FINAL SUMMARY REPORT

Grant agreement number:	603016
Project acronym:	MATRICS
Project title:	Multidisciplinary Approaches to Translational Research in Conduct Syndromes
Funding scheme:	FP7-CP-FP
Funding duration:	1st March 2014 – 30th November 2019
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1 Executive summary

Aggression is part of human nature and is a way humans control their environment. Most juveniles tend to socialize and learn to inhibit these aggressive behaviours and adopt more socially acceptable forms of defending one's rights and goods, and expressing one's wishes. The key mechanisms involved in this process of socialization are the development of cognitive control of impulses, sensitivity to negative feedback and in particular punishment through conditioning, and the development of empathy and a sense of morality. MATRICS sought to understand the neural, (epi)genetic mechanisms underlying these control mechanisms and their failure in some juveniles leading to psychiatric disruptive behaviour disorders, notably oppositional defiant disorder (ODD) and conduct disorder (CD). This failure may due to a complex constellation of aetiological factors including genetic predisposition, temperamental factors, early prenatal and post-natal adversities, exposure to severe trauma, abuse and neglect, inadequate, inconsistent and harsh parenting and negative effects from the neighbourhood and peer group. MATRICS focused on ODD and CD as the two central juvenile disorders characterized by aggression and antisocial behaviour. MATRICS sought to characterise these behaviours in terms of their molecular and neural substrates and addressed both the efficacy and mechanistic underpinnings of pharmacotherapy and bio-/neuro-feedback approaches. The outcomes of MATRICS have highlighted the loss of inhibitory GABAergic cingulate cortical control mechanisms in aggression, their phenotypic remediation by methylphenidate and oxytocin by altering attention, fear processing and empathy regulation respectively. MATRICS further highlighted a role for opioid signalling and RBFOX1 (methylation) as key regulators of the aggressive phenotype. The regulation of salience networks by feedback approaches in human CD may also be useful as will the targeting of immune-related attentional mechanisms which appear to be causal to the aggressive phenotype in multiple ODD/CD and population cohorts. Partners of the MATRICS consortium will seek to further advocate and utilise these findings in the future within cross-consortia initiatives such as the European Brain Research Area (EBRA) consortium as next steps to enable these insights for the improved clinical management of ODD/CD.

2 Summary description of project context and objectives

Aggressive behaviours as hitting, pushing, slapping, biting, kicking, spitting, and hair pulling are rather universal in young children. Growing older, most children tend to socialize and learn to inhibit these aggressive behaviours. Interactions with caregivers play an important role in shaping children's behavioural repertoire towards more socially acceptable forms of defending one's rights and goods, and expressing one's wishes. The key mechanisms involved in this process of socialization are the development of cognitive control of impulses, sensitivity to negative feedback and in particular punishment through conditioning, and the development of empathy and a sense of morality. Some children, however, fail to follow this path of socialization and continue frequently manifesting aggressive and rule breaking behaviours. These children may fall within the categories of psychiatric disruptive behaviour disorder, of which the most severe one is conduct disorder (CD). This failure may due to a complex constellation of aetiological factors including genetic predisposition, temperamental factors, early prenatal and post-natal adversities, exposure to severe trauma, abuse and neglect, inadequate, inconsistent and harsh parenting and negative effects from the neighbourhood and peer group. MATRICS focused on CD as the central paediatric disorder characterized by severe aggression and antisocial behaviour.

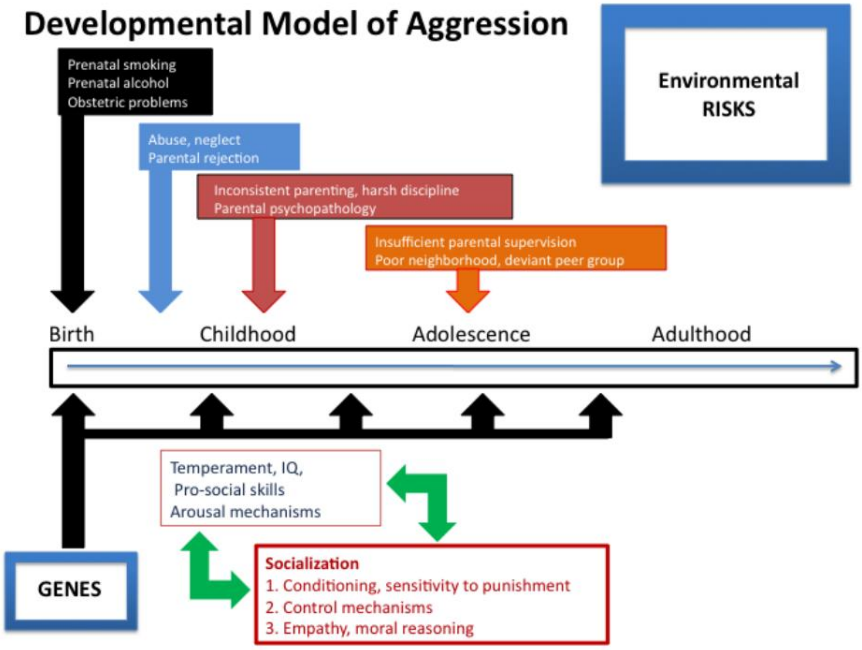

Figure 1. Developmental - aetiological model of aggression

The aim of MATRICS was to identify, over the duration of 5 years and 9 months, the neural, genetic and molecular factors involved in the pathogenesis of aggression and antisocial behaviour in (i) normally developing adolescents, (ii) high risk samples, i.e. children and adolescents with Attention-Deficit Hyperactivity Disorder (ADHD), and (iii) children and adolescents with CD. In order to identify these factors, MATRICS pursued the translational approach of matching preclinical studies on animals to clinical studies by basing them on identical paradigms and methodology. This allowed for optimal cross-validation of findings from animal models to humans, and vice versa. In addition to the identification of risk factors, we focussed on the translation of results to clinical practice by (i) focussing on longitudinal cohorts to identify neural, genetic, cognitive and molecular markers that predict the development of aggression and antisocial behaviour through adolescence, its persistence and/or remission in adulthood, and its clinical manifestations in various domains (school, peer interactions, family), (ii) developing and pilot testing preventative biofeedback interventions in very young children at very high risk (those with callous-unemotional (CU) traits), and (iii) performing proof-of-concept clinical studies with medication shown to be promising in our animal studies. In order to reach these goals, MATRICS assembled a strong multidisciplinary team of preclinical and clinical top researchers, both from academia and SMEs.

MATRICS was based on the principle that aggression can be understood based on aberrant arousal mechanisms altering top-down control mechanisms of aggressive behaviour, empathy and the balance between rational and emotional decision making. This premise was characterized in a series of preclinical and clinical work packages (WPs) which applied this principle to aggression in juvenile conduct disorder (CD) using both current and novel arousal-modifying pharmacological strategies and preventative bio-/neuro-feedback approaches to remediate aggression. MATRICS

dissected aggression not only in terms of reactive and instrumental aggression, but also examined the role of the modifier CU traits. This “CU traits modifier” is an important addition to existing diagnostic constructs of CD in the DSM-V and is associated with increased aggression and antisocial behaviour with high criminal impact. The improved clinical management of aggression in CD+/-CU traits was an important aspiration of MATRICS.

In a series of 11 multidisciplinary scientific work packages and 3 supporting WPs (project management, ethics, and dissemination) the following objectives were assessed:

- **WP1 and WP2** focussed on animal models of aggression and altered stress reactivity (during early life and adolescence) both in terms of their aggressive and cognitive phenotype (WP1) and their multiparametric magnetic resonance imaging (MRI) and proton magnetic resonance spectroscopy (1H-MRS) assessment (WP2). Together, they identified the neural circuits underlying aggressive phenotypes and its relationship to altered arousal, anxiety and cognition. WP1 additionally assessed pharmacological strategies to remediate aggression.
- **WP3 and WP4** explored existing imaging genetic datasets (NeuroIMAGE; IMAGEN) to validate the findings from WP1 and WP2, and also established new data collection in CD+/-CU cohorts encompassing phenotypic, MRI, 1H-MRS bio-sampling and arousal measures.
- **WP5** generated a genetic landscape of CD, based on existing genome-wide association study (GWAS) data sets. The top-ranked targets have been confirmed in the brain / blood biomaterial from animal models in WP1 and in the blood / saliva human CD samples from WP4 and WP5. This material was also used in WP5 to assess the ability of environmental triggers to leave epigenetic (methylomics / microRNA) marks which are associated with increased aggression.
- **WP6** focussed on implementing phase IIb clinical trial studies of medication identified and tested in WP1, and aimed to assess clinical and phenotypic changes in CD cohorts.
- **WP7** aimed to alter arousal and stress reactivity in a series of bio- / neuro-feedback studies in childhood and adolescent CD cohorts, respectively, as preventative strategies to reduce aggressive behaviour.
- **WP8** utilised hair samples to produce immunopluripotent stem cells (IPSCs) to simulate stress challenges responses relevant during foetal development. These IPSCs were later epigenetically profiled by WP5.
- **WP9** explored gene-environment relationships in existing population datasets and in those data sets that emerged from W3 and WP4. It was assessed how different environmental risk factors for CD translate into genetic and molecular mechanisms, using a candidate gene approach.
- **WP 10**’s key objective was the causal modelling of aggression with other psychological markers in existing datasets. WP10 also utilised Bayesian machine learning approaches to integrate multi-parametric data sets collected across WPs 1-8.
- **WP11** had the responsibility for maintaining ethical oversight over all studies in WP1-10 and also coordinated training activities for those WPs involved in clinical studies.
- **WP12** coordinated the dissemination of MATRICS output to different stakeholders: patient groups, profession societies, journals and conferences, the media, and the general public.
- **WP12** was responsible for valorisation of MATRICS output with regard to intellectual property and exploitation of the results.
- **WP13** ensured professional project management of MATRICS by coordinating scientific content, finances, meetings, and administrative tasks.

3 Description of the main Science & Technology results / foregrounds

3.1 Summary of the main results / foreground of MATRICS

WP1 has established an aggressive and antisocial phenotype in the BALB/cJ mouse which is accompanied by high anxiety, inattention and reversal learning deficits. These aggressive, inattentive and anxiety changes are reversible by administration of the psychostimulant methylphenidate (also under investigation in humans in WP06) and points towards an improvement in sustained attention underlying anti-aggressive effects. In vivo MRI / 1H-MRS studies of this mouse model demonstrate decreased fractional anisotropy diffusion tensor imaging (DTI) markers and GABA tone in the anterior cingulate cortex and striatum. These changes in cortical inhibition are confirmed in (WP05) transcriptomic expression changes in GABA anabolism and catabolism in two independent animal models (BALB/cJ mice and (peripubertal stress) PPS rats). Furthermore, BALB/cJ mice demonstrate cingulate cortical GABA interneuron cell loss, particularly of parvalbumin and somatostatin classes. Peripubertal stress is not only associated with increased aggression but also changes in cortical and amygdala markers of GABAergic activity. However ex-vivo brain MRI imaging in WP02 of the BALB/cJ model has documented marked volumetric and white matter changes in regions involved with 'valuation' such as the anterior cingulate and insular cortices and registration of (autonomic) information via the periaqueductal grey (PAG) matter region. Documentation of WP02 ex vivo MRI changes in structural, volume or DTI measures in the PPS model demonstrates clear changes in regions involved in stress reactivity. WP01 has also demonstrated that BALB/cJ mice display insensitivity to punishment learning (analogous to that seen in humans with high callous unemotional traits). Furthermore, decreased fear conditioning in BALB/cJ mice that show less 'empathic-like' licking behaviour in response to the distress of another animal is normalised by treatment of oxytocin that has also been paralleled in human clinical pharmacology studies in WP06 where oxytocin administration has clear effects on emotional recognition. Interestingly, further studies on methylphenidate in the low-empathy like BALB/cJ mice demonstrate it normalised sociality, aggression and behavioural stereotypies, thereby ameliorating the phenotype of mice characterised by deficient empathy-like behaviour. Besides, MPH produced the expected dose-dependent increment of locomotor activity and anxiety-like behaviour, while positively modulating the performance in the attentional set-shifting task (CDR stage) in control mice. As such MPH may constitute a valid therapeutic approach in disturbances characterised by abnormal aggression and poor sociability. Clonidine was tested in the aggressive STX knockout model and found to have no effect on aggressive behaviour without inducing hypo-locomotion. Furthermore, knockout of KCNQ1, a gene involved in the inhibition of insulin release had no effect on anxiety or aggressive behaviour.

WP2 Amongst significant findings we identified a pattern of volumetric ("morphometric") regional brain changes in all four animal models, only some of which were common to all. Thus, in the rat peripubertal stress model, the predominant result was an abnormal tissue microstructure in the brain areas associated with aggression including hippocampus and amygdala. Similarly, in the high/low corticosterone reactivity rats (CAST), hippocampus was the area of the brain most significantly associated with aggression-related abnormalities. Both findings represent an important step towards establishing a causal link between development and aberrant glucocorticoid adaptation to stress as an underlying mechanism. We also detected widespread changes including the hippocampus, in the mouse models of aggression and high-low empathy like traits. Additional brain areas that were affected point toward circuits governing olfactory-based fear response (to e.g. predatory odour), communication of pain and autonomic stress (in high/low empathy-like trait mice), as well as striato-limbic systems. The similarities between abnormal brain areas corroborate that common circuits are indeed affected in these models, but the differences point toward the more specific and nuanced array of developmental brain alterations that arise in response to a particular combination of genetic, environmental, or combined factors that are responsible for emergence of CD-related phenotype.

WP3 demonstrates differences between children/adolescents with ODD and/or CD and typically developing controls with regard to several neuroimaging modalities. Using the NeuroIMAGE cohort, we showed reduced fractional anisotropy in fronto-temporal and striatal white matter to be associated with anti-social behaviour independent of ADHD symptoms. Additionally, neurocognitive functioning appeared to be more severely impaired with the presence of ODD and/or CD within ADHD participants. Using the new cohort with ODD/CD collected within MATRICS, we have been able to use aggression subtype-specific analyses, showing the importance of including callous unemotional traits and the distinction between reactive and proactive aggression. Structural and functional imaging analyses revealed differences regarding these subtype specific measures. In addition, for the first time, we showed an involvement of glutamatergic neurotransmission to be related to continuous measures of aggression. Our findings support the idea of subtype-specific impairments in aggression, where different brain regions are involved in empathy, threat response and decision making

which are in turn more associated with either proactive aggression, reactive aggression and CU-traits. This may have implications for designing targeted intervention strategies, which needs to be further explored in future studies.

WP4 findings suggest that dysfunctional levels of glutamate within the brain play a role in CU traits and proactive aggression. As these behaviours represent particularly severe and difficult to treat subtypes of CU traits,5 these findings may form the basis of novel pharmacological targets for treatment resistant individuals. Our work also highlights the need to further dissociate CU traits and proactive aggression future studies, as they are associated with glutamate concentrations in different regions. The WP4 findings in the anterior cingulate cortex / medial prefrontal cortex are of particular translational promise, as they support an emerging neurochemical theory of aggression. Specifically, it has been suggested that aggression is associated with differences in the balance of chemical excitation and inhibition in this region that favours increased excitation. We provide evidence for this hypothesis in the present study, and in an animal model of CU traits analysed within the same consortium. This suggests that such joint animal and human work will play an important role in developing novel treatments for the treatment-resistant aggression subtypes that we have examined in WP4.

WP5 has sought to indentify DNA sequence variation in rodent models of aggression. WGS identified 11,019 robust SNVs between the BALB/cJ and BALB/cByJ strains. Importantly, three of these variants are predicted to have a 'high impact' on protein function or structure (i.e. variants in the coding regions of Acvr1c, Gm13030, and Nlrp5-ps). These represent excellent candidates for future characterization and can be considered novel candidate genes for aggression that can be explored in human and other model datasets. 76 of the identified SNVs are predicted to have a 'medium impact' and the remaining SNVs have low or no predicted effect on protein function or structure. Of note, we found 22 variants annotated to 10 genes previously implicated in aggression, ADHD and compulsivity, including Mecom, Shank2, Avpr1a and Rbfox1. In the CORT rat model, we identified ~16,000 SNVs between the 'low' and 'high' CORT lines, which were annotated to 770 genes. Strikingly, these genes are highly enriched for pathways and functions relevant to aggression and childhood psychiatric disorders. For example, the top ranked OMIN disease category enriched amongst these genes is 'autism' (odds ratio: 4.11; P = 0.0347). Furthermore, KEGG pathway analysis showed a significant enrichment for pathways relevant to CD and aggression including 'long term potentiation' (odds ratio = 3.11; P = 0.004) and 'dopaminergic synapse' (odds ratio = 2.31; P = 0.006). The integration of these gene lists with the Human Cell Atlas demonstrated that the genes harbouring SNVs between the 'low' and 'high' CORT strains were enriched for multiple brain regions including the amygdala (P = 0.0002) and prefrontal cortex (P = 0.01). Similarly, we identified ~20,000 SNVs annotated to 783 genes between the 'low' and 'intermediate' CORT strains. Again, these genes are highly enriched for pathways and functions relevant to aggression and childhood psychiatric disorders. They are significantly enriched for amygdala-expressed genes (P = 0.03). Taken together, our WGS findings suggest that the genetic differences identified in rodent models of aggression directly reflect pathways relevant to CD and neuropsychiatric traits in human cohorts. In addition to providing a novel reference genome resource for the wider research community, these data highlight genetic differences between the strains that potentially underpin the differences observed in aggressive behaviour.

Reduced representation bisulphite sequencing identified epigenetic variation in the brain associated with aggression: We identified multiple differentially methylated positions (P<0.001) between the BALB/cJ and BALB/cByJ mouse strains in each of the three brain regions profiled using RRBS: 368 (annotated to 28 genes) in the VMH, 143 (annotated to 10 genes) in the MCC and 26 (annotated to 9 genes) in the ACC. The majority of the DMPs were not in proximity to any of the between-strain SNVs identified in the WGS analyses, indicating they are not likely to be influenced by single nucleotide genetic variations acting in cis. Of note, there were several common loci showing strain-specific DNA methylation patterns across the three brain regions. Furthermore, several of the genes annotated to these sites have been previously implicated in intellectual disability and aggression – for example Jarid2 and Trio. We also used bioinformatics approaches to explore broader differentially methylated regions (DMRs) between the two BALB strains. The most striking DMR, covering multiple adjacent DMPs, was identified on chromosome 17 in both the VMH and MCC. The region spans 273 (VMH) / 99 (MCC) hypomethylated CpGs covering two CpG islands and overlapping the Gm26917 and Gm42418 predicted genes and AY036118, which encodes a transcription factor. We also identified widespread methylomic differences in the prefrontal cortex (PFC), hippocampus (HC) and hypothalamus (HPT) of BALB/cJ mice exposed to two doses of corticosterone: 298 in the hippocampus, 13 in the hypothalamus and 1 in the frontal cortex. Genes annotated to the top-ranked loci in the hippocampus included a number of genes previously implicated in aggression and related traits such as Rbfox and Kcnn3. Finally, we also identified multiple differentially methylated positions between the three rat lines with differential aggressive response to corticosterone: 11 in the hippocampus, 454 in the hypothalamus and 396 in the

frontal cortex. Genes annotated to the top-ranked loci in the hippocampus included a number of genes previously implicated in aggression and related traits such as Foxp2.

Integration of rodent genomic data with GWAS and GXE findings from human cohorts: The interplay between environmental and genetic factors related to disruptive behaviour is expected to play an important role and contribute to the associations of environmental factors with aggression and disruptive behaviour. Therefore, in collaboration with WP9 (UMCG), we conducted research to investigate specific ways by which genetic and environmental factors could affect each other. These mechanisms include 'gene-environment correlation' and 'gene-environment interaction'. Gene-environment correlation assumes that the genetic make-up of an individual is linked to certain environmental risk factors for disruptive behaviour. This way, one could observe an association between the environment and child behaviour, while in fact this effect of the environment (also) represents a genetic predisposition. Further, in gene-environment interaction the genotype of an individual affects its response or vulnerability to certain environments. This mechanism may therefore help to explain why some individuals appear to be more susceptible to certain environmental factors than others. In our studies of gene-environment interplay, we focussed on environmental factors both before and after birth, using data from the ALSPAC-cohort. In summary, we used the ALSPAC data to design a study in which we considered gene-environment interactions (and correlations) between all approximately 20,000 protein-coding genes in the human genome and a number of previously identified environmental factors for disruptive behaviour. The results of this study (currently still unpublished) indicate that indeed a multitude of genes interact with environmental factors in relation to disruptive behaviour rather than only a few genes. Different genes interacted with different environmental factors, yet also overlap in interacting genes was seen. In addition, we obtained evidence pointing to certain biological pathways that may underlie the observed gene-environment interactions in disruptive behaviour. Further – again in collaboration with WP9 and WP1 – we have compared the genetic and epigenetic results from the other WP animal models of aggression with our findings in humans. We found that the genes that were implicated through both animal and human studies of aggression are involved in specific biological pathways, and point to proteins that have been recently implicated in stress-related and behavioural psychiatric disorders.

The data from WP6 examining the acute effects of drugs confirm the efficacy of methylphenidate and atomoxetine on the modulation of attentional skills and inhibition, improving the accuracy of performance and the ability to discriminate affective stimuli as well. Further analyses might clarify the results relating to the administration of D-2 modulating agents. However, no administered drug resulted in a significant deterioration in performance compared to baseline and placebo. These findings are in line with those from our data-mining research, which highlighted the efficacy of these medications (mainly Methylphenidate and Risperidone) on aggressive symptoms in the context of CD. In addition, in ODD/CD adolescents living in residential youth care, empathy was increased after oxytocin administration compared to placebo and this effect was specific to individuals with high callous-unemotional traits. There was no effect of intranasal oxytocin on the overall emotion recognition, but there was a positive effect on accuracy of fear recognition. Trauma and dissociation did not moderate the oxytocin effect on empathy or emotion recognition. Our findings provided evidence of a beneficial effect of oxytocin on empathy and fear recognition in residential youth. Taken together, these results contributes to validating the indications of the most important guidelines on the management of antisocial behaviour and conduct disorders in children and young people (NICE Clinical guideline, 2017), which state to offer, when psychosocial interventions are not significantly effective and pharmacological treatment is needed, methylphenidate or atomoxetine for the management of ADHD in children and young people with oppositional defiant disorder or conduct disorder, and to consider risperidone for the short-term management of severely aggressive behaviour in young people with a conduct disorder. Furthermore, the data from WP6 confirm that clinical studies involving oxytocin are warranted to further evaluate its clinical utility in light of its ability to improve empathy and fear recognition in residential youth with ODD/CD.

WP7 demonstrated the feasibility and efficacy of both individualized skin conductance biofeedback (SC-BF) as well as individualized real time function magnetic resonance imaging neurofeedback (rtfMRI-NF). Effects were larger in the SC-BF trial, which may partly be attributed to the younger age of participants in this trial and selection for high CU-traits and thus more severe cases in the rtfMRI-trial.

The time course of clinical outcomes up to 6 month follow-up in both the rtfMRI-NF as well as the SC-BF group was comparable to their respective TAU control groups, suggesting non-inferiority of the experimental treatments. Results suggest partly sustained clinical improvement for SCL-BF, rt-fMRI NF and TAU mainly in older individuals with elevated CU traits as included in the rt-fMRI NF trial. None of the post-hoc tests show a significant deterioration from treatment end to FU, but further controlled investigation in larger samples is needed as some scales descriptively show non-significant

increase of symptom scores at FU compared to post-treatment assessment. Further, the statistics from the small subgroups with completed FU questionnaires are preliminary and clearly highly exploratory. Still, it is encouraging that the results for the MOAS as the primary study outcome suggested that overt aggression was reduced at follow-up compared to baseline in the rt-fMRI NF trial, consistent with sustained or delayed improvement following the treatments.

Given the increasing evidence for impaired parental care in the aetiology of ODD/CD, WP8 examined the impact of maternal immune activation (MIA) during foetal development which leads to behavioural abnormalities in offspring that are associated with psychopathy and psychiatric disorders. Using iPSCs derived from typically developing individuals, we have developed and characterised a human cellular model of MIA. Specifically, WP8 has differentiated iPSCs towards a forebrain fate and have tested the hypothesis that exposure to specific antiviral agents at specific time points alter the development of neuronal cells and causes a transcriptomic signature similar to that seen in neurodevelopmental disorders. Exposure of iPSC-derived neural progenitor cells to IFNγ activates an antiviral transcriptional state involving dramatic upregulation of the antigen presentation (MHC class I; MHCI) pathway and leads to enduring morphological abnormalities in postmitotic neurons. MHCI overexpression is a hallmark of MIA in rodent models. Interestingly, exposure of iPSC-derived neural progenitor cells to IFNγ also resulted in a neurite outgrowth phenotype similar to that observed in iPSC-neuron derived from individuals with ASD. Namely, a transient exposure to IFNγ increases neurite outgrowth. IPSC-derived neural progenitor cells exposed to IFNγ also demonstrated a persistent increase in the expression of MHCI genes. The persistent increase in MHCI gene expression is dependent on the formation of nuclear entities known as “PML bodies”. These protein rich nuclear complexes coordinate the expression of MHCI gene in response to IFNγ. The expression of PML bodies is persistently increased following transient IFNγ exposure. Interestingly, we found that MHCI protein expression at growth cones was further enriched following exposure to IFNγ exposure. Critically, disruption of PML bodies using As2O3 concurrent to IFNγ treatment prevents IFNγ-dependent abnormalities in neuronal morphology and MCHI enrichment in growth cones. Similar effects were seen when we blocked the expression of B2M – an adaptor protein required for surface expression of MCHI proteins, either using an shRNAi approach or in human ES lines where B2M has been knocked out by gene-editing methods. We also examined the relevance of IFNγ-induced transcriptional effects for neurodevelopmental disorders. The immediate and persistent transcriptional signature induced by a transient exposure to IFNγ is highly enriched in differentially expressed genes that significantly increase risk of developing ASD or schizophrenia. Moreover, IFNγ-induced differentially regulated genes are also differentially expressed in ASD and schizophrenia, but not other disorders, based on post-mortem work. Finally, we found that MHCI and PML expression is increased in post-mortem cortex from ASD and schizophrenia individuals, and moreover that iPSC-derived neural progenitor cells have increased expression of PML and furthermore, demonstrated an exacerbated response to IFNγ. Furthermore, in utero exposure to cellular stressors such as cortisol are thought to increase risk of developing neurodevelopmental disorders including ADHD/CD. Using induced pluripotent stem cells (iPSC) derived from healthy individuals, we have developed and characterised a human cellular model to study the impact of exposure to in utero of stress, and furthermore the impact of methylphenidate either alone or following co-treatment with cortisol. Treatment with cortisol on neural progenitor cells or neurons from healthy iPSCs, results in altered expression of genes involved in neural differentiation and synaptic function. Acute treatment with methylphenidate alone does not appear to alter the expression of synaptic glutamatergic gene expression. Co-treatment with methylphenidate attenuates cortisol-driven changes in some genes but does not affect the expression of others. Treatment of neural progenitor cells derived from ASD/ADHD iPSCs results in exacerbated expression of genes involved in MHCI signalling and synaptic function; these effects are blunted by methylphenidate co-treatment.

WP9 has demonstrated that the genetic liability for aggression is related to structural connectivity in some of the major white-matter tracts. In addition, genetic risk scores for aggression also appeared to affect the relation between environmental stressors in childhood and structural connectivity in the brain. WP9 further investigated the genetics and biological mechanisms involved in gene-environment interactions in disruptive behaviour. To this end, we have compared the genetic and epigenetic results from the WP1 animal models of aggression with our findings in humans. We found that the genes that were implicated through both animal and human studies of aggression are involved in specific biological pathways, and point to proteins that have been recently implicated in stress-related and behavioural psychiatric disorders. Summarizing, these results show that the ethology of aggression and disruptive behaviour disorders is multifactorial and involves a broad range of both genetic and environmental risk factors. Genetic risk factors may act on certain pathways, may affect each other, and as we have showed in particular, may affect an individual’s response to the environment (i.e. gene-environment interaction). As such, an individual’s genetic make-up and environmental exposures should not only be considered independently but also in relation to each other. Furthermore, genetic liability for aggression appears to be related to variation in structural connectivity in the brain, which may point to neural abnormalities that could play a role in

behavioural problems. Still, much work remains to be done and future studies should be conducted to validate and extend some of the current findings, to gain further insights into the ethology of aggression and disruptive behaviour in children and adolescents.

The main contribution of WP10 undoubtedly lies in the hypothesised integrated causal model of aggression based on human clinical and population datasets. It shows the key target variables of the MATRICS project: impulsive (reactive) vs. instrumental (proactive) aggression and their relation to various forms of conduct problems, in combination with important behavioural and personality traits and other contextual factors. A summary of the most important insights and conclusions captured by the global model:

- there is strong and consistent statistical evidence for a causal link from inattention to aggressive behaviour that is persistent over different age groups and different cohorts,
- this link is present for both reactive and proactive types of aggression, where reactive aggression is most strongly linked to oppositional behaviour (ODD), and proactive aggression to more serious conduct problems (CD): together they point to the fact that two different types of inattention may be in play,
- the link from inattention to reactive aggression seems to originate from a misreading of social cues, whereas the link to proactive aggression signifies a disinterest in social norms / other people, that in turn is influenced by callous/unemotional traits and (partially) mediated by a rule breaking disposition,
- perhaps surprisingly both substance abuse and anxiety appear mainly as side-effects of aggressive behaviour and psychological problems rather than as driving factors in themselves,
- in contrast, contextual factors such as age, gender, and crucially familial circumstances are seen to play an important role in driving or moderating various forms of aggression and behavioural problems,
- gene analysis (GWAS) resulted in a number of genome wide significant candidate genes, most importantly CTD-3194G12.2 (linked to ODD/reactive aggression) and SLC12A8 (linked to CD/personal aggression);
- causal results from the animal experiments suggest that the brain metabolites GABA and glutamine play a mediating role between genetic variation and aggression related behavioural traits.

We are particularly excited by the discovery and confirmation of the different types of inattention that play a role in the relation to both proactive and reactive aggression. Furthermore, the mediating traits on the separate causal paths should provide promising starting points for new treatment strategies. Naturally, we want to stress that the hypothesised global causal model presented here is only the most likely coherent explanation for the statistical patterns observed in WP10: additional experimental verification is required to validate our results. However, the main conclusions proved extremely stable over all our largest and most reliable data sets, and we are confident that such confirmation can eventually be found.

3.2 Main results / foreground of the different work packages

WP01: Animal models

Foreground

WP1 aims to assess the underlying mechanistic, epigenetic and MRI constructs linking discrete neural substrates to aggression in preclinical animal models (WP01-02, 05). In this period, we aimed to build on the earlier observations of the aggression phenotype across different preclinical models (BALB/cJ mice; peripubertal stress (PPS) rat; STX knockout mouse, rat lines with low / medium / high levels of endogenous corticosterone tone (CORT lines), high and low empathy-like subgroups of BALB/cJ mice and differential perinatal corticosterone administration in mice) by additional phenotyping (e.g. in attention tasks) and characterising the neural (MRI), metabolic (1H-MRS), and epigenetic profiles (WP01-02, 05). By the utilisation of high-field magnetic resonance in vivo and ex vivo MRI / 1H-MRS studies in the BALB/cJ versus BALB/cByJ mice, the Tph2 knockout mouse (in collaboration with Prof. K.P. Lesch, Aggressotype consortium), the PPS rat, low, intermediate and high corticosterone (CORT) expression lines and low / high empathy-like behaviour in BALB/cJ mice (WP01-02).

Results

WP01 has established an aggressive and antisocial phenotype in the BALB/cJ mouse which is accompanied by high anxiety, inattention and reversal learning deficits. These aggressive, inattentive and anxiety changes are reversible by administration of the psychostimulant methylphenidate (also under investigation in humans in WP06) and points towards an improvement in sustained attention underlying anti-aggressive effects. In vivo MRI / 1H-MRS studies of this mouse model demonstrate decreased fractional anisotropy diffusion tensor imaging (DTI) markers and GABA tone in the anterior cingulate cortex and striatum. These changes in cortical inhibition are confirmed in (WP05) transcriptomic expression changes in GABA anabolism and catabolism in two independent animal models (BALB/cJ mice and (peripubertal stress) PPS rats). Furthermore, BALB/cJ mice demonstrate cingulate cortical GABA interneuron cell loss, particularly of parvalbumin and somatostatin classes. Peripubertal stress is not only associated with increased aggression but also changes in cortical and amygdala markers of GABAergic activity. However ex-vivo brain MRI imaging in WP02 of the BALB/cJ model has documented marked volumetric and white matter changes in regions involved with ‘valuation’ such as the anterior cingulate and insular cortices and registration of (autonomic) information via the periaqueductal grey (PAG) matter region. Documentation of WP02 ex vivo MRI changes in structural, volume or DTI measures in the PPS model demonstrates clear changes in regions involved in stress reactivity. WP01 has also demonstrated that BALB/cJ mice display insensitivity to punishment learning (analogous to that seen in humans with high callous unemotional traits). Furthermore, decreased fear conditioning in BALB/cJ mice that show less 'empathic-like' licking behaviour in response to the distress of another animal is normalised by treatment of oxytocin that has also been paralleled in human clinical pharmacology studies in WP06 where oxytocin administration has clear effects on emotional recognition. Interestingly, further studies on methylphenidate in the low-empathy like BALB/cJ mice demonstrate it normalised sociality, aggression and behavioural stereotypies, thereby ameliorating the phenotype of mice characterised by deficient empathy-like behaviour. Besides, MPH produced the expected dose-dependent increment of locomotor activity and anxiety-like behaviour, while positively modulating the performance in the attentional set-shifting task (CDR stage) in control mice. As such MPH may constitute a valid therapeutic approach in disturbances characterised by abnormal aggression and poor sociability. Clonidine was tested in the aggressive STX knockout model and found to have no effect on aggressive behaviour without inducing hypo-locomotion. Furthermore, knockout of KCNQ1, a gene involved in the inhibition of insulin release had no effect on anxiety or aggressive behaviour.

WP02: Animal imaging readouts

Foreground

Various brain structures have been implicated in aggressive and non-aggressive antisocial behaviours and in conduct disorders (CDs) in humans, such as the prefrontal cortex, amygdala, striatum and the hippocampus. Although many imaging studies have to date been conducted, they seldom utilise specific clinical sub-populations that would enable us to make inference about specific mechanisms underlying aggression, antisocial behaviour and CDs, and that could deconstruct different types of aggression by relating these to the specific brain systems that underlie each type based on e.g. different arousal mechanisms. The work packages in MATRICS have attempted to achieve this by also undertaking additional parallel examinations of equivalent behaviours and phenotypes in experimental animal models of aggression

and CDs. Firstly, the animal models were carefully chosen and extensively characterised for their behavioural, genetic and molecular phenotype (in WP1). Then, four models were chosen to represent various facets of human aggression/CDs in which to test hypotheses related to development and mechanism of the disorders as well as to evaluate experimental pharmacological interventions. In WP2, we performed high resolution 3D MR imaging of the brains of all four models to ascertain which brain circuits might be implicated, and to see how these data relate to the findings from human clinical populations. To this end, multiparametric MRI was used to identify the neural underpinnings of aggressive behaviour and to reveal potential targets for therapy. Importantly, the structural imaging protocols were closely aligned to the equivalent imaging protocols utilised in clinical work packages within MATRICS. The four rodent models tested were: peripubertal rat stress model, corticosterone adaptation stress test ("CAST") rat model, BALB/cJ and BALB/cByJ mouse model of aggression, and a mouse model of high and low empathy-like traits within the BALB/c strain. The objectives were:

- To characterise changes in a) morphometry, b) white matter integrity, and c) tissue microstructure in the fronto-striatal and fronto-limbic circuits in four rodent models of aggression and/or stress, as determined in WP1, with similar neuroimaging protocols as in WP3-4.
- To determine whether and which of the behavioural & autonomic responses, and histological markers, are associated with changes in tissue morphology, regional volumetric changes or relaxivity parameters as determined by multi-parametric MRI.
- To test the MRI data for their ability to predict and describe the aggressive phenotype, using machine learning, as outlined in WP10.

During this investigation we explored the link between genetic and developmental stress/aggression and changes in brain volumes (global and regional), relaxometry (T1, T2) and diffusivity (MD, FA) parameters in both grey and white matter. We detected structural changes in brain regions associated with aggression in all models, which highlights the link between stress/aggression phenotype and the brain microstructure. This information is currently being used to construct a combined fingerprint of imaging and corroborative readouts as biomarkers of aggression related brain circuits.

Results

Amongst significant findings we identified a pattern of volumetric ("morphometric") regional brain changes in all four animal models, only some of which were common to all. Thus, in the rat peripubertal stress model, the predominant result was an abnormal tissue microstructure in the brain areas associated with aggression including hippocampus and amygdala. Similarly, in the high/low corticosterone reactivity rats (CAST), hippocampus was the area of the brain most significantly associated with aggression-related abnormalities. Both findings represent an important step towards establishing a causal link between development and aberrant glucocorticoid adaptation to stress as an underlying mechanism. We also detected widespread changes including the hippocampus, in the mouse models of aggression and high-low empathy like traits. Additional brain areas that were affected point toward circuits governing olfactory-based fear response (to e.g. predatory odour), communication of pain and autonomic stress (in high/low empathy-like trait mice), as well as striato-limbic systems. The similarities between abnormal brain areas corroborate that common circuits are indeed affected in these models, but the differences point toward the more specific and nuanced array of developmental brain alterations that arise in response to a particular combination of genetic, environmental, or combined factors that are responsible for emergence of CD-related phenotype.

WP03: Human Neuroimaging

Foreground

Various brain structures and functions have been implicated in aggressive and non-aggressive antisocial behaviour in human studies, such as the prefrontal cortex (with abnormalities localized to the orbitofrontal cortex, anterior cingulate and dorsolateral prefrontal cortex), and the amygdala, insula and the ventral striatum. However, findings are heterogeneous, based on small samples, and to a large extent based on studies of adults with antisocial personality disorder, psychopathy and/or violent behaviours. It is unclear whether these findings generalize across the various aggression phenotypes in children and adolescents with CD/antisocial behaviour. For example, the classic distinction between childhood onset and adolescent-onset aggression does not map onto clear differences in functional and structural brain imaging data. Male adolescents with both early onset and adolescent onset CD displayed grey matter volume reductions in the bilateral amygdala, extending into the insula, relative to healthy comparison subjects. Further, both CD subtypes displayed reduced responses in the amygdala, when comparing angry vs neutral faces, and had an increased amygdala response to neutral faces. Only for sad vs neutral faces, reduced amygdala activation was observed in early relative to adolescent

CD, which might indicate why early onset-CD is more severe and persistent than adolescent-CD. Female adolescents with CD showed about similar structural abnormalities as males with CD. However, the contrast high vs low CU traits was found to be associated with differential activations of the amygdala, with high CU traits within CD linked to amygdala hypoactivity to consciously and even pre-attentive perceived fear, and low CU traits linked to amygdala hyperactivity. Patients with CD and high psychopathic traits had lower orbitofrontal responsiveness both to early stimulus-reinforcement exposure and to rewards, as well as less caudate response to early stimulus-reinforcement exposure. There are no imaging studies that have examined the differential correlates of impulsive versus affective aggression in CD.

Other neural correlates of CD are reduced cortical thickness and folding deficits over various cortical areas, and white-matter microstructural abnormalities in the anatomical tract that connects the amygdala and orbitofrontal cortex. The imaging literature thus suggests that the integrated functioning of the orbitofrontal cortex, anterior cingulate, amygdala, and striatum is abnormal in CD.

Thus, in summary, human neuroimaging studies on the neural correlates of CD in the presence and absence of CU traits, and on reactive vs. impulsive aggression are absent or few, and based on small samples.

Given this foreground, WP03 has pursued the following specific objectives:

1. Identify neural, neurocognitive, and biomarker mechanisms underlying aggressive / antisocial behaviour and CU traits in a longitudinal population-based cohort of adolescents.
2. Identify neural, neurocognitive, and biomarker mechanisms underlying aggressive / antisocial behaviour and CU traits in high-risk children and adolescents (subjects with ADHD) and controls cross-sectionally and longitudinally.
3. Collect a new cohort of children with CD, adolescents with CD, and controls (N=180) and identify neural, neurocognitive, and biomarker mechanisms underlying the aggressive / antisocial behaviour and CU traits.
4. Integrate findings from objectives 1-3, and examine the common (cross-disorder) and the disorder-specific correlates of the aggressive / antisocial behaviour and CU traits.

Results

Objective 1:

- A population-based neuroimaging study found that hyperactive amygdala responses and regions critical for top-down emotional processing to social-emotional stimuli were associated with high levels of psychosocial stress in individuals with greater conduct and hyperactivity/inattention symptoms. This work highlights the importance of studying how psychosocial stress affects functional brain responses to social-emotional stimuli, particularly in adolescents with externalizing symptoms (Quinlan et al., 2017).
- We examined whether childhood family adversity (CFA) and the presence of conduct disorder (CD) would converge in influencing affective processing in the ventral striatum (VS) and amygdala. Findings indicate that CD mediated the effect of CFA on brain activity in the social-emotional brain (Holz et al., 2017).

Objective 2:

- Comorbid ODD/CD is associated with lower FA in left fronto-temporal and striatal WM, which appeared independent of ADHD symptoms, and is dimensionally associated with antisocial behaviour in ADHD+ODD/CD, but not in ADHD-only (van Ewijk et al., 2016).
- We also compared ADHD to ADHD plus ODD/CD on neurocognitive functioning: ADHD+ODD was associated with more and more severe abnormalities in tests of cool EF, hot EF and temporal processing. (Noordermeer et al., 2015)
- We have analysed the relation between CU traits, antisocial behaviour, and brain activation on the reward anticipation task. The hypothesis about the link between aggression and striatal activity has not been confirmed. In healthy controls, high CU traits predicted reduced hippocampal engagement (Veroude et al., 2016).
- In the analysis of the structural MRI VBM data of the NeuroIMAGE cohort, we found that ADHD+ODD/CD and ADHD-only showed volumetric reductions in several, mainly frontal, brain areas. Stepwise significant volumetric reductions (ADHD+ODD/CD<ADHD-only<Controls) were found in the orbitofrontal gyrus, middle frontal gyrus, right superior frontal gyrus, and left inferior parietal gyrus. ADHD+ODD/CD-specific volumetric reductions were found in the right precuneus and left middle temporal gyrus (Noordermeer et al., 2017).

Objective 3:

- We have established a database of the neuroimaging study in children and adolescents with aggressive behaviour and controls, with a total of 158 cases (mean age 13.0 years, SD 2.8, 130 boys, 28 girls) and 96 controls (mean age 13.5 year, SD 2.6), 55 boys, 41 girls). Data include an extensive phenotype battery, MRI (all modalities including MRS), and cognitive tests.
- Analysis of the structural MRI data from the new cohort showed differential associations for reactive and proactive aggression with the volumes of neural structures. Reactive aggression was negatively associated with insula volume, while proactive aggression was negatively associated with amygdala volume. Thus, the findings support the hypothesis that reactive and proactive aggression was differentially related to brain regions involved in threat response and empathy, respectively (Naaijen et al., submitted).
- Magnetic resonance spectroscopy results from the new cohort showed a positive association between callous unemotional traits and glutamate concentrations in the anterior cingulate cortex as well as a negative association between proactive aggression and glutamate concentrations in the dorsal striatum. This suggests a central role for the neurotransmitter glutamate in regulating aggressive behaviour as part of the fronto-striatal network (Craig & Muller et al., 2019).
- Resting state functional connectivity, as measured in the new cohort, was shown to be altered in cases with ODD and/or CD as compared to controls. The default mode network (DMN) and salience network (SN) showed reduced functional connectivity with left hemispheric frontal clusters. Additionally, aggression subtype-specific patterns were found; reactive and proactive aggression correlated with distinct DMN and SN seed based functional connectivity patterns, while CU traits showed different connectivity patterns with frontal, parietal and cingulate areas (Werhahn et al., 2020, submitted).
- Negative emotional face recognition, as shown before, was associated with higher amygdala activity in children and adolescents with ODD/CD. Using median-split cut-offs for callous unemotional traits showed decreased amygdala activity in the high CU group. In addition, skin conductance was lower in the ODD/CD group and was negatively associated with CU traits. These analyses highlight the importance of taking CU traits into account to address subtype-specific amygdala activation and physiological responses (Aggensteiner et al., 2020, submitted).
- Using CANTAB tests, visual working memory and inhibitory control were impaired in youth with ODD and CD compared to healthy controls. Impaired recognition of disgust, fear, happiness, and sadness were specific to CD, whereas impaired anger recognition was also present in ODD. Worse visual working memory was associated with worse recognition of all basic emotions. The impairments were not explained by comorbid ADHD or internalizing symptoms (Kleine Deters et al. 2020, submitted).

Objective 4:

- We conducted integrative analyses across multiple data-sets to investigate aggressive behaviour as part of an ADHD sample (NeuroIMAGE), a population sample of adolescents (IMAGEN) and a data-set with participants with conduct disorder and oppositional defiant disorder (CD and ODD), the MATRICS new cd-cohort. We found no associations of aggressive behaviour with decision making processes but found a role for ADHD symptomatology (Portengen et al., 2020, submitted).
- In the population sample, two symptom groups, consisting of anxiety/depression and executive dysfunction symptoms, respectively, were correlated with distinct sets of brain regions and inter-regional connections, measured by structural and functional neuroimaging modalities. These neural correlates showed case–control differences in corresponding psychiatric disorders, depression and attention deficit hyperactivity disorder in independent clinical samples (Ing et al., 2019).

Conclusions

Results from WP03 show differences between children/adolescents with ODD and/or CD and typically developing controls with regard to several neuroimaging modalities. Using the NeuroIMAGE cohort, we showed reduced fractional anisotropy in fronto-temporal and striatal white matter to be associated with anti-social behaviour independent of ADHD symptoms. Additionally, neurocognitive functioning appeared to be more severely impaired with the presence of ODD and/or CD within ADHD participants.

Using the new cohort, we have been able to use aggression subtype-specific analyses, showing the importance of including callous unemotional traits and the distinction between reactive and proactive aggression. Structural and

functional imaging analyses revealed differences regarding these subtype specific measures. In addition, for the first time, we showed an involvement of glutamatergic neurotransmission to be related to continuous measures of aggression. Our findings support the idea of subtype-specific impairments in aggression, where different brain regions are involved in empathy, threat response and decision making which are in turn more associated with either proactive aggression, reactive aggression and CU-traits. This may have implications for designing targeted intervention strategies, which needs to be further explored in future studies

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WP04: Human Neurochemistry

Foreground

Disruptive Behaviour Disorders (DBDs) and aggression, represent a significant social economic and personal burden, with a 5 to 10-fold risk of later substance abuse, criminality, employment and early death.[1,2] It is increasingly recognised that individuals with DBD are not a homogenous group, and that these problems may arise from distinct traits and mechanisms. To date several trait markers and behaviours have been consistently associated with poorer outcome. In particular, callous-unemotional (CU) traits, including limited empathy, reduced guilt and shallow affect, have been linked to particularly persistent and violent patterns of antisocial behaviour.[3] The utilisation of proactive aggression, 'cold' purposeful and deliberative aggression employed to achieve one's goals, has also been highlighted as a risk factor. Further, both of these risk factors have been shown to co-occur in individuals.[4] Problematically, such individuals also

appear to be marked by resistance to current treatments.[5] Understanding the chemical basis for these problems within the brain has therefore been deemed a priority, as this could be used to lay the groundwork for future pharmacological interventions.

Previous work has implicated several brain regions in DBDs, in particular the amygdala, the anterior cingulate/medial prefrontal cortex (ACC/mPFC), the striatum and the insula.[6,7] Glutamate is the primary major excitatory neurotransmitter in these regions, and can be measured *in vivo* using proton magnetic resonance spectroscopy (1H-MRS). We therefore aimed to assess neurochemical differences in these regions in youths with DBDs.

We included 245 participants (*n* = 148 cases, and *n* = 97 typically developing) aged 8-18. These children were diagnosed with either a specific DBD (i.e. conduct disorder or oppositional defiant disorder), or met a clinical threshold or aggressive or rule breaking behaviours.[8] We also measured CU traits[9] and proactive aggression[10] to see whether these related to measures of glutamate. We measured glutamate concentration in the ACC/mPFC, the striatum, insula and amygdala using 1H-MRS.

Results

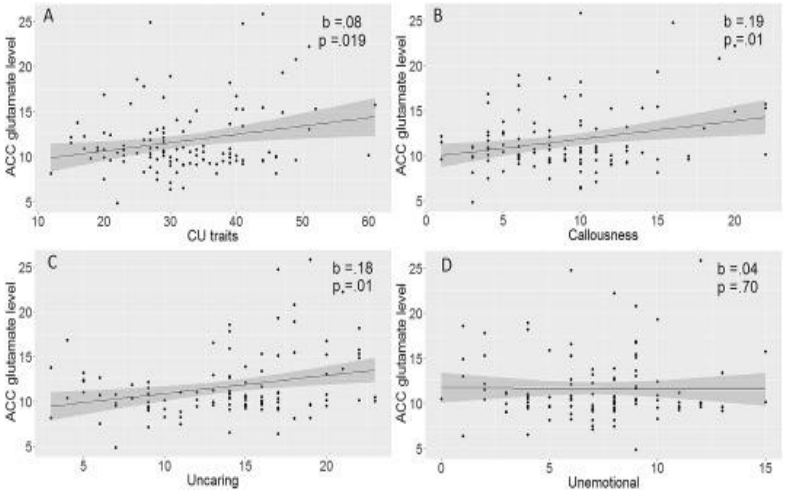

Figure 2. Positive association between CU traits and glutamate levels in the ACC/mPFC in cases only.

Relationship between glutamate, callous-unemotional traits and proactive aggression: We first examined the relationship between glutamate and our two clinical measures of interest, CU traits and proactive aggression in the DBD group. We found a positive association between CU traits and glutamate in the ACC/mPFC (b = .08, t (113) = 2.38, p = .019, r = .22). As CU traits are actually constituted by several 'subscales', we also measured the relationship between these and glutamate in the ACC/mPFC. This suggested that glutamate was specifically related to 'callous' (b = .19, t (108) = 2.63, p = .01, r = .25) and 'uncaring' (b = .18, t (108) = 2.59, p = .011, r = .24), but not 'emotional' CU traits (b = .04, t (108) = .39, p = .70; Figure 2).

We also found a negative association between proactive aggression and glutamate concentration in the striatum (b = −.23, t (28) = -3.02, p = .005, r = .50; Figure 3) in the DBD group.

We found no associations between our clinical measures of interest and glutamate in the insula or amygdala.

Group differences: We found a trend towards increased glutamate in the ACC/mPFC in the DBD group compared to controls (*p*=0.08), and a trend towards decreased glutamate in the striatum in the DBD group when compared to controls (*p*=0.06). Neither of these met full statistical significance however. See[11] for further results and methodological information.

Discussion

These findings suggest that dysfunctional levels of glutamate within the brain play a role in CU traits and proactive aggression. As these behaviours represent particularly severe and difficult to treat subtypes of CU traits,[5] these findings may form the basis of novel pharmacological targets for treatment resistant individuals. Our work also highlights the need to further dissociate CU traits and proactive aggression future studies, as they are associated with glutamate concentrations in different regions.

Our findings in the ACC/mPFC are of particular translational promise, as they support an emerging neurochemical theory of aggression. Specifically it has been suggested that aggression is associated with differences in the balance of chemical excitation and inhibition in this region that favours increased excitation.[12] We provide evidence for this hypothesis in the present study, and in an animal model of CU traits analysed within the same consortium.[12] This suggests that such joint animal and human work will play an important role in developing novel treatments for the treatment-resistant aggression subtypes that we have examined in WP4.

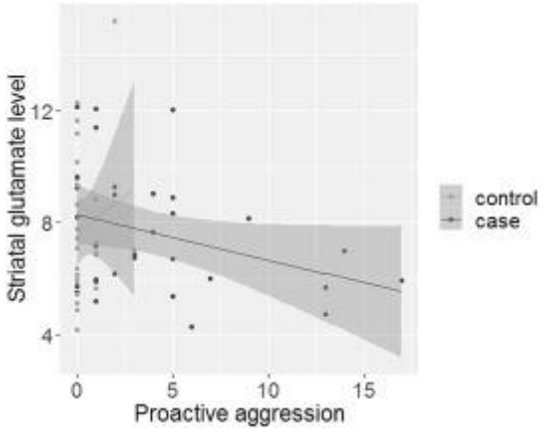

Figure 3. Association between proactive aggression and glutamate levels in the striatum in cases and controls separately.

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WP05: Genomic, epigenomic, transcriptomic and microRNA markers

Foreground

Aggressive behaviour is a common feature of several child and adolescent psychiatric disorders including attention-deficit/hyperactivity disorder (ADHD), conduct disorder (CD) and oppositional defiant disorder (ODD). These complex disorders are highly polygenic and epidemiological research suggests that prenatal environmental insults or stressful events during childhood also play an important role. WP05 aimed to explore genomic variation underlying aggressive behaviour using studies in human cohorts and the three validated rodent models described in WP01. First, we used whole genome sequencing (WGS) to identify DNA sequence differences and reduced representation bisulphite sequencing (RRBS) to identify methylomic variation across multiple brain regions associated with aggression in these models. We profiled multiple brain regions from the three rodent models of aggression: a) the anterior cingulate cortex (ACC), middle cingulate cortex (MCC) and ventromedial hypothalamus (VMH) from BALB/cByJ and from BALB/cJ mice; b) the prefrontal cortex (PFC), hippocampus (HC) and hypothalamus (HPT) of BALB/cJ mice exposed to two doses of corticosterone; and c) the PFC, HC and HPT from three rat lines with a differential aggressive response to corticosterone. This work represents the most systematic investigation of gene regulation in rodent models in aggression, and highlights novel mechanistic pathways that can be explored in future research. As a resource to the community, we have generated a list of genomic sites associated with aggression that can be further interrogated for a role in aggression. Second, we explored overlaps between the genes and networks highlighted in our analyses of rodent models with those identified in studies of human cohorts, building on the work undertaken in the WP09 studies together with WP01.

Results

Identification of DNA sequence variation in rodent models of aggression. WGS identified 11,019 robust SNVs between the BALB/cJ and BALB/cByJ strains. Importantly, three of these variants are predicted to have a 'high impact' on protein function or structure (i.e. variants in the coding regions of *Acvr1c*, *Gm13030*, and *Nlrp5-ps*). These represent excellent candidates for future characterization and can be considered novel candidate genes for aggression that can be explored in human and other model datasets. 76 of the identified SNVs are predicted to have a 'medium impact' and the

remaining SNVs have low or no predicted effect on protein function or structure. Of note, we found 22 variants annotated to 10 genes previously implicated in aggression, ADHD and compulsivity, including *Mecom*, *Shank2*, *Avpr1a* and *Rbfox1*. In the CORT rat model, we identified ~16,000 SNVs between the 'low' and 'high' CORT lines, which were annotated to 770 genes. Strikingly, these genes are highly enriched for pathways and functions relevant to aggression and childhood psychiatric disorders. For example, the top ranked OMIN disease category enriched amongst these genes is 'autism' (odds ratio: 4.11; P = 0.0347). Furthermore, KEGG pathway analysis showed a significant enrichment for pathways relevant to CD and aggression including 'long term potentiation' (odds ratio = 3.11; P = 0.004) and 'dopaminergic synapse' (odds ratio = 2.31; P = 0.006). The integration of these gene lists with the Human Cell Atlas demonstrated that the genes harbouring SNVs between the 'low' and 'high' CORT strains were enriched for multiple brain regions including the amygdala (P = 0.0002) and prefrontal cortex (P = 0.01). Similarly, we identified ~20,000 SNVs annotated to 783 genes between the 'low' and 'intermediate' CORT strains. Again, these genes are highly enriched for pathways and functions relevant to aggression and childhood psychiatric disorders. They are significantly enriched for amygdala-expressed genes (P = 0.03). Taken together, our WGS findings suggest that the genetic differences identified in rodent models of aggression directly reflect pathways relevant to CD and neuropsychiatric traits in human cohorts. In addition to providing a novel reference genome resource for the wider research community, these data highlight genetic differences between the strains that potentially underpin the differences observed in aggressive behaviour.

Reduced representation bisulphite sequencing identified epigenetic variation in the brain associated with aggression. We identified multiple differentially methylated positions (*P*<0.001) between the BALB/cJ and BALB/cByJ mouse strains in each of the three brain regions profiled using RRBS: 368 (annotated to 28 genes) in the VMH, 143 (annotated to 10 genes) in the MCC and 26 (annotated to 9 genes) in the ACC. The majority of the DMPs were not in proximity to any of the between-strain SNVs identified in the WGS analyses, indicating they are not likely to be influenced by single nucleotide genetic variations acting in cis. Of note, there were several common loci showing strain-specific DNA methylation patterns across the three brain regions. Furthermore, several of the genes annotated to these sites have been previously implicated in intellectual disability and aggression – for example *Jarid2* and *Trio*. We also used bioinformatics approaches to explore broader differentially methylated regions (DMRs) between the two BALB strains. The most striking DMR, covering multiple adjacent DMPs, was identified on chromosome 17 in both the VMH and MCC. The region spans 273 (VMH) / 99 (MCC) hypomethylated CpGs covering two CpG islands and overlapping the *Gm26917* and *Gm42418* predicted genes and *AY036118*, which encodes a transcription factor. We also identified widespread methylomic differences in the prefrontal cortex (PFC), hippocampus (HC) and hypothalamus (HPT) of BALB/cJ mice exposed to two doses of corticosterone: 298 in the hippocampus, 13 in the hypothalamus and 1 in the frontal cortex. Genes annotated to the top-ranked loci in the hippocampus included a number of genes previously implicated in aggression and related traits such as *Rbfox* and *Kcnn3*. Finally, we also identified multiple differentially methylated positions between the three rat lines with differential aggressive response to corticosterone: 11 in the hippocampus, 454 in the hypothalamus and 396 in the frontal cortex. Genes annotated to the top-ranked loci in the hippocampus included a number of genes previously implicated in aggression and related traits such as *Foxp2*.

Integration of rodent genomic data with GWAS and GXE findings from human cohorts. The interplay between environmental and genetic factors related to disruptive behaviour is expected to play an important role and contribute to the associations of environmental factors with aggression and disruptive behaviour. Therefore, in collaboration with WP9 (UMCG), we conducted research to investigate specific ways by which genetic and environmental factors could affect each other. These mechanisms include 'gene-environment correlation' and 'gene-environment interaction'. Gene-environment correlation assumes that the genetic make-up of an individual is linked to certain environmental risk factors for disruptive behaviour. This way, one could observe an association between the environment and child behaviour, while in fact this effect of the environment (also) represents a genetic predisposition. Further, in gene-environment interaction the genotype of an individual affects its response or vulnerability to certain environments. This mechanism may therefore help to explain why some individuals appear to be more susceptible to certain environmental factors than others. In our studies of gene-environment interplay, we focussed on environmental factors both before and after birth, using data from the ALSPAC-cohort. In summary, we used the ALSPAC data to design a study in which we considered gene-environment interactions (and correlations) between all approximately 20,000 protein-coding genes in the human genome and a number of previously identified environmental factors for disruptive behaviour. The results of this study (currently still unpublished) indicate that indeed a multitude of genes interact with environmental factors in relation to disruptive behaviour rather than only a few genes. Different genes interacted with different environmental factors, yet also overlap in interacting genes was seen. In addition, we obtained evidence pointing to certain biological pathways that may underlie the observed gene-environment interactions in disruptive behaviour. Further – again in collaboration with WP9 and WP1

– we have compared the genetic and epigenetic results from the other WP animal models of aggression with our findings in humans. We found that the genes that were implicated through both animal and human studies of aggression are involved in specific biological pathways, and point to proteins that have been recently implicated in stress-related and behavioural psychiatric disorders.

WP06: Drug treatment for aggressive behaviours in CD

Foreground

Problems of aggression, oppositionality, and impulsivity, with or without attention deficit or hyperactivity, constitute the most prevalent psychopathology in children and adolescents: these disorders are among the most common and highly impairing mental and behavioural problems in children and young people, implying a significant impact on functioning and quality of life with strong long term negative effects on the individual and on families, and on society in general. In the last decade, many efforts have been made to obtain a better neuropsychological characterization of subtypes of Conduct Disorder (CD) and there has been a rapid progress in understanding the neurobiology of psychopathic traits, in particular of the Callous-Unemotional (CU) component.

We systematically reviewed the available published scientific literature relating to the efficacy of pharmacotherapy in reducing aggression in in children and adolescent with CD and, where data were adequate, we conducted a meta-analysis on the efficacy of medication on aggression considering the impact of CU traits.

- Few studies have investigated patients with CD as primary diagnosis, and few of these have discriminated between different types of aggression or reported measures of CU traits, thus providing inconclusive results on the modulating role of CU traits on the efficacy of medications.
- Methylphenidate and risperidone showed the largest effects on aggression in randomized controlled trials.
- Other antipsychotics showed clinical efficacy on CD but this evidence is mainly revealed by open label trials.
- There is some low quality evidence to support a small effect of mood stabilizers and other agents.

Considering heterogeneity of the disorder, we concluded that more proof-of-concept clinical studies are needed to define effects of medication and role of CU traits.

The manuscript “The pharmacological treatment of aggression in children and adolescents with conduct disorder. Do callous-unemotional traits modulate the efficacy of medication?” has been published in the journal Neuroscience & Biobehavioral Reviews (Elsevier) [*Balia C, Carucci S, Coghill D, Zuddas A.* ***The pharmacological treatment of aggression in children and adolescents with conduct disorder. Do callous-unemotional traits modulate the efficacy of medication?*** *Neurosci Biobehav Rev. 2018 Aug;91:218-238. doi: 10.1016/j.neubiorev.2017.01.024. Epub 2017 Jan 27. Review].*

In order to understand how aggressive subjects suffering from CD or Oppositional Defiant Disorder (ODD) distinguish from control subjects with typical development in terms of neuropsychological functioning (attention, working memory, social cognition, capacity decision-making, understanding of emotions, motivation, etc.) and specific parameters of autonomic regulation (heart rate and skin conductance) we designed **a multicentre case-control study (MATRICS_WP6-1) involving a CD/ODD cohort and a Typically Developing (TD) controls cohort aged 10-17 years old**. Following we also included a **single-blind, placebo controlled, acute dose, cross-over, randomized medication challenge (involving the ODD/CD cohort only)** in order to investigate the acute effects of medications, known to impact positively on aggression in the context of CD/ODD, on specific neuropsychological and physiological features possibly underlying different types of aggression.

In total, 68 aggressive subjects with CD/ODD and 40 TD controls have been enrolled. For this preliminary analysis a partial sample of the CD/ODD group (63) has been included. To date, only neuropsychological outcome measures have been analysed; further analysis will produce knowledge on autonomic profiles of these subjects and on effects of medications on autonomic measures. Preliminary results from the MATRICS_WP6-1 are summarised below.

Results

Case-control study (baseline testing and comparison between a CD/ODD group and a TD controls group)

The main aim was to investigate the neuropsychological and autonomic profiles that differentiate children and adolescents with clinically relevant levels of aggression and diagnosis of ODD or CD compared to TD controls, and distinguish subjects with CU traits.

The preliminary results of this study show that, compared to TDC, aggressive subjects with CD/ODD with normal IQ show:

- deficit in all the investigated cognitive domains (memory, language, attention and visual-spatial skills), and in the executive functions, especially inhibition, planning, flexibility skills, problem solving and behavioural control.
- difficulty in recognizing emotions, especially negative ones (fear and sadness);
- altered voluntary control over reactivity to external stimuli, due to impairment of abilities essentials for this mechanism (attention, planning and inhibition);
- anomalies in emotions reactions and moral judgment of the situation: they feel less guilt and shame and feel greater well-being when they do wrong (acting as a victimizer); they experience less sense of annoyance when they suffer (being a victim).

When considering aggressive CD/ODD patients only, preliminary results show that:

- younger patients show greater impairment in attention skills, while older ones are more compromised in terms of empathy and moral judgment of situations;
- patients with higher CU traits have more difficulties in emotion recognition, in particular the negative ones as sadness.
- no significant differences were observed between high and low CU traits when assessing "cold" executive functions

Single-blind, placebo controlled, acute dose, cross-over, randomized medication challenge (involving the ODD/CD cohort only)

The main objective was to investigate the acute effects of medications, known to impact positively on aggression in the context of CD/ODD, on specific neuropsychological and physiological features possibly underlying different types of aggression. For this purpose, we specifically explored the responses to an acute medication challenge by the administration of a single dose of a stimulant (Methylphenidate), a not stimulant SNRI (Atomoxetine) and two antipsychotic medications (Risperidone and Aripiprazole) according to the current evidences from literature.

The preliminary results of this phase of the study show that:

- in response to a single dose of methylphenidate and atomoxetine, there is a significant improvement in measures of affective attentional control in terms of accuracy (higher percentage of correct responses and less errors). Improvement of attentional abilities and the reduced impulsivity by methylphenidate, can explain the improved ability to discriminate the affective stimuli.
- Aripiprazole resulted effective in improving the risk assessment of win, within the New Cambridge Gambling Task; the underlying mechanism is possibly related to the reduction of impulsive behaviours by the study drug.
- The administration of Risperidone resulted in an increase of mean reaction times both for correct and incorrect responses and to a slight improvement of accuracy (although not significant) both compared to baseline and placebo assessments, evidencing a possible positive effect of this drug in helping to manage impulsivity rather than determining an adverse event causing a slowdown as for mild sedation.
- No significant worsening of performance was found in group A neither B, showing that none of the selected medication can determine, by a single administration, any worsening of cognitive function.

In conclusion, data on acute effects of drugs confirm the efficacy of methylphenidate and atomoxetine on the modulation of attentional skills and inhibition, improving the accuracy of performance and the ability to discriminate affective stimuli as well. Further analyses might clarify the results relating to the administration of D-2 modulating agents. However, no administered drug resulted in a significant deterioration in performance compared to baseline and placebo.

These findings are in line with those from our data-mining research, which highlighted the efficacy of these medications (mainly Methylphenidate and Risperidone) on aggressive symptoms in the context of CD.

Taken together, these results contributes to validating the indications of the most important guidelines on the management of antisocial behaviour and conduct disorders in children and young people (NICE Clinical guideline, 2017), which state to offer, when psychosocial interventions are not significantly effective and pharmacological treatment is needed, methylphenidate or atomoxetine for the management of ADHD in children and young people with oppositional defiant disorder or conduct disorder, and to consider risperidone for the short-term management of severely aggressive behaviour in young people with a conduct disorder.

The WP6 second study: a study on one novel medication for aggression treatment

Previous research has revealed a positive effect of oxytocin administration on several social behaviours especially in individuals with social-affective deficits. However, it is still unknown whether intranasal oxytocin administration (OT-IN) can be beneficial to residential youth who exhibit severe social-affective impairments including those with ODD and CD diagnoses. We conducted a randomized, double-blind, placebo-controlled, within-subjects, sequential study to examine the effect of OT-IN on empathy and emotion recognition in 100 male adolescents living in residential youth care facilities. We also explored the moderating role of callous-unemotional traits, trauma, and dissociation in the oxytocin effect. Participants self-administered one dose of 24 IU of oxytocin or placebo and performed experimental tasks on empathy and emotion recognition before and after the administration. The same procedure was performed one week later with the other substance. We found that empathy was increased after oxytocin administration compared to placebo and this effect was specific to individuals with high callous-unemotional traits. There was no effect of OT-IN on the overall emotion recognition, but there was a positive effect on accuracy of fear recognition. Trauma and dissociation did not moderate the oxytocin effect on empathy or emotion recognition. Our findings provided evidence of a beneficial effect of OT-IN on empathy and fear recognition in residential youth. We propose that a combination of OT-IN and psychological interventions merits further exploration, as it might be a novel promising direction for more tailored approaches and better treatment outcomes.

Although oxytocin administration influences behaviour, its effects on peripheral oxytocin circulation are mixed and derived from studies on healthy subjects. Additionally, trauma attenuates the behavioural effects of oxytocin, but it is unknown whether it also influences its effect on peripheral circulation. This study examined whether salivary oxytocin increased after oxytocin administration and whether trauma attenuated this effect. We conducted a randomized, double-blind, placebo-controlled, within-subjects study in 100 male adolescents living in residential youth care facilities. Participants self-administered intra-nasally 24 IU of oxytocin and placebo (one week later) and provided a saliva sample before and 15 min after administration. Salivary oxytocin increased significantly after oxytocin administration but not after placebo. Trauma did not moderate this effect. Our findings suggest that trauma did not attenuate the effect of oxytocin administration on salivary oxytocin in residential youth

Key points:

• Performed as a randomized, double-blind, placebo-controlled, within-subjects study.

• Tested the effect of oxytocin administration on empathy and emotion recognition.

• 100 male adolescents from residential youth care facilities were recruited.

• Oxytocin increased empathy in boys with high callous-unemotional traits.

• Oxytocin improved fear recognition in the total sample.

• Earlier trauma exposure does not affect the bioavailability of oxytocin following administration.

WP07: Behavioural management training and neurofeedback approaches in clinical paediatric population

Foreground

Available nonpharmacological treatments of CD/ODD problems in children and adolescents show small clinical effects (Bakker et al., 2016). Early proposals to use biofeedback of arousal to normalize physiological deviance as in neurofeedback treatment of ADHD have not been followed by controlled biofeedback trials for paediatric aggression. The purpose of WP7 was to develop and test two very innovative approaches to personalized prevention/intervention of aggression: 1) arousal modulation in young children at high risk for CD and with CD, and 2) real-time functional magnetic resonance imaging neurofeedback (rtfMRI-NF) in adolescents with a severe form of CD. The two corresponding

randomized controlled trials were registered under ClinicalTrials.gov identifiers NCT02485587 and NCT02563145, respectively. The approaches in this WP complemented and extended those in the FP7 Aggressotype project.

WP7 had three primary objectives:

1. Datamining of available arousal and fMRI data sets to validate the relation between arousal and aggression network imbalance, depending on CU traits (Task 1)
2. Developing innovative, individualized and specific physiological autonomous (arousal) based prevention and treatments in young children at high risk for/with CD to improve prevention over conventional behavioural approaches (prevention and treatment as usual) (Task 2),
3. Supplement behavioural approaches with brain based real-time-fMRI (Caria & Birbaumer 2011, 2012) self-regulation in adolescents with CD/aggression, personalized by CU traits and arousal profiles, to target their individual dysfunctional arousal and brain connectivity patterns linked to their imbalance of control, value and empathy processing (Task 3).

Results

During the course of the project, WP7 achieved all three objectives.

Datamining (Task 1) clarified that:

- Responses in the VS and OFC during reward anticipation were decreased in participants with previous CD diagnoses and with high impulsivity.
- Caudate activity during reward delivery was increased as a function of aggression during later life.
- The amygdala was less active during emotion processing in participants with previous CD diagnoses and high CU traits.
- Reactive aggression was inversely related to SCL and SCR during faces and shapes.
- Aggression during later life was negatively correlated with the SCR during reward anticipation.

Treatment development and implementation of the arousal-based and brain-based clinical trials (Tasks 2 and 3) was completed by:

- Validation of emotional video clips (n= 120 clips, N=20 subjects 8-18y)
- Feedback and subtyping paradigms optimization, programming, and SOPs finalization
- Registering the randomized controlled trials (RCTs) under ClinicalTrials.gov:

  https://clinicaltrials.gov/ct2/show/NCT02485587 and https://clinicaltrials.gov/ct2/show/NCT02563145
- The arousal-based skin conductance biofeedback (SC-BF) trial was implemented at two sites (CIMH, UZH), and rtfMRI-NF trial at CIMH.
- Major recruitment efforts which proved successful in increasing inclusion to compensate for the drop out of the Nijmegen site. These additional efforts included:

  1.1. updates of the homepages to make both studies and ongoing recruitment highly visible;

  1.2. a full day of lectures and workshops for referring institutions, clinics and local authorities on Aggression and related studies in Mannheim;

  1.3. intensified personal contacts and site visits with referring clinicians, external clinics and institutions in Zurich and Mannheim at all levels;

  1.4. personal letters from the clinic director in Mannheim sent in April 2018 regarding successful cooperation and prolonged recruitment.
- Subtyping results across both RCTs showed higher heart rate during resting state in participants with ODD/CD diagnosis when compared with typical developing children and adolescents while no difference for skin conductance was found. Overall, cases showed increased slow wave EEG activity, and fronto-central alpha and beta activity decreased with increasing aggression-related behaviours. (Task 2 and Task 3)

- 39 participants were randomized, 32 started, and 24 finished treatment in the SC-BF trial. (Task 2)
- In line with the hypothesis of non-inferiority, both the SC-BF as well as the treatment as usual (TAU, individualized behavioural therapy) group showed significant clinical improvement and no group differences in the primary outcome measure (modified overt aggression scale) and secondary outcomes (CBCL subscales ODD and CD). (Task 2)
- No significant changes were found in the inventory of callous-unemotional traits, and the Reactive and Proactive Questionnaire, suggesting that CU traits and proactive aggression are stable traits over time. (Task 2)
- Self-regulation within the SC-BF training showed successful differentiation between up-and downregulation and a trend for improving performance across time. However, performance and clinical improvement were unrelated. (Task 2)
- A final sample of 23 patients performed both pre- and post-treatment assessment in the rtfMRI-NF trial. (Task 3)
- No significant changes were found in the in the primary outcome measure (modified overt aggression scale) and secondary outcome inventory of callous-unemotional traits. (Task 3)
- In line with the hypothesis of non-inferiority, both the rtfMRI-NF as well as the TAU group (individualized behavioural therapy) showed significant clinical improvement and no group differences in secondary outcomes of aggression (CBCL subscales ODD and CD). (Task 3)
- Reactive aggression, as measured by the Reactive and Proactive Questionnaire, improved only in the TAU group. (Task 3)
- There was no difference in clinical outcome between the rtfMRI-NF target regions. (Task 3)
- Self-regulation within the rtfMRI-NF training showed overall higher activity in the amygdala target region (AMG-NF) compared to the insula target region (INS-NF). Successful differentiation between up-and no regulation increased across time was observed in the right amygdala in the AMG-NF group during video-supported feedback and in the bilateral insula in the INS-NF group during transfer. (Task 3)
- Analyses of changes in emotional face processing at an explorative uncorrected threshold yielded higher activity in several brain areas related to emotion regulation, such as the dorsolateral prefrontal cortex, across the rtfMRI-NF and TAU groups before treatment, which may indicate more efficient affective processing after treatment in both groups. Further, the TAU group showed higher activity for positive faces compared to the NF group after treatment in a cluster comprising the caudatus and the anterior insula, possibly due to effects of habituation to this type of task in the rtfMRI-NF group or indicating that TAU treatment may effectively improve affective processing. (Task 3)
- A 6-month follow up revealed sustained improvement in the CD subscale of the CBCL that was mainly driven by the SC-BF group. No effects were observed for the primary outcome, ICU or RPQ. For The ODD subscale of the CBCL, scores did improve from baseline to post-treatment assessment, but were not significantly different from baseline at follow-up. (Task 2)
- For the rtfMRI-NF trial, improvement in the primary outcome (MOAS) was observed from baseline to follow-up across both treatment groups, while no sustained effects were observed for CBCL subscales ODD and CD or ICU and RPQ. (Task 3)

Conclusions

In summary, feasibility and efficacy of both individualized SC-BF as well as individualized rtfMRI-NF was demonstrated. Effects were larger in the SC-BF trial, which may partly be attributed to the younger age of participants in this trial and selection for high CU-traits and thus more severe cases in the rtfMRI-trial.

The time course of clinical outcomes up to 6 month follow-up in both the rtfMRI-NF as well as the SC-BF group was comparable to their respective TAU control groups, suggesting non-inferiority of the experimental treatments. Results suggest partly sustained clinical improvement for SCL-BF, rt-fMRI NF and TAU mainly in older individuals with elevated CU traits as included in the rt-fMRI NF trial. None of the post-hoc tests show a significant deterioration from treatment end to FU, but further controlled investigation in larger samples is needed as some scales descriptively show non-significant increase of symptom scores at FU compared to post-treatment assessment. Further, the statistics from the small

subgroups with completed FU questionnaires are preliminary and clearly highly exploratory. Still, it is encouraging that the results for the MOAS as the primary study outcome suggested that overt aggression was reduced at follow-up compared to baseline in the rt-fMRI NF trial, consistent with sustained or delayed improvement following the treatments.

References

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WP08: Clinical induced pluripotent stem cell derivates

Foreground

Increasing evidence points to conduct disorder (CD) and related disorders having a polygenetic genetic basis. In addition to genetic contributions, environmental factors such as maternal infection, prenatal exposure to recreational drugs or 'stress' during critical periods of development are thought to increase the likelihood of abnormal behaviours in children. This includes aggressive, anti-social behaviours as well as increased irritability, inattentiveness and abnormal social behaviour. Concurrent with this, preclinical animal studies also support prenatal development as being a critical period when the underlying mechanisms contributing to abnormal social behaviours and antisocial/aggressive behaviours may occur. At a clinical level, attention deficit hyperactivity disorder (ADHD) is comorbid with CD in about 50% of cases. Interestingly, ADHD is also comorbid with autism spectrum disorders (ASD). Moreover, individuals diagnosed with ASD can also be reported to display poor empathic functioning, associated with impaired emotional processing. Poor empathic functioning is thought in part to contribute to abnormal behaviours seen in CD. Critically, there is increasing evidence that ADHD and ASD share overlapping genetic risk. Therefore, modelling the underlying genetic mechanisms associated with disorders related to CD and determining how environmental factors influence these genetic risk factors, is key in developing a more comprehensive understanding of the underpinnings of abnormal behaviours associated with CD and related disorders.

Advances in stem cell biology now make it possible to generate induced pluripotent stem cells (iPSCs) from adult somatic cells. IPSCs can be generated from a range of somatic cells including skin fibroblasts as well as hair keratinocytes. Critically, these iPSCs retain the genetic makeup of the host donor. This makes this cellular system an ideal platform from which to study how multiple genetic factors converge to increase risk of developing specific disorders or diseases. IPSC-based system also make it possible to study previously inaccessible human neuronal cell types, understand how genetic and environmental factors can influence neurodevelopment as well as investigating the cellular and molecular underpinnings of potential therapeutic compounds.

In WP8, we have utilized this approach to investigate how gene and environmental factors impact prenatal development, thereby increasing risk for developing disorders related to CD. Specifically, the overall objectives for WP8 were to:

1) Generate and establish a biobank of hair biopsies and induce pluripotent stem cells (iPSCs) from patients diagnosed with CD and related disorders (patient-iPSCs);
2) Compare gene expression profiles between iPSC-derived neurons from typically developing individuals (control-iPSCs) and patient-iPSCs;
3) Establish a cellular model relevant for CD and related disorders, with an emphasis on modelling the impact of environmental factors on neurodevelopment;
4) Examine cellular phenotypes associated in a cellular model for CD and related disorders and to search for common underlying mechanisms of CD and related disorders;
5) Investigate the mechanisms underlying the actions of behaviourally modifying compounds.

Results

Objective 1: Hair keratinocytes (hair biopsies) were collected by the clinical groups involved. A total of 40 samples from 3 different sites were collected. Participants were recruited and methods carried out in accordance to the 'Patient iPSCs for Neurodevelopmental Disorders (PiNDs) study' (REC No 13/LO/1218). Informed consent was obtained from all subjects for participation in the PiNDs study. Ethical approval for the PiNDs study was provided by the NHS Research Ethics Committee at the South London and Maudsley (SLaM) NHS R&D Office.

We have established multiple clonal lines from individuals diagnosed with ASD comorbid with ADHD. In addition, multiple clonal iPSC lines were established individuals carrying the 22q11.2 chromosomal deletion. Individuals with this deletion are at high risk of developing ADHD. Finally, we established multiple clonal lines from typical developing individuals. All iPSC lines were expanded and bio-banked. Each line underwent extensive quality control (QC) characterisation. This includes QC of genetic stability by karyotyping, exome sequencing, and SNP array analysis. Functional characterisation was achieved by carrying out Pluritest arrays, Alkaline phosphatase testing for pluripotency, assessment of pluripotency marker expression and spontaneous differentiation of iPSCs into cells of three embryonic germ layers (endoderm, mesoderm, and ectoderm lineages) from embryoid bodies.

Objective 2: To compare gene expression profiles between control and patient iPSCs, we first performed SNP array analysis (PsychArray chips) on 25 controls and patient (diagnosed with ASD, 22q11.2 deletion and related disorders) iPSC lines. These data were then used to identify common polymorphisms and to generate polygenic risk scores (PRS) where possible.

Increasing evidence indicates a critical role of altered epigenetic marks in risk of developing neurodevelopmental disorders, including ASD, ADHD and schizophrenia. Patient-derived iPSCs offer a unique opportunity to assess the epigenetic signature associated with specific diagnoses, without the confounding impact of "lifestyle" on the epigenome. To this end, we have examined the methylome of iPSCs derived from 50 individuals with diagnoses of ASD, ASD/ADHD, schizophrenia; carriers of copy number variants (CNVs) for *SHANK3*, *NRXN1* or 22q11; or typically developing and apparently healthy individuals. A total of 100 samples (multiple clones from each line) have been run (collaboration with J. Mill, University of Exeter). These data have already demonstrated that it is possible to age the samples based on DNA methylation as demonstrated by an assessment of donor sample cell type (hair keratinocyte) vs reprogrammed iPSCs. Analysis has identified several hundred differentially methylated sites between control and ASD iPSCs. Loci demonstrating differential DNA methylation status include genes involved in synaptic function as well as major histocompatibility class 1 (MHCI) genes.

To complement these studies, we have also performed RNA sequencing (RNASeq) on iPSc cells differentiated towards forebrain neuronal cell fates at multiple time points. In addition, we have carried out RNASeq analysis on iPSC-neurons from typically developing and ASD/ASD comorbid with ADHD individuals. These studies have highlighted that iPSC-neurons from ASD individuals have a transcriptomic profile highly similar to that obtained from post-mortem studies. Furthermore, RNASeq analysis highlights early prenatal neurodevelopment as a critical period where pathophysiology associated with ASD and related disorders may emerge. Differentially regulated genes were highlight enriched for gene terms including immune and inflammatory responses, DNA-binding and transcriptional regulation, chromosome organisation, epigenetic regulation, neurodevelopment, and synaptic structure, development and plasticity.

Objectives 3 & 4: Prenatal exposure to infections and compounds including recreational drugs have been linked with increased risk of CD, ADHD as well as ASD and even schizophrenia. Therefore, we established 3 cellular models to study the impact of environmental factors on neurodevelopment.

Model 1: Prenatal exposure to cannabis, and in particular the psychoactive component of cannabis Δ9-tetrahydrocannabinol (THC) has been linked with altered neurodevelopment and increased chances of developing abnormal behaviours. Using iPSCs derived from typically developing individuals, we have developed a novel cellular system to study the impact of THC and other endocannabinoids on the development of human neurons. Specifically, we have differentiated iPSCs towards a forebrain fate and have tested the hypothesis that exposure to THC or specific endocannabinoids alter the development of neuronal cells. This study has revealed that iPSC-derived neural progenitor cells and neurons express the key genes required for endocannabinoid synthesis and signalling. Exposure to THC or endocannabinoids causes a reduction in neurite outgrowth in iPSC-neurons. Moreover, exposure to THC or endocannabinoids causes a differential reduction in the activity of ERK1/2 and Akt-signalling pathways. Finally, we found that treatment with a cannabinoid receptor 1 inverse agonist blocks THC/endocannabinoid induced reduction in neurite outgrowth and ERK1/2 activity.

Model 2: Maternal immune activation (MIA) during foetal development leads to behavioural abnormalities in offspring that are associated with psychopathy and psychiatric disorders, including deficits in sensorimotor gating, prepulse inhibition and startle reflex. Critically, MIA has been strongly associated with increased risk of developing neurodevelopmental disorders including ASD, ADHD and schizophrenia. Using iPSCs derived from typically developing individuals, we have developed and characterised a human cellular model of MIA. Specifically, we have differentiated iPSCs towards a forebrain fate and have tested the hypothesis that exposure to specific antiviral agents at specific time points alter the development of neuronal cells and causes a transcriptomic signature similar to that seen in neurodevelopmental

disorders. Exposure of iPSC-derived neural progenitor cells to IFNγ activates an antiviral transcriptional state involving dramatic upregulation of the antigen presentation (MHC class I; MHCI) pathway and leads to enduring morphological abnormalities in postmitotic neurons. MHCI overexpression is a hallmark of MIA in rodent models. Interestingly, exposure of iPSC-derived neural progenitor cells to IFNγ also resulted in a neurite outgrowth phenotype similar to that observed in iPSC-neuron derived from individuals with ASD. Namely, a transient exposure to IFNγ increases neurite outgrowth. IPSC-derived neural progenitor cells exposed to IFNγ also demonstrated a persistent increase in the expression of MHCI genes. The persistent increase in MHCI gene expression is dependent on the formation of nuclear entities known as "PML bodies". These protein rich nuclear complexes coordinate the expression of MHCI gene in response to IFNγ. The expression of PML bodies is persistently increased following transient IFNγ exposure. Interestingly, we found that MHCI protein expression at growth cones was further enriched following exposure to IFNγ exposure. Critically, disruption of PML bodies using As2O3 concurrent to IFNγ treatment prevents IFNγ-dependent abnormalities in neuronal morphology and MCHI enrichment in growth cones. Similar effects were seen when we blocked the expression of B2M – an adaptor protein required for surface expression of MCHI proteins, either using an shRNAi approach or in human ES lines where B2M has been knocked out by gene-editing methods. We also examined the relevance of IFNγ-induced transcriptional effects for neurodevelopmental disorders. The immediate and persistent transcriptional signature induced by a transient exposure to IFNγ is highly enriched in differentially expressed genes that significantly increase risk of developing ASD or schizophrenia. Moreover, IFNγ-induced differentially regulated genes are also differentially expressed in ASD and schizophrenia, but not other disorders, based on post-mortem work. Finally, we found that MHCI and PML expression is increased in post-mortem cortex from ASD and schizophrenia individuals, and moreover that iPSC-derived neural progenitor cells have increased expression of PML and furthermore, demonstrated an exacerbated response to IFNγ.

Model 3: To expand upon our human cellular model of MIA, we have performed a number of additional studies using different cytokines thought to be critically involved in the MIA response, in iPSC-neural progenitor cells or neurons derived from healthy iPSCs or those generated from individuals diagnosed with ASD, schizophrenia or that carry copy number variants thought to increased risk of neurodevelopmental disorders. These studies have shown that exposure of iPSC-neural progenitor cells to IL1beta causes alterations in gene expression similar to IFNγ – with a particular enrichment in MHCI and synaptic genes. Alteration in gene expression induced by exposure to IL1beta or IFNγ are exacerbated in neural progenitor cells differentiated from iPSC-derived from schizophrenic patients. IPSC-neural progenitor cells generated from individuals with 22q11 chromosomal deletions and either with a diagnosis of ADHD, ASD and risk of psychosis, showed altered response to IL1beta and IFNγ. The transcriptomic signature of neural progenitor cells derived from healthy iPSCs and treated with IL1beta show significant overlap with altered gene expression associated with ASD and schizophrenia. Treatment of neural progenitor cells derived from schizophrenia iPSCs and treated with IL1beta are enriched for gene involved in neurodevelopment, MHCI pathway and synaptic function.

Objective 5: *In utero* exposure to cellular stressors such as cortisol or corticotrophin release factor (CRF) are thought to increase risk of developing neurodevelopmental disorders including ADHD. Using induced pluripotent stem cells (iPSC) derived from healthy individuals, we have developed and characterised a human cellular model to study the impact of exposure to in utero of stress, and furthermore the impact of methylphenidate either alone or following co-treatment with cortisol. Treatment with cortisol or CRF on neural progenitor cells or neurons from healthy iPSCs, results in altered expression of genes involved in neural differentiation and synaptic function. Acute treatment with methylphenidate alone does not appear to alter the expression of synaptic glutamatergic gene expression. Co-treatment with methylphenidate attenuates cortisol-driven changes in select genes but does not affect the expression of other cortisol-driven genes. Treatment of neural progenitor cells derived from ASD/ADHD iPSCs results in exacerbated expression of genes involved in MHCI signalling and synaptic function; these effects are blunted by methylphenidate co-treatment.

WP09: Environmental risk factors and gene-environment interactions

Foreground

Aggression and disruptive behaviour disorders such as opposition-defiant and conduct disorder represent multifactorial traits and disorders that are caused by both genetic (i.e. heritable) and environmental factors. In WP9 we have investigated the role of environmental risk factors, the interaction between genetic and environment factors, and genetic liability and brain connectivity in relation to aggression and disruptive behaviour disorders in children and adolescents.

Results

First, we have systematically investigated the existing literature with regard to common, pregnancy-related environmental risk factors for disruptive behaviour. We found most evidence supporting a relation between maternal smoking during

pregnancy and offspring disruptive behaviour. Further evidence pointed to maternal alcohol use during pregnancy as a risk factor for offspring disruptive behaviour, but we did not find sufficient evidence suggesting a link between cannabis or caffeine intake during pregnancy and offspring disruptive behaviour (Ruisch, Dietrich, *et al.*, 2018). Extending the findings from the existing literature, we subsequently investigated a more comprehensive range of early pregnancy-related environmental factors in relation to offspring disruptive behaviour using data from the Avon Longitudinal Study of Parents and Children (ALSPAC - https://www.bristol.ac.uk/alspac/). ALSPAC is a U.K.-population based, longitudinal birth cohort study which contains (epi)genetic data, biological samples and an extensive range of phenotypic data including information about environmental exposures and disruptive behaviour disorders symptomatology (Golding *et al.*, 2001; Boyd *et al.*, 2013; Fraser *et al.*, 2013). In this study, we have adjusted potential effects of multiple pregnancy-related factors for each other, we also adjusted for co-occurring disruptive behaviour and attention-deficit/hyperactivity symptoms, and adjusted for some genetic risk factors related to disruptive behaviour. A link between maternal smoking during pregnancy and offspring disruptive behaviour was confirmed, and in addition we observed that maternal paracetamol use, life events and depressive symptoms during pregnancy were also linked to disruptive behaviour in the offspring (Ruisch, Buitelaar, *et al.*, 2018).

Still, interplay between environmental and genetic factors related to disruptive behaviour is expected to play an important role and contribute to the observed associations regarding environmental factors. Therefore, we conducted further research to investigate specific ways by which genetic and environmental factors could affect each other. These mechanisms include 'gene-environment correlation' and 'gene-environment interaction'. Gene-environment correlation assumes that the genetic make-up of an individual is linked to certain environmental risk factors for disruptive behaviour. This way, one could observe an association between the environment and child behaviour, while in fact this effect of the environment (also) represents a genetic predisposition. Further, in gene-environment interaction the genotype of an individual affects its response or vulnerability to certain environments. This mechanism may therefore help to explain why some individuals appear to be more susceptible to certain environmental factors than others. In our studies of gene-environment interplay we focussed on environmental factors both before and after birth, using data from the ALSPAC-cohort. First, we investigated potential gene-environment interplay involving genetic risk factors that were identified in more recent genetic studies of disruptive behaviour (i.e. (Rautiainen *et al.*, 2016; Tielbeek *et al.*, 2017)) and a previously well-studied susceptibility gene for disruptive behaviour (*monoamine oxidase A (MAOA)*; e.g. (Byrd and Manuck, 2014)). Since these genetic factors were identified in males and females separately, we also investigated gene-environment interplay separately for males and females. We found evidence for gene-environment interactions involving maternal smoking during pregnancy in males, and gene-environment interactions involving childhood maltreatment in females (Ruisch *et al.*, 2019). Nevertheless, comprehensively investigating the genetic liability for disruptive behaviour in gene-environment interplay remains a challenge. Therefore – in collaboration with the WP5 SME Drug Target ID, Ltd. (DTID) – we performed genetic studies of disruptive behaviour and designed a study in which we considered gene-environment interactions (and correlations) between all approximately 20,000 protein-coding genes in the human genome and a number of previously identified environmental factors for disruptive behaviour. The results of this study (currently still unpublished) indicate that indeed a multitude of genes interact with environmental factors in relation to disruptive behaviour rather than only a few genes. Different genes interacted with different environmental factors, yet also overlap in interacting genes was seen. In addition, we obtained evidence pointing to certain biological pathways that may underlie the observed gene-environment interactions in disruptive behaviour.

Using data from the NeuroIMAGE-study (https://www.ru.nl/donders/vm-site/collaborations/projects/neuroimage/), we further investigated the genetics of aggression and the link with brain structure (more specifically, the connections between different parts of the brain). NeuroIMAGE is the Dutch follow-up of the International Multisite ADHD Genetics (IMAGE) project and contains a wide range of data including genetic, neuroimaging and phenotypic (i.e. environmental and behavioural) data.(von Rhein *et al.*, 2015) First, we have investigated evidence for a shared genetic background among different subtypes of aggression and disruptive behaviour. To this end, we investigated whether genetic liability for aggression (as represented by 'genetic risk scores' that were calibrated based on a large genetic study of aggression in children (Pappa *et al.*, 2016)) is linked with a more severe subtype of disruptive behaviour ('callous-unemotional traits'). Callous-unemotional traits describe a lack of guilt, limited empathy and a shallow affect and are related to more severe and persistent behavioural problems.(Blair, Leibenluft and Pine, 2014) We found evidence for genetic sharing between more general aggressive behaviours and callous-unemotional traits in children. In addition, we confirmed involvement of certain neurotransmitter systems as potential shared biological pathways, and identified an interaction between genetic risk scores for aggression and childhood stressful life events in relation to callous-unemotional traits. As such, genetic liability for aggression appears to affect the vulnerability for stressful life events during childhood in relation to callous-unemotional traits.(Ruisch *et al.*, 2020) Furthermore, the relation between genetic liability for aggression and the structural

connections between different areas of the brain (i.e. 'connectivity') was investigated. Such structural connections in the brain are composed of the nerve fibres between different brain areas, also referred to as 'white-matter' tracts. According to previous literature, potential structural differences across a range of white-matter tracts has been linked to aggression and disruptive behaviour. Therefore, in this study we investigated across the whole brain (i.e. across all large white-matter tracts) whether genetic risk scores for aggression are related to structural connectivity. Results (currently still unpublished) indicated that genetic liability for aggression is related to structural connectivity in some of the major white-matter tracts. In addition, genetic risk scores for aggression also appeared to affect the relation between environmental stressors in childhood and structural connectivity in the brain.

Further – in collaboration with DTID and the Complex Disease Epigenetics Group from the University of Exeter UK (WP5) – we have further investigated the genetics and biological mechanisms involved in gene-environment interactions in disruptive behaviour. To this end, we have compared the genetic and epigenetic results from the other WP animal models of aggression with our findings in humans. We found that the genes that were implicated through both animal and human studies of aggression are involved in specific biological pathways, and point to proteins that have been recently implicated in stress-related and behavioural psychiatric disorders.

Summarizing, these results show that the ethology of aggression and disruptive behaviour disorders is multifactorial and involves a broad range of both genetic and environmental risk factors. Genetic risk factors may act on certain pathways, may affect each other, and as we have showed in particular, may affect an individual's response to the environment (i.e. gene-environment interaction). As such, an individual's genetic make-up and environmental exposures should not only be considered independently but also in relation to each other. Furthermore, genetic liability for aggression appears to be related to variation in structural connectivity in the brain, which may point to neural abnormalities that could play a role in behavioural problems. Still, much work remains to be done and future studies should be conducted to validate and extend some of the current findings, to gain further insights into the ethology of aggression and disruptive behaviour in children and adolescents.

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WP10: Machine learning & bioinformatics

Foreground

The MATRICS project delivered many new and different sources of information related to the prediction of aggression and antisocial behavioural subtypes. The key challenge from a machine learning perspective was to integrate all this information into a single coherent, unified view that could explain the mechanisms behind these observations and deliver new insights and potential new avenues of treatment opportunities to both practitioners and researchers in the field of neuropsychology and paediatric conduct disorders. Key focal point in this endeavour was to elucidate the recently uncovered link between *inattention* (the 'attention deficit' component in ADHD) and various forms of *aggression*, as well as their subsequent relations to the development of behavioural problems like *oppositional defiant disorder* (ODD) and more serious forms of *conduct disorder* (CD). Other important objectives were to uncover new predictive genetic and molecular biomarkers for conduct disorders and investigate the role of other comorbidities like ADHD and potentially crucial contextual factors like *age*, *gender*, *substance abuse* and *family problems*.

To this end WP10 employed state-of-the-art machine learning techniques for causal discovery from complex real-world data sets. Since the early nineties it has been known that, despite the familiar adage 'correlation does not imply causation', under suitable assumptions modern statistical methods *can* be able to validly infer the existence of causal relations from both experimental and observational data. An attractive feature of these methods is that they can build this into an *intuitive graphical model network* that visually captures crucial elements of the underlying system, in our case: human behaviour related to aggression and its many contributing and co-occurring factors. Of course, not all relations can always be found, and any positive identification remains subject to experimental validation, but the resulting hypothesised causal network provides a unique insight into the complex interplay between all related elements. Most importantly, it allows distinguishing between crucial driving factors and merely correlated side-effects of a given target. As such it can be an invaluable resource for researchers to develop and confirm new hypotheses that ultimately may lead to novel, effective treatment strategies.

However, before we could apply these methods to the tasks required by the MATRICS project important challenges needed to be overcome. The other work packages and external sources provide a vast array of wildly different types of information, ranging from questionnaires, to genetic information, brain metabolites, animal behavioural studies, and medical trials. Bringing all these sources together in a single unifying view was beyond existing state-of-the-art of causal discovery methods at the start of the project. We successfully managed to tackle and resolve these issues, ultimately leading to the desired hypothesised causal model. We hope this model may prove insightful to researchers and help to further the cause of developing effective clinical treatments for young children and adolescents at risk of getting trapped in a downward spiral of aggressive behaviour and social impairment leading to ever more serious conduct problems.

Results

Out of the many results we would like to showcase three major contributions of WP10:

1. Development of a new, multi-source causal discovery method in the form of the Joint Causal Inference (JCI) framework
2. Implementation of this method in the form of a free, publicly available user-friendly software package for causal discovery called 'RUcausal'

3. A coherent, integrated causal model that shows the key processes linking the driving factors behind aggression and conduct disorders

Below we will discuss each of these in detail, followed by some opportunities for future research.

Novel multi-source causal discovery method

The MATRICS project was designed to be a comprehensive multi-disciplinary approach to tackle. However, the very diverse collection of data obtained through the MATRICS project posed unique challenges to available methods for causal discovery. Existing methods either analyse data sets in isolation, or have to rely on assumptions that are unlikely to hold in practice, making the output unreliable and/or incomplete. We developed the *Joint Causal Inference* (JCI) framework in conjunction with leading researchers from the University of Amsterdam, precisely to bring such important and ubiquitous data sets into the domain of state-of-the-art causal discovery methodology. It provides a generic approach for combining results from different experiments into a single encompassing causal model that effectively subsumes current solution strategies to the problem. Experimental validation proved that our new approach significantly outperformed existing methods, both in terms of robustness and reliability, but also in the number of positively identified presence or absence of causal relations between target variables. The method is accepted for publication in the leading *Journal for Machine Learning Research.*

Open-source software package RUcausal

A major objective for WP10 was not just to develop a new causal discovery method suitable for challenging data sets such as those encountered in MATRICS, but also to help broaden the appeal and accessibility of such methods to other researchers in the field of neuropsychology and beyond. To that end we implemented our approach in a publicly available open-source software package using the popular language R. The algorithm is built around the award-winning Bayesian Constraint-based Causal Discovery (BCCD) algorithm in the context of the JCI framework. It is designed to be fast and easy to use, even for researchers not familiar with causal modelling theory. The package can be downloaded for free from https://gitlab.science.ru.nl/gbucur/RUcausal, and comes with an easy two-step installation guide, a data pre-processing toolbox, and demonstration examples to help the user get started. The package will be actively maintained and extended over the foreseeable future to keep adding new and exciting developments such as the ability to handle cyclic feedback interactions.

Integrated causal model

However, the main contribution of WP10 undoubtedly lies in the hypothesised integrated causal model, a summary version of which is depicted in Figure 1. It shows the key target variables of the MATRICS project: impulsive (reactive) vs. instrumental (proactive) aggression and their relation to various forms of conduct problems, in combination with important behavioural and personality traits and other contextual factors.

A summary of the most important insights and conclusions captured by the global model:

- there is strong and consistent statistical evidence for a causal link from *inattention* to *aggressive behaviour* that is persistent over different age groups and different cohorts,
- this link is present for both *reactive* and *proactive* types of aggression, where reactive aggression is most strongly linked to *oppositional behaviour* (ODD), and proactive aggression to more serious *conduct problems* (CD): together they point to the fact that *two different types of inattention* may be in play,
- the link from inattention to reactive aggression seems to originate from a *misreading of social cues*, whereas the link to proactive aggression signifies a *disinterest in social norms / other people*, that in turn is influenced by *callous/unemotional* traits and (partially) mediated by a *rule breaking* disposition,
- perhaps surprisingly both *substance abuse* and *anxiety* appear mainly as side-effects of aggressive behaviour and psychological problems rather than as driving factors in themselves,
- in contrast, contextual factors such as *age*, *gender*, and crucially *familial circumstances* are seen to play an important role in driving or moderating various forms of aggression and behavioural problems,
- gene analysis (GWAS) resulted in a number of genome wide significant candidate genes, most importantly CTD-3194G12.2 (linked to ODD/reactive aggression) and SLC12A8 (linked to CD/personal aggression);

- causal results from the animal experiments suggest that the brain metabolites GABA and glutamine play a mediating role between genetic variation and aggression related behavioural traits.

We are particularly excited by the discovery and confirmation of the different *types* of inattention that play a role in the relation to both proactive and reactive aggression. Furthermore, the mediating traits on the separate causal paths should provide promising starting points for new treatment strategies. Naturally, we want to stress that the hypothesised global causal model presented here is only the most *likely* coherent explanation for the statistical patterns observed in WP10: additional experimental verification is required to validate our results. However, the main conclusions proved extremely stable over all our largest and most reliable data sets, and we are confident that such confirmation can eventually be found.

WP11: Business development and dissemination

Foreground

Early-onset disruptive behaviour disorders (DBDs) such as Oppositional Defiant Disorder (ODD) and Conduct Disorder (CD) are a common reason for referral to child and adolescent mental health services and carry long-lasting negative consequences. DBDs carry risks of delinquency, substance abuse, self-harm and suicide, depressive disorders, personality and maladaptive social functioning. Severe and enduring DBDs are a challenge to families, teachers, educators and therapists. DBDs are characterized by multiple environmental and individual risk factors and involve complex developmental trajectories resulting in clinical and etiological heterogeneity. Typically, childhood onset puts individuals at increased risk of developing antisocial personality disorder in adulthood and when coupled to callous-unemotional traits they present with more severe treatment aggression and antisocial behaviour.

Evidence-based treatments are scarce and of modest effect when ODD/CD symptoms are severe and pervasive; most treatments are symptomatic or target environmental risk-factors without direct insight on the neurobiological developmental mechanisms. Psychosocial interventions such as parent management training and therapeutic programmes to develop self-control and socialization are central aspects of current therapeutic plans for children and adolescents. However some situations require complementary approaches such as medications. Children and adolescents with severe DBD often need help from different professionals and institutions. A better understanding of mechanisms involved in common dimensions of these disorders (i.e. maladaptive aggressive behaviour, impulsivity, and callous-unemotional traits) is needed to develop better treatment strategies.

Therapeutic innovation in ODD/CD requires a shift from symptoms towards the identification of biological phenotypes leading to personalized treatments. MATRICS aims at the identification of biomarkers with diagnostic and therapeutic value using a translational approach. The main objective of MATRICS is to deconstruct current diagnostic schemes of DBD by adopting a bottom-up approach, mapping neural functions onto observed clinical signs and symptoms, using different levels of analysis (e.g., genes, proteins, physiological activity, brain imaging, behaviour, and self-reports of symptoms) to define the key constructs. It is unlikely that biomarkers directly map into existing clinical categories; however, they can be used to define biologically-based phenotypes and innovative targets for drug development.

Results

Objective 1: Make MATRICS known to the scientific community and the public

This objective was achieved through different initiatives:

- creation of the MATRIC logo, corporate identity and website
- dissemination of MATRICs using social media (e.g. Twitter, scientific blog, publication of the project on ResearchGate…)
- teaching activities directed at young clinicians and researchers both during the General Assembly meetings and during the annual school of child and adolescent psychopharmacology organized by the European College of Neuropsychopharmacology (ECNP). This school brings together junior clinicians from a variety of EU-countries in a programme featuring plenary sessions and workshops involving WP1, WP6 and WP11 personnel.

Each work package involved in MATRIC has its specific contribution to scientific communication. Below are some of the most significant outputs. The MATRICS consortium proposed a special issue of Neuroscience & Biobehavioral Reviews about aggression in children/adolescents. The different topics addressed in this issue are multivariate description of

conduct disorders: phenotypes, endophenotypes and biological determinants (9 manuscripts), preclinical models for the study of conduct disorders (6 manuscripts), and therapeutic approaches (4 manuscripts).

A focused issue on conduct disorders has been published in the European Journal of Child and Adolescent Psychiatry (Freitag, C.M., Boomsma, D., Glennon, J.C. et al. Eur Child Adolesc Psychiatry (2018) 27: 1231. https://doi.org/10.1007/s00787-018-1216-y) about the output of MATRICS and other consortia working on different aspects of conduct disorder and aggression.

Several symposia proposals have been submitted and accepted at both national and international congresses including ECNP 2017, the World Congress of ADHD 2017, WCPG 2017 and ECNP 2018. In 2017 the MATRICS consortium was invited to a policy workshop in Brussels organized by the EC's health research directorate to present research outputs that 'call for translation' in terms of guideline development regarding treatment recommendations, and uptake by public health, policy makers, and/or by industry for the development of new products or product development tools.

For the general public, concentris performed an interview with Dr. Jeffrey Glennon, and used the film material to produce an animated official project video (https://youtu.be/avC6E5h33zg) which was uploaded to YouTube in October 2018 to present the aims of the MATRICS project.

The WP11 team leader and team have also been involved in various media outputs about conduct disorder and specifically intrafamilial violence, for example a movie about intrafamilial DBDs was filmed with parents participating in a therapeutic programme at the CHU Montpellier. It has been released in July 2019 on the 3rd channel of the French public TV https://france3-regions.francetvinfo.fr/occitanie/emissions/doc-24-midi-pyrenees-et-languedoc-roussillon/mon-enfant-cet-etranger-recits-bouleversants-parents-demunis-face-au-comportement-tyrannique-leur-enfant-1738469.html

Objective 2: Disseminate the results to the scientific community in the academic, healthcare and pharmaceutical sectors and foster interaction and exchange with the scientific community and the public (Task 2 and 3)

Results of the MATRICS studies have been published in high-impact journals (Front Psychiatry, Behav Brain Res, Eur Arch Psychiatry, Clin Neurosci, Brain Struct Funct, and Neuropharmacology).

As an additional initiative led by the European Brain Council, the Horizon 2020 EBRA proposal identified aggression as one of three thematic areas in neuroscience in which further collaborative initiatives should be facilitated. The MATRICS coordinator is the theme leader for this domain within the EBRA consortia and further efforts to consolidate cross-consortia efforts for both PIs and early career scientists will be made.

Objective 3: Identify and valorise the intellectual property rights (IPR) generated within WP1-10

MATRICS output relevant for IP issues have been obtained from WP leaders and a report on putative biomarkers for potential further investigation has been submitted. Furthermore, the algorithm for the causal modelling interface used in WP10 will be made available to the R community via the publically available source code sharing forum CSCAN. A spin-out service company Machine2Learn formed as output from the MATRICS, TACTICS and OPTIMISTIC consortia is at present valorising the causal discovery efforts resulting in its participation in a number of ventures including the Aggressotype consortium.

Objective 4: Initiate next steps for full scale clinical trials of the most promising behavioural scenarios identified in WP6 to treat childhood DBDs

The current output of MATRICS emphasizes two different aspects : 1) novel applications of drugs already in use e.g. the role of methylphenidate in fear processing 2) the role of comorbidity and dimensional approaches in psychopharmacology moving from categories of disorders towards transnosographic drug targets such as aggression, impulsivity or chronic irritability.

Members of the MATRICS consortium (Prof A Zuddas and Prof D Purper-Ouakil) are now members of an expert group of child psychopharmacology within conect4children, a collaborative European network that aims to facilitate the development of new drugs and other therapies. A Multi Stakeholders Meeting (MSM) about child and adolescent psychopharmacology is in preparation about issues relevant to conduct disorder (e.g. chronic irritability).

MATRICS output is expected to foster research on new drugs or novel indications but also on mitigation of risk factors such as prenatal exposure to toxins or other facets of maternal immune activation. Another central therapeutic domain is optimisation of the environment. In this context, a national grant was recently obtained by Prof. Purper-Ouakil to conduct a RCT comparing the efficacy of two behavioural parent programs on children's and adolescent's chronic irritability in 12

centres across France. This project also emphasizes the growing acceptability of dimensional targets relevant for novel therapeutic approaches of DBDs.

WP12: Ethics and training

Foreground

MATRICS can be considered an extremely complex and ambitious project in every respect. Research groups from all over Europe had set themselves the task of understanding aggressive behaviour from new perspectives in order to understand the basis for modified and more effective therapeutic approaches. The special feature of the undertaking was primarily the translational method. This means that not only basic researchers and clinicians got to talk to each other, but also that the results of preclinical and clinical research were put into a meaningful relationship.

Results

A DSMB roadmap was drawn up at the beginning of the project work. In cooperation with the coordinator, a Data Monitoring Safety Board was set up and standardised procedures were defined in this context. In addition to the respective study designs, the basis for all studies was to ensure formal and ethical standards in joint research across national borders. Furthermore, the aim was to achieve the greatest possible agreement in the methods used.

Thus, on the one hand, it was a matter of the complete receipt of ethical votes for all studies and all study centres (D12.03: “Ethics and regulatory approvals from the submitted protocols provided to the EU commission”).

It was ensured that preclinical investigators comply with legislation and local guidelines regarding the ethical use of animals. Moreover, the importance of protecting confidentiality in clinical studies was declared a maxim. The members of the Ethics Advisory Board had previously been provided with all relevant study documents in order to check them for possible inadequacies or errors.

While joint SOPs (D12.02: “Training procedures and documents for clinical studies”) were drawn up and trained for the preclinical and imaging studies, training workshops on the application of the psycho-diagnostic instruments were held prior to the clinical studies (D12.04: “Training procedures and documents for clinical studies”). In detail, cross-site training related to MRI, MRS was carried out, genetics protocols were compiled and a dissemination of SOP for blood sampling has been done.

It was also ensured that research assistants who were added later were able to "catch up" on their training with the help of written instructions. In addition and with the help of case vignettes, the inter-rater reliability at all relevant sites was determined in a comprehensive process and forwarded to all research assistants who were involved in the collection of data.

During the course of the studies, the up-to-datedness of the respective GCP certificates was repeatedly queried and checked.

WP13: Project management

Foreground

Work package 13 was dedicated to project management and to take care of all administrative and coordinating tasks. In order to support the scientific coordinator in monitoring the compliance of all beneficiaries with their obligations under the grant agreement, the project management office at concentris, together with the coordinator, kept a close eye on all partners’ performance

Results

WP13 ensured

- that tasks assigned to the beneficiaries were performed correctly and in a timely manner
- that reports were submitted according to the guidelines and in time
- that funds were used and claimed according to the rules
- that the partners fulfilled their obligations regarding dissemination and funding acknowledgements

- that any changes to the work plan were communicated to the EC swiftly
- that any changes were compliant with the ethical requirements and regulations

Conclusion

The project management office acted as a helpdesk for all participants. It was the central node of communication on a day-today basis and communicated with the European Commission on behalf of the Coordinator regarding administrative and managerial issues (i.e. contracts, amendments, 4 periodic reports, and the final report).

4 Potential impact, the main dissemination activities, and exploitation of results

4.1 Socio-economic impact and the wider societal implications of MATRICS

Persistent antisocial behaviour, aggression and antisocial disorders, particularly conduct disorder (CD) and associated callous unemotional (CU) traits have significant clinical and societal impact across the EU. CD is defined as a repetitive and persistent pattern of behaviour through which the basic rights of others and major age-appropriate societal norms or rules are violated. CD is often comorbid with other disorders (Attention-deficit Hyperactivity Disorder-ADHD in 50% of cases) and is associated with significant levels of functional impairment and high burden for the patients, their families and society in general. CD has a lifetime persistent course in half of the cases, with increased risk of criminality. By their nature, these disorders can have a chronic persistent course far into adulthood with approximately 50% of all cases presenting with antisocial personality disorder or psychopathy as adults with increased risk of criminality. It is precisely this combination of high prevalence and strong persistence which poses a major medical and socioeconomic problem in our society, and leads to substantial economic costs. These financial costs of antisocial behaviour/CD are high. Antisocial behaviour/CD in childhood and adolescence is a major predictor of how much an individual will cost society. By age 28, costs for individuals with CD were nearly 10 times higher than for those with no CD problems. Delivering new approaches to diagnosis, prevention and treatment will alleviate substantially the burden for patients, family and society, and also impact on broader issues such as feeling safe in public places and society in general. Furthermore, these strategies in at-risk and CD individuals will be a cost-effective way to reduce the public health burden of aggressive and antisocial behaviour that is at risk of extension into adulthood with negative risks for the individual with CD (e.g. criminal activity, imprisonment and poor socioeconomic prospects) and society. This negative impact extends not only to our society at large but also to the victims of aggression and imposes burdens on the executive and judiciary legal systems. Systematically meta-analysing the frequency of psychiatric disorders in adolescent detainees reveals a striking finding: CD (46-53%) is the most frequently observed. Current treatments have limited efficacy or only benefit a subsample of the clinical population. Designing new and more effective interventions requires a much better understanding of the neural, genetic, cognitive and biomarker mechanisms involved in paediatric aggression and antisocial behaviour.

MATRICS already created major impact in the identification of new genetic, neural and autonomic biomarkers associated with aggression and antisocial behaviour in CD with and without CU traits. It also addressed aggression as cross-disorder problem by addressing CD comorbidity with ADHD in paediatric populations. In the future, early identification of integrated biomarkers in CD and population may allow for stratification and early intervention in at risk groups and may assist in acting as surrogate clinical biomarkers for diagnostic, prognostic and treatment outcome use. MATRICS results were disseminated to patient organisations, clinical professionals and industrial end-users for improvement in the clinical management of these comorbidities and directing future therapeutic trials. Eventually, we hope that MATRICS will have the following social and socio-economic impact:

1. Availability of more effective medication for reactive and instrumental aggression in subjects with CD +/- CU traits.

2. The development of specific preventive interventions targeting reactive and instrumental aggression in CD +/- CU traits in childhood will reshape our way of thinking about developmental issues of brain and behaviour, and may lead starting interventions earlier, before adolescence.

3. The identification of neural, cognitive, genetic and biomarkers predicting antisocial behaviour and aggression in CD +/- CU traits will facilitate research by allowing for stratification and early intervention in at risk groups.

4. The development of clinically feasible Risk Assessment Charts to be used in routine clinical practice will provide clinicians with the tools to prioritize and identify youngsters with increased risks and in need of early interventions.

5. Eventually, the impact will be further in lowering the persistence of reactive and instrumental aggression in CD +/- CU traits over age by lowering the burden of adult psychiatric disease, which will result in substantial benefits for patients, families and society.

4.2 Main dissemination activities of MATRICS

In addition to its online representation via the website (www matrics-project.eu), its twitter account (https://twitter.com/MATRICS_EU) and general dissemination items such as the project flyer (https://matrics-project.eu/images/MATRICS_Folder.pdf), and the project video (https://youtu.be/avC6E5h33zg), the entire MATRICS consortium has been extremely active in communicating the project's scope and objectives as well as its results and outcomes to the general public, to patients and stakeholders, and to scientific and clinical professionals. In total, MATRICS produced, organised, or contributed to the following dissemination outputs:

- **118 peer-reviewed publications** in scientific journals, ranking as high as Science, Nature, Nature Neuroscience, Translational Psychiatry, Molecular Psychiatry, and Neuropsychopharmacology

- About **250 other dissemination activities**:

    - 151 oral presentations at scientific events
    - 18 oral presentations to a wider public
    - 35 posters
    - 10 articles published in the popular press
    - 10 workshops (organisation)
    - 8 TV clips, videos, or films
    - 6 press releases or media briefings
    - 5 papers in proceedings of a conference or workshop
    - 4 radio interviews
    - 4 conferences (organisation)
    - 2 articles for edited books

Ongoing news were communicated on a regular basis via the MATRICS website, Twitter, and the project's newsletter. Senior scientists within the MATRICS consortium have also supervised the completion of 11 Bachelor's, Master's, and PhD thesis projects.

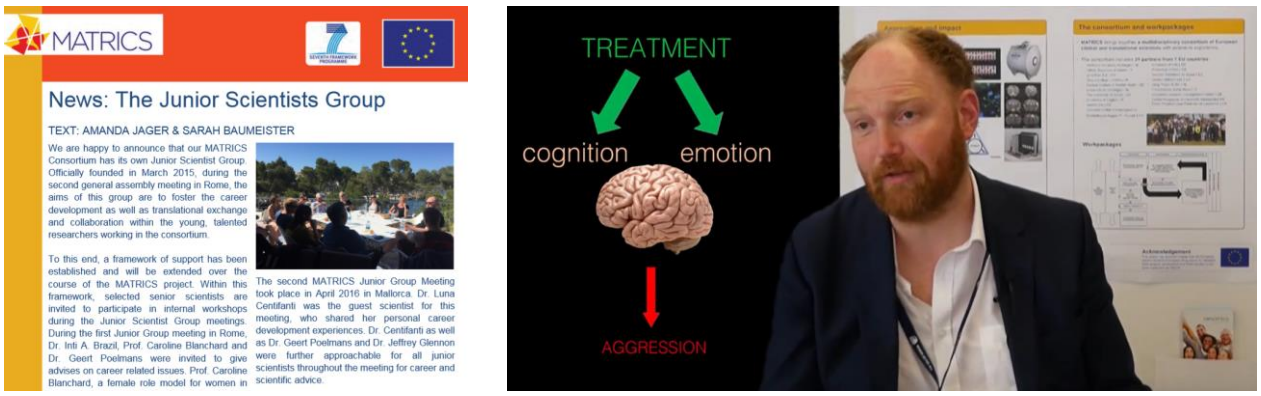

Figure 4. Exemplary excerpts from a MATRICS newsletter (left) and the MATRICS project video on YouTube (right).

4.3 Exploitation of results of MATRICS

On a yearly basis, all partners were asked to identify and valorise the knowledge and the intellectual property that they had produced within the realm of the MATRICS project. As a result, a report on putative biomarkers for potential further investigation has been submitted. Furthermore, the algorithm for the causal modelling interface used in WP10 will be made freely available to the research community (on the online platform CSCAN). In addition, a spin-out service company (Machine2Learn, https://machine2learn.com/) was formed as output from the MATRICS, TACTICS and OPTIMISTIC consortia, and is at present valorising the causal discovery efforts resulting in its participation in a number of ventures including the Aggressotype project.

5 Address of the project website and relevant contact details

Website address: https://matrics-project.eu/

Below is a list of all MATRICS beneficiaries and contact details of the team leaders at each of these institutions. Contact details for each beneficiary can also be found on the MATRICS website under "About MATRICS => Members".

Beneficiary	Title	First Name	Last Name	Email
01 RUN	Dr.	Jeffrey	Glennon	Jeffrey.Glennon@radboudumc.nl
02 EPFL	Prof. Dr.	Carmen	Sandi	carmen.sandi@epfl.ch
03 ISS	Prof. Dr.	Giovanni	Laviola	gianni.laviola@iss.it
04 Genoway	Dr.	Angelique	Heckmann	heckmann@genoway.com
05 KCL	Prof. Dr.	Steve	Williams	steve.williams@kcl.ac.uk
06 CIMH	Prof. Dr.	Daniel	Brandeis	daniel.brandeis@zi-mannheim.de
07 UNIVDUN	Prof. Dr.	David	Coghill	d.r.coghill@dundee.ac.uk
08 UMCG	Prof. Dr.	Pieter	Hoekstra	p.hoekstra@accare.nl
09 UNEXE	Prof. Dr.	Jonathan	Mill	j.mill@exeter.ac.uk
10 UNICA	Prof.	Alessandro	Zuddas	azuddas@unica.it
11 neuroConn	Dipl.-Ing.	Klaus	Schellhorn	klaus.schellhorn@neuroconn.de
12 UULM	Dr.	Ulrike	Schulze	Ulrike.Schulze@uniklinik-ulm.de
13 CHUM	Prof. Dr.	Diane	Purper-Ouakil	d-purper_ouakil@chu-montpellier.fr
14 Phenosys	Prof. Dr.	York	Winter	winter@phenosys.com
15 concentris	Dr.	Nina	Donner	nina.donner@concentris.de
16 IDIBAPS	Prof. Dr.	Josefina	Castro Fornieles	jcastro@clinic.ub.es
17 SERMAS	Prof. Dr.	Celso	Arango	carango@hggm.es
18 CI	Mr.	Greg	Prescott	gp@cixyz.co.uk
19 DTID	Dr.	Geert	Poelmans	g.poelmans@donders.ru.nl
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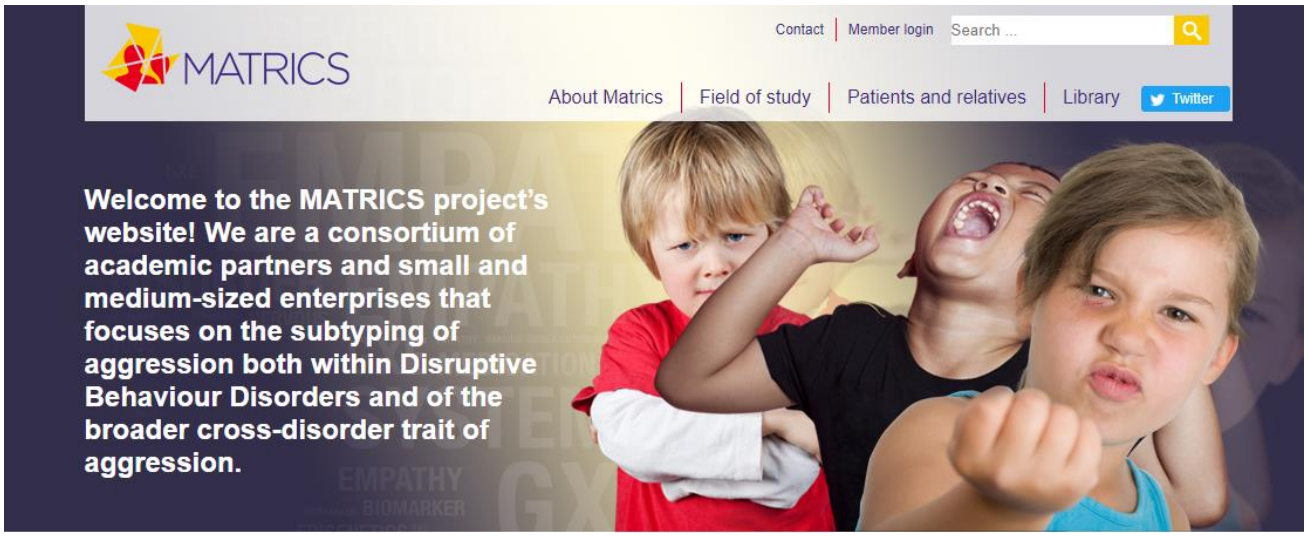

Figure 5. Screenshot from the official website of the MATRICS project.