Quality Assurance (QA)
Head of Department Approval and Declaration Form

This form MUST be completed AND submitted with the entire QA application to the Office for Research.

Section A: To be completed by Principal Investigator

Short Project Title:	Genotype-phenotype link between NTHL1 and NTHL1 tumour syndrome: a review
Principal Investigator:	Prof Finlay Macrae
Brief description of the aim of the study:	This project aim to provide genotype-phenotype correlation for NTHL1 polyposis syndrome.
Estimated duration of the project:	~ 2 Year

Note: this QA activity, which is exempted from ethics committee review, must still comply with the *National Statement on Ethical Conduct in Human Research* and *The Australian Code for Responsible Conduct of Research*.
As Principal Investigator I confirm that to the best of my knowledge, and based on the information provided in the QA application, this project meets the criteria for a quality assurance activity.

Name: Prof Finlay Macrae Signature: Date: 9 Jan 2023

Section B: To be completed by HOD(s)

(Divisional Director to sign where the HOD is a researcher on the application)

Note: this QA activity, which is exempted from ethics committee review, must still comply with the *National Statement on Ethical Conduct in Human Research* and *The Australian Code for Responsible Conduct of Research*.

Declaration

I certify that I have reviewed and discussed the study protocol and applicable project documentation *with the principal researcher or his/her delegate* and:

- The protocol and aims are acceptable I support the project going forward;
- I have reviewed the budget for the project and confirm that this is appropriate;
- I certify that this research can be conducted under the auspices of Melbourne Health utilising the resources outlined in the protocol;
- All investigators/students involved in this project at Melbourne Health have the skills, training and experience necessary to undertake their respective roles;
- I have reviewed the Statements of Approval and am satisfied that all relevant departments who may be impacted by this project have been appropriately consulted;

Head of Department
Department: Medical Services
HOD Name: Kirsty Buising
Designation: Medical Director
Signature:
Date: 11-1-23

** Divisional Director to sign where the HOD is a researcher on the application.*

MN **Nusrat, Maleeha** 5 May 2023 at 1:34 pm
RE: QA2023005 - Acknowledgement Email
To: Khan, Zafirah, Cc: Macrae, Finlay, Weilun Alan Gao Details

Dear Alan,

RE: QA2023005 – Genotype-Phenotype associations NTHL1 polyposis syndromes

We write in response to your submission of the above named project for review via the RMH Quality Assurance process.

The project has been reviewed against the tenets of the National Statement on Ethical Conduct in Research 2007 (updated 2018) and the NHMRC Ethical Considerations in Quality Assurance and Evaluation Activities (March 2014).

This project has been assessed by a member of the Office for Research Ethics & Governance team and a member of the Royal Melbourne Hospital HREC, as evaluation activity not requiring Human Research Ethics Committee (HREC) review.

Site - Melbourne Health

Documents noted:
- QA Application Form, signed 15 December 2022 (amended copy provided 03 April 2023)
- Protocol, Version 1, 20 December 2022 (amended copy provided 28 April 2023)
- Head of Department Approval and Declaration Form signed by Kirsty Buising dated 11 January 2023
- Excel Data Collection Spreadsheet -NTHL1 Working Group

Other information about your QA approval:
- Your project number is **QA2023005.** Please quote this number in future correspondence.
- If there are any changes to the project a new QA project will need to be submitted
- This QA approval covers the period **05 May 2023** and will expire on **05 May 2025**. If the study is to continue beyond 2 years, a new QA project will need to be submitted.
- Annual progress reports are not required for QA projects.
- All documentation regarding this project, including this email, must be kept for 12 months from completion.
- However if you intend to publish the results, documentation must be kept for 5 years post publication or 5 years from the decision not to publish.

Note for multisite projects:
- This assessment is for RMH sites only. For other sites, the organisation may accept this assessment or choose to undertake another assessment.
- Site Governance Authorisation is required at each site participating in the study before the research project can commence at that site.

PLEASE FILE THIS EMAIL WITH THE QA PROJECT RECORDS.

All the best with your project.

Kind regards,

Maleeha

Maleeha Nusrat
Research Governance and Ethics Officer, Office for Research
RMH City Campus, Level 2 South West
300 Grattan Street I Parkville Victoria 3050
maleeha.nusrat@mh.org.au I **thermh.org.au**
The Royal Melbourne Hospital acknowledges the Kulin nations as the Traditional Custodians of the land on which our services are located. We are committed to improving the health and wellbeing of Aboriginal and Torres Strait Islander peoples.

Project Title: Genotype-Phenotype associations NTHL1 polyposis syndromes

3. Protocol Version No: 1

4. Protocol Date: 20/12/2022

5. Project Lead Institution: Royal Melbourne Hospital

6. Project Lead Person *(repeat if more than lead, ie at multiple institutions:* Dr Weilun Gao

7. Other Key Personnel: Professor Finlay Macrae, Professor Richarda De Voer, Mr John Paul Plazzer

8. Contact Information:

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8. Background:

a) What activity has been undertaken in this subject area before?

In 2015 Weren et al. described a hereditary cancer syndrome caused by biallelic mutations in the DNA base excision repair gene NTHL1, characterized by attenuated adenomatous polyposis and increased colorectal cancer (CRC) risk, largely resembling the recessive syndrome caused by MUTYH mutations (1). A common cause of DNA damage is reactive oxygen species (ROS) that are generated

from normal cellular metabolism, inflammation, or through exogenous sources such as radiation. Importantly, tumor cells experience an increased ROS burden from these sources, potentially facilitating more mutations. Base excision repair (BER) is the main pathway for repairing ROS-induced DNA damage and is initiated by the N- glycosylase proteins (NTHL1). Familial cancer genes have been well circumscribed by the ACG and AMP guidelines (2).

There is extensive work to better characterize which pathogenic variants along the putative genes are correlated in disease and which are benign. This information is key for the multidisciplinary care team offering diagnostic, prognostic and interventional information for patients and their families.

b) What are the limitations of this previous activity?

No investigations have been completed to formulate genotype-phenotype correlations between NTHL1 gene and polyposis syndrome or to provide meta-analysis of the phenotypic manifestations of this syndrome.

c) Why is this project important and what will it add to the literature or how will it improve patient care?

This project forwards ClinGen's goals of producing a centralized genomic database and software to improve accuracy, classification yield and dissemination of knowledge into the field of medical genetics. We plan to formulate evidence-based and expert-guided criteria to improve classification concordance, accuracy, and transparency. Advancing the ClinGen initiative is significant to improve community knowledge, diagnosis, and management for those with disease-causing variants.

Methods of developing gene/disease-specific ACMG modifications for other genes have involved curation and consensus discussions between curators and expert panels (3,4,5) This research design utilizes existing evidence by expert and non-expert curators. We employ quantitative methods and qualitative methods to determine the genotype and phenotype correlations of NTHL1 polyposis syndrome. This design ensures we can better counsel and diagnose patients with biallelic NTHL1 mutations.

9. Project Aim and Objective/s:

Please describe the specific study aim/s, and/or question/s being investigated.

This project aims to:

1. To provide genotype-phenotype correlation for NTHL1 polyposis syndrome

Objective:

1. Curation of variant-phenotype data base of families and linked individuals with biallelic mutations in NTHL1 including age of diagnosis, symptoms, location and histopathology of polyposis, extracolonic manifestations.
2. Provide statistical analysis and correlation for NTHL1 polyposis syndrome including age of diagnosis, gender distribution and frequency of intestinal/extraintestinal phenotypes.

10. Project Design and Methods:

The project involves systematic review of existing literature, unpublished data collected from diagnostic laboratories of deidentified genotype-phenotype information. Will involve independent reviewers working with covidence (literature filtering software) according to the PRISMA search guidelines and quality assurance criteria.

The phenotype information will be segregated according to variant type and location to provide correlation.

Phenotype information will be extracted and tabulated for quantitative analysis:

Age at diagnosis	Country of origin / ethnicity	Colonic manifestations					Colorectal cancer (CRC)	Extracolonic manifestations								Family hx of transmission
		Polyps count				Number of polyps reported of A adenomas, H hyperplastic polyps, TVA tubulovillous adenomas		Breast Ca	Duodenal Polyps	Duodenal Ca	Endometrial Ca	Cervical Ca	Urethelial Bladder Ca	CNS (meningioma etc)	Others: (BCC, SCC, Pancreatic, benign cysts)	
		0	"1-19"	20-99	≥100	≥1000										

10.1 Participant recruitment:

Where, by whom and who will be asked to participate (Inclusion/Exclusion criteria)?

1. Literature search and access to public databases for cases with biallelic NTHL1 mutations on germline testing
2. Access to deidentified phenotypic information from collaborators' networks subject to their ethical considerations of the jurisdiction of collaborators
3. Access to deidentified and centralized data housed by familial cancer clinic through the ICCON research collaboration including the RMH and PMCC (a network of researchers associated with Australia's Familial cancer clinics, relating to phenotypic and genotypic information) This approach has been suggested by Professor Paul James, Director of Parkville Familial Cancer Clinics and Chair of the ICCON network.
    a. Only patients with prior consent to sharing their genotypic data for research publication will be used. This will be specified to each clinician alongside our request prior to data is collected.
    b. For example: collection of genotype level data from Melbourne Colorectal Oncogenetics Group, Via Professor Daniel Buchannan, a collaborator to the research has independent ethics already collected through HREC#1955946. The case data we collect have already been consented for use for research, which will also become public/published via their paper. Furthermore, approval for centralization of variant and associated clinical data is part of the consent process that applies across the Parkville precinct.
        i) Regarding sharing and centralisation of variant data across the familial cancer clinics in Parkville is covered in the consent clause which

patients sign. This is through the INHERITED CANCER CONNECT PARTNERSHIP (ICCON). This data has has been curated by the familial cancer clinics or genetic clinics for the purpose of research.

ii) https://www.petermac.org/research/clinical-research/clinical-research/familial-cancer-research-centre/iccon-database

c. RMH/PeterMac includes in their consent a mandatory tick box that allows variant and associated clinical data to be forwarded to local and international registries. We will try contact these registries for genotypic/phenotype data surrounding NTHL1. Only cases that have already been consented for research purposes will be requested according to quality assurance project.

i) The consent of familial cancer clinic patients for research is similarly covered through the ICCON consent for research purposes. We will use the consented data through the ICCON through a central ICCON coordinator.

4. Access to genotypic and phenotypic information through private DNA laboratories in USA (through existing collaborations within the InSiGHT-ClinGen variant curation expert panels- Through John Paul Plazzer and Professor Macrae as manager of the InSiGHT-ClinGen networks).
   a. Regarding InSiGHT we will contact through the manager of registries Prof Macrae for genotype/phenotype records insofar as patients have already consented for their data to be published for research purposes. No new consent will be sought.
   b. Regarding USA laboratories we will contact the laboratories specifically asking, "insofar as your laboratory has consent for variant and associated de-identified clinical information to be passed on research publication". No new consent or patient contact will be sought.
5. Access to genotypic and phenotypic information collected in European diagnostic laboratories through our collaboration with Professor Richarda De Voer.
   a. Professor De Voer has Dutch ethics approval from 2019 for screening of *NTHL1* and collecting clinical information of cases. We will use data that has been collected for research from that source. I.e. We will use the original patient information that patients consented to be published. As the data has already been prepared by her.
   b. The genotype data we collect for use in this study will be from already collected patient information that patients have consented for use in publication/research.
6. Access to genotype and phenotype information through circulation of request through UK Cancer Genetics Group (CGG) and the Association for Clinical Genomic Science (ACGS) courtesy of Professor Ian Frayling (clinical molecular geneticist, Cardiff UK)
   a. A request will be sent to Professor Ian Frayling for individual clinician contacts in UK given there is a few to our knowledge. In contacting the geneticists/clinicians, we will specify the data we collect will only include patients who have already consented for their genotype/phenotype status to be published for research purposes. As patient presenting with rare NTHL1 to familial clinics are routinely consented for using their data for research publications.
   b. Again, deidentified information will be collected from the participating clinicians.

10.2 Project Procedures:

How will the specific project be carried out?

As part of Dr Gao's Master of Philosophy's research project, Dr Gao will be responsible for initiating and managing the communications with our collaborators and public data bases e.g. ClinVar and LOVD.

This will involve:

-Contacting the treating clinician for more phenotypic information

-Contact of clinicians or other informed sources e.g diagnostic laboratories.

-For patients information collected from non-RMH patients through relevant clinicians, or other informative sources, ethic will be sought under their respective jurisdictions. Our experience with European colleagues is that approval from a formal ethic committee is helpful for that purpose.

a) Specifically, patients who are included in our quality assurance project will have previously been consented to have their genotype/phenotype and clinical data to be used and published for research purposes. We are not reapproaching patients or requiring new consent for this process.

b) In contacting the clinicians, we will specify that this is regarding previously consented patients that they wish to contribute to our review of genotype and phenotype. These clinicians will also have their own ethics approval for collecting patient information for research purposes.

b) If there are participants, what will they have to do during the study, when and how often?

No

c) What will the project lead do, where and when?

Support and further clarification in genotype-phenotype NTHL1 biallelic gene carriers, importantly this will inform the work of a variant curation panel and INSIGHT-ClinGen variant expert panel addressing NTHL1. Especially in so far relating to phenotypic descriptions, which is an important component of ACMG criteria for pathogenicity assessment.

10.3 Data Collection and Storage:

a) What data will be collected and how (from medical records, questionnaires, survey)?

Genetic variation data will be collected in the HGVS nomenclature or as reported in the literature/reporting laboratories.

Phenotype information pertaining to age of diagnosis, method of diagnosis, gender, colonic/extracolonic manifestations, number of polyps, histopathology of polyp types. This information will be collected depending on availability in published or recorded patient medical records and will be deidentified.

b) How will data be stored (electronically, paper, etc)?

The student researcher will collect the data in the format of excel spreadsheets. This will begin in February 2023 until December 2023.

Data will primarily be stored in an electronic form on a shared drive on the MH server. The student researcher will have remote access to the server. Working copies will temporarily be stored on the student-researchers password-protected hard drive and destroyed after it is re-uploaded on the MH server. The data is non-identifiable, will not require coding and is not required to stay on-premises as per the Melbourne Health (MH) Data Management in Research Guidelines (2015). During the active phase of the project, only the team members listed on the title page will have access to the data.

c) How long will data be stored and how will it be destroyed?

On completion of the project, the data will be archived for 12 months on the MH server unless required otherwise as per the Australian Code for the Responsible Conduct of Research (2018). Data will be disposed of by reformatting under the authorization of the Head of Department (HOD) according to the Melbourne Health Policy MH05 Documentation and Records Management (2020) and the Archiving, retention, and disposal of data guidelines (2019).

If the PI has left MH, the HODs will be the custodian of the data.

10.4 Sample Collection and Storage:

N/A

11. Data Analysis

11.1 Justification of sample size:

Sample size will depend on convenience sample of all patients who have recorded genotype/phenotype information collected in various sources mentioned in 10.1.

Currently there are 20 families with 33 individuals with documented genotype and phenotype data according to Kuiper et al (1). To power our genotype-phenotype correlations, we predict that 50-100 individuals with biallelic NTHL1 mutation will be enough according to historical correlation studies in other published literature of hereditary cancer genes

11.2 Proposed means for analyzing the data citing specific statistical techniques:

What statistical techniques will be used to analyze the data? Descriptive statistics such as percentages and means or medians may be sufficient dependent on the project.

Pearson correlation coefficient will be used to estimate the correlation of genotype and phenotype in NTHL1 polyposis syndrome

Additionally, percentages will be used to estimate prevalence of different phenotype amongst NTHL1 biallelic population.

11.3 Proposed means for analyzing the samples citing the specific techniques:

Data will be analyzed using Microsoft excel spread sheet to obtain key data specifiers such as percentages, mean age of onset, mean polyp count, and correlation coefficient of phenotypes and genotypes.

11.4 Dissemination of Results

The results of phenotype and genotype correlation will be published as systematic review and meta-analysis in genomic medical journals to improve awareness and help NTHL1 VCEPs and clinicians to better classify and manage patients with NTHL1 mutations.

12. References:

Note any literature or web references that may have been cited.

1. Kuiper RP, Nielsen M, De Voer RM, et al. NTHL1 Tumor Syndrome. 2020 Apr 2. In: Adam MP, Everman DB, Mirzaa GM, et al., editors. GeneReviews® [Internet]. Seattle (WA): University of Washington, Seattle; 1993-2022.
2. Richards, S., et al. (2015). "Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics

and Genomics and the Association for Molecular Pathology." Genetics in Medicine 17(5): 405-424.
3. Kountouris, P., et al. (2021). "Adapting the ACMG/AMP variant classification framework: a perspective from the ClinGen Hemoglobinopathy Variant Curation Expert Panel." Human Mutation 12: 12.
4. Oza, A. M., et al. (2018). "Expert specification of the ACMG/AMP variant interpretation guidelines for genetic hearing loss." Hum Mutat 39(11): 1593-1613.
5. Romanet, P., et al. (2019). "Proposition of adjustments to the ACMG-AMP framework for the interpretation of MEN1 missense variants." Human Mutation 40(6): 661-674. 5.