Schizophrenia and Your Metabolism

Glia Club - Dennis T., Ella O., Erika P, Subathra R.
Overview

Introduction - Leptin, Vitamin D, Schizophrenia

Methods

Results

Wrap it up
What is schizophrenia?

- discovered in 1887 by Emile Kraepelin
- 0.7% lifetime prevalence
- **Broad** clinical syndrome - including subjective experiences + loss of function
- Patients may be described as having “lost touch with reality”
- **Nature + nurture**
  - Genetic predisposition assessed by PRS
- **Known environmental factors**
  - High latitude
  - Urban residence in early life
  - Winter/spring season birth

!!!Prenatal + Early Life Matter!!!
Negative Symptoms

Definition: present in healthy individuals, but not in schizophrenia patients

1. Flat affect
2. Decreased everyday pleasure
3. Difficulty beginning + sustaining activities
4. Decreased speaking
Positive Symptoms

Definition: present in schizophrenia patients, but not in healthy individuals

1. Hallucinations
2. Delusions
3. Thought disorders
4. Movement disorders
Cognitive Symptoms

Definition: cognitive processes affected in schizophrenic patients

1. Poor executive functioning
2. Trouble focusing + paying attention
3. Decreased working memory capabilities
Vitamin D (Calcitriol) - D2 + D3 Forms

Sources

- Steroid hormone
- Measurement Method: 25OHD
- Critical for bone mineralization
Vitamin D Metabolization

- hydroPHOBIC
- transported into blood bound to carrier proteins (primarily Vitamin D-binding protein)
Vitamin D -> Ca++ Regulation

- Multiple Sclerosis
- Alzheimer’s
  - Protein aggregates
  - Amyloid clearance
- Parkinson’s
  - Protein aggregates
  - Amyloid clearance
- Dementia
- Neurocognitive Disorders
- Amyotrophic Lateral Sclerosis
Leptin

- Released by adipocytes
- Food intake + metabolic process regulation
- Amyloid B -> inhibition of leptin production (mTORC1 pathway) + inhibition of IGF-1 production (JAK/STAT pathway)
  - Leptin -> JAK/STAT pathway
  - IGF-1 -> decreased amyloid B production + tau phosphorylation - Parkinson’s
- Leptin -> proliferation of neuronal progenitors - depression + Alzheimer’s
  - Progenitors -> neuronal division
  - Neurogenesis -> inhibition of schizophrenia
- VDR Vitamin D Receptors in brain - especially dopaminergic neuron rich regions
- protein aggregation
- decreased amyloid clearance
- neonatal levels

- Increased Vitamin D -> Increased leptin levels
- Decreased Vitamin D -> Decreased leptin levels -> obesity

- Across BBB -> hypothalamus, hippocampus, frontal cortex (astrocytes)
- Leptin especially low in violent schizophrenic patients
- Decreased sirtuin-1 -> increased Alzheimer’s + depression susceptibility
Methods
Type of Study

- What is a case-controlled study?
  - An observational study where two existing groups differing in outcome are identified and compared on the basis of some supposed casual attribute.
    - This means that they are done to identify possible predictors of outcome.
Subjects

- Subjects come from the Danish National Registry
  - Used newborn dried blood spots as they were randomly selected (to avoid bias responses/errors)
  - These dried blood spots came from people who were diagnosed with schizophrenia according to the standards of the International Statistical Classification of Diseases
  - Developed between September 2005 and December 2008

- Controls were set by matching an individual with schizophrenia to one that was alive without schizophrenia based on sex and date of birth to compare
3.2 mm DBS (samples and calibrants) were hydrated and the Vitamin D species extracted with acetonitrile and its metabolites 25 (OH) D2 and D3 which are serums associated with vitamin D.

- Named 25- Hydroxy Vitamin D as it is an indicator of vitamin D in the blood as it is the active form of Vitamin D (used as standard controls).
- Acetonitrile is an organic nitrite that is used to extract fatty acids and vegetable oils. Also, it can be used to detect materials within chemical labs such as pesticide residues since Vitamin D is a fat soluble vitamin.
Mixing and Filtering

- After mixing all of these reagents with the supernatant, the supernatants were subjected to solid-phase ion exchange to remove ion suppressing phospholipids
  - Solid phase ion exchange is done based on electrostatic interactions between molecules and the ion exchanger as the stationary phase and sample must be charged beforehand
    - Was done to filter out any ion-suppressing phospholipids (lipids that contain phosphate groups)
  - Samples that were retained at the end as the impurities (ion-suppressing phospholipids) were washed out were then evaporated and derivatized using PTAD or 4-phenyl-1,2,4-triazoline-3,5-dione and was left to dry again to get the solid product
    - Derivatized is a technique to transform a chemical compound into another product of similar properties/structure (sometimes it is safer than other like for example how lidocaine is a derivative of cocaine)
Reformation and Assays

● Product was then reformed in 1:3 acetonitrile to water and using the analytical chemistry technique called liquid chromatography-tandem mass spectrometry to filter out for the product ions and analyze.

● When doing Liquid Chromatography- Tandem Mass Spectrometry, you need to use a standard to compare to.
  ○ The researchers prepared an “in house” dried blood spot sample for the inter-assay variance.

● Interassay and Intra Assay coefficients of variability were 6.9% for 25-hydroxyvitamin D2 and 11.6% for 25-hydroxyvitamin D3.
Liquid Chromatography and Tandem Mass Spectrometry

- Combining the power of liquid chromatography with the selective mass analysis capability of mass spectrometry
- Sample solution of analytes are pumped through a stationary phase by a mobile phase at high pressures
- (1) After elution (washing and extraction), they put the sample into a mass spectrometer as the precursor ion is passed through the first quadrupole (filter step to exclude mass/charged particles)
- (2) Selected ion is fragmented by colliding it with an immobile gas phase
- (3) The last step is to take the remaining isolated particles and use an electron multiplier, which
- Does this with high specificity and is very flexible (lots of separation options)
Liquid

LC Mobile Phase
- Liquid
- Water, Methanol, Acetonitrile, etc.
- Pump

LC Chromatograph

LC pump

Mass Spectrometer
- Quadrupoles
- Collision cell
- Ion source
- LC column
- Photomultiplier
Genotyping and Generation of the PRS

- **PRS** or polygenic risk score
  - Serves as a prediction for the person to likely have the disease
- DNA extracted and the whole genome amplified using a mini kit (DNA extraction kit)
- Using SNPs (single nucleotide polymorphism, very useful as a genetic marker as it is a position in the genome where individual in a population **inheres** a change in a single nucleotide)
  - To compare with a reference genome (other genome and databases) to see if there is a change to what was thought or predicted
- 8 subjects were excluded due to ambiguous sex and 1 was removed as an outlier due to having a very high concentration of 25 OHD2
- Control Study Design
  - When generating the PRS in the Danish sample, the danish samples were excluded as they selected SNPs that were present in the combined data set and the data from the Psychiatric Genomics Consortium
- **Linkage Disequilibrium** used to filter the data and standardize the data set
  - Refers to the non-random association of alleles at two or more loci in a general population
    - If these two alleles do occur randomly in a population, the two alleles are said to be in linkage equilibrium
    - If not, then they are in linkage disequilibrium
Statistical Analyses & Results
Seasonal Variation and Vitamin D.

- Study was able to show that the mean monthly 25OHD concentrations displayed the expected winter/spring low levels, which is consistent with the epidemiology of vitamin D deficiency.

- 25OHD concentration varied by parents place of birth. The mean (95% confidence intervals) for 25OHD subjects with both parents born in Denmark was 38.3 (37.3 -39.3) mol/L, while those with one or both parents born outside of Denmark had lower 25OHD concentrations (33.9, 31.2-36.6 mol/L) (results not shown in figure).

Figure 1. Mean monthly 25 hydroxyvitamin D in nmol/L (95% confidence intervals). Note the characteristic seasonal variation, with lower 25 hydroxyvitamin D concentrations in winter and spring born infants (coincident with seasons of increased risk of schizophrenia).
More than just pea plants...

What is a Polygenic Risk Score?

Def: A number based on variation in multiple genetic loci and their associated weights. It serves as the best prediction for the trait that can be made when taking into account variation in multiple genetic variants.

- Used when considering more than one variant at a time.
- “Poly” = many

Why do we care?

- B/c there needs to be a group of genes, not just one, to be predisposed to schizophrenia!
- used to predict future disease susceptibility!
But first...

**Incidence Rate**: A measure of the probability of occurrence of a given medical condition in a population within a specified period of time.

\[
\text{Incidence Rate} = \frac{\text{events}}{\text{person time}}
\]

**Incidence Rate Ratio (IRR)**: a relative difference measure used to compare the incidence rates of events occurring at a given point in time.

\[
\text{Rate Ratio} = \frac{\text{Incidence Rate 1}}{\text{Incidence Rate 2}}
\]

- Used to search for a causal association between a certain risk factor and an outcome.
Influence of neonatal 25OHD and risk of schizophrenia

- Quintiles for 25OHD based on the control sample were derived, and used conditional logistic regression (IRR) to assess the relationship between neonatal 25OHD concentration and risk of schizophrenia.
- Compared to the reference category (fourth quintile), those in the lowest quintile had an increased risk of schizophrenia.
- The general pattern of findings was identified in the native Danes, but not in second generation migrants.

![Graph showing Incidence Rate Ratio and 95% confidence intervals for schizophrenia by quintiles of 25 hydroxyvitamin D concentration.](image)

**Figure 2.** Incidence Rate Ratio and 95% confidence intervals for schizophrenia by quintiles of 25 hydroxyvitamin D concentration, a nested case-control study of 1301 cases and 1301 controls. There was a significantly increased Incidence Rate Ratio for those in the lowest quintile versus the fourth (reference) quintile (IRR = 1.44, 95% CI: 1.12–1.85, p = 0.004). None of the other comparisons were statistically significant.
The influence of neonatal 25OHD and PRS and risk of schizophrenia in the combined samples.
Logistic regression formula (1):

**Full model:** \( Y \sim \text{PRSres} + \text{vitDres} + \text{vitDres} \times \text{PRSre} \)

**Null model:** \( (Y \sim 1 + E) \)

Levels of significance were tested using the **Likelihood Ratio Test (LRT)**

- An LR test is used for comparing the goodness of fit of two statistical models, a null model against an alternative model.

- Use LRT to know if the full model is actually telling us that the two factors: PRS score and vitamin D actually do influence schizophrenia.
Logistic regression formula (2):

Advanced model:

\[(Y \sim PRSres.vitDq1 + PRSres.vitDq2 + PRSres.vitDq3 + PRSres.vitDq3 + PRSres.vitDq4 + PRSres.vitDq5 + Q + E)\]

reduced (null) model: \(Y \sim PRSres + Q + E\).

Q= Quintiles

- The advanced model was used because it controls for all quintiles, which will give us a more powerful result!
- Also used Likelihood Ratio Test
More results...

- Based on the combined sample, both the lowest and second lowest 25OHD quintiles were associated with an increased risk of schizophrenia compared to the reference fourth quintile (IRR=1.52, 95% CIs 1.20–1.93; IRR=1.31, 95% CI 1.03–1.67 respectively).

- Main effect analyses confirmed the influence of (a) PRS on schizophrenia risk (IRR=1.33, 95% CI 1.24–1.43; higher PRS associated with higher risk) and (b) 25OHD concentration on schizophrenia risk when included as a continuous variable (IRR=0.92, 95% CI 0.86–0.99; higher 25OHD associated with lower risk).
Neonatal vitamin D deficiency was associated with a significantly increased risk of Schizophrenia.
Main Takeaway

- Bottom quintile (<20.4 nmol/L) had a 44% increased risk of Schizophrenia
  - Reference group was fourth quintile (40.1 nmol/L-53.5 nmol/L)
- So make sure my baby gets a lot of vitamin D?
  - No
  - Individuals with normal Vitamin D levels did not benefit from extra vitamin D
- Association between neonatal vitamin D and risk of Schizophrenia has been replicated
- Evidence linking high levels of vitamin D and Schizophrenia is mixed
  - Last study found a U-shaped curve, and this study had the power to identify the same thing but it didn’t
Other Takeaways

- Maternal vitamin D deficiency is also a risk factor for Schizophrenia
  - Neonatal vitamin D levels at birth are strongly correlated with maternal vitamin D levels during pregnancy
  - Fetus is entirely reliant on the mother’s vitamin D stores
Other Takeaways Continued

- Seasonal fluctuations
  - Babies born in the winter and spring have lowest levels of vitamin D
- No interaction between PRS and vitamin D concentration
Other Implications

● Possible that neonatal vitamin D deficiency affects other neurodevelopmental disorders
  ○ Another study found that it was associated with autism-related traits
● Developmental vitamin D deficiency alters dopaminergic pathways
● Variants in voltage-gated calcium channels have been found to be associated with an increased risk of Schizophrenia
  ○ The active form of vitamin D increases the activity of those channels
Limitations

- Link between vitamin D deficiency and schizophrenia was limited to ethnic Danes (was not found in second generation migrants)
- Only had one measure of vitamin D from neonatal blood
  - Other studies have found that early postnatal exposure is also critical
- Only studied Denmark, which has a high latitude, so seasonal fluctuations may not be as severe at lower latitudes
- Diagnosis of Schizophrenia were based on Danish psychiatric registry
  - High validity, but still probably not as good as research instruments
Polygenic risk score for schizophrenia is more strongly associated with ancestry than with schizophrenia. https://www.ncbi.nlm.nih.gov/pubmed/30160659


Vitamin D (Calcitriol). http://www.vivo.colostate.edu/hbooks/pathphys/endocrine/otherendo/vitamind.html

Vitamin D and Neurological Diseases: An Endocrine View. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5713448/


A meta-analysis of the relationship between vitamin D deficiency and obesity.
More References

Methods:

- "LC-MS-MS | Liquid Chromatography." *EAG Laboratories*, [www.eag.com/techniques/mass-spec/lc-ms-ms/](http://www.eag.com/techniques/mass-spec/lc-ms-ms/).

Discussion:

*Womb-babies-daily.jpg*

*pregnant-2-qty-er-171120_16x9_992.jpg*