A Feasibility Study: Pregabalin for the treatment of CANVAS associated chronic cough

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Chapter 1 Rationale

Cerebellar Ataxia with Neuropathy and Vestibular Areflexia Syndrome (CANVAS) is a newly recognised recessively inherited ataxia. Genetic ataxias have been clinically divided into pure ataxias which present with just ataxia and those with additional clinical features. Common additional features include cognitive impairment, spastic paraparesis, neuropathy, and oculomotor involvement (1). CANVAS was one of the first ataxias where the involvement of the vestibular system was recognised and, after the third cardinal feature of neuropathy was recognised, it was named CANVAS for Cerebellar Ataxia, Neuropathy and Vestibular Areflexia Syndrome in 2011 (2, 3).

The underlying cause of CANVAS – a pathogenic intronic biallelic expansion in the Replication Factor C subunit gene (*RFC1*) was identified by two independent groups in 2019 (4, 5) with subsequent studies showing genetic variants specific to New Zealand and Cook Island Māori and the Asia-Pacific region (6, 7). Additional studies have shown that the combination of a pathological expansion and a pathological single nucleotide variant in the same gene can also cause the disease (8). Brain imaging and postmortem studies have shown atrophy of the anterior cerebellar vermis and crus 1 of the cerebellar hemispheres, of multiple cranial nerve ganglia, and the dorsal root ganglia (4, 9). Consistent with this latter finding,

patients are often found to have absent sensory nerve action potentials on nerve conduction studies (10, 11). Additionally, the autonomic system may be involved (12).

In 2014 our group first described chronic cough as a feature of CANVAS syndrome, and subsequently other studies have replicated this with 71.8% of patients in one cohort experiencing it (12, 13). Some even consider it as a diagnostic criterion for CANVAS (13). This cough may be the first sign of CANVAS, preceding the onset of ataxia by years. The impact of chronic cough on patients with CANVAS syndrome is also significant, with many describing sleep disturbance, and issues of social stigmatisation and cough interfering with conversations affecting quality of life (14). The latter issues being magnified by concerns about COVID.

Chronic cough is defined as a cough that persists for longer than eight weeks (15). Chronic cough unattributable to underlying respiratory or gastric disease is often termed *neurogenic cough*. The normal cough reflex is a complex pathway that involves stimulation of the larynx and tracheobronchial sensory receptors passing to the brainstem via the vagal nerve. The efferent pathway passing by the vagal, spinal and phrenic motor nerves stimulate the diaphragm, intercostal and laryngeal muscles to produce the complex sequence of contractions which combine to make a cough. This output is modified by complex central nervous system processing. Neurogenic cough is thought to occur either through local hypersensitisation of the receptors to ordinarily non-tussive stimuli or due to central sensitisation, where feedback loops between the various pathways associated with coughing are altered (16). Some authors have speculated that the chronic cough observed in CANVAS is the result of both central and peripheral nervous system dysfunction; though the exact aetiology and pathology of neurogenic cough in general, and in CANVAS specifically, remains unknown (8, 16).

There have been no formal interventional trials for chronic cough in CANVAS syndrome. Three out of eight patients in a study of cough in CANVAS syndrome by Tatineni *et al* who were treated for cough with neuromodulator medications (amitriptyline, gabapentin, and pregabalin) reported benefit (14). However, the specific medication regimens that these patients used are not described in the paper, so it is difficult to fully put these patient experiences into the context of neurogenic cough treatment as described above.

Recent guidelines published by the French Respiratory, Otolaryngology, Speech Pathology and Gastroenterology Societies have reviewed medications used for chronic cough in general and

conclude that they are likely to be effective (15). Medications that have been subjected to randomised clinical trials for this condition include amitriptyline, gabapentin, pregabalin, erythromycin, azithromycin, morphine and ketamine, however none of these has randomised more than 60 patients and most were considerably smaller (17-31). Recently a large study of gefapixant, a $P2X_3$ inhibitor, has been shown to be effective in a large phase three study with over 2000 patients randomised (28).

In considering which medication to trial we were guided by the current clinical experience of one of our clinicians who manages chronic cough on a daily basis (JA) and has extensive experience of gabapentin and, more recently, pregabalin, together with clinical reports of the efficacy of individual patients with CANVAS responding to *ad hoc* trials of medication (RR personal communication).

Pregabalin and gabapentin were devised as analogues of γ-aminobutyric acid (GABA); however, they have no GABAergic activity (32). Rather they have a high affinity for the voltage gated calcium channel $\alpha_2\delta$ subunit expressed in the nervous system and peripheral nervous system, attenuating nerve depolarisation and possibly neurotransmitter release - though the exact mechanism or mechanisms of action have yet to be fully elucidated. (33) (32). Gabapentin was approved in 1993 in Europe, and in the search for increased efficacy, was followed by pregabalin, approved in 2004 (34). Both medications have a broad range of indications including neuropathic pain, and epilepsy.

When gabapentin and pregabalin are compared, pregabalin has more favourable pharmacokinetics. (32). The oral bioavailability of gabapentin is around 80% at a dose of 100 mg every 8 h. Doses of gabapentin above this amount decrease bioavailability due to rapid saturation of the uptake pathway, resulting in diminishing returns where dose escalation is concerned. At 1,600 mg bioavailability is reduced to just 27%. Pregabalin has near non-saturable uptake and the average bioavailability of oral doses between 70 – 900 mg per day is ≥ 90%. The T_{max} (the time taken to reach the maximum concentration of a drug in the systemic circulation) of pregabalin is also shorter than gabapentin; the maximum concentration of pregabalin (C_{max}) is reached after approximately an hour, whereas gabapentin doses above 100 mg have a T_{max} of around 2 hours, with T_{max} increasing to 3 – 4 hours as dose is increased (32). These pharmacokinetic differences are reflected in the different dosing regimens for these drugs. Gabapentin is typically prescribed three times a day (TID) and a starting dose of 300 mg

is common. Pregabalin, however, is typically prescribed only twice a day (BID) at a starting dose of 75 mg. Both are registered for use in Aotearoa/New Zealand.

Both medications are generally well tolerated although in the double-blind trial of pregabalin there were reports of blurred vision, altered cognition, dizziness and weight gain – all of which resolved after medication was stopped (30).

Impact Statement:

Chronic cough is a significant burden to patients who have CANVAS syndrome. This study will lay the foundations for developing treatments for this chronic cough and indicate whether further testing of this medication is worthwhile. Although pregabalin treatment has been tried in the general population with chronic cough this is the first time any medication has been tried in a group of patients with a specific genetic cause of cough. It is possible that patients with a specific genetic cause for their condition (*RFC1* expansion) could have a gene specific response to treatment.

Chapter 2 Study Aims and Outcomes

2.1 Aims

1. Determine the efficacy of pregabalin for the treatment of chronic cough associated with RFC1 expansion/CANVAS
2. Determine the tolerability and safety of pregabalin in this population

2.2 Primary Endpoints

1. The difference in the reduction in frequency of daytime CANVAS cough in participants while on pregabalin compared with while on placebo, as measured by cough monitoring software Cough Pro

2.3 Secondary Endpoints

1. The difference in the reduction in frequency of nighttime CANVAS cough in participants while on pregabalin compared with while on placebo, as measured by cough monitoring software Cough Pro

2. Patient reported effectiveness of pregabalin treatment for CANVAS associated cough by use of the Leicester Cough Questionnaire (LCQ), Cough Visual-analogue scale (Cough VAS), Cough Quality of Life questionnaire (CQLQ).

3. Patient reported tolerability of pregabalin in the treatment of CANVAS associated cough by use of the Glasgow Antipsychotic Side-Effect Scale (GASS)

4. Patient reported global Quality of Life (QOL) measured by the 36 Item Short Form Survey (SF-36)

Chapter 3 Study Design and Protocol

3.1 Participants

3.1.1 Inclusion and Exclusion Criteria

Table 1: Inclusion and Exclusion Criteria:

Inclusion	Exclusion
• Participants with neurological symptoms attributable to *RFC1* pathology (neuropathy, vestibular failure, ataxia) • Positive *RFC1* genetic test (either biallelic pathological expansion or pathological expansion and pathological variant). • > 1 year of chronic cough • Over 18 years old. • Can give informed consent. • Has access to a smart phone.	• History of cancer (other than skin SCC or BCC). • History of severe renal impairment (GFR < 30) • History of intolerance to pregabalin • Pregnancy*/breastfeeding. • Active respiratory disease. • Current or recently quit (< 6 months) smokers. • ACE inhibitor use. • Productive cough. • Use of any pregabalin, gabapentin within 3 months of baseline visit • Comorbid medical condition which, in the opinion of the Principal Investigator (PI) will either confound the outcome of the study, or place the participant at risk • Blood test abnormalities at screening indicating severe liver or kidney dysfunction

*Pregabalin may be present in breastmilk, the effects on breastfeeding infants are not clear.

3.1.2 Recruitment

Participants with genetic diagnosis and chronic cough will be identified through Pūnaha Io, the New Zealand NeuroGenetic Registry and BioBank, the Neurogenetics Research Clinic, and by word of mouth. Participants will also be asked whether they have access to a Smart phone and are happy to download the cough app to the phone. A Participant Information Sheet (PIS) will be provided to prospective study participants.

3.2 Study Size and Power Calculation

No group has documented cough frequency in CANVAS using objective measures such as Cough Pro, so it is not possible to make precise power calculations. Even the collection of these data will be useful for future studies and therefore the study will have value even if no difference between treatment and placebo is seen.

However, to estimate the power of the study to detect a difference we can consider studies of undifferentiated chronic cough. Study power for the cross-over trial was calculated based on the following assumptions: 18 participants available; baseline geometric mean (geometric SD) of 55 (6.9) DAYTIME coughs/hour; an estimated correlation of untransformed (original) pairs of data of 0.3; and an estimated ratio of the standard deviation to the mean on the original data scale of 0.85 or lower (29). Based on these assumptions, the cross-over trial would have 80% power (alpha = 5%) to detect a change in the geometric mean of DAYTIME coughs/hour from 55 to 30 following treatment (i.e., geometric mean difference of 25).

3.3 Data Capture

The Research Electronic Data Capture (REDCap) database system will be used to store and capture longitudinal data during participant visits (35). This system is hosted securely at the Faculty of Medical and Health Sciences at the University of Auckland. After the participant visit is complete, the REDCap visit will be locked to preserve the data. Paper Case Report Forms (CRFs), if required, will be stored securely at the University of Auckland Clinical Research Centre.

When participants are entered into the database their REDCap ID will be used as their code. This code will serve as a pseudonym for the labelling of their medication.

3.4 Randomisation

Participants will be randomly assigned to either Arm A or Arm B using REDCap's randomisation function. In the active part of the study, Arm A will receive 12 weeks of pregabalin (75 mg, BID) followed by a washout period of four weeks, and then an inactive control for a further 12 weeks. Arm B will be the reverse: they will receive 12 weeks of inactive control followed by washout and then 12 weeks of pregabalin (see Figure 1). Acknowledging

the possibility of seasonal differences in environmental cough stimulants, randomisation will be in blocks of 10 and 8.

3.5 Blinding

Both drug and control are administered orally and have an identical appearance. To make sure that the study team does not know which group the participants have been assigned to, an unblinded pharmacist, Compounding Labs Ltd, will dispense the medication.

Safety bloods will be assessed by the PI without communication with the Student Researcher and Study Coordinator, to avoid unblinding.

Unblinding will occur once the data collection part of the study has been competed i.e. the last participant has finished the final visit.

Premature unblinding may also occur if there is a serious adverse reaction that in the opinion of the PI requires knowledge of the participant's treatment arm.

3.6 Trial Registration

This trial will be registered with the Australian New Zealand Clinical Trials Registry (ANZCR) prior to enrolling participants.

3.7 Visits

The study will involve six in-person assessment visits and six telephone visits (***Figure 1***),

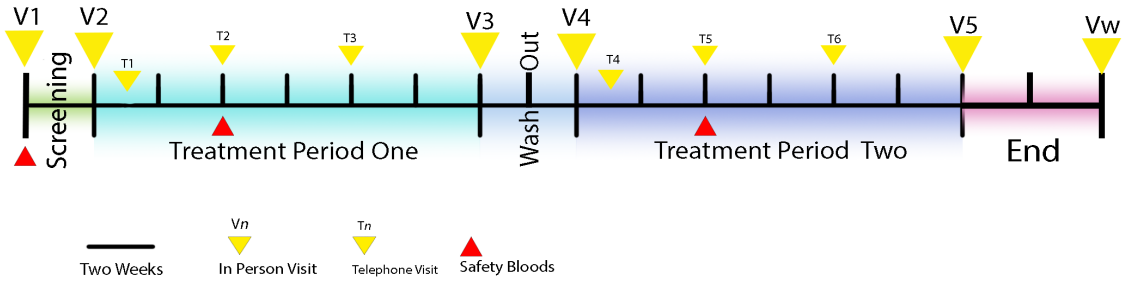

Figure 1: The study is divided into three stages of data collection. Firstly, immediately after the screening participant will install and use the cough monitoring software to collected baseline measurements of cough frequency. All visits labelled V(χ) are in person visits whilst visits labelled T(χ) are telehealth visits conducted remotely.

Assessment visits will occur at screening, at the beginning and end of each treatment period and four weeks after washout. All in-person visits will be conducted at the University of Auckland's Clinical Research Centre. Regular visits will be conducted by video call or telephone depending on participant resources and preference.

3.7.1 Screening

After obtaining informed consent, participants will have a baseline medical history with particular attention to previous investigation and treatments of chronic cough.

Physical examination will be performed to confirm and document neurological involvement of CANVAS and exclusion of other serious illness including respiratory disease.

Baseline blood tests including Full Blood Count (FBC), renal function and liver function test will be performed. These will be performed through community laboratories.

Spirometry to assess Forced Vital Capacity (FVC) and Forced Expiratory Volume (FEV1), and Electrocardiogram (ECG) screening, including for AV block, will be performed.

Participants who meet clinical inclusion and do not have clinical exclusion criteria will be shown how to install the cough monitoring app on their Smart Phone and instructed on how to use it. Participants will be asked to use the app between Screening and Baseline to make sure

they are familiar with it and to establish their baseline cough rate. Participants will be contacted to check that they are managing the software competently. Participants will be encouraged to activate the app for two weeks prior to baseline until the end of the study period. Participants will then be randomised to either study arm using the REDCap randomisation function.

3.7.2 Baseline/Start of Phase 1

Participants will fill in the cough frequency and severity questionnaires (LCQ and CoughVAS) and the quality-of-life questionnaire (CQLQ) at baseline. They will also undergo standardised neurological assessments (SARA and INAS scores), performed by the research team to document their neurological signs. Pregabalin or inactive control will be dispensed.

3.7.3 Telephone follow up

The Student Researcher will contact the participant at the end of the first week to confirm that they are well and that they are recording their coughing.

If the participant is experiencing intolerable adverse drug reactions then the dose of medication can be reduced to 1 tablet once a day.

The participants will be invited to contact the Student Researcher in case of adverse drug reactions or adverse events (4.2.3).

The participants will undergo formal assessments by telephone at weeks 4 and 8 of the first phase, which will include the GASS questionnaire.

Participants will have safety bloods taken at week 4 of the first phase, which will include FBC, Coagulation screening, renal function and liver function tests.

At week four, if participants are tolerating the study medication and blood tests are not deranged, but they are still experiencing ongoing cough then they can increase the medication to two tablets twice a day (or from 1 tablet once-a-day back to twice a day if they had previously reduced their medication).

At week eight, participants are given a further opportunity to increase their medication, up to a possible maximum of three tablets twice-a-day.

Patients experiencing significant adverse drug reaction after increasing the dose will be instructed to contact the team and can return to the previous dose they were taking at either of the time points.

3.7.4 End of Phase 1 in-person visit

Participants will have a general examination (performed by the PI or by the Student Researcher under supervision of PI), and a targeted neurological examination using the SARA and the INAS assessments (performed by Student Researcher after specific training).

3.7.5 Wash Out:

After phase one/treatment period one there will be a four week washout period where neither drug nor control will be administered to the participants. Cough recording by Cough Pro will still occur.

3.7.6 Commencement of Phase 2 - in person visit

Participants will have a general examination (performed by the PI or by the Student Researcher under supervision of PI), and a targeted neurological examination using the SARA and the INAS assessments (performed by Student Researcher after specific training).

3.7.7 Phase 2 telephone assessments

The participants will undergo the same assessments at weeks 1,4 and 8 as in the first treatment period.

Participants will have safety bloods taken at week 4 of the second phase, which will include FBC, Coagulation screening, renal function and liver function tests.

3.7.8 End of Phase 2-in person visit

Participants will have a general examination (performed by the PI or by the Student Researcher under supervision of PI), and a targeted neurological examination using the SARA and the INAS assessments (performed by Student Researcher after specific training).

3.7.9 Post study safety review

Four weeks after each participant has completed both phases of the study a follow up visit will be conducted to ensure that there are no long lasting, negative effects. This will take the form of a general medical examination but may include routine blood testing or other investigations if necessary in the opinion of the PI.

3.8 Premature Withdrawal

Participants may withdraw at any time without a reason. Data collected up to that point will remain as part of the study to maintain scientific validity. Participants will be invited to take part in a Premature Exit Visit which would include the same assessments as an end of treatment visit.

Where participants are unable to tolerate even the lowest dose of treatment during a treatment phase, the treatment phase will be prematurely ended. If this occurs during the first treatment phase and they have not completed at least four weeks' treatment, then they will be exited from the study with a request to complete a Premature Exit Visit.

If this occurs during the first treatment phase and they have completed at least four weeks' treatment, then the end of Phase 1 visit will be brought forward and they will proceed to the other arm of the study after a four-week washout.

3.9 Delegation Log

	Duties
PI: A/Prof Richard Roxburgh	○ Study design ○ Data analysis ○ Ethics applications ○ Funding applications ○ Manuscript preparation ○ Clinical oversight and training ○ Academic supervision
Study Coordinator: Juno Barnett Collins	○ Study design ○ Data analysis ○ Ethics applications ○ Funding applications ○ Manuscript preparation ○ Participant Recruitment ○ Database management ○ Clinical coordination ○ Data collection
Student Researcher: Rory Burnell	○ Data collection ○ Data analysis ○ Manuscript preparation

3.10 Functional Assessments for Cough Severity

The functional assessments to be use in this study to assesses the impact of CANVAS associated chronic cough include:

3.10.1 Cough Frequency analysis

Hyfe A.I based app ‘Cough Pro’ will be used to record the frequency of coughing during the course of the study. The app works by detecting explosive sounds which triggers the SmartPhone to record using its built in microphone 0.5 second snippets. These are sent to a confidential cloud-based system which analyses and discards non-cough explosive sounds. If there is no connection to the internet, explosive sounds are saved to be analysed once the device is reconnected.

The sampling range for cough frequency recording varies from study to study, from limited recording during visits to continuous 24 h ambulatory monitoring [OBJ][OBJ]. To ensure the best possible chance of detecting pregabalin induced alterations in cough frequency, cough monitoring will be conducted continuously. Participants will be instructed to keep their smartphones on or near their person for the duration of the study period.

At the end of each week, or more frequently if it suits the participant, the cough report will be sent to the study team. This report will detail the frequency of coughs per day. The Study Team cannot remotely access participant’s devices but can contact participants to remind them to upload the cough data every Monday if the participant desires or forgets.

3.10.2 Leicester Cough Questionnaire

A frequently used questionnaire which has been validated, for the assessment of cough. The LCQ is not specific to any one condition (36).

19 Questions: 1 (every time/all of the time) to 7 (never)

3.10.3 Cough Visual Analogue Scale (Cough VAS)

A frequently used scale which has been validated, for the assessment of the severity of cough. CoughVAS is not specific to any one condition (37).

Severity ranked by marking a 100 mm line (0 – 100 mm)

3.10.4 Cough Quality of Life Questionnaire

This has also been validated in chronic cough. It assesses the impact of cough on daily life and social perception (38).

28 Item, ranked 1 – 4; from strongly disagree to strongly agree

Note this study will modify one question to improve its relevance for this participant population: the question, “I fear I might have AIDS or tuberculosis (Q7)” will be modified to, “I fear people think I have AIDS, tuberculosis or COVID-19”

3.10.5 36 Item Short Form Survey (SF-36)

This is a widely used questionnaire which has been used in thousands of studies and is validated across the board for assessing quality of life in relation to general health

It consists in a 36 item questionnaire, which looks at how a person’s ability to perform various activities impacts their participation and perceptions of their health.

3.11 General and Neurological Examination

A general examination will be undertaken to confirm that the participant does not have a serious illness which would preclude them taking part in the study. This exam will also be conducted appropriately in response to adverse events.

A general screening neurological examination to document the patient's neurological status what features of CANVAS syndrome are present in each candidate. Specific examinations for ataxic patients – SARA score and INAS score, will also be undertaken. The Student Researcher will do training on these scales to ensure consistency with international methodology. These exams will be performed at the beginning and end of each phase to explore whether pregabalin has any other effects – the condition is indolent and would not be expected to progress significantly on these scales in a 12 week period.

Chapter 4 Safety Pharmacology, Drug Tolerability and Adverse Events

4.1 Formulation and Excipients of Concern

The pregabalin used in this study will be prepared by taking commercially available 75 mg pregabalin capsules and encasing them within a larger, nondescript capsule for the purpose of blinding. The only excipient of potential concern is lactose monohydrate. The control formulation will be made from generic fillers using an identical capsule to that of the pregabalin and is not bioactive.

Formulation of the pregabalin and control will be performed by Compound Labs Ltd in accordance with all national regulations. They will also dispense the medications and maintain the master document.

4.2 Functional Assessments for Tolerability and Adverse Drug Reactions

An Adverse Drug Reaction (ADR) is defined as any harmful or potentially harmful response to drug therapy that can be causally linked to the administration of that drug but is not a component of the therapeutic use of the drug. Pregabalin has a list of commonly experienced ADR ranging from mild: xerostomia and/or somnolence to rarer and more severe reactions:

suicidal ideation, AV block, etc. A full description of potential ADR is available in the New Zealand Formulary monograph on pregabalin.

As patients perceived tolerability is as essential to successful pharmacotherapy as pharmacologically efficacy, the GASS assessment tool will be used to determine the impact of pregabalin ADR.

No new pregabalin or control ADR are expected to be observed in this study. To ensure participant safety, however, the Liverpool ADR Causality Assessment Tool will be used when a participant presents with a new symptom unattributable to any pre-existing condition that presents during the course of this study. The decision to employ this tool rests with the Investigators.

4.2.1 Glasgow Antipsychotic Side-Effect Scale (GASS)

This scale is for assessing the impact of drug ADR. It covers CNS, Cardiovascular, extra-pyramidal, GI and genitourinary symptoms (39).

4.2.2 Liverpool ADR Causality Assessment Tool

This tool will be used when participants present with a suspected ADR not currently listed as likely for the test drug/control.

4.2.3 Adverse Event Reporting

An Adverse Event (AE) is defined as an untoward medical event or investigation in a study participant with the potential to cause harm or indicate harm has occurred. This may or may not be related to the administration of a study drug or control. A Serious Adverse Event (SAE) is any life-threatening medical occurrence requiring hospitalisation that may or may not be

related to the administration of the study drug or control. During all visits a review of AE and SAE will be conducted as part of the medical interview. AE recording is essential to participant safety and also to contextualising the result of the study and preventing potential confounders such as Upper Respiratory Tract Infections from going unrecorded by the study team.

4.2.4 Pregnancy and Use of Pregabalin in Those of Childbearing Potential

Individuals who are currently pregnant or planning on becoming pregnant during the study period must be excluded from this study. If a participant becomes pregnant during the study they must be withdrawn. Pharmacovigilance studies report contradictory findings on the teratogenicity of pregabalin. Pregabalin may have teratogenic effects, although the risk of birth defects appears only marginally higher than the basal risk of birth defects (40).

Sexually active individuals who have childbearing potential must agree to use a highly effective form of contraception from the screening visit until one month after completing the study. Highly effective contraception includes both barrier and hormonal contraceptive methods Sexual abstinence can be used if it is consistent with the participant's usual practice.

4.2.4.1 Investigator's Disclaimer for Fertile Individuals without Childbearing Potential

The effect of pregabalin on sperm or the pharmacokinetics of pregabalin in seminal/prostatic fluids is unknown. The potential teratogenic effects have only been studied in those of childbearing potential. Pregabalin is not suspected of changing sperm count or altering the composition or function of seminal and/or prostatic fluids however the presence of pregabalin within said fluids cannot be commented on. If participants are concerned around pregabalin and its effects on teratogenesis they should consult with a fertility expert.

Chapter 5 Data Management

5.1 Data Management Plan

5.1.1 Storage and Capture

After data is collected, a working data set will be created by exporting the data from REDCap to an excel or similar file. This is to preserve data integrity of the original REDCap system. Study Team members involved with data analysis will have access to these files. These files will be securely stored in the Centre for Brain Research Neurogenetics Clinic servers, hosted in FMHS.

The Cough Pro app stores cough frequency measurements and reports locally on the device. Participants will need to treat this data with the same degree of confidentiality as any other personal information stored on their mobile device. At the end of every week participants will be instructed to send their cough reports to the study team by email or drop box. This email or drop box will only be accessible by the study team and is securely hosted at the University of Auckland.

Data collection at bedside and during telephone visits will be performed using hardcopy case report forms generated from the REDCap database. These hardcopies will be scanned and uploaded to REDCap after data entry is complete.

5.1.2 Data Access

Study Team members listed on the Delegation Log (3.1) will have read/write access to the REDCap database. Only the PI and Co-Investigator will have authority to lock and unlock REDCap visits that have been completed.

5.1.3 Anonymisation and Privacy

All data will be de-identified before publication.

5.2 Statistical Analysis

The primary endpoint of this study will be the frequency of cough as determined by cough monitor. The mean number of coughs per hour measured on two days a week apart at baseline, at the end of Phase one and at the end of washout and at the end of Phase two will be calculated. The effect of pregabalin will be measured as the difference between the change in cough frequency from baseline to the end of the pregabalin treatment, compared with the change in cough frequency from baseline to the end of the placebo phase. Student's t-test will be used to assess the statistical significance at a 95% confidence level.

Secondary endpoints will be reported individually, as the score each participant received at Baseline, the end of Phase one, Washout, and the end of Phase two. Additionally, the difference in scores at these timepoints will be reported as a percentage increase or decrease from baseline.

All statistical analysis will be performed in GraphPad Prism v.9.

Chapter 6 Student Involvement

The Student Researcher will perform a literature review regarding the pathogenesis of chronic neurogenic cough, the understanding of cough in CANVAS and medications that have been used for cough and their purported mechanisms of action. This review will serve to further contextualise any results in terms of pharmacology and physiology and build upon the theoretical underpinnings outlined by this document.

The Student Researcher will collect all the primary data during all Phases of this study. This will be their principal research component. The Student Researcher will perform the interviews

and baseline assessments of participants during their visits. Following the primary data collection phase, the Student Researcher should assist/engage with data analysis of trial results. This phase nominally includes writeup of dissertation.

At each of these three stages the Student Researcher(s) will be exposed to the three major components of scientific research; A) soft research/literature reviews and critical appraisal of scientific literature, B) primary data collection within a clinical environment, basic data management and participant management, C) clinical data analysis and scientific writing.

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