Project Final Report

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Name, title and organisation of the scientific representative of the project's coordinator:

Prof. Dr. Christine M. Freitag - Johann Wolfgang Goethe Universitaet Frankfurt am Main

Tel: +49 - 69 / 6301-5408

Fax: +49 - 69 / 6301-5843

E-mail: C.Freitag@em.uni-frankfurt.de

Project website address: www.femnat-cd.eu

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FemNAT-CD

Logo

Project title: Neurobiology and Treatment of Adolescent Female Conduct Disorder: The Central Role of Emotion Processing

Website: www.femnat-cd.eu

Contractors involved (FemNAT-CD consortium):

Prof. Dr. Christine M. Freitag - Johann Wolfgang Goethe Universitaet Frankfurt am Main.

Other partners and team leaders:

[Partner 02]	UKAACHEN	UNIVERSITAETSKLINIKUM AACHEN
[Partner 03]	VUA	STICHTING VU-VUMC
[Partner 04]	UOS	UNIVERSITY OF SOUTHAMPTON
[Partner 05]	UNIBAS/UPK	UNIVERSITAET BASEL
[Partner 06]	UKL-HD	UNIVERSITAETSKLINIKUM HEIDELBERG
[Partner 07]	UoB	THE UNIVERSITY OF BIRMINGHAM
[Partner 08]	TCD	THE PROVOST, FELLOWS, FOUNDATION SCHOLARS & THE OTHER MEMBERS OF BOARD OF THE COLLEGE OF THE HOLY & UNDIVIDED TRINITY OF QUEEN ELIZABETH NEAR DUBLIN
[Partner 09]	UREG	UNIVERSITAET REGENSBURG
[Partner 10]	UTB	FUNDACIO MUTUA DE TERRASSA PER A LA DOCENCIA I RECERCA BIOMEDICA I SOCIAL FUNDACIO PRIVADA CATALANA
[Partner 11]	BIOEF	FUNDACION VASCA DE INNOVACION E INVESTIGACION SANITARIAS
[Partner 12]	SU	SZEGEDI TUDOMANYEGYETEM
[Partner 13]	UoA	NATIONAL AND KAPODISTRIAN UNIVERSITY OF ATHENS
[Partner 14]	GEN	GENEWAKE GMBH
[Partner 15]	VAR	VARIONOSTIC GMBH
[Partner 16]	DAA	DAACRO GMBH & CO KG
[Partner 17]	GABO:mi	GABO:MI GESELLSCHAFT FUR ABLAUFORGANISATION:MILLIARIUM MBH & CO KG
[Partner 18]	CU	CARDIFF UNIVERSITY
[Partner 19]	ART	ARTTIC SAS
[Partner 20]	GenXPro	GENXPRO GMBH
[Partner 21]	VUmc	STICHTING VUMC

1.1 Executive summary

The FemNAT-CD study (www. femnat-cd.eu) on "Neurobiology and Treatment of Adolescent Female Conduct Disorder: The Central Role of Emotion Processing" focuses on conduct disorder (CD) in females during late childhood and adolescence by, first, a cross-sectional and, second, a longitudinal approach. It aims at describing phenotypic subgroups, their underlying neurobiological and neurocognitive mechanisms leading to pathological aggression and delinquent behaviour in females compared to males, the longitudinal course of CD in females during puberty, and to apply a new psychotherapeutic approach in adolescent females within a randomised controlled trial. In addition, innovative pharmaco-challenge studies have been performed in animals and humans. The present consortium comprises several highly experienced clinical sites, a core database, monitoring and biostatistics unit as well as several basic scientists from different disciplines who have an outstanding track record on studies related to the aims and topics of this proposal.

1) Clarify the phenomenology and neurobiology of female CD from pre-puberty to post-puberty. We study:

1.1 the role of complex main and interacting genetic and environmental risk factors on female CD, related psychopathology, brain function and structure, HPA axis and ANS disturbances to identify CD specific endophenotypes and related biomarkers;

1.2 female CD specific epigenetic changes and associated early psychosocial and biological environmental risk factors;

1.3 the clinical, neural and neurocognitive phenotype of female CD from pre- to postpuberty and related neuroendocrine and ANS function as well as moderating, mediating and direct risk factors to identify neurobiologically and phenotypically distinct homogeneous subtypes to guide future, targeted treatment approaches.

2) Translate knowledge of neuropsychological and neurobiological characteristics into targeted intervention

2.1 Based on the central role of EP in female CD, we will study the effect of a DBT-CD-A psychological treatment program focusing on improving emotion regulation, emotion recognition, distress tolerance and interpersonal effectiveness.

2.2 Based on the central role of oxytocin and serotonin in emotion recognition, representation and regulation, their differential effects on neural functions underlying emotion processing and aggressive behaviour will be studied in a female animal model, and two proof-of-concept pharmaco-challenge studies with intranasal oxytocin and the serotonin precursor L-tryptophan (TRP) will be performed in human female CD.

2.3 Results of the pharmaco-challenge and epigenetic studies will be explored for their pharmaceutical potential.

3) Societal and educational objectives

3.1 Provide clinicians, researchers, youth welfare and forensic services with training on assessment instruments, different CD subtypes, and psychotherapeutic methods, and with information on therapeutic interventions to be effectively used in female CD.

3.2 Provide adolescents with CD and their families with evidence based diagnostic and therapeutic approaches, to explain risk and protective factors to these families, and to improve the long-term course of the disorder by targeted treatment.

1.2 Summary description of project context and objectives

Background and Aims

The FemNAT-CD study (www. Femnat-cd.eu) focuses on conduct disorder (CD) in females during late childhood and adolescence by, first, a cross-sectional and, second, a longitudinal approach. It aims at describing phenotypic sub-groups, their underlying neurobiological and neurocognitive mechanisms leading to pathological aggression and delinquent behaviour in females compared to males, the longitudinal course of CD in females during puberty, and to apply a new psychotherapeutic approach in adolescent females within a randomised controlled trial. In addition, innovative pharmaco-challenge studies will be performed in animals and humans. The present consortium comprises several highly experienced clinical sites, a core database, monitoring and biostatistics unit as well as several basic scientists from different disciplines who have an outstanding track record on studies related to the aims and topics of this proposal.

CD is one of the most common reasons for referral to Child and Adolescent Mental Health Services and has a highly negative impact on the affected individual as well as their families, teachers, and society (Scott, Knapp, Henderson, & Maughan, 2001). It is one of the major reasons for school dropout, which is a major concern to the EU and affects approximately 15% of all adolescents in Europe. Children and adolescents under the care of the state shower a higher rate of CD and other psychiatric disorders than their non-institutionalised counterparts (Schmid, Goldbeck, Nuetzel, & Fegert, 2008). Although the number of females exhibiting serious aggressive and dissocial behaviours is growing, the majority of studies on biomarkers, neurocognitive phenotypes, and therapeutic treatment of CD have focused on male subjects only, despite strong evidence for a differential aetiology and neurobiology of female CD (Berkout, Young, & Gross, 2011). As a consequence, female CD remains a highly neglected research area resulting in a significant gap of knowledge on neurobiological mechanisms underlying the development of the disorder in females leading to an absence of sex-specific targets for prevention and intervention. Teenage pregnancies are common in females with CD, and the children of females with CD are also at greater risk for CD (Pedersen & Mastekaasa, 2011). Further individual and societal problems strongly associated with female adolescent CD are difficulties in integration into the working life, teenage prostitution, chronic health problems, substance abuse, and delinquency (Bardone et al., 1998). Over the last decades the prevalence of CD characterized by aggressive and antisocial behaviours violating the rights of others and societal has increased in the western industrialized world (Collishaw, Maughan, Goodman, & Pickles, 2004). European and North American studies have reported a prevalence of CD of around 1-3% in girls and 2-5% in boys, with rates increasing during puberty (Maughan, Rowe, Messer, Goodman, & Meltzer, 2004). Conduct problems (including subclinical symptoms) are observed in approximately 14% of girls and 16% of boys in Europe (Ravens-Sieberer et al., 2008). Interestingly, a strong persistence of CD has especially been observed in girls (Costello, Mustillo, Erkanli, Keeler, & Angold, 2003). Associated costs for society are tremendous and of permanent duration (Scott et al., 2001; Bonin, Stevens, Beecham, Byford, & Parsonage, 2011).

A comprehensive model of CD: emotion processing at the core of the disorder in females

Clinically, CD is a phenotypically and aetiologically heterogeneous disorder characterised by aggressive (e.g. fighting, bullying) and non-aggressive, rule breaking (RB, e.g. lying, truancy) behaviours (Lahey & Waldman, 2012). Here, we focus on the description and study of aggressive behaviour (AB) in CD, as this is more strongly genetically determined than RB (Burt & Klump, 2012; Bezdjian, Tuvblad, Raine, & Baker, 2011) and of strong influence on the individual, their family, peers and society. The neurobiological underpinnings of AB have not been delineated in adolescent females (Berkout et al., 2011). CD related AB can be subdivided into instrumental/proactive (IA) and reactive (RA) aggression. RA and IA differ with regard to their genetic and environmental underpinnings, related neurobiology and brain function, as well as different abnormalities of emotion processing (EP). Blair et al. (Blair, Mitchell, & Blair, 2005; Blair, 2012) described two distinct, but overlapping neurobiological pathways underlying CD and AB, the first associated with threat-related RA, the second with IA combined with frustration-related RA, and highly correlated with psychopathy (in adults) and callous-unemotional traits (CU) in children and adolescents. The underlying neurobiology is expected to strongly differ between females and males. Briefly, the model posits a distinction between three separate elements of emotion processing (EP) and the neural substrates underlying RA and IA: emotion recognition (including cognitive empathy), mediated in the superior temporal gyrus, the temporo-parietal junction, and the fusiform face area; emotion regulation, involving the prefrontal cortex; and emotion representation (related to affective empathy, emotional learning and attention selection), with the amygdala, hypothalamus and brain stem mediating different outputs in relation to distress cues, e.g. avoidance/flight, reactive aggression/fight, and arousal. As healthy females and males strongly differ with regard to the neural basis of EP (Stevens & Hamann, 2012), and sex hormones modulate EP (Kret & de Gelder, 2012), this model is particularly suited for studying the neurobiology of adolescent female CD (Bezdjian et al., 2011; Baker,

Raine, Liu, & Jacobson, 2008). The neurobiological basis of the model has not yet been fully specified. Thus, FemNAT-CD has studied and expanded the emotion processing model by Blair, and delineate its underlying neurobiology in females and males with CD.

Major limitations of previous research are the lack of integrated genetic, epigenetic, neurobiological and neuroendocrinological studies in combination with neuropsychological, brain imaging and clinical phenotyping studies to delineate biomarkers of female CD, female specific CD subtypes and predictors of persistence and remission. In addition, there is a lack of neuropsychological and neurobiological mechanism-based, new treatment approaches beyond parent training and neuroleptic treatment. Indeed, no female specific therapeutic approaches have been studied in adolescents with CD despite evidence of sex-specific treatment responses with e.g. smaller effects of parent training in females compared to males. FemNAT-CD aims at overcoming these limitations by implementing an integrated and multidisciplinary research approach.

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Work strategy and general description

First, using a large case-control sample, we carried out a cross-sectional study from pre-puberty to post-puberty to examine neurobiological (including genetic, neuroendocrinological, autonomic nervous system function) and neurocognitive mechanisms underlying female adolescent CD. In a subgroup, we also performed the first prospective longitudinal study across adolescence to study environmental and neurobiological factors related to persistence and remission of female CD. The cross-sectional and longitudinal samples were recruited over 6 WPs (WP1 – WP6). Sample size calculation for the target sample at start of the study was as follows: (1) Cross sectional study on 9 – 18 year old females and matched controls (females 2 x N=620, males 2 x N=290). The matched case-control sample size of 1860 is sufficient to detect a main genetic effect of ≥1.2 (MAF ≥0.20), a main environmental effect of ≥2.0, and an interaction effect of ≥2.0 (Caspi et al., 2003; Retz et al., 2008) with a power of 1-□=0.8 and□□□0.001. The genomic control method will be used for candidate gene association statistics, which even has a higher power to observe true associations while controlling for false positives (Devlin & Roeder, 1999; Bacanu, Devlin, & Roeder, 2000). For quantitative measures, a balanced sample size of, e.g., 4 x 200 f/m cases and controls will detect medium effect sizes with a power >90% and □=0.001; (2) Longitudinal study in 12-16 year olds: The target sample size of N=200 (4 x N=50: females and males with CD, matched female and male controls) will allow to detect medium effects (f=0.24) by a repeated measures analysis of variance, with a power of 1-□=0.8 and□□□0.01, correlation among repeated measures 0.5.

Second, FemNAT-CD investigated new treatment approaches capitalising on the central role of impaired EP in the aetiology of female CD and AB. A new psychological treatment, CD adapted group-based dialectic behaviour therapy (DBT-CD-A), has been studied in a randomised controlled trial in female adolescents. Two proof-of-concept studies assessed the effect of oxytocin and tryptophan challenge on different aspects of EP in females and males. A complementary animal model added pre-clinical neurobiological evidence especially with regard to the interaction of oxytocin, the stress axis, and the serotonergic system on female aggression. This has been achieved by WP 5, 7 & 8.

Third, FemNAT-CD has disseminated knowledge on the impact of female adolescent CD on society and on the necessity of standardised clinical assessment as well as new treatment options by distributing information about the study design and results within the consortium, to collaborating youth welfare institutions, and to local as well as international medical, psychological, educational, forensic and political communities. The DBT-CD-A manual has been refined and published. Conference talks and publications were accepted and published, and a final international public conferences has been organised at Frankfurt. Partners of the consortium have been involved in the development of clinical guidelines on diagnosis and treatment of CD. This has been achieved by WP 9 & 10.

Partners of the FemNAT-CD consortium have specific expertise and an outstanding scientific track record in genetic (Frankfurt, GU, GenXPro; Ulm: Varionostic), epigenetic (Frankfurt, Ulm), phenotypic and clinical (Aachen, Amsterdam, Athens, Basel, Bilbao, Birmingham, Frankfurt, Heidelberg, Southampton, Szeged), neurocognitive (Aachen, Birmingham, Heidelberg, Frankfurt, Southampton), brain imaging (Aachen, Amsterdam, Basel, Birmingham, Heidelberg, Frankfurt, Southampton), neuroendocrinological (Aachen, Amsterdam, Basel, Heidelberg, Frankfurt, Regensburg, Southampton, Trier), and animal model (Regensburg) research, and have successfully performed randomised controlled psychotherapy trials (Aachen, Basel, Heidelberg, Frankfurt) as well as phamaco-challenge studies (Aachen, Heidelberg). In addition, monitoring of studies, online database development, and the broad range of statistical methods necessary to analyse the complex dataset will be provided by Frankfurt and Heidelberg in cooperation with all partners.

FemNAT-CD has been endorsed by the World Health Organization as a project that will significantly impact our understanding of CD characterised by aggressive traits. FemNAT-CD has brought together powerful multidisciplinary expertise that has improved our understanding of the role of complex main and interacting genetic and environmental risk factors implicated in the development of different subtypes of CD and their underlying neurobiology, and, for the first time, examined sex differences in the pathophysiology of paediatric CD. In parallel to generating and utilising new knowledge about the clinical phenotype and subtypes of child and adolescent female CD and associated risk factors, we also developed novel psychological and pharmacological interventions for female adolescents with CD, thereby translating knowledge on neurocognitive and neurobiological characteristics into targeted intervention.

Management structure and procedures

The Project Coordinator ensured the smooth operation of the project and guaranteed that all efforts were focused towards the objectives. She submitted all required progress reports, deliverables, financial statements to the European Commission, and, with the assistance of a management company, she was responsible for the proper use of funds

and their transfers to participants. The first and highly effective management company GABO:mi unfortunately went insolvent during the course of the project, and was eventually taken over by ARTTIC, which supported the coordinator in organising meetings, the final public conference and with the final reporting. The Project Office at the Coordinator was concerned with the scientific management and the co-ordination of all research activities. The Project Office at ARTTIC was responsible for administrative, financial and contractual management and the organisational co-ordination of the project activities.

The Steering Committee was in charge of the political and strategic orientation of the project. It met twice a year in person, and regularly once per month by phone conferences. It consisted of all work package leaders and the Coordinator and was in charge of monitoring all activities towards the objective of the project in order to deliver as promised, in due time and in the budget. In addition, the General Assembly, comprising all partners of the project, met once a year and discussed the scientific progress of the project. It also received regular advice by the Scientific Advisory Board, which's members strongly emphasized the importance and good progress of the project.

Objectives of FemNAT-CD

The FemNAT-CD study on "Neurobiology and Treatment of Adolescent Female Conduct Disorder: The Central Role of Emotion Processing" focuses on paediatric conduct disorder (CD) in females during late childhood and adolescence by, first, a cross-sectional and, second, a longitudinal approach. It aims at studying the phenotypic subgroups, their underlying neurobiological and neurocognitive mechanisms leading to pathological aggression in females compared to males, the longitudinal course of CD in females, and to apply a new psychotherapeutic approach in adolescent females within a randomised controlled trial. In addition, innovative pharmaco-challenge studies are performed in animals and humans.

1) Clarify the phenomenology and neurobiology of female CD from pre-puberty to post-puberty. We study:

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2.1 Based on the central role of EP in female CD, we will study the effect of a DBT-CD-A psychological treatment program focusing on improving emotion regulation, emotion recognition, distress tolerance and interpersonal effectiveness.

2.2 Based on the central role of oxytocin and serotonin in emotion recognition, representation and regulation, their differential effects on neural functions underlying emotion processing and aggressive behaviour will be studied in a female animal model, and two proof-of-concept pharmaco-challenge studies with intranasal oxytocin and the serotonin precursor L-tryptophan (TRP) will be performed in human female CD.

2.3 Results of the pharmaco-challenge and epigenetic studies will be explored for their pharmaceutical potential.

3) Societal and educational objectives

3.1 Provide clinicians, researchers, youth welfare and forensic services with training on assessment instruments, different CD subtypes, and psychotherapeutic methods, and with information on therapeutic interventions to be effectively used in female CD.

3.2 Provide adolescents with CD and their families with evidence based diagnostic and therapeutic approaches, to explain risk and protective factors to these families, and to improve the long-term course of the disorder by targeted treatment.

1.3 Description of the main S&T results/foregrounds of FemNAT-CD

Several studies have been highly successfully performed within FemNAT-CD. First, the largest sample on CD in European adolescents and age/sex matched controls with a multitude of phenotypic and neurobiological measures has been ascertained (CD: 855; controls: 933). Second, 155 adolescents (74 CD) were followed longitudinally. Third, the first randomised controlled trial in female adolescents with CD or ODD within the youth welfare sector in Europe was performed. Fourth, pharmaco-challenge studies have achieved their recruitment goals. Fifth, a new female rat model was established; and neurobiological results match the findings in humans. Sixth, a multitude of dissemination activities were completed to inform the public about female adolescent CD, its underlying neurobiology, and the current status of evidence based diagnosis and intervention.

It has to be emphasized, that the consortium has achieved most of its objectives despite the insolvency of 3 companies during the consortium duration, which created a lot of additional work for the coordinator, and heavily delayed WP2. Still, the vast majority of the planned scientific and dissemination activities has been achieved by the FemNAT-CD consortium. Extremely high quality data were obtained, which are the basis of FemNAT-CD related and future publications. With regard to *all* scientific and dissemination objectives of FemNAT-CD, publications are currently prepared or have been submitted or accepted.

Summary of main results:

1. Cross-sectional study

Genetic, environmental, basal neuroendocrinological, basal and reactive measures of the autonomic nervous system, neurocognitive markers, and a broad range of phenotypic characteristics have been collected in the full sample. In large, matched subsamples, the following additional data were obtained: epigenetic data, stress reactive neuroendocrinological markers, functional and structural MRI/DTI data.

Several original papers have already been published on community violence as possible risk factor (Kersten at al., 2017), and on different MRI based measures (Smaragdi et al., 2017; Raschle et al., 2017). Also, the consortium has published several review papers (Freitag et al., 2014; Biskup et al., 2015; Waltes et al., 2016; Bernhard et al., 2016 epub; Rogers & de Brito, 2016; de Jong & Neumann, 2017; Menks et al., 2017).

2. Longitudinal study

The original objective of the longitudinal study was to study the longitudinal course of female pre-pubertal CD into adolescence. This had to be adjusted during the course of the project (accepted amendment), because in Europe (in contrast to the US), a lower prevalence of CD in pre-pubertal girls was observed than expected. Therefore, the longitudinal neurobiology of adolescent CD was studied. The target sample was 200, and the consortium has achieved to include 81% of the target sample, which is a major achievement given the difficult to include population with CD.

3. Randomised-controlled trial START Now

Of this innovative study, the study protocol has been published (Kersten et al., 2016). The target sample, which posed many challenges to the conduct of the study, has been randomised; most of the T1, T2, and T3 measures have been collected. The trial will be completed and analysed within 2018.

4. Pharmaco-challenge studies

The oxytocin challenge study has been completed as planned. One article has been published (Timmermann et al., 2017). With regard to the tryptophan challenge study, an amendment was accepted, to include only healthy female subjects, because CD individuals did not sufficiently volunteer with study participation. Data collection has been completed.

5. Female aggression rat model

The study has been completed, studying the effect of early social stress on adult aggressive behaviour, and the underlying neurobiology focussing on oxytocin, vasopressin, and the serotonergic system.

6. Dissemination

FemNAT-CD consortium members have shown results of the data at a multitude of international scientific and public conferences. In January 2018, the consortium has organised its own public conference in Frankfurt to present results to all relevant stakeholders, including parents and patients. In addition, many PhD, MD and master´s theses as well as

scientific articles have been submitted or accepted based on FemNAT-CD data. Several meetings with the other FP7 conduct disorder and aggression consortia have taken place. In addition, two talks were given at a meeting of all consortia which was organised by the responsible EU officer in Brussel to discuss the societal implications of the study results.

Results of Work Package 1 (Partner: GU): Ethics, training, standardisation, and data collection

The first main tasks of WP1 was the monitoring of all ethical applications and approvals of the cross-sectional and longitudinal study performed by WP2-WP6 as well as the ethical applications and approvals of the randomised – controlled trial performed by WP7. In addition, a systematic assessment of ethical issues was done at the FemNAT-CD project meetings, such as the meetings of the steering committee and the General Assembly. All ethical applications were received by all partners prior to the local start of any study or data collection. Ethical aspects were mainly discussed with regard to the in- and exclusion criteria for all trials, and the chance to reach to most affected individuals by all involved clinical sites.

The second main task of WP1 was to establish SOPs for any kind of data collection for WP2-WP7 during the studies. This was successfully achieved, and SOPs were written with regard to any kind of data collected within the cross-sectional and longitudinal study, and provided to all clinical sites collecting data. The implementation of the SOPs was closely monitored by GU Frankfurt in cooperation with IMBI and KKS at UKL-HD Heidelberg. The implementation of the SOPs resulted in the collection of an extremely high data quality in the large cross-sectional and longitudinal samples.

The third main task of WP1 was to organise trainings in all relevant procedures at the General assembly meetings. This has successfully done an repeated for phenotypic measures (K-SADS-PL, questionnaires, medical history etc.), the ANS assessment, related data pre-processing and analysis as well as the implementation of the relevant SOPs.

The fourth main task of WP1 was an ongoing monitoring of the data collection with regard to sample size and data quality. Regular clinical phone conferences were done every two weeks. In addition, together with IMBI and KKS at UKL-HD Heidelberg GU did a vast check of data consistency of the data entered into the central database in 2017 and 2018. This structured and intensive query process eliminated many contradictory data entry issues and again resulted in a very high quality of the data for data analysis and publications.

Data collection for the cross-sectional study reached 98% of the total expected sample size, even despite having two data collecting sites dropping out during the course of the project. In half of the sample groups, the recruited sample exceeded the originally planned sample size (female controls and male cases 9-12 years, male cases and controls 13-15 years, and male cases and controls 16-18 years) by more than15%. For the longitudinal study >80% of the target sample could be achieved, which also is a major success given the many exclusion criteria for adolescents regarding MRI assessment, and the very hard to reach target population.

It has to be noted (see above), that by our strict data quality strategy, the FemNAT-CD consortium has achieved to collect a very large sample with an extremely high data quality regarding the phenotypic measures. In addition, the monitoring also helped very early in the process to detect saliva sampling errors, and ensured an overall high and consistent use of the study related SOPs. Numerous consortium publications based on data collected on the centralised phenotypic database have been completed (see WP9) and are in preparation. In addition, all PhD, MD and Master's students (with the exception of WP8 related work) who have passed or will submit their theses have been able to use the high quality data from the well maintained database.

Results of Work Package 2 (Partner: GU): Genetics, gene x environmental risk factors, and epigenetics

The achievement of the main targets of WP2 was severely delayed by the insolvency of two companies during the course of the project, which originally had planned to provide the analysis of genetic and epigenetic data for this WP. The coordinator and the lab based scientist, Dr. Andreas G. Chiocchetti, had to re-distribute tasks and find another company (GenXPro), which successfully provided all lab-based analyses of the epigenetic data. The bioinformatics analysis which was originally planned to be performed by one of the insolvent companies was completely taken over by the team at GU. Thus, the scientific aims of the project eventually could be achieved, albeit with a lot of additional effort and working time of involved staff.

The first main task of WP2 was the establishment of a standardized measurement battery for environmental risk factors, which was successfully completed very early during the course of the study. All data collected within this measurement battery were entered into the central project database. In addition, standard operating procedures (SOPs) for DNA collection and standardized assessment of environmental risk factors (such as pre-, peri-, postnatal and medical risk factors, history of early life stress and trauma, acute life events, parenting measures, socio-economic status, migration status, neighborhood quality, peer influences etc.) were developed for all recruiting partners.

The second main task of WP2 was the collection of the DNA samples together with data on environmental risk factors for the cross-sectional sample. This has been highly successfully achieved. We have collected 1920 samples of which 1651 samples of individual study participants passed quality check and had sufficient database entries to be included (status March 22nd 2018). This cohort includes 1157 females (608 controls and 549 cases) and 539 males (274 controls and 265 cases).

Several consortium publications have been completed (see WP9) and are in preparation, using some of the environmental data collected here. In addition, all PhD, MD and Master's students (with the exception of WP8 related work) who have passed or will submit their theses have been able to use the environmental data collected within this WP.

The third main task of WP3 was the pilot study to identify differentially methylated gene loci in female CD. Successfully (despite the insolvencies of GeneWake and Varionostic) GU together with GenXPro has achieved the completion of this part of the project. Of the 102 samples sent to GenXPro all passed additional QC. After successful methylation analysis 50 individuals per group passed stringent QC, each with more than 500 000 measured methylated loci that were mapped to the human reference genome. Subsequent statistical analysis was performed by Partner 01 (GU). Regions with potential technical artifacts due to genetic variation and with no variance were excluded resulting in a total of 259 742 regions that were finally analyzed for association with CD. A total of 13 050 regions have shown nominally significant differences between cases and controls. Methylation signatures specifically influenced genes involved in the development of the emotion regulation and reward system (amygdala, hippocampus and thalamus). We further observed that environmental risk factors have a differential effect on the epigenetic signatures of these brain regions e.g. the effect of trauma was specifically mediated by methylation signatures of the emotion regulation associated differentially methylated genes. Finally, we selected top-20 regions for follow up in Task 4. See also detailed results in section below.

The fourth main task of WP2 was the re-evaluation and exploration of environmental correlates of methylation pattern in female CD. Here, we also applied the same alternative study design as described above including study participants independent of pubertal status. With respect to sample collection, we have met the target for collecting a female replication cohort, as well as the same number of male study participants. Overall, high quality DNA from blood is available from additional 435 cases and 541 controls not yet included in the pilot study to select a total of 100 cases (50 males and 50 females) and 100 controls (50 males and 50 females) all matched for pubertal status and age to the initial cohort. We have also collected 19 duplicated samples with at least 2 weeks between blood collection (with full phenotype data and high quality DNA) for a potential reliability analysis of epigenetic methods.

Partner 01 (GU) functionally re-evaluated the top 20 methylation signatures for their regulatory potential at mRNA expression level in lymphoblastoid cell lines using real-time PCR. Of the 20 loci 7 showed a significant correlation between methylation levels and gene expression of the adjacent gene and will be further investigated in functional studies. A publication is already in advanced preparation. In addition, Partner GXP has analysed the genome wide methylation signatures of 50 males with and 50 males without conduct disorder following the same protocols and quality controls as implemented for the females. Again, all samples passed primary quality controls and over 500 000 loci are available. To investigate gender specific effects (group x gender effects) we have now methylation data of 2x50 cases + 2x50 controls available which has a statistical sensitivity to detect effect sizes of f = 0.46 in a 2x2 ANOVA correcting for 200 000 independent methylation loci tested (as identified in the pilot study) or effect sizes f = 0.22 at a nominal level with an alpha threshold of 0.05. Final statistical analysis is currently ongoing and results will be available in July 2018. A second publication is planned for 2018.

The fifth main task of WP2 was the investigation of genetic, environmental and g x e interaction in female CD. We have selected 164 functional variants in relevant biological systems likely involved in CD. Variants were selected based on literature findings and predicted genetic functions. Reviewing of the literature has been published as review (Waltes et al., 2015, AJMG B). Additionally, we included 50 variants that allow estimating ethnicity with high accuracy (accuracy >90%). These SNPs were selected combining the SNPs published by Samson et al. (2007) and a random forest machine learning approach. SNPs were tested on the human HapMap 3 reference panel.

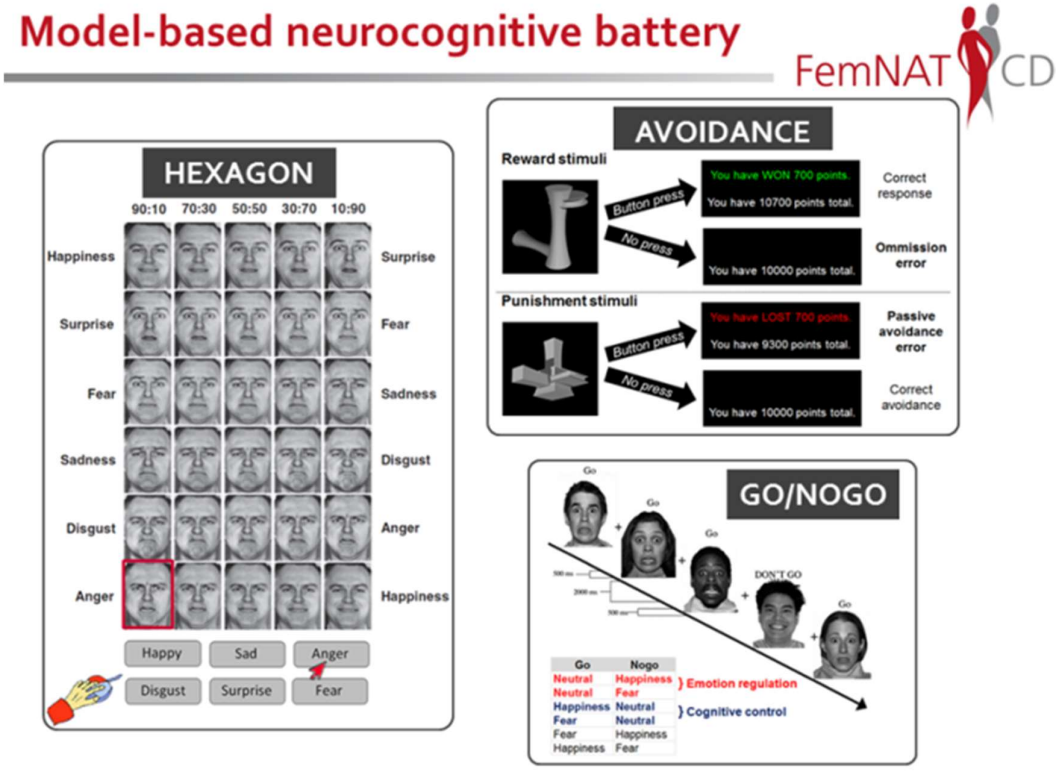
Genotyping Assays were successfully established by Varionostic (Partner 15) for 195 variants and initial experiments testing validity of SNP detection have been started. Varionostic successfully genotyped 195 variants of which 189 passed QC. This included 149 functional variants and 40 variants to detect ethnicity. This still allowed us to estimate ethnicity with an accuracy of >92%. Genotyping has been completed in October 2017 and quality checks were finished in January 2018. Due to insolvency of Partner 15 dropouts and samples recruited after the first batch was sent in July 2017 could not be genotyped. Initial data analysis successfully shows a significant association of 20 out of the 112 genetic variants of interest with conduct disorder. Two of these variants also showed a significant interaction with smoking during pregnancy on the risk for CD. One PhD student at GU has done quality checks and statistical analysis of this task.

Results of Work Package 3 (Partner: UKAACHEN): Clinical & neurocognitive phenotype

The first main task of WP3 was the establishment of a standardized measurement battery for phenotypic measures, which was successfully completed very early during the course of the study. All data collected within this measurement battery were entered into the central project database.

The second main task of WP3 was the establishment of a neurocognitive measurement battery according to the tested model, putting emotion regulation at the core of the disorder. The cognitive test battery was successfully established and distributed to all partners who collected data within WP2-WP6.

Fig. 1 Implemented cognitive test battery in FemNAT-CD



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The third main task of WP3 was to compare clinical and neurocognitive functions related to emotion processing (EP) in matched females and males, and to describe phenotypically and neurocognitively defined subtypes of female CD within a large cross-sectional study. Altogether, phenotypic data from 1827 participants have been collected and entered into the central database (completeness: 98% of N=1860). All clinical centres (CR1, CR2, CR3, CR4, CR5, CR7, CR10, CR11, CR12, and CR13) have successfully contributed to data collection and data entry. CR1 has updated the respective SOP after the second periodic report, as well as all relevant phenotypic measures for the clinical assessments.

Table 1. Final cross-sectional sample with clinical and neuropsychological data

Overall N = 1827 (Target: N=1860)	Female CD	Female controls	Male CD	Male controls
	Recruited / Target (completeness in %)			
9-12 years old	83 / 120 (69.2%)	149 / 120 (124.2%)	117 / 100 (117%)	98 /100 (98%)
13-15 years old	236 / 260 (90.8%)	255 / 260 (96.1%)	123 / 100 (123%)	120 / 100 (120%)
16-18 years old	216 / 260 (83.1%)	221 / 260 (85.0%)	105 / 90 (116.7%)	104 / 90 (115.6%)

As shown in Table 1, some individual recruitment targets (e.g., 9-12 year old females with CD) have not been fully reached as it was continuously challenging to recruit those specific subsamples despite great efforts among all involved clinical partners. Although we were able to include individuals with a diagnosis of Oppositional Defiant Disorder (ODD) and at least 1 CD symptom (age group 9-12 years old) or 2 CD symptoms (age group 13-18 years old) after the approved amendment in April 2016, we still encountered problems specifically reaching our recruitment targets for females with CD and/or ODD. In contrast, other individual recruitment targets, specifically for males with CD, were over-achieved, as we agreed in the consortium to include as many available CD cases as possible.

Diagnostic data using the KSADS from 75 participants (94% of N=80) have additionally been entered into a separate database in order to calculate the inter-rater reliability (IRR) for our main diagnostic measure across all clinical sites. IRR of CD was excellent (Cohen's κ = 0.91, CI 95%: 0.82 – 1.0), with an agreement rate of 94.7%. IRR of other disorders, including ADHD, ODD, major depressive disorder (MDD), and generalized anxiety disorder (GAD), was also in an excellent rage (Cohen's κs ≥ 0.84, agreement rates ≥ 92%).

For the cross-sectional part of the study, a total of 1579 participants (i.e., 87% of N=1827) have provided a complete neuropsychological data set on emotion processing (EP), including the passive avoidance learning task, the emotion hexagon task and the emotional go/nogo task: 440 female cases, 565 female controls, 290 male cases, and 312 male controls. Only 116 participants (i.e. 6% of N=1827) have not provided any neuropsychological data. At least one or two – instead of three – data sets of EP measures are available from 132 participants (i.e., 7% of N=1827) due to non-compliance or other (e.g., technical) issues. Quality control on the neuropsychological test battery has been performed on a continuous basis by CR2. In the case of issues (e.g., mislabelled logfiles, export errors), feedback has been emailed to the respective clinical centre in order to solve the problem. It was our target to obtain a comprehensive neuropsychological data set on EP from at least 50% of the included females and 80% of the included males. As shown in Table 2, we achieved both of these targets.

Table 2. Overview of complete neuropsychological data sets on emotion processing for the cross-sectional and longitudinal part of the study

	Cross-sectional (t1)	Longitudinal (t2)	Cross-sectional and Longitudinal (t1+t2)
Female CD	440 / 505 (87.1%)	26 / 32 (81.3%)	22 / 26 (84.6%)
Female controls	565 / 625 (90.4%)	55 / 55 (100%)	48 / 55 (87.3%)
Male CD	290 / 345 (84.1%)	21 / 32 (65.6%)	20 / 21 (95.2%)
Male controls	284 / 312 (91.0%)	34 / 38 (89.5%)	27 / 34 (79.4%)
Overall	1579 / 1827 (86.5%)	136 / 157 (86.6%)	117 / 136 (86.0%)

In order to describe phenotypically and neurocognitively defined subtypes of female CD, we applied a more person-centered, bottom-up analytic approach by sub-categorizing female youths with CD as "impaired" versus "unimpaired" with regard to the three emotion domains: emotion recognition, emotion learning, and emotion regulation. This allowed us to reveal and compare neurocognitive subgroups of conduct-disordered females with pervasive versus those with deficits in two or just one emotion domain or no meaningful deficits at all. A similar approach has been tremendously informative in the field of attention deficit hyperactivity disorder (ADHD), and has led to dual or triple pathway models of ADHD aetiology with fundamental clinical (e.g., treatment) implications. Similar to ADHD, we expected to find

subgroups of CD females without impairment in any domain versus those who show severe deficits across all three domains. For preliminary findings, see below "Significant results".

The fourth main task of WP3 was the longitudinal assessment of clinical and neurocognitive changes in female CD. Altogether 157 participants have been enrolled and thoroughly characterized in the longitudinal study, and their phenotypic data (32 female cases, 55 female controls, 32 male cases, 38 male controls) have been entered into the central database. In this part of the study, individual recruitment targets were set as follows: 60 females with CD, 60 healthy female controls, 60 males with CD and 60 healthy male controls. Only participants who were successfully scanned using functional and/or structural MRI as part of the cross-sectional study (WP6) were eligible for the longitudinal study. We did not reach our intended recruitment goals for any of the four groups despite great efforts among all involved partners. According to the five partners involved in this task (CR1, CR2, CR4, CR5, and CR7), recruitment has been challenging due to several factors, such as (a) unwillingness of subjects to participate again, (b) incorrect contact information for re-recruitment, or (c) MRI contraindications (e.g., braces). All partners repeatedly discussed ways on how to improve recruitment efforts during regular phone conferences. CR1 has updated the respective SOP as well as all relevant phenotypic measures for this task after the second periodic report. Analysis of the longitudinal data is currently under way.

The fifth main task of WP3 was the assessment of treatment-related changes of EP measures in female CD, across the centres involved in WP7 (CR1, CR2, CR3, and CR5). 128 girls with CD/ODD have already been tested with neuropsychological EP measures. Given the design of this task as an RCT, which is completely blinded until the closure of the database, EP will be analysed after completion of data collection after summer 2018.

Results of Work Package 4 (Partner: VUA, task lead GU): Psychophysiology & Neuroendocrinology

The first main task of WP4 was to study sex specific associations between ANS and neuroendocrine parameters and CD, IA, RA in the overall cross-sectional study. A large sample with complete ANS data, comprising heart rate (HR), respiratory sinus arrhythmia (RSA), pre-ejection period (PEP) and skin conductance (SCL/SCRs) was obtained in 1651 subjects (473 female CD cases, 588 female controls, 298 male CD cases, 292 male controls). Data on both, basal ANS activity and ANS reactivity was collected. Data collection was conducted by 10 clinical sites (GU, RWTH Aachen, VUA, UOS, UNIBAS/UPK, UOB, UTB, BIOEF, SU, UoA). The ANS data collection was coordinated by VUA which included the logistics of supplying all sites with the required hardware and software, as well as providing standardized operating procedures, and consultation for both practical issues and technical problems. All sites were successful in collecting ANS data during the study and no major technical issues, nor any deviations from the standard operating procedures occurred. After collection, the ANS data were sent to VUA and were checked and processed by VUA. Two consortium papers have been written on the baseline psychophysiological data, which both have been accepted in a special issue of the Journal of Criminal Justice (see dissemination report). Currently, analysis of the ANS reactivity data is under preparation, which is planned to result in at least another three papers that will be submitted in 2018. Basal neuroendocrinological measures from around 1500 of overall 1827 (82%) participants have been collected. In a subset of N=400 age and puberty status matched individuals (100 females with CD, 100 healthy females; 100 males with CD, 100 healthy males), the basal neuroendocrinological measures have been analyzed in 2016. An article with first results of this data is currently in preparation with planned submission in May 2018. A subsample of participants has additionally performed a psychosocial stressor protocol (Trier Social Stress Task (TSST)) to measure neuroendocrinological reactivity. We have collected data from 410 subjects at four sites (GU, UKAACHEN, VUA, UNIBAS/UPK, UOS). The total planned subsample size was overachieved and now presents the largest sample of adolescent females and males with CD worldwide. In a subset of N=360 age and puberty status matched individuals (100 females with CD, 100 healthy females; 80 males with CD, 80 healthy males), the reactive neuroendocrinological measures of cortisol, testosterone and oxytocin have been analyzed in 2017. An article with first results of this data is currently in preparation with a planned submission in June 2018.

The second main task of WP4 was the collection of ANS and endocrinological data in the longitudinal sample. ANS data has been collected from 146 participants (27 female CD cases, 56 female controls, 28 male CD cases, 35 male controls). Neuroendocrinological measures have been collected in around 130 participants in the longitudinal study (LTT) collected at five sites (GU, UKAACHEN, UNIBAS/UPK, UOB, UOS). Longitudinal ANS data are currently analysed.

The third main task of WP4 was to collect ANS and endocrinological data as predictors of treatment response of the RCT psychotherapy trial (WP7). ANS measures also collected repeatedly as secondary outcomes. As stated in the DOW we expected around 50% of girls participating in the RCT (50% of N=128: 32 per treatment condition) to complete

the ANS assessment. In total 115 participants (97%) completed the ANS assessment at T1. Of those, 70 participants were allocated to the intervention (DBT-CD-A) condition, and 45 were allocated to the Treatment As Usual condition (TAU). The targeted number of ANS assessments was thus reached. At T2 83 participants (82.8%) completed the ANS assessment: 52 from the DBT-CD-A condition and 31 from the TAU condition, and at T3 and 74 (79.6%) completed the ANS assessment: 47 from the DBT-CD-A condition and 27 from the TAU condition. See Table 4.3 for an overview of the ANS assessment on the different time points. As for the neuroendocrine measures, a psychosocial stressor protocol was applied in a subsample to measure endocrinological stress reactivity. 15 participants (13.0%) completed the protocol at T1: 5 from the DBT-CD-A condition and 10 from the TAU condition.

Results of Work Package 5 (Partner: UKL-HD): Data management, monitoring and statistics

The first main tasks of WP5 were the establishment of the secure online databases and the data-management for the cross sectional and longitudinal studies (WP1-6) as well as the psychotherapy RCT (WP7). Electronic case report forms (eCRF) were developed based on the SOPs and the assessment batteries provided by WP1-WP3. A beta version of the database was available in July 2014, three of the participating centres were asked to test the database. After some adaptations, the database was accessible for productive use since September 2014. In order to guarantee a high quality of the data, predefined edit checks for data validation were implemented in the eCRF together with partner 1 (GU). By this, the person performing the data entry is automatically forced to correct or explain the data already at the time of data entry. Furthermore, data that is reproducible is checked for completeness. In order to facilitate the data entry, an investigator user guide, a description of data entry options, and annotated questionnaires were provided for the data entry staff. During the 2nd general assembly meeting, a workshop on data entry was provided. Regular status updates informed the partners about recruitment and status of data entry. A feature to download data was configured within the online database system. The access to this feature has been restricted to certain users in the participating centres. In May 2016 the database had to be updated because the data collection volume for longitudinal study was modified. Study data for analyses prior to consortium publications were provided on demand. Additional validation of K-SADS-PL (diagnostic interview) data was implemented in October 2017. In cooperation with WP7 leader UNIBAS/UPK, the items for data collection were defined. Paper based CRF and electronic case report forms (eCRF) were finalized after a planned protocol amendment. Validation rules were implemented according to the data validation plan in order to guarantee best data quality for statistical analyses. Status updates concerning recruitment and completeness of documentation are provided regularly as well. The system used for data management is validated and is compliant with FDA 21 CFR part 11. All data transmission is encrypted with secure socket layer (SSL) technology. All changes to the data are logged with a computerized timestamp in an audit trail which includes the name of the author/editor, the date/time of the change, the reason for the change (if applicable), and the new information. The database server of the IMBI with the stored data is located in a secure data centre and is protected by a firewall. The system provides an infrastructure to support user roles and rights. Users can apply for access to the database by a special user account application form to be sent to the account administrator. The access is restricted to data of the participants in the respective centre. Backups are performed regularly.

The second main tasks of WP5 were the monitoring of data collection for the cross-sectional and longitudinal studies (WP1-6) as well as the psychotherapy RCT (WP7). All sites were visited at least once to check whether the sites work according to the protocol and to offer help if necessary. Three sites (Birmingham, Southampton, Athens) were visited twice as they had difficulties at the beginning with the proper conduct of the study (insufficient knowledge about ICH-GCP). However, all 3 sites were highly motivated and dedicated to the study and improved. Reports were generated for all visits and forwarded to the study coordinator. The sites received detailed follow-up lists. Also, for the RCT (WP7), all sites were visited several times for onsite-monitoring. Monitoring reports and follow-up lists were generated and forwarded to the coordinating investigator resp. the sites. Informed consent forms were available for all subjects, the primary endpoint was verified for all subjects, additional items were checked randomly. In general, the sites delivered good quality.

The third main task of WP5 was statistical support and statistical analysis. Statistical support was given to several partners which includeded critical review of the planned statistical analysis. Additionally, statistical analyses for partner 1 were conducted. This comprises statistical modelling for a proper analysis of the data. Inter-rater reliability for the diagnoses of the K-SADS was computed and a report on the results was provided for all partners. The results were used in the different publications. A solution for the imputation of missing data in the questionnaires was presented and appropriate imputation models were set up. As a result, missing values in the items of the different questionnaires were imputed and the imputed data sets were provided. Additionally, the scoring algorithms for each questionnaire were implemented so that scores for the raw and the imputed data could be provided as well. Significant results

Results of Work Package 6 (Partner: UOS, task lead UOB): Structural and functional neuroimaging of emotion processing in CD

The first main tasks of WP6 were to identify structural and functional neuroimaging biomarkers of CD, and to apply multivariate analysis to the longitudinal sMRI and fMRI datasets. For the grey matter, we have identified differences between CD and healthy control subjects in ventromedial prefrontal cortex cortical thickness, as well as alterations in cortical folding and surface area. These results have now been published (Smaragdi et al., 2017, JACAAP). For the white matter, we have analysed the diffusion tensor imaging data collected within the study in two different ways – the first used Tract-Based Spatial Statistics to examine for differences in structural connectivity across the entire white-matter 'skeleton' in an unbiased, hypothesis-free way (see 'significant results' section below; paper currently under review at JACAAP), whereas the second used deterministic tractography to test for main effects of diagnosis or sex-by-diagnosis interactions in specific white-matter tracts that make up the extended limbic system. We also have performed analyses of the functional MRI data for 6 different paradigms: (1) conscious face processing, (2) reward/punishment learning, (3) empathy for pain in others, (4) subliminal face processing, (5) deliberate emotion regulation (reappraisal), and (6) cognitive/affective theory of mind. Except for (5) where only females were tested, across these paradigms, both main effect of diagnosis (i.e., CD has a group show difference compared to the healthy controls) and sex-by-diagnosis (females and males exhibit distinct pattern of brain response relative to their healthy control counterparts) have been observed. Altogether, we have collected the sMRI and fMRI data and the detailed clinical data required to perform multivariable analyses. Also, we have started to use some of these innovative approaches in preliminary analyses (e.g. machine learning). However, it has taken a considerable amount of time to collect the cross sectional and longitudinal MRI datasets (N = 700+ at T1 and 126 at T2), and run the classic and most-established analyses (e.g. VBM, SBM, fMRI analyses using general linear models) that it has not been possible to extensively investigate the value of multivariate analyses. Those multivariate analyses will use methods such as Angle-based Generalised Matrix Learning Vector Quantisation (Angle-GMLVQ; Bunte, Baranowski, Arlt & Tino, 2016; Ghosh et al., 2017), which is a prototype-based machine learning classifier. Angle-GMLVQ predicts class membership by positioning prototypes as class exemplars, or class representatives. It then assigns each data point to the class of the nearest prototype. (Here, the similarity between a data point and a class prototype is quantified through their angle.) Angle-GMLVQ has the additional advantage that it provides information on the relevance of each feature (i.e. variable) to the model. We are currently conducting those analyses using the grey matter volume of several regions of interest derived from our VBM pipelines. Finally, we are aiming to use graph theory methods to analyse the DTI data, to complement TBSS and tractography approaches that have been used to date.

The second main task of WP2 is to predict treatment response in the psychotherapy RCT (WP7). This task has been more difficult to achieve than expected, because the randomised controlled trial that it relies upon has not finished yet and the treatment response data will not be available until several months after the RCT closes. However, we do have clear plans to relate the structural MRI and fMRI data (particularly from the emotion regulation task) with treatment outcomes in the RCT. We hypothesise that the individuals with emotion regulation difficulties will benefit most from the Dialectical Behaviour Therapy intervention, which focuses heavily on controlling impulses and identifying and accepting strong and painful emotions.

Results of Work Package 7 (Partner: UNIBAS/UPK): Randomized controlled trial (RCT): Psychological treatment by DBT-CD-A

The first task of WP7 was to adopt an existing DBT-based intervention for female CD and ODD patients. NOW manuals as well as workbooks were successfully adapted and tailored to the specific needs of conduct-disordered/oppositional-defiant girls within youth welfare institutions and will be published within the next year. All intervention material (workbook, manual, trainings) is additionally provided for trainers and adolescents on the webpage istartnow.ch with additional online material (film clips, life-exercises, self-tests, etc.).

The second main task of WP7 is to run the START-NOW RCT and investigate the efficacy of this DBT-CD-A approach in female adolescents with CD and/or ODD. So far as planned, all 128 participants have been included in the RCT trial (see figure below, status: February 2018). This is a major achievement for all collecting sites, due to the difficult to recruit sample as well as the organisation of the intervention at different youth welfare institutions.

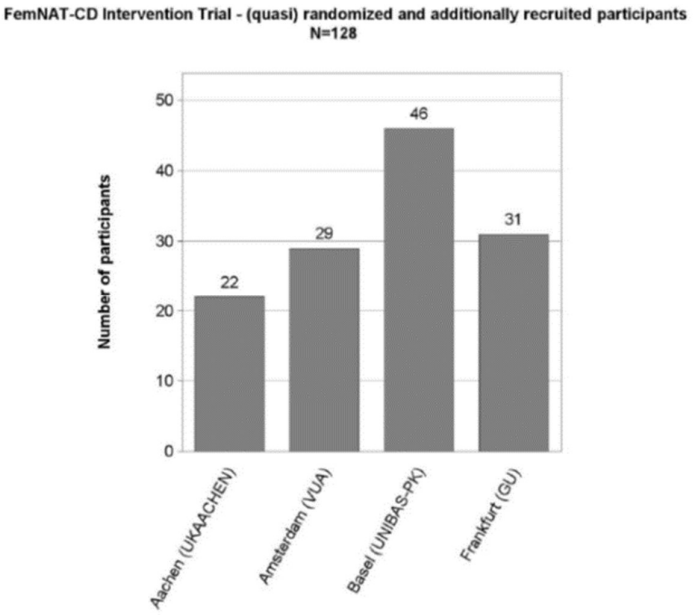

Figure 2: Data collection for the START-Now randomised controlled trial

The third main task of WP7 was to investigate biological predictors of treatment. The rate of participants of the RCT who gave consent to this additional testing was very high (>80%). Results are pending.

Additional results of WP7:

- The Study was registered:
  - ✔ Deutsches Register Klinischer Studie

    ID:DRKS00007524

    http://drks-neu.uniklinik-freiburg.de/drks_web/navigate.do?navidationId=trial.HTML&TRIAL_ID=DRKS00007524
  - ✔ WHO

    http://apps.who.int/trialsearch/Trial2.aspx?TrialID=DRKS00007524
  - ✔ Kofam: Koordinationsstelle Forschung am Menschen

    ID:SNCTP000001697

    http://www.kofam.ch/studienportal.html
- The study protocol is published:

Kersten, L., Prätzlich M., Mannstadt, S., Ackermann, K., Kohls, G., Oldenhof, H., Saure, D., Herpertz-Dahlmann, B., Popma, A., Freitag, D.M., Trestman, R., Stadler, C., (2016) START NOW: Study protocol of a quasi-randomised clinical trial testing a comprehensive skills training for adolescent females with oppositiona defiant and conduct disorders.

- Additional Newsletters have been published on femnat-c.eu and were sent to all participating institutions. In addition, the newsletters serve as a supportive advertising campaign in recruiting new institutions.
- Data Safety Monitoring Board: The independent Data Safety Monitoring Board (DSMB) was informed regarding current conduct of the trial and especially SAEs in all sites. In the final DSMB report (Prof. H.-C. Steinhausen) no concerns in terms of continuation of the RCT trial were mentioned.
- Monitoring Visits: The following visits have been conducted:

  Aachen: 9th September 2016 and 27th June.2017

  Amsterdam: 14th September 2016 and 27th July.2017

  Basel: 19th November 2015 and 26th July 2016 and 26th October.2017

  Frankfurt: 9th December 2015 and 20th July 2016 and 5th July 2017

  All sites have corrected deficiencies that have been discovered during the monitoring visits, thus all sites are working in accordance to the ICH-GCP standards.

Results of Work Package 8 (Partner: UKL-HD): Pharmaco-challenge and neurobiological studies on oxytocinergic and serotonergic transmission

The first main task of WP8 was to study the effects of single OXT challenge on facial threat perception in CD. The primary objective was to investigate the oxytocinergic modulation of early attentional processing (emotion representation and recognition) of threatening facial cues. To this end, we have conducted a randomized double-blind placebo-controlled within-subject repeated measures study at UKL-HD. Ethical approval was received in October 2013 from the ethical commission of the Medical Faculty at Heidelberg University, last amendment was added in April 2016. Participants took part in an emotion classification task in which they viewed facial expressions (fear, anger, and happiness) after intranasal administration of 24 IU of oxytocin or placebo. Participants were young adult females and males (aged 18-30 years) with antisocial personality disorder (ASPD) and a history of CD in adolescence and healthy controls. W completed recruitment and included 102 participants, of whom, 96 have completed the two within-subject fMRI scans and provided with within-subject neuroendocrine assessments (recruitment process of 100%; n=24x4). After pre-processing the MRI data, we obtained full 79 data sets (drop-out rate of 21.5%) with 20 being males and 19 being females with ASPD in addition to 20 male healthy controls and 20 female healthy controls. All participants were assessed by the standardized assessment battery of WP3 (phenotypical and neurocognitive characterization) and model-based computerized battery (the Passive Avoidance Task, Hexagon task, Go/Nogo task). In addition to the comprehensive data collection, we applied the Approach-Avoidance task. For the behavioural data of the emotion classification task, we have collected full data sets of 51 participants which we already published (Timmermann et al., 2017). The results of the fMRI study are currently analysed and will be submitted in 2018.

The second main task of WP8 was to study the effects of a single tryptophan challenge on perceived threat in typically developing, healthy adolescent females. This task combines a tryptophan loading challenge procedure with fMRI neuroimaging techniques. The participants take part in a TRP-related challenge study, involving a within-subject repeated measures design with two study days of per participant (500 mg TRP and a placebo are administered on separate study days [one day for TRP; and one day for placebo]). Both study days had an identical schedule. For each study day the participants were requested to not consume any protein-rich foods after 8 p.m. on the day before each study day, and to not consume any caffeine and to arrive at the study site without breakfast on the study day (overnight protein fast). After a standardized breakfast and intake of the TRP challenge procedure condition or the placebo, the participants completed various assessments and tests. Three hours after challenge intake an fMRI experiment session took place during which several scans were obtained (anatomical scans, emotion regulation task, resting state). After completion of the fMRI sessions, a test on attentional performance was administered. At the beginning (baseline = before challenge administration) and after completion of all tests and assessments blood samples were taken in order to be able to calculate the influx of TRP across the blood brain barrier. The two study days were spaced several days apart (between 7 and 35 days). In addition, several baseline questionnaires to assess different patient characteristics

were administered as part of the study (aggression and impulsivity related questionnaires, anxiety as well as temperament and character related assessments, mood etc.). The recruitment of healthy female controls has been completed (N = 24 subjects with 2 datasets, TRP challenge & control condition). The results of the fMRI study are currently analysed and will be submitted in 2018.

The third main task of WP8 was to study animal models of female AB, especially with regard to the correlation of individual female AB with endogenous blood / brain OXT/AVP/5HT levels, and neuronal effects of pharmacological interference. In order to reveal the neurobiological mechanisms underlying adolescent and adult female AB and alterations in EP, participant 09 (I. Neumann) focused on a novel Wistar rat model of high versus low spontaneous female AB. Based on our pilot findings, we aimed to correlate the level of spontaneous female AB with local neuronal activity patterns (c-fos, pERK as neuronal activity markers), and with the local expression of OXT, AVP (for specificity), 5-HT and their receptors (OXTR, V1A, 5-HT1A/1BR) using in-situ hybridization (ISH) and receptor autoradiography (RAR), respectively. We will further compare local and systemic (as in humans) release patterns of OXT and 5-HT before, during and after the female RI-test using intracerebral microdialysis and chronic jugular vein catheterization between spontaneous aggressive and non-aggressive females. This approach was expected to importantly support interpretation of human saliva data. Animal numbers: neuronal parameters: 24 high/low =40, neuroendocrine parameters: 24 high/low x 2 (blood, microdialysis) = 48, total = 72 female rats. Based on our pilot studies that icv OXT effectively inhibits high female AB in the female RI-test, we aimed to localize these pharmacological effects and reveal the involvement of the local endogenous OXT system in female AB by local infusion of OXT and its receptor antagonist via stereotaxically implanted guide cannulas, respectively. In order to extend the results of corresponding pharmaco-challenges in young women with CD we further planned to monitor behavioural effects on female AB after systemic OXT and TRP (as in humans) and local infusion of 5-HT and its se-lective receptor (ant) agonists (5-HT1A agonist/antagonist: 8-OH-DPAT/WAY-100635; 5-HT1B ago-nist/antagonist:anpirtoline/GR-127935; both agonists are anti-aggressive in males). Pharmacological studies on peripheral OXT and TRP (as in humans) were planned to be complemented by the characterization of the endogenous OXT/5HT brain system, in particular by quantification of OXT and 5HT receptor binding using RAR in order to exclude treatment-induced down-regulation of receptor expression in selected brain regions. Animal numbers: 12 high AB x 2 regions x 6 treatments (OXT/OXTA, 5HT agonist/antagonist, 2 vehicle) = 144, 3 systemic treatments (OXT, TRP, vehicle) x 10 high AB = 30; total = 174 female rats. To characterize sex-dependent effects of early life adverse experiences on adolescent and adult AB, female and male offspring exposed to either maternal separation (days 2-14) or social isolation after weaning, 250 were intended to be tested in the specific RI-test established for male and female rats at the age of 7 and 11 weeks; plasma (repeated blood sampling via chronic jugular vein catheter) and selected brain OXT, AVP and 5-HT (using ISH and RAR in dependence on results in 3.1.) markers were estimated. Animal numbers: 10 dams, offspring: 2 sexes x 2 parameters (plasma, neuronal) x 12 = 48, total = 58 female rats. At the final stage of animal experiments of WP8, for further validation of human pharmaco-challenge studies, two models for environmentally modified (early life stress) and genetically determined (LAB rats) high female AB, respectively, were planned to be employed. According to our study design, adult female rats exposed to early lifestress hypothesized to show increased AB (using either maternal separation or social isolation) as well as adult rats from our genetic model of high female AB (LAB females) should be used as a final proof of concept for neuropharmacological intervention to inhibit high female AB by OXT or systemic TRP not only in unselected, spontaneously high AB rats. but also in individuals with environmentally modified and genetically determined high AB. Animal numbers: 2 models x 4 treatments (OXT/vehicle; TRP/vehicle) x 12; total= 96 female rats. Results of all studies are currently analysed and will be published.

Results of Work Package 9 (Partner: UNIBAS/UPK): Dissemination

The first main task of WP9 was to organise all relevant dissemination and exploitation activities of the consortium with the support of all partners and the coordinator. All partners of the consortium highly effectively disseminated study information to all relevant stakeholders. The various dissemination activities (phone contacts, talks, flyers, home-page, interviews, press releases, conference talks, publications etc.) resulted in the impressive data collection achieved by the FemNAT-CD consortium. All necessary dissemination strategies were implemented to reach local institutions via local media. The list of dissemination strategies includes the following:

- Local internal websites of clinical departments and other medical or educational institutions
- Flyers and posters at medical and educational institutions
- Ads in local newspapers, including ads in student newspapers and local professional newspapers

Regular press releases

- Personal contact to employees in educational, medical, psychological and forensic institutions

On 26th January 2018, in Frankfurt, a well received and highly successful conference was organized by partner 1 (GU) and partner 19 (ARTTIC SAS). All relevant stakeholders as well as several international guests were invited (see program and speakers below). Especially the panel discussion gave additional feed-back to the current FemNAT-CD results, and added many ideas for the improvement of care for female adolescents with CD (such as employing evidence based treatments throughout the youth welfare and healthcare sector). Through the set-up of a professional PR campaign performed by ARTTIC, the conference attracted a large audience after having been widely announced in regional and national news and reached a number of ca. 160 participants.

FemNAT CD

GOETHE UNIVERSITÄT FRANKFURT AM MAIN

Conduct Disorder in Female Adolescents: Underlying mechanisms and innovative interventions

Frankfurt am Main, 26th January 2018, 9am – 4pm

Agenda and Speakers

9:00 a.m.	**Welcome** Prof. Brigitte Haar, Vice president of the Goethe University – Frankfurt am Main
9:06 a.m.	**Adolescents with conduct disorder – girl specific aspects** Prof. Dr. Christine Freitag, Goethe University - Frankfurt am Main
9:30 a.m.	**(Epi)genetic signatures and environmental risk factors in female conduct disorder** Dr. Andreas Chiocchetti, Goethe University - Frankfurt am Main
9:56 a.m.	**What do we know about the brain in conduct disorder, and what has FemNAT-CD told us so far?** Dr. Graeme Fairchild / Dr. Stephane de Brito, University of Bath / University of Birmingham
10:30 a.m.	**Coffee break**
11:00 a.m.	**Emotion processing in girls and boys with conduct disorder** Prof. Dr. Kerstin Konrad / Prof. Dr. Arne Popma, University Hospital Aachen / Stichting VUmc Amsterdam
11:26 a.m.	**New pharmacological approaches to treat conduct disorder** Dr. Katja Bertsch, University Hospital Heidelberg
11:50 a.m.	**Motivating and treating female adolescents with conduct disorder: a comprehensive cognitive behavioral approach** Prof. Dr. Christina Stadler, University of Basel
12:16 m.	**Promising interventions for adolescents with conduct disorder - an international perspective** Prof. Dr. Sigrid James, University of Kassel
1:00 p.m.	**Lunch**

FemNAT-CD has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under Grant Agreement n° 602407.

FemNAT CD

GOETHE UNIVERSITÄT FRANKFURT AM MAIN

Conduct Disorders in Female Adolescents: Underlying mechanism and innovative interventions

Frankfurt am Main, 26th January 2018, 9am – 4pm

Agenda and Speakers

2:00 p.m.	**Panel discussion with 6 invited guests** Moderator - Verena von Scharfenberg: ARTTIC, Munich (Germany): 1. Prof. Dr. Christine Freitag: Coordinator of the project, Goethe University Frankfurt am Main (Germany) 2. Andrea Billow: ADHD parent organisation, London (UK) 3. Polly Wright: Children's charity Barnardo's, Bristol (UK) 4. Prof. Dr. Christina Stadler: Department of Child and Adolescent Psychotherapy, University of Basel, (Switzerland) 6. Prof. Dr. Sigrid James: Department of Social Work and Social Policy, University Kassel (Germany)
3:00 p.m.	**Open discussion with audience**
4:00 p.m.	**End of the conference**

Registration
Please register HERE

Contact
FemNAT-CD Project Office
+49 (0) 89 248 8303 – 38| mass@arttic.eu
www.femnat-cd.eu

Venue
Goethe-Universität, Frankfurt am Main
Casino - Campus Westend
Nina-Rubinstein-Weg 1
60323 Frankfurt am Main, Germany

Please click Hotels for itinerary

http://www.goethe-university-frankfurt.de/en?locale=en

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Further dissemination activities:

- Consortium meetings: Five General Assembly Meetings were held during the project. The meetings lasted 2-3 days, and consisted of highlights of scientific achievements within FemNAT-CD, symposia and keynote lectures to introduce and discuss new concepts and technological advances. Members of the scientific advisory board were regularly present and enjoyed the impressive achievements of the FemNAT-CD consortium.
- Conference presentations: Results of FemNAT-CD's work were regularly presented at national and international well attended conferences in the field. Local 'information days' were organized regularly in order to inform a large and broad audience. Meetings with other FP7 conduct disorder / aggression consortia (ACTION, Aggressotype, MATRICS) have been organized successfully, and in Dec 2017, the EU officer in charge of all consortia has organized a meeting in Brussels to discuss the societal impact. The coordinator, Christine Freitag, and Christina Stadler from UNIBAS/UPK attended the meeting and gave talks about result of FemNAT-CD and the development of the German AWMF S3 evidence based and broadly consented clinical guideline on ODD and CD.
- Peer-reviewed international publications: To date, a preliminary list of articles which either have been published or are currently under review, comprises 26 manuscripts which are directly related to the project (see list of publications). Hartzing´s meta search engine "Publish or Perish" lists at least 56 publication in total which directly or indirectly refer to the project. To date, these publications have been cited 256 times.
- PhD, MD, and Master's thesis: The consortium has resulted in > 20 PhD, >5 MD, and >10 Master's theses, which have been or will in the near future be submitted to the relevant University authorities.

- Press releases and (social) media activity: Several press releases regarding FemNAT have been published on the project website and in local or international press. To date, Google lists 13,400 web entries referring to FemNAT-CD's project website illustrating the widespread activity related to the project. The project is also present on Twitter with 93 tweets to date. Regarding communication within the scientific community the project has also been added to Research Gate.

In addition, as second main task of WP9, the START-NOW manual was translated, adjusted and made available for youth welfare organisations. The manual and the workbook for adolescents are currently available for youth welfare institutions and in addition for schools (see: https//:istartnow.ch and istartnow.de). After the successful implementation of the RCT in several youth welfare institutions, an increasing interest in the START NOW intervention occurred also from outside youth welfare institutions. Thus, training for therapists, social workers and educators regularly is provided at the University Hospital of Basel. After the end of the RCT, the START NOW intervention will be provided by youth welfare institutions but also at Child and Adolescent Psychiatry Hospitals (University Hospital Basel, University of Zürich) and schools (see also exploitation).

The third main task of WP9 was the planning and coordination of publications. An internal review and coordination process has been installed within the FemNAT-CD consortium to facilitate publications. Research units within the consortium were asked to submit research proposals to the steering committee (SC) before planning detailed research activities. Our publication guideline aims to initiate comprehensive scientific discussion of results in the consortium before a manuscript is submitted and to avoid research groups to compete on identical research topics. Based on this practice, several research papers have been developed within the consortium already before data collection was finished (see publication list). The same procedure will stay in place after the funding period. The planning of publications was in close alignment with the distribution of work packages within the consortium. Different research groups had different focuses based on their involvement in work packages. Thus, research groups could work on different aspects of the data collected, potentially increasing the variety of published findings. Within the publication process, several within-consortium feedback loops were installed, to achieve highest quality before submission.

Results of Work Package 10 (Partner: GU): Management

The management of FemNAT-CD was efficiently done by the scientific and administrational Project Office, i.e. the coordinator and her administrative assistant at Johann Wolfgang Goethe Universitaet Frankfurt am Main, and the management companies GABO:mi until June 2016 and ARTTIC since July 2016. The first main task of WP10 was to ensure an integrated and flexible management of the project and to support the participants to achieve the objectives, complete the milestones in time and deliver the deliverables. The coordinator and the respective management company closely worked together to ensure, that the consortium's contractual duties were carried out, and the respective EU regulations and their contractual and legal requirements were met. Five amendments were submitted to and granted by the EC during the course of the project. All reports were submitted in time, including the financial reporting. In addition, a close monitoring of data collection, including performance related payments, was established within the project. The Coordinator and ART were responsible for central finance control. The payments were coordinated and transferred to the participants; costs vs. budgets were monitored on an 18 months basis and on demand. All partners were supported with issuing Form Cs and obtaining audit certificates in due time.

The second main task of WP10 was to establish an effective communication between partners and beyond. The information flow in place within the FemNAT-CD project was very effective. Regular phone calls were installed for the SC (once monthly) and for the sites involved in WP1-6 (every two weeks), the sites involved in WP7 (at least once monthly). Additional task specific phone calls were organised by the involved partners and the management companies. The coordinator and the Management Office at the coordinating site were constantly informed by the management companies (e-mails, phone calls). In addition, :milliarium, a web-based project management and communication tool, has been used to remind partners of deadlines and as a central platform for important documents as well as the project's internal part of the website. Several consortium meetings were organised by GABO:mi/ARTTIC and the coordinator: yearly meetings of the General Assembly, and half-yearly meetings of the Steering Committee. At the end of the FemNAT-CD consortium activities, a public, international conference was organised (see WP9) to disseminate study results to the broad public. A professional PR strategy was set up, in order to achieve a high public awareness and to reach highest resonance possible. At the end 160 participants attended the conference. German and English press releases were prepared and distributed via SuperMailer to all contacts: Professional/private network of each partner and his/her institution (collected in advance), Press Office of partner institutions, the project consortium, the panel guests, ARTTIC press contacts. Altogether, about 1000 contacts were addressed by ARTTIC and ca. 3000

contacts through the distribution over the Press Offices of the partner institutions. A project twitter account (https://twitter.com/FemNATCD) was set up, and an effective communication network was implemented to disseminate information on the study to the broad public. The conference attracted a large audience after having been widely announced in regional and national news, incl. e.g.:FOCUS (the second largest print news magazine in Germany after DER SPIEGEL).

1.4 The potential impact

Socio-economic impact and the wider societal implications of the project

Given that conduct disorder especially in adolescent females has a severe impact on the individual, her/his family, but also their school environment, peers, and society as a whole, the topic of the FemNAT-CD consortium in itself already has a strong societal impact.

The knowledge about the presentation of CD in females, its longitudinal course over adolescence, and predictors of change is very limited. Thus, by studying the clinical and cognitive phenotype of female adolescent CD and its underlying neurobiology will ultimately help to improve diagnosis and intervention of this severe disorder. FemNAT-CD also is a project, which has studied 3 different potential interventions for female adolescent CD. No such studies have been performed to date, as the few intervention studies in the hard to motivate individuals with CD have mainly focussed on males. Especially, the pilot phase IIa-RCT which was successfully performed in collaboration with youth welfare institutions in Germany and Switzerland as well as youth detention facilities in the Netherlands is a step forward in implementing evidence based approaches within existing care models, and help to improve the course of the disorder in the often highly disadvantaged and criminalised population of female adolescents with CD. Also, results of the oxytocin challenge study have been promising with regard to the behavioural data. The study of the underlying neurobiology has already given highly interesting results, which also will lead to new ideas of effective intervention in adolescents with CD.

If results of the FemNAT-CD study will help to improve outcome of any single CD case, this is of utmost importance to European societies on the individual, social and financial level, as these “recovered” individuals will better be able to finish school, to achieve a proper education; they also will have less comorbid psychiatric disorders, such as substance abuse, PTSD or depressive disorders. In addition, “recovered” females likely will have less early pregnancies. Also, costs of detention will be far lower, and criminality rates will decrease.

The FemNAT-CD study has also published one paper on the impact of community violence on CD related behaviour in typically developing and CD adolescents (Kersten et al., 2017). Further publications are planned to delineate individual, family related and societal risk factors for CD in children and adolescents. The results of FemNAT-CD thus will support politicians in issuing preventive strategies. Many effective preventive strategies are already in place in European societies, such as pregnancy related medical support for mothers (reducing smoking, alcohol use, infectious diseases and partner violence during pregnancy, which all have detrimental effects on the mental health of the offspring). FemNAT-CD has also spread this knowledge to all relevant stakeholders working with pregnant women. In addition, education is provided for all children living in Europe, and parents can seek help to get support with parenting. Positive parenting practises and environmental enrichment especially during early childhood has been shown to be highly effective in reducing aggressive and rule-breaking behaviour in children. This may be one of the reasons, that the FemNAT-CD consortium had great difficulties to include young females with CD in the group aged 9-12. The estimated number of individuals in this age group in the application was based on US-American data. In the US, public health care and early intervention is far less available than in most European countries, and community violence is much higher than in most European Societies. Thus, results of the study strongly point towards the important role of social policies (including strict regulations regarding weapons) and the health care system on child development and child protection.

Still, the CD individuals of our study often had a history of aversive parenting, insufficient support and education by parents, early kindergarten education, and often showed strong difficulties at school. The current approach to either evict these children from school or to put them into special education or even youth detention facilities together with other aggressive children does not seem to be helpful for many adolescents with CD. FemNAT-CD has shown that puberty plays a strong role in adolescent CD in females and males. The longitudinal study also reports data on several individuals who have lost their previous diagnosis of CD after 12-24 months. FemNAT-CD will be able to add more information on the characteristics of the group who has lost their CD diagnosis. Within FemNAT-CD, the consortium

has encountered many different ways how to deal with aggressive, dissocial, and delinquent behaviour in adolescents across the different involved countries. From the preliminary data of our study, it seems that empowering approaches (in contrast to restriction and detention) are the best way to improve outcome of the disorder. In addition, the implementation of evidence based educational and psychotherapeutic approaches needs to be strongly improved, especially in the school, social welfare, and healthcare sector as well as forensic settings.

On the project level, the project has resulted in many PhD, MD, and master thesis with many physicians, psychologist, or biologist additionally employed through the funding of the study. These individuals have learned to effectively collaborate within a large European project, and will be a future network for CD related research.

Contribution to Community and social objectives

See part above. In addition, the FemNAT-CD project has resulted in many highly relevant findings, which will strongly change the knowledge about CD. This will be relevant for the individuals with CD themselves, their families and peers, teachers at school, the healthcare and social welfare sector as well as judges dealing with adolescent delinquent individuals.

With regard to phenotypic characteristics of female adolescent CD, the consortium has analysed data on psychiatric comorbid disorders, aggressive and dissocial behaviour characteristics, callous-unemotional traits, empathy and emotion regulation in females compared to males with CD. Additional studies on the behavioural phenotype are planned aiming at clarifying female specific behavioural characteristics of CD. Regarding the neurocognitive phenotype, no differences between females and males with CD were observed. In contrast, many CD individuals did not differ from controls, just a small subgroup was characterised by severely impaired cognitive abilities. Cross-sectional neurobiological studies have been completed and partly published regarding structural and functional brain imaging, neuroendocrinology and autonomic nervous system (ANS) assessment. The role of genetic, epigenetic and environmental factors in female adolescent CD is currently studied. In addition, societal aspects, such as community violence and its role on CD traits in typically developing and CD adolescents are studied. Longitudinal brain imaging, cognitive and ANS measures have been obtained in a large subsample.

Together, given the multitude of phenotypic and neurobiological measures studied cross-sectionally as well as longitudinally, FemNAT-CD has already changed the evidence regarding adolescent CD considerably. For example, female specific aspects are found for comorbid psychiatric disorders, namely a higher rate of internalising disorders, including PTSD, and a lower rate of ADHD. Also, endocrinological, cognitive and brain imaging findings were sex specific. Surprisingly, the expected basal ANS and stress hormone attenuations in CD were not observed. Additional future data analyses of the large FemNAT-CD sample will especially focus on multi-level analyses, integrating several neurobiological and cognitive measures.

A further huge success has been the completion of the randomisation of the full target sample for the START-Now RCT. The results are still pending, but the study already had a large impact on increasing knowledge about female CD in the youth welfare sector, and also has empowered staff at specific institutions to run evidence based interventions locally. Study results will allow estimating the effect size of the intervention on aggressive behaviour, but also will give many qualitative results, such as how to increase motivation to participate in interventions in females with CD.

Also, the animal model and human adult pharmaco-challenge studies have been fully completed. Data analyses and publications are still pending. However, the results of the pharmaco-challenge studies point towards a positive effect on emotion recognition in adult antisocial personality disorder (ASPD). In the aggression rat models, oxytocin also reduced aggressive behaviour in aggressive female and male models, pointing towards oxytocin as a possible innovative future intervention option in CD and ASPD.

By informing all relevant stakeholders about the study via flyers, visits, talks, public conferences etc., evidence based information on adolescent female CD has been given to all relevant stakeholders. In addition, adolescents with CD and their families received information on the condition and were offered treatment. The participants in the START NOW trial directly received a likely effective intervention, which they overall liked and enjoyed; and staff at youth welfare institutions was trained in evidence based intervention. The pending study results will be distributed to the public and will be included into evidence based clinical guidelines, such as the German AWMF-S3 guidelines or the UK NICE guidelines.

Main dissemination activities and exploitation of results

See above, report on WP9. In addition, also, see table on publications, conferences, and talks all consortium partners

have continuously provided to a broad and divers audience.

Possible exploitation:

Based on our huge physiological data base we currently work on publishing norm sample data on ANS measures. This will be of major interest for all research groups working with physiological data and might be of clinical interest. The exact details will be discussed among the members of the SC. Other norming data (such as basal endocrinological measures throughout puberty) also can be established, after having obtained additional funding for the respective saliva analysis.

A topic of fundamental importance is related to storing the huge amount of data collected within FemNAT-CD and making the source ready to be used by current and future scientists. A data storage plan (storage of all collected data at GU, parts of the dataset additionally stored locally) is in place. In addition, the SC has agreed to continue regular phone conferences; and a data sharing plan will be developed.

Based on the positive feedback from youth welfare institutions that conducted the DBT-oriented START NOW intervention the Swiss Health Promotion, responsible for national health prevention programs, further supports the implementation of START NOW in residential care facilities and additionally in schools (https://gesundheitsfoerderung.ch/). Until the end of 2019 schools and residential care institutions from the cantons Basel-city and Basel-Country will have free training and supervision in START NOW. All intervention material (workbook, manual, trainings) is provided on the webpage istartnow.ch. Both trainers and trainees can enter the START NOW portal, where they will find the material that they need to teach or exercise the START NOW skills. Further, additional online exercises, films and sounds are provided at the portal. Trainees can do self-tests and see their progress in their START NOW skills.

The results of the tryptophan loading challenge study suggest that attenuated tryptophan availability in the central nervous system may have a critical role in the modulation of the so-called Default Mode Network (DMN). The DMN is a network in the human brain is related to different aspects of emotion regulation. Integrating the findings from previous publications (Biskup et al., 2016) and the analyses of the present investigation (manuscript in preparation), it is suggested that loading and depletion of tryptophan in healthy adolescents may impact different brain areas with regard to the functional connectivity of the DMN as a network involved in emotion regulation. This is a finding that, once replicated, could be of interest for relevant industry partners. These aspects will be presented at the 15th Conference of the International Society for Tryptophan Research (ISTRY, www.istry.org) in Kyoto, Japan (September 18th - 21st 2018, www.istry2018.com). The meeting will allow discussing the implications of the above-mentioned findings with representatives of the neuropharmacological industry focusing on various aspects of tryptophan in clinical contexts.

Results from the oxytocin challenge study as well as the endocrinological studies also indicate hormones and neuro-peptides as interesting targets for future pharmaceutical potential. As study findings need to be replicated, contact with the pharmaceutical industry has not yet been established.

Outlook and future research

The consortium has applied for an European Training Network (ETN) within the Marie-Sklodowska-Curie-Actions of the EU (January 2018), named iCONNECT (proposal number 813333; SEP210389017; coordinator Christine M. Freitag). The main aim of this ETN is to translate basic science findings on adolescent CD into the clinical and social welfare setting with the aim of improving the current standard of diagnosis and care. This will be done based on innovative multi-level analyses of FemNAT-CD data, but also within additional projects testing new interventions which were developed based on the results of FemNAT-CD. To effectively translate basic science findings into clinical care and the youth welfare sector, Europe is in need of a highly motivated workforce who knows the needs of patients and parents, all relevant stakeholders, clinicians, the healthcare and social welfare sector, who can employ a range of clinical and scientific methods in interdisciplinary teams, and who knows about the industrial sector. One of the most under-researched mental disorders with an increasing prevalence in adolescence (>5% of the population) is Conduct Disorder (CD), especially in females CD is characterized by aggressive and antisocial behavior, and a high rate of delinquency, having a highly negative impact on the affected individual as well as their families, teachers, and society. The overall aim of the current iCONNECT European Training Network is to educate the next generation of early stage researchers (ESRs) to do innovative, interdisciplinary, and integrated multi-method research on female adolescent CD to (1) gain a better understanding of this severe, heterogeneous mental disorder; to (2) identify and characterise clinically relevant homogeneous subtypes based on their underlying neurobiology and risk factors; and to (3) translate these findings into improved diagnostic and therapeutic approaches as well as service models for female adolescents with CD. The highly successful FP7 EU-project FemNAT-CD has generated a very large, multi-modal dataset on female

and male adolescents with CD and matched controls, which will be a strong basis of iCONNECT. A *broad range of experts* from diverse academic fields, parent organisations, social welfare institutions, schools, and industry will bring specific theoretical and methodological approaches to create a unique training program that goes strongly beyond the current state of the art. iCONNECT beneficiaries/partners have successfully collaborated for several years within other EU projects and ETNs. All supervisors have long-standing experience in establishing research projects, providing excellent training, and supervising and mentoring ESRs (PhD students) successfully.

In addition, all partners are currently planning to obtain local funding, which at least will allow to analyse some of the rich FemNAT-CD dataset. However, for the consortium to maintain their highly effective work, a successful joint EU project will be most helpful.

All PIs of FemNAT-CD will go proceed with their scientific as well as clinical work with children and adolescents with CD and will train future clinicians as well as scientists about the necessary diagnostic procedures, evidence based intervention and scientifically based aetiological models which comprise genetic, epigenetic as well as environmental risk factors, which include biological, familial, school, peer group related as well as societal factors which are often understudied with regard to CD.

Section 2 – Use and dissemination of foreground

Please see PARTICIPANT PORTAL.

Section 3 – Report on societal implications

Please see PARTICIPANT PORTAL.