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TITLE: Detecting Missing Heritability for Risk Stratification and Clinical Management of the Neurofibromatoses

PRINCIPAL INVESTIGATOR: Dr Miriam Smith

CONTRACTING ORGANIZATION: The University of Manchester

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6. AUTHOR(S)

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7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

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14. ABSTRACT

Neurofibromatosis type 1 (NF1), NF2 and schwannomatosis are genetically distinct tumour predisposing conditions. However, many cases display significant clinical overlap and genetic diagnosis is critical in these cases, due to their differing prognoses and clinical management protocols. We will use an extended range of genetic techniques to identify the missing heritable elements in our cohort of families with NF1, NF2 or schwannomatosis. In our first year we have recruited, inducted and carried out initial training of a postdoctoral research associate (PDRA) for this project. She has begun work on functional studies, originally intended for year three, while we await HRPO approval for use of patient samples. In addition, due to the coronavirus pandemic, we have been working remotely from home for over four months and during this time the postdoctoral researcher has been writing a systematic review on an area that is relevant to the project for publication.

15. SUBJECT TERMS
Neurofibromatosis, Schwannomatosis, NF1, NF2, SMARCB1, LZTR1, pathogenic variant, genetic predisposition, genetic diagnosis

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1. **INTRODUCTION:**
   Neurofibromatosis type 1 (NF1), NF2 and schwannomatosis are autosomal dominant neurogenetic disorders that predispose affected individuals to develop tumours of the nervous system. While these disorders are both clinically and genetically distinct, there are many cases with significant clinical overlap, particularly between NF2 and schwannomatosis. Genetic diagnosis is critical in these cases, as the disorders have different prognoses and require differing clinical management protocols. Routine genetic testing currently identifies >90% of non-mosaic pathogenic variants in *NF1* and *NF2* genes, but there remain families with a clinical diagnosis of NF1 or NF2 in which no causative variant has been identified. In addition, the two known schwannomatosis predisposition genes, *SMARCB1* and *LZTR1*, currently only account for around 50% of cases. Our aim is to use an extended range of genetic techniques to identify the underlying cause of disease in well characterized families with a clinical diagnosis of NF1, NF2 or schwannomatosis, in whom no underlying pathogenic mutation has yet been found. We also aim to investigate the mechanism of action of these variants to aid future research into therapeutic drugs.
2. **KEYWORDS:** Neurofibromatosis, Schwannomatosis, *NF1*, *NF2*, *SMARCB1*, *LZTR1*, pathogenic variant, genetic predisposition, genetic diagnosis
3. **ACCOMPLISHMENTS:**
   What were the major goals of the project?
   The major goals for year 1, were to:
   1. Obtain HRPO ethics approval (months 1-3. Institutional approval obtained, awaiting final approval from HRPO)
   2. Recruitment, induction and initial training of postdoctoral researcher in the lab (months 1-3, completed)
   3. Selection of patient samples for the study (months 1-3. Awaiting HRPO approval)
   4. Preparation and submission of samples for genome wide Axiom SNP arrays (months 4-5. Awaiting HRPO approval)
   5. Targeted in-house Next Generation Sequencing for all three aims (months 4-9. Awaiting HRPO approval)
   6. Analyses of initial experiments and preparation for subsequent steps, depending on findings (months 10-12. Analyses of initial experiments postponed as awaiting HRPO approval. Preparation for subsequent steps is under way.)

   What was accomplished under these goals?
   We have successfully recruited a postdoctoral research associate (Dr Perez-Becerril) and have carried out inductions and initial training. We obtained institutional ethics approval prior to the application being made. However, the process of obtaining HRPO approval has taken significantly longer than anticipated, which has delayed the start of our major activities. While this process has been ongoing, we have initiated preliminary experiments originally intended for year 3 (Functional assessment of novel variants), which do not require ethical approval. This was possible as we have identified

schwannomatosis-associated variants of uncertain significance that have been published recently. Dr Perez-Becerril has carried out molecular cloning to create plasmid constructs for these experiments.
In addition, the current global pandemic has meant hindered our progress and our laboratory has been out of operation for four months. During this time Dr Perez-Becerril has been planning for experiments to be initiated on our return to the lab and writing a systematic review article on genetic variants in the neurofibromatoses, which we plan to publish and which will act as an introduction to the work that is planned for the remainder of this project. In addition, Dr Perez-Becerril has been able to attend several online training courses which will be useful for facilitating data analysis once the preliminary genotyping experiments are complete. We have recently been approved for a phased return to the laboratory from August and we plan to prioritise preparation of DNA samples for sequencing and SNP arrays as once generated, this data can be analysed remotely from home in the event of a second lockdown.

- **What opportunities for training and professional development has the project provided?**

The new PDRA, Dr Perez-Becerril, has undergone initial training in the laboratory under the supervision of Dr Smith. She has also attended regular lab meetings, departmental seminars and has given a presentation at a recent journal club. She has also attended bioinformatics training courses and workshops, which are relevant to the data analysis aspects of her project, such as use of Linux, Python and the University high-performance computer cluster. She has also recently undertaken a large amount of self-study during the preparation of her systematic review.

- **How were the results disseminated to communities of interest?**

Nothing to report.

- **What do you plan to do during the next reporting period to accomplish the goals?**

Once we have returned to the laboratory and received HRPO permission to begin using patient samples, we will prioritise preparation and submission of these samples to generate genotyping data. Once laboratory research is back to full capacity, we will also continue with the functional assessment of variants of uncertain significance

4. **IMPACT:**
   - **What was the impact on the development of the principal discipline(s) of the project?**

   Nothing to report.
   - **What was the impact on other disciplines?**

   Nothing to report.
   - **What was the impact on technology transfer?**

   Nothing to report.
   - **What was the impact on society beyond science and technology?**

   Nothing to report.
5. **CHANGES/PROBLEMS:**
   - **Changes in approach and reasons for change**

   No change.
   - **Actual or anticipated problems or delays and actions or plans to resolve them**

   The major delays encountered during this reporting period have been due to a) the significant amount of time it has taken to obtain HRPO approval to begin the major work activities and b) the global pandemic which has disrupted all non-Covid19 related laboratory based research. We have recently obtained permission to return to the laboratory part time under reduced occupancy conditions and we will prioritise generation of data that can be analysed from home in the event of a second lockdown period. Once the laboratory is back to full capacity, we will also resume functional work to assess three recently reported schwannomatosis-associated variants of uncertain significance.
   - **Changes that had a significant impact on expenditures**

   The delays described above (HRPO approval & global pandemic) have significantly affected year 1 spending, as we have been unable to begin the major research activities described in the SOW or to attend conferences in person.
   - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

   No changes were made to the anticipated use of human samples. We obtained institutional Research Ethics Committee (REC reference 10/H1008/74; R&D Pin: R10257) prior to the start of the funding period.
   - **Significant changes in use or care of human subjects**

   None.
   - **Significant changes in use or care of vertebrate animals.**

   None.
   - **Significant changes in use of biohazards and/or select agents**

   None.
6. **PRODUCTS:**
   - **Publications, conference papers, and presentations**
     - **Journal publications.** Nothing to report.
     - **Books or other non-periodical, one-time publications.** Nothing to report.
     - **Other publications, conference papers, and presentations.** Nothing to report.
   - **Website(s) or other Internet site(s)**

     N/A
   - **Technologies or techniques**

     N/A

- **Inventions, patent applications, and/or licenses**
  N/A
- **Other Products**
  N/A

7. **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**
   - **What individuals have worked on the project?**

Name:	Miriam J Smith
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-3184-0817
Nearest person month worked:	2
Contribution to Project:	Dr. Smith has advertised for, recruited and trained a PDRA to work on the project.
Funding Support:	N/A

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Name:	Cristina Perez-Becerril
Project Role:	PDRA
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0003-1630-1943
Nearest person month worked:	10
Contribution to Project:	Dr. Perez-Becerril has been inducted into the University, undertaken relevant training courses, attended relevant seminars and undertaken molecular cloning work to create four plasmid constructs to begin functional work for the project.
Funding Support:	N/A

Name:	Prof D. Gareth Evans
Project Role:	Co-I
Researcher Identifier (e.g. ORCID ID):	https://orcid.org/0000-0002-8482-5784
Nearest person month worked:	1
Contribution to Project:	Professor Evans has helped to recruit the PDRA and advised on clinical aspects of the project work.
Funding Support:	N/A

   - **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

     Nothing to report
   - **What other organizations were involved as partners?**
     - **Organization Name:** National Health Service
     - **Location of Organization:** Manchester
     - **Partner's contribution to the projec**t: Our laboratory space is housed within a National Health Service building.
     - **Financial support;**
       - **Facilities** Project staff use the partner's facilities for project activities.

8. **SPECIAL REPORTING REQUIREMENTS**
   - **COLLABORATIVE AWARDS:** N/A
   - **QUAD CHARTS:** N/A
9. **APPENDICES:** N/A