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: Plan for this talk

* Objectives
* Methods
* Status of project

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)

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. Leverage results from previous aim and extract medication treatment effects from disorder-specific differences in methylation.
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. Role of DNAm 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
* Methylation patterns associated with brain volume, structure and function; social & cognitive function
* MZ twins discordant for SCZ/BPD show DNAm associated differences (Dempster et al., 2011)
* Overtime changes as a function of age and chronicity of disorder

Slide 8: Circadian rhythms and “chrono-epigenetics”

* Circadian rhythms are disrupted in serious mental disorders
* Methyl cycle and circadian clocks co-evolved and affects biological rhythms from bacteria to humans
* DISC1 is involved in neuronal signaling and neurodevelopment. It has been shown to oscillate with circadian rhythms in the brain´s main pacemaker (SCN) – and is associated with sleep behaviors. DISC1 contributes to the core circadian system by regulating BMAL1 stability - DISC1 in SCZ has a protective role for BMAL1

Slide 9: Time-naïve DNAm measurement

Slide 10: Time-sensitive DNAm measurement

Mention something about cases and controls being out of phase.

Slide 12: 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 13: Methods

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

2. Estimated cell-type proportions from DNAm data \*Salas \_et al.,\_ 2018\*

3. Compared linear regression models when adjusting for blood draw time.

4. Compared full day vs half day

5. Sub-analyses: Neutrophil-to-lymphocyte ratio / Medication-free SCZ

Slide 14: Distribution of time and models

Slide 15: Results

Check results for NLR – Medication did not have an affect

Conclusion: Overlooking time-of-blood draw may lead to false association in omics studies of Schizophrenia

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, have cognitive slowing, 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: How we think AP work

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

- 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 (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.

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

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 22: 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 23: DNAm findings from the literature

Pai, et al, 2019, reported that significant hypomethylation of an enhancer in the insulin-like growth factor 2 (IGF2) gene was observed in major psychosis neurons. This enhancer targets the tyrosine hydroxylase (TH) gene which is responsible for dopamine synthesis (Ibid). Read what Sudderman found about adolescents – is this an indicator of a gene that points to development?

BP patients using mood stabilizers have a higher 5mC level compared to patients using atypical antipsychotics (Burghardt et al., 2019).

Make a demographic table like Zong’s Table 1 but for SCZ, BPD and MDD

Slide 24: Methods Project 3

Select SCZ and BPD cases from Project 2

1. Extract CpGs associated with antipsychotics from Project 2

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

3. ENCODE reference

4. GSEA analysis

5. Pathway analysis (GREAT)

Slide 25: References

Slide 26: Acknowledgements

### notes

Project 1 - Time of Blood Sampling

It is standard practice in EWA- studies to adjust for the heterogeneity of cell-type proportions. Cell-type proportions change during the course of the day and are influenced by many factors, including diet, exercise, smoking, ill-health and medication use. In this study, we looked at the association of schizophrenia versus healthy controls on cell-type proportions. It is well documented that patients with schizophrenia have higher neutrophil-to lymphocyte ratios than controls, suggestive of a low-grade inflammatory process. But recent studies report on the influence of time-of-day on DNA methylation levels. Here the natural process of methylation/demethylation in white blood cells is oscillating with circadian rhythms. Overlaps of these oscillating methylated cytosines with methylated sites associated with SCZ. The Function of the Circadian Clock Is Tissue Speciﬁc.Yeung and Feliz, 2018

Project 2

To date there is evidence that antipsychotics ameliorate the symptoms of psychosis by … affecting neurotransmitter systems, like gaba and glutamate, as serotonin. Evidence from DNA methylation studies ….

Therefore we wish to see identify the common effects of antipsychotics in part one of the study, followed by the specific effects in part 2.

We can validate the risperidone.

Thefirst antipsychotic medication was introduced in the 1950s. Al-though this was thefirst medical treatment to reduce psychotic symp-toms, it was not until 20 years later that the underlying mechanism ofaction—blockade of the dopamine D2 receptor—was identified. Themost consistent and influential theory to date to explain the develop-ment of psychosis is that the disease is related to dopaminergic alter-ations. (Devi Treen)

Effects of AP on neutrotransmitter levels (Bojesen, 2020)

Slide 13

Project 3

Peripheral blood DNAm of SZ and BPD compared to controls has shown that the promoter methylation of the serotonin receptor type-1 (HTR1A) gene is significantly increased (Carrard et al, 2011). Recently, Sugawara et al. suggested that gene hypermethylation of FAM63B and intergenic region on chromosome 16 may be a common epigenetic risk factor in the pathogenesis of these diseases (Sugawara et al., 2018).

Point out the difference between SCZ and BPD findings – are any associated with development.

Check Hannon papers – and Mill

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