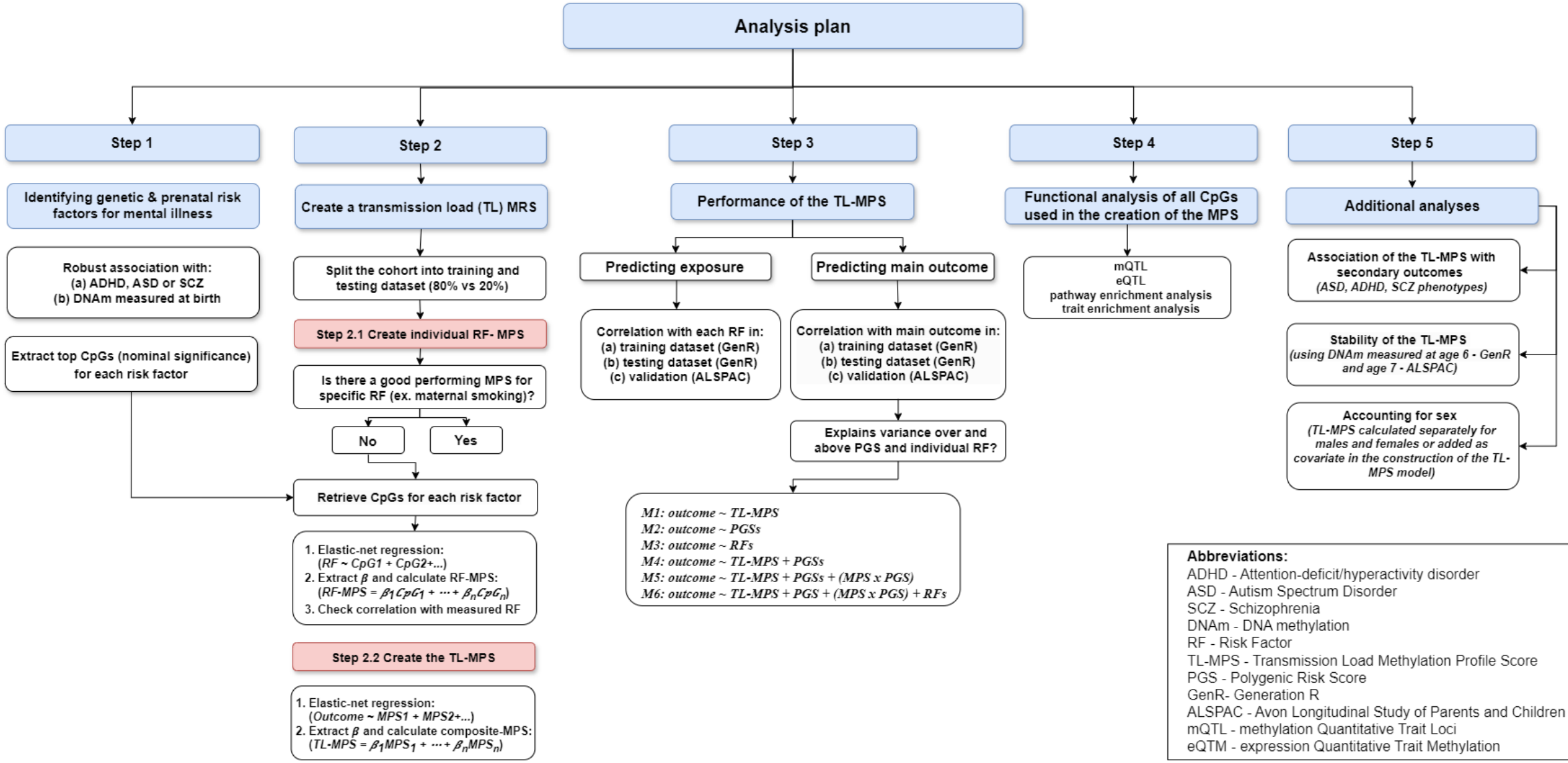
- **Cord blood DNA methylation**
  - 450K array (N=1317 after QC)
  - EPIC array (N=1097 after QC)
- **Exposures:** genetic (PGS) & prenatal exposure data
- **Outcome:** Child Behavior Checklist (CBCL)
  - Total problems score at 6 yrs
- Divided in training set / testing set (80% and 20%)

## ALSPAC – validation

- **Cord blood DNA methylation**
  - 450K array (N~ 900 after QC)
- **Exposures:** genetic (PGS) & prenatal exposure data
- **Outcome:** Strengths and Difficulties Questionnaire
  - Total difficulties score at 7 yrs

# Methods

## *Analysis plan - outline*




**Abbreviations:**
ADHD - Attention-deficit/hyperactivity disorder
ASD - Autism Spectrum Disorder
SCZ - Schizophrenia
DNAm - DNA methylation
RF - Risk Factor
TL-MPS - Transmission Load Methylation Profile Score
PGS - Polygenic Risk Score
GenR- Generation R
ALSPAC - Avon Longitudinal Study of Parents and Children
mQTL - methylation Quantitative Trait Loci
eQTM - expression Quantitative Trait Methylation

# Methods

## Step 1 Identify “transmission load“ variables

- **Genetic liability**
  - ADHD-PGS
  - ASD-PGS
  - SCZ-PGS
- **Maternal characteristics**
  - Pre-pregnancy BMI
  - Smoking in pregnancy
- **Pregnancy complications**
  - Gestational diabetes
  - Pre-eclampsia
- **Birth complications**
  - Preterm birth
  - Low birthweight
  - Cesarean delivery

## Step 2 Create transmission load MPS using elastic net

***Why elastic net?***

- Machine learning
- Combines penalties of both lasso and ridge
- Reduces overfitting
- Balances between feature selection and preservation
- Works well with inter-correlated variables
- Used in the creation of previous MPSs

- *glmnet* R-package
- Lambda and the alpha parameter chosen through a tenfold cross validation

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# Methods

## Step 2 Create transmission load MPS using elastic net (details)

Top CpGs (nominal p<0.05) associated with the risk factor in previous meta-analysis

*Elastic-net* RF1 ~ $CpG_1 + CpG_2 + \ldots + CpG_n$

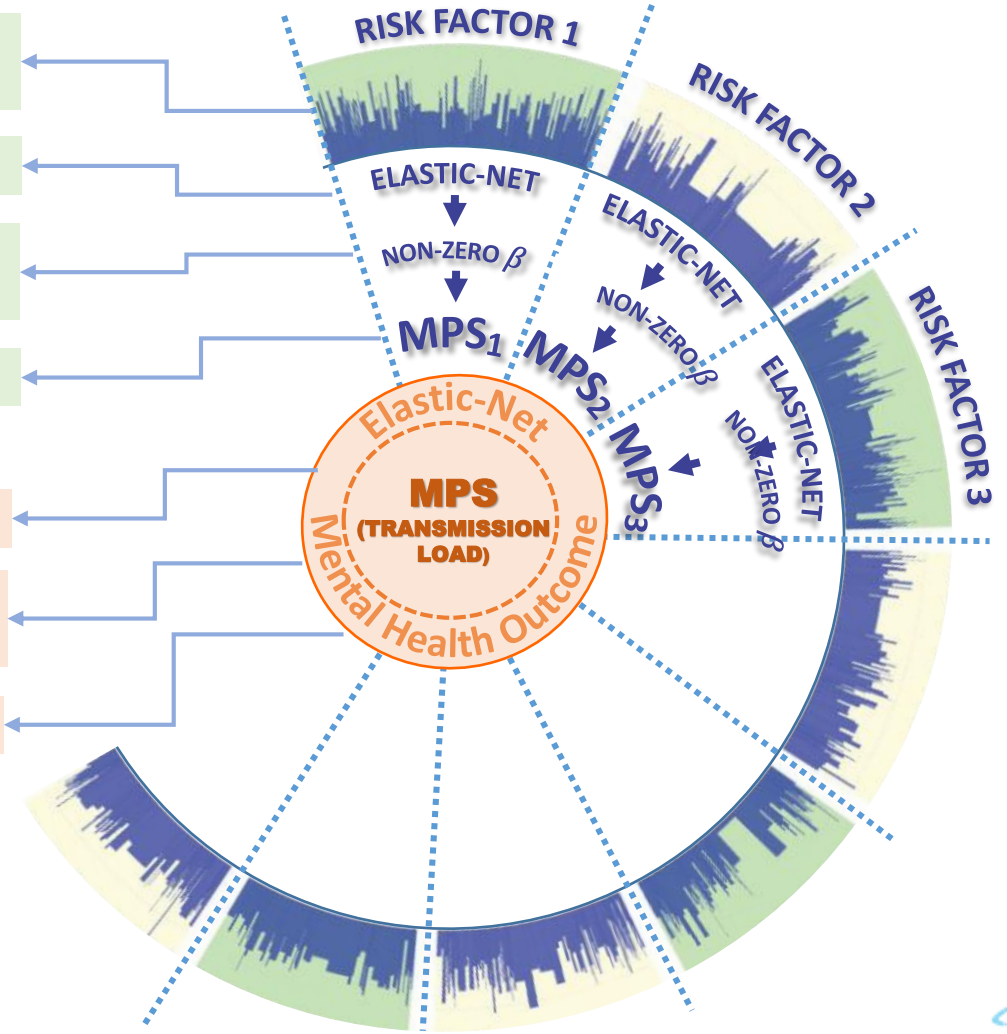
Extract betas and CpGs with non-zero coefficients (relevant for prediction)

*Individual RF- MPS* $MPS_1 = \beta_1 CpG_1 + \beta_2 CpG_2 + \cdots + \beta_n CpG_n$

*Elastic-net* Outcome ~ $MPS1_1 + MPS_2 + \ldots + MPS_n$

Extract betas and MPSs with non-zero coefficients (relevant for prediction)

*Transmission load MPS* $MPS = {}_1MPS_1 + \beta_2 MPS_2 + \cdots + \beta_n MPS_n$




*Covariates: batch, sex, cell types*

# Methods

## Step 3 Performance of the MPS

- **Correlations** with each PGS & prenatal **risk factors**.
- **Predictive ability** for **mental health outcome** over and above PGSs & prenatal risk factors:

*Model 1: outcome ~ MPS*
*Model 2: outcome ~ PGSs*
*Model 3: outcome ~ risk factors*
*Model 4: outcome ~ MPS + PGSs*
*Model 5: outcome ~ MPS + PGSs + (MPS X PGS)*
*Model 6: outcome ~ MPS + PGS + (MPS X PGS) + risk factors*

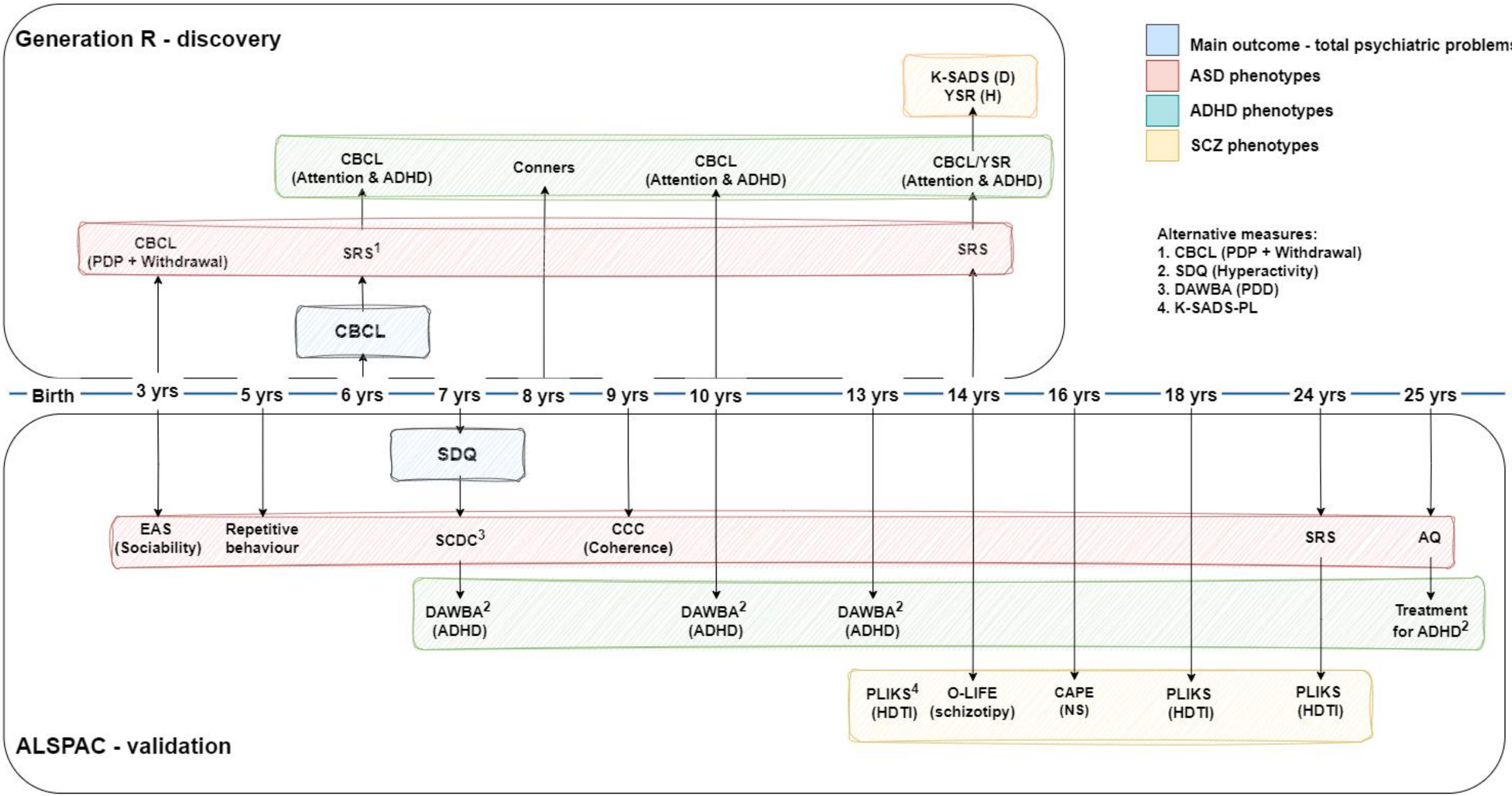
## Step 4 Exploratory functional analysis (causality???)

- **Genetic influences** *(mQTL, GoDMC database)*
- **Gene expression** *(eQTM, HELIX web catalogue)*
- **Pathway enrichment** *(GOmeth, missMethyl R-package)*
- **Trait enrichment** *(EWAS toolkit)*

# Methods

## Step 5 Additional analyses for secondary objectives

- **Association with secondary outcomes**
  - ADHD, ASD and SCZ phenotype-related measures assessed at different ages using different instruments
- **Stability of the MPS**
  - Calculating the MPSs using DNAm measured in childhood (age 6) and test its association with genetic and prenatal risk factors & concurrent/future phenotypes.
- **Accounting for sex**
  - Calculate MPSs separately for males and females, compare predictive ability to the main model


Generation R - discovery
Main outcome - total psychiatric problems
ASD phenotypes
ADHD phenotypes
SCZ phenotypes
K-SADS (D)
YSR (H)
CBCL
(Attention & ADHD)
Conners
CBCL
(Attention & ADHD)
CBCL/YSR
(Attention & ADHD)
CBCL
(PDP + Withdrawal)
SRS1
SRS
CBCL
Alternative measures:
1. CBCL (PDP + Withdrawal)
2. SDQ (Hyperactivity)
3. DAWBA (PDD)
4. K-SADS-PL
Birth
3 yrs
5 yrs
6 yrs
7 yrs
8 yrs
9 yrs
10 yrs
13 yrs
14 yrs
16 yrs
18 yrs
24 yrs
25 yrs
SDQ
EAS
(Sociability)
Repetitive
behaviour
SCDC3
CCC
(Coherence)
SRS
AQ
DAWBA2
(ADHD)
DAWBA2
(ADHD)
DAWBA2
(ADHD)
Treatment
for ADHD2
PLIKS4
(HDTI)
O-LIFE
(schizotipy)
CAPE
(NS)
PLIKS
(HDTI)
PLIKS
(HDTI)
ALSPAC - validation

# Current status

- Prepared OSF for pre-registration
- Data access was obtained from Generation R on May 16th, 2024.
- ALSPAC has accepted the project proposal. Data request is under review at the time of pre-registration.
- Plan to start analyses by the end of June…

# Questions?

# Any feedback is appreciated ☺