Eat2beNICE

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

H2020 - 728018

D 7.1– Manuscript: Epigenetically moderated candidate genes for impulsive/compulsive and addictive behaviour in selected human cohorts.

Dissemination level	PU
Contractual date of delivery	31/08/2020
Actual date of delivery	26/10/2021
Type	Report
Version	1
Workpackage	WP7 – Epigenetic modification profiles for impulsivity and compulsivity
Workpackage leader	UKW, Partner 04

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 728018.

Author list

Organisation	Name	Contact information
UKW	Klaus-Peter Lesch	kplesch@mail.uni-wuerzburg.de
RUMC	Barbara Franke	Barbara.Franke@radboudumc.nl
VHIR	Toni Ramos	ramosquiroga@gmail.com
UB	Bru Cormand	bcormand@gmail.com
UT	Jaanus Harro	jaanus.harro@ut.ee
SU	Janos Rethely	rethelyi.janos@med.semmelweis-univ.hu

Executive Summary

A considerable number of epigenetically moderated candidate genes for impulsive/compulsive and addictive behaviour have been identified in several selected human cohorts. The most relevant gene are CDH13, APOB, LPAR5, KMT5A, KLF4, LRRFIP1, RAB11, FIP1, SLA, SNORA38, EGR2, IL7R, CTNNBL1, SCP2, ECHDC2, DRD4, KLDR1, TARBP1, GNG7, TNXB, ADAMTS2, CD247, CAPN2, HDAC4, TRIM10 PTPRN2, CACNA1D, SLC3GAL3 and LMX1B. 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.

1. Deliverable report

The following manuscripts are published or submitted:

EWAS on persistent ADHD, as well as callous behaviour and impulsivity in a longitudinal cohort (NeuroIMAGE) of adolescents (Maijer et al., Front Genet, 2020, 11:16)

Abstract: Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder that often persists into adulthood. ADHD and related personality traits, such as impulsivity and callousness, are caused by genetic and environmental factors and their interplay. Epigenetic modifications of DNA, including methylation, are thought to mediate between such factors and behavior and may behave as biomarkers for disorders. Here, we set out to study DNA methylation in persistent ADHD and related traits. We performed epigenome-wide association studies (EWASs) on peripheral whole blood from participants in the NeuroIMAGE study (age range 12-23 years). We compared participants with persistent ADHD (n = 35) with healthy controls (n = 19) and with participants with remittent ADHD (n = 19). Additionally, we performed EWASs of impulsive and callous traits derived from the Conners Parent Rating Scale and the Callous-Unemotional Inventory, respectively, across all participants. For every EWAS, the linear regression model analyzed included covariates for age, sex, smoking scores, and surrogate variables reflecting blood cell type composition and genetic background. We observed no epigenome-wide significant differences in single CpG site methylation between participants with persistent ADHD and healthy controls or participants with remittent ADHD. However, epigenome-wide analysis of differentially methylated regions provided significant findings showing that hypermethylated regions in the APOB and LPAR5 genes were associated with ADHD persistence compared to ADHD remittance ($p = 1.68 * 10\text{-}24$ and $p = 9.06 * 10\text{-}7$, respectively); both genes are involved in cholesterol signaling. Both findings appeared to be linked to genetic variation in cis. We found neither significant epigenome-wide single CpG sites nor regions associated with impulsive and callous traits; the top-hits from these analyses were annotated to genes involved in neurotransmitter release and the regulation of the biological clock. No link to genetic variation was observed for these findings, which thus might reflect environmental influences. In conclusion, in this pilot study with a small sample size, we observed several DNA-methylation-disorder/trait associations of potential significance for ADHD and the related behavioral traits. Although we do not wish to draw conclusions before replication in larger, independent samples, cholesterol signaling and metabolism may be of relevance for the onset and/or persistence of ADHD.

Transcriptome analysis in ADHD (Mortimer N, et al., Eur Neuropsychopharmacol, 2020, 41:160-166)

Abstract: Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder with an estimated heritability of around 70%. Although the largest genome-wide association study (GWAS) meta-analysis on ADHD identified independent loci conferring risk to the disorder, the molecular mechanisms underlying the genetic basis of the disorder remain to be elucidated. To explore ADHD biology, we ran a two-step transcriptome profiling in peripheral blood mononuclear cells (PBMCs) of 143 ADHD subjects and 169 healthy controls. Through

this exploratory study we found eight differentially expressed genes in ADHD. These results highlight promising candidate genes and gene pathways for ADHD and support the use of peripheral tissues to assess gene expression signatures for ADHD.

Association analysis of SNPs that influence methylation on substance use disorder. Genetic variants that correlate with differential methylation and expression changes of the CTNNBL1, SCP2 and ECHDC2 genes have been identified. (Pineda-Cirera L, et al., J Psychiatr Res, 2021, 136:474-482).

Abstract: Drug dependence is a neuropsychiatric condition that involves genetic, epigenetic and environmental factors. Allele-specific methylation (ASM) is a common and stable epigenetic mechanism that involves genetic variants correlating with differential levels of methylation at CpG sites. We selected 182 single-nucleotide polymorphisms (SNPs) described to influence cis ASM in human brain regions to evaluate their possible contribution to drug dependence susceptibility. We performed a case-control association study in a discovery sample of 578 drug-dependent patients (including 428 cocaine-dependent subjects) and 656 controls from Spain, and then, we followed-up the significant associations in an independent sample of 1119 cases (including 589 cocaine-dependent subjects) and 1092 controls. In the discovery sample, we identified five nominal associations, one of them replicated in the follow-up sample (rs6020251). The pooled analysis revealed an association between drug dependence and rs6020251 but also rs11585570, both overcoming the Bonferroni correction for multiple testing. We performed the same analysis considering only cocaine-dependent patients and obtained similar results. The rs6020251 variant correlates with differential methylation levels of cg17974185 and lies in the first intron of the CTNNBL1 gene, in a genomic region with multiple histone marks related to enhancer and promoter regions in brain. Rs11585570 is an eQTL in brain and blood for the SCP2 and ECHDC2 genes and correlates with differential methylation of cg27535305 and cg13461509, located in the promoter regions of both genes. To conclude, using an approach that combines genetic and epigenetic data, we highlighted the CTNNBL1, SCP2 and ECHDC2 genes as potential contributors to drug dependence susceptibility.

DNA methylation study focusing on adult ADHD and the different symptom domains (inattention and hyperactivity/impulsivity) was performed on target genes and published (Weiss et al., Neuropharmacology, 2021, 184:108370).

Abstract: Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterized by age-inappropriate symptoms of inattention and/or hyperactivity and impulsivity. ADHD is highly prevalent in childhood and often persists into adulthood. Both genetic variants and environmental factors play a role in the onset and persistence of ADHD, and epigenetic changes, such as DNA methylation are considered as a link for their interplay. To investigate this, we studied DNA methylation in 37 candidate genes by performing targeted bisulfite sequencing of DNA isolated from whole blood of N = 88 individuals diagnosed with adult ADHD and N = 91 unaffected individuals (mean age 34.2 years). Differentially methylated sites were assessed by generalized linear models testing ADHD status and ADHD symptoms, accounting for a methylation-based smoking score, age, sex, and blood cell count. DNA

methylation of single sites within DRD4 and KLDR1 was associated with adult ADHD status, and multiple DNA methylation sites within TARBP1 were associated with ADHD symptoms in adulthood and childhood. Awaiting replication, findings of this pilot study point to TARBP1 as a new candidate gene for ADHD symptoms. Our work also stresses the need for research to further examine the effects of environmental factors, such as nicotine exposure, on epigenetic modifications associated with psychiatric traits.

Study on the genetic and causal relationship between ADHD and Substance Use Disorder (Vilar-Ribó L, et al., Am J Med Genet B Neuropsychiatr Genet. 2021, 186:140-150)

Abstract: Substance use disorder (SUD) often co-occur at high prevalence with other psychiatric conditions. Among them, attention-deficit and hyperactivity disorder (ADHD) is present in almost one out of every four subjects with SUD and is associated with higher severity, more frequent polysubstance dependence and increased risk for other mental health problems in SUD patients. Despite studies suggesting a genetic basis in the co-occurrence of these two conditions, the genetic factors involved in the joint development of both disorders and the mechanisms mediating these causal relationships are still unknown. In this study, we tested whether the genetic liability to five SUD-related phenotypes share a common background in the general population and clinically diagnosed ADHD individuals from an in-house sample of 989 subjects and further explored the genetic overlap and the causal relationship between ADHD and SUD using pre-existing GWAS datasets. Our results confirm a common genetic background between ADHD and SUD and support the current literature on the causal effect of the liability to ADHD on the risk for SUD. We added novel findings on the effect of the liability of lifetime cannabis use on ADHD and found evidence of shared genetic background underlying SUD in general population and in ADHD, at least for lifetime cannabis use, alcohol dependence and smoking initiation. These findings are in agreement with the high comorbidity observed between ADHD and SUD and highlight the need to control for substance use in ADHD and to screen for ADHD comorbidity in all SUD patients to provide optimal clinical interventions.

Review of transcriptomic and genomic analyses on ADHD (Pujol-Gualdo N, et al., Eur Neuropsychopharmacol, 2021, 44:1-13)

Abstract: Attention-deficit/hyperactivity disorder (ADHD) is a highly heritable condition that represents the most common neurodevelopmental disorder in childhood, persisting into adulthood in around 40-65% of the cases. ADHD is characterised by age-inappropriate symptoms of inattention, impulsivity, and hyperactivity. Mounting evidence points towards ADHD having a strong genetic component and the first genome-wide significant findings have recently been reported. However, the functional characterization of variants unravelled by genome-wide association studies (GWAS) is challenging. Likewise, gene expression profiling studies have also been undertaken and novel integrative approaches combining genomic and transcriptomic data are starting to be conducted, which offers an exciting way that might provide a more informative insight towards the genetic architecture of ADHD. In this review, we summarised current knowledge on genomics, transcriptomics and integrative approaches in ADHD, focusing on GWAS and GWAS meta-analyses (GWAS-MA)- as genomics analyses-

microarray and RNA-seq- as transcriptomics analyses-, and studies integrating genomics and transcriptomics data. In addition, current strengths and limitations of such approaches are discussed and further research avenues are proposed in order to face unsolved issues. Although important progress has been made, there is still a long way ahead to elucidate the biological mechanisms of ADHD, which eventually may lead to more personalized approaches in the future. Large- scale research efforts and new technological and statistical approaches are envisaged as important means towards deciphering ADHD in the upcoming years.

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

Abstract: Methylomic markers of life history of aggression in a longitudinal population-based study: 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 (Pishva E, et al., manuscript submitted).

Review and combined analysis on epigenetics in externalizing behaviours: (Maijer M, et al., Mol Psychiatry. In revision).

Abstract: Review and combined analysis on epigenetics in externalizing behaviours: Both genetic and environmental factors contribute to externalizing behaviours and related disorders, and epigenetic modifications, such as DNA methylation (DNAm), are considered as a mechanism for their interplay. Previous epigenome-wide association studies (EWAS) for

externalizing behaviours have been limited in sample size, and, therefore, candidate genes and biomarkers with robust evidence are still lacking. Here, we performed a systematic literature review of EWAS of attention-deficit/hyperactivity disorder (ADHD)- and aggression-related behaviours conducted in peripheral tissue and cord blood. For the most strongly associated DNAm sites observed in individual studies (p<10-3), we combined data across studies to identify candidate genes and biological systems for ADHD and aggressive behaviours. We performed enrichment analyses for Gene Ontology (GO) terms and cell markers, and we linked the EWAS findings with genome-wide association studies of ADHD. In total, we identified 14 EWAS of ADHD or ADHD symptoms and 10 EWAS of aggression-related behaviours. Only 10 out of 24 studies reported DNAm sites that were epigenome-wide significantly associated with externalizing behaviour, without any overlap in CpG sites. While promising results have been reported, the majority of current studies are limited by methodological differences in study design and analysis methodology, as well as small sample sizes. Of these studies, five EWAS for ADHD and ADHD symptoms as well as three aggression EWAS met inclusion criteria for the combined analysis. CpG sites were annotated to genes that were enriched in granulocyte cell markers for both ADHD and aggression, but studies for aggression also showed an enrichment for astrocyte cell markers. Only 1% of the top epigenetic findings for ADHD/ADHD symptoms were likely to be directly explained by genetic factors involved in ADHD. By comparing individual studies, GNG7, TNXB, ADAMTS2, and CD247 were found to be associated with ADHD in more than half of the EWAS, while CAPN2 and HDAC4 were identified in all three EWAS for aggression. This review shows the power of combining results of multiple studies and how the field would greatly benefit from larger sample sizes and harmonization of assessment instruments. We also discuss what animal studies can teach us. Addressing all existing EWAS of selected externalizing behaviours, our combined analysis prioritized several candidate genes. We also detected distinct biological enrichments and cellular substrates for ADHD and aggression (Maijer M, et al., Mol. Psychiatry. In revision).

2. Tables and other supporting documents where applicable and necessary

Not applicable.

3. Acknowledgement and Disclaimer

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 728018.