Title Slide1

Slide 2 – skip

Slide 3

Welcome to my Midway Evaluation. I hope to convey to you my interest in my PhD 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 5: 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 6. Characteristics of DNAm/EWAS advances

### Characteristics of DNAm

* DNAm is highly tissue and cell-type specific (ref) Jaffee? And plays an essential role in
* tissue differentiation (Reik)
* Key role in determining transcription factor binding and enhancer function during development (Ziller)
* DNAm is highly malleable, and is influenced by many environmental exposures, including diet, levels of *in utero*  nutrients and smoking (15-19)
* Over time the DNAm landscape changes as a function of age (20), cancer (20) and complex diseases (3)

Slide 7

### Advances in Epigenomic Wide Association Studies (EWAS)

Epigenome-wide association studies (EWAS) seek to identify epigenetic alterations, which are associated with disease predisposition or disease itself.

1st generation: Case-Control

2nd generation: Cellular heterogeneity (Jaffe & Irizarry, 2014, Lappalainen & Greally, 2017)

3rd generation: Temporal dimension: chrono-epigenetics (Oh, E. & Petronis, 2021)

Slide 8: Circadian rhythms and chrono-epigenetics

* Methyl cycle is the universal pathway that provides methyl groups to nucleic acids and thereby regulates all aspects of cellular activity.
* Methyl cycle and circadian clocks co-evolved and affects biological rhythms from bacteria to humans
* DISC1 was associated with SCZ over 20 years ago and is involved in neuronal signaling and neurodevelopment. It has been shown to oscillate with circadian rhythms in the brain´s main pacemaker (SCN) – with expression late nighttime and early daytime. DISC1 knockout in mice decreases the expression of circadian genes.

It is associated with sleep behaviors and was found to exhibit daily oscillating pattern – and is regulated by binding to CLOCK and BMAL1 to E-boxes in its promoter. oscillation of *DISC1*expression is under the control of CLOCK and BMAL1, and that DISC1 contributes to the core circadian system by regulating BMAL1 stability. Low expression of DISC-1 showed reduced expression of other circadian genes.

Slide 9: Time-naïve DNAm

Slide 10: Time-sensitive DNAm

Mention something about cases and controls being out of phase.

Slide 11: Project 1

So 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 entrains these rhythms in cells in major organs in the body to activate physiological processes. White blood cells also show circadian rhythms which is has been shown to influence DNA methylation studies.

* SCZ is associated with alterations in cell-type proportions – which may reflect many factors, including diet, exercise, smoking, ill-heath and medication.
* It is well documented that patients with SCZ have higher NLR than controls, suggestive of a low-grade inflammatory process.
* Psychiatric disorders are associated with circadian rhythm disruptions – as evidenced by sleep disorders and preference for evening, or feeling better in the evening.

Slide 11: 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 12: Distribution of time and models

Slide 13: 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 14: 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 15: 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 16: What do studies of DNAm tell us?

Slide 17: Correlation blood-brain DNAm

Candidate genes:

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 genes

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 18: Methods

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

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

1. Common: Compare AP use vs non AP use

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

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

1. Identify CpGs, associated genes, and DMRs

1. GSEA and pathyway analysis (i.e.GREAT)

Slide 19: Project 3 - Identify differential DNAm in SCZ versus BPD

There is considerable interest in whether the neurobiological abnormalities underlying the psychotic symptoms of [schizophrenia](https://www.sciencedirect.com/topics/neuroscience/dementia-praecox" \o "Learn more about schizophrenia from ScienceDirect's AI-generated Topic Pages) are unique to the disorder or if there is a common mechanism that underlies psychosis across disorders such as bipolar and schizophrenia. There is some evidence from neuroimaging studies that dopamine abnormalities are present in [bipolar disorder](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/bipolar-disorder" \o "Learn more about bipolar disorder from ScienceDirect's AI-generated Topic Pages), in particular patients with mania show increases in dopamine D2/3 receptor availability and appear to have hyper-responsive reward systems in the [ventral striatum](https://www.sciencedirect.com/topics/neuroscience/ventral-striatum" \o "Learn more about ventral striatum from ScienceDirect's AI-generated Topic Pages) ([Ashok et al., 2017](https://www.sciencedirect.com/science/article/pii/S002839081930262X?via%3Dihub" \l "bib17)). Moreover, dopamine synthesis capacity appears to be elevated in patients with bipolar psychosis to a similar degree to that seen in schizophrenia ([Jauhar et al., 2017a](https://www.sciencedirect.com/science/article/pii/S002839081930262X?via%3Dihub" \l "bib144)). More studies that compare aspects of dopamine function across psychotic disorders would help determine if there are differences in the nature of the [dopaminergic](https://www.sciencedirect.com/topics/pharmacology-toxicology-and-pharmaceutical-science/dopamine-receptor-stimulating-agent" \o "Learn more about dopaminergic from ScienceDirect's AI-generated Topic Pages) dysfunction between disorders. These indicate that both first- and second-generation antipsychotics are effective in treating acute mania in bipolar disorder, with effect sizes similar to those seen in schizophrenia ([Cipriani et al., 2011](https://www.sciencedirect.com/science/article/pii/S002839081930262X?via%3Dihub" \l "bib56)). Furthermore, antipsychotics are being explored as treatments for maintenance therapy in bipolar disorder ([Jauhar and Young, 2019](https://www.sciencedirect.com/science/article/pii/S002839081930262X?via%3Dihub" \l "bib143), [Prajapati et al., 2018](https://www.sciencedirect.com/science/article/pii/S002839081930262X?via%3Dihub" \l "bib275)). However, it remains to be determined if the therapeutic effects of antipsychotic drugs in bipolar disorder or depression are mediated by D2 occupancy, although the relationship between D2 occupancy and extra-pyramidal side-effects in bipolar seems to be the same as that seen in schizophrenia ([Attarbaschi et al., 2007](https://www.sciencedirect.com/science/article/pii/S002839081930262X?via%3Dihub" \l "bib18)).

(Kaar)

Up to 80% of patients with SCZ have some form of cognitive impairment (Reichenberg,\_et al.\_, 2009), which may predate the onset of schizophrenia (Bora and Murray, 2014)

Take info from report about BP

Slide 20: 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. I anticipate that we will have to correct for several medications in the model – this would be lithium, anticonvulsants and antidepressants. We correct for age, sex, smoking, cell types and perhaps one technical batch. Does the model get too big??

Lithium (Li) is the cornerstone maintenance treatment for bipolar disorders (BD), but response rates are highly variable.

Slide 21: DNAm findings from the literature

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

Effects of anticonvulsants on DNA methylation in Bipolar Disorder

Recently, another study on the blood and brain tissues of SZ patients emphasized that DDR1 hypermethylation in leukocytes and brain tissue could be associated with psychosis, physiological stress and inﬂammation (Garcia-Ruiz et al., 2020).

The most important questions likely to be addressed by new studies in this context are whether epigenetic changes can be used to diagnose subtypes of major psychosis

Promisingly, studies on abnormal gene expression in the postmortem brain and peripheral blood sample have revealed that epigenetic mechanisms may be viable tools for early diagnosis for some cases of SZ ve BD (Akbarian and Huang, 2009; Fachim et al., 2019; Liu et al., 2017). However, there is limited research data to examine the eﬀects of DNMT and HDAC inhibitors in reducing cognitive deﬁcits and the role of antipsychotic drugs on epigenetic changes in the brain (Abel and Zukin, 2008; Bowden, 2007; Deutsch et al., 2008; Dong et al., 2008).

Another gene shown to contribute to the etiopathology of the major psychosis of the methylation change pattern is the membrane encoding catechol-O methyl transferase (MB−COMT) that is responsible for the dissimilation of neurotransmitters such as dopamine and noradrenaline. The evidence that the MB−COMT gene promoter methylation is more common in the postmortem brains of patients with SZ and BD compared to controls shows that this may increase the risk of major psychosis (Abdolmaleky et al., 2006

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?

The initial studies investigating the relationship between DNA methylation alterations and major psychosis were focused on disease-related genes such as DRD2, MB−COMT, RELN, GAD1, and SOX10 (Grayson and Guidotti, 2013; Zhao et al., 2015). Among these genes, RELN and GAD1 have been most associated with abnormal methylation in major psychosis.

Among these genes, RELN and GAD1 have been most associated with abnormal methylation in major psychosis. The RELN gene expresses an extracellular matrix glycoprotein reelin, an important function in directing neurons and radial glial cells to their correct position in the developing brain (Lee and D’Arcangelo, 2016). GAD1 expresses the glutamic acid decarboxylase 67 (GAD67) which catalyzes the conversion of L-glutamate to the inhibitor neurotransmitter γ-amino butyric acid (GABA) (Magri et al., 2018).

But we are interested in the AP

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 22: 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 23: References

Slide 24: 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

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

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