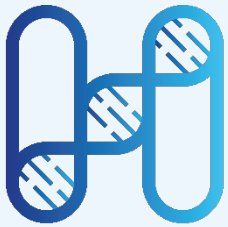

HEREDITARY

HetERogeneous sEmantic Data integratIon for the guT-bRain interplaY

Deliverable 2.20

RUMC clinical studies documentation

This project has received funding from the European Union's Horizon Europe research and innovation programme under grant agreement No GA 101137074. Views and opinions expressed are however those of the author(s) only and do not necessarily reflect those of the European Union. Neither the European Union nor the granting authority can be held responsible for them.

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EXECUTIVE SUMMARY

This deliverable provides comprehensive documentation on the regulatory, ethical, and legal approvals required for the clinical studies conducted by RUMC as part of the HEREDITARY project. It details the procedures followed to secure the necessary approvals, ensuring full compliance with relevant regulatory bodies, ethical standards, and data protection laws.

The document covers ethical approvals, regulatory compliance, and data access associated with the clinical Use Cases led by RUMC under the HEREDITARY project. Data from four previous studies will be included in the HEREDITARY project that obtained previous authority from the regulating ethical authority depending on the Wet Medisch-Wetenschappelijk Onderzoek (WMO[1]) (Table 1). RUMC is committed to the FAIR principles of data management[2]. We strive to adhere to those whenever possible.

Table 1 Data from previous projects included in HEREDITARY

Previous project	Cases	Data types	Data privacy	Ethical authority
EXAMODE	**10000**	Histopathology images	Pseudonymized	CMO RUMC (non-WMO)
BaCo-study	**120**	Histopathology images Gut&Tissue microbiome Clinical data	Pseudonymized	CMO Arnhem/Nijmegen (WMO)
MIND-set	**200**	fMRI images Gut microbiome Subject related data collected in questionnaires	Pseudonymized	CMO Arnhem/Nijmegen (WMO)
HBS	**750**		Pseudonymized	CMO RUMC (non-WMO)

The results of this project are relevant to understand histopathological-microbe interactions at mucosal sites and distant sites such as brain diseases. Linking multiple modalities will help to understand the complex relationship between pathology, the microbiome and the brain creating new insights in biology. In addition, the linkage of the data in these Use Cases within HEREDITARY serves as an example for federated learning. For all RUMC datasets in question, there is a minimal risk of incidental findings by the analyses we will perform. Although the risk for incidental findings is low, due to the type of analysis performed, the policy provides clear guidelines on how to manage such findings if they occur.

Overall, this deliverable demonstrates HEREDITARY's commitment to ethical research practices and the protection of data subjects in the design of RUMC Use Cases 4 and 5 of linking gut and brain and extracting clinical value from this linkage. By aligning with GDPR, upholding rigorous ethical standards, and implementing comprehensive data protection measures, the project ensures that its research contributes to scientific knowledge while respecting the privacy and autonomy of participants.

[1] https://wetten.overheid.nl/BWBR0009408/2025-01-01#Paragraaf1
[2] https://www.radboudumc.nl/en/research/technology-centers/data-stewardship

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1.0	25.06.2025	Anna Romanovych	Final formatting of the deliverable

Contents

List of Tables

List of Abbreviations

Abbreviation	Original version
ADHD	Attention deficit hyperactivity disorder
BMI	Body Mass Index
CMO	Centrale Commissie Mensgebonden Onderzoek (Central Committee Human Research)
DTA	Data transfer agreement
DTI	Diffusion Tensor Imaging
EGA	European Genome-Phenome Archive
ENA	European Nucleotide Archive
fMRI	Functional Magnetic Resonance Imaging
GBA	Gut-Brain Axis
GDPR	General Data Protection Regulation
HBS	Healthy Brain Study
IBD	Inflammatory bowel disease
OSF	Open Science Framework
PEP	Polymorphic Encryption and Pseudonymisation
PD	Parkinson's disease
RUMC	Radboud University Medical Center
SSCAI	Simple Clinical Colitis Activity Index
SOP	Standard Operating Procedure
WGBO	Wet op de Geneeskundige Behandelovereenkomst (Dutch Medical Treatment Contract Act)
WMO	Wet Medisch-Wetenschappelijk Onderzoek (medical research involving human subjects act)
UC	Ulcerative Colitis

1 Introduction

This deliverable provides the legal context and documentation for the gut microbiome related Use Cases within the HEREDITARY project, specifically **Use Case 4** (Phenotyping of the gut-brain axis in healthy individuals to understand deviations in disorders) and **Use Case 5** (Gut-Brain linkage and disease relevance). These Use Cases are at the core of the HEREDITARY project, which aims to enhance the understanding of the gut-brain axis and deviations from a healthy gut brain axis. Potential influential factors from the gut microbiome in neurological, stress-related and neurodevelopmental disorders such as PD, ADHD and anxiety disorders are investigated using multi-modal data integration.

The HEREDITARY project addresses the limited understanding of the interactions in the gut-brain axis and makes use of different RUMC cohorts to capture the biological heterogeneity in healthy subjects (Use Case 4) using multi-modal data integration - including microbiome, fMRI, health related data (e.g., food intake, BMI and stress), clinical data (e.g., disease indexes and severity) and histopathological data. The approach used in Use Case 4 will then be applied to several existing samples of clinical disorders in Use Case 5 such as IBD, neurodevelopmental disorders such as ADHD, and stress related disorders such as anxiety. By integrating data, the project seeks to refine factors in the gut microbiome that are important for health and/or gut-brain related diseases which may indicate relevance of specific probiotic supplements for patients with specific symptoms.

- **Use Case 4: Phenotyping of the gut-brain axis in healthy individuals to understand deviations in disorders**. The Use Case focuses on the relationship between gut microbiome alterations, environmental and other health related data in a population sample as reference. It aims to detect variations in gut-brain linkage in healthy individuals to establish deviations from a healthy gut-brain axis.
- **Use Case 5: Gut-Brain linkage and disease relevance.** This Use Case aims to establish deviations from a healthy gut-brain axis in several gut and brain related disease backgrounds such as gut related conditions (IBD, spirochaetosis) and brain-related conditions (neurodevelopmental and stress-related disorders).

1.1 Legal and ethical framework

Given the sensitive nature of the data involved and the scale of the project, it is crucial to follow the legal and ethical framework to ensure compliance with applicable regulations and to protect the rights of data subjects. This document outlines the **key legal considerations**, primarily focusing on the General Data Protection Regulation (GDPR)[3] and relevant national legislation, which govern the processing of personal data in scientific research within the context of these gut-brain related Use Cases.

Use Cases 4 and 5 will be conducted using data from the RUMC; therefore, this deliverable particularly focuses on adherence to Dutch and European legislation regarding data privacy and security. Specifically, (1) the Wet Medisch-wetenschappelijk Onderzoek (WMO) with human subjects valid from 01-01-2025[1]; and (2) the Code of

[3] https://gdpr-info.eu/

Conduct for Health Research version 2022[4]. The research proposal must receive a favourable opinion from the competent Ethics Committee and be submitted for prior consultation with the Privacy Authority in accordance with the Dutch law[1,4] and the GDPR[3]. For research with human subjects at RUMC the authority providing approval is the Central committee for research with human subjects (CMO) RUMC that examines the research protocol against Dutch and European law and attests whether the interests of participants are protected. The WMO[1] only applies to medical-scientific research in which people are subjected to (medical) procedures or rules of conduct are imposed on them. When the WMO applies the regulatory authority providing approval is CMO Arnhem/Nijmegen. The CMO RUMC tests the research protocol and rules whether the WMO[1] applies and, if not, refers to the Code of Conduct[4] for health research in the Netherlands that provides a practical guideline for all Dutch health research considering personal data and human materials. The Code of conduct is based on the GDPR[3] and the Dutch Medical Treatment Contracts Act (WGBO)[5] and is applicable when medical research is not covered by the scope of the WMO[1].

1.2 Processing personal data

In this context, "personal data" refers to "any information relating to an identified or identifiable natural person ('data subject')." A natural person is considered identifiable if they can be identified, directly or indirectly, by reference to an identifier such as a name, identification number, location data, online identifier, or one or more factors specific to their physical, physiological, genetic, mental, economic, cultural, or social identity of that natural person (GDPR art. 4[3]). The use of personal data for scientific research in the medical, biomedical, and epidemiological fields is permitted, provided that the data subject's consent is obtained or exemptions are in place as set out in the Code of Conduct[4] article 4 (collection of new data) and article 5 (re-use of previously collected data), and GDPR art 9 (2)[3].

Processing refers to any operation or set of operations which is performed on personal data or on sets of personal data as set out in Article 4 of the GDPR[3]. When data is used or re-used in new research the Code of Conduct[4] article 9 states that the original rules apply and re-use of data should be built in the consent for follow-up studies signed by participants, which needs to be reviewed and approved by the researchers and ethical authority before starting the new study. However, consent for re-use is not required when data is fully anonymized for secondary use as described in Article 5 paragraph 1 of the Code of Conduct[4] or informing the data subjects is impossible or involves a disproportionate effort, or when it would make it impossible or seriously impair the achievement of the research purposes. More details are available in Article 5 paragraph 1 of the Code of Conduct[4].

1.3 Data minimization

The principle of data minimization is crucial, stipulating that data must be "adequate, relevant, and limited to what is necessary in relation to the purposes for which they are

[4] https://www.coreon.org/wp-content/uploads/2023/06/Code-of-Conduct-for-Health-Research-2022.pdf
[5] https://english.ccmo.nl/investigators/legal-framework-for-medical-scientific-research/laws/dutch-medical-treatment-contracts-act#:~:text=The%20Dutch%20Medical%20Treatment%20Contracts,between%20patients%20and%20care%20providers.

processed" (GDPR Art. 5(1)(c))[3]. In accordance with this principle, the processing of personal data for scientific research in the medical, biomedical, or epidemiological fields may include health-related data.

According to the principle of storage limitation, data should be "kept in a form which permits identification of data subjects for no longer than is necessary for the purposes for which the data are processed." Personal data may be stored for longer periods if they are processed solely for archiving purposes in the public interest, scientific or historical research purposes, or statistical purposes, subject to the implementation of appropriate technical and organizational measures required by this Regulation to safeguard the rights and freedoms of the data subjects (GDPR Art 5(1)(e)[3] and Chapter 8 of the Code of Conduct[4] and the declaration of Helsinki[6]).

Among the principles applicable to processing established in Article 5 of the GDPR, the principle of accountability is particularly significant. It requires that "the data controller must comply with and be able to demonstrate compliance with the principles and obligations set out in the Regulation" (GDPR Art 5(2) and Art 24[3]), and to ensure that data protection rights and regulations are safeguarded (Article 25 of the GDPR[3]). The data controller must adopt appropriate technical and organizational measures designed to effectively implement data protection principles and integrate the necessary safeguards into the processing to comply with data protection regulations and protect the rights and freedoms of the data subjects.

1.4 Outline of HEREDITARY Use Cases

In the following chapters, we will provide a detailed examination of how the gut-brain axis Use Cases within the HEREDITARY project are designed and implemented to meet the ethical and legal requirements outlined in this chapter. We will explore the specific measures taken to ensure compliance with GDPR and Dutch law. Furthermore, we will illustrate how these Use Cases adhere to the ethical standards necessary for the responsible processing of personal data, ensuring the protection of the rights and freedoms of the data subjects involved in these crucial research activities.

2 Data to be included in the study

As outlined in the description of the gut-brain axis Use Cases 4 and 5 (see deliverable D2.3[7] and D2.4) the HEREDITARY project will re-use (See also Table 2 on page 14):

- Data collected from previous EU-funded **EXAMODE** project in 2018 by RUMC department of Pathology, and which has received prior waiver for compliance to WMO from the CMO RUMC (reference number CMO 2018-4764).
- Data collected from the NWO funded **BaCo-study** collected by RUMC department of Pathology 2016-2018, and which has received prior approval from the CMO Arnhem/Nijmegen following regulations within WMO (reference number CMO 2016-2616).

[6] WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Participants – WMA – The World Medical Association

[7] https://zenodo.org/records/14627764

- Data collected from the **MIND-set** study executed by the Donders Institute for Brain, Cognition and Behaviour and the department of Psychiatry from RUMC in 2015, and which has received prior approval from the CMO Arnhem/Nijmegen following regulations within WMO (reference number CMO 2015-1972).
- Data collected from **Healthy Brain Study** (HBS), a longitudinal cohort study, carried out by Radboud University, RUMC, and the Max Planck Institute for Psycholinguistics in 2018. The Institutional Review Board of RUMC waived compliance to WMO for the HBS (reference number CMO 2018–4894)

For HEREDITARY a request to re-use data and generate new data from these cohorts has been submitted and approved by the CMO with reference number CMO 2024-17669 on 20-11-2024. In addition, for Use Case 5 additional pathology data regarding spirochaetosis have been collected, similar to EXAMODE and approved under CMO 2024-17669 on 20-11-2024.

2.1 Summary of data to be used in Use Cases 4 and 5

The study population will include all subjects who gave consent for re-use of the collected data which includes all participants from HBS (pseudonymized data). Cases included in EXAMODE (pseudonymized data) and HEREDITARY that opted in for re-use of body materials at the time of collection of data for the EXAMODE project have been included in HEREDITARY. Subjects that have given previous informed consent for re-use of their data from the BaCo-study and MIND-set (both pseudonymized data) are included. For each dataset, the inclusion, exclusion and type of data included in HEREDITARY are described in their respective sections below.

2.1.1 EXAMODE

The original objective of the EXAMODE project was to allow easy and fast, weakly supervised knowledge discovery of exa-scale heterogeneous data in the field of digital pathology [1]. In this project, archived paraffin embedded tissue sections and blocks from the RUMC pathology archive and their corresponding pathology reports were collected including colorectal, lung and uterine cervix cancer cases, as well as duodenal biopsies for celiac disease diagnosis between 2000 and 2021. Patients who did not authorize the use of their data for scientific research (i.e., they decided to opt-out) were not included in EXAMODE. Complete pseudonymization (de-identification) of images and pathology reports was carried out by the data manager of the computational pathology group of RUMC. The de-identified histopathology slides (10000 cases) are re-used in HEREDITARY.

Inclusion criteria

- Digital pathology images of colon biopsies and polyps from the EXAMODE dataset

Exclusion criteria

- Diagnosis of hyperplasia, dysplasia (low- and high-grade) or neoplasia

List of data to be included in HEREDITARY

Clinical data

- Histopathological data
    - cases with normal histopathology

- Histological subtype

2.1.2 BaCo-study

The original objective of the BaCo-study was to identify pathogenic bacteria or bacterial traits within biofilms in patients with ulcerative colitis (UC), an inflammatory bowel condition, which are predictive of neoplasia in a prospective cohort study compared to controls [2]. These bacteria and bacterial traits are distinctive among UC-patients without neoplasia in comparison with control patients (120 subjects total). Furthermore, the expression of known oncotraits was detected in tissues with bacterial biofilms and histopathological and immunohistochemistry images were evaluated for precancerous changes such as hyperplasia, increased cell proliferation or dysplasia. The histopathology images of the colon biopsies and the microbiome data from both biopsies and faecal samples are re-used for HEREDITARY for multi-modal assessment of histopathology and microbiome data.

Inclusion criteria

Inclusion criteria UC-patients:

- Left-sided colitis or pancolitis (Montreal score E2 or E3)
- > 8 years of disease duration
- At time of colonoscopic surveillance in clinical remission (Montreal score S0)

Inclusion criteria controls:

- Routine scheduled colonoscopy for iron deficiency anemia, changed stool pattern, or unexplained bowel complaints.
- Irritable bowel syndrome or status post-diverticulitis
- No abnormal findings during colonoscopy

Exclusion criteria

Exclusion criteria for UC-patients and controls:

- Oral antibiotics in last 3 months before colonoscopy (drastically affects the mucosal microbiota)

Exclusion criteria for controls only:

- History of colon cancer and UC or other inflammatory disease of the intestine

List of data to be included in HEREDITARY

Demographics and clinical data

- Age
- Sex
- BMI
- Disease activity index (Mayo-score)
- Medication
- Antibiotic use

Data obtained from biosamples

- Histopathology (H&E) from right- and left-sided colon
- Biopsy microbiome (metagenomics) from right- and left-sided colon
- Faecal microbiome (re-use of faecal material to generate 16s rRNA amplicon microbiomics)

Questionnaires

- SSCAI (standardized questionnaire for IBD)
- Stool questionnaire
- Risk factors for cancer (smoking, family history)

2.1.3 HBS

The original objective of the Healthy brain study (HBS) is to discover how the brain works and affects our daily lives [3]. The HBS monitored and tested over 750 thirty to forty-year-old volunteers from Nijmegen, Arnhem and the surrounding area. People in their thirties were selected because their brains are no longer developing, while the aging process had yet to start. Moreover, this group often has to deal with important life events that affect their well-being such as a career, a burnout, buying a house, having children or getting a divorce. The HBS is characterized by a broad range of repeated assessments, a large number of participants, both laboratory and real-world assessments, and a population-based sample. Moreover, data are managed to allow data sharing with scientists worldwide while maintaining participants' privacy[8]. With the HBS resource, the scientific community can take the next step in understanding the human brain and how it dynamically and individually operates in its bio-social context. Within HEREDITARY we make use of the HBS resource which allows pseudonymized data and biosample sharing upon request. Data are accessible for the wide research community and pseudonymized with unique IDs per researcher using Polymorphic Encryption and Pseudonymisation (PEP)[9].

Inclusion criteria

- Age 30–39 years
- Living in the Nijmegen region (<15km)
- Willingness and ability to follow the study protocol.

On top of this, a special effort was made to create a group of participants that would accurately represent the average of the Nijmegen region. For example, nearing the end of data collection more emphasis was placed on recruiting citizens of low and middle level of education, as those were underrepresented compared to the average of the Nijmegen region at that time.

Exclusion criteria

- Not speaking, reading, and/or understanding the Dutch language (minimum B1 level)
- a prior history of significant psychiatric or neurological illness (self-report)
- a current disease that affects the brain
- a current medication that is therapeutically targeted at the brain (e.g., antidepressants, methylphenidate)

[8] https://osf.io/jzwrg/
[9] https://pep.cs.ru.nl/

- pregnancy
- contra-indication for MRI (metal or devices in the upper body (cardiac pacemaker, cochlear implant, aneurism clip))
- previous brain surgery
- moderate to severe claustrophobia
- contra-indication for the submaximal Åstrand cycle test (current use of beta-blockers, a current disease that hinders physical exercise)
- contra-indication for the cold pressor test (Raynaud's phenomenon, chronic pain syndrome in shoulder or arm, open wounds on arm or hand, scleroderma, arteriovenous fistula or shunt, presence of (unstable) angina pectoris).

List of data to be included in HEREDITARY

Demographics and clinical data

- age
- sex
- handedness
- education
- income
- occupation
- menstrual cycle
- smoking history
- alcohol use
- food frequency
- sedentary behaviour
- sleep quality index
- satisfaction with life scale
- cantril ladder

Physiological assessments

- body composition

Biosamples

- faecal gut metabolomics (re-use of faecal samples to generate metabolomics)
- faecal microbiome (re-use of faecal samples to generate 16s rRNA amplicon sequencing data)
- salivary cortisol levels
- blood cell count and white blood cell differential
- blood proteomics
- blood metabolomics
- hair cortisol levels

Neuroimaging data

- fMRI
- gray/white matter

Questionnaires

- stool questionnaire
- over-the-counter medication
- depression questionnaire

- anxiety questionnaire
- stress questionnaires
- burnout questionnaire
- life events
- COVID questionnaires

2.1.4 MIND-set

The original objective of MIND-set was to investigate psychiatric disorders, both neurodevelopmental disorders and stress-related disorders [4]. The disorders were compared in commonalities and dissimilarities in biological factors in biosamples (blood markers and faecal markers, hair cortisol), neuropsychological factors (computer tasks and questionnaires on mental wellbeing) and imaging data (MRI). Within HEREDITARY we will re-use multi-modal data from MIND-set to capture changes in the gut-brain axis in Use Case 5. The data in the MIND-set study have been pseudonymized.

Inclusion criteria

- Age >17 in- and out-patients at the department of Psychiatry (RUMC)
- Stress-related disorder (mood, anxiety and addiction disorders, comorbid somatic disorders)
- Neurodevelopmental disorders (autism spectrum disorders, ADHD)
- Group of control patients without a lifetime history of psychiatric disorders

Exclusion criteria

- Full scale IQ estimate <70
- Sensorimotor handicaps
- Current psychosis
- Inadequate command of the Dutch language
- Mentally incompetent to sign informed consent

Exclusion criteria specifically for the MRI section of the MIND-Set study:

- Metal objects in the body (excluding dental fillings)
- Jewellery or piercings that cannot be removed
- Brain surgery
- Epilepsy
- Claustrophobia
- Pregnancy
- Ferromagnetic implants or pacemakers
- Inability to lie still for more than one hour

Additional exclusion criteria for controls:

- Neurological disorders of the central nervous system
- Fifteen or more units of alcohol per week
- Recreational drug use of once or more per week
- Benzodiazepine use of once or more per week
- Malignancies
- Rare, chronic somatic disorders, for which matching will not be possible
- First degree relatives with severe psychiatric disorders

List of data to be included in HEREDITARY

Demographics and Clinical data

- Age
- Sex
- Medication
- Food intake
- BMI
- Smoking
- Somatic status

Biosamples

- Hair cortisol
- Faecal microbiome (16s rRNA amplicon sequencing data)

Neuroimaging data

- fMRI scans
- Diffusion Tensor Imaging (DTI)

Questionnaires

- ADHD rating scale (CAARS)
- Autism-spectrum Quotient (AQ-50)
- Depressive symptoms (IDS-SR)
- Anxiety sensitivity index (ASI)

3 Ethical conduct of the study

To ensure the quality and integrity of research, all Clinical Use Cases led by RUMC will be conducted under the Declaration of Helsinki[6] and its amendments, the code of conduct for Health Research 2022[4] and any applicable national regulations and guidelines[1] as well as the GDPR[3].

3.1 Ethical approvals

The HEREDITARY project plans to use data and biosamples that have been collected in previous studies for gut-brain axis Use Cases (see section 2). The gut-brain axis Use Cases 4 and 5 within the HEREDITARY project plan to analyze multi-modal pseudonymized data from EXAMODE, HBS, BaCo-study and MIND-set. Below is a brief justification for why a waiver of specific informed consent is warranted within the scope of HEREDITARY and which has been approved by CMO RUMC (CMO 2024-17669) – (see Annex 1-5):

- No data directly identifying the patient (e.g., name, initials, institutional identification numbers, medical record number, full date of birth, full date of exams or evaluations, etc.) will be collected or transferred.
- The data in all data-sets are pseudonymized and cannot be traced back to individual subjects by researchers in HEREDITARY. The pseudonymization keys of the data are archived according to data management procedures within RUMC and not directly accessible to researchers within HEREDITARY.
- Patients and controls within WMO-studies BaCo and MIND-set gave specific informed consent for re-use of data and biosamples within the informed consent.

Participants who did not agree for re-use of the data have been removed from the data set. Participants of HBS gave informed consent for usage of their data for researchers through an encrypted system (PEP). Cases included in EXAMODE gave consent for re-use of their data at hospital admission.

- There is a minimal risk of unexpected findings (see chapter 5) since we are only processing biosamples for faecal microbiome and metabolites which will not impact clinical diagnosis or outcomes for patients in these studies. Within HBS fMRI images are being studied which have a minimal risk for incidental findings for which a standard operating procedure is in place (see chapter 5).

3.1.1 Ethical approval documentation

All data collected within the HEREDITARY project have received the necessary ethical approvals for research purposes, in compliance with GDPR[1] and relevant ethical standards. Ethical oversight for HEREDITARY has been provided by the local RUMC ethics committee or the CMO Arnhem/Nijmegen as explained in section 1, ensuring that all procedures for data collection, processing, and sharing comply with established guidelines and regulations. Specifically, data processed in HEREDITARY were collected under the following projects approved by RUMC and CMO Arnhem/Nijmegen ethics committee (Table 2).

Table 2 Ethical approval of projects included in Use Cases 4-5

Project	Protocol Number	Date of Approval	Annex
EXAMODE	CMO 2018-4764	02-04-2019	1
BaCo-study	CMO 2016-2616	12-09-2016	2
MIND-set	CMO 2015-1972	10-02-2016	3
HBS	CMO 2018-4894	09-04-2019	4
HEREDITARY	CMO 2024-17669	20-11-2024	5

These waivers and approvals (attached as Annexes 1-5) confirm that all data collection processes meet the ethical requirements for protecting the rights and well-being of the data subjects involved. Furthermore, to promote transparency and advance scientific research, microbiome and clinical data collected through previous projects have been or will be made accessible upon request. These datasets are available on designated platforms (e.g. ENA for BaCo (ENA Browser PRJE87602) and will be uploaded for FAIRification to EGA before the end of the HEREDITARY project. HBS data can be requested and will be provided in anonymous form to the data requestor: How to get access? - Healthy Brain Study. This means that the data are provided with renamed subject IDs and the key is destroyed. De-identified EXAMODE histopathology images will be made available through BIGPICTURE: A central repository of digital pathology slides to boost the development of artificial intelligence | Bigpicture), providing researchers and stakeholders with the opportunity to utilize the data while ensuring compliance with data protection regulations. MIND-set data are at the moment only available on request from the study coordinator at the department of Psychiatry of the RUMC.

4 Data Transfer Agreements

A Data Transfer Agreement (DTA) for HBS data is in place for RUMC. The DTA is based on the data transfer agreement which is publicly available in Open Science framework (OSF)[10]. The DTA allows access and processing of data listed in 2.1.3 at the premises of RUMC. Sharing with third parties is not allowed in this DTA, and to share it with the HEREDITARY partners another DTA would have to be signed through the website of HBS (see section 2.1.3). For the other datasets a DTA is not necessary as the data will not be directly shared with other partners within HEREDITARY, but made available through the designated platforms (see section 3.1.1).

5 Incidental Findings Policy

When conducting research involving human participants, it is crucial to anticipate the potential discovery of incidental findings and establish a procedure in advance for handling such situations. This preparation includes obtaining consent from participants and clarifying how any incidental findings may be managed (e.g., confidentiality, communication to research participants). RUMC follows an Opt-in procedure for re-use of clinical data and patient materials which has been approved and explained to all patients upon hospital admission[11]. For pseudonymized data, it remains important to outline the theoretical approach to dealing with these findings. More information about re-use of biomaterials and clinical data for patients of the RUMC is explained on the website, including incidental findings policy of research conducted at RUMC[12]. The study participants in MIND-set and BaCo have been informed about incidental findings in the initial studies performed according to WMO[1]. Healthy participants undergoing fMRI for the HBS at the Donders institute were informed in the initial informed consent and a standard operating procedure for incidental findings during fMRI processing is present[13]. Incidental findings are unexpected results that arise during research but are unrelated to the study's primary objectives. We do not expect any incidental findings from biosample re-use within HEREDITARY. The only data that is being generated with biosample re-use within HEREDITARY is gut microbiome sequencing and metabolome data from faecal samples. This newly generated microbiome data is very unlikely to result in clinically relevant results that may lead to incidental findings for participants in HEREDITARY. In the unlikely case that incidental findings do occur the respective authority (CMO RUMC) is consulted and if necessary, the medical doctor involved is consulted via the data controller (access to the archived de-identification key) of each respective study.

[10] https://osf.io/4uktr
[11] https://www.radboudumc.nl/en/research/technology-centers/radboud-biobank/news/patient-must-give-explicit-consent
[12] https://www.radboudumc.nl/en/patientenzorg/uw-afspraak/meer-informatie/patient-in-een-umc/use-medical-data-and-biomaterial
[13] https://elsi.health-ri.nl/sites/elsi/files/2020-09/Donders%20beleid%20Incidental_Findings_SOP_DCCN_version%201.7.pdf

6 Conclusion

This deliverable outlines the comprehensive approach taken by the HEREDITARY consortium to ensure that all data processing activities related to Use Cases 4 and 5 comply with the stringent legal and ethical standards set by the GDPR and relevant EU and national legislation. We have provided detailed information on the gut-brain axis Use Cases 4 and 5 adhere to ethical and legal requirements, provided the specific ethical approvals obtained, and the data protection measures implemented to safeguard the rights and freedoms of data subjects. By aligning with GDPR principles—including the lawful basis for processing, transparency and data minimization—the HEREDITARY project is committed to maintaining the highest ethical standards in research. The project's ethical framework, as applied to Use Cases 4 and 5, further underscores the integrity and responsibility that underpin all research activities within HEREDITARY.

References

The bibliographic entries are arranged in lexicographical order based on the key, following the APA style. This enables us to place the entries and citations in the table and text in any sequence, allowing for later sorting while ensuring consistency.

Key	Reference
[1]	Menotti, L., Silvello, G., Atzori, M., Boytcheva, S., Ciompi, F., Di Nunzio, G. M., Fraggetta, F., Giachelle, F., Irrera, O., Marchesin, S., Marini, N., Müller, H., & Primov, T. (2023). Modelling digital health data: The ExaMode ontology for computational pathology. Journal of Pathology Informatics, 14, 100332. https://doi.org/https://doi.org/10.1016/j.jpi.2023.100332
[2]	Bruggeling, C. E., Garza, D. R., Achouiti, S., Mes, W., Dutilh, B. E., & Boleij, A. (2021). Optimized bacterial DNA isolation method for microbiome analysis of human tissues. Microbiologyopen, 10(3), e1191. https://doi.org/10.1002/mbo3.1191
[3]	Healthy Brain Study, c., Aarts, E., Akkerman, A., Altgassen, M., Bartels, R., Beckers, B., Bevelander, K., Bijleveld, E., Davidson, E. B., Boleij, A., Bralten, J., Cillessen, T., Claassen, J., Cools, R., Cornelissen, I., Dresler, M., Eijsvogels, T., Faber, M., Fernandez, G.,…Willemsen, A. (2021). Protocol of the Healthy Brain Study: An accessible resource for understanding the human brain and how it dynamically and individually operates in its bio-social context. PLoS One, 16(12), e0260952. https://doi.org/10.1371/journal.pone.0260952
[4]	van Eijndhoven, P., Collard, R., Vrijsen, J., Geurts, D. E. M., Vasquez, A. A., Schellekens, A., van den Munckhof, E., Brolsma, S., Duyser, F., Bergman, A., van Oort, J., Tendolkar, I., & Schene, A. (2022). Measuring Integrated Novel Dimensions in Neurodevelopmental and Stress-Related Mental Disorders (MIND-SET): Protocol for a Cross-sectional Comorbidity Study From a Research Domain Criteria Perspective. JMIRx Med, 3(1), e31269. https://doi.org/10.2196/31269

Annexes

Annex	Name
1	Ethical waiver of the EXAMODE study
2	Ethical approval of the BaCo-study
3	Ethical approval of the MIND-set study
4	Ethical waiver of the HBS study
5	Ethical waiver of the HEREDITARY study