PROJECT FINAL REPORT

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Final publishable summary report

1 An executive summary

The 'Aggression subtyping for improved insight and treatment innovation in psychiatric disorders' (Aggressotype) project was a five year project funded in the context of the HEALTH.2013.2.2.1-3 call for projects addressing paediatric conduct disorders characterised by aggressive traits and/or social impairment: from preclinical research to treatment. The consortium consisted of 27 partners from multiple disciplines, forming a team to address aggression research in a highly interdisciplinary manner.

Aggressotype was aimed at understanding the mechanisms underlying impulsive and aggressive behaviour in individuals with childhood the psychiatric disorders attention-deficit/hyperactivity disorder (ADHD) and conduct disorder (CD). Importantly, we argued that the mechanisms underlying aggressive behaviour can only be sufficiently understood if aggression subtypes are considered, that might have different aetiologies. Extrapolating from research in animals, a subtyping into impulsive (reactive) and instrumental (proactive, premeditated) was postulated. Based on the increased insights, we aimed at improving treatment and prevention of aggressive behaviour, working on the optimization of existing treatments and the development of novel pharmacological and non-pharmacological ones.

To achieve its aims and objectives, Aggressotype employed a multi- and interdisciplinary design, connecting across different levels of investigation, and balanced work in existing samples with new data collection for optimal use of resources. The project consisted of 12 work packages (WPs), including 10 scientific ones, as well as 2 coordinating WPs, one for ethics, training, and dissemination (WP11) and one for the general management of the project (WP12). WPs 1-6 were preclinical WPs, in which the investigation of mechanisms underlying aggressive behaviour is central. WP01 and WP02 were geared towards identifying the neural circuits contributing to aggression and clarifying differences in neural plasticity and neurochemistry underlying impulsive and instrumental aggression in human cohorts and mouse models. In WPs 3-6, it was our objective to define the genetic factors, their interaction with environmental factors, and epigenetic factors involved in aggression, and increase our understanding of the biological pathways through which these factors act. This was done through large genetics studies, and by using zebrafish, mouse, and human cellular models as well as neuroimaging genetics studies in humans to define biological pathways. WPs 7-10 worked towards improving treatment and prevention of aggression in ADHD and CD, by developing statistical prediction algorithms, by developing a new, non-pharmacological treatment for aggressive behaviour based on biofeedback training, and by testing the effectiveness of methylphenidate in treating aggressive behaviour in young adult prison inmates diagnosed with ADHD. In addition, we devised a zebrafish model to test novel compounds for their effect on aggressive behaviour, as a first step to develop improved pharmacological treatments.

We performed data collection for a multicentre study on children with conduct disorder (CD). Our analyses showed shared and separate neural substrates for proactive and reactive forms of aggression. In addition, in several cohorts as well as in mouse models, we found evidence that both inattention and hyperactivity/impulsivity drive aggression in adolescents with ADHD. In the mouse models, we showed that aggressive mice deficient for the Tph2 gene present with altered neurotransmitter levels in fronto-striatal brain circuits. In addition, we found that BALB/cJ (compared to BALB/cByJ) mice showed increased aggression, anxiety, and rule-breaking behaviour, associated with a number of MRI volumetric and white matter changes in regions associated with learning / memory. The stimulant methylphenidate reduced aggressive behaviour in BALB/cJ mice at low but not at high doses. We performed the world-wide largest genome-wide association study (GWAS) of ADHD in >20.000 cases, identifying 12 novel risk loci for the disorder. In a second GWAS of similar size, we showed a strong overlap of 80% in the genetic contribution to ADHD in children and adults. We also finished preliminary GWAS of CD in ADHD in >4500 cases and completed genotyping of 3000 additional CD cases. In our work in the model systems, we characterized the molecular biology linking several candidate genes to aggression-relevant phenotypes through analyses in models based on zebrafish, mice, and induced human neurons. We found monoamine oxidase (the product of the archetypical aggression gene MAOA) to be altered in its expression in relevant parts/cells of the brain; in neuroimaging genetics studies, we could deduce that MAOA genetic variation causes alterations in a broad network of functional connections in the brain. This was in line with our findings in the dopaminergic induced neurons, where we also observed an uncoordinated pattern of functional connectivity among the neurons. We also generated a new mouse model for the RBFOX1 gene, which had been identified in our human genetics studies; we could show that this model has a striking, non-social phenotype upon knock-out. In genome-wide genetic overlap studies for ADHD and human brain volumes, we found genetic links of this aggression-relevant disorder with total brain volume; genes involved in neurite outgrowth played a role in this overlap. We noticed distinct influence of environmental adversity on the severity of aggression, and identified epigenetic patterns that may mediate the genetic and environmental influences. Some of the gene-environment interactions could be validated in human cohorts.

Working towards improving treatment and prevention, we developed a novel method for data reduction of genome-wide genetic data and developed correlation engines for data integration to predict aggression. We have finalized inclusion into our newly developed novel biofeedback protocol for training of self-regulation. We observed aggression-reducing effects of methylphenidate in prisoners diagnosed with ADHD and subsequently initiated a larger, placebo-controlled randomized trial, which is currently recruiting. Based on our findings, we have initiated diverse activities to improve treatment of offenders with ADHD. We also developed a platform for high-throughput testing of new compounds for anti-aggressive properties in zebrafish, and performed testing of 108 potentially aggression-reducing compounds in larval zebrafish. A total of four compounds were taken forward, and several – including methylphenidate - were confirmed in mouse models.

Our work has provided important progress in the field of biological research of aggression, a field that has been lacking behind – despite the large societal impact of aggression. We have employed activities to help contribute to improve treatment and prevention in the foreseeable future, based on our findings.

2 A summary description of project context and objectives

Concepts

Aggression, overt behaviour with the intention of inflicting physical damage, is a physiological trait with important roles throughout evolution, both in defence and predation. When expressed in humans in the wrong context however, aggression leads to social maladjustment and crime. Maladaptive aggression is commonly observed across childhood conduct (disruptive behavioural) disorders, in particular in attention-deficit/hyperactivity disorder (ADHD) and conduct disorder (CD). Aggression in these disorders is a heterogeneous trait, which scientists have tried to subdivide in diverse ways. Such subdivision is a prerequisite for the development of effective prevention and treatment strategies, as currently available treatment options for maladaptive aggressive behaviour have limited efficacy. The most promising candidate subdivision, derived from animal studies, defines an emotionally labile, reactive impulsive and a low emotional, instrumental ('predatory') subtype (Lesch and Merschdorf, 2000). Evidence from human studies supports this subdivision (e.g., Heinz et al., 2011), which may finally open up possibilities for developing effective prevention and treatment strategies for vulnerable children.

The main aims of this project were to (1) build a knowledge chain for the aetiology of maladaptive aggression, from the molecular level via cellular, brain-network and cognitive levels to behaviour, and (2) improve the subtyping of aggression. This was done in children most vulnerable to pathological aggression, i.e. those with CD (prone to impulsive and instrumental aggression) and ADHD (prone to impulsive aggression) and in individuals from the general population. We argued that only through such rigorous characterisation will a better prediction of developmental course and outcome of aggression be achieved and that this would open new horizons for individualising prevention of pathological aggression and improving and personalising aggression treatment.

To achieve its aims and objectives, Aggressotype employed a multi- and interdisciplinary design, connecting across different levels of investigation, and balanced work in existing samples with new data collection for optimal use of resources. The project consisted of 12 work packages (WPs), including 10 scientific ones, as well as 2 coordinating WPs, one for ethics, training, and dissemination and one for the general management of the project.

WPs 1-6 were preclinical WPs, in which the investigation of mechanisms underlying aggressive behaviour is central. WP01 and WP02 were geared towards identifying the neural circuits contributing to aggression and clarifying differences in neural plasticity and neurochemistry underlying impulsive and instrumental aggression. WP01 used existing and newly collected data from human samples of individuals with ADHD and CD for this purpose, WP02 investigated these points in mouse models. In WPs 3-6, it was our objective to define the genetic factors and their interaction with environmental factors involved in aggression, and increase our understanding of the biological pathways through which these factors act. WP03 worked on gene identification using large, existing samples, WP04 used zebrafish and mouse as well as cellular models to define biological pathways. In WP5, we used neuroimaging genetics models to investigate, how genetic risk factors for aggression act on the human brain. WP06 performed epigenetic studies in both animals (mouse) and existing human cohorts to clarify the interplay between genetic and environmental contributors to aggressive behaviour.

WPs 7-10 were working towards improving treatment and prevention of aggression in ADHD and CD. In WP07, we worked on the prediction of aggressive behaviour based on biomarkers using the biological insights gained in WPs 1-6. In WP08, it was our aim to develop a new, non-pharmacological treatment for aggressive behaviour based on biofeedback training. WP09 tested the effectiveness of existing medication for ADHD, methylphenidate, in treating aggressive behaviour in young adult prison inmates, which have been diagnosed with ADHD. WP10 developed and used a zebrafish model to test novel compounds for their effect on aggressive behaviour, as a first step to develop improved pharmacological treatments.

WP11 had the responsibility for ensuring the ethical conduct of all studies within the Aggressotype project, covering both studies in humans and in animals. This WP also coordinated the dissemination activities of the project, in which we aimed to reach different target groups including clinicians, researchers, the general public, but also policy makers. For this, we worked in close collaboration with the patient organisation ADHD-Europe. In addition, this WP was responsible for training; in this, we saw ourselves as responsible for training a new generation of researchers, which would need to be able to work in an interdisciplinary manner. WP12 was responsible for effective management of the Aggressotype consortium and its activities as well as for the contact with the EC.

The set-up of the project was geared towards addressing 11 key objectives in three major themes, (I) increasing insight, (II) improving patients' quality of life, and (III) training, dissemination, and valorisation. The main results of the project will be reported here by addressing the 11 key objectives of Aggressotype:

(I) Gaining insight into the mechanisms underlying pathological aggression:

Objective 1: To identify the neural circuits contributing to aggression and clarify differences in neural plasticity and neurochemistry underlying impulsive and instrumental aggression in childhood-psychiatric patients
Using neuroimaging studies in humans in combination with diverse study designs in mouse models, we aimed to understand overlap and differences in the neural architecture underlying impulsive and instrumental aggression subtypes. For the human part of this work, we enriched existing cohorts with longitudinal data and collected a new multicentre cohort of conduct disorder. In addition, we selected several mouse models based on monogenic and multifactorial aggression models.

Objective 2: To identify the genetic pathways/networks involved in impulsive and instrumental aggression through studies in humans and animals, and to understand how they interact with preventable environmental influences
We investigated the world-wide largest genetic cohorts to identify new genetic risk factors for ADHD and CD, as well as studying their overlap. We subsequently employed zebrafish and mouse models as well as human models to study the networks involved. We studied those models at the levels of genetics, epigenetic modifications, and transcriptomic profiles.

Objective 3: To understand the biological mechanisms by which genetic and epigenetic networks exert effects on the aggression subtypes
Using different environmental stressors, and investigating different candidate genes for aggression, we studied the biological mechanisms underlying aggression at different levels of organismal complexity. We employed zebrafish and mouse models as well as two types of human models for this work, i.e. induced dopaminergic neurons derived from induced pluripotent stem cells extracted from individuals carrying mutations in the archetypical aggression gene 'MAOA', and neuroimaging genetics studies based on existing and newly collected cohorts with MRI and (genome-wide) genotyping data. We studied those models at the levels of genetics, epigenetic modifications, and transcriptomic profiles.

(II) Translation of preclinical findings to therapies for the benefit of the patient

Objective 4: To develop predictors of aggressive outcome in children and adults for prevention and prediction of treatment outcome by integrating multilevel data
Together with a commercial partner, we developed several algorithms to predict aggression based on input from genetics, neuroimaging, and behavioural studies. This included the definition of the variables to include in these algorithms through data reduction methods.

Objective 5: To perform pilot studies on efficacy and safety of novel non-pharmacological, biofeedback-based treatment in paediatric clinical populations to support future large-scale clinical trials according to these strategies
We aimed to develop and test a biofeedback protocol for the treatment of aggression problems in youth, distinguishing between the different subtypes of aggression.

Objective 6: To perform a clinical trial of ADHD medication in a sample characterized by negative young adult outcome of aggression, a prison sample
We aimed to study the effectiveness of methylphenidate in the treatment of aggression in male young adult offenders in a prison setting. In first instance, we planned an open label trial, but also aimed to obtain follow-up funding for a larger, randomized placebo-controlled trial.

Objective 7: To develop a novel animal model for a first pharmacological screen and identify novel candidate treatment leads
We planned to devise a model based on zebrafish larvae, using a high-throughput platform to be newly developed as part of Aggressotype. In this setting, we planned to investigate at least 100 candidate pharmacological compounds for their anti-aggressive properties.

(III) Training, dissemination, and valorisation

Objective 8: To develop a central database on aggression risk factors and make it available to the research community to maximize opportunities for research in this area and increase speed of discovery
We aimed to integrate available and newly generated knowledge on individual risk genes for aggression into a database for the purpose of making it an infrastructure available to all researchers.

Objective 9: To educate a new generation of interdisciplinary researchers in the field of aggression, one which has knowledge of basic scientific approaches to understand the mechanism of action of aggression risk factors but also knows how to translate these findings into clinically relevant information
We aimed to develop a training program involving the definition of clear training and supervision plans, reflecting the multidisciplinary nature of the research. We also planned to hold regular Master classes in key-areas of Aggressotype research to provide the early career scientists with a basis in both fundamental research and translational approaches towards clinical and societal use of research findings. In addition, we planned to develop a mentoring program for both male and female researchers, with female researchers to be mentored by a female senior researcher with the goal of enhancing the retention of the junior females in (academic) research.

Objective 10: To disseminate our protocols, procedures, results and findings as widely as possible. Our target groups are all parties involved in paediatric disruptive behavioural disorders, including scientists, patients and their representative organizations, clinicians, nurses, medical authorities, teachers and the general public as well as industrial companies
We aimed to make protocols, procedures, and our findings publicly available in the spirit of open science. This included the database of integrated findings on aggression genes, but also protocols and procedures for different studies used in the project.

Objective 11: To valorise predictive algorithms for clinical use, identified pharmacological treatment leads by attracting pharmaceutical companies and implement biofeedback into clinical aggression treatment
We aimed to have a significant impact on society based on our findings, by developing implementation plans for our findings and products. In particular, we aimed at disseminating/valorising the findings from the biofeedback study, the methylphenidate study in young offenders, and the platform and findings from the zebrafish screen of pharmacological compounds. In addition, we planned to disseminate the algorithms and methods for data integration aimed at predicting aggression.

3 A description of the main S&T results/foregrounds

3.1 Summary of the main results/foreground of Aggressotype

In WP01, we performed data collection for a multicentre study on children with conduct disorder (CD) as well as an enrichment of an existing cohort with longitudinal data. Our analyses show shared and separate neural substrates for proactive and reactive forms of aggression. In several cohorts, we found evidence that both inattention and hyperactivity/impulsivity drive aggression in adolescents with ADHD. This finding was confirmed in mouse model work in WP02.

In WP02, we showed that aggressive mice deficient for the Tph2 gene present with altered neurotransmitter levels in fronto-striatal brain circuits (in collaboration with WP06). In addition, we found that BALB/cJ (compared to BALB/cByJ) mice, showed increased aggression, anxiety, and rule-breaking behaviour, and had a number of MRI volumetric and white matter changes in regions associated with learning / memory. The stimulant methylphenidate reduced aggressive behaviour in BALB/cJ mice at low but not at high doses.

In WP03, we performed a genome-wide association study (GWAS) of ADHD in >20.000 cases, identifying 12 novel risk loci for the disorder. In a second GWAS, we showed a strong overlap of 80% in the genetic contribution to ADHD in children and

adults. We also finished preliminary GWAS of CD in ADHD in >4500 cases and completed genotyping of 3000 additional CD cases.

In WP04, we characterized the molecular biology linking several candidate genes to aggression-relevant phenotypes through analyses in models based on zebrafish, mice, and induced human neurons. We found monoamine oxidase (the product of the archetypical aggression gene MAOA) to be altered in its expression in relevant parts/cells of the brain. We also generated a new mouse model for the RBFOX1 gene, identified in WP03, which had a striking phenotype upon knock-out.

In WP05, we could deduce that MAOA genetic variation causes alterations in a broad network of functional connections in the brain. This was in line with our findings in the dopaminergic induced neurons, where we also observed an uncoordinated pattern of functional connectivity among the neurons. For AVPR1A, we showed in influence on amygdala volume, and for NOS1, we found white matter connectivity to be altered. In genome-wide genetic overlap studies for ADHD, we found genetic links of this aggression-relevant disorder with total brain volume; genes involved in neurite outgrowth played a role in this overlap.

In WP06, we noticed distinct influence of environmental adversity on the severity of aggression, and identified epigenetic patterns that may mediate the genetic and environmental influences. Some of the gene-environment interactions could be validated in human cohorts.

WP07, responsible for the development of predictive algorithms for aggression, has developed a novel method for data reduction of genome-wide genetic data. With a new consortium partner, we have developed correlation engines for data integration to predict aggression.

In WP08, we have finalized inclusion into our newly developed novel biofeedback protocol for training of self-regulation. Preliminary findings of one of the outcome measures shows very promising results, comparable to existing treatments of aggression.

In WP09, we observed aggression-reducing effects of methylphenidate in prisoners diagnosed with ADHD in a first trial. Based on this, we started a second, placebo-controlled randomized trial, which is currently recruiting. Based on our promising findings, we have initiated diverse activities to improve treatment of offenders with ADHD.

In WP10, we performed testing of 108 potentially aggression-reducing compounds in larval zebrafish. A total of four compounds were taken forward, and several – including methylphenidate - were confirmed in mouse models.

In WP11, responsible for ethics, training, and dissemination, we developed a training program for our early career scientists, and organized activities to communicate our research to patients and the public. We worked together with the other 3 EU-funded consortia on aggression, through collaborative activities for young researchers, symposia, and special issues in scientific journals.

WP12 has successfully managed Aggressotype, keeping communication optimal through regular meetings and clear reporting schedules.

Since cross-site collaboration and interdisciplinary work were highly important in Aggressotype, we also founded several working groups across different WPs. In this way, WP01 and WP08 worked closely together in an ‘imaging working group’, WP02, WP04, and WP06 formed an ‘animal working group’, and WP03, WP04, WP05, WP06, and WP07 worked together in a ‘genetics working group’. Interdisciplinary work was done by combining data across different model systems and approaches, leading to several publications describing results for e.g. individual genes across model systems, and publications integrating data from multiple sources to identify patterns and mechanisms from gene to disorder. Among others, this interdisciplinary work was the basis for the successful selection of a new animal model for the Aggressotype work, and formed the basis for the interdisciplinary training of the early career scientists in Aggressotype.

3.2 Main results/foreground of the different work package

WP01: Neural correlates of aggression – human studies

Background

Studies available at the start of Aggressotype mainly consisted of small studies in male adults, implicated the limbic system (especially amygdala, hippocampus and ventral striatum) and various subregions of prefrontal cortex in aggression. A large body of animal and human literature showed that the prefrontal regions are strongly linked to the limbic structures identified as abnormal in violence, forming regulatory circuits. However, no systematic attempt had been made to characterize these extended circuits using neuroimaging in paediatric populations. Furthermore, both structural and, especially, functional studies showed inconsistencies, and no specific alteration could be assigned reliably to one of the aggression subtypes. The majority of studies had employed only a single functional task, and thus could not disambiguate between functional state and subject variables related to violence or diagnoses. In Aggressotype, we pursued a circuit-focused, transdiagnostic approach to define the neural substrates of aggression for the different subtypes. We used an established robust and reliable battery of tasks, and associated quality control procedures, to study neural circuits in large samples of children with ADHD and CD at rest and during several tasks behaviourally related to aggression and involving the prefrontal cortex and limbic structures. We aimed to assess the structural integrity and connectivity of participants through high-resolution structural and diffusion tensor imaging scans. In this way, we aimed to gain new insights into the neural mechanisms underlying pathological aggression by improving subtyping and using advanced imaging methods.

Overall Objectives

WP01 had four primary objectives:

1. Identify neural, neurocognitive and biomarker mechanisms underlying aggressive / antisocial behaviour in high-risk children and adolescents (subjects with ADHD) and controls cross-sectionally and longitudinally
2. Collect a new sample of children with Conduct Disorder (CD), adolescents with CD, and controls and identify neural, neurocognitive, and biomarker mechanisms underlying aggressive / antisocial behaviour and establish standard operating procedures and quality control of MRI acquisition across sites
3. Measure cognitive, physiological (skin conductance and heart rate) and motor (EMG) components of empathy in children and adolescents with CD and examine whether different empathy components are differentially affected across the different aggression subtypes
4. Integrate findings from the WP tasks, and examine the common (cross-disorder) and the disorder-specific correlates of the aggressive / antisocial behaviour

Results

During the course of the project, WP01 has achieved the following results:

1. 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[1].
2. 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[2].
3. 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.
4. The presence of ADHD+ODD and ADHD-only is associated with stepwise significant volumetric reductions in the orbitofrontal gyrus, middle frontal gyrus, right superior frontal gyrus, and left inferior parietal gyrus. ADHD+ODD-specific volumetric reductions were found in the right precuneus and left middle temporal gyrus
5. The positive bias effect present in healthy controls (having significant difficulty in contrasting positive with neutral pictures compared to negative-neutral or negative-positive pictures) was absent in the ODD/CD (and ASD) group.
6. ODD/CD is associated with higher levels of cortisol and testosterone and lower levels of oxytocin, compared to the healthy control situation and ASD.
7. 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[3].

8. 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 yr, 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.
9. 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).
10. 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., in preparation).
11. 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., submitted).
12. Negative emotional face recognition, as shown before[4,5], 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., in preparation).

Conclusions

Results from WP01 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.

References

1. van Ewijk H, Noordermeer SDS, Heslenfeld DJ, et al. The influence of comorbid oppositional defiant disorder on white matter microstructure in attention-deficit/hyperactivity disorder. *Eur Child Adolesc Psychiatry*. 2016. doi:10.1007/s00787-015-0784-3.
2. Noordermeer SDS, Luman M, Buitelaar JK, et al. Neurocognitive Deficits in Attention-Deficit/Hyperactivity Disorder With and Without Comorbid Oppositional Defiant Disorder. *J Atten Disord*. 2015. doi:10.1177/1087054715606216.
3. Noordermeer SDS, Luman M, Greven CU, et al. Archival Report Structural Brain Abnormalities of Attention- De fi cit / Hyperactivity Disorder With Oppositional De fi ant Disorder. *Biol Psychiatry*. 2017;82(9):642-650. doi:10.1016/j.biopsych.2017.07.008.
4. Blair RJ. Psychopathy: cognitive and neural dysfunction. *Dialogues Clin Neurosci*. 2013;15(2):181-190.
5. Veroude K, von Rhein D, Chauvin RJM, et al. The link between callous-unemotional traits and neural mechanisms of reward processing: An fMRI study. *Psychiatry Res - Neuroimaging*. 2016;255:75-80. doi:10.1016/j.pscychresns.2016.08.005.

WP02: Neural correlates of aggression – animal studies

Background

The regulation of aggression relies on the interplay between rational versus emotional decision making and its response to environmental challenges and stressors. In humans, regional abnormalities in individuals diagnosed with disorders having a strongly increased risk for aggression are reported. Brain structural analyses have shown reduced volumes of the amygdala, the posterior hippocampus and the frontal and superior temporal cortex, and increased volume of the striatum and the corpus callosum. Connectivity changes such as reduced structural integrity of the uncinate fasciculus have been reported. In functional neuroimaging, reduced activity has been observed in the amygdala, hippocampus, ventral striatum and insula as well as in anterior and posterior cingulate gyri, although findings, especially in limbic structures, have been inconsistent. Taken together, these studies implicated the limbic system (especially amygdala, hippocampus and ventral striatum) and various sub-regions of prefrontal cortex in aggression. The core circuit we propose to be involved in violence and abnormal social behaviour includes interactions between orbitofrontal and cingulate cortex with amygdala, hippocampus, insula and ventral striatum, and interactions between dorsolateral and medial prefrontal cortices with orbitofrontal and cingulate cortex. As such, we propose that instrumental and impulsive aggression both reflect deficient regulation of limbic and other subcortical structures by prefrontal cortex, but those they differ in the directionality of limbic activity. While the neurochemistry of aggression has strongly focussed on neuropeptides such as oxytocin and monoamines such as dopamine / serotonin, proton spectroscopy (1H-MRS) enables the measurement of other metabolites including the primary excitatory / inhibitory neurotransmitters glutamate and GABA as well as the acetylcholine precursor choline. Their role in aggression and antisocial behaviour is largely unestablished. The role of corticolimbic / cortico-striatal transmission can be tested in animal models, phenotyped for aggression, anxiety, and social behaviours matched to magnetic resonance imaging (MRI) / 1H-MRS of brain to elucidate changes in brain volume, white matter integrity, functional connectivity, and metabolism.

Overall Objectives

WP02 had three primary objectives:

1. To characterize developmental changes in a) morphometry, b) white matter integrity, and c) functional connectivity (resting state fMRI) in the brain circuits including fronto-striatal circuits across early adulthood in three rodent models, as determined in WP04, with similar neuroimaging protocols and analysis tools as in WP01.
2. To ascertain whether changes in the blood oxygen level dependent (BOLD) signal (or its tissue oxygen correlate) are coupled to alterations in concurrent measurements of gene expression of candidates identified in WP03.
3. To determine the structural and functional neural and molecular correlates of behavioural improvement in aggressive behaviour after pharmacological treatment with the most promising drug candidate, as identified in WP10, in one of the three rodent models.

Results

During the course of the project, WP02 has achieved the following results:

1. Set-up and validation of mouse resident-intruder paradigm with BALB/cJ or BALB/cByJ residents vs. C57/Bl76 (intruder) mice. Robust increases in aggression observed. Attack latency decreases over successive days of testing with BALB/cJ mice biting more than their BALB/cByJ controls. BALB/cJ mice demonstrate an atypical bite profile during their attacks (rule breaking behaviour: biting vulnerable body parts) while also demonstrating increased (ratio of closed versus open arm entries) anxiety in the elevated plus maze compared to the BALB/cByJ controls.
2. Implementation of mouse protocols for the acquisition and analysis of magnetic resonance imaging data at 11.7T. Protocols implemented for sequences for arterial spin labelling (to measure arterial blood flow), structural 3D analysis, diffusion tensor imaging (DTI) to examine local connectivity (fractional anisotropy / apparent diffusion coefficient), T1 imaging (as an index of myelin status), proton spectroscopy (1H-MRS) to examine changes in glutamate turnover and neuronal integrity (N-acetylaspartate; NAA), resting-state MRI (rs-MRI) with analysis of default mode networks.
3. MRI data has been generated in the BALB/cJ mouse model of aggression. In MRI-DTI studies, these mice demonstrate reduced fractional anisotropy (white matter integrity) in the anterior cingulate cortex (ACC) and dorsomedial striatum (DMS). No change in the apparent diffusion coefficient is seen or 1H-MRS Glx markers is seen.
4. The BALB/cJ mouse model shows aggression coupled to increased anxiety-like behaviour. BALB/cJ mice show increased brain gray matter volume coupled to lower body weight. Analysis of morphometry demonstrates regional brain volume increases are seen in striatum, hypothalamus, hippocampus while decreases in regional volume are apparent in the frontal, insular and sensory cortices in aggressive BALB/cJ mice compared to BALB/cByJ controls. Furthermore, 1H-MRS of the aggressive BALB/cJ mouse model demonstrates no change in anterior cingulate cortex ACC excitatory glutamate but a reduction in total ACC choline levels which may reflect reduced excitatory

acetylcholine and phosphocholine concentrations. At the same time, the BALB/cJ mouse demonstrates decreased attention performance compared to its BALB/cByJ control which may be related to reduced ACC cholinergic tone (as a clear relationship between attention and mPFC acetylcholine tone has been demonstrated elsewhere in rodents performing the same attention (5CSRTT) task as tested in the BALB/cJ mice.

5. In the BALB/cJs, there is a negative correlation between omissions in the 5CSRTT (inattention) and MRI T2 maps in the cerebral aqueduct, 4th ventricle, midbrain and periaqueductal grey matter. Furthermore, there was a positive correlation between omissions (inattention) and unilateral differences in the VBM of the inferior coliculus and thalamus, FA of the retroflex fasc-iculus and T1 map of the vagus, 4th ventricle.
6. Reduced cortisol levels are seen in blood samples from aggressive BALB/cJ mice both before and following the resident intruder task compared to their BALB/cByJ controls; this is linked to reduced ACC expression of corticotrophin-releasing hormone (Crh) mRNA in BALB/cJ mice. Interestingly, qPCR based mRNA expression of the biological target of testosterone, the androgen receptor demonstrate a 3 fold upregulation of the AR in the ACC of BALB/cJ mice compared to the BALB/cByJ substrain.
7. BALB/cJ (compared to BALB/cByJ mice) show increased aggression, anxiety and rule-breaking behaviour. In addition, they show an inability to learn from punishment reinforces (in contrast to BALB/cByJ mice), similar to juvenile CU traits (which also is punishment-insensitive).
8. BALB/cJ mice show a number of MRI volumetric changes compared to BALB/cByJ mice; both whole brain (increased) and in the anterior cingulate, mid-cingulate cortices as well as hippocampal regions associated with learning / memory. MRI-DTI in BALB/cJ mice suggests altered white matter integrity markers with changes in fractional anisotropy in the ACC with no changes in the apparent diffusion coefficient. 1H-MRS demonstrates a reduction in GABA in the ACC, which may be related to both a) a reduction in parvalbumin (PV) and somatostatin (SOM) positive (PV+ / SOM+) GABAergic interneuron number and b) increased GABA catabolism in this region. The reduction in PV+ / SOM+ interneurons strongly correlates with both sociability and aggression in BALB/cJ mice.
9. BALB/cJ mice show impaired global attention (increased omissions) in the 5-choice serial reaction time task compared to BALB/cByJ mice but show no strain dependent differences in the continuous performance task.
10. Methylphenidate decreases anxiety and aggression and restores fear conditioning in BALB/cJ mice with low dose (3mg/kg i.p) MPH being the most effective (similar to clinical observations that low but not high dose MPH is effective against aggression in children with conduct disorder). The same dose improves error detection in BALB/cJ mice in the continuous performance task of sustained attention.
11. In total 11 heterozygous and 11 homozygous Tph2 knockout mice arrived from the UKW laboratory. These were tested in the resident intruder task for aggression, the elevated plus maze / open field for anxiety measures followed by multiparametric MRI data acquisition.
12. Aggressive Tph2 knockout mice show alterations in Glx, PEA, and NAA levels in fronto-striatal circuits. In particular, Tph2 knockout (-/-) mice show context-specific alterations in aggression: clear aggression was seen towards C57/Bl6 but not towards DBA/2 mice. Analysis of 1H-MRS data demonstrates changes decreases in n-acetyl-aspartate (NAA; a marker of neuronal integrity) and phosphoethanolamine (PEA; a marker of lipid metabolism, choline turnover and endocannabinoid signalling) in the striatum of aggressive Tph2 knockout mice. Furthermore, in the same mice, an increase in Glx (a composite signal of glutamate and glutamine) is apparent in the anterior cingulate cortex (ACC) in Tph2 knockout mice compared to controls. These changes were only seen in aggressive Tph2 mice (versus C57/Bl6 mice) and not in those that non-aggressive to DBA/2 mice.
13. Tph2 knockout mice show rule-breaking behaviour (abnormal bite pattern) in addition to aggression.
14. Analysis of MRI-DTI data collected has largely demonstrated a reduction in fractional anisotropy (a marker of white matter integrity) across a number of brain areas including the mid-cingulate cortex, dorsal striatum, striatal fundus, globus pallidum, parietal-temporal lobes and inter-peduncular nucleus in homozygous Tph2 knockout mice compared to controls. Heterozygous Tph2 knockout mice were largely similar to wildtype controls and did not show gene-dose effects. In only one region of interest was an increase in fractional anisotropy observed, namely in the white matter tract, the fimbria (which together with the fornix) connects prefrontal and hippocampal regions.
15. The reduction in white matter integrity in the dorsal processing stream (large associated with rational decision making namely the mid-cingulate cortex, dorsal striatum and globus pallidum) but not in the ventral processing stream (the anterior cingulate cortex, the orbitofrontal cortex, the ventral striatum and ventral pallidum) suggests that the inhibitory control exerted by the dorsal stream over emotional decision making is impaired in the homozygous Tph2 knockout mice.
16. Our WP04 colleagues (Reif laboratory) have characterised the behavioural profile of RBFOX1 knockout mice as demonstrating anxiety and compulsive behaviours. No effect in the resident intruder task has been observed. A new batch of mice is currently being phenotyped and ex vivo MRI will be performed in Q1/2 2019.

Conclusions

Analysis of behavioural data demonstrates a clear aggressive phenotype of both BALB/cJ and homozygous Tph2 knockout mice in the resident intruder task versus C57/BL6 intruders. Both strains demonstrate anxiety in the elevated plus maze / open field suggesting that increased anxiety to threats in the environment may play a role in reactive aggression in both strains. The BALB/cJ strain is also accompanied by global attention deficits which may impair its sensitivity to environmental changes. Furthermore, methylphenidate may exert in part its anti-aggressive effect by (i) reducing anxiety, (ii) re-instating fear conditioning and (iii) improving error detection in BALB/cJ mice. Analysis of morphometry demonstrates regional brain volume increases are seen in striatum, hypothalamus, hippocampus while decreases in regional volume are apparent in the frontal, insular and sensory cortices in aggressive BALB/cJ mice compared to BALB/cByJ controls suggesting changes in regions involved in learning, stress-regulation, executive function and salience to environmental stimuli. Both the BALB/cJ and homozygous Tph2 knockout mice demonstrate altered fronto-striatal white matter changes. BALB/cJ mice demonstrate reduced fractional anisotropy in the anterior cingulate cortex and dorsomedial striatum but do not demonstrate changes in the orbitofrontal cortex. In contrast, Tph2 knockout mice demonstrate reduced fractional anisotropy in the mid cingulate cortex, dorsal striatum and globus pallidus. This suggests that both models of aggression are associated with white matter changes but in different regions of cingulate cortex, subserving emotional versus rational decision making. In BALB/cJ mice, a loss of inhibitory GABAergic tone is documented in the cingulate cortex with no change in glutamate tone while aggressive Tph2 knockout mice show increased glutamatergic tone with no change in GABA in the cingulate cortex. In both cases, excitatory (E) / inhibitory (I) balance is disturbed such that there is a relative increase in excitation/inhibition (E/I) balance in the cingulate cortex compared to non-aggressive controls.

WP03: Genetic studies of common and rare variants in aggression: genes and pathways

Background

Genome-wide association (GWA) scans, based around common SNPs, have proved to be a powerful tool for identifying variants with an impact on susceptibility to various common disorders including neuropsychiatric conditions. However, limited GWA scans on conduct disorder (CD) and aggressive traits have been published and no whole-genome association studies including rare and low frequency variants for aggressive/antisocial traits had been reported at the start of the project. Rare variants may have larger effects on aggressive traits than common variants, providing important new insights into the mechanisms underlying pathological aggression. Aiming towards improving the understanding of the neurobiology of paediatric conduct disorders characterised by aggressive traits and/or social impairment, this WP aims at analysing existing and expanding GWA scans available to the partners involved. For the discovery of genetic variants, this WP has leveraged a unique resource of GWA data generated by the Psychiatric Genomics Consortium (PGC) and the Danish iPSYCH consortium. iPSYCH contributes with more than 2,000 cases with a conduct disorder diagnosis, and some additional 3,000 subjects with CD from the Danish New Born Screening Biobank have been genotyped in the context of Aggressotype. Although most subjects showing aggression phenotypes had already been recruited by the team members at the beginning of the project, the analyses performed have required commensurating phenotypes across different sites and creating a joint data set that have allowed joint analyses across studies.

Overall Objectives

WP03 had three primary objectives:

1. Testing the association of candidate genes with conduct disorder (CD) in large sample sets with pre-existing and new genome-wide association (GWA) data.
2. Using hypothesis-free approaches in large sample sets with pre-existing GWA data to search for genes or pathways, not previously implicated in aggressive traits.
3. Validation of variants.

Results

During the course of the project, WP03 has achieved the following results:

1. WP03 promoted a Special Issue of the American Journal of Medical Genetics B (Am J Med Genet B 171(5): 557–760, 2016) edited by Aggressotype members including 13 papers, seven of them from the Aggressotype group.
2. Review of existing GWAS studies as well as other types of genetic and genomic studies to select the *RBFOX1* gene for study by Aggressotype animal model WPs. RBFOX1 is a neuron-specific RNA splicing factor that regulates expression of large genetic networks during early neuronal development and is expressed in the developing forebrain. It has been previously related to several neurodevelopmental and neuropsychiatric disorders. *Rbfox1* knockout mice show neuronal hyperexcitability, but aggressive behaviour has not yet been assessed. As the direction of effect of genetic variation in

RBFOX1 on gene activity/expression is not known yet and could not be deduced based on existing human data, we worked on both knock-out and overexpression models of Rbfox1 (see WP04). The integration of the previous GWAS results was published in [1] and [2]. We summarized all evidence supporting a role of the *RBFOX1* gene in aggression, including genetic and epigenetic studies in humans, neuroimaging genetics, gene expression and several animal models [3] (see References section below).

3. We have systematically examined all aggression phenotypes catalogued in Online Mendelian Inheritance in Man (OMIM), identifying 95 human disorders that have documented aggressive symptoms linked to 86 causal genes. Although most of these genes had not been implicated in human aggression by previous studies, the most significantly enriched canonical pathways had been so (e.g., serotonin and dopamine signalling). The study was based on the hypothesis that studies of rare, functional genetic variations can lead to a better understanding of the molecular mechanisms underlying complex multifactorial disorders such as aggression. Our findings provide strong evidence to support the causal role of these pathways in the pathogenesis of aggression and point at additional mechanisms underlying the origins of human aggression [4].
4. Using a cross-species approach, we integrated existing genetic data on aggression and identified enriched common pathways such as the G-protein coupled receptor (GPCR) signalling pathway, axon guidance, reelin signalling in neurons and ERK/MAPK signalling. Also, we defined a list of 40 top-ranked and highly interconnected genes for aggression [5].
5. We have completed the world-wide largest GWAS of ADHD and related population traits in in over 20.000 cases and over 35.000 controls. We identified 12 genome-wide significant loci for ADHD [6]. ADHD is often comorbid with conduct disorder (CD), with a possible shared genetic background that we have subsequently investigated. We found strong genetic overlap between ADHD and aggressive behaviour in the population and antisocial behaviours. ADHD and aggression: genetic correlation = 0.7; ADHD and antisocial behaviour: genetic correlation = 0.5 [5].
6. We have performed a GWAS in around 4.500 ADHD patients with comorbid conduct disorder (CD) or oppositional defiant disorder (ODD) using pre-existing data from the Psychiatric Genomics Consortium (PGC) and iPSYCH. This allowed identification of a first genome-wide significant hit in the gene *STIM1*. SNP-based heritability was estimated as h^2=0.34 for ADHD+CD in the iPSYCH sample, but it was only 0.20 for ADHD without comorbid CD, indicating that common variants explain more of the genetic architecture of ADHD+CD than for ADHD alone. Also, we observed an increased polygenic risk in individuals with ADHD+CD than in subjects having only ADHD. These analyses are currently being combined with the results from 3.000 CD cases primarily without a comorbid ADHD diagnosis.
7. We assessed the combined effect on aggressive traits in the general population of common genetic variants in two gene sets that cover the monoaminergic and the neuroendocrine systems. This study allowed us to identify distinct (but correlated) subtypes of aggression. We also found association between genetic variation in the neuroendocrine and serotonergic signalling in frustration-based reactive aggression in females [7].
8. We identified three genes (*RRM1, ZNF544, DDX28*) showing differential expression in ADHD+CD versus controls using SNP weights derived from models trained on reference transcriptome datsets from GTEx using metaXcan.

Conclusions

We have performed genome-wide analyses in ADHD and also in individuals with ADHD and comorbid CD, identifying specific risk genes in both groups. We have found that the SNP-based heritability is higher in ADHD+CD than in ADHD, indicating a higher common genetic burden in the first phenotype. Finally, ADHD and several aggression/antisocial phenotypes are genetically correlated. A systematic analysis of single-gene aggression phenotypes catalogued in the Online Mendelian Inheritance in Man (OMIM) identified near 100 causal genes. Most of them are new, but they point at canonical pathways that had previously been linked to aggression. We have identified *RBFOX1*, a neuron-specific RNA splicing factor, as a candidate gene for aggression by reviewing and analysing previous data from different sources: genetics and epigenetics in humans, neuroimaging genetics, gene expression and animal models. In addition, we used a cross-species approach to identify common pathways in aggression and defined a list of 40 top-ranked genes. Gender-specific effects have been found in a subtype of aggression (frustration-based reactive aggression) in females, involving the neuroendocrine and serotonergic signalling pathways. Altered gene expression is observed in ADHD+CD versus controls.

References

[1] Fernàndez-Castillo N, Cormand B. Aggressive behavior in humans: Genes and pathways identified through association studies. Am J Med Genet B Neuropsychiatr Genet. 2016 Jul;171(5):676-96. doi: 10.1002/ajmg.b.32419. Review. PubMed PMID: 26773414.

[2] Veroude K, Zhang-James Y, Fernàndez-Castillo N, Bakker MJ, Cormand B, Faraone SV. Genetics of aggressive behavior: An overview. Am J Med Genet B Neuropsychiatr Genet. 2016 Jan;171B(1):3-43. doi: 10.1002/ajmg.b.32364. Review. PubMed PMID: 26345359.

[3] Fernàndez-Castillo N, Gan G, van Donkelaar MMJ, Vaht M, Weber H, Retz W, Meyer-Lindenberg A, Franke B, Harro J, Reif A, Faraone SV, Cormand B RBFOX1, encoding a splicing regulator, is a candidate gene for aggressive behaviour. Eur Neuropsychopharmacol. 2017 Nov 23. pii: S0924-977X(17)32003-5. doi: 10.1016/j.euroneuro.2017.11.012. [Epub ahead of print]
[4] Zhang-James Y, Faraone SV. Genetic architecture for human aggression: A study of gene-phenotype relationship in OMIM. Am J Med Genet B Neuropsychiatr Genet. 2016 Jul;171(5):641-9. doi: 10.1002/ajmg.b.32363. PubMed PMID: 26288127.
[5] Zhang-James Y, Fernàndez-Castillo N, Hess J, Malki K, Glatt S, Cormand B, Faraone SV. An integrated analysis of genes and functional pathways for aggression in human and rodent models. Mol Psychiatry, 2018 [in press]
[6] Demontis, Walters, et al., Discovery of the first genome-wide significant risk loci for ADHD. Nature Genetics, 2018 [in press] – see also http://biorxiv.org/content/early/2017/06/03/145581
[7] van Donkelaar MMJ, Hoogman M, Shumskaya E, Buitelaar JK, Bralten J, Franke B. Monoamine and neuroendocrine gene-sets associate with frustration-based aggression in a gender-specific manner. Eur Neuropsychopharmacol. 2017 Nov 27. pii: S0924-977X(17)32007-2. doi: 10.1016/j.euroneuro.2017.11.016. [Epub ahead of print]

WP04: Neurobiological mechanisms of risk genes for aggression subtypes: animal and in vitro studies

Background:
The overall objective of WP04 was to examine the neurobiological mechanisms of aggression as a result of genetic variation in known and novel candidate genes. We started with known risk genes to ensure immediate beginning of the project and the implementation of pertinent structures and added the best candidate genes from WP03/WP05, which we studied later using identical procedures. The set of pre-defined candidate genes comprised genes with high validity that feature at least two independent lines of evidence (human genetics, rodent studies, other lines of research) linking these genes to aggressive / impulsive behaviour. The selected genes code for monoamine oxidase A (*Maoa*), latrophilin 3 (*Lphn3*), cadherin 13 (*Cdh13*), neuronal tryptophan hydroxylase (*Tph2*), and neuronal nitric oxide synthase (*Nos1*). Additionally, we studied the role of expressional variation in a novel candidate gene coding for RNA binding protein FOX-1 (*Rbfox1)* in aggressive / impulsive and social behaviours. The mechanisms linking known and novel candidate genes to aggression were studied on several levels to identify converging pathways. Finally, we compiled molecular, circuit-level, and behavioural data from zebrafish, mouse, and human studies within Aggressotype into a comprehensive data matrix.

Overall Objectives
WP04 had four primary objectives:

1. To investigate the biochemical consequences of variants in coding regions of candidate genes.
2. To assess the behavioural impact of candidate gene knockout in different species with emphasis on subtyping aggressive behaviours.
3. To develop a multi-level neurobiological database of candidate-gene mediated predisposition towards aggressive behaviour that can later be further validated in humans.
4. To delineate neural networks that specifically and primarily mediate aggressive behaviour.

Results
During the course of the project, WP04 has achieved the following results:

1. Behavioural screening of the *Nos1, Cdh13, Tph2, Lphn3*, and *Snap25* mouse lines was completed and results summarized in the central Aggressotype database.
   i. Homozygous *Nos1*-knockout mice display reduced aggression in the resident-intruder task, and hyperactivity in the open field test without changes in the anxiety-related measures. There is a marked decrease in social investigation and social novelty preference in these mice, possibly related to the impairments in the detection and discrimination of social odours. Increase in the number of perseverative responses in the 5-choice serial reaction time task (5-CSRTT) in the Nos1-knockout animals suggests cognitive inflexibility.
   ii. *Tph2*-knockout mice have decreased attack latency in the resident-intruder task, and increased impulsivity in the 5-CSRTT. No genotype differences were found in social preference or anxiety-like behaviour. However, open field test revealed hyperactivity in the knockout males, which was also replicated in a non-aversive setting (red-light illuminated open field).
   iii. *Cdh13*-knockout mice do not present attentional deficits or impulsivity in the 5-CSRTT but show mild cognitive impairment during the initial training stages. Cdh13-ko mice also have a mild spatial memory impairment as measured in the Barnes maze, and a fear-learning deficit in a fear conditioning paradigm. No genotype differences were found in anxiety-like behaviour, but *Cdh13*-knockout animals present mild hyperactivity in the open field test.

iv. *Lphn3*-knockout mice were found to have increased horizontal and reduced vertical locomotor activity without concomitant changes in anxiety measures in the open field and light/dark box test. Aggression and impulsivity testing will be done in early 2017 for these mice.
v. Heterozygous male *Snap25* mice do not display genotype-dependent differences in either locomotor activity (open field test), anxiety-like, aggressive or social behaviour.

2. Homozygous *nos1* zebrafish mutants have been thoroughly phenotyped and were found to be much less aggressive than the other two genotypes. They also swim slower and a shorter distance than controls. They spend more time at the bottom of a novel tank and do not interact differently with a novel object compared to controls. There is no shoaling phenotype in these fish. *Nos1* mutant zebrafish display a reduction of aggression and increased anxiety-like behaviour. These alterations correlate with reduced monoamine neurotransmitter breakdown in the brain, and pharmacological stimulation of 5-HT signalling can rescue the behavioural phenotype.
3. Cross-model characterization of aggression and related behavioural traits in NOS1 knock-out models of zebrafish and mouse: Carreño Gutiérrez H, O'Leary A, Freudenberg F, Fedele G, Wilkinson R, Markham E, van Eeden F, Reif A, Norton WHJ. Nitric oxide interacts with monoamine oxidase to modulate aggression and anxiety-like behaviour. Eur Neuropsychopharmacol. 2017 Sep 23. pii: S0924-977X(17)30906-9. doi: 10.1016/j.euroneuro.2017.09.004. [Epub ahead of print] PubMed PMID: 28951000.
4. Completion of behavioural characterization of several zebrafish lines, including *lphn3.1* and the novel genes *hrh3*, *ywhaz*, *libra*, and *reelin*. A paper describing the libra mutants was published in 2018 (Bitetti et al., 2018, MicroRNA degradation by a conserved target RNA regulates animal behaviour; Nature Structural & Molecular Biology 25, 244-251).
5. Creation of a mouse line overexpressing the cytoplasmic isoform of *Rbfox1* at genOway. Completion of behavioural screening of animals with heterozygous neuron-specific overexpression of *Rbfox1*. We have back-translated the human genetic evidence that RBFOX1 has a role in aggression to mice, where we could establish a role for *Rbfox1* in aggression, anxiety, and social behaviour
   i. Mice with neuronal-specific deletion of *Rbfox1* are non-social and non-aggressive, display marked persistent hyperactivity, impaired fear learning and deficits in sensorimotor gating.
   ii. *Rbfox1* knockout mice were tested in the visual discrimination task to assess their cognitive performance. We found that the mice were able to acquire two-stimuli discrimination as well as the controls. However, they displayed mild inflexibility in the reversal phase. Additionally, *Rbfox1*-KO mice were found to be impulsive and unable to sustain attention throughout the testing session.
   iii. Mice with heterozygous or homozygous overexpression of the cytoplasmic isoform of *Rbfox1* did not differ from controls in tests of locomotor activity, anxiety, or fear learning.
6. Generation of a new *Nos1*-Cre.ERT2 mouse line at genOway. Crossing these mice with Channelrhodopsin-2 (ChR2) transgenic mice allows for precise and specific ChR2 expression in *Nos1*-positive neurons; these mice can then be used for aggression testing where optogenetic activation the *Nos1*-positive ChR2-expressing neurons could lead to changes in aggressive behaviour.
7. Optogenetic activation of mouse *Nos1*-positive ventromedial hypothalamic neurons did not lead to altered locomotor activity, social behaviour, or increased aggression.
8. Reprogramming of fibroblast cell lines and peripheral monocyte blood cells into induced pluripotent stem cells (iPS) using integrating lentiviral STEMCCA vector and non-integrating Sendai virus vector and differentiation of iPS cells into dopaminergic neuronal cells successful.
9. Generation of iPSCs from fibroblasts of 3 independent patients with Brunner Syndrome carrying different mutations in the aggression gene MAOA was successfully performed. Successful implementation of a protocol for dopaminergic differentiation of the iPSC cultures, and differentiation MAOA iPSCs into dopaminergic cells and identification of altered neuronal activity in the absence of (consistent) morphological alterations. (Shi et al., manuscript in preparation)
10. The druggable genome based on GWAS data across ADHD and comorbid disorders has been explored in a manuscript in preparation: “Druggable Genome in Attention Deficit/Hyperactivity Disorder and its Co-morbid Conditions. New avenues for treatment”. Many promising druggable genes were identified in this study, including SLC6A9, encoding the sodium- and chloride-dependent glycine transporter 1 (GlyT1), the protein phosphatase PTPRF and KCNH3, encoding a brain-expressed voltage-dependent potassium channel (known as KCNH3, BEC1 or Kv12.2) (Hegvik et al., in preparation).
11. We have completed the multi-level, multi-species aggression database summarizing molecular, circuit-level, and behavioural findings from zebrafish, mouse, and human studies within Aggressotype.

Conclusions

We have successfully assessed the aggressive, social, and affective-like behaviours in zebrafish and mice with constitutive or inducible deletion or overexpression of known and novel candidate genes and have confirmed the role of these genes in the

regulation of aggressive behaviour and/or other psychiatrically-relevant behaviours. Further, identification of novel druggable targets could lead to better treatments of psychiatric disorders for which aggression is a frequent comorbid symptom. Although optogenetic activation of the *Nos1*-positive in the hypothalamus did not lead to behavioural changes, our efforts to delineate neural circuits which directly control aggressive behaviour in mice will continue in the future.

References

- Carreño Gutiérrez H, O'Leary A, Freudenberg F, Fedele G, Wilkinson R, Markham E, van Eeden F, Reif A, Norton WHJ. (2017) Nitric oxide interacts with monoamine oxidase to modulate aggression and anxiety-like behaviour. European Neuropsychopharmacology, Sep 23. pii: S0924-977X(17)30906-9. doi: 10.1016/j.euroneuro.2017.09.004. [Epub ahead of print] PubMed PMID: 28951000.

- Bitetti A, Mallory AC, Golini E, Carrieri C, Carreño Gutiérrez H, Perlas E, Pérez-Rico YA, Tocchini-Valentini GP, Enright AJ, Norton WHJ, Mandillo S, O'Carroll D, Shkumatava A. (2018) MicroRNA degradation by a conserved target RNA regulates animal behaviour. Nature Structural & Molecular Biology 25, 244-251.

WP05: Neurobiological correlates of aggression subtypes: neuroimaging genetics studies

Background

Understanding the neurobiological mechanisms underlying the effects of aggression-related genetic and environmental factors is a prerequisite for translating knowledge from fundamental science into clinical practice. Animal models are an essential tool in this, but understanding how the risk factors affect functioning of the human brain can provide additional critically important information. WP5 concentrated on the evaluation of effects of aggression risk genes and gene-networks on brain structure, brain activity and brain connectivity in people with ADHD and other aggression-related disorders. As aggression is a cross-disorder trait and continuous with aggression in the general population, WP5 also validated the observed effects of genes and networks on brain phenotypes in healthy individuals' brain structure and function, using the world-wide largest data sets.

Overall Objectives

WP5 had three primary objectives:

1. Evaluate effects of known aggression genes and novel candidates identified in WP3 as well as aggression gene-networks identified in WP7 on brain phenotypes of individuals with ADHD and other aggression-related disorders. In this objective, we investigated effects on brain structure (Task 1), brain activity (Task 2), and brain connectivity (Task 3).
2. Validate the observed effects of genes and networks on brain phenotypes in individuals from the general population (Task 4).
3. Identify brain structural and functional characteristics that mediate the effects of genetic and environmental risk factors on impulsive, aggressive behaviour and/or social impairment in the different samples (Task 5).

Results

WP5 has resulted in nearly 30 publications, highlights among which were the following:

In Task 1:

1. DAT1 is differentially associated with caudate nucleus volume across the lifespan (Onnink et al., J Neural Transm 2016)
2. Variation in neuroendocrine and serotonergic signalling is involved in the aetiology of frustration-based reactive aggression in a gender-specific manner (van Donkelaar et al., Eur Neuropsychopharmacol. 2017 Nov 27)
3. We identified significant overlap between genetic risk factors for ADHD and those for intracranial volume (ICV), which was partly explained by a set of genes involved in neurite outgrowth (Klein et al., 'Genetic markers of ADHD-related variations in intracranial volume', Am J Psychiatry, in press; see also a non-reviewed manuscript on bioRXiv https://www.biorxiv.org/content/early/2017/09/12/184192). Overlap of ADHD risk genes and genetic contribution to aggression-related brain volume is focal rather than global.

In Task 2:

4. We published two brain imaging genetics review papers: van Donkelaar et al., 2017, 'Imaging genetics in neurodevelopmental psychopathology', Am J Med Genet Part B 174 (5), 485-537; Klein et al. 2017, 'Brain imaging genetics in ADHD and beyond - Mapping pathways from gene to disorder at different levels of complexity', Neurosci Biobehav Rev. 80, 115-155.

5. Based on the work in this WP, in combination with the other WPs, we defined several promising targets for treatment development: based on the work of WP03 and WP05, RBFOX1 appears particularly promising. However, being a regulator of gene expression, its network is highly complex. Promising also seems AVPR1A, which has been investigated in WP03 and WP05, a gene known to be associated with social behaviour in animal models.

In Task 3:

6. Resting-state brain connectivity in the cerebellum is different between adult patients with ADHD and healthy controls (Mostert et al., Prog Neuropsychopharmacol Biol Psychiatry. 2016; 67:82-91).
7. Widespread differences in the white matter structure exist between the brains of adults with ADHD and healthy individuals. The pattern of results suggest aberrant myelination as a potential cause for such differences (Onnink et al., Prog Neuropsychopharmacol Biol Psychiatry, Epub May 5 2015).
8. We found that NOS1 genetic variation was associated with total ADHD and hyperactivity-impulsivity symptoms, but not inattention; this effect was independent of gender. NOS1 variation was also associated with mean diffusivity values in several white matter tracts in females, but not males (van Ewijk, Bralten et al., J Child Psychol Psychiatry. 2017;58 (8), 958-966).
9. Network-based statistics of MAOA genetic variation identified a significant brain cluster showing increased functional connectivity in the low expression genotype group during emotional face processing within a large, distributed subnetwork, an overlapping network was identified during resting state fMRI (Harneit et al, in preparation).

In Task 4:

10. Genome-wide analysis of subcortical brain structures identified novel genes for brain structures involved in ADHD and aggression. Such genes are novel candidates for analysis in behaviour (Hibar et al., Nature 2015).
11. Genetic factors underlying brain volumes linked to aggression have been identified (for details please also see publications Hibar et al., Nature 2015; Adams et al., Nature Neuroscience 2016; Hibar et al., Nature Communications 2016)
12. Aggression gene AVPR1A shows significant gene-wide association both with amygdala volume and hippocampal volume in >13,000 individuals from ENIGMA consortium (van Donkelaar et al., Front Behav Neurosci. 2018;12:61).
13. A manuscript on the largest genome-wide analyses of subcortical volumes worldwide (in over 40.000 individuals) is currently in revision in Nature Genetics (Satizabal et al., Nature Genetics, in revision, also see bioRXiv https://www.biorxiv.org/content/early/2017/08/28/173831).
14. A manuscript on a large, international study of GWASs of cortical brain structures is currently under review in Nature (Grasby et al., Nature, under review); the unreviewed version is available at biorXiv (https://www.biorxiv.org/content/early/2018/09/09/399402).
15. Analysis of the Nijmegen Longitudinal Study showed that early-life and pubertal stressors code for altered growth in subcortical and frontal brain structures during adolescence. In addition, the serotonin transporter HTTLPR S-allele was associated with deviant (longer and shorter) infant freezing behaviour, which in turn was related to increased chance on developing internalizing symptoms. The work in this sample has been described in 5 papers.

In Task 5:

16. Mediation analysis for the serotonin transporter shows that grey matter volume in the orbitofrontal cortex mediates between the genetic variant and ADHD severity (van der Meer et al., Am J Psychiatry, Am J Psychiatry. 2015; 172(8):768-75). This analysis also indicates that not all imaging genetics findings are relevant for the phenotypes of interest.
17. Causal modelling shows that genetic variation in the dopamine transporter gene is linked to reward anticipation-related brain activity through inattentive behaviour rather than impulsivity (Sokolova et al., Am J Med Genet Part B, Epub Apr 2 2015).

Additionally:

18. Two PhD-theses have resulted from Aggressotype WP5 analyses, one by Marjolein van Donkelaar (Title: Genetic and neurobiological mechanisms underlying aggression subtypes; ISBN: 978-94-6284-134-5, defended successfully on June 15 2018), and one by Marieke Klein (Title: From gene to disorder in ADHD: Mapping mechanisms at different levels of complexity, to be defended on February 1 2019).
19. Local and national funding was obtained to continue work on aggressive behaviour in clinical and population samples in The Netherlands.
20. Effects of aggression candidate genes investigated in local and international GWAS data and added to the Aggressotype Database established by WP4.

Conclusions

We have been able to link genes for aggression and ADHD to brain structure and function in multiple different approaches, covering both candidate gene-based and whole genome-based analyses on the one hand and both brain region-specific and brain-wide analyses on the other. Our findings show that candidate genes for aggression affect structure and function of areas of the brain that have previously been linked to aggression. Our mediation analyses indicate that such brain alterations can be causally linked to the behavioural phenotype, but that not all brain alterations linked to a single gene (variant) are relevant for the trait/disorder of interest. Specifically for ADHD, we find that brain structure is generally affected, and that its genetic overlap is strongest with a general measure of total brain volume (intracranial volume); this suggests that the brain processes affected in ADHD act on the entire brain rather than on very specific individual brain regions. More generally, the genetic overlap between brain volumes and cortical thickness/surface area is more limited than expected, and our findings suggest that network-based analyses of brain connectivity may be the most promising way forward. In terms of potential differences between effects observed in patients and healthy individuals, our findings suggest that most effects of genetic risk factors will be similar in cohorts of patients and general population samples. In summary, brain imaging genetics approaches are an excellent approach to unravel the molecular pathways from (variant) gene to behavioural trait/disorder.

WP06: Gene-by-environment interaction in aggression subtypes and translational epigenetics

Background

Meta-analyses of twin studies as well as animal studies show that aggressive traits are significantly heritable and have also significant shared and unique environmental contributions. Twin studies provide some evidence for gene-environment correlation; for example, negative parenting is correlated with the genetic liability for aggressive/antisocial traits. However, studies of discordant identical twins have shown evidence for specific environmental risk factors such as expressed emotion, which cannot be accounted for by gene-environment correlation. Although gene-by-environment interaction seems likely, more work is needed before definitive statements can be made. Twin and adoption studies also suggest that family adversity and social disadvantage interact with genetic predisposition to produce aggressive/antisocial behaviours.

Animal model studies, often in combination with studies in humans, have implicated serotonergic, dopaminergic and noradrenergic pathways in aggression and antisocial behaviour. Genetic variation in the *TPH2* gene (coding for the rate-limiting enzyme in CNS serotonin biosynthesis, tryptophan hydroxylase 2), for example, has been shown to go along with increased intermale aggression. Similarly, the *LPHN3* gene, which was identified as an ADHD gene through combined linkage and association approaches in humans, has subsequently been linked to impulsive-aggressive behaviour in humans as well as zebrafish. As another example, deleting the *Maoa* gene in mice leads to aggressive behaviours. Strong evidence also suggests involvement of a common promoter variant of the *MAOA* gene in human aggression, especially in combination with childhood adversity, an example of gene-by-environment (G x E) interaction.

However, eight additional human genome-wide linkage studies have not found any replicated, genome-wide significant loci. This suggests that common DNA risk variants must have small effects on the expression of the phenotype, and/or that aggression is highly genetically heterogeneous. Environment therefore may act as a strong modulator of the effects of genetic risk factors on aggressive behaviour.

Studying the aggression in animal models such as mice permits the identification of underlying mechanisms and the discovery of novel drugs for extreme aggressive behaviour. Although those paradigms developed in rodent models do not directly translate to human paradigms, they have the huge advantage of measuring ethologically valid and relevant intra-species aggression in an experimental context. Studies using specific behavioural paradigms have provided evidence for several genes and critical brain areas linked to aggression. Very importantly, the use of genetically tractable model organisms is an invaluable means to the validation and functional characterization of candidate genes from human genetic studies and their interaction with environmental risk factors. In WP, we aimed to understand the molecular pathways underlying aggression, with a particular emphasis on gene-by-environment interactions.

Overall Objectives

WP06 had four primary objectives:

1. Develop and maintain an epidemiological database of G x E in selected human cohorts.
2. Investigate and validate epigenetic programming by early-life stressors in genetically modified mouse models subjected to maternal deprivation that simulate neurobehavioural characteristics of human aggression.
3. Translate and validate novel epigenetically regulated aggression risk genes derived from G x E mouse models in human cohorts characterised for environmental adversity that exhibit aggressive traits/behaviour and determine their utility as biomarkers.

4. Use translational resources from WP06 tasks to conceptualise and initiate testing the effects of preventive strategies and therapies for aggression on epigenetic modification patterns.

Results

During the course of the project, WP06 has achieved the following results:

Objective 1:

1. The ECPBHS database has been tuned to fit the analyses of Aggressotype. A new data and biomaterial collection wave has been completed. This latest data collection wave included a large battery of specific aggression-related measurement instruments that were selected to meet the objectives of Aggressotype and adapted for use in this sample.
2. The EPBTB sample has been further increased by 1,324 subjects recruited at traffic schools. These subjects have been randomly allocated to control and intervention groups as in previous data collection waves.
3. Consistent G x E interaction was found across a variety of aggressiveness measures for the *HCRTR1* genotype in the human studies: The rs2271933 A-allele was associated with higher aggressiveness, and in particular in female A/A homozygotes who had experienced either inferior family relationships in childhood or more of stressful life events. Another interesting finding is the consistent association of the *HTR1A* genotype with aggressiveness in response to life stress, while the aggressiveness profile in response to stress qualitatively differs between males and females.
4. Analysis, integration and interpretation of database of G x E in human cohorts complete.
5. Methylation profiling of the *MAOA* gene suggests differences in G x E in subjects with alcohol use disorders.
6. Further functional variants of candidate genes (*VMAT1* Thr136Ile, *DAT1* 3UTR VNTR, *DIRAS* rs1412005 G/T, *CRHR1* A/G, *FKBP5* rs1360780 C/T, *HCRTR1* rs2271933, *HTR1B* rs6296, *GABRA2* rs279858, *HTR1A* C-1019G, *OPRM* A118G, *AVPR1B* rs35369693, *CACNA1C* rs1006737, *TPH1* rs1800532, *SLC2A1* rs710218, *CNR1* rs1406977, *TRPV1* rs222747, *HTR2C* rs6318, *KTN1* rs945270 and rs8017172, *OXTR* rs1488467 and rs7632287, *FAAH* rs324420, *DRD2* rs6277, *RBFOX1* rs809682, rs6500744, rs8062784, rs12921846; *TSPO* rs6971; *FTO* rs1421085) have been genotyped in the original samples of the ECPBHS and EPSTB studies, and genotyping of variants with more interesting outcomes (*NPSR1* Asn107Ile, *TPH2* G-703T, *NRG1* rs6994992, *VMAT1* Thr136Ile, *CACNA1C* rs1006737, *HCRTR1* rs2271933, *CRHR1* rs17689918) have been genotyped in their extension subsamples. A variety of gene-by-environment interactions have been found and are being examined in depth for mediation mechanisms.

Objective 2:

8. *Tph2*-deficient mice display strong impulsivity-related behaviour and escalating aggression with decreased attack latency and increased time of fighting in the resident-intruder paradigm.
9. The hypothesis that such aggression-like phenotypes result from epigenetic programming prompted us to analyse the expression and DNA methylation profiling completed in the *Tph2*-modified mouse model. *Tph2*-/- male mice display deficits in appraisal and developmental stress shows different regulatory effects on gene expression in the amygdala of *Tph2*+/+ males, when compared to *Tph2*-/- and *Tph2*+/-. Enriched terms in the gene ontology analysis showed a significant enrichment of terms related to epigenetic gene expression regulation in the amygdala of *Tph2*+/+ males, while predator stress induces robust increases in impulsivity and aggression-related behaviour. This leads to novel G x E-regulated novel candidate genes currently being explored for differential methylation. In conclusion, 5-HT seems to mediate developmental programming by early-life and later-life stress via changes in epigenetic regulation.
10. A differentially methylated region in the gene encoding myelin basic protein (*Mbp*) was associated with its expression and was dependent on serotonin transporter (*5-Htt*) genotype, prenatal stress (PS) and the interaction of both (*5-Htt* x PS). Subsequent fine-mapping of this *Mbp* locus linked the methylation status of two specific CpG sites to Mbp expression.
11. The expression of CDH13 (a gene associated with ADHD and comorbid disorders, as well as violent behaviour) in the mouse hippocampus is confined to distinct classes of interneurons. Specifically, CDH13 is expressed by numerous parvalbumin and somatostatin-expressing interneurons located in the stratum oriens, where it localizes to both the soma and the presynaptic compartment. *Cdh13*-/- mice show an increase in basal inhibitory, but not excitatory, synaptic transmission in *cornu ammonis* field 1 (CA1) neurons. Associated with these alterations in hippocampal function, *Cdh13*-/- mice display deficits in learning and memory. Our results indicate that CDH13 is a negative regulator of inhibitory synapses in the hippocampus and provide insights into how CDH13 dysfunction may contribute to the excitatory/inhibitory imbalance observed in neurodevelopmental disorders, such as ADHD.
12. CDH13 deficiency in mice increases dorsal raphe 5-HT neuron density and prefrontal cortex innervation in the mouse brain. This might be related to serotonergic dysfunction leading to deficits in learning and memory associated with neurodevelopmental disorders. Thus, CDH13 appears to be a guidance molecule for serotonergic fibres to target regions.

13. Maternal separation as a model for early life stress resulted in increased stress resilience, increased exploration and an overall anxiolytic behavioural phenotype in male *Cdh13*+/+ and *Cdh13*+/- mice. CDH13 deficiency, however, obliterated most of the effects caused by early-life stress; with *Cdh13*-/- mice exhibiting delayed habituation, no reduction of anxiety-like behaviour and decreased fear extinction. These findings indicate a critical role of CDH13 in the programming of and adaptation to early-life stress. Moreover, our transcriptomic data support the view of CDH13 as a neuroprotective factor as well as a mediator in cell-cell interactions, with an impact on synaptic plasticity.
14. LPHN3 deficiency in mice reduces aggressive behaviour, increases locomotor activity and alters gait, and affects learning and memory. However, anxiety-like behaviour is not altered in LPHN3-deficient mice.
15. Varying social experiences during life history impacts behaviour, gene expression, and vasopressin receptor gene methylation in mice.
16. Social withdrawal behaviours and memory deficits result from Western diet in mice.

Objective 3:

17. TPH2 genotype-related serotonergic modulation of 'waiting impulsivity' is mediated by the impulsivity phenotype in humans.
18. Cholesterol levels throughout childhood, starting from age 9, predict maladaptive impulsivity in adulthood.

Objective 4:

19. Thiamine compounds are able to counteract stress-induced aggression, which is likely to be mediated by normalizing brain oxidative stress and expression of molecular regulators of emotionality and neural plasticity.
20. Thiamine and benfotiamine improve cognition and ameliorate GSK-3β-associated stress-induced behaviours in mice.
21. The insulin receptor sensitizer dicholine succinate prevents emotional changes, including anxiety and depression, induced by a high-cholesterol diet in mice.
22. Low-dose lipopolysaccharide (LPS) inhibits aggressive and augments depressive behaviours in a chronic mild stress model in mice.
23. Pro-neurogenic, memory-enhancing and anti-stress effects of a novel fluorine gamma-carboline derivative, DF-302, with multi-target mechanism of action.

Conclusions

The findings of WP06 support the view that the genes involved in the aetiology of impulsivity and aggression are of varied nature and impact on several brain functions involved in cognition, emotional regulation, and plasticity of several brain regions such as the prefrontal cortex, the dorsal raphe, and the amygdala. Remarkably, the role of those genes In maladaptive changes in impulsivity and social behaviour can only be understood when considering environmental factors, such as early life stress, social experiences during life, or high cholesterol diet. Indeed, drugs that act over glucose (dicholine succinate) or lipid (LSP) metabolism can positively modify aggressive behaviour. Similarly, a second group of drugs that counteract different stressors such as oxidative stress has also been shown to improve aggressive conditions in mice.

References (most relevant publications)

1. Schraut KG, Jakob SB, Weidner MT, Schmitt AG, Scholz CJ, Strekalova T, El Hajj N, Eijssen LM, Domschke K, Reif A, Haaf T, Ortega G, Steinbusch HW, Lesch KP, Van den Hove DL. Prenatal stress-induced programming of genome-wide promoter DNA methylation in 5-HTT-deficient mice. Transl Psychiatry. 2014 Oct 21;4:e473. doi: 10.1038/tp.2014.107. PubMed PMID: 25335169; PubMed Central PMCID: PMC4350514.
2. Rivero O, Selten MM, Sich S, Popp S, Bacmeister L, Amendola E, Negwer M, Schubert D, Proft F, Kiser D, Schmitt AG, Gross C, Kolk SM, Strekalova T, van den Hove D, Resink TJ, Nadif Kasri N, Lesch KP. Cadherin-13, a risk gene for ADHD and comorbid disorders, impacts GABAergic function in hippocampus and cognition. Transl Psychiatry. 2015 Oct 13;5:e655. doi: 10.1038/tp.2015.152. PubMed PMID: 26460479; PubMed Central PMCID: PMC4930129.
3. Neufang S, Akhrif A, Herrmann CG, Drepper C, Homola GA, Nowak J, Waider J, Schmitt AG, Lesch KP, Romanos M. Serotonergic modulation of 'waiting impulsivity' is mediated by the impulsivity phenotype in humans. Transl Psychiatry. 2016 Nov 8;6(11):e940. doi: 10.1038/tp.2016.210. PubMed PMID: 27824354; PubMed Central PMCID: PMC5314122.
4. Forero A, Rivero O, Wäldchen S, Ku HP, Kiser DP, Gärtner Y, Pennington LS, Waider J, Gaspar P, Jansch C, Edenhofer F, Resink TJ, Blum R, Sauer M, Lesch KP. Front Cell Neurosci. 2017 Sep 26;11:307. doi: 10.3389/fncel.2017.00307. eCollection 2017. PMID: 29018333).
5. Bodden C, van den Hove D, Lesch KP, Sachser N. Impact of varying social experiences during life history on behaviour, gene expression, and vasopressin receptor gene methylation in mice. Sci Rep. 2017 Aug 18;7(1):8719. doi: 10.1038/s41598-017-09292-0. PubMed PMID: 28821809; PubMed Central PMCID: PMC5562890.

6. Kiser DP, Popp S, Schmitt-Böhrer AG, Strekalova T, van den Hove DL, Lesch KP, Rivero O. Early-life stress impairs developmental programming in Cadherin 13 (CDH13)-deficient mice. Prog Neuropsychopharmacol Biol Psychiatry. 2018 Aug 28;89:158-168. doi: 10.1016/j.pnpbp.2018.08.010. [Epub ahead of print] PubMed PMID: 30165120.
7. Kiive E, Laas K, Vaht M, Veidebaum T, Harro J. Stressful life events increase aggression and alcohol use in young carriers of the GABRA2 rs279826/rs279858 A-allele. Eur Neuropsychopharmacol. 2017 Aug;27(8):816-827. doi: 10.1016/j.euroneuro.2017.02.003. Epub 2017 Feb 23. PubMed PMID: 28237505.
8. Markova N, Bazhenova N, Anthony DC, Vignisse J, Svistunov A, Lesch KP, Bettendorff L, Strekalova T. Thiamine and benfotiamine improve cognition and ameliorate GSK-3β-associated stress-induced behaviours in mice. Prog Neuropsychopharmacol Biol Psychiatry. 2017 Apr 3;75:148-156. doi: 10.1016/j.pnpbp.2016.11.001. Epub 2016 Nov 5. PubMed PMID: 27825907.
9. Veniaminova E, Cespuglio R, Cheung CW, Umriukhin A, Markova N, Shevtsova E, Lesch KP, Anthony DC, Strekalova T. Autism-Like Behaviours and Memory Deficits Result from a Western Diet in Mice. Neural Plast. 2017;2017:9498247. doi: 10.1155/2017/9498247. Epub 2017 Jun 8. PubMed PMID: 28685102; PubMed Central PMCID: PMC5480052.
10. Roy A, Laas K, Kurrikoff T, Reif A, Veidebaum T, Lesch KP, Harro J. Family environment interacts with CRHR1 rs17689918 to predict mental health and behavioral outcomes. Prog Neuropsychopharmacol Biol Psychiatry. 2018 Aug 30;86:45-51. doi: 10.1016/j.pnpbp.2018.05.004. Epub 2018 May 14. PubMed PMID: 29772307.
11. Auth CS, Weidner MT, Popp S, Strekalova T, Schmitt-Böhrer AG, van den Hove D, Lesch KP, Waider J. Differential anxiety-related behaviours and brain activation in Tph2-deficient female mice exposed to adverse early environment. Eur Neuropsychopharmacol. 2018 Nov;28(11):1270-1283. doi: 10.1016/j.euroneuro.2018.07.103. Epub 2018 Aug 23. PubMed PMID: 30146458.
12. Strekalova T, Bahzenova N, Trofimov A, Schmitt-Böhrer AG, Markova N, Grigoriev V, Zamoyski V, Serkova T, Redkozubova O, Vinogradova D, Umriukhin A, Fisenko V, Lillesaar C, Shevtsova E, Sokolov V, Aksinenko A, Lesch KP, Bachurin S. Pro-neurogenic, Memory-Enhancing and Anti-stress Effects of DF302, a Novel Fluorine Gamma-Carboline Derivative with Multi-target Mechanism of Action. Mol Neurobiol. 2018 Jan;55(1):335-349. doi: 10.1007/s12035-017-0745-6. PubMed PMID: 28856531.

WP07: Multilevel data integration

Background

The heterogeneous nature of aggression has prevented the estimation of the effects of its etiological components hampering the prediction of clinical outcome. The principle controversy surrounding aggression subtypes, however, has not been with their existence but with their number. The yet undescribed variability in aggression subtypes comes from the fact that, despite the fact that previous findings are indicative of meaningful etiologic distinctions between aggression subtypes, currently applied methodological strategies do not include causal processes that underlie antisocial behaviours. WP07 aimed to integrate data accumulated across the different disciplines studied in WPs 1-6 in order to develop, through machine learning approaches, predictive algorithms for aggression subtypes for use in prevention and the prediction of adult outcome.

Overall Objectives

WP07 had three primary objectives:

1. To apply state-of-the-art analysis techniques to combine different sources of information on aggression. We will combine neurobiological, genetic and environmental markers with aggression subtypes and use sparse factor analysis and multilevel/longitudinal modelling that allow the contribution of multilevel class variables in different factors (shared patterns). We will extend the methodology to a probabilistic approach for multi-source learning to determine the relevance of (aggression) markers by combining different sources of information. We will use these combined variables (factors) as input for our correlation engine and our prediction pipeline.
2. To establish novel, accurate predictive algorithms for aggression subtyping using neurobiological, genetic and environmental markers and validate them in paediatric and adult populations. We will use a novel, highly efficient and unbiased correlation engine to transform data from different sources into a uniform format and apply data selection, which will determine causality correlations across data sources and we will create a multilevel data prediction / classification pipeline for aggression. Importantly, our prediction will be based on different combinations of aggression markers to make the pipeline more flexible and adaptable to a particular patient profile (increasing the probability of accurate aggression subtyping and the identification on novel aggression subtypes).
3. To generate business development possibilities by marketing the predictor for clinical use and uncovering novel causal therapeutic interventions. The pipeline will be validated in two longitudinal childhood samples and tested for adult outcome: A) the Nijmegen Longitudinal Study and B) The Estonian Children Personality Behaviour and Health Study

(ECPBHS) (Task 5). The validation step consists of running the prediction pipeline in these samples together with a cross validation step. In addition, we will test the relationship with adult outcomes using the IMpACT adult ADHD sample.

Results

During the course of the project, WP07 has achieved the following results:

1. We have been able to secure participation of the multiple collaborating WPs.
2. We initiated the selection of adequate and informative variables to be investigated.
3. We completed a data overview of the Aggressotype consortium, 7 sites in The Netherlands, Germany, UK, and USA have suitable data available.
4. In cooperation with GENALICE, we have created a data combination approach that transforms data from different modalities (genetics, behavioural, environmental, and neurological) into binary events in a single search space.
5. In cooperation with GENALICE, we created a univariate search method to identify informative binary events in common data space.
6. We extended our analysis capabilities and included non-human data in our analysis pipeline.
7. Causal discovery to determine whether conduct problems mediate the risk of substance use disorders in ADHD adolescents showed that (a) causal discovery can be applied successfully to complex neurological phenotypes to distinguish causal and pleiotropic effects; (b) conduct problems mediate risk for substance use, however conduct problems do not mediate risk for gaming addiction. Paper on causality learning „Role of Conduct Problems in the Relation between Attention-Deficit Hyperactivity Disorder, Substance Use, and Gaming“ was published in journal European Neuropsychopharmacology (Schoenmacker et al., 2018)
8. A novel genetic analysis method to leverage multi-level organisation of genome for data reduction and classification. Human genome datasets consisting of 50000 to 150000 samples with psychiatric phenotypes are being leveraged, with first results showing that the data reduction technique retains key genetic information, which captures a different portion or variance than traditional SNP-based techniques. Publication in preparation.
9. Data reduction method „Quantitative genetic scoring“ presented at the World Congress of Psychiatric Genetics in Orlando, Florida (Schoenmacker et al.).
10. Software implementation for reducing genetic data „Quantitative genetic scoring“, alpha version available at https://bitbucket.org/GS42/qgs.
11. Causality models applied to aggression subtype data, brain imaging, gene-level variables.
12. Aggression subtypes in RPQ questionnaire verified using data-driven causality approach, showing good overlap with existing subtypes as well as room for improvement.
13. Performed statistical data analysis for WP10 (zebrafish), resulting in robust selection of compounds as well as publishable statistical analyses.
14. We implemented a correlation engine designed to combine these variables together with our partners in Machine2Learn.
15. Replication of variable selection and machine learning models to predict police contact in The Estonian Children Personality Behaviour and Health Study. Publication in preparation
16. In cooperation with Machine2Learn a causal correlation engine was created which leverages subscaling correlation structures of questionnaire data to robustly create factors and find causal structure in cross sectional data.
17. In cooperation with Machine2Learn, a web interface was created for the causal correlation engine which makes the software deliverables from the project easily available to other researchers.

Conclusions

We determined that data reduction approaches are useful tools to detect subtypes of aggression, and created new domain-specific methods for reducing data for machine learning approaches. Our data supports that there are different types of aggression that can be better characterized by the integration of (e.g.) genetic, MRI-derived, and behavioural variables. This characterization can be done via causal network analysis methods such as the ones we applied for the project and made available for others to use. Our results suggest that many of the different measures associated with aggression (e.g. behaviour, neurobiological, genetic, environmental) are correlated and that this correlation can be detected robustly. The correlation of this multi-level data can be used to subtype aggression. Antisocial behaviour can be robustly predicted in the population by analysing retrospective questionnaire data using feature selection methods, which resulted in a list of risk factors for antisocial behaviour at a later (20+ years) age.

WP08: Biofeedback treatment of arousal in impulsive and instrumental aggression

Background

Available nonpharmacological treatments of CD/ODD problems in children and adolescents show small clinical effects (Bakker et al., 2016). The purpose of this study was to determine whether individualized biofeedback of arousal (SC) is effective in the treatment of aggressive behavior problems in children and adolescents with either predominantly impulsive (reactive) and/or high callous-unemotional traits (proactive) subtypes of aggression when compared to treatment as usual (TAU).

Overall Objectives

WP08 had three primary objectives:

1. Establish the most consistent markers of distinguishing between instrumental and impulsive aggression.
2. Develop an innovative biofeedback training protocol for patients to learn self-regulation of their individual specific physiological deficits in various naturalistic situations to up- or downregulate arousal, depending on the initial arousal level reflecting their physiological "aggressotype".
3. Evaluate the effects of such personalized, deficit -specific biofeedback training for both forms of aggression in a controlled multicentre trial.

Results

During the course of the project, WP08 has achieved the following results:

1. Decreased activation response was observed in the VS and OFC during reward anticipation in participants with previous CD diagnoses and with high impulsivity.
2. Increased brain activity in the caudate nucleus during reward delivery as a function of aggression during later life.
3. Less activity in the amygdala during emotion processing in participants with previous CD diagnoses and high CU traits.
4. Reactive aggression was inversely related to Skin Conductance Level (SCL) and Skin Conductance Response (SCR) during a task viewing faces and shapes.
5. Aggression during later life was negatively correlated with the SCR during reward anticipation.
6. Protocols for neurofeedback training were optimized.
7. Validation of emotional video clips (n= 120 clips, N=20 subjects 8-18y) was achieved.
8. Arousal GSR biofeedback and subtyping paradigm – system programmed, SOP finalized.
9. Randomized controlled trial (RCT) was registered under ClinicalTrials.gov Identifier: NCT02485587 https://clinicaltrials.gov/ct2/show/NCT02485587
10. Feedback trial was implemented at two sites (CIMH, UZH).
11. Major recruitment efforts proved successful in increasing inclusion to compensate for the drop out of the RUN site. The additional efforts in 2018 included:
    a. updates of the homepages to make the Aggressotype study and ongoing recruitment highly visible;
    b. a full day of lectures and workshops for referring institutions, clinics and local authorities on Aggression and related studies in Mannheim;
    c. intensified personal contacts and site visits with referring clinicians, external clinics and institutions in Zurich and Mannheim at all levels;
    d. personal letter from the clinic director in Mannheim sent in April 2018 regarding successful cooperation and prolonged recruitment.
12. Patients included in biofeedback training, So far (as of 6.12.2018), 34 participants were randomized, 30 started, and 22 finished treatment.
13. Subtyping results showed higher heart rate during resting state and higher skin conductance in response to negative pictures in participants with ODD/CD diagnosis when compared with typical developing children and adolescents.
14. Randomized controlled trial will still be ongoing in the framework of the MATRICS project.
15. Preliminary results for secondary outcomes showed large improvement of aggression-related symptoms, independent of treatment group.

Conclusions

This pilot RCT was designed to evaluate personalized treatment options and to demonstrate if this new Biofeedback approach can show the same clinical improvement as the active control condition. With regard to the intermediate results, the experimental condition is not inferior to the established treatment as usual, and continuation of the trial within the extended MATRICS project will further add clinical data for the primary outcome.

References
Bakker, M. J., Greven, C. U., Buitelaar, J. K., & Glennon, J. C. (2016). Practitioner Review: Psychological treatments for children and adolescents with conduct disorder problems - a systematic review and meta-analysis. *Journal of Child Psychology and Psychiatry, and Allied Disciplines*. https://doi.org/10.1111/jcpp.12590

WP09: The treatment of aggression in young male prisoners with attention-deficit/hyperactivity disorder

Background
Despite the known effectiveness of ADHD medication in reducing aggression and the high rates of young adults with ADHD and impulsive forms of aggression within the criminal justice system (Young et al., 2014), ADHD is rarely diagnosed and treated among young offenders across Europe. Furthermore, there has been no systematic evaluation in offender samples, of the effects of methylphenidate, the first line treatment of ADHD, on aggressive or violent behaviour. Such studies are now required to support clinical guideline implementation. The overall objective of WP09 was to investigate the use of methylphenidate as a treatment for aggression in young adult male prisoners with ADHD and high levels of impulsive behaviour. These individuals reflect a group who have persistent problems with impulsive aggression and criminal behaviour by adulthood. The age group (18-24 years) was selected in order to evaluate the effectiveness of methylphenidate treatment on established conduct disorder and aggression in a group that has not been previously studied for this primary outcome, and where demonstration of benefits can lead to a stepped change in care with obvious benefits to society.

Overall Objectives
WP09 had four primary objectives:

1. Conduct a 3-month open label study of Concerta XL (an extended release formulation of methylphenidate) on aggression, in 100 male offenders with ADHD, aged 18-24.
2. Investigate additional secondary outcome measures of engagement with educational, occupational and offender reduction programmes. These outcomes reflect the overall function of the individuals concerned, and engagement in activities designed to improve long-term outcomes and prevent further violent and criminal behaviour.
3. Complete a 6-month open label extension of initial study period, following further optimisation of medication using standard clinical protocols, to maximise potential long-term benefits.
4. Develop and Initiate follow-up studies: first a longer term follow-up study of participating prisoners on release, to investigate the impact on aggressive behaviour and social and occupational function in the community; second a large multi-site study with a placebo arm, to provide definitive data required for guideline implementation by prison mental health teams.

Results
During the course of the project, WP09 has achieved the following results:

1. The estimated prevalence of ADHD in the prison was 19%. This is a large over- representation compared with 2 to 4% in general adult populations but falls slightly short of previous studies that suggest rates of around 26% in convicted young offenders (1).
2. 78% of participants met criteria for the combined subtype of ADHD. This indicates a higher level of hyperactive and impulsive symptoms in this prison population than in community samples of adult ADHD.
3. Data from 267 cases with ADHD was compared to 147 who screened negatively for ADHD. Among the ADHD group we found significantly greater levels of cannabis use ($p<.00001$); daily cannabis use ($p<.00001$); alcohol use ($p<.0002$): alcohol abuse ($p<.02$); CNS depressants ($p<.02$); CNS stimulants ($p<.00001$). There was no increase in the use of opiates or hallucinogens.
4. The group with ADHD were LESS likely to be involved with or sentenced for crimes relating to drug dealing ($p<.0001$).
5. The ADHD screener had an estimated sensitivity of 79% and specificity of 78%. Screen-positive cases were defined as scoring 4 or more in either domain of the Barkley DSM-IV self-rating scale for current ADHD symptoms.
6. The primary outcome was the total number of adjudications reported by prison officers in the electronic prison records. The effects were $d=0.53$ using the per-protocol (pp) analysis but only $d=0.29$ using the intention-to-treat (ITT) analysis. Here, the confidence that such changes are accounted for by the study medication is uncertain. Adjudication rates are relatively low, and it may be that the prison regime is good at responding to adjudications events once they occur. Taking part in the clinical trial with weekly visits from research staff may further impact in a beneficial way on aggressive or antisocial behaviour. However, we did not select on the primary outcome, so adjudications rates could have gone up as well as down. Taking into account the small effect on the primary outcome for the ITT analysis, and

the moderate effect in the pp analysis it is clear that no conclusions can be drawn from the analysis here. Overall the positive effects on behavioural outcomes (aggression, antisocial behaviour, and engagement with education) indicate the need for a larger, placebo-controlled, definitive trial powered and designed to address these questions.

7. The results reported here show a decrease of 25.0 points on the CAARS ADHD symptoms scale with a standard deviation of 9.1. This suggested a standardised effect size of d=2.75. It could reasonably be assumed that 20% of this effect might be attributed to the effects of MPH. On this basis we estimated that a sample size of 200 in a randomised placebo controlled trial (with 25% drop out rate) would be powered to detect a standardised effect size of d=0.55. This effect size is consistent with the results of a recent meta-regression analysis of the effects of methylphenidate in ADHD, which estimates the effect of treatment to be d=0.49 (95% CI 0.08, 0.64).
8. Treatment of ADHD with long-acting methylphenidate (Concerta XL) was shown not only to reduce core symptoms of ADHD (d=2.78, p<.001) and emotional dysregulation (d=1.71, p<.001), but also attitudes towards violence (d=0.98, p<.001), total sum of all adjudications (e.g. verbal and physical aggression, number of (p<.001), as well as increasing the number of positive incidents earning awarded privileges (p<.001).
9. Emotional dysregulation had a mediating effect on the sum of adjudication scores (F (3, 68) =5.130, P=0.003, R^2 =.185), while baseline emotional dysregulation and IQ has a moderating effect (F(2,69)=6.768, p= 0.002, R^2= .164)
10. Feedback from prison inspectorate: All prisoners were offered screening for attention deficit hyperactivity disorder (ADHD) through the specialist Concerta (an ADHD treatment) in adult offenders (CIAO) trial…" "Some prisoners on the CIAO programme to whom we spoke were experiencing some stability of behaviour for the first time in their lives". "There should be efforts to ensure the continued prescribing of medication and ongoing specialist support for prisoners started on the Ciao trial following their release."
11. Compliance with medication was adequate for 80% of the prisoners treated for ADHD.
12. We found no evidence of abuse of prescribed stimulants. Of the 121 participants who took part in medication trial, 27 (22.3%) were titrated to a stable dose of 18mg, 41 (33.9%) to 36mg, 24 (19.8%) to 54mg, 24 (19.8%) to 72mg and 5 (4.1%) to 90mg. Overall there was an unanticipated preference toward lower doses, a trend we had not anticipated, indicating a potentially heightened sensitivity to side effects but also minimal diversion/drug seeking behaviour.
13. The trial procedures and medication were found to be safe. Titration was to lower doses with no evidence of drug-seeking behaviour. Adverse effects were those commonly seen when treating ADHD with methylphenidate. No serious adverse events occurred.
14. Overall, this study demonstrated the feasibility of conducting clinical trials of ADHD in young adult prisoners. Clinical effects were observed supporting the need for a definitive clinical trial.
15. The report on the open label study was submitted and published at the clinical trial registry (EudraCT 2012-000517-37, see https://www.clinicaltrialsregister.eu/ctrsearch/search?query=2012-000517-37).
16. The data presented was successfully used in an application to the National Institute of health Research (NIHR), through which follow-up funding was obtained for a randomised placebo-controlled trial of 200 adult offenders with ADHD, following similar procedures to those used here. The study started on the 1st of August 2016.
17. A long-term follow-up study of participants after leaving the prison was also initiated and is currently ongoing. Results to date indicate that follow-up of individual participants to gather clinical or personal reports on progress is not feasible – since it has not been possible to contact most participants on release from prison. Thus, this study and future studies will have to use available data from databases (e.g. criminal or medical records) or take a different recruitment strategy (e.g. recruiting via probation officers who maintain links with offenders released from prison).
18. A consensus meeting was held to clarify the further needs towards implementing ADHD treatment in offender health care, initiated by collaborator Susan Young and Professor Asherson.
19. Recommendations on how to diagnose and treat prisoners with ADHD were published following a consensus meeting. This work was led by Aggressotype members Susan Young and Professor Asherson was the senior author (2).
20. NICE guidelines for ADHD treatment were adapted in 2018 to put emphasis on the need to treat ADHD in offender health care.

Conclusions

We successfully completed a pilot study of methylphenidate in young adult prisoners with ADHD. Treatment of ADHD in this population led to marked reductions in symptoms of ADHD, emotional dysregulation, and aggression. There were reductions in critical incidents leading to adjudications for antisocial and aggressive behaviour, and an increase in positive behaviours and engagement with the prison rehabilitation regime. These data enabled us to obtain follow-up funding for the initiation of a definitive placebo-controlled trial that is due for final report in August 2019. We further initiated a long-term follow-up study to inform about the feasibility and future design of treatment trials for prisoners released back into the community. Overall, we conclude that treating ADHD in prisoners does appear to reduce level of irritability, anger, and aggression. Recognising treating

ADHD is an important clinical activity as there is now converging evidence from different study designs that this can lead to reduced aggression and criminal behaviour. Furthermore, aggression and criminal behaviour could potentially be prevented in individual patients and reduced in its societal impact through early recognition and treatment of ADHD during childhood, as ADHD is a treatable condition.

References

1. Young, S., Moss, D., Sedgwick, O., Fridman, M. & Hodgkins, P. (2014). A meta-analysis of the prevalence of attention deficit hyperactivity disorder in incarcerated populations. Psychol Med, 1-12.
2. Young, S., Gudjonsson, G., Chitsabesan, P., Colley, B., Farrag, E., Forrester, A., Hollingdale, J., Kim, K., Lewis, A., Maginn, S., Mason, P., Ryan, S., Smith, J., Woodhouse, E. & Asherson, P. (2018). Identification and treatment of offenders with attention-deficit/hyperactivity disorder in the prison population: a practical approach based upon expert consensus. BMC Psychiatry 18, 281.

WP10: Novel treatment targets for pharmacological intervention in pathological aggression

Background

Although aggression is a common symptom of psychiatric disorders, the drugs available to treat it are non-specific and can have unwanted side effects. The zebrafish is an ideal model for aggression research. Zebrafish are small, amenable to genetic and pharmacological manipulation, and agonistic behaviour can be measured reliably. In WP10, we aimed at using a behavioural platform to screen for drugs that can reduce aggression without affecting locomotion.

Overall Objectives

WP10 had five primary objectives:

1. Establish a novel larval zebrafish aggression model, building upon validated data from adult fish and taking advantage of pre-existing genetic tools.
2. Develop novel behavioural software for automatic quantification of zebrafish aggression levels.
3. Identify small molecules which reduce aggression levels in larval zebrafish.
4. Verify the ability of novel drugs to reduce aggression in fish and mouse.
5. Create a database cataloguing the behavioural profile of the small molecules screened.

Results

During the course of the project, WP10 has achieved the following results:

1. Identification of 28 days as the optimum time point to screen for changes in aggression in zebrafish.
2. Design, construction, and validation of novel aggression arenas and software. The instrument has been described in the following publication: Carreño Gutiérrez H, Vacca I, Pons AI, Norton WHJ. Automatic quantification of juvenile zebrafish aggression. *J Neurosci Methods*. 2018 Feb 15;296:23-31. doi: 10.1016/j.jneumeth.2017.12.012. Epub 2017 Dec 21. PubMed PMID: 29274793.
3. Screening of 108 compounds completed. Second round rescreen of 25 compounds completed, and four compounds chosen for verification.
4. Caffeine, cannabidiol, and sildenafil validated in juvenile zebrafish.
5. Caffeine and sildenafil derivatives screened in juvenile fish. Paraxanthine also reduces aggression.
6. A paper describing the aggression screen in zebrafish is in preparation.
7. Screening set-up commercialised by our partner; the SME ViewPoint has started promoting the system after the first paper published. The systems was promoted at the European Neuroscience meeting (FENS) in Berlin and SFN in San Diego (http://viewpoint.fr/app.php/en/p/equipment/zebra-aggression-box-zab).
8. Discussion initiated with industrial partners to develop caffeine into treatment for aggression, with possible target of fish farming industry.
9. Successful translation into higher vertebrate mouse model of two compounds in collaboration with WP02 (methylphenidate) and WP06 (DF302).

Conclusions

We have demonstrated that one month-old juvenile zebrafish are a suitable model to screen for novel drugs that can reduce aggression. Due to the automated screening set-up established by Viewpoint (Partner 15) it was possible to screen over 120 compounds in our zebrafish model. This set-up has been commercialised. In a three tier screen of ninety-four drugs we identified caffeine and sildenafil reduce as drugs that selectively reduce zebrafish aggression. Caffeine also decreased attention

and increased impulsivity in the 5-choice serial reaction time task, whereas sildenafil showed the opposite effect. Both caffeine and sildenafil are active in the zebrafish brain, with prominent roles in the thalamus and cerebellum. They also interact with 5-HT neurotransmitter signalling. We have validated two compounds for their ability to alter aggression and impulsivity and identified the brain areas that they activate. In summary, we have demonstrated that juvenile zebrafish are a suitable model to screen for novel drugs to reduce aggression, with the potential to uncover the neural circuits and signalling pathways that mediate their behavioural effects. Future plans include developing caffeine as a possible food supplement for the fish farming industry and exploring whether sildenafil could be applied to human patients.

References

Carreño Gutiérrez H1 Vacca I, Pons AI, Norton WHJ. Automatic quantification of juvenile zebrafish aggression. J Neurosci Methods. 2018 Feb 15;296:23-31.

WP11: Ethics, training, and dissemination

Background

WP11 had the tasks of organizing all ethical issues relevant to the whole process of preparing and carrying out the Aggressotype program. Moreover, one of the project's general aims was to perform all of its studies at the highest scientific levels. To achieve this, regular training of the researchers in key-areas of the program (i.e. through dedicated master classes, training on general procedures of multicentre studies) was to be conducted. A mentoring program was to be established, giving especially female early career scientists the possibility to benefit, as females are still strongly underrepresented in the higher ranks of scientific research. In addition to the ethics and training aims, this WP dealt with the dissemination of the project results to different target groups, including patients and the general public, scientists, policy makers, and commercial parties.

Overall Objectives

WP11 had three primary objectives:

1. Ethics: Aggressotype will comply with all legal regulations and recommendations of the European Union, national legislation and local animal and human ethics committees. In addition we will adhere to the Declaration of Helsinki in its latest accepted version, ICH Good Clinical Practice as well as the European Directives 86/609/EEC and 2010/63/EU and the Federation of European Laboratory Animal Science Associations (FELASA) 2012 guidelines for the protection of animals used for scientific purposes. (See also section B.4 for more discussion of the ethics aspects of the Aggressotype programme.) Ethical conduct of all parts of the program will be ensured by the formation of an internal ethics board, which will support partners in obtaining ethical approval for their work and monitor that the use of already available data and material occurs according to ethics regulations.
2. Training: It is our aim to perform all our studies at the highest scientific levels. To achieve this, we will standardize and provide regular training for multicentre studies and train our researchers in key-areas of the program through dedicated master classes, particularly concentrating on interdisciplinary education. As females are still strongly underrepresented in the higher ranks of scientific research, we will establish a coaching program for female early career researchers within the Aggressotype programme.
3. Dissemination: Reaching out to the scientific community, patient organizations, industry and the general public through the development of a communication plan for Aggressotype.

Results

During the course of the project, WP11 has achieved the following results, according to the three primary objectives:

1. Ethics:
    1. An Ethics Board was established.
    2. Lists of data protection officers and data controllers were prepared for all Aggressotype sites performing human studies.
    3. All ethics documentation for animal studies were completed.
    4. Ethics documentation for human original set of observational studies complete, also including new additions to the project, and new studies of existing members was collected.
    5. Ethics approvals for all new human studies were completed.
2. Training:
    6. Early career Scientist training procedures were prepared (i.e. a training and supervision plan).
    7. Active Early Career Scientist (ECS) working group was established and maintained throughout the entire period of Aggressotype.

8. Active and successful mentorship program for ECS was run, giving female ECS the opportunity to choose a female role model as their mentor.
9. Master classes were held at 5 General Assembly meetings of Aggressotype.
10. A 2-day training workshop was held in conjunction with General Assembly meeting in 2016, organized and coordinated by the ECS.
11. Training activities with very active participation of and self-organization by Early Career Scientists held throughout the funding period of Aggressotype.

3. Dissemination:
    12. The website for Aggressotype was designed and launched: https://www.aggressotype.eu/ to disseminate Aggressotype to the general public, also including an informative Aggressotype movie.
    13. Dissemination to scientific community through symposia and presentations on Aggressotype at scientific congresses.
    14. 'Scientist of the Season' item was set-up and maintained by ECS on the Aggressotype website.
    15. Regular newsletter were being sent to all members of the Aggressotype Consortium.
    16. Special Issues were published in different scientific journals, including American Journal of Medical Genetics Part B, Neuroscience and Biobehavioural Reviews, European Neuropsychopharmacology, and European Journal of Child and Adolescent Psychiatry.
    17. An active collaboration and interaction between EU-funded aggression consortia established (Aggressotype, MATRICS, ACTION, and FemNAT-CD).
    18. Two dissemination hotspots were defined for Aggressotype: the work of WP09 on methylphenidate treatment of ADHD patients in the prison system and the work of WP10 on the zebrafish screen for potential new treatment targets for aggression.
    19. A policy workshop on implementation of methylphenidate treatment for ADHD patients in prison and definition of roadmap towards implementation was organized.
    20. The publication of the zebrafish screen of WP10 (Carreño Gutiérrez et al., J Neurosci Methods. 2018) facilitated the marketing of the screen equipment and IP.
    21. Paper on novel promising compound DF302 (Strekalova et al., Mol Neurobiol. 2018) was published.
    22. Promising targets for anti-aggression treatment identified from the zebrafish screen and additional testing were: methylphenidate, caffeine, sildenafil, DF302.
    23. Symposia for patients and their representatives were organized in Dublin (March 2018, over 300 participants were present), Barcelona in May 2018 (over 200 participants), and at the location of the Aggressotype GA Meeting 2018 in Rome in September, which was also received very favourably.
    24. Together with the MiND consortium, we produced a short Youtube documentary on ADHD, aimed at increasing awareness for the disorder. This documentary was released at all sites of Aggressotype and MiND on the last day of the ADHD Awareness Month (October 31 2018), and has already been viewed over 17.000 times: https://www.youtube.com/watch?v=XmS7jUhB74A.
    25. ECS regularly posted blogs on our cross-consortium blog Mind the Gap (https://mind-the-gap.live/).
    26. Two Aggressotype partners were invited to join the Scientific Board of the ADHD patient organization ADHD-Europe.
    27. Multiple follow-up actions were employed to work towards implementation of methylphenidate treatment into offender health care. Those include a consensus meeting and publication providing information on the current limitations and needs for implementation and defining necessary steps and stakeholders, adaptation of the NICE guidelines for the treatment of people with ADHD, and training sessions to prison mental health services and prison psychiatrists.

Conclusions

We have successfully managed all ethics requirements in Aggressotype. Training activities have been multiple, and were well received by the Early Career Scientists (ECS). The mentorship program proceeds beyond the end of the Aggressotype funding period for many of the ECS. In addition, through the collaborative activities, they formed a close, multidisciplinary network, which will be of value to them beyond their time as members of Aggressotype. The ECS learned to organize meetings, present their findings to scientific and lay audiences, in addition to doing high-quality scientific research. Dissemination activities were effective in reaching all target groups. Dissemination to the scientific audience included over 170 publications in peer-reviewed journals and several special issues, as well as presentations and symposia at multiple scientific congresses. Importantly, several Aggressotype findings that can be further developed into products for implementation into society. Among those, we have been working intensely towards implementation of methylphenidate treatment into offender health care as a way to reduce

and prevent aggression. Those actions included a consensus meeting and publication providing information on the current limitations and needs for implementation and defining necessary steps and stakeholders, adaptation of the NICE guidelines for the treatment of people with ADHD (to include treatment in prison), and training sessions to prison mental health services and prison psychiatrists. Additional, new leads for treatment development have been identified through our compound screen in zebrafish. Our dissemination activities to the general public include a science blog (Mind the Gap: https://mind-the-gap.live/), several symposia for patients and their representatives, and the Youtube documentary 'Shine a light on ADHD' (https://www.youtube.com/watch?v=XmS7jUhB74A).

References

- Aggressotype website: https://www.aggressotype.eu/.
- ADHD Youtube documentary 'Shine a light on ADHD': https://www.youtube.com/watch?v=XmS7jUhB74A.
- Science blog Mind the Gap: https://mind-the-gap.live/.
- Carreño Gutiérrez H, Vacca I, Pons AI, Norton WHJ. Automatic quantification of juvenile zebrafish aggression. J Neurosci Methods. 2018 Feb 15;296:23-31
- Strekalova T, Bahzenova N, Trofimov A, Schmitt-Böhrer AG, Markova N, Grigoriev V, Zamoyski V, Serkova T, Redkozubova O, Vinogradova D, Umriukhin A, Fisenko V, Lillesaar C, Shevtsova E, Sokolov V, Aksinenko A, Lesch KP, Bachurin S. Pro-neurogenic, Memory-Enhancing and Anti-stress Effects of DF302, a Novel Fluorine Gamma-Carboline Derivative with Multi-target Mechanism of Action. Mol Neurobiol. 2018 Jan;55(1):335-349

WP12: Project management

Effective project management is a central element of successful research. This is because large research projects often entail a lot of administrative work which needs to be dealt in an efficient and timely manner. In view of this, the purpose of WP12 was project management for the Aggressotype project. This WP took care of all administrative and coordinating tasks.

- To ensure compliance by beneficiaries with their obligations under the grant agreement, the project management office at concentris routinely supported the Coordinator in monitoring the partners' performance based upon the following:
- To make sure that tasks assigned to them were correctly and timely performed.
- Reports were submitted according to the guidelines and on time.
- Funds were used and claimed according to the rules.
- The partners fulfilled their obligations regarding dissemination and funding acknowledgements.
- Any changes to the work plan were communicated to the European Commission (EC) efficiently.
- Compliant to ethical regulations.

The project office at concentris acted as a helpdesk for all participants; it was the central node of communication on a day-by-day basis and communicated with the EC on behalf of the Coordinator regarding administrative and managerial issues (i.e. contract, amendment, reporting etc.).

4 The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results

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

Socio-economic impact and the wider societal implications:

Maladaptive aggression is a commonly observed trait in psychiatric disorders, especially in the paediatric conduct disorders of interest to this project, ADHD and CD. These two disorders are also the most frequent psychiatric disorders in childhood and adolescence, with over 5% and up to 10% of the population below the age of 18 years affected. This means that more than 5.4 million affected children and adolescents in the European Union. Costs of treatment and direct non-medical health care amount to over 6.2 billion Euro per year for this group. Importantly however, the disorders are linked to lifelong serious impairment in social and occupational functioning, as ADHD persists into adulthood in 65% of cases and both disorders are strong predictors of the development of antisocial and substance use disorders, which underscores their huge impact on society. Costs associated with the adult outcome of the ADHD and CD are at least 10 times higher than those for childhood disorders. ADHD and CD thus place large personal and economic burdens on individuals and society including the family and schools. The numbers above, computed from studies of youth, do not account for the impact of aggression and criminality in adulthood that accompanies the disorders. Additional impact on our society is thus caused on the victims of this aggression and also on the executive and judiciary legal systems. Crime statistics from Eurostat indicate that in 2009 (the most recent assessment date

available), 127 in every 100000 individuals from the adult and juvenile European population had been imprisoned for a crime (http://epp.eurostat.ec.europa.eu/statistics_explained/index.php/Crime_statistics). More detailed statistics available from the US show that approximately 30% of imprisonments are due to violent crimes (homicide, kidnapping, rape and other sexual assaults, robbery and assault) (Bureau of Justice Statistics, http://bjs.ojp.usdoj.gov/index.cfm?ty=tp&tid=13). Strikingly, when systematically meta-analysing the frequency of psychiatric disorders in adolescent detainees, CD (46-53%) and ADHD (12-18%) are the most frequently observed diagnoses. It can thus be assumed that persistence of CD and ADHD, under certain negative environmental conditions, opens up a detrimental developmental trajectory leading towards aggression, violence and crime. Appropriate counteractive measures should ideally be prophylactic in nature, i.e. provided early on after definite diagnoses of the disorders, in order to prevent the very first violent-aggressive act. However, current behavioural and pharmacological treatment options for ADHD and CD are insufficiently effective in treating aggression.

Having an impact on society requires a deeper understanding of the molecular, cellular and brain-circuit-based underpinnings of aggression (subtypes) than is currently available. Strategies to prevent (escalation of) aggression in those susceptible to it require better markers of susceptibility at the genetic, epigenetic and brain-level, which have not yet been forthcoming. Understanding the mechanisms underlying aggression subtypes will also lead to the design of better and more effective treatment strategies – be it pharmacological or behavioural.

To address these challenges, Aggressotype aimed to make advances in several key areas affecting the societal burden caused by aggression:

1. *We improved aggression subtype characterization for the benefit of more effective research into underlying mechanisms in order to enable development of more individualized treatment strategies.*
   As described in more detail below, we published over 170 scientific papers on aggression and ADHD research, which deal with aggression mechanisms at different levels of organismal complexity, from molecule to behaviour, investigating known and novel genes, gene-networks and their epigenetic interactions, and mapping their mode of action from the molecular via the cellular to the brain-circuit level. This was accompanied by analyses of the neural substrates of aggression subtypes. Biological research of aggression had been very limited in the decade before Aggressotype started its science, which makes our findings even more important to the progress of this field, and provided a positive impetus to increasing research efforts in this field.
2. *We developed best-practice guidelines for aggression treatment in prison inmates.*
   In WP09, we successfully performed an open label trial of methylphenidate in young, male prisoners with ADHD, which lead to strong reductions in aggressive behaviour. This study allowed us to obtain follow-up funding for a second, more definitive, placebo-controlled randomized trial, which is currently ongoing. We have been working with multiple stakeholders to improve prisoner health care based on our findings. This work has already led to adaptations in the NICE guidelines for ADHD treatment (NICE 2018), and has produced a consensus paper on the steps necessary to implement ADHD treatment into offender health care (Young et al., BMC Psychiatry 2018). We have developed training courses for prison staff and other target groups to help implementation.
3. *We developed a model system as well as a testing platform for the efficient and cost-effective development of more effective pharmacological treatments for paediatric conduct disorder patients at high risk of aggressive behaviour using zebrafish as a model.*
   In WP10, we developed a zebrafish larvae model for fast and high-throughput screening of pharmacological compounds for anti-aggressive properties. Our partner ViewPoint developed the platform for automated analysis of fish behaviour, which has been commercialized (see below and Carreño Gutiérrez et al, J Neurosci Methods 2018). We determined that fish at 21 weeks of age could already be used to reliably test aggression, which limits costs for maintenance. The model was validated using compounds with known anti-aggressive properties.
4. *We identified new leads for more effective pharmacological treatment of aggression as a first step towards more mechanism-based treatment.*
   Using the model described above in WP10, we performed a screen of 108 compounds using a selection of promising drugs already used for other indications. The four most promising of those were selected for further study, and two were also tested and validated in mouse models. For those promising leads, we are now looking for follow-up funding for further development, not only for the treatment of aggression in humans, but also in farmed fish (e.g. salmon, where aggression can have a substantial financial impact).

5. *We developed a novel non-pharmacological treatment program for children based on neurofeedback, placed strategically at an early time point of disorder manifestation in order to lastingly prevent escalation of aggression.*
   In WP08, we developed a biofeedback treatment program to determine whether individualized biofeedback of arousal (SC) is effective in the treatment of aggressive behavior problems in children and adolescents with either predominantly impulsive (reactive) and/or high callous-unemotional traits (proactive) subtypes of aggression when compared to treatment as usual (TAU). We recruited 34 participants. Intermediate clinical outcome (Secondary clinical outcome) showed a large improvement for externalizing symptoms, but independent of treatment group. Although final results are pending, this clinical trial, at least for the secondary outcomes, shows promising effects comparable to an established treatment as usual.
6. *We developed flexible predictive algorithms based on combinations of molecular, environmental, neural, and/or cognitive/behavioural information allowing a stratification of risk groups for more effective, individualized treatment approaches and prediction of adult outcome.*
   In WP07, we developed correlation engines enabling the prediction of aggressive behaviour in suitable data sets. Different types of data, including genetic, neuroimaging, and behavioural variables can be included for the prediction. Validation was successful, and the software is being made publicly available.

Impacts on the biological understanding of aggression:
With our work in Aggressotype WPs 1-6, we improved aggression subtype characterization for the benefit of more effective research into underlying mechanisms in order to enable development of more individualized treatment strategies. Our studies in WP01 identified overlap and differences in the brain substrates for impulsive and instrumental aggression subtypes. WP03 identified genetic factors involved in aggression and showed the surprisingly high overlap of the genetic risk factors between ADHD and different definitions of aggressive behaviour in general population samples. WP02 and WP01 showed that such overlap might be due to symptoms of both inattention and hyperactivity/impulsivity present in ADHD. WP04, WP05, and WP06 unravelled the molecular pathways from aggression genes to behavioural outcome, by integration of findings across several model systems, including different animal models and human cellular and in vivo neuroimaging models. WP06 additionally identified several molecular pathways by which environmental factors contribute to aggression by studying epigenetic factors.
A total of over 170 scientific publications resulted from our work, and Aggressotype researchers delivered over 260 oral presentations on their work. Through this, Aggressotype significantly advanced the field of biological aggression research.

Impacts on health economics:
Earlier work in the Swedish registries the impressively showed how effective treatment of ADHD reduces crime (Lichtenstein et al, N Engl J Med. 2012; 367(21):2006-2014): The authors showed that compared with nonmedication periods, among patients receiving ADHD medication, there was a significant reduction of 32% in the criminality rate for men and 41% for women. ADHD has a prevalence of approximately 2.4% in the adult population, and affects 5% of children. ADHD has a prevalence of approximately 20% in European prisons (Young et al, Psychol Med. 2015; 45(2):247-58). In this light, and including the cost estimates given above, we expect that our efforts to implement effective ADHD treatment in offenders will have a substantial impact on health economics in Europe.

Impacts on research collaborations:
Aggressotype successful brought together a group of complementary researchers across Europe. They formed a closely collaborating team, and since the start of the Aggressotype project have also worked together in additional EU-funded projects, including the MiND Marie Curie European Training Network on ADHD and autism comorbidity, the CoCA project on comorbidity of ADHD with substance use disorders, depression, anxiety, and obesity, and the more recent Eat2beNICE project, which investigates the link of nutrition and the microbiome with impulsive, aggressive, and compulsive behaviour. Aggressotype researchers also run additional research collaborations successfully, like the ECNP Network on ADHD across the Lifespan, the ADHD Working Group of the Psychiatric Genomics Consortium, and the ENIGMA ADHD Working Group on neuroimaging and genetics. Beyond the role of researchers in research collaborations, the Aggressotype results shape the field, based on their scientific content, but also based on the availability of data. We have made many of our protocols, procedures, as well as results available and will continue to do so. For example, we made available the database of integrated findings for several aggression candidate genes, and will provide summary statistics for the CD genetic data produced in WP03 as soon as the analysis is complete.

Impacts on the EU economy:
SME's played an important role as partner organizations of the Aggressotype project. ViewPoint contributed its expertise on zebrafish testing to the project. During Aggressotype, this company developed and successfully tested the high-throughput platform for zebrafish aggression testing, which has been commercialized. We anticipate that ViewPoint will profit by selling this equipment to other research groups (see below). Machine2Learn contributed expertise on prediction testing, and developed the correlation engine for use in prediction of aggressive behaviour. This correlation engine will be made available to other researchers, and we anticipate that Machine2Learn will profit from the visibility created by this. GenOway provided the mouse models of the Rbfox1 gene, and publication of the findings will provide them with increased visibility, too. Lastly, concentris performed project management of the IMAGEMEND project and will profit both from the increased visibility, contacts and the experience gained throughout the project. We further anticipate IMAGEMEND findings to be the groundwork for developments in the area of personalized psychiatry, with substantial positive long-term impact on diagnostics and pharmaceutical industry in the European economic area.

4.2 The main dissemination activities of Aggressotype

Early on in the Aggressotype project, the Ethics & Dissemination Board of Aggressotype defined the different target groups that should be reached by Aggressotype dissemination activities as the following:
1) Scientific audience
2) Patients and the general public
3) Society/policy makers and commerce
For each of these groups, we subsequently devised a number of activities for the members of the consortium, which are summarized with examples in the following. A complete list of dissemination activities can be found online in the EC online tool.

1) Scientific audience
The Aggressotype partners have been highly productive in providing new knowledge for scientific audiences in the 5 years of its duration. A few numbers are listed below:

- Peer-reviewed publications in scientific journals: 173 so far (please see a selection below)
- Special Issues in peer-reviewed scientific journals: 4 (American Journal of Medical Genetics, Part B: Neuropsychiatric Genetics (2016), Neuroscience Biobehavioral Reviews (2018), Eur Child Adolsc Psychiatry (2018), and Eur Neuropsychopharmacol (2019))
- Oral presentations at scientific meetings: 250 (including symposia organized by Aggressotype at major conferences, e.g. International College of Neuropsychopharmacology (CINP 2014), World Congress of Psychiatric Genetics (WCPG 2014 and 2017), and European College of Neuropsychopharmacology (ECNP 2018))
- Posters presented at scientific meetings: 79

One of Aggressotype's strengths is the interdisciplinary work within the project. In addition Aggressotype actively promoted the interaction with the other three aggression-related EU projects (ACTION, FemNAT-CD, and MATRICS). Please find here a selection of Aggressotype publications, highlighting those points in particular:

No	Title	Main author	Title of the periodical or the series	Number, date or frequency	Year of publication	Relevant pages	Permanent identifies / Doi
1*	Genome-wide analyses of aggressiveness in attention-deficit hyperactivity disorder	E.J. Brevik	American Journal of Medical Genetics, Part B	Vol. 171/Issue 5	2016	733 – 747	10.1002/ajmg.b.32434
2*	Aggressive behavior in humans: Genes and pathways identified through association studies	N. Fernàndez-Castillo	American Journal of Medical Genetics, Part B	Vol. 171/Issue 5	2016	676 - 696	10.1002/ajmg.b.32419
3*	Genetics of aggressive behavior: An overview	K. Veroude	American Journal of Medical Genetics, Part B	Vol. 171/Issue 5	2016	3 – 43	10.1002/ajmg.b.32364
4*	Aggression in non-human vertebrates: Genetic mechanisms and molecular	F. Freudenberg	American Journal of Medical	Vol. 171/Issue 5	2016	603 - 640	10.1002/ajmg.b.32358

No	Title	Main author	Title of the periodical or the series	Number, date or frequency	Year of publication	Relevant pages	Permanent identifies / Doi
	pathways		Genetics, Part B				
5*	Gene-set and multivariate genome-wide association analysis of oppositional defiant behavior subtypes in attention-deficit/hyperactivity disorder	M. Aebi	American Journal of Medical Genetics, Part B	Vol. 171/Issue 5	2016	573 – 588	10.1002/ajmg.b.32346
6*	Genetic architecture for human aggression: A study of gene-phenotype relationship in OMIM	Y. Zhang-James	American Journal of Medical Genetics, Part B	Vol. 171/Issue 5	2016	641 - 649	10.1002/ajmg.b.32363
7	The genetics of aggression: Where are we now?	P. Asherson, B. Cormand	American Journal of Medical Genetics, Part B	Vol. 174/Issue 5	2016	559-561	10.1002/ajmg.b.32542
8	The role of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors in depression: Central mediators of pathophysiology and antidepressant activity?	F. Freudenberg	Neuroscience and Biobehavioral Reviews	Vol. 52	2015	193 - 206	10.1016/j.neubiorev.2015.03.005
9*	Gene x environment interactions in conduct disorder: Implications for future treatments	N.E. Holz	Neuroscience and Biobehavioral Reviews	Vol. 91	2018	239 - 258	10.1016/j.neubiorev.2016.08.017
10*	Aggressive behavior in transgenic animal models: A systematic review	A. Jager	Neuroscience and Biobehavioral Reviews	Vol. 91	2018	198 - 217	10.1016/j.neubiorev.2017.09.028
11*	Neuro-cognitive system dysfunction and symptom sets: A review of fMRI studies in youth with conduct problems	R.J.R. Blair	Neuroscience and Biobehavioral Reviews	Vol. 91	2018	69 - 90	10.1016/j.neubiorev.2016.10.022
12	Maternal substance use during pregnancy and offspring conduct problems: A meta-analysis	I.H. Ruisch	Neuroscience and Biobehavioral Reviews	Vol 84	2018	325 - 336	10.1016/j.neubiorev.2017.08.014
13	Brain imaging genetics in ADHD and beyond – Mapping pathways from gene to disorder at different levels of complexity	M. Klein	Neuroscience and Biobehavioral Reviews	Vol. 80	2017	115 - 155	10.1016/j.neubiorev.2017.01.013
14	Genetic Overlap Between Attention-Deficit/Hyperactivity Disorder and Bipolar Disorder: Evidence From Genome-wide Association Study Meta-analysis	K.J.E: van Hulzen	Biological Psychiatry	Vol. 82, Issue 9	2017	634–641	10.1016/j.biopsych.2016.08.040
15	Ventral striatum and amygdala activity as	N.E. Holz	Social Cognitive and Affective	Vol. 12, Issue 2	2016	261–272	10.1093/scan/nsw120

No	Title	Main author	Title of the periodical or the series	Number, date or frequency	Year of publication	Relevant pages	Permanent identifies / Doi
	convergence sites for early adversity and conduct disorder		Neuroscience				
16*	Comparing the DSM-5 construct of Disruptive Mood Dysregulation Disorder and ICD-10 Mixed Disorder of Emotion and Conduct in the UK Longitudinal Assessment of Manic Symptoms (UK-LAMS) Study.	I. Sagar-Ouriaghli	European Child and Adolescent Psychiatry	Vol. 27 / Issue 9	2018	1095 – 1104	10.1007/s00787-018-1149-5
17	Are Proactive and Reactive Aggression Meaningful Distinctions in Adolescents? A Variable- and Person-Based Approach	K.C. Smeets	Journal of Abnormal Child Psychology	Vol. 45, Issue 1	2016	1-14	10.1007/s10802-016-0149-5
18*	RBFOX1 , encoding a splicing regulator, is a candidate gene for aggressive behavior	N. Fernàndez-Castillo	European Neuropsychopharmacology	Epub ahead of print	2017		10.1016/j.euroneuro.2017.11.012
19	An integrated analysis of genes and functional pathways for aggression in human and rodent models	Y. Zhang-James	Molecular Psychiatry	Epub ahead of print	2018		10.1038/s41380-018-0068-7
20	Predicting attention-deficit/hyperactivity disorder severity from psychosocial stress and stress-response genes: a random forest regression approach	D. van der Meer	Translational Psychiatry	Vol. 7/Issue 6	2017		10.1038/tp.2017.114
21	Discovery of the first genome-wide significant risk loci for ADHD	D. Demontis	Nature Genetics	Vol. 51/Issue 1	2019	63-75	10.1038/s41588-018-0269-7
22	Structural Brain Abnormalities of Attention-Deficit/Hyperactivity Disorder With Oppositional Defiant Disorder	S.D.S. Noordermeer	Biological Psychiatry	Vol. 82/Issue 9	2017	642-650	10.1016/j.biopsych.2017.07.008
23	Mapping cortical brain asymmetry in 17,141 healthy individuals worldwide via the ENIGMA Consortium	X. Kong	Proceedings of the National Academy of Sciences of the United States	Vol. 115/Issue 22	2018	E5154-E5163	10.1073/pnas.1718418115
24	Variation in a range of mTOR-related genes associates with intracranial volume and intellectual disability	M.R.F. Reijnders	Nature Communications	Vol.8/Issue 1	2017		10.1038/s41467-017-00933-6
25	Autism spectrum disorders and autistic traits share genetics and biology	J. Bralten	Molecular Psychiatry	Vol. 23/Issue 5	2018	1205 - 1212	10.1038/mp.2017.98
26	Novel genetic loci associated with hippocampal volume	D.P. Hibar	Nature Communication	Vol. 8	2017	13624	10.1038/ncomms13624
27	Vitamin levels in adults with	E.T.	British Journal of	Vol.2/Issue	2016	377 - 384	10.1192/bjpo.bp.116.003

No	Title	Main author	Title of the periodical or the series	Number, date or frequency	Year of publication	Relevant pages	Permanent identifies / Doi
	ADHD	Landaas	Psychiatry	6			491
28	MicroRNA degradation by a conserved target RNA regulates animal behavior	A. Bitetti	Nature Structural and Molecular Biology	Vol. 25/Issue 3	2018	244 - 251	10.1038/s41594-018-0032-x
29	Genetic Overlap Between Attention-Deficit/Hyperactivity Disorder and Bipolar Disorder: Evidence From Genome-wide Association Study Meta-analysis	K.J.E. van Hulzen	Biological Psychiatry	Vol.2/Issue 9	2017	634 - 641	10.1016/j.biopsych.2016.08.040

*part of special issues initiated for the Aggressotype project, often together with the other EU-funded aggression consortia.

Oral presentations by Aggressotype members given at important international conferences, a selection:

No	Title	Date/Period	Place
1*	Symposium: Paediatric disorders characterised by aggression and/or social impairment: from preclinical research to clinical subtyping Presentation: Overlap and differences between ADHD and conduct disorder: Imaging genetics approaches	23.06.2014	CINP, Vancouver, Canada
2*	Genetics of adult ADHD and antisocial personality disorder	23.06.2014	CINP, Vancouver, Canada
3*	Gene-by-environment interaction and epigenetic mechanisms in aggression: lessons from animal models	23.06.2014	CINP, Vancouver, Canada
4*	Genetic determinants in aggression	23.06.2014	CINP, Vancouver, Canada
5*	"Genetics and aggression: The Aggressotype project": Symposium in the XXII World Congress of Psychiatric Genetics	15.10.2014	WCPG, Copenhagen, Denmark
6*	Aggression in ADHD and Conduct Disorder: Impulsive and Instrumental Subtypes	15.10.2014	WCPG, Copenhagen, Denmark
7*	'Genetics and Aggression: The AGGRESSOTYPE PROJECT'	15.10.2014	WCPG, Copenhagen, Denmark
8*	Genetics of ADHD and Aggression	15.10.2014	WCPG, Copenhagen, Denmark
9	The interaction effect of the tryptophan hydroxylase-2 G-703T genotype and stressful life events on inattentive and hyperactive behaviour in adolescents	13.10.2014	WCPG, Copenhagen, Denmark
10*	Screening for novel aggression therapeutics in zebrafish	15.10.2014	WCPG, Copenhagen, Denmark
12	ADHD diagnosis, treatment and research in forensic settings	04.03.2015	Glasgow, UK
13	Treatment of ADHD in young adult offenders	05.03.2015	Glasgow, UK
14	Consortium Science: how can international collaboration move forward our research in psychiatry and ADHD in particular?	28.05.2015	5th World Congress on ADHD Glasgow, UK
15	ADHD across the lifespan: brain structure	28.05.2015	5th World Congress on ADHD Glasgow, UK
16	Brain Structure and ADHD across the Lifespan: an ENIGMA collaboration	29.08.2015	European College of Neuropsychopharmacology 2015, Amsterdam
18*	RBFOX1, a splicing regulator, is a candidate gene for aggressive behavior	15.10.2017	WCPG 2017, Orlando USA
19*	An integrated and network-based analysis of genes for aggression in human and rodent models	15.10.2017	WCPG 2017, Orlando USA
20*	Identification of genetic risk factors for conduct disorder/oppositional defiant disorder in the context of ADHD	15.10.2017	WCPG 2017, Orlando USA
21*	Linking inattention to aggression? Data from the BALB/cJ mouse model of aggression	15.10.2017	WCPG 2017, Orlando USA
22*	Lunch session: ADHD in the Prison System (presentations and discussion)	14.01.2018	APSARD 2018, Washington, USA

No	Title	Date/Period	Place
23	Screening for novel aggression therapeutics in zebrafish	13.02.2018	University of Toronto Canada
24	Genome-wide significant risk loci and the emerging role of rare variants in ADHD	29.05.2018	PGC/iPSYCH Pathways to Drugs meeting, Copenhagen, Denmark
25*	How ADHD changes its presentation over the lifespan	11.06.2018	CINP Congress, Vienna, Austria
26	Novel targets in psychiatric disorders: Focus on Rbfox1	06.06.2018	Institute of Medical Sciences at the University of Aberdeen
27	Treatment of aggression in prisoners with ADHD	26.09.2018	EUNETHYDIS, Edinburgh, UK
28	How to be a successful clinical scientist; Debate - Evidence based findings in the diagnosis of paediatric mood disorders	06.10.2018	ECNP 2018, Barcelona, Spain
29*	The neurobiology of aggression: Genes and beyond.	07.10.2018	ECNP 2018, Barcelona, Spain
30*	From man to fly: convergent evidence links FBXO25 to ADHD and comorbid psychiatric phenotypes	14.10.2018	WCPG 2018, Glasgow UK

*part of symposia initiated by the Aggressotype project, often together with the other EU-funded aggression consortia.

2) Patients and general public

We have worked closely together with the patient organization ADHD-Europe. In fact, two Aggressotype partners, Philip Asherson and Barbara Franke, have been elected to join the Scientific Board of ADHD-Europe. Both have been visiting the General Assembly Meetings of the organization. In the last year, we have made it a tradition to organize symposia for the patients and their representatives at every location of our GA meetings, together with the local patient ADHD-Europe partner organization (this is a cross-consortium effort of the Aggressotype, CoCA, MiND, and Eat2beNICE consortia lead by SC members of Aggressotype). The first meeting was held in Dublin in March 2018 (Aggressotype SC Meeting, CoCA GA Meeting), with over 300 participants. A second one was carried out at the location of the MiND GA Meeting, Barcelona, in May 2018 (again, over 200 participants were present), and a third one, at the location of the Aggressotype GA Meeting 2018 in Rome in September, was also received very favourably.

We produced an information film about Aggressotype early on in the project, which is available on the Aggressotype website (www.aggressotype.eu). In addition, together with the MiND consortium, we produced a short Youtube documentary on ADHD, aimed at increasing awareness for the disorder. This documentary was released at all sites of Aggressotype and MiND on the last day of the ADHD Awareness Month (October 31 2018), and has already been viewed over 19.000 times: https://www.youtube.com/watch?v=XmS7jUhB74A.

In addition, two members of Aggressotype, Phil Asherson and Barbara Franke, took part in the filming of the BBC Horizon documentary 'ADHD and Me' with Rory Bremner, on April 25th 2017. The TV clip can be viewed at https://myshare.box.com/s/3b9djvx50oi5ihanhg7lhakopapi4qsb (password: adhd17)

Aggressotype members provided several interviews to radio, television, and press, and took part in documentaries. A selection is shown here:

- 'Genes for aggression discovered' Radio Interview BNR Radio, October 31st 2014
- 'Radio Interview on zebrafish and Aggressotype research' March 11th 2014, https://soundcloud.com/university-of-leicester/will-norton
- 'New study to tackle ADHD in prisoners' Website application at KCL, October 21st 2016
- 'Studying ADHD on a large scale: Discussing ENIGMAtters with Martine Hoogman & Barbara Franke', Video production April 29th 2017
- 'ADHD and the Brain' May 1st 2017, Interview for Balans Magazine, a magazine for people with ADHD
- 'Lifting the red mist with research on aggression.' Horizon Magazine, Dec.5th 2017 https://horizon-magazine.eu/article/lifting-red-mist-research-aggression.html
- 'The world's largest set of brain scans are helping reveal the workings of the mind and how diseases ravage the brain', Interview, January 28th 2018 http://www.sciencemag.org/news/2018/01/world-s-largest-set-brain-scans-are-helping-reveal-workings-m
- 'Vom Zappelphilipp zum Fall für die Polizei' by Andreas Reif (GUF), Frankfurter Allgemeine Zeitung (FAZ), May 1st 2018

- '?TDAH. Viviendo deprisa? ?ADHD. Living quickly?', Interview on Radio Nacional de España (RNE) https://www.ivoox.com/entrevista-doctora-maria-jose-penzol-audios-mp3, May 6th 2018
- News at the public Catalan TV (TV3): Dissemination of the results of the scientific work by Zhang-James et al (Mol Psychiatry 2018, 10.1038/s41380-018-0068-7)', TV Clip in Catalan television, July 15th 2018
- 'Researchers have found the first risk genes for ADHD' Press release, November 27th 2018 sent to broad range of media, with subsequent interviews to over 10 different newspaper and other media outlets
- '¿Pueden los genes convertirte en un asesino?' Interview on the genetics of aggressive behaviour: https://www.quo.es/ser-humano/a25639205/pueden-los-genes-convertirte-en-un-asesino/, December 20th 2018

3) Society/policy makers and commerce

In order to use our findings and products to improve society, we defined two dissemination hotspots: the work of WP09 on methylphenidate treatment of ADHD patients in the prison system and the work of WP10 on the zebrafish screen for potential new treatment targets for aggression. Unfortunately, the biofeedback approach in WP08 could not be finished in time to form a third dissemination hotspot, but the promising preliminary findings may warrant dissemination after the end of Aggressotype.

For WP09, based on the CIAO trial performed as part of Aggressotype, there have already been a considerable number of follow-up actions taken and others planned, to work towards implementation of methylphenidate treatment into offender health care. Those include:

- The value of our CIAO treatment program, which was led by Prof. Asherson was recognised by the UK prison inspectorate, who stated in their report: "All prisoners were offered screening for attention deficit hyperactivity disorder (ADHD) through the specialist Concerta (an ADHD treatment) in adult offenders (CIAO) trial…Some prisoners on the CIAO programme to whom we spoke were experiencing some stability of behaviour for the first time in their lives." The inspectorate then supported the need for continued treatment in the community: "There should be efforts to ensure the continued prescribing of medication and ongoing specialist support for prisoners started on the CIAO trial following their release" (Her Majesty's Inspectorate of Prisons' report carried out in February of 2014; www.imb.org.uk/wp-content/uploads/2015/01/isis-2013.pdf). A more recent report is in preparation (due late 2018). Initial feedback is that the inspectors specifically highlighted learning disability and ADHD services as areas of good practice.
- Prof. Asherson and his team have published a consensus guideline on the recognition and treatment of ADHD among prisoners (Young S, Gudjonsson G, Chitsabesan P, Colley B, Farrag E, Forrester A, Hollingdale J, Kim K, Lewis A, Maginn S, Mason P, Ryan S, Smith J, Woodhouse E, Asherson P. Identification and treatment of offenders with attention-deficit/hyperactivity disorder in the prison population: a practical approach based upon expert consensus. BMC Psychiatry. 2018 Sep 4;18(1):281). The paper was a follow-up of a meeting hosted by the United Kingdom ADHD Partnership (UKAP; www.UKADHD.com) in November 2016, where researchers, prison staff, clinicians and patient representatives with expertise in offender mental health and ADHD convened to discuss identification and treatment of youth and adult offenders with ADHD in the prison population. Each author attended the meeting. The authors represent a multidisciplinary group including both prescribing and non-prescribing clinical and academic experts, with extensive experience working with individuals with ADHD, including prisoners. The consensus reported reflected the views of the co-authors based on their experience, along with cited research, to provide practical guidance advice to health care professionals working with prisoners with ADHD. Experts at the meeting addressed prisoners' needs for effective identification, treatment, and multiagency liaison, and considered the requirement of different approaches based on age or gender. The authors developed a consensus statement that offers practical advice to anyone working with prison populations. They identified specific barriers within the prison and criminal justice system such as the lack of adequate staff and offender awareness of ADHD symptoms and treatments; trained mental health staff; use of appropriate screening and diagnostic tools; appropriate multimodal interventions; care management; supportive services; multiagency liaison; and preparation for prison release. Prof. Asherson was involved in the UKAP workshop and is the senior (last) author of the paper.
- The findings that ADHD is an under-recognised problem within prisons was indicated in NICE 2018. Philip Asherson was on the committee for this adaptation of the NICE guidelines. NICE now recommend enhanced awareness that among people known to the Youth Justice System or Adult Criminal Justice System there is an increased prevalence of ADHD compared to the general population. They explain that evidence showed that the prevalence of ADHD is higher in some groups than in the general population, and the committee agreed that a recommendation was needed to raise awareness of these groups among non-specialists to help them avoid missing a diagnosis of ADHD.
- Prof. Asherson and his team have delivered four training sessions to different prison mental health services and prison psychiatrists.

- The team of Prof. Asherson is providing training for prison mental health services across Scotland. A 1-day meeting delivers training on recognition and treatment of prisoners with ADHD to prison adult mental health services; based on the findings from the CIAO study and experience during the ongoing RCT. Similar training events are planned in England and other regions of the UK. We would then further aim to extend the training program to different countries/regions across the EU and to support local development of training programs.
- Following the completion of the current randomised controlled trial (CIAO II), we will complete a report (due September 2019) that can be used to support regional and national change in practice within prison settings.
- With regard to the long term plans, the team of Prof. Asherson has recruited a consultant psychiatrist from October 2018, who will assist with completion of the ongoing randomised controlled trial and will be part of future dissemination activities. The consultant will be supported to develop future research program focused on the integration of community mental health and probation services and treatment/support for offenders with ADHD in the community.

For WP10, we defined three routes of dissemination, **(1)** the marketing of the screen equipment (by ViewPoint), **(2)** the dissemination of results of the screen, and **(3)** the dissemination of the screen itself. While we did not manage to obtain follow-up funding for the latter yet, we have made considerable progress on the other two routes:

- For aim **(1)**, the first publication of the zebrafish aggression screen (Carreño Gutiérrez H, Vacca I, Pons AI, Norton WHJ. Automatic quantification of juvenile zebrafish aggression. J Neurosci Methods. 2018 Feb 15;296:23-31.) already delivered several interested clients.
- For aim **(2)**, we have progressed as follows: out of the >100 compounds we tested in zebrafish, we selected 3, which had a promising profile: methylphenidate, sildenafil, and caffeine. The methylphenidate finding was further investigated in mouse models, and the implementation into society is pursued as described above in WP09. The aggression-related profile of caffeine is quite complex, with only the low doses reducing aggression. The Aggressotype members felt it not sufficiently promising to pursue this compound further in humans, as nearly everybody drinks more caffeine than the dose required for reducing aggression. However, aggression is a big problem in the farming of consumption fish (e.g. salmon), which is not normally exposed to caffeine. The WP10 leader therefore has submitted a grant to pursue the use of caffeine to reduce aggression in the fish. Sildenafil is the active compound in Viagra. We have investigated the interest of clinicians to start a clinical trial on this compound, though without immediate success. We have also tested an additional compound, the novel compound DF302, for its effect on aggressive (as well as related) behaviour and on the brain. This work involved WP6, and a paper describing the findings has been published (Strekalova T, Bahzenova N, Trofimov A, Schmitt-Böhrer AG, Markova N, Grigoriev V, Zamoyski V, Serkova T, Redkozubova O, Vinogradova D, Umriukhin A, Fisenko V, Lillesaar C, Shevtsova E, Sokolov V, Aksinenko A, Lesch KP, Bachurin S. Pro-neurogenic, Memory-Enhancing and Anti-stress Effects of DF302, a Novel Fluorine Gamma-Carboline Derivative with Multi-target Mechanism of Action. Mol Neurobiol. 2018 Jan;55(1):335-349.). The promising results can be exploited towards further testing and potential later implementation in the future.
- For aim **(3)**, we have already been in contact with different pharmaceutical companies, but successful pilot projects, identifying promising compounds as well as the mechanisms underlying their effect, seem to be required to create interest from such companies. The WP10 leader and his group will continue working on those.

4.3 Exploitation of results of Aggressotype

An important Aggressotype result, which can be further exploited is **the implementation of ADHD treatment into offender health care**. We expect that this will be an important product of societal impact. To facilitate implementation, we have published a consensus guideline on the recognition and treatment of ADHD among prisoners (Young S, Gudjonsson G, Chitsabesan P, Colley B, Farrag E, Forrester A, Hollingdale J, Kim K, Lewis A, Maginn S, Mason P, Ryan S, Smith J, Woodhouse E, Asherson P. Identification and treatment of offenders with attention-deficit/hyperactivity disorder in the prison population: a practical approach based upon expert consensus. BMC Psychiatry. 2018 Sep 4;18(1):281), following a consensus meeting hosted by the United Kingdom ADHD Partnership (UKAP; www.UKADHD.com) in November 2016, where researchers, prison staff, clinicians and patient representatives with expertise in offender mental health and ADHD convened to discuss identification and treatment of youth and adult offenders with ADHD in the prison population. In addition, the findings that ADHD is an under-recognised problem within prisons was indicated in NICE 2018. NICE guidelines now recommend enhanced awareness that among people known to the Youth Justice System or Adult Criminal Justice System there is an increased prevalence of ADHD compared to the general population. They explain that evidence showed that the prevalence of ADHD is higher in some groups than in the general population, and the committee agreed that a recommendation was needed to raise awareness of these groups among non-specialists to help them avoid missing a diagnosis of ADHD.

An Aggressotype product that has been commercialized is the **platform for the analysis of zebrafish behaviour** developed by ViewPoint.in WP10. The purpose of this tool is to speed up analysis of zebrafish aggression, making it possible to carry out high-throughput experiments. This foreground of Aggressotype can be exploited by academics or companies that are interested in using zebrafish as a model to search for drugs or genes that control vertebrate aggression. For the company ViewPoint itself it enlarges the developments for use with other applications. The tool is not patented for this application, as the market is a niche and the technology is unlikely to be copied. The foreseen market for exactly the same application is 20 to 30 units. Re-use of hardware developments will allow to address a market of 60 to 100 units. Overall turnover expected following Aggressotype project is 1.5 to 2 M€ in the next 5 years.

A third Aggressotype product of value for exploitation is the **software developed for the gene scoring method (QGS)** developed in WP07. This software will, however, be exploited in the sense of Open Science, i.e. it will be made available as open source and thus free of charge. Exploitable results may come from the application of the software to solve biological problems related to the genetic architecture of complex behavioral phenotypes, including aggression. The software is readily available and it can already be used for the detection of interesting genetic signals (at the gene level) as well as to enhance the amount of genetic variance that can be detected using common (genetic) variance. In summary, this gene scoring method (QGS) is a new tool for genetic analysis of common variants that, in combination with standard analytical methods (e.g. GWAS) facilitates the selection of candidate genes for downstream analysis including functional assays in cells and model organisms.

The Aggressotype results have led to the development of Machine2Learn's **Galaina software** in WP07. Galaina can be used to extract and visualize causal interactions from datasets. It has a wide range of possible future applications, within and outside of the medical domain. It can e.g. be used to identify latent factors between different types of (class) aggression (related) variables (e.g. measured from different distributions, like brain function/structure, behavior, genetics, etc.) based on their (yet unknown) correlation patterns in order to identify (neurobiology based) aggression subtypes. It also has applications outside of the medical domain, e.g. behaviour monitoring in public spaces, traffic behaviour monitoring, and cause-effect analysis in industry. Galaina will fall under a proprietary commercial software licensing scheme. The Galaina computer code of the correlation engine will be made open source, freely available for academic research. This foreground can be exploited by academic centers (public and private), health professionals, and companies.

Lastly, the **biofeedback study** (WP08) might provide an exploitable product in the future, though more research is needed to decide, whether effectiveness of the treatment can support implementation into health care.

5 The address of the project public website, if applicable as well as relevant contact details

The project website is called: http://www.aggressotype.eu/ and will be kept alive even after the official end of Aggressotype. Please see Annex A for project logo.