Eat2beNICE

Effects of Nutrition and Lifestyle on Impulsive, Compulsive, and Externalizing Behaviours

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D 7.2 – Manuscript: Validation of selected epigenetically modified candidate genes derived from human cohorts profiling in the BALB/cJ and Tph2-modified mouse models.

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Executive Summary

The Tph2-deficient mouse model was validated in a human cohort of patients with ADHD. The impact of varying social experiences during murine life history was confirmed by demonstrating adaptive behaviour as well as differential gene expression and DNA methylation. A considerable number of epigenetically moderated candidate genes for impulsive/ compulsive and addictive behaviour have been identified in several selected human cohorts (see also D7.1). LMX1B links the findings in human cohorts to the mouse models genetically modified for genes of the brain serotonin system, such as Tph2 and Sert. Other epigenetically regulated candidate gene identified by GWAS meta-analysis and/or EWAS in ADHD and ASD, disorders high in impulsivity/compulsivity or substance use, are CDH13 and ST3GAL3. Since investigation of BALB/cJ had to be abandoned, BALB/cJ mice were replaced by alternative mouse models displaying dysregulation of the brain serotonin system, such as Sert-, Cdh13, St3gal3-deficient mice. Particularly Tph2- and Sert -deficient mice provide practical models to study the effects of nutritional factors and other interventions.

1. Deliverable report

The following manuscripts are published or submitted:

The gene LMX1B, a transcription factor, which plays a critical role in the development of the brain serotonin system, was identified as a GxE-moderated and epigenetically regulated candidate gene for impulsive and aggressive behaviours in Estonian Children Personality, Behaviour and Health Study (ECPBHS) cohort.

Pishva E, et al., Methylomic markers of life history of aggression in a longitudinal population-based study. Manuscript submitted.

Abstract: Making use of blood obtained from 96 individuals participating in the Estonian Children Personality Behaviour and Health Study (ECPBHS) study, the present study examined genome-wide DNA methylation at age 15 and 25 in view of varying levels of aggressive behaviour. We first identified differentially methylated positions (DMPs) and differentially methylated regions (DMRs) associated with life history of aggression (LHA) scores assessed at age 25, after which we examined whether the LHA-associated variation in DNA methylation at CpG sites identified at age 25 could be used as early markers of LHA, making use of DNA methylation data obtained from the blood samples collected from the same individuals at age 15. Subsequently, in order to examine the predictive accuracy of the early (age 15) DNA methylation markers identified, we used elastic net regression, a type of machine learning, to determine the optimum number of probes from the CpG sites showing a significant association with total LHA scores at age 15. Finally, the CpG sites with the highest accuracy in discriminating between, i.e. predicting, high and low LHA scores using the DNA methylation data generated from the samples collected at age 15, were identified. As such, the 768 LHA-associated DMPs at age 25 that displayed a p-value <1.0E-4 were examined at age 15 in order to see whether they also represented nominally significant LHA-associated DMPs earlier in life. In total, 156 out of the original 768 LHA-associated DMPs displayed a similar association at age 15, suggesting these epigenetic marks were already obtained earlier in life. Among those probes were four CpG sites within TRIM10 as well as four CpG sites belonging to PTPRN2. Examples of other top hits were CACNA1D and LMX1B. To examine whether the DNA methylation patterns at age 15 could accurately predict the degree of LHA, we used elastic net regression, and generated a summative methylomic score per individual and assessed the predictive accuracy of the models, which yielded promising results that warrant further attention. A set of 71 probes was identified as an optimal classifier of low versus high LHA scores.

Study of DNA methylation of the SERT (5-HTT, SLC6A4) gene in a patient cohort with panic disorder, a mental disorder high in Neuroticism, a personality trait associated with stress reactivity and increased impulsivity/compulsivity/aggression.

Leibold NK, et al., DNA methylation in the 5-HTT regulatory region is associated with CO2-induced fear in panic disorder patients. Eur Neuropsychopharmacol, 2020, 36:154-159.

Abstract: A polymorphism in the gene encoding the serotonin (5-HT) transporter (5-HTT) has been shown to moderate the response to CO_2 inhalation, an experimental model for panic attacks (PAs). Recurrent, unpredictable PAs represent, together with anticipatory anxiety of recurring attacks, the core feature of panic disorder (PD) and significantly interfere with patients' daily life. In addition to genetic components, accumulating evidence suggests that epigenetic mechanisms, which regulate gene expression by modifying chromatin structure, also play a fundamental role in the etiology of mental disorders. However, in PD, epigenetic mechanisms have barely been examined to date. In the present study, we investigated the relationship between methylation at the regulatory region of the gene encoding the 5-HTT and the reactivity to a 35% CO_2 inhalation in PD patients. We focused on four specific CpG sites and found a significant association between the methylation level of one of these CpG sites and the fear response. This suggests that the emotional response to CO_2 inhalation might be moderated by an epigenetic mechanism, and underlines the implication of the 5-HT system in PAs. Future studies are needed to further investigate epigenetic alterations in PD and their functional consequences. These insights can increase our understanding of the underlying pathophysiology and support the development of new treatment strategies.

TPH2 genotype-related serotonergic modulation of 'waiting impulsivity' is mediated by the impulsivity phenotype in humans. We investigated the translational capacity of a mouse model of Tph2 deficiency in relation to the impact of TPH2 variation on fMRI parameters in ADHD.

Akhrif A, et al., REVERSE phenotyping - Can the phenotype following constitutive Tph2 gene inactivation in mice be transferred to children and adolescents with and without adhd? Brain Behav, 2021, 11:e02054.

Abstract: Experimental models of neuropsychiatric disorders, for example, ADHD, are used to mimic specific phenotypic traits of a complex human disorder. However, it remains unresolved to what extent the animal phenotype reflects the specific human trait. The null mutant mouse of the serotonin-synthesizing tryptophan hydroxylase-2 (Tph2-/-) gene has been proposed as experimental model for ADHD with high face validity for impulsive, aggressive, and anxious behaviors. To validate this ADHD-like model, we examined the Tph2-/- phenotype in humans when considering allelic variation of TPH2 function ("reverse phenotyping"). 58 participants (6 females, 8-18 years) were examined, of whom 32 were diagnosed with ADHD. All participants were phenotyped for impulsivity, aggression, and anxiety using questionnaires, behavioral tests, and MRI scanning while performing the 4-choice serial reaction time task. Additionally, participants were genotyped for the TPH2 G-703T (rs4570625) polymorphism. To analyze the relation between TPH2 G-703T variants and the impulsive/ aggressive/ anxious phenotype, mediation analyses were performed using behavioral and MRI data as potential mediators. We found that the relation between TPH2 G-703T and aggression as part of the

reverse Tph2-/- phenotype was mediated by structure and function of the right middle and inferior frontal gyrus. At the example of trait aggression, our results support the assumption that the Tph2 null mutant mouse reflects the TPH2 G-703T-dependent phenotype in humans. Additionally, we conclude that "reverse phenotyping" is a promising method to validate experimental models and human findings for refined analysis of disease mechanisms.

Investigation of varying social experiences during murine life history reveals adaptive behaviour as well as differential gene expression and vasopressin receptor-1a gene (Avpr1a) methylation.

Bodden C, et al., Impact of varying social experiences during life history on behaviour, gene expression, and vasopressin receptor gene methylation in mice. Sci Rep, 2017, 7:8719.

Abstract: Both negative and positive social experiences during sensitive life phases profoundly shape brain and behaviour. Current research is therefore increasingly focusing on mechanisms mediating the interaction between varying life experiences and the epigenome. Here, male mice grew up under either adverse or beneficial conditions until adulthood, when they were subdivided into groups exposed to situations that either matched or mismatched previous conditions. It was investigated whether the resulting four life histories were associated with changes in anxiety-like behaviour, gene expression of selected genes involved in anxiety and stress circuits, and arginine vasopressin receptor 1a (Avpr1a) gene methylation. Varying experiences during life significantly modulated (1) anxiety-like behaviour; (2) hippocampal gene expression of Avpr1a, serotonin receptor 1a (Htr1a), monoamine oxidase A (Maoa), myelin basic protein (Mbp), glucocorticoid receptor (Nr3c1), growth hormone (Gh); and (3) hippocampal DNA methylation within the Avpr1a gene. Notably, mice experiencing early beneficial and later adverse conditions showed a most pronounced downregulation of Avpr1a expression, accompanied by low anxiety-like behaviour. This decrease in Avpr1a expression may have been, in part, a consequence of increased methylation in the Avpr1a gene. In summary, this study highlights the impact of interactive social experiences throughout life on the hippocampal epigenotype and associated behaviour.

Cholecystokinin gene identified by genome-wide expression and methylation profiling as potential mediator of serotonin-dependent behavioral effects of maternal separation in the murine amygdala.

Weidner MT, et al., Identification of cholecystokinin by genome-wide profiling as potential mediator of serotonin-dependent behavioral effects of maternal separation in the amygdala. Front Neurosci, 2019, 13:460.

Abstract: Both negative and positive social experiences during sensitive life phases profoundly shape brain and behaviour. Current research is therefore increasingly focusing on mechanisms mediating the interaction between varying life experiences and the epigenome. Here, male mice grew up under either adverse or beneficial conditions until adulthood, when they were subdivided into groups exposed to situations that either matched or mismatched previous conditions. It was investigated whether the resulting four life histories were associated with changes in anxiety-like behaviour, gene expression of selected genes involved in anxiety and

stress circuits, and arginine vasopressin receptor 1a (Avpr1a) gene methylation. Varying experiences during life significantly modulated (1) anxiety-like behaviour; (2) hippocampal gene expression of Avpr1a, serotonin receptor 1a (Htr1a), monoamine oxidase A (Maoa), myelin basic protein (Mbp), glucocorticoid receptor (Nr3c1), growth hormone (Gh); and (3) hippocampal DNA methylation within the Avpr1a gene. Notably, mice experiencing early beneficial and later adverse conditions showed a most pronounced downregulation of Avpr1a expression, accompanied by low anxiety-like behaviour. This decrease in Avpr1a expression may have been, in part, a consequence of increased methylation in the Avpr1a gene. In summary, this study highlights the impact of interactive social experiences throughout life on the hippocampal epigenotype and associated behaviour.

SERT (5-HTT, SLC6A4) is an epigenetically regulated candidate gene identified by numerous studies in humans and exeprimental animals. Complementary to the study in patients with panic disorder it was demontrated that CO2 induces panic-like behavior in Sert-deficient mice, thus establishing a valid translational mouse model.

Leibold NK, et al., Effect of serotonin transporter genotype on carbon dioxide-induced fear-related behavior in mice. J Psychopharmacol, 2020, 34:1408-1417.

Abstract: Inhaling 35% carbon dioxide induces an emotional and symptomatic state in humans closely resembling naturally occurring panic attacks, the core symptom of panic disorder. Previous research has suggested a role of the serotonin system in the individual sensitivity to carbon dioxide. In line with this, we previously showed that a variant in the *SLC6A4* gene, encoding the serotonin transporter, moderates the fear response to carbon dioxide in humans. To study the etiological basis of carbon dioxide-reactivity and panic attacks in more detail, we recently established a translational mouse model.

Cadherin-13 (CDH13) is an epigenetically regulated candidate gene identified by GWAS meta-analysis in ADHD and ASD. Our work links CDH13 to the brain serotonin system, thus validating several related genes including TPH2, SERT and LMX1B. Cadherin-13 deficiency increases dorsal raphe 5-HT neuron density and prefrontal cortex innervation in the mouse brain.

Forero A, et al., Cadherin-13 deficiency increases dorsal raphe 5-HT neuron density and prefrontal cortex innervation in the mouse brain. Front Cell Neurosci, 2017, 11:307.

Abstract: During early prenatal stages of brain development, serotonin (5-HT)-specific neurons migrate through somal translocation to form the raphe nuclei and subsequently begin to project to their target regions. The rostral cluster of cells, comprising the median and dorsal raphe (DR), innervates anterior regions of the brain, including the prefrontal cortex. Differential analysis of the mouse 5-HT system transcriptome identified enrichment of cell adhesion molecules in 5-HT neurons of the DR. One of these molecules, cadherin-13 (Cdh13) has been shown to play a role in cell migration, axon pathfinding, and synaptogenesis. This study aimed to investigate the contribution of Cdh13 to the development of the murine brain 5-HT system. For detection of Cdh13 and components of the 5-HT system at different embryonic developmental stages of the mouse brain, we employed immunofluorescence

protocols and imaging techniques, including epifluorescence, confocal and structured illumination microscopy. The consequence of CDH13 loss-of-function mutations on brain 5-HT system development was explored in a mouse model of Cdh13 deficiency. Our data show that in murine embryonic brain Cdh13 is strongly expressed on 5-HT specific neurons of the DR and in radial glial cells (RGCs), which are critically involved in regulation of neuronal migration. We observed that 5-HT neurons are intertwined with these RGCs, suggesting that these neurons undergo RGC-guided migration. Cdh13 is present at points of intersection between these two cell types. Compared to wildtype controls, Cdh13-deficient mice display increased cell densities in the DR at embryonic stages E13.5, E17.5, and adulthood, and higher serotonergic innervation of the prefrontal cortex at E17.5. Our findings provide evidence for a role of CDH13 in the development of the serotonergic system in early embryonic stages. Specifically, we indicate that Cdh13 deficiency affects the cell density of the developing DR and the posterior innervation of the prefrontal cortex (PFC), and therefore might be involved in the migration, axonal outgrowth and terminal target finding of DR 5-HT neurons. Dysregulation of CDH13 expression may thus contribute to alterations in this system of neurotransmission, impacting cognitive function, which is frequently impaired in neurodevelopmental disorders including attention-deficit/hyperactivity and autism spectrum disorders.

Forero A, et al., Serotonin (5-HT) neuron-specific inactivation of Cadherin-13 impacts 5-HT system formation and cognitive function. Neuropharmacol, 2020, 168:108018.

Abstract: Genome-wide screening approaches identified the cell adhesion molecule Cadherin-13 (CDH13) as a risk factor for neurodevelopmental disorders, nevertheless the contribution of CDH13 to the disease mechanism remains obscure. CDH13 is involved in neurite outgrowth and axon guidance during early brain development and we previously provided evidence that constitutive CDH13 deficiency influences the formation of the raphe serotonin (5-HT) system by modifying neuron-radial glia interaction. Here, we dissect the specific impact of CDH13 on 5-HT system development and function using a 5-HT neuron-specific Cdh13 knockout mouse model (conditional Cdh13 knockout, Cdh13 cKO). Our results show that exclusive inactivation of CDH13 in 5-HT neurons selectively increases 5-HT neuron density in the embryonic dorsal raphe, with persistence into adulthood, and serotonergic innervation of the developing prefrontal cortex. At the behavioral level, adult Cdh13 cKO mice display delayed acquisition of several learning tasks and a subtle impulsive-like phenotype, with decreased latency in a sociability paradigm alongside with deficits in visuospatial memory. Anxiety-related traits were not observed in Cdh13 cKO mice. Our findings further support the critical role of CDH13 in the development of dorsal raphe 5-HT circuitries, a mechanism that may underlie specific clinical features observed in neurodevelopmental disorders.

ST3GAL3 is an epigenetically regulated candidate gene identified by GWAS meta-analysis and EWAS in ADHD. Generation of a new mouse models of ST3GAL3 and ST3GAL5 deficiency. Study of the impact of St3gal3 deficiency on ADHD-like behaviour and marker of myelination. ST3GAL3 is coding for a sialyltransferase, which is a critical nutritional component of breast feeding. St3gal3-deficient mice display profound cognitive dysfunction caused by dysmyelination.

Rivero O, et al. Haploinsufficiency of the ADHD risk gene St3gal3 in mice causes alterations in cognition and expression of genes involved in myelination and sialylation. Front Genet, 2021, 12:688488.

Abstract: Genome wide association meta-analysis identified ST3GAL3, a gene encoding the beta-galactosidase-alpha-2,3-sialyltransferase-III, as a risk gene for attention-deficit/hyperactivity disorder (ADHD). Although loss-of-function mutations in ST3GAL3 are implicated in non-syndromic autosomal recessive intellectual disability (NSARID) and West syndrome, the impact of ST3GAL3 haploinsufficiency on brain function and the pathophysiology of neurodevelopmental disorders (NDDs), such as ADHD, is unknown. Since St3gal3 null mutant mice display severe developmental delay and neurological deficits, we investigated the effects of partial inactivation of St3gal3 in heterozygous (HET) knockout (St3gal3 ±) mice on behavior as well as expression of markers linked to myelination processes and sialylation pathways. Our results reveal that male St3gal3 HET mice display cognitive deficits, while female HET animals show increased activity, as well as increased cognitive control, compared to their wildtype littermates. In addition, we observed subtle alterations in the expression of several markers implicated in oligodendrogenesis, myelin formation, and protein sialylation as well as cell adhesion/synaptic target glycoproteins of ST3GAL3 in a brain region- and/or sex-specific manner. Taken together, our findings indicate that haploinsufficiency of ST3GAL3 results in a sex-dependent alteration of cognition, behavior and markers of brain plasticity.

2. Tables and other supporting documents where applicable and necessary

Not applicable.

3. Acknowledgement and Disclaimer

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