Study Title: Distribution of cell-cell junction proteins in arrhythmic disorders

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CHIEF INVESTIGATOR (CI):
Angeliki Asimaki, PhD
Senior Lecturer/ Scientist; Cardiology Clinical Academic Group
Cardiovascular and Genomics Research Institute
St. George's, University of London
Cranmer Terrace, London, SW17 0RE

Phone: 02087252817
Email: aasimaki@sgul.ac.uk

SPONSOR: City St George's University of London

SPONSOR REPRESENTATIVE:

Name Lauren Thomson
Address: City St George's Joint Research Enterprise Office
Hunter Wing, Ground Floor,
Cranmer Terrace SW17 0RE
Phone: 020 8725 5013
Email: researchgovernance@sgul.ac.uk
Fax: 020 8725 0794

Funding Source:
British Heart Foundation/private donation: consumables for conduction of the analysis.
The NHS will provide service support for the materials required to obtain the patient samples.

Statement

The Chief Investigator (CI) and the Sponsor representative have discussed this protocol version. The investigators agree to perform the investigations and to abide by this protocol except where departures from it are mutually agreed in writing.

The Investigator agrees to conduct the trial in compliance with the protocol, GCP, the Data Protection Act (1998), the Trust Information Governance Policy (or other local equivalent), the Research Governance Framework (2005 2nd Edition), the Sponsor's SOPs, and other regulatory requirements as appropriate.

This protocol has been written in accordance to the Sponsor's procedure identified as: JREOSOP0039 "Protocol Design" and is intended for use at UK sites only

Chief Investigator Dr. Angeliki Asimaki Senior Lecturer/ Scientist, Cardiology Clinical Academic Group City St George's, University of London	Signature 	Date 12/01/2024

Acknowledgements and Protocol contributories

Dr. Angeliki Asimaki (Cardiology Clinical Academic Group, Molecular and Clinical Sciences Research Institute, City St. George's, University of London, Cranmer Terrace, London, SW17 0RE), conceived and designed the study. Dr. Asimaki is the grant holder, will perform the sample analysis, manage the data and the statistical analysis. If senior statistical support is required, Dr. Asimaki will work with Dr. Chis Ster, an experienced medical statistician who is working closely with the cardiology clinical academic group. Only the study team members will have access to the patients' medical records and to the file that connects the patients with the de-identified, coded samples. All study team members have contributed to the refinement of the study protocol and

approved the final manuscript. The sponsor institution has the final decision about collection, management, analysis and data interpretation as well as submission of results reports.

Contents

1 List of abbreviations

ACM	Arrhythmogenic Cardiomyopathy
CI	Chief Investigator
Cx43	Connexin 43
HRA	Health Research Authority
ICD	Inherited Cardiac Condition
ICF	Informed Consent Form
ISF	Investigator Site File
NHS R&D	National Health Service Research & Development
PI	Principal Investigator
PIS	Participant Information Sheet
REC	Research Ethics Committee
SCD	Sudden Cardiac Death

Roles and Responsibilities –

Principal Investigator (PI): Dr. Angeliki Asimaki
Cardiology Clinical Academic Group
Molecular and Clinical Sciences Research Institute
City St. George's, University of London
Cranmer Terrace, London, SW17 0RE

E-mail: aasimaki@sgul.ac.uk

Statistician: Dr. Irina Chis Ster
Senior Lecturer in Biostatistics
City St. George's, University of London
Cranmer Terrace, London, SW17 0RE

E-mail: i.chisster@sgul.ac.uk
Phone: 020 8725 5636

Medical Experts

Study synopsis

Brief title:	Distribution of cell-cell junction proteins in arrhythmic disorders
Official title:	Analysis of distribution and expression levels of cell-cell junction proteins in buccal cell samples from patients with arrhythmic disorders and family members at risk as a means for diagnosis
Sponsor reference number:	16.0099
Study type & Phase	Basic science study involving procedures with human participants
Study design	Laboratory study/ Non-experimental confirmatory correlational research
Study Population/disease condition	Individuals diagnosed with heritable arrhythmic disorders and family members at risk of sudden cardiac death.
Eligibility criteria:	***Inclusion criteria:*** • Participants will include patients diagnosed with a heritable arrhythmic disorder (including arrhythmogenic, hypertrophic and dilated cardiomyopathy, cardiac sarcoidosis as well as cardiac

	channelopathies; Long QT syndrome, Brugada syndrome and catecholaminergic polymorphic ventricular tachycardia • Family members of victims of SCD evaluated for risk assessment and diagnosis. These groups include both individuals with clear disease manifestation (termed "affected") as shown by conventional diagnostic approaches (electrocardiography, echocardiography, cardiac MRI, Holter monitoring) as well as potential carriers of disease-causing mutations who, however, may not/not yet manifest any overt sign of cardiovascular abnormalities (termed "carriers"). These are typically family members of probands diagnosed with a heritable arrhythmic disorder or family members of a sudden cardiac death victim. • All individuals that fall in the above categories will be included regardless of their management (medication, devices, and surgical procedures). • Individuals with co-existing conditions will also be included and their medical history will be taken into account when interpreting the results of the immunohistochemical analysis. • Adult individuals (>18 years of age). • Pregnant women will be included as the approach used is not in any way harmful or uncomfortable. • All individuals must have provided the study team with a signed informed consent in order to participate in the study.
	Exclusion criteria: • Children under 18 years of age • Individuals lacking decisional capacity. • Individuals with non-heritable, non-arrhythmic cardiac disorders (such as ischemic heart disease or inflammatory disorders) Non-English speakers will be excluded from the study unless a translator is present who can thoroughly explain to them the research question/plan in order for them to provide an informed consent.
Target number of participants	We anticipate to recruit 800 participants for this study over the course of 4 years

Criteria for evaluation	**Primary outcome measure(s):** The primary outcome of the study is to evaluate if specific heritable arrhythmic disorders are associated with changes in selected protein distribution/expression levels at cell-cell junctions within the buccal mucosa. Our preliminary studies suggest that protein distribution/expression alterations in the buccal mucosa mirror equivalent changes that occur in the myocardium of patients with ACM. This study will evaluate the use of this "novel diagnostic tool" in individuals at risk of sudden cardiac death due to other, more common forms of arrhythmic disorders including the cardiac channelopathies. The processing approach (laboratory technique: immunocytochemistry) for protein distribution visualization has been optimized to be qualitative rather than quantitative. In this sense - each sample will be characterized by "presence" or "absence" of a specific protein at the cell-cell connections. We deem that the qualitative approach is not subject to bias and will ensure accuracy and reproducibility of the results.
	Secondary outcome measure(s): A potential secondary outcome of the research is whether we can correlate protein distribution in the buccal mucosa with specific genotypes. Our preliminary results on the cohort of patients with ACM suggest that mutations in different genes result in different protein distribution patterns within the buccal mucosa. Being able to predict the gene bearing the disease-causing mutation will significantly reduce the time and cost of genetic sequencing. Identifying the specific gene underlying a heritable arrhythmic disorder is of pivotal importance as increasingly developing genotype/phenotype correlations help individualized risk identification and patient management. Another potential secondary outcome of the research has to do with the effect changes in the patients' treatment plans may have on protein distribution. An example has to do with a protein known as Connexin43 (Cx43). Cx43 is the major gap junction protein expressed in the heart and is responsible for the electrical coupling of the cells and the smooth propagation of electricity throughout the myocardium. Cells at the buccal mucosa also express Cx43. It has been shown that patients with arrhythmic disorders show decreased distribution of Cx43 at the cell borders in their hearts and this "Cx43 remodeling" has been associated with an increased risk for arrhythmias. Our preliminary studies show that patients with ACM, as well as patients with different types of cardiomyopathy (including dilated, hypertrophic and ischemic) show Cx43 remodeling in their buccal mucosa. If a patient shows Cx43 remodeling during initial sampling and in a follow-up visit his clinical care team deems necessary to change his treatment plan (for instance, if he/she is to be started on a different anti-arrhythmic drug) we would like to study Cx43 distribution again during one of his/hers follow up visits. If the new treatment plan results in restoration of Cx43 at the buccal mucosa cell connections, and given how protein distribution in the buccal epithelium mirrors protein distribution in the heart, this finding may be an indication of successful arrhythmia management. It could thus, potentially, provide us with a non-invasive, straightforward and risk-free means to assess therapy efficacy in the future.

	Finally, a secondary outcome of the research has to do with the effect mutations have on protein expression levels. We are aware that mutations change the distribution of proteins but to date we do not know whether the proteins of interest are actually expressed at lower levels, overly degraded or just shifted from a concentrated membrane localization to a more diffuse cytoplasmic distribution pattern. Western immunoblotting can help us answer this question and establish the actual mode of action of disease-causing mutations. Establishing the mechanism of disease is of pivotal importance both for diagnosis and for future mechanism-based therapies.
Sources of funding	British Heart Foundation/private donation
Anticipated start date:	02/01/2017
Anticipated primary completion date:	30/06/2026
Sponsor/Co-Sponsor	City St George's, University of London
Key Contact names	Sponsor representative: Lauren Thomson Email: researchgovernance@sgul.ac.uk Tel: 020 8725 5013 Fax: 020 8725 0794 Chief Investigator: Dr. Angeliki Asimaki Email: aasimaki@sgul.ac.uk

Background

Analysis of myocardial samples has provided us with novel diagnostic markers for cardiomyopathies[1] and inflammatory heart disorders.[2] However, myocardial samples are difficult and risky to obtain from patients and impossible to obtain from family members, who do not manifest evidence of the disease but may still be carrying genetic alterations that put them at risk of SCD. We showed that the buccal mucosa expresses similar proteins to those expressed in the heart and may thus be used as a surrogate for the myocardium.[3] We also showed that alterations in protein distribution occurring in the heart of patients with cardiomyopathies, also occur in their buccal cells, potentially providing us with a quick, easy, and totally risk-free means of diagnosis.[3] We now want to apply this method to another group of heritable arrhythmic disorders; the cardiac channelopathies. In a sub-study we want to establish the mode of action of disease-causing mutations. By examining the actual expression levels (in addition to the distribution) of key proteins we can establish whether the mutations cause disease through reduced expression, increased protein degradation or merely protein re-distribution. Establishing the mechanism of disease pathogenesis will improve diagnosis, prognosis and form the basis for mechanism-based therapies.

Study Rationale

This study will not have a direct benefit to the participants. However, if patients/family members decide to participate, they will help individuals at risk of sudden cardiac death (SCD) in the future. The goal of the study is to evaluate a novel, totally non-invasive, risk-free method for diagnosis of inherited heart diseases. This will aid cascade screening with the potential to diminish the burden of SCD in our society. The results of the study will also help us gain insights into mechanisms of disease pathogenesis improving our understanding and management of arrhythmic disorders. The efficacy of this approach in diagnosing and understanding ACM was published in 2016.[3]

Study objectives

Primary objective

The primary objective of this study is to use buccal smear samples to diagnose arrhythmic disorders. We know that heritable heart diseases affect the distribution of proteins at the cardiac intercalated disk – the area where heart cells are coupled mechanically and electronically. In the past, we have analyzed specific protein distribution patterns in heart samples to aid with patient diagnosis. Obtaining a biopsy sample from the heart, however, is an invasive, potentially risky procedure, which is not performed unless absolutely necessary and only in specialized centers. We recently showed that the buccal mucosa shows protein distribution abnormalities equivalent to those exhibited by the heart. This has provided us with a fast, inexpensive, totally risk-free way to potentially identify those individuals at risk of SCD. Moreover, the majority of the heritable heart diseases are characterized by incomplete penetrance; this means that even members of

the same family bearing the same genetic alteration may not manifest the disease to the same degree. Some family members may be “silent carriers” of the genetic change, and may thus not exhibit heart abnormalities measurable by conventional tests (such as electrocardiography and echocardiography). But they may still be at risk of SCD. It is not possible to justify a heart biopsy in a “carrier” showing no evidence of disease. The approach we are proposing, however, is giving us a totally risk-free means to cascade screen family members of individuals diagnosed with an arrhythmic disorder. This approach will also facilitate cascade screening of individuals involved in competitive, high endurance sports, who are known to be at increased risk of SCD.

Secondary objectives

Our preliminary studies suggest that we can use a buccal smear sample not only to identify those at risk of SCD but also to predict the genotype – which protein is bearing the disease-causing alteration. This correlation would allow us to significantly increase time-and cost-effectiveness of DNA sequencing by targeting analysis to those candidate proteins that appear to be mis-localized in the buccal mucosa. Timely identification of the disease-bearing protein is of pivotal importance as it allows for genotype-phenotype correlations; associations between a specific location/type of mutation and the course of the disease, thus improving risk stratification and patient management.

Another objective of this study could potentially be to evaluate the effect of treatment changes/ interventions on protein distribution in the buccal mucosa, which we anticipate would mirror equivalent changes in the heart. For instance, the main electrical coupling protein in the heart (and the buccal mucosa) is Cx43. Patients with heart disease have been shown to have abnormal distribution of this protein at the cell-cell connections in both tissues. Remodelling of this protein has been previously associated with arrhythmias, and it in certain cases it is considered a primary factor in their generation. If a patient is decided by his consultant cardiologist to start treatment with a new antiarrhythmic drug, it would be very informative to see whether distribution of Cx43 at the buccal mucosa is altered in response to the new treatment. This would allow us to make predictions in terms of risk stratification and treatment efficacy.

Finally, an objective of our sub-study, is to evaluate the mode of action of disease-causing mutations. We know that disease-causing mutations alter the distribution of key proteins. What we do not know is whether mutations affect the expression levels of key proteins. The approach we will use (Western immunoblotting) can show whether key proteins are expressed at normal or reduced levels. This will be of pivotal importance to establish the efficacy of mechanism-based therapies such as AAV.

Trial design

Overall design

Individuals will be recruited by their regular medical care providers. Once they have read the information sheet and understood the process entirely, if they agree to participate, they will sign

an informed consent form, a copy of which they will get to keep. If the sampling is taking place at home, the patient needs to return the signed consent form to City St George's, University of London along with their samples. If seen on site, the patient will then provide a study team member with 6 buccal smear samples. These samples will be transferred to Dr Asimaki's laboratory at City St George's, University of London. If using the at-home kit, the patient will obtain the buccal smears himself and then mail them to SGUL. The samples will be identified by a study ID number. There shall be no personal or medical information noted on them. Once at Dr. Asimaki's laboratory at City St George's, University of London, they will be subjected to immunohistochemical analysis to characterize specific protein distribution. Dr. Asimaki and her lab team members will be blinded to the patient information. There shall be a single copy of a file linking the study ID number to a specific patient, accessible only by the patients' regular medical care providers.

Patients participating in the protein expression sub-study will provide a study team member with buccal cell samples to be transferred to Dr Asimaki's laboratory at City St George's, University of London. Such samples can only be obtained on-site and not by the patients themselves at-home.

Intervention plan and rationale

If seen at the hospital, the study participants will just be asked to open their mouth. A study team member will rub a soft brush a few times at the inside of their cheek and smear the brush on a microscopy slide. The slide will be sprayed with 70% ethanol to preserve the material and taken to the research laboratory. The patient will have a total of 6 smears taken (3 from each cheek). For the majority of the patients, only a single sampling will be enough which will take place during one of their regular follow-up appointments at the cardiology clinic or using the at-home kit. There is no pain and no discomfort associated with the procedure and it lasts only a few seconds. In selected cases, however, if for instance we want to use the buccal smear to evaluate the effect of a change in medical treatment on protein distribution, the patient might be asked to provide the study team with another sample, again during one of his scheduled regular follow-up visits or using the same self-sampling kit at home. If the at-home kit is being used, the patient will be asked to obtain their own buccal smear samples using the materials provided and mail the slides back to City St George's, University of London.

Exfoliated buccal mucosa cells have been used as a source of material for various genetic tests and in studies of oral cancer but their use in studies of cardiovascular disease has been limited. Previous applications have included analysis of telomere length in buccal cells from patients with ischemic heart failure and measurements of intracellular magnesium levels in patients undergoing radiofrequency catheter ablation for atrial fibrillation. To the best of our knowledge, our study published at Circulation Arrhythmia and Electrophysiology 9(2) in 2016, was the first analysis of buccal mucosa cells in patients with a heritable form of heart disease.[3] This analysis included 39 patients diagnosed with ACM, a primary myocardial disease characterized by an unusually high burden of arrhythmias and SCD as well as 15 carriers of disease-causing mutations without overt disease manifestation. In a subsequent analysis (unpublished data), 55 additional individuals affected by ACM were sampled and the positive predictive value of our approach in diagnosing the disease was 91.9%. Although highly arrhythmogenic, ACM is a

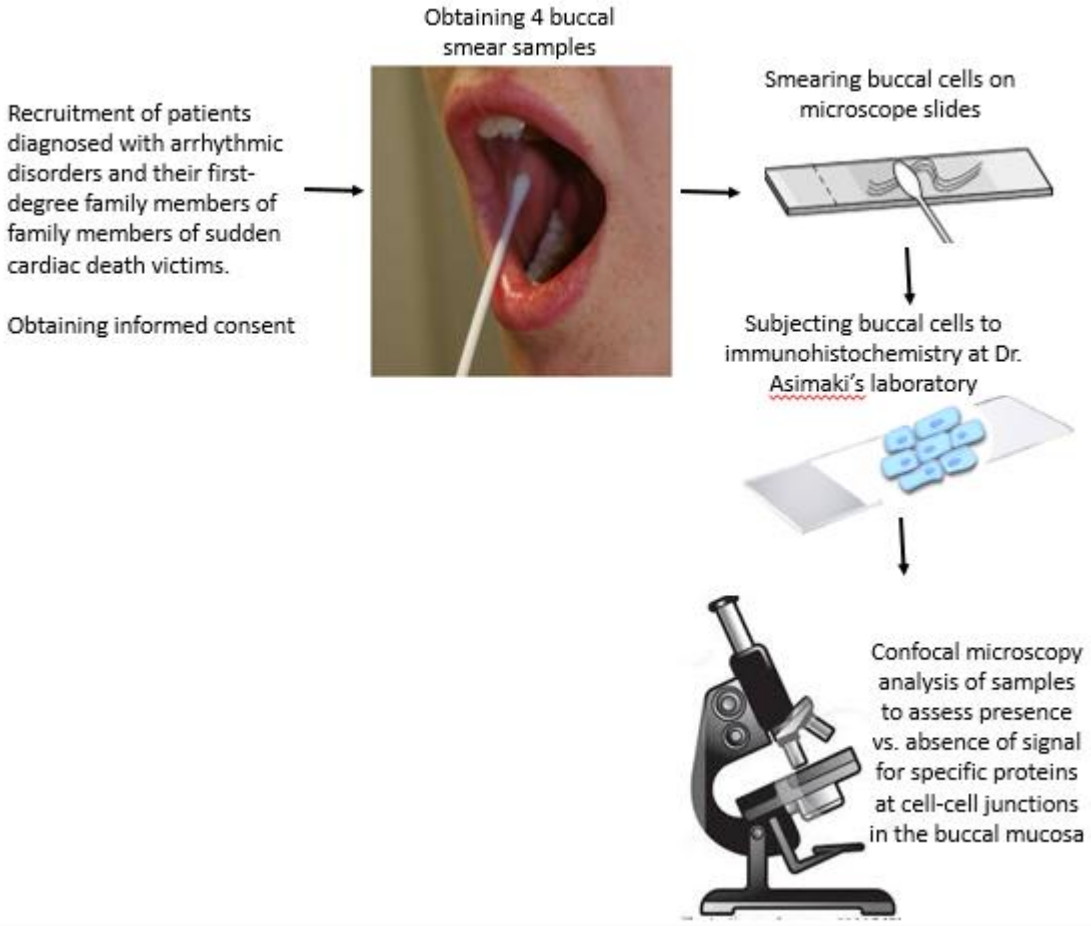
relatively rare disorder. We are now at a position to apply this simple, totally risk-free approach to help identify those individuals at risk of SCD due to more common forms of heritable arrhythmic disorders including the cardiac channelopathies. The cardiologists at centres included evaluate more than 500 families of SCD victims per year for diagnosis and risk assessment. The large number of individuals evaluated at these sites provides the unprecedented opportunity to use this novel diagnostic approach to aid significant numbers of those at risk of developing life-threatening arrhythmias. There are no risks or potential discomfort associated with the study for the volunteer participants. The outcomes, however, may prove highly beneficial for prevention of SCD, timely and accurate diagnosis and management of arrhythmic disorders.

Those participating in the sub-study, will be asked to open their mouth. A study team member will rub a toothbrush at the inside of their cheeks for 30 seconds. The toothbrush will then be placed in a tube of de-ionised water for the cells to be released and taken to the research laboratory. In the majority of the cases, a single collection will provide sufficient cell numbers. In selected cases, when not enough cells have been collected, we may need to rub the toothbrush against the cheek's inside for an additional 30 seconds. There are no risks or potential discomfort associated with the study for the volunteer participants. The outcomes, however, will provide much-needed insights into actual mechanisms of disease pathogenesis.

Additional treatment/ interventions

Not applicable

Schematic of Study design



Participation selection criteria

There will be no exceptions (waivers) to eligibility criteria prior to participant inclusion into the study. Any questions raised about eligibility should be addressed prior to entering the participant.

The eligibility criteria have been carefully considered and are standards used to ensure the trial results can be appropriately used to make future treatment decisions for other people with similar disease or medical condition. It is therefore vital exceptions are not made to the following detailed selection criteria.

All participants that are screened for inclusion into the study must be entered onto the Sponsor screening log JREOLOG0001 and will be assigned a unique study sequential number. Participants will be considered eligible for enrolment into this trial if they fulfil all of the inclusion criteria and none of the exclusion criteria as defined below.

Eligible participants will be entered onto the Sponsors Subject ID log JREOLOG0002 and assigned a unique study specific Identification number in a pre-agreed format in accordance with Site identifier and next sequential numerical value e.g. SG001

Inclusion criteria

- Participants will include patients diagnosed with a heritable arrhythmic disorder (including arrhythmogenic, hypertrophic and dilated cardiomyopathy, cardiac sarcoidosis as well as cardiac channelopathies; Long QT syndrome, Brugada syndrome and catecholaminergic polymorphic ventricular tachycardia).

- Family members of victims of SCD evaluated at the same clinic for risk assessment and diagnosis. These groups include both individuals with clear disease manifestation (termed "affected") as shown by conventional diagnostic approaches (electrocardiography, echocardiography, cardiac MRI, Holter monitoring) as well as potential carriers of disease-causing mutations who, however, may not/not yet manifest any overt sign of cardiovascular abnormalities (termed "carriers"). These are typically family members of probands diagnosed with a heritable arrhythmic disorder or family members of a sudden cardiac death victim.

- All individuals that fall in the above categories will be included regardless of their management (medication, devices, and surgical procedures).

- Individuals with co-existing conditions will also be included and their medical history will be taken into account when interpreting the results of the immunohistochemical or Western immunoblotting analysis.

- Adult individuals (>18 years of age).

- Pregnant women will be included as the approaches used is not in any way harmful or uncomfortable.

- All individuals must have provided the study team with a signed informed consent in order to participate in the study.

Exclusion criteria

- Children under 18 years of age

- Individuals lacking decisional capacity.

- Individuals with non-heritable, non-arrhythmic cardiac disorders (such as ischemic heart disease or inflammatory disorders).

- Non-English speakers will be excluded from the study unless a translator is present who can thoroughly explain to them the research question/plan in order for them to provide an informed consent.

Participant Recruitment process

Patient recruitment at a site will only commence once evidence of the following approval/essential documents are in place:

1. HRA and REC approval,

2. Final sponsorship and host site confirmation of capacity and capability,

Potential participants will be selected by their direct care team Selection will be done based on whether they have been diagnosed with an inherited arrhythmic disorder or are related to a diagnosed patient/victim of SCD and considered to be potentially at risk of SCD. Potential participants will involve only individuals regularly followed-up at the arrhythmia service of the centres involved whose medical information is already known to their direct care team.

Study procedures

Informed consent

Informed consent from the participant will be obtained following explanation of the aims, methods, benefits and potential hazards of the trial and before any trial-specific procedures are performed. All this information is contained in an information sheet, which will be given to the potential participants. They will then be allowed as much time as they need to read through all the sections and get all of their questions answered prior to making a decision. The only procedures that may be performed in advance of written informed consent being taken are those that would have been performed on all participants as routine clinical practice.

Potential participants who do not speak English, will only be included in the study if a translator is present who can translate the information sheet to them and with the aid of the medical providers answer any questions they may have. The delegated study team members will explain that the patients are under no obligation to enter the trial and that they can withdraw at any time during the trial, without having to give a reason. All study team members responsible for obtaining informed consent are GCP trained and will have completed the Sponsor STAFF delegation of responsibility log JREOLOG0004

A copy of the signed Informed Consent Form (ICF) along with a copy of the most recent approved Patient Information Sheet (PIS) will be given to the study participant. An original signed & dated consent form will be retained in the ISF and a copy will be placed in the medical notes.

If sampling is being performed at home, informed consent will be obtained remotely following the below procedure: The principal investigator or a member of the direct care team will contact the participant by telephone to introduce the study. If the participant is amenable, the study team will mail a copy of the PIS and the ICF to him/her and agree for a time to call back. The PIS will then be discussed over the phone. Informed consent will then be taken over the phone, the participant will sign the ICF and the team member will note that consent was obtained in the study documents. The participant will then send the partially signed ICF to City St George's, University of London. The study team will then re-confirm consent over the phone, countersign the ICF and return a fully signed copy to the participant.

A separate PIS and ICF has been created for those participating also in the sub-study.

Discontinuation/withdrawal of participants and stopping rules

If a volunteer decides to participate in the study and later changes his/her mind, he/she will have the right to withdraw without providing a reason. Withdrawing from the study will have no effect on their follow-up care by their medical providers. If they have already provided the research team with a buccal smear sample, they may instruct the study team to:

- Destroy the sample and remove the results of the analysis from the study
- Destroy the sample but maintain the results of the smear analysis in the study
- Maintain both the sample and results in the study

Based on preliminary data, we anticipate that there shall be a strong correlation between specific protein distribution abnormalities at the buccal mucosa and specific arrhythmic disorders. Unlikely as it appears, if following the first year of the study, it appears that we are unable to predict "arrhythmic status" using such samples, we shall stop recruiting patients with cardiac channelopathies and will only focus on patients with cardiomyopathies, where the correlation is already proved.

Based on our pilot data from myocardial samples, mutations acting through haploinsufficiency lead to decreased protein expression levels as shown by western immunoblotting. We anticipate that we will be able to show this also in buccal cells from patients carrying such mutations. If, however, for unpredictable technical reasons we are unable to show changes in key protein expression levels using buccal samples after the first year, we shall stop recruiting participants for the sub-study.

9.3 Definition of the End of Trial

We define the End of the Trial as the day when the last buccal cell sample is obtained and analysed from a volunteer.

Study Procedures

Screening assessments

Participants will fall in either of the following three categories:

1. Patients already diagnosed with a heritable arrhythmic disorder (ACM, hypertrophic cardiomyopathy, dilated cardiomyopathy, Brugada syndrome, Long QT syndrome, catecholaminergic polymorphic ventricular tachycardia). The individuals will only be asked to participate in the study based on their already established diagnosis. No baseline screening assessment will be required.

2. Family members of patients diagnosed with a specific heritable arrhythmic disorder regardless of whether they show disease manifestation or not. These individuals will be asked to participate in the study based on the diagnosis of the proband. No further screening assessment will be required.

3. Family members of SCD victims. If pathology examination of the SCD victims' heart suggests that the individual has died due to a heritable arrhythmic disorder, when his/her family members attend the arrhythmic services at City St George's, University of London for medical evaluation/ risk stratification, they may be asked to participate at the study regardless of whether they show evidence of disease manifestation. No further screening will be required other than the clinical screening that is regularly offered to SCD victim relatives for risk stratification.

Baseline assessments

No baseline assessments are required after the participant has entered the study.

Interventions

The study participants will just be asked to open their mouth. A study team member will rub a soft brush a few times at the inside of their cheek and smear the brush on a microscopy slide. The slide will be sprayed with 70% ethanol to preserve the material and taken to the research laboratory. The patient will have a total of 6 smears taken (3 from each cheek). For the majority of the patients, only a single sampling will be enough which will take place during one of their regular follow-up appointments at the cardiology clinic. There is no pain and no discomfort associated with the procedure and it lasts only a few seconds.

If sampling is being done at-home, the participant will be provided with a kit containing two soft brushes, 6 microscopy slides, a small spray bottle with the fixative, and slide boxes. The

participant will then be asked to obtain the buccal smear samples following instructions on this youtube video: https://www.youtube.com/watch?v=3hIHtaSuijs&feature=youtu.be

Briefly, the participant will be asked to open the packaging of one sterile brush and open their mouth. The participant will then pass the brush several times at the inside of one cheek. The brush will then be smeared across a slide from one edge to the other and sprayed with the fixative 3 times from a distance of 20 centimeters. The slide will he be left at room temperature for 1-2 minutes to dry, following which it can be placed in the slide box provided. This needs to be repeated 2 more times from the same cheek and then an additional 3 times from the other cheek for a total of 6 slides.

For those participating in the sub-study; they will be asked to open their mouth. A study team member will rub a toothbrush at the inside of their cheeks for 30 seconds. The toothbrush will then be placed in a tube of de-ionised water for the cells to be released and taken to the research laboratory. In the majority of the cases, a single collection will provide sufficient cell numbers. In selected cases, when not enough cells have been collected, we may need to rub the toothbrush against the cheek's inside for an additional 30 seconds.

Subsequent assessments

Regardless of the outcome of the baseline analysis, all participants will be asked to provide the study team members with additional samples up to 3 times/year following their appointment, using a self-sampling kit, and then at their next regular follow-up appointment. If the interim results yielding from the analysis of the self-obtained samples, shows abnormal protein distribution, the medical providers may contact the participant and discuss with them the possibility of bringing their next regular appointment forward for discussion and further evaluation. Regarding the sub-study, the majority of participants will only be asked to provide buccal cells once. In selected cases we may invite them to provide an additional sample at their next regular appointment. Our buccal smear work has shown that one protein in particular, Connexin43, shows restored distribution in response to clinically effective anti-arrythmic medication. Yet, to date, we do not know what is the mechanism of action through which drugs fix Connexin43 localization and hence restore normal cardiac rhythm. Our sub-study would show whether the drugs act by increasing protein production or merely shifting the protein to junctional sites facilitating electrical conduction.

Methods

Laboratory procedures (if applicable)

The samples obtained will be mailed to Dr. Asimaki's research laboratory. The fixed slide will be dipped in blocking buffer (97 ml phosphate buffered saline (PBS), 3 ml normal goat serum, 1 gram of bovine serum albumin and 150 microliters of Triton-X for a total of 100ml). The slides shall be left to incubate for 45 minutes at room temperature. The primary antibody will then be added (I use a liquid-repellent pen to draw a circle around the area where the cells are) diluted in blocking

buffer. For plakoglobin the optimal dilution is 1: 1500 and I use the mouse monoclonal antibody from sigma P8087. For Cx43 the optimal dilution is 1:1000 and the antibody is a rabbit polyclonal from Abcam ab11370. For desmoplakin the optimal dilution is 1: 50 and the antibody is a mouse monoclonal from Fitzgerald 10R-D109a. For PKP1 the optimal dilution is 1:20 and the antibody is a mouse monoclonal from santa cruz biotechnology sc-59880. Further protein targets/optimal dilutions will be assessed throughout the length of the study. The primary antibody stays on the slides overnight at 4 ºC. The next day the slides will be equilibrated at room temperature for ~ 30 minutes, and washed 3 times (5 mins each) in PBS + 1% Triton-X. The secondary antibody is diluted in PBS (I use goat anti-mouse or anti-rabbit Cy3 conjugated antibodies from Jacksons ImmunoResearch at a dilution of 1:400) and the slides left in a covered box at room temperature for 2 hours. Following incubation with the secondary, again the slides are washed 3 times in PBS + 1% triton-X and mounted in mounting medium containing 4',6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI).The immunostained slides will then be analysed by laser confocal microscopy at the Image Facility Core of City St George's, University of London.

No samples will be shipped outside Dr. Asimaki's laboratory. Following imaging, the samples will be stored at a 4 ºC fridge in Dr. Asimaki's laboratory and they shall be safely discarded at the end of the study. All samples will be de-identified; there shall be no way to link a particular slide with a specific patient. No sample will be used for any further study other than the one described in the information sheet. A study ID log containing participants name and unique study identification number will be kept separately from any other information related to the study in the ISF. Any samples and test results will only bear the unique participant ID number. To ensure data confidentiality, only authorized persons will be granted access to study data, passwords and system IDs shall not be shared, physical security of the workstations/files will be maintained, adequate back-up plans will be in effect and staff will be trained on data entry systems and importance of security procedures. Only the participants' direct care team will have access to personal data or medical files throughout the duration of the study. Professional auditors and regulatory inspectors (NHS offices) may need to get access to participants' information to ensure the study is compliant with regulations. The participants will be informed of this possibility by their medical care providers, it shall be stated on the information sheet and they will consent to it as part of their informed consent.

For the sub-study: tubes containing the buccal cells will be transferred to Dr Asimaki's laboratory. The tubes will be centrifuged and the cell pellet will be rinsed with PBS. The solution will be centrifuged again, PBS will be discarded and the sample will be resuspended in 10mM Tris-HCl with 1% SDS. Cell lysis will be achieved by sonication (15 pulses, 40% power output, 30% duty cycle). Sonicated samples will be centrifuged at 4 degrees for 20 minutes and the supernatant transferred to a new tube. Protein concentration will be measured using a BCA assay. 20 micrograms of protein solution will be mixed with laemelli buffer, boiled at 95 degrees for 5 minutes and loaded on a Tris gel. It will then be subjected to electrophoresis (150 volts, 40 minutes). Proteins separated on the gel will be transferred on a nitrocellulose membrane, which will be incubated with an antibody against the protein of interest. Membranes will then be incubated with a suitable secondary antibody, washed and exposed with the aid of chemiluminescent reagents. Images will be obtained via a Chemidoc 18 software to be kept in Dr Asimaki's notebook. All

samples will be de-identified. There shall be no way to link a tube containing a participant's cells with his identity. No sample will be used for any further study other than the one described in the information sheet. A study ID log containing participants name and unique study identification number will be kept separately from any other information related to the study in the ISF. Any samples and test results will only bear the unique participant ID number.

Safety Events

Definitions

Adverse Event (AE)—any untoward medical occurrence in a participant whether it is considered to be related to the intervention or not, that includes a clinical sign, symptom, or condition and /or an observation of a near incident. (This does not include pre-existing conditions recorded as such at baseline; continuous persistent disease or a symptom present at baseline that worsens following administration of trial intervention

Serious Adverse Event (SAE)—any Adverse Event or untoward medical occurrence in a trial participant that can be wholly or partly to the intervention which resulted in any of the following:

- Results in death; or
- Is life-threatening (places the participant, in the view of the Investigator, at immediate risk of death)
- Requires hospitalisation or prolongation of existing hospitalisation (hospitalisation is defined as an inpatient admission, regardless of length of stay; even if it is a precautionary measure for observation; including hospitalisation for an elective procedure, for a pre-existing condition)
- Results in persistent or significant disability or incapacity (substantial disruption of one's ability to conduct normal life functions)
- Consists of a congenital anomaly or birth defect (in offspring of participants regardless of time of diagnosis).
- Or is another important medical condition

Important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the outcomes listed in the definition of serious will also be considered serious.

1.1 Recording Adverse Events (AEs)

No adverse events are anticipated. There is no harm or risk associated with obtaining a buccal smear sample.

All Adverse Events will be recorded in the hospital notes in the first instance. A record of all AEs will also be kept in the CRF and the Sponsor's AE Log JREOLOG0007.

1.2 Investigator Responsibilities relating to Safety Reporting

Collection, recording and reporting of AEs (including serious and non-serious events and reactions) to the Sponsor will be done according to the Sponsor's Safety reporting for non-CTIMP studies SOP JREOSOP0033. All SAEs will be recorded in the hospital notes and the CRF, and the Sponsor's AE Recording Log JREOLOG0007. The AE Log will be sent to the Sponsor on request and every 2 months.

All SAEs will be reported both to the Sponsor via the JREO & REC *using the SAE report form for research other than CTIMPs (non-CTIMPs) published on the HRA website* The Chief or Principal Investigator at any participating site will complete the SAE form which will be faxed both to the JREO on 020 8725 0794 or E-mailed to adverseevents@sgul.ac.uk, within 48hrs of the Investigator becoming aware of the event, and via email to the relevant REC.

The Chief or Principal Investigator will respond to any SAE queries raised by the Sponsor as soon as possible. Follow up reports must continually be completed within acceptable time-frames and sent as detailed above until the reportable event is considered resolved

Events will be followed up until resolution; any appropriate follow-up information will be clearly marked as such and reported to the sponsor via the JREO as above in a timely manner.

Full reports should be completed and submitted to REC within 15 days of the event

1.3 Notification of deaths

All deaths will be reported to the Sponsor irrespective of whether the death is related to disease progression, the intervention, or an unrelated event. These reports shall be made immediately.

Data management and quality assurance

Confidentiality

All data will be handled in accordance with the Data Protection Act 1998.

The Case Report Forms (CRFs) will not bear the participant's name or other directly identifiable data. The participant's trial Identification Number (ID) only, will be used for identification. The sponsor Subject ID log JREOLOG0002 can be used to cross reference participant's identifiable information.

1.2 Data collection tool

Case Report Forms will be designed by the CI. All data will be entered legibly in black ink with a ball-point pen. If the Investigator makes an error, it will be crossed through with a single line in such a way to ensure that the original entry can still be read. The correct entry will then be clearly

inserted. The amendment will be initialled and dated by the person making the correction immediately. Overwriting or use of correction fluid will not be permitted.

It is the Investigator's responsibility to ensure the accuracy of all data entered and recorded in the CRFs. The Staff Delegation of Responsibilities Log JREOLOG0004 will identify all trial personnel responsible for data collection, entry, handling and managing the database.

The raw data generated from the study will be in TIF format (as images of protein distribution) and they shall be stored, de-identified, in Dr. Asimaki's computer at City St George's, University of London. Observations regarding protein-protein distribution will be noted in the CRF.

Incidental Findings

We are looking at the distribution of proteins at cell-cell junctions, the areas where cells are coupled together, which may only indicate a potential propensity to an arrhythmic disorder. Incidental findings are not relevant to this type of study.

Data handling and analysis

The data will be stored in TIF (images) format. The observations of the images will be logged on excel spreadsheets. City St George's, University of London is developing an institutional data repository as part of a Jisc-funded RDM Shared Service (April 2016 - September 2017). This will provide discoverability, secure storage and long-term preservation for all research data deposited in it. The PI will not have access to patient data/medical records. Slides will be labelled by a unique study participant ID code. There shall be no hard copy of the key linking the code to patients kept in the laboratory. There will be a linked document stored in the ISF JREOLOG0002. The study will be conducted in compliance with SOPs, GCP, all relevant laws of the EU and the UK including but not limited to: the Human Rights Act 1998, the Data Protection Act 1998, the Human Medicines Regulations 2012, ICH, the World Medical Association Declaration of Helsinki (2008), the NHS Research Governance Framework for Health and Social Care (2005). At the end of the study all coded samples shall be disposed of in accordance with the Human Tissue Authority's code of practice. Data generated will be kept on a password-protected workstation within a controlled-access office at City St George's, University of London. Computer softwares and firewall protection are up to date. City St George's, University of London servers are protected by line-interactive uninterruptible power supply (UPS) systems. No data will be kept in cloud-based storage. Only secure City St George's, University of London -controlled FTP servers will be used to share data with other team members. No sensitive data will be emailed according to ISO 27001. Digital data kept on City St George's, University of London servers are backed up overnight, every night, to hard disk and then cloned to tape storage. Full backups of all the data will be carried out monthly. The cloned tapes are stored separately in a fireproof and bombproof safe off-site. Laptops will be backed up and data removed from them as soon as practicable. This is in accordance with the City St George's, University of London Information Security and Removable Media policies: https://portal.sgul.ac.uk/org/lis/computing-services/infosec-external

Archiving arrangements

The trial essential documents along with the trial database will be archived in accordance with the sponsor SOP JREOSOP0016. The agreed archiving period for this trial will be 10 years.

Statistical design

Statistical input in trial design

Endpoints

Primary endpoints

The primary end point will be the presence of signal for a specific protein at the junctions of the buccal smear sample. No quantification of immunoreactive signal will be performed as this approach may increase bias. Accordingly, the results for each protein stain will be categorized as "present" or "absent".

The end point of the sub-study will be whether a protein of interest is expressed at control levels or significantly decreased levels in the presence of a mutation. Accordingly, the results for each proteins examined will be categorized as 'control' or 'reduced'.

Secondary endpoints

Not applicable for this study.

Sample size and recruitment

Sample size calculation

The sample size was calculated based on the size of the St. George's and satellite centres' Arrhythmia Services. The number selected constitutes only a fraction of the individuals evaluated and regularly followed at the hospitals involved and will allow us to obtain statistically significant results for each arrhythmic disorder.

Planned recruitment rate

Based on the number of patients/family members evaluated at the arrhythmic services at City St George's, University of London, we estimate that we shall be recruiting approximately 200 individuals per year over the 4 year course of the study.

Statistical analysis plan

Summary of baseline data and flow of patients

The participants will include individuals from 3 groups:

1. Patients already diagnosed with an arrhythmic disorder

2. Relatives of patients diagnosed with arrhythmic disorders to establish risk status

3. Relatives of SCD victims for risk stratification.

There shall be no randomization of the participants and no differential treatment amongst the population. All individuals enrolled with only provide a buccal smear sample.

Primary endpoint analysis

Both for the primary study (buccal smear immunostaining) and the sub-study (buccal cell western immunoblotting) only frequencies and associations will be assessed. No complex statistical analysis is required for completion of this study. Differences between disease groups in the distribution of immunoreactive signals and levels of protein expression will be assessed in a pairwise fashion with the Fisher exact test. Bonferroni correction will be used to correct P values because of multiple comparisons as appropriate. A p value of less than 0.05 will be considered significant. SPSS 21.0 software shall be used for all analyses. This statistical analysis will be performed by Dr. Angeliki Asimaki. If expert statistical help is required, the research team will consult medical statisticians within St. George's University of London (Dr Irene Chis Ster) who shall not have access to the participants' personal information.

To describe the precise methods of analysis, I shall use ACM as an example. We have previously shown that in the hearts of patients with ACM a protein known as plakoglobin is dislocated from the cell-cell junctions regardless of the underlying pathogenic mutation or even in cases where no mutation has been identified but the clinical or pathological examination are consistent with a diagnosis of ACM. We used the "presence" or "absence" of plakoglobin from the cell junctions in the buccal mucosa to predict which individuals had ACM in a blinded fashion. Of 39 patient samples obtained, plakoglobin was absent from the cell junctions in 34. It was not, however, absent in samples from patients diagnosed with other forms of cardiomyopathy including hypertrophic, dilated or ischemic cardiomyopathy. This indicated that using plakoglobin distribution in the buccal mucosa as a means to diagnose ACM had sensitivity of 87.1% and specificity of 100%.

Secondary endpoint analysis

Regarding the secondary outcome of the analysis: patients with ACM bear mutations in genes encoding desmosomal proteins. These include desmoplakin, desmoglein2, desmocollin2, plakoglobin and plakophilin2 (PKP2). Buccal cells do not express PKP2. Instead they express a different isoform of the protein; plakophilin1 (PKP1). 15/15 ACM patients bearing mutations in PKP2 showed loss of PKP1 signal in their buccal mucosa. No patients bearing mutations in different genes showed this abnormality. Moreover, only patients bearing mutations in desmoplakin, desmoglein2 or desmocollin2 showed loss of desmoplakin signal in their buccal mucosa. Accordingly, using PKP1 distribution to predict the presence of mutations in PKP2 showed 100% specificity and sensitivity. This is a remarkable finding given how PKP1 and PKP2 are expressed by different genes localized on different chromosomes. And yet, a mutation in one

isoform expressed in one tissue affects distribution of a different isoform expressed in a different tissue. Similarly, using desmoplakin distribution to predict the presence of mutations in desmoplakin, desmoglein2 and desmocollin2 showed 100% specificity and sensitivity. We are now at a position to use the same approach in other, more common forms of arrhythmic disorders including the cardiac channelopathies.

We shall use the presence or absence of signal for specific proteins at buccal smears to:

1. predict clinical diagnosis for heritable arrhythmic disorders

2. predict the genes bearing disease-causing mutations

3. assess the effect of potential changes in treatment plan on the distribution of proteins (specifically Cx43)

We shall use the expression levels of specific proteins to establish

1. If mutations act through haploinsufficiency
2. If anti-arrhythmic medication corrects the distribution of connexin43 by increasing its production levels.

Sensitivity and other planned analyses (if applicable)

Please see explanation above

Interim analysis

If following the first year of the study, it appears that we are unable to predict "arrhythmic status" using such samples, we shall stop recruiting patients with cardiac channelopathies and will only focus on patients with cardiomyopathies, where the correlation is already proved

Committees in involved in the trial

A trial management group (TMG) will be in place throughout the duration of the study

This will include those individuals responsible for the day-to-day management of the study, such as the CI, research nurse and medical care providers. The role of the group will be to monitor all aspects of the conduct and progress of the study, ensure that the protocol is adhered to and take appropriate action to safeguard participants and the quality of the study itself.

Direct access to source data

The Investigator(s)/institution(s) will permit trial-related monitoring, audits, REC review, and regulatory inspection(s), providing direct access to source data/documents. Trial participants are

informed of this during the informed consent discussion. Participants will consent to provide access to their medical notes.

Ethics and Research Governance requirements

Before any site can enrol patients into the trial, the Principal Investigator must ensure written permission to proceed has been granted by that Trust Research & Development (R&D). If conducting the study at City St Georges contact the governance team within the JREO for any assistance.

The site must conduct the trial in compliance with the protocol as agreed by the Sponsor and, which was given favourable opinion by the Research Ethics Committee (REC).

The Chief Investigator will be provided (via the Sponsor) with file indexes E.G. JREODOC0003 TMF index and JREODOC0004 ISF index for use with SOP JREOSOP0019 'Preparation and Maintenance of the TMF' The CI will be responsible for the maintenance of the TMF and may delegate the responsibility of ISF file maintenance to the PI at each participating site.

It is the responsibility of the Principal Investigator to ensure that all subsequent amendments gain the necessary approval. Refer to JREOSOP0011 'Management of Amendments'.

Within 90 days after the end of the trial, the CI and Sponsor will ensure that the REC is notified that the trial has finished. If the trial is terminated prematurely, those reports will be made within 15 days after the end of the trial. Refer to JREOSOP0015 'End of study declaration'

The CI will supply an End of Study report of the clinical trial to the REC within one year after the end of the trial. The sponsor can provide JREODOC0059 End of study Report template

17.1 Annual Progress Reports (APRs)

The Chief Investigator will prepare the APR in accordance with JREOSOP0043. Following review by the Sponsor the report will be sent to the REC. The APR is due for submission annually within 30 days of the anniversary date on which the favourable opinion was given by the Ethics committee, until the trial is declared ended.

1.2 Notification of Serious Breaches of GCP and/or the protocol

Any Protocol Deviations, Violations will be documented using JREODOC0061, and entered onto the Sponsor's log JREOLOG0005. Potential Serious Breaches and Urgent Safety Measures will be recorded both on the Sponsor's Log JREOLOG0005 and processed according to JREOSOP0012 and where necessary JREOSOP0032

A "serious breach" is a breach which is likely to effect to a significant degree:

(a) The safety or physical or mental integrity of the participants of the trial; or
(b) The scientific value of the trial.

The CI will notify the Sponsor immediately of any case where there exists a possible occurrence of a serious breach

Finance

The PI is funded by the British Heart Foundation as well as a private donation to cover the costs of the immunohistochemical and confocal microscopy analysis. The NHS is providing the study team with an annual stipend to cover the costs of the materials required to obtain the sample (soft brushes and microscope slides). There shall be no monetary gain for the participants or the medical care providers involved in the study.

Insurance and indemnity

City St George's, University of London holds insurance to cover participants for injury caused by their participation in the clinical trial. Participants may be able to claim compensation if they can prove that City St George's has been negligent. This includes negligence in the writing of the protocol, or selection of trial resources.

Where the Trial is conducted in a hospital, the hospital has a duty of care to participants. City St George's, University of London will not accept liability for any breach in the hospital's duty of care, or any negligence on the part of hospital employees. Hospitals selected to participate in this clinical trial shall provide clinical negligence insurance cover for harm caused by their employees and a copy of the relevant insurance policy or summary shall be provided to City St George's, University of London, upon request.

Participants may be able to claim compensation for injury caused by participation in this Trial without the need to prove negligence on the part of City St George's, University of London or another party.

If a participant indicates that they wish to make a claim for compensation, this needs to be brought to the attention of City St George's, University of London immediately.

Failure to alert City St George's, University of London without delay and to comply with requests for information by the sponsor or any designated Agents may lead to a lack of insurance cover for the incident.

IP and development policy

Unless otherwise specified in agreements, the following guidelines shall apply: All Intellectual Property Rights and Know How (IP) related to the Protocol and the Trial are and shall remain the property of the Sponsor excluding

1) Pre-existing IP related to clinical procedures of any Hospital.

2) Pre-existing IP related to analytical procedures of any external laboratory.

All contributors

shall assign their its rights in relation to all Intellectual Property Rights and in all Know How, not excluded above to the Sponsor and at the request and expense of the Sponsor, shall execute all such documents and do all such other acts as the Sponsor may reasonably require in order to vest fully and effectively all such Intellectual Property Rights and Know How in the Sponsor or its nominee.

shall promptly disclose to the Sponsor any Know How generated pursuant to this Protocol and not excluded above and undertake treat such Know How as confidential information jointly owned between it and the Sponsor

Nothing in this section shall be construed so as to prevent or hinder and medical professional from using Know How gained during the performance of the Trial in the furtherance of its normal business activities, to the extent such use does not result in the disclosure or misuse of Confidential Information or the infringement of any Intellectual Property Right of the Sponsor.

Publication policy

Publication: “Any activity that discloses, outside of the circle of trial investigators, any final or interim data or results of the Trial, or any details of the Trial methodology that have not been made public by the Sponsor including, for example, presentations at symposia, national or regional professional meetings, publications in journals, theses or dissertations.”

All scientific contributors to the Trial have a responsibility to ensure that results of scientific interest arising from Trial are appropriately published and disseminated. The Sponsor has a firm commitment to publish the results of the Trial in a transparent and unbiased manner without consideration for commercial objectives.

To maximise the impact and scientific validity of the Trial, data shall be consolidated over the duration of the trial, reviewed internally among all investigators and not be submitted for publication prematurely. Lead in any publications arising from the Trial shall lie with the Sponsor in the first instance.

Before the official completion of the Trial,

All publications during this period are subject to permission by the Sponsor. If an investigator wishes to publish a sub-set of data without permission by the Sponsor during this period, the Steering Committee shall have the final say.

Exempt from this requirement are student theses that can be submitted for confidential evaluation but are subject to embargo for a period not shorter than the anticipated remaining duration of the trial.

Up to 180 days after the official completion of the Trial

During this period the Chief Investigator shall liaise with all investigators and strive to consolidate data and results and submit a manuscript for peer-review with a view to publication in a reputable academic journal or similar outlet as the Main Publication.

- The Chief Investigator shall be senior and corresponding author of the Main Publication.
- Insofar as compatible with the policies of the publication outlet and good academic practice, the other Investigators shall be listed in alphabetic order.
- Providers of analytical or technical services shall be acknowledged, but will only be listed as co-authors if their services were provided in a non-routine manner as part of a scientific collaboration.
- Members of the Steering Group shall only be acknowledged as co-authors if they contributed in other capacities as well.
- If there are disagreements about the substance, content, style, conclusions, or author list of the Main Publication, the Chief Investigator shall ask the Steering Group to arbitrate.

Beyond 180 days after the official completion of the Trial

After the Main Publication or after 180 days from Trial end date any Investigator or group of investigators may prepare further publications. In order to ensure that the Sponsor will be able to make comments and suggestions where pertinent, material for public dissemination will be submitted to the Sponsor for review at least sixty (60) days prior to submission for publication, public dissemination, or review by a publication committee. Sponsor's reasonable comments shall be reflected. All publications related to the Trial shall credit the Chief and Co-Investigators as co-authors where this would be in accordance with normal academic practice and shall acknowledge the Sponsor and the Funders.

Statement of Compliance

The trial will be conducted in compliance with the protocol, Sponsor's Standard Operating Procedures (SOPs), GCP and the applicable regulatory requirement(s).

The study conduct shall comply with all relevant laws of the EU if directly applicable or of direct effect and all relevant laws and statutes of the UK country in which the study site is located including but not limited to, the Human Rights Act 1998, the Data Protection Act 1998, the Human Medicines Regulations 2012, ICH GCP, the World Medical Association Declaration of Helsinki entitled 'Ethical Principles for Medical Research Involving Human Subjects' (2008 Version), the NHS Research Governance Framework for Health and Social Care (Version 2, April 2005).

This study will be conducted in compliance with the protocol approved by the REC and according to GCP standards. No deviation from the protocol will be implemented without the prior review and approval of the Sponsor and REC except where it may be necessary to eliminate an immediate hazard to a research subject. In such case, the deviation will be reported to the Sponsor and REC as soon as possible.

23 List of Protocol appendices

Not applicable to this protocol

24 References

1. Asimaki A, Tandri H, Huang H, Halushka MK, Gautam S, Basso C, Thiene G, Tsatsopoulou A, Protonotarios N, WcKenna WJ, Calkins H, Saffitz JE: A new diagnostic test for arrhythmogenic right ventricular cardiomyopathy. N Eng J Med, 2009; 360:1075-1084. PMID: 19279339

2. Asimaki A, Tandri H, Duffy ER, Winterfield JR, Mackey-Bojack S, Picken MM, Cooper LT, Wilber DJ, Marcus FI, Basso C, Thiene G, Tsatsopoulou A, Protonotarios N, Stevenson WG, McKenna WJ, Gautam S, Remick DG, Calkins H, Saffitz JE. Altered desmosomal proteins in granulomatous myocarditis and potential pathogenic links to arrhythmogenic right ventricular cardiomyopathy. Circ Arrhythm Electrophysiol, 2011; 4:743-752. PMCID: 3293529

3. Asimaki A, Protonotarios A, James CA, Chelko SP, Tichnell C, Murrat B, Tsatsopoulou A, Anastasakis A, te Riele A, Kleber AG, Judge DP, Calkins H, Saffitz JE. Characterizing the molecular pathology of arrhythmogenic cardiomyopathy in buccal mucosa cells. Circulation: Arrhythmia and Electrophysiology. 2016 Feb;9(2): e003688. PMID: 26850880

25 Research Data Protection Impact Assessment (DPIA)

Data Protection Impact Assessments (DPIAs) are a tool which can help organisations identify the most effective way to comply with their data protection obligations under the Data Protection Act 2018 (DPA 18) and meet individuals' expectations of privacy.

A DPIA helps identify data privacy risks when planning new, or revising existing, projects and to identify actions to mitigate these risks. In the rare cases where risks cannot be mitigated at all it may be necessary to consult with the Information Commissioner's Office (ICO). Under data protection legislation it is a legal requirement to complete a DPIA in the following circumstances:

- • where data processing is likely to result in a high risk of harm to individuals, e.g. new, invasive technology is proposed
- • when large volumes of personal data are processed, e.g. use of behavioural profiles based on website usage
- • when processing special category personal data on a large scale, e.g. healthcare data, genetic tests to assess and predict the disease/health risks

• where publicly accessible areas are monitored, e.g. CCTV or when filming public areas

Therefore a DPIA will be carried out for both internal and partnership projects which require the collection/processing of personal data in any format for the purpose of research.

The DPIA should be carried out towards the start of the project, in order to identify any associated information risks and mitigate in the early stages, before you start processing.

Study Title/Acronym:	Distribution of cell-cell junction proteins in arrhythmic disorders
JRES Reference Number:	16.0099
Chief Investigator Name:	Dr Angeliki Asimaki
Chief Investigator Email Address:	aasimaki@sgul.ac.uk

PROJECT DETAILS
Project / process description: **- include / attach processing operations (include a flow diagram or another way of explaining data flows), the purpose and, where applicable, what City St George's lawful basis is for the processing of the information.**
Analysis of myocardial samples has provided us with novel diagnostic markers for heritable cardiac disorders and inflammatory heart disorders. However, myocardial samples are difficult and risky to obtain from patients and impossible to obtain from family members, who do not manifest evidence of the disease but may still be carrying genetic alterations that put them at risk of sudden cardiac death. We showed that the buccal mucosa expresses similar proteins to those expressed in the heart and may thus be used as a surrogate for the myocardium. We also showed that alterations in protein distribution occurring in the heart of patients with cardiomyopathies or cardiac channelopathies also occur in their buccal cells, potentially providing us with a quick, easy, and totally risk-free means of diagnosis. Individuals will be recruited by their regular medical care providers. Once they have read the information sheet and understood the process entirely, if they agree to participate, they will sign an informed consent form, a copy of which they will get to keep. If the sampling is taking place at home, the patient needs to return the signed consent form to City St George's, University of London along with their samples. If seen on site, the patient will then provide a study team member with 6 buccal smear samples. These samples will be transferred to Dr Asimaki's laboratory at City St George's, University of London. If using the at-home kit, the patient will obtain the buccal smears himself and then mail them to City St George's, University of London. Dr Asimaki will subject the smears to immunohistochemical analysis to characterize specific protein distribution. If seen at the hospital, the study participants will just be asked to open their mouth. A study team member will rub a soft brush a few times at the inside of their cheek and smear the brush on a microscopy slide. The slide will be sprayed with 70% ethanol to preserve the material and taken to the research laboratory. The patient will have a total of 6 smears taken (3 from each cheek). For the majority of the patients, only a single sampling will be enough which will take place during one of their regular follow-up appointments at the cardiology clinic or using the at-home kit. There is no pain and no discomfort associated with the procedure and it lasts only a few seconds. In selected cases, however, if for instance we want to use the buccal smear to evaluate the effect of a change in medical treatment on protein distribution, the patient might be asked to provide the study team with another sample, again during one of his scheduled regular follow-up visits or using the same

self-sampling kit at home. If the at-home kit is being used, the patient will be asked to obtain their own buccal smear samples using the materials provided and mail the slides back to City St George's, University of London.
We anticipate that the study will be highly beneficial for prevention of sudden cardiac death, timely and accurate diagnosis and management of arrhythmic disorders. A few patients will be invited to participate into the sub-study. The purpose of this is to examine expression levels of key proteins via western immunoblotting and hence establish the precise mechanism of pathogenesis of selected mutations. These participants will be asked to open their mouth and a study team member will obtain buccal cells by gently rubbing a toothbrush at the inside of their cheek.

What personal data do you intend to use, and why? (List all categories)

The samples will be identified solely by a study ID number.
A data collection form will accompany each set of samples. This will include the arrhythmia syndrome the participant has been diagnosed with, whether there is history of sudden cardiac death in the participant's family, whether the participant has had a mutation identified, whether the participant is on any regular medication and whether s/he has been diagnosed with any additional clinical conditions. No personal information will be shared.

Will the personal data be identifiable, pseudonymised or anonymised (if a mix tick accordingly)

Identifiable		
*Pseudonymised	x	
Anonymised	x	

**Confirm that the key to this data is kept securely away from the used data with strict controlled access*
The key linking the study ID number to a specific participant will be held in secure workstations at or Bart's Hospital, only accessible by the participant's regular medical team.

List all organisations / agencies which will have access to the personal data collection used for this project / process

Only the regular medical care providers of each participant will have access to the participants' personal data. No personal data will be collected for the purpose of this study.

Length of the study – include an assessment of the necessity and proportionality of the processing in relation to the purpose. Also include who, internally & externally, has been consulted in the preparation of this DPIA.

The current end date of the study is 30/06/2026. All eligible participants have regular follow-up appointments every 6 or 12 months. In order for us to recruit enough participants for the study's outcomes to reach statistical significance, at least 1 year is required.

Rosie Jacobs, the City St George's, University of London representative has been consulted for the preparation of this DPIA.

If external organisations / agencies are involved, is there a contract or information sharing agreement in place with suitable clauses for data protection and data incident reporting,? If not why not?

Not applicable.

RISK			
Can you achieve your objectives using anonymised data? – see ICO Code of Practice on Anonymisation			
Yes	x		
No		Why not?	

What are the benefits to the individual of their personal data being used for this purpose?

Not applicable

What are the organisational benefits of the individual's personal data being used for this purpose?

Not applicable

What are potential negative impacts to the individual of their personal data being used for this purpose in the event of a Data Breach occurring?
Not applicable
How will you avoid causing unwarranted or substantial damage/distress to the individual when using their personal data for this purpose?
Not applicable

Is the data already held by City St George's?			
Yes	X		
No			
Is it held by one of the partner organisations / agencies involved in this process/project?			
Yes			
No	X	Which agency will be collecting the data	
Have you told the individuals whose personal data you want to use for this purpose, how and why you intend to use their data?			
Yes			
No			
If not, are you intending to tell them?			
Yes			
No		Why not?	
Do you already have the individual's consent to use their data for this purpose?			
Yes			
No	X	Why not?	Informed consent will be sought
If not, are you going to ask for their permission?			
Yes	X		
No		Why not?	

Have individuals been given the opportunity to refuse us permission to use their data for this purpose?		
Yes	X	
No		

How will you make sure that the personal data you are using is kept accurate and up to date?

Not applicable

What steps or controls are you taking to minimise risks to privacy?
Please tick those which apply and provide details of how each is ensured

• X Risks to individual privacy are minimal • Personal data is pseudonymised • Encryption of data at rest, i.e. when stored • Encryption used in transfers • Information compliance training for staff has been completed - data protection, information security, FOI • X Adherence to privacy by design principles • Special category personal data is not used • X Participant opt out at any stage of the research • Personal data kept in the UK • Research is not used to make decisions directly affecting individuals • Short retention limits • Restricted access controls • X Other (please specify)	Samples will be identified by a study ID number only. No personal data will be noted on the slides. A key linking the study ID numbers to specific individuals will be kept in protected workstations at respective hospital sites only accessible by the participants' regular medical team.

How long will you need to hold the personal data for after the study has completed?

Not applicable

How will you make sure that you are holding data for the appropriate length of time and no longer?

Not applicable

How will the data be held /stored?

Personal data for all patients/family members are routinely held in medical files and electronically by the individuals' regular medical care providers. There shall not be shared with the principal investigator.

Will you be using any electronic and/or paper Case Report Forms (CRFs) to collect data? If so what are these and how will they be held securely and managed at the end of the project?

A data collection form, not including any personal data, will accompany each set of slides or cell suspension sample. This will include:

- The arrhythmia syndrome the participant has been diagnosed with

- Whether the participant has a family history of sudden cardiac death
- Whether the participant is taking any regular medication
- Whether the participant has been diagnosed with additional medical conditions
- Whether the participant is bearing a known disease-causing mutation
- The number of slides obtained (or if participating in the sub-study whether a single or double round of toothbrush rubbing was required to obtain sufficient cells)
- The team member obtaining the smears/cell sample (or the participant if using the at-home kit)

These will be completed by the participant's regular medical providers.

Following completion of the immunohistochemical/western immunoblotting analysis the principal investigator will complete the second part of the form with any changes observed in the distribution/expression levels of key proteins investigated.

There shall be no personal data noted on the form.

The forms will be held in Dr Asimaki's office at City St George's, University of London. The office is locked and accessible only by the PI.
Upon completion, they shall also be scanned and an electronic copy held in Dr Asimaki's City St George's, University of London workstation accessible by the PI only.

Will personal data be transferred/shared between the organisations involved in this project? If so how?

No

Will you be transferring personal data to a country or territory outside of the UK? *If yes, name countries and receiving parties.*

Yes – within EEA		
Yes – outside of EEA		
No	x	

How will you ensure that third parties will comply with data protection obligations?

Not applicable

What measures are in place to ensure only appropriate and authorised access to and use of, personal data?

All members of the participant's regular medical team have been trained in security procedures.
The study will be conducted in compliance with SOPs, GCP, all relevant laws of the EU and the UK including but not limited to: the Human Rights Act 1998, the Data Protection Act 2018, the Human Medicines Regulations 2012, ICH, the World Medical Association Declaration of Helksinki (2008), the NHS Research Governance Framework for Health and Social Care (2005). At the end of the study, all coded buccal samples will be disposed according to the Human Authority's code of practice.

How will technical and organisational security be monitored/audited?

The workstations where data will be stored are password-protected within controlled-access offices at the hospitals involved. Computer softwares and firewall protection are up to date. Hospital servers are protected by line-interactive uninterruptible power supply systems. Secure - controlled FTP servers will be used to share data with other team members.

All data stored on the university network are backed up overnight, every night, to hard disk and then cloned to tape storage. Full backups of all the data are carried out monthly. The cloned tapes are stored separately in a fireproof location off-site. City St George's, University of London conducts penetration tests and social engineering tests annually to ensure network integrity. If access to the data is required while off-site it will only be provided by means of a managed VPN service and remote desktop services. Password-protected laptops will be backed up on the network and data removed from them as soon as practicable.

Declaration

I confirm that the information recorded on this form is, to the best of my knowledge, an accurate and complete assessment of the potential privacy impacts of this study.

Name: Dr Angeliki Asimaki

Signature: 

Date: 12/01/2024

Institute Director (City St George's, University of London) or Care Group Lead (SGHFT)

Name: Guy Whitley

Signature: 

Date: 11/10/2022

JRES Reviewer

Name: Rosie Jacobs

Signature:

Date: 11th October 2022