Title Slide1

Welcome to my Midway Evaluation.

Slide 2 – skip

Slide 3: The tentative title of my project is An Epigenetic Study of Treatment Effects and Environmental Factors in SCZ and BPD.

I hope to convey to you my interest for this project which has held my attention these last two years -and for which I am still greatly enthused. I am working under the supervision of Stéphanie Le Hellard and Anne-Kristin Stavrum here in Bergen. I am also co-supervised by Tetyana Zayats at the Broad Institute and Ingrid Melle at NORMENT/UiO.

Slide 4: Financing for PhD project

Slide 5: Plan for this talk

* Objectives
* Methods
* Status of project

Slide 6: Formal training components completed

Slide 7: Scientific communication requirements to be completed

FORMIDL901 to be completed

Slide 8: Plan for project completion

Slide 5: Objects of this PhD work

The focus of my project is to identify differentially methylated positions and regions associated with environmental exposure in individuals with SCZ, BPD, and MDD. These disorders are characterized by abnormalities of thought, behavior, cognition, and mood. They are thought to have similar etiologies and affect over ~2 % of the world´s population (Steardo, 2019, Yu, 2019). Medication is the primary treatment.

1. Evaluate whether the association of SCZ versus controls on cell-type proportions is impacted by time-of-blood draw.
2. EWAS: to identify common effects of AP polypharmacy and the specific effects of monotherapy with four AP drugs: Aripiprazole, Olanzapine, Quetiapine, and Risperidone.
3. Extract medication treatment effects from disorder-specific effects.
4. Evaluate whether any specific effects are associated with developmental timing.

Slide 6: Epigenetics image from Smigielski Show complex picture of epigenetic landscape

This is a complex picture of the epigenetic landscape that portrays cellular activities from histone modifications, microRNAs and DNA methylation. There is crosstalk between the different mechanisms, and DNAm, posttranslational histone modifications and non-coding RNA all have an important role in the etiology of major psychosis (Gerscher et al., 2018). DNA methylation however is the most widely studied epigenetic mechanism and is the subject of this talk. And specifically, I will be talking about DNA methylation at cytosines that sit adjacent to guanines. (ref from Gurel)

Slide 7. Epigenetics in psychiatric disorders

* Development: prenatal environmental factors encountered *in utero* (maternal diet, stress, smoking, etc. All of these prenatal exposures can act through the epigenome to alter gene expression related to neuronal function – and thereby brain development (Starnawska )
* Dynamic: influenced by many environmental exposures – from adverse childhood events, trauma stress, diet, smoking alcohol. These exposures are strongly associated with psychiatric disorder risk
* MZ twins discordant for SCZ/BPD show DNAm associated differences (Dempster et al., 2011) greater variance than unaffected twin (Bryn, 2013)
* Methylation patterns associated with brain volume, structure and function; social & cognitive function
* Overtime changes as a function of age and chronicity of disorder

Slide 8: The Data (N = 2365

TOP cohort (NORMENT): recruitment and blood samples

- DNA sent in three batches to Bonn for typing

- Illumina EPIC 850K microarray

- First PCA showed big batch effects

- QC: each batch separately and then merged the three

- PCA: technical replicates in each batch clustered close to each other

Removal of probes with detection p-value > 0.01, FunnNorm, Combat

Functional normalization for clustering of samples

SVD visualization of variables that may be responsible for the variation in a sample

Slide 9: Project 1

We decided to look closer at this issue of time, in relation to our samples. I am introducing a painting here as a bridge to the clinic. The painter Eugene Gabritschevsky was a Russian biologist and geneticist who spent half of his adult life in an institution being treated for psychosis. In this painting he portrays light at the horizon – it is not certain if he saw this light when he arose in the morning, or if he had been up all night. What we do know is that light is a strong external cue that entrains circadian rhythms in the central pacemaker in the brain which then directs rhythms in cellular activity throughout the body, affecting physiology and behavior (Hastings, et al, 2018). Virtually all cells display circadian rhythms, including white blood cells and DNA methylation contributes to the precision of the molecular clock – as I mentioned with the example of the BMAL clock gene.

Slide 10: Circadian rhythms and “chrono-epigenetics”

* Methyl cycle co-evolved with circadian clocks and affects biological rhythms from bacteria to humans (Fustin)
* Circadian rhythms are disrupted in serious mental disorders (Walker II) DISC1 gene regulates a core clock gene (BMAL)
* Chrono-epigenetics:
  + temporal dynamics of epigenetic processes (Oh E & Petronis)
* oscillating cytosines contribute to epigenetic variability but not to mean methylation levels.

For instance, the mesor, the average value around which oscillations occur, can be the same between the tested groups, but any minute changes to oscillatory parameters, such as acrophase, amplitude and duration of the period, can introduce additional variability.

Hypothetical sampling times for the epigenetic oscillations in part a, as if they were collected from real- life cross- sectional investigations (red dots). The assumption was that sampling occurred between regular work hours (9 a.m.–5 p.m.; grey background) and that both cohorts visited at the same time.

Slide 11: Epigenetic oscillations

figure b

- blood collection time: 09-16

- example time: 12:00

- black lines: oscillation profiles

- red dots: collection time

- figure c

- cpg density at 12:00

Slide 12: Method of analysis

Pre-step: Estimated cell-type proportions from DNAm data (Salas, \_et al.\_, 2018)

1. Selected European SCZ cases & controls with recorded time-of-blood draw (n=729)

2. Calculated variable “hours from 07:00 baseline”

3. Samples matched for age and gender

4. Compared full day vs. half day

5. Sub-analyses: Neutrophil-to-lymphocyte ratio (NLR) and medication-free SCZ cases

Slide 13: Distribution of time and models

Slide 14: Results

Full day –

* P-values reduced significant in Model 2
* Effect sizes diminished in Model 2

Half day

* P-values no significant change
* Effect sizes: no change

Slide 15: Results: Sub-analysis

NLR:

- significant association in all analyses (\_p\_ <.001);

- modest increase in mean NLR in half-day medication-free samples; i.e.

- unadjusted mean NLR (.93) vs. adjusted mean NLR (1.14)

- Medication-free cases: significant for neutrophils and CD4T adjusted/unadjusted (\_p\_ <.01)

In DNAm studies, correcting for cell-type proportions will reduce epigenetic variation associated with time-of-blood draw and circadian cycling of white blood cells.

Slide 16: Project 2 - Common and Specific effects of antipsychotics on DNA methylation in Psychosis

Antipsychotics are central to the treatment of psychosis and reduce distressful symptoms in most patients. But for many, the side-effects can challenge compliance. Physical side-effects include weight gain, but also sedation, and patients report feeling lethargic, slowed thinking, and reduced motivation. I think this painting by Gabritschevsky portrays the emotional blunting that he may have felt. Gaining a better understanding of these medications is important to improving treatment and providing an effective treatment option to the approximately 30% who do not respond well to these drugs.

Slide 17: AP and corresponding receptors

The key role for clinical response is dopamine D2 receptor blockade in the striatum – but this blockade is also associated with endocrine and potentially serious motor side-effects. Second generation Aps are formulated with a balance between D2&D3 blockade that is still effective for psychosis but has reduced risk of motor side-effects. These 2nd generation drugs also have affinity for serotonergic, histaminergic, cholinergic, and adrenergic receptors. The degree of affinity and receptor occupancy plays a role in therapeutic effects, for example, levels of histamine H1 receptor occupancy with Olanzapine and Quetiapine is helpful in acute phases of psychosis as sedation can be beneficial– but over time can lead to lethargy which challenges medication compliance.

Patients can be prescribed multiple antipsychotic drugs. Polypharmacy leads to greater dopamine D2 occupancy and blockade that may possibly be explained by:

1. increased efficacy,
2. or a reduction in side effects which increases tolerability,
3. or finally the additional drugs may induce beneficial effects via actions at other receptors.

But more work is needed to identify which of these three contribute to the mechanism of actions. We will look at common effects in this study.

For the specific effects of antipsychotics, we will be looking at four 2nd generation AP that are prescribed at monotherapies: Aripiprazole, Olanzapine, Quetiapine and Risperidone.

D2/D3 receptor blockade is necessary for the therapeutic action of these drugs, although it does not guarantee response.

Slide 18: Pharmacoepigenetic findings in psychiatry

- Early stages still

- Study design, technology, patient population, lack of replication

- No uniform identification of hyper- or hypo-methylation (i.e. \_COMT\_, \_5-HTT\_, \_HTR2A\_) (Burghardt, \_et al.\_, 2020, Zhou \_et al\_, 2021)

- Lacking studies of AP monotherapy (Burghardt, \_et al.\_, 2020)

- DNAm associated with both the pathology and treatment (Jaffe, \_et al.\_, 2016)

- Tissue issue - relevance of peripheral blood (Bakulski, \_et al.\_, 2016)

Slide 19: Correlation blood-brain DNAm

Candidate genes studies: Theory-driven tests of association between phenotypes and variants of genes which have been identified as related or relevant to these phenotypes, typically based on their known biological properties.

NR3C1 – codes for glucocorticoid receptor

BDNF: promotes neuronal survival in adult brain, plays a role in the regulation of the stress response and in the biology of mood disorders.

SLC6A4 – codes for serotonin transporter

GWAS studies scan large numbers of commonly occurring genetic variants (SNPs) across the entire genome of many individuals and test for associations between phenotypes and a large number of genetic variants. – Represents a blind (hypothesis-free) search for evidence of association.

KCNJ13 potassium channel allows for greater influx than outward flux of potassium (SCZ)

CACNA1C – brain tissue and long QT in cardiac tissue

(MDD) RNA binding gene whose pathway is related to methyl-CpG binding domain (MBD)

Slide 20: Methods

1. Select European SCZ, BPD and MDD cases being treated with antipsychotics

2. Select samples with blood drawn within 4 months of interview date

3. Common: Compare AP use vs non AP use

4. Specific: Contrast mono-therapy against non-target polytherapy.

5 Model: DNAm ~ Psychosis\_Cases + Age + Sex + Smoking + eCells + Technical +𝜺

6. Identify CpGs (limma), associated genes (Illumina Annotation lib), and DMRs (DMRcate)

7. GSEA and pathway analysis (i.e.GREAT)

Slide 21: Project 3 - Identify differential DNAm in SCZ versus BPD

Painting of “twins”

Let´s say that SCZ and BPD appear here as twins. There are many genetic overlaps. But they are also heterogenous disorders. On a clinical level however, many of the symptoms are different.

Slide 22: Characteristics

Similarities

- Early age of onset ~ 20-30 years old

- Heritability ~ 80%

- Periods of stability and relapse

- Dysregulated circadian rhythmicity: both a trait and state marker (Walker, \_et al.\_, 2020)

- Medication primary form of treatment

Slide 23: Differences

Differences

- Polygenic risk score (PRS) differed significantly (Ruderfer, \_et al., 2014)

- Two genome-wide significant SNPs (BPD&SCZ Working Group of the PGC., 2018)

- Clinical differences behavioral, cognition, intelligence (Reichenberg,\_et al.\_, 2009)

Slide 24: Clinical Course of SCZ and BPD

We receive patients in the research project when they are stable. So what are we measuring? We have removed CpGs with AP effects.

Slide 25: Methods

1. Select SCZ and BPD cases from Project 2

2. Extract CpGs associated with antipsychotics from Project 2

3. Model: DNAm ~ SCZ\_BDP + Age + Sex + Smoking + Cells + Technical +𝜺

4. GSEA and pathway analyses (GREAT)

5. Compare annotated genes to GWAS findings

Slide 26: References

Slide 27: References

Slide 28: References

Slide 29: Acknowledgements