# Clinical Research Information Sheet

## What is the PERSYST study about?

### Quick Introduction

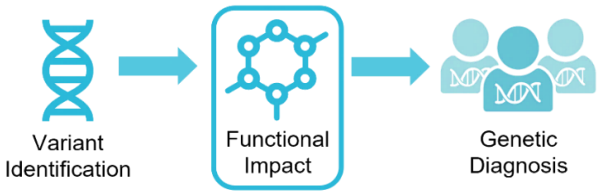
PERSYST is a clinical research study that will provide RNA-based evidence for Variants of Uncertain Significance (VUS) predicted to be involved in splicing.

PERSYST's main focus will be to study splicing variants from genes not expressed in skin or blood that can be transactivated in patient skin biopsies.

### Introduction

PERSYST stands for Pathogenic Evaluation of Recalcitrant variants by SYStematic Transactivation.

PERSYST will evaluate Variants of Uncertain Significance (VUS) predicted to alter splicing that are recalcitrant to current diagnostic practice.




Over 1500 Mendelian genes are not expressed in clinically accessible tissue: skin and blood.

PERSYST can study the functional impact of these 1500 organ-specific genes that are usually unavailable while the patient is living (e.g., brain sample). This is done using systematic gene activation technology (transactivation) on clinically accessible patient skin biopsy tissue to "wake up" the silent genes. The study will then produce a research report for the referring clinical team to determine if the RNA-based evidence can reclassify a VUS to a clinically actionable outcome (e.g., likely pathogenic.)

The study will determine how useful transactivation and RNA-based functional genomic testing is in providing evidence supportive of a damaging effect on the gene product (ACMG-AMP guidelines). This can allow clinical teams to initiate the reclassification of a VUS to enable clinical action for the patient and family.

**Our study allows individuals with a genetic medical condition(s) to potentially gain a genetic diagnosis elevated to clinical utility due to our functional follow-up and close the diagnostic odyssey for your patients and their families.**

### Study Aim

PERSYST aims to resolve the impact and mechanism of splicing VUS to increase the diagnostic rate. This will be done by providing RNA-based functional evidence by transactivating silent genes in patient cell lines.

### Funding

MRFF/GHFM Funding from October 2022 – October 2027.

### Are there any costs?

The study will financially cover the following:

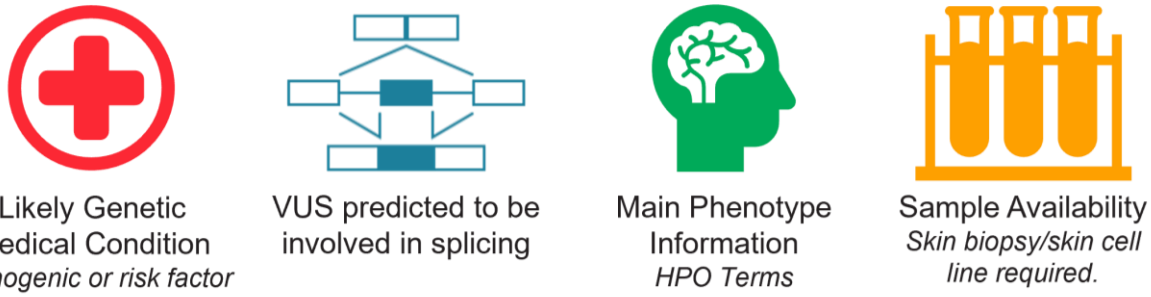
- Shipping for sample collection.
- All research conducted within the study.

The study will not financially cover the following:

- Time for enquiry and recruitment of participants.
- Time to return research report to participant/s.
- The resources to reclassify the VUS.

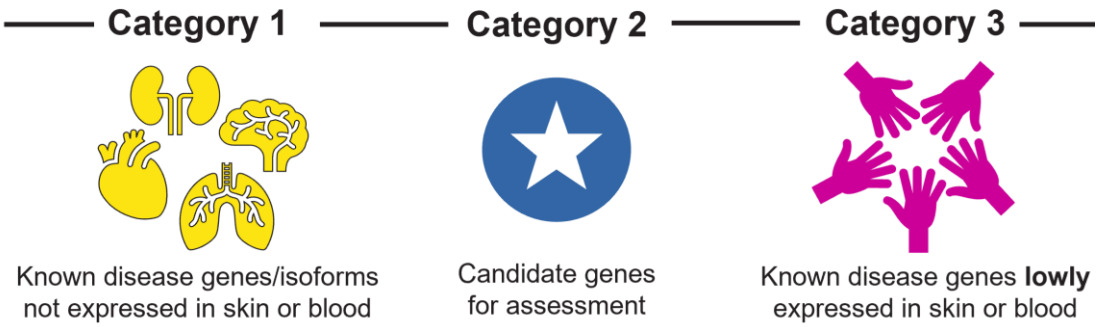
## Who is eligible for the study?

### Minimum Criteria




- **Suspected variant of major effect (monogenic or risk factor**): A patient's gene diagnostic report highlights a gene variant classified as a "Variant of Uncertain Significance" (VUS) which raises suspicion that the gene variant may be responsible for the patient's medical condition(s) but requires additional evidence to be certain.
- **Putative splicing VUS:** The gene variant is a type that has been predicted to change the splicing of the gene RNA and, as such, the fidelity of the gene's mRNA.
- **Phenotype information:** Main clinical phenotypes of the patient are available and described using Human Phenotype Ontology (HPO) terminology.
- **Patient Sample Availability and Consent:** Patients with suspected genetic medical condition(s) (or their legal guardian) consent to provide a sample of their living cells (e.g., from blood and/or skin).

### Categories




- **Category 1, Variant gene or gene isoform is not expressed in skin or blood**: The gene or gene isoform within a gene in which the variant is found is also not expressed (is not active) in skin or blood and requires transactivation to sufficiently study RNA splicing. See the list here.
- **Category 2, Variant in a novel candidate condition gene:** The gene in which a VUS exists has not yet been implicated in a medical condition but is still a rational candidate for a novel condition gene.
- **Category 3, Variant lowly expressed in skin or blood:** The gene in which the variant is found is lowly expressed in blood or skin samples and requires further transactivation may or may not be required to sufficiently study RNA splicing.

### Exclusion Criteria

- Not consenting to the study.
- Already existing functional interpretation of the variant that does not require further validation.
- Variants in genes causing late-onset disorders which are not clinically actionable.
- Variant origin not validated by a Human Research Ethics approved project.

# How does PERSYST Work?

1. **Submit VUS Enquiry.** Submit **deidentified information** of case details, including a **redacted clinical report,** via the PERSYST REDCap Enquiry submission portal: www.redcap.link/persystenquiry

2. **PERSYST Review.**

The study's Data Curation Team will review each case for eligibility and priority using in silico analysis. An email will be sent to you with the outcome (accepted/rejected).

**If we have rejected your case at a given time**, we will keep your enquiry submission just in case at another given time; we accept the case, depending on demand and the development of the study.

**If your case is accepted and we have a gene kit for your variant,** an email will be sent with a participant information pack and details on how to consent and register the participant(s) for the study via the REDCap Registry form. Please see step 4.

**If your case is accepted and we don't have a gene kit for your variant**, please see step 3.

3. **Gene Kit Creation**

If a gene kit has been made, we will ask you to commence recruitment. Please see step 4.

If a gene kit has not been made, we will ask you to ask the patient and/or family if they would like to be a part of the study and if they would be motivated to provide a skin biopsy sample. Then fill in the PERSYST Pre-Recruitment Survey (2 mins).

If the patient is motivated to provide a skin biopsy, PERSYST will commence making a gene kit. This may take around 3 months to make. However, we would prefer to make sure we can achieve transactivation of the gene before asking your patient and/or family for a skin biopsy.

4. **Recruit Participant/s.**

**Consent.** See Consenting Notes on Page 4.

**Register Participant/s**
Immediately after consent, please register all participants within the REDCap Registry Form (further clinical and medical details). At this point, participants are considered a part of the study.

**Collect Sample/s**
Immediately after the registry, please organise a time for participant sample collection. Please also discuss the skin biopsy procedure using the skin biopsy fact sheet with the patient (if relevant) and family so that they can mentally prepare and strategize for the scheduled sample collection.

If your state requires it, please also upload your non-regenerative tissue/skin biopsy certificate as a record of completion.

Please refer to the PERSYST Sample Submission Guidelines for details on how to store and pack the sample(s).

5. **PERSYST Assay.**

PERSYST will transactivate the silent gene from a patient skin cell line and then perform follow-up RNA-based experiments, such as short-read RNA Sequencing, Long-Read Oxford Nanopore Sequencing, and RT-qPCR, to determine the functional outcome of the VUS.

6. **Clinical Team Review and Report.** See Page 5 for Research Report notes for further information.

**Review Research Report.**
PERSYST will aim to return a research report via email in one year from a patient's sample collection with an outcome and possible recommended interpretation of the VUS.

**Return Study Outcomes to the Participant.**
Please communicate the study outcome to the participant to ensure they are aware if this study changes anything in the participant's clinical management.

**Clinical Impact Survey.**
Please complete a 5 min survey after returning the study outcome to the participant to measure study impact on participant's clinical management.

# Consenting Notes

## Expectations of the Clinical Team

- Prioritise the patient's and family's care and health over the study preferences.
- Provide options in a non-biased manner when the patient and family are considering consenting.
- When participants are enrolled in the study during this appointment, plans (current or prospective) are discussed with the participants about sample collection.
- If the sample collection process causes psychological or physical distress to the participant/s, a follow-up appointment is required to ensure the skin biopsy wound is healing and if further treatment is required. **Where a patient experiences distress, you must notify the study team within 24 hrs.**
- All outcomes from the study for a patient will be communicated back to the patient by their clinical team.

## Participant Requirements and Options

Please ensure participants have had time to consider, ask questions, and ask others about participating in the study. Please go through the associated fact sheets on Variants of Uncertain Significance (VUS), Genetic and Genomic Testing (RNA). Please get in touch with the study team if you have any queries.

| Required Sample | Optional Sample | Required Sample Type | Optional Sample Type |
|---|---|---|---|
| Proband* | Proband's adult parents and/or adult relatives | Skin Biopsy (For a skin cell line) | Blood (For blood cells and DNA) |

*Where parents are carriers of the proband's variant, a skin biopsy can be taken from them alternatively.

Please encourage the patient's family (if relevant) to participate in the study and provide skin biopsies when using the Parent/Guardian Consent Form.

An increase in controls will strengthen the validity of the PERSYST transactivation assay. One encouragement is that parents could go through this process with their children so they are not doing it alone.

**Please note our current HREC approval only permits the additional relative controls from adults.**

The study requires a minimum of a skin biopsy (min 2 mm x 2 mm) from the patient to perform the assay. We will accept if a cell line from a skin biopsy from the patient already exists or utilise clinical care pathways where possible (i.e., surgery). We optionally would like patient blood for further clinical research.

Before consent, the REDCap Database will ask who and how many people consent as a prerequisite. The study will require basic identifiers (name, email, age, nature of participant's consent ability and site) to determine the suitable consent and information forms.

## Consent Types

- E-consent via REDCap, where the consent will be emailed to the participants to follow prompts from REDCap for completion. (see PERSYST Consent Sop for further details)

OR

- Paper Consent to print for signing, scan and upload a copy of these documents to the REDCap database.

| Consent Types | Consenter | Proband Capable of Consent | Proband Mature Minor |
|---|---|---|---|
| **Parent/Guardian PGICF** | Parent/Guardian | No | N/A |
| **Adult Participant PICF** | Proband | Yes | N/A |
| **Mature Minor MICF** | Parent/Guardian (as greater than low risk) Mature minor to provide accent | Yes, but aged 13-17 and deemed a MM according to state law | Yes |

# Research Report Notes

**Review Research Report.**

PERSYST will aim to return a research report via email in one year from a patient's sample collection with an outcome and possible recommended interpretation of the VUS.

Please note the participant will receive one of the three possible outcomes:

- Data to support that my DNA VUS causes my medical condition(s).
- Not enough data to support that my DNA VUS causes my medical condition(s).
- No data as the study was inconclusive.

However, it will be the responsibility of the submitting clinical and diagnostic teams to determine:

- The ACMG-AMP weighting of the outcome.
- If the VUS is reclassified.

As there currently are no standard guidelines (only recommendations) for interpreting RNA functional genomic assays, any outcome deemed by the study is considered a recommendation and ultimately will be up to the clinical and diagnostics teams to agree/ disagree with this recommendation based on their expertise.

If the clinical team determine the outcome is clinically actionable for the patient (evidence can reclassify the VUS), this will be up to the clinical team to resource.

If the clinical team determine the outcome is not clinically actionable:

- The study can continue research using the patient sample to find additional and stronger evidence to work towards a clinically actionable outcome (funding dependent).
- The study can refer the case to other ethically approved research studies (if the patient consents) that can provide additional, stronger evidence to work towards a clinically actional outcome.

Independent of outcome from the study:

- The study can meet with the clinical team to further explain the results.
- The study can continue researching the case on the patient sample (e.g., precision medicines).

**Report Study Outcome.** We assume in good faith that all clinical teams will report the study outcome to the patient and family.

**Clinical Impact Survey.**

Once you have returned and actioned (if applicable) the research report research outcomes to the patient and family, please provide an assessment of the clinical outcome, your experience and feedback on the study via the **Clinical Impact Survey.** A link will be sent to you when the research report is returned.