STUDY TITLE:

Effect of Subtle Energy Transmission and Tao Calligraphy Mindfulness Practice on Mitochondrial DNA (mt DNA) Content in Peripheral Blood Leucocytes

A Follow-up Pilot Study

PRINCIPAL CLINICAL INVESTIGATOR:

Name: Peter Hudoba De Badyn, MD, FRCS
Department: Sha Research Foundation, San Francisco, California

PRINCIPAL RESEARCH INVESTIGATOR:

Name: Cynthia Hamilton, PhD
Department: Sha Research Foundation, San Francisco, California

VERSION DATE:

Version 3 of 30th September 2025

RELATED STUDIES:

Effect of Subtle Energy Transmission and Tao Calligraphy Mindfulness Practice on mt DNA in Peripheral Blood Leukocytes

1.0 **Purpose and rationale of the study:**

OBJECTIVES

The goal of this study is to measure the effects of a unique form of Tao Calligraphy mindfulness practice that involves engaging with Tao art (tracing the Tao Calligraphy "Greatest Love" while listening to or singing the Tao Music or Tao Song "Greatest Love") together with subtle energy transmission on Mitochondrial DNA (mt DNA) in peripheral blood leucocytes.
In our previous study, the participants who regularly practiced, shown an increase of the length in telomeres in peripheral blood granulocytes. In our other studies, participants reported a decrease in symptoms of their illness as perceived subjectively, improved signs reported by treating clinicians and an improvement of well-being as measured by standardized scientific questionnaires.

HYPOTHESIS

The research null hypothesis is that individuals who receive subtle energy transmission for mitochondrial DNA in hemato-lymphoid tissue, and who will engage in daily Tao Calligraphy mindfulness practice for 12 months, will have no significant change in Mitochondrial DNA (mt DNA) content in peripheral blood leucocytes in follow-up analyses and will show no improvement of well-being as measured by standardized scientific questionnaire Rand SF 36(s) at 3, 6 and 12 months.

For statistical analysis of the data from laboratory assessment and scores obtained from questionnaires, the Anova, T-Test and regression analysis will be used to evaluate the null hypothesis. The p value will represent how unlikely the observed data would be if the null hypothesis were actually true and investigators will use them to reach conclusions. The confidence level is set at 95% and if we receive $p < 0.05$, then H0 is rejected. The correlation coefficient will be used to determine any correlation between various factors (e.g. effects of age, sex, length, and frequency of mindfulness practices and other) on outcome and regression analysis will be used to determine the relation of independent and dependent variables.
Tools such as the Minitab version 14 and or PSPP/SPSS will be used.

BACKGROUND

Mitochondria

Mitochondria are membrane bound, self-replicating organelles present in almost all eukaryotic cells. Mitochondrial energy production is essential for all cellular processes. In general, each human cell contains several hundred to 1,000 mitochondria, each with 2-10 copies of Mitochondrial DNA (mt DNA) encoding 13 proteins essential for respiratory chain function (Zhang et al., 2015). Thought to have originated from symbiotic ancestors, mitochondria contribute to many processes central to cellular function and dysfunction including calcium signalling, cell growth and differentiation, cell cycle control and cell death. Mitochondrial shape and positioning in cells is tightly regulated by processes of fission and fusion, biogenesis and autophagy, ensuring a relatively constant mitochondrial population (Osellame et al., 2012). Mitochondrial inheritance is generally accepted to be maternal although small amounts of paternally transmitted mitochondria have been discovered on rare occasions (Hu et al., 2025).

Mitochondrial dysfunction is considered one of the hallmarks of aging (López-Otín et al., 2023) and age-associated diseases (Pahal et al., 2025). The mitochondrial genome is more vulnerable to oxidative damage and undergoes a higher rate of mutation compared with nuclear DNA (nDNA) (Copeland et al., 2002, Lan et al., 2008). Additional underlying causes of mitochondrial dysfunction include inadequate mitochondrial quantity, exposure to environmental toxins, reduction in mitochondrial membrane permeability, impaired transport of essential metabolites, and malfunctioning of the electron transport chain and ATP synthesis (Zong et al., 2024)

Unfortunately, no measure is currently available that globally assesses the ability of mitochondria to perform normal biological functions. Mitochondrial functions include but are not limited to oxidative phosphorylation and energy production in the form of ATP, reactive oxygen species (ROS) production, cell death signalling, as well as steroid hormone synthesis (Selvaraj et al., 2018), and systemic signalling (Forsstrom et al., 2019; Lehtonen et al., 2016).

It has been proposed that the number of mt DNA copies per cell (mt DNA copy number; mt DNAcn) reflects mitochondrial health. A major driver of the popularity of mt DNAcn as a potential marker of mitochondrial health lies in its ease of measure from stored DNA, or indirectly from genotyping/sequencing data. Compared to direct assays of mitochondrial function, which require fresh tissue, the scalability of mt DNAcn assessments is appealing for biomarker studies.

However, mt DNAcn does not directly reflect respiratory chain (RC) function or energy production capacity (Picard, 2021). According to the theory of “biochemical threshold”, only when mt DNAcn decreases by 60 to 80% of normal levels does RC function and energy production capacity decrease (Boulet et al., 1992; Rossignol et al., 2003). This means that in many cases 20-40% of the baseline mt DNAcn is sufficient to produce the 13 proteins necessary to sustain respiratory capacity. But this level of mt DNA depletion seems to occur only in rare mitochondrial diseases (Basel, 2020) or in isolated single cells in diseased organs (Grunewald et al., 2016). The uncoupling of mt DNAcn and respiratory capacity may be accounted for by the fact that up-regulation of transcription and translation from pre-existing mt DNA copies can increase the levels of mRNA, protein subunits, RC function, and energy production capacity, without a change in mt DNAcn. This notably occurs in response to exercise, where mitochondrial content and RC activity in human muscle increases within days to weeks without a change in mt DNAcn (Egan et al., 2013; Puente-Maestu et al., 2011). Thus, in human tissues, mt DNAcn is not directly coupled to, and does not directly reflect mitochondrial bioenergetics.

Furthermore, the picture is somewhat complicated in that while low blood mt DNAcn has been associated with neurodegenerative disease (Yang et al., 2021), cardiovascular disease (Ashar et al., 2017), and both cognitive and physical performance in aging (Mengel-From et al., 2014); other conditions such as diabetes, major depression, some cancers, and mitochondrial disorders are associated with elevated mt DNAcn (Cai et al., 2015; Hagg et al., 2020; Kim et al., 2015). Regarding exposure to environmental toxicants, mt DNAcn has been associated with either higher or lower mt DNAcn (Roubicek and Souza-Pinto, 2017, Meyer et al., 2018).

Nevertheless, the value and specificity of blood mt DNAcn can be increased in several ways as described by Picard, 2021 and summarized below.

1. Use data from complete blood counts with differential (Hagg et al., 2020; Moore et al., 2018), enhanced using flow cytometry methods that quantify immunologically-defined cell subpopulations (Patin et al., 2018), then analyses with multivariate models (i.e., deconvolution method) to understand the proportion of variance in mt DNAcn attributable not just to general immune cell categories (e.g., granulocytes and lymphocytes) but to specific cell subtypes (naïve and memory CD4 and CD8 T cells, B cells, subtypes of monocytes, etc).

2. Further enhance the sensitivity and interpretability of mt DNAcn in relation to health related phenotypes by quantifying mt DNAcn directly in molecularly-defined subtypes of immune cells. For example, CD4+ Naive T cells, monocytes, or other specific immune subtype exist in sufficient abundance in circulation to be isolated by either flow cytometric cell sorting (also known as fluorescence-activated cell sorting, FACS) or by negative/positive selection by magnetic activated cell sorting (MACS) (Kramer et al., 2014). Compared to cell mixtures, cell-specific mt DNAcn quantification add biological specificity to detect meaningful mitochondrial associations related to exposures, other biomarkers, and possibly age and sex-related differences (Rausser et al., 2021)

3. Measure other markers of mitochondrial content and/or function in parallel with mt DNAcn, e.g. live assays of mitochondrial function. Some examples include i) citrate synthase (CS) activity, cardiolipin, or mitochondrial protein abundance to estimate mitochondrial content (McLaughlin et al., 2020); ii) mt DNA integrity, such as DNA damage, point mutations, or deletions (Lujan et al., 2020; Ye et al., 2014); or iii) mitochondrial respiratory capacity, such as oxygen consumption by respirometry (Gumpp et al., 2020; Osto et al., 2020), which can and should be performed in specific cell types (Kramer et al., 2014), as well as respiratory chain enzymatic activities that reflect energy production capacity on either a per-cell or per-mitochondrion basis (Rausser et al., 2021). In the context of direct measurements of respiratory chain function, mt DNAcn becomes a more biologically interpretable feature of mitochondrial health.

4. Measure circulating markers of mitochondrial stress accessible in plasma or other bio fluids, including GDF15 (Sharma et al., 2021), cf-mt DNA (Trumpff et al., 2021), or other emerging mitokines. However, these may lack specificity. E.g. GDF15, cf-mt DNA, and other circulating markers can be induced by a number of stressors not necessarily reflecting mitochondrial RC capacity or stress.

Picard, 2021 concludes that although the biological interpretation of differences in mt DNAcn is tenuous, in combination with relevant markers assessed in homogenous or well-defined cell populations, continuing to add mt DNAcn to existing studies with rich sets of outcomes is likely to contribute valuable insights into the role of mitochondria in human health, aging, and resilience.

Additional Tests:

5. **There is a negative correlation between the copy number of mt DNA, number of mutations in mitochondrial deoxyribonucleic acid (mt-DNA) and longevity.** Higher rates of somatic mt-DNA mutations are consistently associated with aging phenotypes, increased risk of age-related diseases, and reduced lifespan in both animal models and human populations. (Krishnan et al 2007; Green et al 2025; Sanchez-Contreras et al 2023; Mikhailova et al 2022).

6. **Whole mt-DNA sequencing (via next-generation sequencing or Sanger sequencing)** can identify single nucleotide polymorphisms (SNPs), haplogroups, and mutational burden associated with longevity. This approach enables detection of specific SNPs (e.g., C5178A, G9055A, A10398G, C8414T) and haplogroups (e.g., D4, U, J) that have been associated with increased lifespan.

7. **The mitochondrial-to-nuclear genome ratio (mt-DNA content) in tissues and body fluids correlates with the size and number of mitochondria.** PCR-based molecular test to determine mitochondrial-to-nuclear genome ratio in peripheral blood leukocytes is described in paper by J. Knez et all 2015.

Mindfulness Practices and Mitochondria

Psychologically, mindfulness reduces cognitive and emotional reactivity, rumination, and worry, while increasing self-awareness and acceptance, which mediate improvements in mental health and facilitate healthier behaviours. These psychological changes are tightly linked to the observed biological effects.

The biological mechanisms by which mindfulness practices improve health involve down-regulation of stress and inflammatory pathways, enhancement of immune function, and neoplastic changes supporting self-regulation and adaptive coping.

Mindfulness practices may contribute to prolonging human life by improving mitochondrial function, reducing cellular stress, and promoting adaptive stress responses that enhance cellular maintenance and resilience. Chronic psychological stress accelerates mitochondrial dysfunction, increases oxidative damage, and impairs cellular repair mechanisms, all of which are associated with aging and reduced lifespan. Mindfulness-based interventions have been shown to mitigate these effects by reducing stress-induced inflammation and oxidative stress, thereby supporting mitochondrial health.

Mitochondria regulate key longevity pathways through energy production, redox signalling, and mitonuclear communication. Mindfulness practices can activate beneficial mitochondrial stress responses, such as mitohormesis, which involves mild mitochondrial stress that triggers adaptive cellular repair and maintenance mechanisms, ultimately promoting longevity in animal models and potentially in humans. These responses include enhanced osteoporosis, increased expression of overprotective factors, and improved metabolic efficiency.

Chinese calligraphy

Chinese calligraphy handwriting is a dynamic process of integrating visual spatial awareness, cognitive planning and motor skills that is maneuvered with a brush to follow defined configurations of characters.There have been growing empirical studies of Chinese calligraphic handwriting that resulted in improvements in visual attention and span, increased mental concentration, confirming it as an effective treatment for psychosomatic conditions and post-traumatic hyper-arousal symptoms and exhibited significant effects on hypertension and type 2 diabetes. Leisure activities occurring later in life that includes calligraphy may inhibit cognitive decline.

Tao Calligraphy is a unique branch of Chinese calligraphy (Yi Bi Zi) in that it is characterized by one-stroke writing. Every character is written with one continuous stroke with the brush always in contact with the paper. Results from studies confirmed efficacy in tracing in post-acute rehabilitation setting in that patients reported less incontinence, shortened duration of hospital stay, and an increase in overall well-being.Retrospective analysis of data exhibited improvement in general wellbeing, an increase in optimism and energy level, as well as improvement of their symptoms. According to a study measuring

the effects of calligraphy tracing meditation with mantra chanting (repeated sound or word to aid with concentration spoken out loud or silently) of spiritual practitioners, the results exhibited statistically positive improvement in Physical Functioning, Role Limitations due to physical health problems; Role Limitations due to Personal or Emotional Problems; Energy / Fatigue; Emotional Well-being; Social Functioning; Bodily Pain; General Health. In the prospective follow up study of these subjects, following 6 months they continued to improve in functioning in the above-mentioned areas. The authors concluded that tracing calligraphy and mantra chanting was simple to follow, well tolerated, and no complications arose during the study.

The Summary:

Mitochondrial dysfunction is implicated in aging and in pathological processes, such as carcinogenesis and inflammation. Mitochondrial number and shape constantly change in response to energy demands, oxidative stress, and pathological conditions. We postulate that higher mt-DNA copy number (content) is associated with better health status. A single mitochondrion contains on average 2–8 copies of circular mitochondrial DNA (mt-DNA) molecules. Either reduction or increase in the biogenesis or availability of mitochondria in the cells may be markers of primary mitochondrial pathology or of systemic pathology that affects mitochondrial biology. The mitochondrial-to-nuclear genome ratio (mt-DNA content) in tissues and body fluids correlates with the size and number of mitochondria.

Hemato-lymphoid tissue is the source of peripheral blood leucocytes. In our study, the effect of subtle energy transmission to hemato-lymphoid tissue and mindfulness practice with Tao Calligraphy will be measured by mt DNA content in peripheral blood leucocytes.
We will use a PCR-based molecular test to determine mitochondrial-to-nuclear genome ratio in peripheral blood leukocytes as described in paper by J. Knez et all 2015.

Mitochondrial DNA Assessment for our study will be performed by CD Genomics, SUITE 111, 17 Ramsey Road, Shirley, NY 11967.

2.0 **Purpose and rationale of the study:**

3.0 **RATIONALE**

The study aims to acquire both basic and applied knowledge to which the primary purpose is gain the measurable data of individuals to mindfulness practice with Tao Calligraphy together with subtle energy transmission, specifically focused on the mitochondrial DNA content in peripheral blood Leukocytes. Thus, proposing another method of prolonging life expectancy that could be integrated into conventional medical treatment and care.

THEORETICAL FRAMEWORK

Tao Calligraphy art has been intensively studied at Sha Research Foundation in the US and Canada since 2013, producing significant results in improving overall well-being and an efficacious intervention for individuals with chronic conditions. This is a unique style of moving meditation, where mindfulness (heightened awareness) is achieved by the combination of movement and focus on

Calligraphy art. In this practice, the subjects trace the lines of calligraphy with fingers and simultaneously say loudly or silently an affirmation (mantra), which enables them to achieve deep concentration during wakefulness.

No risks or negative side effects of the transmission of pure energy given to human participants or meditation with Tao Calligraphy have been observed in these pilot projects and formal studies.

The proposed research is a pilot project, based on experiential data obtained from several previous pilot and formal studies by our research teams and researchers from other institutions. It focuses on the effects of transmission of subtle energy (blessing) and meditation on improving the Mitochondrial DNA content in peripheral blood leucocytes. We believe, the study will provide further data to offer an integrative intervention for individuals seeking additional treatment that is of minimal risk and to maximize benefits to address condition.

METHOD

This study will determine the effects of transmission of a subtle energy and mindfulness practice with Tao Calligraphy as measured through subjective and objective evaluations of changes in the subjects by Laboratory assessment of Peripheral blood Leucocytes Mitochondrial DNA content and subjective changes in RAND SF 36 Questionnaires.

Design of the Study

The study will be performed as a Pilot follow-up study, should the 0 Hypothesis be successfully rejected, this study will be followed by a formal Double Blind Randomized Crossover Study.

We plan subjects may enter the study as a group and will have all measurements (blood sample for mt DNA analysis and Rand SF 36 Questionnaire) done upon entry into the study – at the baseline 0-time point; at the 3-months time point, at the 6-months time point and at the 12-months time point.

As this is a pilot study, there will be no randomization into treatment and control group and all subjects will receive a transmission and then will be practicing Tao Calligraphy mindfulness daily during the 12 months.
All study participants will be instructed on how to practice and start daily Tao Calligraphy mindfulness practice at baseline zero time point. During mindfulness practice with Tao Calligraphy “Greatest Love” and Tao Song “Greatest Love”, participants will repeatedly trace the lines of calligraphy with fingers while listening to, singing, or chanting with Tao Song. This enables them to achieve deep concentration, while maintaining fully alert state. The practice can be done in sitting or standing, depending on the health status and age, will last 30 minutes and is done daily.

The study is conducted on an outpatient basis and includes:

- a telephone discussion (clarification of inclusion/exclusion criteria)
- signing of the consent/information; instruction in the study design and practicing the Tao Calligraphy-tracing meditation; first data collection questionnaires (0-time point).
- regular weekly meditation practices on Zoom with participants in the treatment arm
- Laboratory assessment of mt DNA in peripheral blood Leucocytes at the baseline zero time point; at the 3-months time point, at the 6-months time point and at the 12-months time point.

- Completion of the questionnaires by the participants at the baseline 0-time point; at the 3-months time point, at the 6-months time point and at the 12-months time point.
- Completion of assessments by a Study principal and co-investigators.

Data collection:

A Laboratory Assessment:
Laboratory assessment of mt DNA in Peripheral Blood Leucocytes:

Blood samples will be drawn at accredited institutions in Life Labs or another accredited institution in Canada and at accredited institutions in USA and will be promptly sent by currier for Mitochondrial DNA Assessment to:
CD Genomics, SUITE 111, 17 Ramsey Road, Shirley, NY 11967
Reports will be issued to clinical Principal Investigator Dr. Peter Hudoba
All participants will have blood samples drawn at the baseline 0-time point; at the 3-months time point, at the 6-months time point and at the 12-months time point.

B Improvement of general well-being or clinical well-being as measured by standardized questionnaires

These questionnaires will be used in the study:
1. John Ware's SF-36 Quality of Life questionnaire
2. Simple Follow up Questionnaire of our design

All participants will complete questionnaires at the baseline 0-time point; at the 3-months time point, at the 6-months time point and at the 12-months time point.

Study Execution

The study will be performed by collecting enrolment data directly from participants personally, over the phone, via email, or on-line (Zoom). The research Questionnaires will be filled on-line using personal ID Codes – to ensure confidentiality. The codes will be emailed by the PI to each of the participants individually.

Organizing data collection, data handling, and data assessment will be the responsibility of the Sha Research Foundation's research team members who have a medical, nursing, and/or scientific background, and will be performed through personal delivery, by email and online collection.
Members of the research team will NOT administer any transmission of subtle energy (blessing) nor any form of medical support to participants of the study.

Transmission of subtle energy and instruction of subjects on how to practice meditations with Tao Calligraphy will be the sole responsibility of Universal Soul Service Corp.
The transmission of pure energy (blessing) will be done to participants for free by Tao Academy certified teacher remotely. There will be no fees for the teaching on how to meditate nor for participation in the study. Conversely, participants will receive no remuneration for participating in the study.

A copy of Tao Calligraphy Greatest Love and Meditation with Tao Calligraphy instruction sheet will be provided to the participants electronically.

Participants of the study will be required to self-meditate using the Tao Calligraphy for thirty minutes twice every day and to record the length of practice and responses during and after each practice. Besides individual meditations on their own, participants will attend twice a week 30-minute-long group meditation practice sessions led by an instructor, done over the Zoom platform.

Participants in the study should continue to follow their physicians' advice and recommendations during their participation in the study and should continue to receive any and all conventional treatment in which they have been participating prior to entry into this study.

Sha Research Foundation will clearly inform participants that the teaching and research teams are not offering any medical diagnosis, guidance, evaluation, or treatment, and that participation in the study is not a replacement for any conventional medical treatments or diagnosis.

Participants are also encouraged to continue their usual spiritual practices in which they have been participating prior to the study.

Participants of the study will be responsible for informing the research team of any and all of their illnesses and medications taken, but NO copies of their medical records from their medical practitioners or specialists will be collected besides Mitochondrial DNA reports.

The study will commence in 2025 and will last for about 3 years. The plan is to accept new subjects into the study at any time from its commencement in 2025 up to 3 years later (from November of 2025 with expected conclusion in December of 2028).

A minimum of 10 and a maximum of 50 subjects may be admitted to this study during the study years. Subjects can withdraw from the study with no penalty at any time for whatever reasons they may have.

4.0 Enrolment Criteria (who can be in your study and who would not be eligible to participate in your study):

Patient Population

Inclusion criteria
- Age 19 and over
- Willingness and ability to comply with data collection requirements
- Submission of required documentation before entering the study, including informed consent and consent to release of information
- Healthy or Ill, with the exception of genetic illnesses and cancer (for which treatments could negatively impact measurement) and Bipolar disorders, other serious mental disorders (e.g. schizophrenia, psychosis),
- Willingness to allow their data to be used for research purposes and published as deemed fit (while conforming to all applicable privacy laws) by Sha Research Foundation.
- Willingness to practice the daily calligraphy meditations and follow the protocol.

Exclusion criteria
- Not meeting any of the inclusion criteria

- Bipolar disorders, other serious mental disorders (e.g. schizophrenia, psychosis), genetic illnesses (primarily affected chromosomes), and cancer (treatment could negatively impact telomere during research period)
- inability to sign consent and follow instructions
- Unwillingness to participate in data gathering
- Unable to follow the practice regimen, including the daily calligraphy meditations
- Pregnant or nursing. Participants who become pregnant during the study will be required to end their participation. (to avoid any, at current time unknown, potential negative effect of the study on the fetus).
- There are no exclusion criteria placed upon potential subjects related to national origin, culture, ethnicity, race, sex, physical disability, sexual orientation, religion, or spiritual practices.

5.0 Sample Size:

A/ Sample Size:

The study will be conducted as a simple follow up study. It will include subjects / patients who are at least 19 old at the time of the examination.

The number of cases estimated was 10 (minimum) <= N <= 50 (maximum).

For the future formal randomized study, we will calculate the power (based on this pilot study).

The evaluation of the changes in the Mitochondrial DNA content and Quality of Life questionnaires is done by means of the program Minitab or SPSS / PSPP using appropriate parametric and non-parametric tests: For statistical analysis of scores obtained from questionnaires we will use Anova, T-test and regression analysis, with confidence level set at 95%.

6.0 Recruitment and Screening Methods:

Recruitment announcement with be placed at:
Sha Research Foundation website: www.ShaResearchFoundation.com
Sha Research Foundation Twitter, Instagram and Facebook social media.
Newspaper and magazine advertisements.
We are submitting previously approved letter and advertisement for the study Pro00031067.

SCREENING METHODS:

Member of the research team will contact candidates for the research study over the phone, set up zoom interviews, conduct discussions (clarification of inclusion / exclusion criteria), help with signing of the consent /Information, and offer instruction in the study design.
Successful applicants will sign the consent and forward it to clinical / research principal investigators.

7.0 Research Locations:

Cynthia Hamilton
943 Beaumont Drive, North Vancouver, BC V7R 1P5
Phone: 778 847-3617 / cynthialhamiltonconsulting@gmail.com

8.0 Multi-site Research (research that involves external collaborating institutions and individuals):

N/A

9.0

10.0 **International Research (where data collection will occur outside the United States and U.S. territories, including online activities)**

This pilot research will take place in Canada and USA exclusively.

11.0

12.0 **Procedures Involved:**

The study is conducted on an outpatient basis and includes:

- A personal or telephone discussion (clarification of inclusion / exclusion criteria) signing of the consent /Information.
- instruction in the study design and practicing the Tao Calligraphy-tracing meditation;
- first data collection questionnaires at the 0-Time point.
- Blood sample drawn and telomere assessment at the 0-Time point.
- regular Zoom practices with participants in the intervention group (or pre-recorded meditation practices with certified teachers as a back-up)
- Completion of the questionnaires by the participants at the 3-months time point.
- Blood sample drawn and telomere assessment at the 3-months time point.
- Completion of the questionnaires by the participants at 6-months time point.
- Blood sample drawn and telomere assessment at 6-months time point.
- Completion of the questionnaires by the participants at the 12-months time point.
- Blood sample drawn and telomere assessment at the 12-months time point.
- Completion of assessments by a Study principal co-investigator.

13.0 **Research with Vulnerable Populations (if children are the ONLY vulnerable population you plan to enrol, do NOT complete this section -- instead fill out Appendix A)**

N/A

14.0

15.0 **Incomplete Disclosure or Deception:**

N/A

16.0

17.0 **Consent Process:**

Trained research team member(s) will follow SRF Consent process V1.0 20230409.
After obtaining the name and contact of potential participant from the PI, research team member will connect with the potential subjects for screening using inclusion and exclusion criteria of the study. This will be done either in person, over the phone, or by Zoom meeting. If the consent is done by phone call or Zoom, subject will sign the consent, witnessed by a second person and mail to Clinical / Research. Participant will be given a study information letter for the participants, explaining the study in more detail.
For consent form see Main ICF.
The signed consent will be stored at Sha Research Foundation for 7 years.

18.0

19.0 **Waiver of Participant Signature on Consent Form:**

N/A

20.0

21.0 **Waiver of Participant Signature on Consent Form:**

N/A

22.0 **Financial Compensation:**

There will be no compensation of research participants. Participants may incur costs related to copying or faxing documents, mailing bloodwork.
Participants will receive free instruction and weekly practices with instructor.
Participants will receive fee for service transmission of subtle energy (blessing) and free electronic version of Tao Calligraphy.
Research involves minimal (no known) risk to the participants; therefore, no compensation will be available to the participants.

23.0

24.0 **Audio/Video Recording/Photography**

We will not be performing any video, audio, or photographic recording of subjects, but may request pictures of analysis from diagnostic laboratory.

25.0 **Potential Benefits of this Research:**

There has been a growing number of empirical studies of Chinese calligraphic handwriting that resulted in improvements in visual attention and span, increased mental concentration, confirmation as an effective treatment for psychosomatic conditions and post-traumatic hyper-arousal symptoms, and exhibiting significant effects on hypertension and type 2 diabetes. Leisure activities occurring later in life that include calligraphy writing may inhibit cognitive decline.
Our own studies clearly documented improvement in Wellbeing Scores (Rand SF-36 questionnaire), improvement in pain scores (McGill pain questionnaire) in subjects suffering from chronic pain, and improvement in depression scores (BDI, PHQ9 and HAM questionnaires) in patients with depression. Our reports have been published and presented at conferences.

26.0 **Potential Risks to Participants:**

No risks or negative side effects of the blessings given to human participants or calligraphy meditation with Tao Calligraphy have been observed in our previous pilot projects and formal studies.

27.0 **Provisions to Protect Participant Privacy and Data Confidentiality:**

All scientific data will be stored in the computer in spreadsheet under ID code numbers according to Tri Council Policy Statement: Ethical Conduct for Research Involving Humans (2005).
A unique ID code number will be assigned to each participant for entry for internal tracking. Once the unique ID number is assigned, no personal identity information is kept with research spreadsheets.
The data collected via website will have only confidential ID assigned and never any names or personal identifiers. As there are no names associated with participants' responses in their electronic research files on the website, it would be impossible to know which subject these responses belong to. These data are periodically downloaded and originals are deleted from the website for extra layer of precaution.
When client provides medical or personal information in paper form, these are scanned and forwarded to research team by email. Paper original documents are returned to participant who provided them. Once the email with documents is received by the research team, downloaded to a dedicated research external hard drive, and confirmed receipt, the emails with files are deleted from email inbox.
All collected personal and medical information is stored on external hard drive that can be accessed only through a password-protected computer.
There will be no hard copies of data printed out, although the results of statistical tests and actual papers for presentation will be in paper form.

28.0 **Recruitment and Screening Methods:**

Recruitment announcement with be placed at:
Sha Research Foundation website: www.ShaResearchFoundation.com
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29.0 **Research Locations:**

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943 Beaumont Drive, North Vancouver, BC V7R 1P5
Phone: 778 847-3617 / cynthialhamiltonconsulting@gmail.com

30.0 **Multi-site Research (research that involves external collaborating institutions and individuals):**
N/A

31.0

32.0 **International Research (where data collection will occur outside the United States and U.S. territories, including online activities)**
This pilot research will take place in Canada exclusively.

33.0

34.0 **Procedures Involved:**

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signing of the consent /Information.

- instruction in the study design and practicing the Tao Calligraphy-tracing meditation;
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- Blood sample drawn and telomere assessment at the 3-months time point.
- Completion of the questionnaires by the participants at the 6-months time point.
- Blood sample drawn and telomere assessment at the 6-months time point.
- Completion of the questionnaires by the participants at the 12-months time point.
- Blood sample drawn and telomere assessment at the 12-months time point.
- Completion of assessments by a Study principal co-investigator

35.0 **Research with Vulnerable Populations (if children are the ONLY vulnerable population you plan to enrol, do NOT complete this section -- instead fill out Appendix A)**

N/A

36.0

37.0 **Incomplete Disclosure or Deception:**

N/A

38.0

39.0 **Consent Process:**

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For consent form see Main ICF.
The signed consent will be stored at Sha Research Foundation for 7 years.

40.0

41.0 **Waiver of Participant Signature on Consent Form:**

N/A

42.0

43.0 **Waivers and Alterations of Consent Information:**
N/A

44.0 **Financial Compensation:**
There will be no compensation of research participants.
Participants may incur costs related to copying or faxing documents.
Participants will receive free instruction and weekly practices with instructor.
Participants will receive free transmission of pure energy (blessing) and electronic version of Tao Calligraphy.
Research involves minimal (no known) risk to the participants; therefore, no compensation will be available to the participants.

45.0

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47.0 **Potential Benefits of this Research:**

There has been a growing number of empirical studies of Chinese calligraphic handwriting that resulted in improvements in visual attention and span, increased mental concentration, confirmation as an effective treatment for psychosomatic conditions and post-traumatic hyper-arousal symptoms, and exhibiting significant effects on hypertension and type 2 diabetes. Leisure activities occurring later in life that include calligraphy writing may inhibit cognitive decline.
Our own studies clearly documented improvement in Wellbeing Scores (Rand SF-36 questionnaire), improvement in pain scores (McGill pain questionnaire) in subjects suffering from chronic pain, and improvement in depression scores (BDI, PHQ9 and HAM questionnaires) in patients with depression. Our reports have been published and presented at conferences.

48.0

49.0 **Potential Risks to Participants:**

No risks or negative side effects of the blessings given to human participants or calligraphy meditation with Tao Calligraphy have been observed in our previous pilot projects and formal studies.

50.0 **Provisions to Protect Participant Privacy and Data Confidentiality:**
All scientific data will be stored in the computer in spreadsheet under ID code numbers according to Tri Council Policy Statement: Ethical Conduct for Research Involving Humans (2005).
A unique ID code number will be assigned to each participant for entry for internal tracking. Once the unique ID number is assigned, no personal identity information is kept with research spreadsheets.

The data collected via website will have only confidential ID assigned and never any names or personal identifiers. As there are no names associated with participants' responses in their electronic research files on the website, it would be impossible to know which subject these responses belong to. These data are periodically downloaded and originals are deleted from the website for extra layer of precaution.
When client provides medical or personal information in paper form, these are scanned and forwarded to research team by email. Paper original documents are returned to participant who provided them. Once the email with documents is received by the research team, downloaded to a dedicated research external hard drive, and confirmed receipt, the emails with files are deleted from email inbox.
All collected personal and medical information is stored on external hard drive that can be accessed only through a password-protected computer.
There will be no hard copies of data printed out, although the results of statistical tests and actual papers for presentation will be in paper form.

51.0 **Data Monitoring Plan to Ensure the Safety of Participants:**

The collected data will remain the property of Sha Research Foundation and only members of the research team will have the access to the research data collected.

Each participant will complete an Application Form. The signed consent forms will be emailed or faxed to the Foundation. Data collection will be executed at 0, 3, and 9-month intervals after receiving the energy transmission, for a total of 9 months.

Research Data

At the time of admission to the study, participants will provide following:

1) Application that includes information of their illnesses and medications, and consent

2) Laboratory assessment of Mitochondrial DNA in Peripheral Blood Leucocytes. Results of analysis will be stored at CD Genomics, SUITE 111, 17 Ramsey Road, Shirley, NY 11967. Reports will be issued to clinical Principal Investigator Dr. Peter Hudoba, will be scanned and electronic copy will be stored on external hard drive, the paper version will be safely destroyed.

3) Data from
1. John Ware's SF-36 Quality of Life questionnaire
2. Simple Follow up Questionnaire of our design

The data collected via website will have only confidential ID assigned and never any names or personal identifiers. As there are no names associated with participants' responses in their electronic research files on the website, it would be impossible to know which subject these responses belong to. These data are periodically downloaded and stored on external hard drive; originals are deleted from the website for extra layer of precaution.

C) Data Processes

There will a Consent Process form filled after consenting procedure. This will be reviewed.

All applications are audited for completeness.

The log of adverse events will be maintained. (This would also include data issues.)

The other logs would be Protocol Deviation Log, Subject Withdrawal and Termination Log, Subject Visit Log spreadsheet, and Telephone Log.

Application and consent will be scanned and stored on large (not flash drive) external drive by principal investigator. After he confirms receipt, any person obtaining these documents will delete them from their computers and from email inbox. Once principal investigator downloads the data from inbox to a hard drive, any email containing data will be deleted from his inbox.

Research Data collected on password protected entry on the website, will have only first name and confidential ID as identifiers, and so even in unlikely event of breach to website, no research data could be attributable to a particular person. These are downloaded to external hard drive and periodically deleted from website.

All data will be stored for 7 years and will be safely deleted afterwards. Any retiring hard drive will be not only safely erased using military protocol 3 wipe repetition and filling drive with Zeros (Program Avast), but also physically damaged so could not be read from anymore (in accordance with Canadian Medical Protective Association guidelines).

52.0 **Long-term Data and Specimen Storage and Sharing:**

All scientific data will be stored on external hard drive for at least 7 years under ID code numbers according to Tri Council Policy Statement: Ethical Conduct for Research Involving Humans (2005) and HIPAA. All data will be deleted after 7 years.
No personal or attributable medical data will be shared outside of Sha Research Foundation.

53.0 **Qualifications of Research Team to Conduct the Research:**

Peter Hudoba De Badyn, MD, FRCS, neurosurgeon, affiliated as researcher with, and prior CEO of Sha Research Foundation. Previously as a As.Professor of Neurosurgery, University of Saskatchewan.

Mailing address: Mt Seymour Clinic, 2nd Floor, 333 Mt Seymour Boulevard, North Vancouver, BC
Sharesearchfoundation@yahoo.ca

Cynthia Hamilton, PhD, Clinical Researcher, former Assistant Research Director Vancouver Coastal Health Research Institute, BC.
cynthialhamiltonconsulting@gmail.com

Monika Hudoba De Badyn, MD FRCP (Canada), hematopathologist at Vancouver General Hospital, Ass. Clinical Professor UBC, former Division Head and former Department Head Vancouver General Hospital, former regional laboratory director Vancouver Coastal Health, former chair Laboratory hematopathology section in Provincial Laboratory Services, British Columbia. Mailing Address:

Department of Pathology, Vancouver General Hospital, 855 W 12th avenue, Vancouver V5G 1M9

Consuelo Fernandez Rahman MD; Specialist in Pain Medicine, USA,
crm1911@yahoo.com

Genevive Julien, PsychD, Licensed Psychologist, USA,
drgenevivejulien@gmail.com

Jaime Chow, BASc, MSc, RN, Clinical Epidemiologist, stationed in UK.
jaimekchow@gmail.com

Stacey Drew OTD. OTR/L, Clinical Occupational Therapist, stationed in San Francisco.
Sdrewotd@gmail.com

54.0 Publication of the Research:

Authorship eligibility guidelines and any intended use of professional writers:
We will follow ICMJE - International standards for reporting research: https://phcogcommn.org/wp-content/uploads/2021/08/icmje-recommendations.pdf

Authorship of the data of this study will be based on:
1. degree of contribution to the conception or design of the study
2. degree of creating any important intellectual content of the study
3. degree of participation on creating final report
4. an agreed role in the conduct of the study.

The results of the study will be published on the Foundation website and in scientific meetings and journals. The audience is expected to be the health care, scientific, and integrative, complementary, and alternative medicine communities.

The research data, data on the reports delivered to PI, and results of the study remain the intellectual property of Sha Research Foundation.
The laboratory data and results of Telomere assessment stored at laboratory site will remain the property of Repeat Diagnostics, North Vancouver, BC, Canada.

1.0 Budget the Research:

Legal and Study overhead cost:

IRB ethics Initial review and approval fee	$5235.00
Advarra Research protocol yearly review fee	$3100.00
TOTAL OVERHEAD 1st Year	**$8335.00**

Specimen handling and testing:

Venipuncture Fee & specimen labeling/handling	$65 (in USA $34 - $54.00)
Specimen packaging*	

**See picture below for details*	$8.10-9.45
UN3373 shipping box: https://www.uline.ca/	
Biological substance category B	
FedEx Priority overnight	$168 CAD (120 USD)
mt-DNA copy number detection,	$168 CAD (120 USD)
gDNA extraction	$34 (25 USD)

TOTAL cost 1 measurement per 1 SUBJECT: $443
TOTAL cost whole study measurements per 10 SUBJECT: $17,724

Appendices and additional documents:

Appendix C: CVs
Informed Consent Form
Information Letter to Subjects
Rand SF 36 Questionnaire (screenshot from website)
Follow up form of our design (screenshot from website)

_____ _____
Allan Chuck Date
President and CEO
Sha Research Foundation

_____ _____
Peter Hudoba De Badyn Date
Principal Clinical Investigator
Director of Research at SRF