FAMILY

"Running in the FAMILY – Understanding and predicting the intergenerational transmission of mental illness"

Grant Agreement number: 101057529

D1.1 1st report about feedback from SEAB

Workpackage:	WP 1
Task:	Task 1.4
Due Date:	30st June 2023 (M9)
Actual Submission Date:	31st October 2023 (M13)
Project Dates:	Project Start Date: October 01, 2022 Project Duration: 60 months
Responsible partner:	Partner 01 EMC Neeltje van Haren
Responsible author:	Neeltje van Haren
Email:	n.vanharen@erasmusmc.nl
Contributors:	Martien Kas, Ghislaine van Thiel, Ali Jawaid

Project funded by the European Commission within HORIZON-HLTH-2021-STAYHLTH-01-02: Towards a molecular and neurobiological understanding of mental health and mental illness for the benefit of citizens and patients'		
Dissemination Level		
PU	Public — fully open (automatically posted online)	X
SEN	Sensitive — limited under the conditions of the Grant Agreement	

Document History:

Version	Date	Changes	From	Review
V0.1	14.3.	Deliverable template	Juliane Dittrich	Neeltje van Haren, Lisanne van Houtum
V1	9.3.	Collection of updates	Martien Kas, Ghislaine van Thiel, Ali Jawaid	Neeltje van Haren
V2	30.10.	Final version	Juliane Dittrich	Neeltje van Haren, Lisanne van Houtum

SUMMARY

This document introduces shortly the composition and tasks of our Scientific and Ethical Advisory Board (SEAB).

Main part is the feedback we received from each member, related to three relevant questions we identified within our coordination team.

TABLE OF CONTENTS

1 INTRODUCTION

1.1 Purpose and Scope

Members of the SEAB:

- Prof. **Martien Kas,** University of Groningen, The Netherlands
- Associate Prof. **Ghislaine van Thiel**, University Medical Centre Utrecht, The Netherlands
- Dr. **Ali Jawaid,** EMBL-Nencki Center of Excellence for Neuronal Plasticity and Brain Disorders (Braincity), Warsaw, Poland

Role and tasks according to our DoA
Contributions to:

- D1.1 – 1st report about feedback from SEAB
- D1.2 – 2nd report about feedback from SEAB
- D1.3 – 3rd report about feedback from SEAB

In WP1, Task 1.4 mentions the SEAB: For key results, additional advice will be sought from the Scientific and Ethical Advisory Board.

And in part B:
Compliance with ethical principles and relevant legislations
All consortium members and researchers in FAMILY are committed to the highest research ethics and integrity standards and will conform to the applicable international and EU law, Horizon Europe standards, and to national law in the countries where the project will be carried out. Consortium members will constantly seek advice from ethics experts involved in the consortium, external ethics experts (in the Scientific and Ethical Advisory Board [SEAB]), specialised ethics departments at their institutions and national ethics bodies, compliance managers, research ethics committees and data protection officers (DPOs). WP8 will have the responsibility to coordinate handling previously identified, as well as any new ethical issues arising from the project.

Feedback report from SEAB
For the deliverable report, we identified three relevant questions that we shared with the SEABs:

- Do you have any suggestions or recommendations related to our scientific approach?
- Do you have any questions related to the project's progress?
- Do you have any concerns, questions or recommendations related to ethical issues (human and animal studies, AI – machine learning)?

We shared their feedback with the FAMILY consortium. These questions will be discussed on a regular basis within the project duration.

1.2 References to other FAMILY Documents

- FAMILY DoA
- Data Management Plan (DMP), deliverable D2.1 1st version of data management, harmonisation, and open science plan

2 FEEDBACK FROM THE SEAB MEMBERS

2.1 Martien Kas

Do you have any suggestions or recommendations related to our scientific approach?

1. How to define mental illness in animals? Keep in mind that the current behavioural testing battery is limited. Have you considered studying social functioning as a general measure for mental illness?

Yes, we have considered social functioning and we do have data showing that social recognition is impaired in exposed animals and their offspring. This was published in Franklin et al. (2011).
In Rotterdam we have a full behavioural battery up and running for different neurological and psychiatric disorders (see Sonzogni et al. (2018) and Rigter et al. (2023)), which we intent to test in our mouse models, including social functioning.

2. Are both male and female animals from the offspring being studied for all outcome measures? Currently, I have seen data on body weight and the elevated plus maze.

Yes, both females and males are being tested in the offspring on most tasks. A full behavioral, metabolic and physiological phenotyping is however not done systematically on each cohort since the results of the phenotyping have been published along the years e.g. van Steenwyk et al., 2018; Franklin et al., 2010; Weiss et al., 2011; Razoux et al., 2017.
Further, due to new restrictions for animal experimentation in Switzerland, it is no longer possible to conduct more than 3 procedures on any given animal, which considerably limits the measurable outcomes. To validate the phenotypes on current cohorts, we classically use a minimal battery based on the weight measure, elevated plus maze and glucose/insulin tolerance tests. Phenotyping of >35 cohorts since the establishment of the model confirmed consistent changes of these measures in females and males. For FAMILY, we do not test females behaviorally or metabolically before collecting oocytes and tissues to avoid any interference of the procedures with the epigenome and transcriptome and obtain clean molecular data.
Also in Rotterdam both males and females will be tested with the different behavioural assays.

References

Franklin, T. B., Russig, H., Weiss, I. C., Gräff, J., Linder, N., Michalon, A., ... & Mansuy, I. M. (2010). Epigenetic transmission of the impact of early stress across generations. *Biological psychiatry*, *68*(5), 408-415.

Franklin, T. B., Linder, N., Russig, H., Thöny, B., & Mansuy, I. M. (2011). Influence of early stress on social abilities and serotonergic functions across generations in mice. *PloS one*, *6*(7), e21842.

Razoux, F., Russig, H., Mueggler, T., Baltes, C., Dikaiou, K., Rudin, M., & Mansuy, I. M. (2017). Transgenerational disruption of functional 5-HT1AR-induced connectivity in the adult mouse brain by traumatic stress in early life. Molecular psychiatry, 22(4), 519-526.

Rigter, P. M., de Konink, C., & van Woerden, G. M. (2023). Loss of CAMK2G affects intrinsic and motor behavior but has minimal impact on cognitive behavior. *Frontiers in Neuroscience*, *16*, 1086994.

Sonzogni, M., Wallaard, I., Santos, S. S., Kingma, J., du Mee, D., van Woerden, G. M., & Elgersma, Y. (2018). A behavioral test battery for mouse models of Angelman syndrome: a powerful tool for testing drugs and novel Ube3a mutants. *Molecular autism*, *9*(1), 1-19.

van Steenwyk, G., Roszkowski, M., Manuella, F., Franklin, T. B., & Mansuy, I. M. (2018). Transgenerational inheritance of behavioral and metabolic effects of paternal exposure to traumatic stress in early postnatal life: evidence in the 4th generation. *Environmental epigenetics*, *4*(2), dvy023.

Weiss, I. C., Franklin, T. B., Vizi, S., & Mansuy, I. M. (2011). Inheritable effect of unpredictable maternal separation on behavioral responses in mice. Frontiers in behavioral neuroscience, 5, 3.

Do you have any questions related to the project's progress?

1. It was not fully clear from the presentation what work was performed at the Erasmus MC in Rotterdam; could you please provide an update, as the presented work seems to be all generated in Switzerland.

The presentation included only work performed in Zürich but no work from Rotterdam. The team in Rotterdam could not attend the meeting or prepare a presentation due to the tragic event in the city that happened the week before (September 28, 2023). Members of the team had to take over teaching duties from colleagues affected by the event and were engaged in emergency measures at EMC following the loss of one of their colleagues. The team will present at the next Steering Committee meeting in November and will share an update after this meeting with the SEAB member.

2. How to integrate the findings from the animal and the human epigenetic studies?

Several approaches are envisaged to integrate findings from animals to human epigenetic studies. The main one is to test the presence of epigenetic markers validated in mouse biofluids and/or tissues in humans. This will be done with miRNAs using signatures obtained in mouse blood, sperm and brain for instance.

3. Is there a possibility to test causality for the human epigenetic and brain findings from the FAMILY project using model systems? Back translation?

Yes, causality can be tested in cell systems by manipulating specific candidates i.e., genes or miRNA identified in the periphery and known to be relevant for brain functions, using CRISPR tools. If done in neuronal cells, we can for instance, overexpress or knockdown given candidate(s), followed by omics analyses e.g., RNA-seq. This can help clarify the molecular pathways altered by changes in the(se) candidates. This can also be done *in vivo* in specific brain areas.

4. If I seem to remember from the kick-off meeting, there is only one deliverable for this complete WP6 at M60; please keep track of internal milestones that have not been defined.

Thanks for the advice, we will keep track of progress accordingly. We have ourselves identified intermediate deliverables to do so.

Do you have any concerns, questions or recommendations related to ethical issues (human and animal studies, AI – machine learning)?

There is an important case for studying the biological mechanisms and causality in animals justifying the experiments proposed.

1. Have proper power analyses been performed prior to the experiments presented to determine sample sizes?

Yes, we did power analyses to estimate the number of mice needed for phenotyping and also considered our past experiences with the mouse model.

2. Is blinding and randomization being applied as part of the animal study design and experimentation?

Yes, all experiments are conducted by experimenters blind to treatment.

3. Implications of the outcome of animal studies (e.g., at the level of germline) for the human perspective?

At the level of the germline, one of the perspectives is to identify molecular signatures e.g. miRNAs, that can be tested in human semen. This is more difficult in female germ cells however due to the lack of accessibility of oocytes.

4. How to translate the preclinical findings to future human diagnostic procedures or putative biomarkers?

This is a big endeavour outside the scope of the FAMILY project. However, the team in Zürich is currently developing a multiplex diagnostic tool based on circulating RNA for potential diagnostic. This

is done in the context of a University Zürich Entrepreneurship grant recently won by Dr Bogdan Mateescu, group leader in the team.

2.2 Ghislaine van Thiel

Do you have any suggestions or recommendations related to our scientific approach?

This is a comprehensive approach to an important (societal) problem. Understandably, the emphasis is on unravelling the genetic and biological mechanisms involved in the transmission of mental illness. This can hopefully inform preventive and treatment strategies. If I understand it correctly, these strategies are currently limited in availability and supporting evidence. Nonetheless, there is a lot of project activity planned to communicate and disseminate the project results (also outside the scientific community). I would recommend that in these communication and dissemination activities, the tension between sharing results and the potential harm of knowledge without offering evidence-based prevention or treatment is explicitly addressed.

Thanks for your advice, we will keep this in mind for any dissemination activity. A FAMILY communication strategy will be discussed to address these concerns.

Do you have any questions related to the project's progress?

D8.1 is an overview of ethical issues related to the project, based on the available literature. It is expected in M36. Would that give the ethics researchers sufficient time left in the project to address ethical issues?

We have finalized the scoping literature review and presented the preliminary results of it during the General Assembly in Lausanne. This presentation was followed by active discussion. Very recently (October 25) we held an online consortium meeting (PARADISE) on the ethics issues, where we had in-depth internal consortium discussion on the results of the literature review, including discussions on how to address ethical issues identified. We will incorporate the suggestions from our consortium partners in our review. In parallel we are working on scientific publication on the literature review which we plan to finalize in the first months of 2024. In this way we already have started the process of addressing ethical issues and will continue it throughout the project development.

Do you have any concerns, questions or recommendations related to ethical issues (human and animal studies, AI – machine learning)?

1. WP2: The data management plan is delivered at M6. My question is how comprehensive this plan is. Does it address aspects of data governance beyond data quality, privacy and consent? For example, is a data access committee part of the structure?

Since submitting the first draft of the Data Management Plan we have drafted and reached consensus on the pre-submission review and approval procedure (now Appendix 3), as follows:

During the Project and for a period of 1 year after the end of the Project, the dissemination of own Results by one or several Parties, including but not restricted to publications and presentations, shall be governed by the procedure of the Grant Agreement Art. 26.2 subject to the following provisions.

Prior notice of any planned publication shall be given to the other Parties at least 45 calendar days before the planned submission of the manuscript to the respective journal. The main author must follow the following procedure:

1. The main authors, first and last authors, fill out the project initiation form (which can be downloaded from KEYWAYS) and send it to the Data Access Committee (as part of WP2; family@erasmusmc.nl) for a first eligibility check (e.g., is form filled out completely, overlap with other ongoing requests, check if permissions to access/use data are in place, can research questions be answered with requested data and is data available). When approved, it will be

sent to the Project Management Office (PMO). The project initiation form summarizes on 1-2 pages the proposed research, including the involved WPs, proposed authors, background, hypotheses, analyzed variables (if applicable), used methods, and key references.

2. The PMO will forward the project initiation form to the Steering Committee (where all partners are represented) who will review it within 14 days. This will guarantee that all "data owners" are able to review and approve use of their data for each new proposal.
3. Once approved by the Steering Committee, the project initiation form will be entered into the FAMILY dissemination tracker by the PMO. An email will be sent to the general assembly to inform the FAMILY community of the new proposal. At this point, any member of the consortium interested in working in this project, not included in the original project initiation form, can contact the PI of the proposal to suggest collaboration.
4. Subsequently, authors analyse the data (if applicable), write the manuscript, and send a final draft, that is approved by all authors, to the PMO. Publication plans may be adjusted to reflect progress of the study. Author list may be adjusted to reflect actual contributions of involved researchers.
5. The PMO informs the team leaders (representatives of a WP, an institution, or a cohort within FAMILY), who have officially 30 days to provide feedback on the manuscript draft.
6. Any objection to the planned publication shall be made in accordance with the Grant Agreement in writing to the Coordinator and to the Party or Parties proposing the dissemination within 7 calendar days after receipt of the notice. If no objection is made within the time limit stated above, the publication is permitted.

2. WP2: Are measures/agreements in place about communicating individual research results to participants (i.e., if actionable findings could be traced back to individual (data-)donors)? Is there a plan for dealing with actionable unsolicited findings in the MRI study?

Participants are given oral and written feedback on findings from clinical tests (psychiatric diagnosis and symptom assessments), which include information on whether a referral for treatment or further tests are indicated. A radiologist will provide a neurodiagnostic evaluation of the collected MRI scans. When the radiologist finds that medical treatment is indicated or that further tests are required, the subject and/or caregivers will be notified, and if necessary, will be helped with a referral. If the experimenter is concerned about the well-being of the participant, this is communicated to the participant and family, and advice may be sought from a clinician (either from within the team or an on-call physician). These procedures may slightly differ per data collection site.

3. WP5: The context of the study (with families involved) requires extra attention for the voluntariness of participation. Are any specific arrangements made to achieve this? Is it possible that incompetent persons are part of the potential participant population? Are there measures in place to avoid potential harms of inclusion (such as the above mentioned knowledge without access to proven intervention and/or (self-)stigmatization of participants)?

Within FAMILY, four sites collect data following local protocols and legislation.
For all sites, participation in the study is on a voluntary basis. Importantly, after being informed about the study, the participants are given time to think about this before having to decide. In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- ✓ provided past consent to be re-contacted for future studies
- ✓ give written informed consent for the current study
- ✓ mentally capacitated (no acute episode of psychosis or mania as determined by the interviewer, no custody measure and no court authorization)

Participation in each of the study modules is voluntary, and a module may be skipped by a participant without justification. Participants may withdraw at any time.

Furthermore, it is important to realise that research has shown that providing patients with limited decisional capacity due to psychotic symptoms, with extensive and thorough information regarding the research project, can compensate for the limited decisional capacity in the informed consent process (Carpenter et al., 2000). These patients can give valuable insights into the etiology of psychotic

disorders. Finally, participants with mental illness are explained that because of the observational, and non-invasive measurements, participating in this research will not interfere with treatment.

> Carpenter et al . Decisional capacity for informed consent in schizophrenia research. Arch Gen Psychiatry. 2000 Jun;57(6):533-8. doi: 10.1001/archpsyc.57.6.533.

For all sites, several other procedures are in place:

- If at any time the participant or the experimenter is uncomfortable with any aspect of the procedure or if the experimenter is uncomfortable with the reaction of the participant to any part of the presented setup, the procedure will be terminated. In the unlikely event that this is necessary, every effort will be taken to ensure that the experience for the subject is still a positive one.
- A radiologist will provide a neurodiagnostic evaluation of the collected MRI scans. When the radiologist finds that medical treatment is indicated, the subject and/or general practitioner will be notified. Radiological reports are archived, accessible for all medical doctors responsible of treating the participant at our hospitals. If the experimenter is concerned about the well-being of the participant, this is indicated to the subject and advice is asked from a psychiatrist on call. When needed, the general practitioner will be notified.

4. WP7: This WP focuses on machine learning and prediction of mental health in familial high-risk offspring. I am concerned about the fact that in this WP aspects of responsible AI, such as explainability are not mentioned. I would recommend strengthening the link between the technical work and the ethics workpackage.

We agree that interpretability of AI methods is crucial for gaining a deeper understanding of prediction models. In WP7 we specifically employ techniques (such as explicit factor models and in particular normative models) that deliver predictive markers that are directly interpretable (i.e., 'explainable') relative to normative cohorts 'by design'. Similarly, during feature extraction our approach is designed to deliver interpretable factor solutions that - through making explicit the relative dependencies in the data - are more rather than less interpretable. Indeed, we have already focused on this interpretability as a main feature of our approach relative to the more common 'black-box' AI approaches in the original proposal and strong interactions between WP7and WP8 have been designed as part of the FAMILY WP structure and formulated as part of WP8 objectives O8.1-O.8.4.

2.3 Ali Jawaid

Do you have any suggestions or recommendations related to our scientific approach?

FAMILY comprises a team of exceptionally qualified researchers with remarkable expertise in molecular biology, genomics and epigenomics, translational neuroscience, psychology, data science and bioethics. The overall project plan is comprehensive and addresses mechanistic, clinical, as well as ethical aspects of intergenerational transmission of mental illnesses with enormous societal relevance. The group has made exceptional progress over the last few months as evident by the presentations and discussions at the GA. My only recommendation is to have greater harmonization between the work packages. For instance, WP3 and WP6 could benefit from each other by 1) validating potential biomarkers identified from the mouse models in samples collected from the human cohorts, 2) integrating potential risk factors identified from the human cohorts in the mouse model. Similar cross-talks can be envisioned for WP7, WP3, and WP6, whereas pathways identified by GWAS approaches in WP7 could be explored in WP3 and WP6.

As is described in the proposal the suggested cross-talk between different WPs is essential to our work. We scheduled a separate session during the recent General Assembly to discuss translation between

animal and human findings with the exact aim as Dr. Jawaid is referring to. We have scheduled follow-up every 6 months to discuss ideas on how to do this.

Do you have any questions related to the project's progress?

WP3: Which biological pathways are potentially affected by the differentially regulated microRNAs?

We do not have much in the way of prior expectations, as there is almost no research on circulating miRNAs in very early life at a population-level (there are a lot of miRNA studies on psychiatric phenotypes, but these are generally very small, focused on specific set of a-priori selected miRNAs, based on clinical samples of adults and scattered in terms of the analyses performed, disorder investigated etc.). So we are taking more of a hypothesis-free approach with our analyses, although we would perform look-ups to examine potential overlap with previously identified miRNAs. Generally, we would be interested to hear from Ali Jawaid (SEAB)& Isabel Mansuy (leading WP6) what kind of pathways emerge from their research: we could then test to what extent these are enriched in our own dataset once we get the data/run the analyses.

WP3 and WP6: Would be interesting to have more information about any behavioral clustering (susceptibility vs. resilience) in the human and animal cohorts after adverse exposures.
We are currently evaluating the existing human cohorts for data about stressor exposure and stress-affected mental health outcomes, in order to calculate resilient vs. non-resilient outcomes in the individuals. This work will focus on cohorts where also biological information with potential predictive or explanatory value for resilient outcomes (e.g., brain data, microbiome, proteome, epigenome) is available. Subsequently, these findings can be integrated with animal work.

Do you have any concerns, questions or recommendations related to ethical issues (human and animal studies, AI – machine learning)?

Dr. van Thiel and Prof. Kas have comprehensively addressed this issue and I have no additional questions or remarks.

3 CONCLUSION

Several suggestions and recommendations regarding the scientific approach, the project's progress, and concerns, questions and recommendations related to ethical issues (human and animal studies, AI – machine learning) are made by the SEAB and have been adequately addressed by the consortium members, as confirmed by the SEAB members.

ACKNOWLEDGEMENT

Funded by the European Union, the Swiss State Secretariat for Education, Research and Innovation (SERI) and the UK Research and Innovation (UKRI) under the UK government's Horizon Europe funding guarantee'. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union, or the European Research Executive Agency (REA), the SERI or the UKRI. Neither the European Union nor the granting authorities can be held responsible for them.