

MY PLAN (FWO DMP)

INTEGRATED, MULTIMODAL PHENOTYPIC ANALYSIS OF PPP2R1A
KNOCKIN MICE: NEW IN VIVO MODELS OF SYNDROMIC PP2A-
RELATED (NEURO)DEVELOPMENTAL DISORDERS
D-2021-1079

ADMIN DETAILS

Project Name: Integrated, multimodal phenotypic analysis of *Ppp2r1a* knockin mice: new *in vivo* models of syndromic PP2A-related (neuro)developmental disorders

Principal Investigator / Researcher: Iris Verbinnen

Institution: KU Leuven

1. GENERAL INFORMATION

Name applicant

Iris Verbinnen

FWO Project Number & Title

12X4222N

Integrated, multimodal phenotypic analysis of *Ppp2r1a* knockin mice: new *in vivo* models of syndromic PP2A-related (neuro)developmental disorders.

Affiliation

- KU Leuven

2. DATA DESCRIPTION

Will you generate/collect new data and/or make use of existing data?

- Generate new data

Describe in detail the origin, type and format of the data (per dataset) and its (estimated) volume. This may be easiest in a table (see example) or as a data flow and per WP or objective of the project. If you reuse existing data, specify the source of these data. Distinguish data types (the kind of content) from data formats (the technical format).

WP1: Behavioral phenotyping of neonatal *Ppp2r1a* M180T/+ and R182W/+ knockin mice

Type of data	Format	Estimated volume	How created
Mouse models (M180T/+ and R182W/+ heterozygous mice)	sperm	50 straws (5 straws per male, 5 males in total)	Sperm cryopreservation is done by the Mouse Expertise Unit (VIB-KU Leuven)
Table with behavioral tests/milestones for each day to evaluate during the preweaning period	.xls	7 MB	Excel and table completed each day during testing (answers: yes/no; +/-; sec; degrees)

WP2: Behavioral phenotyping of adult *Ppp2r1a* M180T/+ and R182W/+ knockin mice

Type of data	Format	Estimated volume	How created
<i>24h cage activity:</i> Table of beam crossings per mouse for every 30 minutes (24h)	.xls	4 MB	Table generated by program: MouseWin
<i>Open field:</i> 1) Table with different parameters 2) ANY-maze backup files 3) ANY-maze data files 4) Track plots (movies and pictures)	1) .xls 2) .szk 3) .szd 4) movies: .mp4 and pictures: .png	1) 700 KB 2) 150 MB 3) 500 MB 4) movies: 200 MB and pictures: 900 KB	All data are generated by ANY-maze
<i>Elevated plus maze:</i> Table with different parameters	.xls	20 MB	Table is generated by EthioVision
<i>Rotarod:</i> Table with different latencies	.xls	1.2 MB	Table is made by researcher in Excel
<i>Grip strength:</i> Table with strength measurements	.xls	532 KB	Table is made by researcher in Excel
<i>Social Preference,</i>	1) .xls	1) 5 MB	All data are generated

<i>Social Novelty (SPSN):</i> 1) Table with different parameters 2) ANY-maze backup files 3) ANY-maze data files 4) ANY-maze video files	2) .szk 3) .szd 4) .szv	2) 400 MB 3) 400 MB 4) 6.5 GB	by ANY-maze
<i>Morris Water Maze:</i> 1) Tables with different parameters (acquisition, probe trials, search strategies) 2) Original files EthioVision 3) Raw data EthioVision	1) .xls 2) .evxt, .pos, .pob, .btn 3) .xls	1) 8 MB 2) 125 MB 3) 800 MB	All data are generated by EthioVision (acquisition, probe trials) and Pathfinder (search strategies)
<i>Passive avoidance:</i> Table with latencies	.xls	1.6 MB	Table is made in Excel
<i>Digigait:</i> Table with different parameters	.xls	800 KB	Table is generated by DigiGait program

WP3: *In vivo* imaging of *Ppp2r1a* M180T/+ and R182W/+ knockin mice

Type of data	Format	Estimated volume	How created
<i>CT scans (SkyScan 1278):</i> 1) Original data 2) Reconstructed data	1) .tif 2) .bmp	1) 120 GB 2) 30 GB	1) Skyscan software 2) NRecon software
<i>MRI scans (Bruker Biospin):</i> 1) Original data 2) Processed data	1) .fid 2) .ascii	1) 200 GB 2) 100 Gb	1) Biospin software 2) Paravision software

WP4: Monitoring of epileptic behavior in *Ppp2r1a* M180T/+ and R182W/+ knockin mice

Type of data	Format	Estimated volume	How created
<i>Video-EEG:</i> 1) Video 2) EEG	1) .png 2) .csv	1) 3 GB 2) 1 GB	Both (1) and (2) are created by the NeuroScore core software

WP5: Long-term and survival follow-up of *Ppp2r1a* M180T/+ and R182W/+ knockin mice

Type of data	Format	Estimated volume	How created
<i>Long term follow-up:</i> 1) Table with weight + follow-up mice 2) Immunostainings	1) .xls 2) .jpeg, .xls	1) 20 KB 2) 20 GB	1) Excel 2) Images made with microscope Pendragon AxioVert 200M, Excel
<i>DMBA/TPA skin carcinogenesis model:</i> 1) Table with weight + follow-up mice 2) Immunostainings 3) qPCR	1) .xls 2) .jpeg, .xls 3) QuantStudio Design & Analysis Software single experiment document and .xls	1) 20 KB 2) 6 GB 3) 300 MB	1) Excel 2) Images made with microscope Pendragon AxioVert 200M, Excel 3) QuantStudio software, Excel
<i>LSL-Kras G12D lung cancer model:</i> 1) Table with weight + follow-up mice 2) Immunostainings 3) qPCR	1) .xls 2) .jpeg, .xls 3) QuantStudio Design & Analysis Software single experiment document and .xls	1) 20 KB 2) 6 GB 3) 300 MB	1) Excel 2) Images made with microscope Pendragon AxioVert 200M, Excel 3) QuantStudio software, Excel

WP6: Effects of *Ppp2r1a* M180T/+ and R182W/+ variants on neuronal and synaptic biology

Type of data	Format	Estimated volume	How created
Immunostainings for several markers of neuronal differentiation	.jpeg, .xls	118 GB	Images made with microscope Pendragon AxioVert 200M, Excel
qPCR	QuantStudio Design & Analysis Software single experiment document and .xls	50 MB	QuantStudio software, Excel
Data micro-electrode arrays	to be completed (collaboration Prof. N. Nadif Kasri)	to be completed (collaboration Prof. N. Nadif Kasri)	to be completed (collaboration Prof. N. Nadif Kasri)

WP7: An integrated transcriptomic and proteomic analysis of *Ppp2r1a* M180T/+ and R182W/+ developing, embryonic brains and adult cortices

Type of data	Format	Estimated volume	How created
<i>Transcriptomics (RNA-seq):</i> 1) RawFastqFiles 2) PreProcessedFastqFiles 3) Mapping ((a) Bamfiles; (b) Reference-Genome; (c) Unmapped) 4) Summarization 5) Statistical analysis	1) .gz 2) .gz 3) (a) .bam; .bai; (b) .gtf; (c) .gz 4) .xls 5) .xls	1) 18 GB 2) 18 GB 3) (a) 12 GB; (b) 60 MB; (c) 1.2 GB 4) 25 MB 5) 70 MB	Data are provided by the Nucleomics Core Facility (VIB-KU Leuven)
<i>(Phospho-)proteomics:</i> Tables with raw and processed data	.raw .xls	20 MB	Data are provided by Sybioma (KU Leuven)

3. LEGAL AND ETHICAL ISSUES

Will you use personal data? If so, shortly describe the kind of personal data you will use. Add the reference to your file in KU Leuven's Register of Data Processing for Research and Public Service Purposes (PRET application). Be aware that registering the fact that you process personal data is a legal obligation.

- No

Privacy Registry Reference:

Short description of the kind of personal data that will be used: N.A.

Are there any ethical issues concerning the creation and/or use of the data (e.g. experiments on humans or animals, dual use)? If so, add the reference to the formal approval by the relevant ethical review committee(s)

- Yes

ECD P129/2020: Karakterisering van muismodellen voor PP2A-gerelateerde syndromen

Does your work possibly result in research data with potential for tech transfer and valorisation? Will IP restrictions be claimed for the data you created? If so, for what data and which restrictions will be asserted?

- No

Do existing 3rd party agreements restrict dissemination or exploitation of the data you (re)use? If so, to what data do they relate and what restrictions are in place?

- No

4. DOCUMENTATION AND METADATA

What documentation will be provided to enable reuse of the data collected/generated in this project?

I will provide detailed descriptions of data acquisition in paper lab notebooks, according to good laboratory practices. All data belonging to a certain work package of the project are properly linked to each other and referred to each other in the lab notebooks and detailed protocols are provided (.doc files + printed and stored in a folder). The complete list of sub-projects is summarized as a final list (.doc file); the detailed protocols are also digitally archived on the lab's dropbox, on an external hard drive and J-drive. In the notes (lab notebook), clear links are provided to (1) digital data (pictures, excel files, movies), and where to find these (Dropbox, J-drive, external hard drive etc); file names, types and dates are provided; and to (2) physical data (mice, tissue samples, new cell lines, plasmids, antibodies) and where/how they are stored. General protocols and information are also stored on the shared J-drive and printed instructions will be stored in a folder. Lists of antibodies and primers for PCR and qPCR are stored in the Dropbox folder of the lab.

The following documentation is available:

WP1: Behavioral phenotyping of neonatal *Ppp2r1a* M180T/+ and R182W/+ knockin mice

- Protocols of the breeding (.docx) and genotyping (.docx) of mice are stored and printed instructions are found in a folder. Genotypes of the mice are collected in an .xls file. Administration of the mice (genotypes, room and cage of housing) are stored on the LAIS website of the KU Leuven animal facility.

- Protocols of neonatal behavioral tests (.docx) and standardized tables (.xls) to complete for each pup are made and stored. Printed versions of protocols are also available. Completed and analyzed tables (written) are pasted in a Lab Notebook and stored on an external hard drive, dropbox and the K-drive.

WP2: Behavioral phenotyping of adult *Ppp2r1a* M180T/+ and R182W/+ knockin mice

Standard operating procedures (SOPs) of adult behavioral tests (.docx), standardized tables for rotarod, grip strength and passive avoidance measurements (.xls) and tables generated by MouseWin, ANY-maze and EthioVision software (.xls) are kept. Completed and analyzed tables (.xls) are stored on specific drives (section 5). Printed protocols are also stored in a folder.

WP3: *In vivo* imaging of *Ppp2r1a* M180T/+ and R182W/+ knockin mice

- Protocols of scanning (.docx) are stored on an external hard drive, shared J-drive and dropbox and printed and stored in a folder.

- The original scans (.tif) and reconstructed images (.bmp) are both stored on specific drives of the MOSAIC core facility (KU Leuven). Each scan is connected to a .txt file in which the parameters of scanning are described.

WP4: Monitoring of epileptic behavior of *Ppp2r1a* M180T/+ and R182W/+ knockin mice

The standardized protocol for implanting telemetry equipment is provided by Datascience International (DSI) and is stored on an external hard drive, shared J-drive and dropbox. Measurement (.csv and .png) files are also stored.

WP5: Long-term survival follow-up of *Ppp2r1a* M180T/+ and R182W/+ knockin mice

- An overview of the mice that participate in the long-term follow-up is available on the shared J-drive, as well as the follow-up of the weight and well-being of these mice. At the end of the experiment, the .xls file will be saved on the K-drive.

- Protocols (.docx) related to the DMBA/TPA skin carcinogenesis protocols and to the LSL-Kras G12D lung cancer model (model, immunostainings, qPCR) are stored on specific drives. For each protocol, the necessary antibodies (stainings) and primers (qPCR) are listed and can be found in the shared .xls files on the Lab dropbox. Storage of the skin and lung samples in -80°C freezer and overview of the boxes with paraffin blocks is listed in an .xls file on an external hard drive, dropbox and K-drive. Printed versions of protocols are stored in a folder.

WP6: Effects of *Ppp2r1a* M180T/+ and R182W/+ variants on neuronal and synaptic biology

Protocols (.docx) related to immunostainings and qPCR are stored on specific drives and are printed and stored in a folder. For each protocol, the necessary antibodies (stainings) and primers (qPCR) are listed and can be found in the shared .xls files on the Lab dropbox. Storage of the brain samples in -80°C freezer and overview of the boxes with paraffin blocks is listed in an .xls file.

WP7: An integrated transcriptomic and proteomic analysis of *Ppp2r1a* M180T/+ and R182W/+ developing, embryonic brains and adult cortices

An overview of the mice that are used for the transcriptomic and proteomic analysis is available on the shared J-drive. Protocols for isolation of mRNA and proteins from brains are saved on several drives, and are printed and stored in a folder. Storage of the brain samples in -80°C freezer and overview of the boxes with paraffin blocks is listed in an .xls file.

Will a metadata standard be used? If so, describe in detail which standard will be used. If no, state in detail which metadata will be created to make the data easy/easier to find and reuse.

- No

No real metadata standard will be used.

Excel files stored within each experiment folder will contain the following information:

1) Experimental design
> Definition of experimental and control groups
> Number within each group (n)
> Researcher
> Date
2) Samples
> Mouse IDs, cage number, birth date, date of sacrificing
> Genotypes
> Type of tissue
> Processing procedure (frozen, fixed)
> Storage location (freezer box, parafin box)
3) Protocol
> Reference to protocol (printed version, drives)
> Adjustments
> Remarks
4) Data analysis
> Computational programs
> Parameters
5) Data storage
> Notebook pages
> Drives (+ sub-folder)

On the K-drive, the data are structured/ordered per paper (separate 'archive': see 5.1). Furthermore, the data of my project are structured/ordered per sub-project (work package) on the K-drive.

5. DATA STORAGE AND BACKUP DURING THE FWO PROJECT

Where will the data be stored?

I will provide detailed descriptions of data acquisition in paper lab notebooks. All data belonging to a certain work package of the project are properly linked to each other and referred to each other in the lab notebooks and detailed protocols are provided (.doc files + printed and stored in a folder). General protocols and information are stored on the shared J-drive, dropbox, external hard drive and printed instructions will be stored in a folder. Lists of antibodies and primers for PCR and qPCR are stored in the Dropbox folder of the lab. Data are stored in dropbox, external hard drive, and the Archive drive (K-drive).

For each work package, the following documentation is stored:

WP1: Behavioral phenotyping of neonatal *Ppp2r1a* M180T/+ and R182W/+ knockin mice

- Protocols of the breeding (.docx) and genotyping (.docx) of mice are stored on an external hard drive, shared J-drive and dropbox (+printed instructions in folder). Genotypes of the mice are collected in an .xls file on an external hard drive and on the shared J-drive. Administration of the mice (genotypes, room and cage of housing) are stored on the LAIS website of the KU Leuven animal facility.
- Protocols of neonatal behavioral tests (.docx) and standardized tables (.xls) to complete for each pup are stored on an external hard drive, shared J-drive and dropbox. Printed versions of protocols are stored in a folder. Completed tables (written) are pasted in a Lab Notebook. Completed and analyzed tables (.xls) are stored on an external hard drive, dropbox and the K-drive.

WP2: Behavioral phenotyping of adult *Ppp2r1a* M180T/+ and R182W/+ knockin mice

Standard operating procedures (SOPs) of adult behavioral tests (.docx), standardized tables for rotarod, grip strength and passive avoidance measurements (.xls) and tables generated by MouseWin, ANY-maze and EthioVision software (.xls) are stored on an external hard drive, shared J-drive and dropbox. Completed and analyzed tables (.xls) are stored on an external hard drive, dropbox and the K-drive. Raw data are stored on an external hard drive. Printed protocols are also stored in a folder.

WP3: In vivo imaging of *Ppp2r1a* M180T/+ and R182W/+ knockin mice

- Protocols of scanning (.docx) are stored on an external hard drive, shared J-drive and dropbox and printed and stored in a folder.
- The original scans (.tif) and reconstructed images (.bmp) are both stored on the Large Volume Storage (L-) drive from the MOSAIC core facility (KU Leuven).

WP4: Monitoring of epileptic behavior of *Ppp2r1a* M180T/+ and R182W/+ knockin mice

The standardized protocol for implanting telemetry equipment is stored on an external hard drive, shared J-drive and dropbox. Measurement (.csv and .png) files are stored on an external hard drive, dropbox and K-drive.

WP5: Long-term survival follow-up of *Ppp2r1a* M180T/+ and R182W/+ knockin mice

- An overview of the mice that participate in the long-term follow-up is available on the shared J-drive, as well as the follow-up of the weight and well-being of these mice. At the end of the experiment, the .xls file will be saved on the K-drive.
- Protocols (.docx) related to the DMBA/TPA skin carcinogenesis protocols and to the LSL-Kras G12D lung cancer model (model, immunostainings, qPCR) are stored on an external hard drive, J-drive and dropbox. For each protocol, the necessary antibodies (stainings) and primers (qPCR) are listed and can be found in the shared .xls files on the Lab dropbox. Storage of the skin and lung samples in -80°C freezer and overview of the boxes with paraffin blocks is listed in an .xls file on an external hard drive, dropbox and K-drive. Printed versions of protocols are stored in a folder.

WP6: Effects of *Ppp2r1a* M180T/+ and R182W/+ variants on neuronal and synaptic biology

Protocols (.docx) related to immunostainings and qPCR are stored on an external hard drive, J-drive and dropbox and are printed and stored in a folder. For each protocol, the necessary antibodies (stainings) and primers (qPCR) are listed and can be found in the shared .xls files on the Lab dropbox. Storage of the brain samples in -80°C freezer and overview of the boxes with paraffin blocks is listed in an .xls file on an external hard drive, dropbox and K-drive.

WP7: An integrated transcriptomic and proteomic analysis of *Ppp2r1a* M180T/+ and R182W/+ developing, embryonic brains and adult cortices

An overview of the mice that are used for the transcriptomic and proteomic analysis is available on the shared J-drive. Protocols for isolation of mRNA and proteins from brains are saved on an external hard drive, J-drive and dropbox, and are printed and stored in a folder. Storage of the brain samples in -80°C freezer and overview of the boxes with paraffin blocks is listed in an .xls file on an external hard drive, dropbox and K-drive. At the end of the experiment, raw data (see section 2) and processed data (.xls) will be saved on the K-drive.

How is backup of the data provided?

Besides regularly provided automated backups by ICTS (of J-drive, K-drive), the data stored on personal PCs and J-drive will be weekly backed up on the personal KU Leuven I-drive, and the own Dropbox of the labs concerned (password-protected). Every two months, additional backups will be made on external hard disks, as an extra security measurement. MS data, RNA-seq data and imaging data will be backed up by copying to an SSD. External SSD hard-disks (up to 1-2 TB storage capacity) keep the storage costs feasible since cost of an external hard drive is cheaper than ICTS storage costs for such big data volumes.

Is there currently sufficient storage & backup capacity during the project? If yes, specify concisely. If no or insufficient storage or backup capacities are available then explain how this will be taken care of.

- Yes

Extra external hard disks will be bought, as appropriate. Same for liquid nitrogen containers or freezers, but sufficient capacity is currently available.

K-drive: up to 100 GB per PI; in addition, extensions of this volume can be asked for at ICTS at all times (for an additional cost).

What are the expected costs for data storage and back up during the project? How will these costs be covered?

- K-Archive drive costs: 20€/year/100 GB; daily back-ups.
- Dropbox: free
- External hard disks: max. 1500€ (=5 disks of 1-2 TB)

Data security: how will you ensure that the data are securely stored and not accessed or modified by unauthorized persons?

The main data archive on the K-drive is a read-only drive for lab members only (password-protected); only the PI of the lab concerned can write on this drive (= add data), but not modify data.

J-drive and I-drive: restricted to account of researcher.

Lab Dropbox: access password-protected.

Paper lab notebooks are kept in locked cabinets.

Digital lab notebooks: password-protected.

6. DATA PRESERVATION AFTER THE FWO PROJECT

Which data will be retained for the expected 5 year period after the end of the project? In case only a selection of the data can/will be preserved, clearly state the reasons for this (legal or contractual restrictions, physical preservation issues, ...).

All generated (raw and processed) data will be preserved, for at least 5 years.

Data underpinning publications: see above (section 5.1, K-drive).

Unpublished data from unfinished work will be kept for longer than 5 years (ideally, until use in a publication, and then preserved or deposited as per our criteria for published data).

Where will the data be archived (= stored for the longer term)?

Raw and processed data: K-drive and external SSD hard disks.

In addition, after publication, all Next Generation Sequencing Data will be uploaded to the Gene Expression Omnibus (GEO) (<https://www.ncbi.nlm.nih.gov/geo/>); and all proteomics data will be deposited to the ProteomeXchange Consortium via the PRIDE partner repository.

Frozen sperm of mouse strains (50 straws (5 straws per male, 5 males in total)) are stored in 2 separated LN2 tanks on 2 different sites on the KU Leuven Gasthuisberg campus (done by Mouse Expertise Unit, VIB-KU Leuven).

Tissue samples of mice are stored in -80 freezers and paraffin blocks in well-defined boxes.

What are the expected costs for data preservation during the retention period of 5 years? How will the costs be covered?

K-Archive drive costs: 20€/year/100 GB

External hard disks: max. 3000€ (=10 disks of 1-2 TB)

Costs are covered by my FWO bench fee and/or by LRD project (Jordan's Guardian Angels foundation) of my PI.

7. DATA SHARING AND REUSE

Are there any factors restricting or preventing the sharing of (some of) the data (e.g. as defined in an agreement with a 3rd party, legal restrictions)?

- Yes. Specify:

Data sharing with peers will exclusively occur through publications, taking into account the Open Access policy of KU Leuven. The planned depositions of data in the relevant responsible repositories (as described above) will only occur **after** publication.

Which data will be made available after the end of the project?

Publications; all data deposited in (public) repositories.

Where/how will the data be made available for reuse?

- In an Open Access repository

Publications; all data deposited in (public) repositories.

When will the data be made available?

Upon publication during the project, or as soon as possible upon publication after the project.

Who will be able to access the data and under what conditions?

Publications and repositories mentioned: all open access.

For unpublished data: only the PI involved (or scientific collaborators who will continue and follow up on the research after the project).

What are the expected costs for data sharing? How will the costs be covered?

Publication costs (Open Access) will be covered by the FWO bench fee.

8. RESPONSIBILITIES

Who will be responsible for data documentation & metadata?

Iris Verbinnen

Who will be responsible for data storage & back up during the project?

Prof. Veerle Janssens

Iris Verbinnen

Who will be responsible for ensuring data preservation and reuse ?

Prof. Veerle Janssens

Who bears the end responsibility for updating & implementing this DMP?

The PI bears the end responsibility of updating & implementing this DMP.