Eat2beNICE

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

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D7.4 Novel epigenetically regulated genes following nutritional and exercise intervention in human cohorts

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Executive Summary

A considerable number of epigenetically moderated candidate genes for impulsive/ compulsive and addictive behaviour have been identified in several selected human cohorts. *LMX1B* links the findings in human cohorts to the mouse models genetically modified for genes of the brain serotonin (5-HT) system, such as *TPH2* and *SERT/5-HTT*. 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*.

Our findings indicated that serum lipid levels may impact the development of Maladaptive Impulsivity starting from an early age. This effect of cholesterol continues throughout adolescence into young adulthood. In a subsample of the ECPBHS cohort genome-wide DNA methylation (DNAm) levels were measured in peripheral blood obtained from individuals at 15 and 25 years of age. We found evidence for co-localization between mQTL SNPs associated with top DMPs and aggression-related traits including serum lipid levels based on individual dietary patterns. Multiple SNPs that are associated with methylation levels of CpG sites within one of our DMRs (*TRIM10*), have been also found to be associated with total cholesterol levels. Since cholesterol levels have been suggested to particularly affect the development of 5-HTergic neurotransmission and also play a major role in synaptogenesis, dendrite differentiation and axonal growth, the contribution of CpG sites annotated to *TRIM10* in the early prediction of aggressive behaviour along with evidence for pleotropic genetic variants associated with the same CpGs and total cholesterol levels might suggest a novel molecular mechanism mediating the early contribution of cholesterol levels toward Maladaptive Impulsivity.

Given the implication of 5-HT system based on the assocation of *LMX1B* methylation and TRIM10/cholesterol with impulsivity/aggression, we assessed the interaction monoamine oxidase A gene (*MAOA*) genotype, methylation and impulsive/antisocial behaviour in the ECPBHS cohort as well as these interactions for the *TPH2* and *SERT/SLC6A4* genes in ADHD and other disorders with comorbid emotional instability, also using functional imaging approaches and environmental challenges.

However, investigation of the relationship between peripheral *SERT/SLC6A4* gene methylation and brain SERT and 5-HT4 availability using positron emission tomography (PET) and the radioligands [11C]DASB and [11C]SB 207145 did not detect an association between peripheral SLC6A4 methylation and brain SERT or 5-HT4 levels. Peripheral *SLC6A4* methylation may only be considered as a peripheral marker, reflecting periphery-related processes such as 5-HT-mediated immune function that may also be related to stress (Bruzzone S. et al., 2023, manuscript in preparation)

In summary, we found a relative lack of consistency between differentially methylated genes in peripheral cells and in the brain of both humans and mice. This failure to identify correlations across tissues and species may also be based on the heterogeneity of neuronal cell in the networks of interest. The focus was therefore shifted to human-derived induced pluripotent stem cells (iPSCs), since they offer unique opportunities for research into the key molecular/ cellular mechanisms, such as gene-by-environment interaction and mechanisms of epigenetic modification of gene expression, specifically the effects of nutritional components on aberrant DNA methylation and miRNA expression.

1. Deliverable report

The following manuscripts are published or submitted:

Tomson-Johanson K. et al., Low cholesterol levels in children predict impulsivity in young adulthood. Acta Neuropsychiatr, 2020, 32:196-205

Abstract: Severe behavioural issues such as impulsive action and suicide have since long been associated with low levels of cholesterol. While it is known that cholesterol plays a role in neural development and hence low levels of serum lipids could have long-term effects on behaviour, no longitudinal studies showed the association of serum lipids levels with impulsivity. We aimed to examine the prognostic properties of serum lipid levels during childhood and adolescence on measures of impulsivity during early adulthood in a representative birth cohort sample. We have investigated whether serum lipid levels measured at 9, 15, 18 and 25 years of age have an association with impulsivity in 25 years old young adults. This analysis was based on data of the birth cohort representative samples of the Estonian Children Personality Behaviour and Health Study (original n = 1238). Impulsivity was self-reported with the Adaptive and Maladaptive Impulsivity Scale. Total and low-density lipoprotein (LDL) cholesterol measured in boys aged 9, 15 and 18 years predicted disinhibition and thoughtlessness in 25-year-old young adults. High scores of disinhibition were associated with low total and LDL cholesterol levels in males but, while less consistently, with high total and LDL cholesterol levels in females. Cross-sectional analysis did not result in systematic outcomes. Serum lipid levels could have an impact on the development of Maladaptive Impulsivity starting from an early age. This effect of cholesterol continues throughout adolescence into young adulthood.

Pishva E. et al., Genome-wide DNA methylation analysis of aggressive behaviour: a longitudinal population-based study. J Child Psychol Psychiatr, 2023, doi: 10.1111/jcpp.13782. Online ahead of print.

Abstract: Human aggression is influenced by an interplay between genetic predisposition and experience across the lifespan. This interaction is thought to occur through epigenetic mechanisms, inducing differential gene expression, thereby moderating neuronal cell and circuit function, and thus shaping aggressive behaviour. Genome-wide DNA methylation (DNAm) levels were measured in peripheral blood obtained from 95 individuals participating in the Estonian Children Personality Behaviours and Health Study (ECPBHS) at 15 and 25 years of age. We examined the association between aggressive behaviour, as measured by Life History of Aggression (LHA) total score and DNAm levels both assessed at age 25. We further examined the pleiotropic effect of genetic variants regulating LHA-associated differentially methylated positions (DMPs) and multiple traits related to aggressive behaviours. Lastly, we tested whether the DNA methylomic loci identified in association with LHA at age 25 were also present at age 15. We found one differentially methylated position (DMP) (cg17815886; $P=1.12 \times 10^{-8}$), and five differentially methylated regions (DMRs) associated with LHA after multiple testing adjustment. The DMP annotated to the *PDLIM5* gene, and DMRs resided in the vicinity of four protein-encoding genes (*TRIM10*, *GTF2H4*, *SLC45A4*, *B3GALT4*), and a long intergenic non-coding RNA (*LINC02068*). We observed evidence for the co-localization of genetic variants associated with top DMPs and general cognitive function, educational attainment, and cholesterol levels. Notably, a subset of the DMPs associated with LHA at age 25, also displayed altered DNAm patterns at age 15 with high accuracy in predicting aggression. Our findings highlight the potential role for DNAm in the development of aggressive behaviours. We observed pleiotropic genetic variants associated with identified DMPs, and various traits previously established to be relevant in shaping aggression in humans. Concordance of DNAm signatures in adolescents and young adults may have predictive value for inappropriate and maladaptive aggression later in life.

Kanarik M, et al., Methylation of an MAOA locus is associated with impulsive and antisocial behaviour: dependence on MAOA uVNTR and adversity in family, and possible regulation by diet. 2023, manuscript submitted

Abstract: We assessed monoamine oxidase A gene (MAOA) methylation and impulsive/antisocial behaviour in a subsample of 121 males from the ECPBHS cohort. In this sample, MAOA-L genotype is not associated with higher aggressive behaviour, but instead with higher educational attainment by age 25. Of all the 12 CpG sites measured, methylation levels at the locus designated CpG3 were statistically significantly lower in subjects with antisocial behaviour involving police contact. Previously peripheral CpG3 methylation has been reported to correlate negatively with MAO-A enzyme levels in the brain. CpG3 methylation was lower in subjects with alcohol use disorder by age 25, but only in MAOA-H genotype. Correlation between MAOA CpG3 methylation levels and adaptive impulsivity was absent at age 15 but in subjects with MAOA-L genotype a positive correlation appeared by age 18; by age 25 this positive correlation was no longer observed in subjects with better family relationships but had increased further with inferior family relationships. In MAOA-H males CpG methylation did not correlate with adaptive impulsivity. In contrast, MAOA CpG3 methylation had a different developmental dynamics in its relation to maladaptive impulsivity. Thus, at age 18 a positive correlation was observed in MAOA-L genotype with inferior family relationships and a negative correlation was found in MAOA-H participants with better family relationships; both of these associations had disappeared by age 25. Higher CpG3 methylation was associated with more assertive conflict solving style. CpG3 methylation was also associated with dietary intake of several micronutrients, most notable was a negative correlation with the intake of zinc, but also with calcium, potassium and vitamin E; a positive correlation was found between CpG methylation and intake of phosphorus. Conclusively, MAOA CpG3 methylation, being temporarily related to both maladaptive and adaptive impulsivity but by young adulthood only to adaptive impulsivity in MAOA-L males from adverse home environment, may serve as a tool for adaptive developmental neuroplasticity. The mechanisms involved remain to be clarified but could include dietary factors.

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.

Akhrif A, et al., Serotonergic modulation of normal and abnormal brain dynamics: the genetic influence of the TPH2 G-703T genotype and DNA methylation on wavelet variance in children and adolescents with and without ADHD. Plos ONE, 2023, manuscript submitted

Abstract: In this (epi)genetic imaging study we examined 144 subjects (74 patients) at rest and while processing the 4-choice serial reaction time task. We addressed the effects of the *TPH2 G-703T* (rs4570625) genotype and methylation in the 5' untranslated region (5'UTR) of *TPH2* on behavioral performance and wavelet variance and found that both, genotype and methylation were associated with wavelet variance in fronto-parietal regions and behavioral performance: whereas comparisons between genotypes of patients and controls revealed highest variance and worst reaction times in patients carrying the T allele [gene-dosage effect, i.e. the WI phenotype is a direct result of the cumulative effect of WI phenotype (ADHD>TDC) and genetically induced tryptophan deficiency (T^+>GG)], regressions revealed a significant effect of methylation on one specific CpG site in ADHD patients but not controls, in terms of a significantly prediction of wVar in fronto-parietal regions as well as premature responses.

We conclude, that with the *TPH2* gene as an example we were able to show a more detailed picture of how genotype and DNA methylation affect the ADHD and/or impulsive endophenotype.

Leibold NK, et al., DNA methylation in the 5-HTT regulatory region is associated with CO_2-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.

van Rhijn J.R. et al., Brunner syndrome associated MAOA mutations result in NMDAR hyperfunction and increased network activity in human dopaminergic neurons. Neurobiol Dis, 2022 163:105587

Jansch C. et al., Serotonin-specific neurons differentiated from human iPSCs form distinct subtypes with synaptic protein assembly. J Neural Transm, 2021, 128:225-241

Abstract: The heterogeneity of neuronal cell in a neural network of interest (e.g., neuronal cells of various specification and developmental stages, glial cells, blood vessel cells) render it challenging to obtain sufficient quantities of target neuronal tissue serving a defined function for epigenetic profiling. The focus was therefore shifted to human-derived induced pluripotent stem cells (iPSCs), since they offer unique opportunities for research into the key molecular/cellular mechanisms, such as gene-by-environment interaction and mechanisms of epigenetic modification of gene expression, specifically the effects of nutritional components on aberrant DNA methylation and miRNA expression. As a proof

of concept, an analysis of MAOA-mutant human iPSC-derived dopaminergic neurons was conducted. It showed that the increased aggression seen in people with MAOA mutations is accompanied by an increase in the activity of dopaminergic neurons through upregulation of NMDA receptor function. Environmental factors, including toxins, that prevent such upregulation, may be able to prevent the overactivity of the dopaminergic neurons.

Similarly, the expression of the ADHD-related risk gene CDH13 was investigated iPSC-derived serotonergic neurons.

Vitale et al., Generation of induced pluripotent stem cell (iPSC) lines carrying a heterozygous (UKWMPi002-A-1) and null mutant knockout (UKWMPi002-A-2) of Cadherin 13 associated with neurodevelopmental disorders using CRISPR/ Cas9. Stem Cell Res, 2021, 51:102169

Ziegler GC, et al., Generation of multiple human iPSC lines from peripheral blood mononuclear cells of two SLC2A3 deletion and two SLC2A3 duplication carriers. Stem Cell Res, 2021, 56:102526

Diouf D. et al., Generation of a ST3GAL3 null mutant induced pluripotent stem cell (iPSC) line (UKWMPi002-A-3) by CRISPR/Cas9 genome editing. Stem Cell Res, 2023, 67:103038

Abstract: We aimed to perform the first systematic analysis of neuronal network activity for a set of risk genes of impulsive/compulsive behaviour, such as TPH2, CDH13, SLC2A3 and ST3GAL3, in interaction with environmental stressors using well-defined microelectrode array (MEA) parameters. Naturally occuring genetic SNP or CNV variants and isogenic CRISPR/Cas9 knockout (KO) lines for several of the chosen risk genes (CDH13, SLC2A3, ST3GAL3) will be investigated for their role in this human model system. ST3GAL3 is an ADHD risk gene, coding for a sialyltransferase. Copy number variants of SLC2A3, which encodes the neuronal glucose transporter GLUT3, are associated with several behavioural traits.

We reprogrammed peripheral blood mononuclear cells from two SLC2A3 duplication and two SLC2A3 deletion carriers and subsequently generated two transgene-free iPSC clones per donor by Sendai viral transduction. All eight clones represent bona fide hiPSCs with high expression of pluripotency genes, ability to differentiate into cells of all three germ layers and normal karyotype. The generated hiPSC lines will be helpful to clarify the role of glucometabolic alterations in patho-physiological processes shared across organ boundaries. Moreover, this will enable us to understand the mechanism-of-action of the risk genes in the context of environmental adversity.

Mossink B. et al., Cadherin-13 is a critical regulator of GABAergic modulation in human stem-cell-derived neuronal networks. Mol Psychiatry, 2022, 27:1-18

Abstract: We investigated the extent to which excitation/inhibition (E/I) balance is disturbed in both human IPSC-derived neural cell and complementary mouse model systems carrying genetic, epigenetic and environmental risk factors. We aim to gain a deeper molecular and neurobiological understanding of mechanisms underlying risk genes interacting with nutrition/life style and how it affects neuronal circuitries, including the role of myelination. We take advantage of our newly developed protocols to generate parvalbumin-positive (PV+) GABAergic neurons for which we have recently demonstrated that these GABAergic neurons exert scalable inhibitory control onto excitatory glutamatergic neurons at the network level.

2. Acknowledgement and Disclaimer

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