Study Protocol and Statistical Analysis Plan

Title: Exploratory Study of Relationships Between Malodor and Urine Metabolomics

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STATEMENT OF COMPLIANCE

The study will be conducted in accordance with the International Conference on Harmonization guidelines for Good Clinical Practice (ICH E6), the Code of Federal Regulations on the Protection of Human Subjects (45 CFR Part 46), and the NIDCR Clinical Terms of Award. All personnel involved in the conduct of this study have completed human subjects' protection training.

INTRODUCTION

Certain body and breath odors are known to indicate serious infections and life-threatening conditions. Malodors without accompanying physical symptoms, however, could be a sign of psychologically but not physically debilitating errors of metabolism. One example is Trimethylaminuria (TMAU) leading to excessive excretion of foul-smelling trimethylamine (TMA) in the sweat and breath. Even this relatively straightforward disorder of choline metabolism exhibits complex genetic and environmental regulation. Trimethylaminuria is diagnosed based on the symptoms, a clinical exam, and a test to measure the level of trimethylamine in the urine after a choline load [1]. Genetic testing can also help confirm the diagnosis, but is not sufficient as not all mutations and all genes responsible for this condition have been mapped. Since clinical exam can miss the symptoms, and urinary TMA levels are abnormal in less than 30% of sufferers with the most severe TMAU-like cases [2], new diagnostic tests are needed.

AIM

The aim of this observational study is to examine diagnostics potential of urine metabolomics for idiopathic body odor of metabolic origin (MEBO) and "People are Allergic to Me" (PATM) syndrome.

RESEARCH PROPOSAL

Urine has been used for medical diagnostics for more than 3000 years. It is easy to obtain, yet chemically complex providing an ideal snapshot of the body's metabolic processes

To identify metabolic signatures associated with malodor conditions, the investigators propose to perform state-of-the-art metabolomics tests and bioinformatic data analytics to explore if conditions leading to malodor can be screened by metabolomic profiling of urine samples.

To capture metabolomic signatures in idiopathic malodor, we will ask participants to continue their usual diet and collect a morning urine sample. At the same time, subjects will be asked to describe observations of daily living, describing diet, quality of life, activities, stress and other environmental exposures.

Urine sample preparation is one of the simplest processes employed in bioanalysis and the complexity of this step usually differs according to the aim of the experiment. We will aim to keep the sample as intact as possible containing all unknown metabolites. When analyzing urine by GC-MS there is a need to pretreat the sample with urease (to reduce urea levels) that can diminish the abundance of some metabolites [3]. We aimed at establishing the most effective sample pretreatment for urea removing while preserving target metabolites in the specimen [4]. The Metabolomics Innovation Centre will send every participant a kit with detailed instructions on collection, handling and storage.

We will then apply a targeted metabolomics appro ach that uses a direct injection and tandem mass spectrometry (DI–MS/MS) coupled with a liquid chromatography–tandem mass spectrometry (LC–MS/MS)-based metabolomics [4]. We will also use metabolite databases [5] diet analytics platform [6], Chenomx NMRSuite v7.0 software (Chenomx, Edmonton, Alberta, Canada), R libraries and VBA Excel macros.

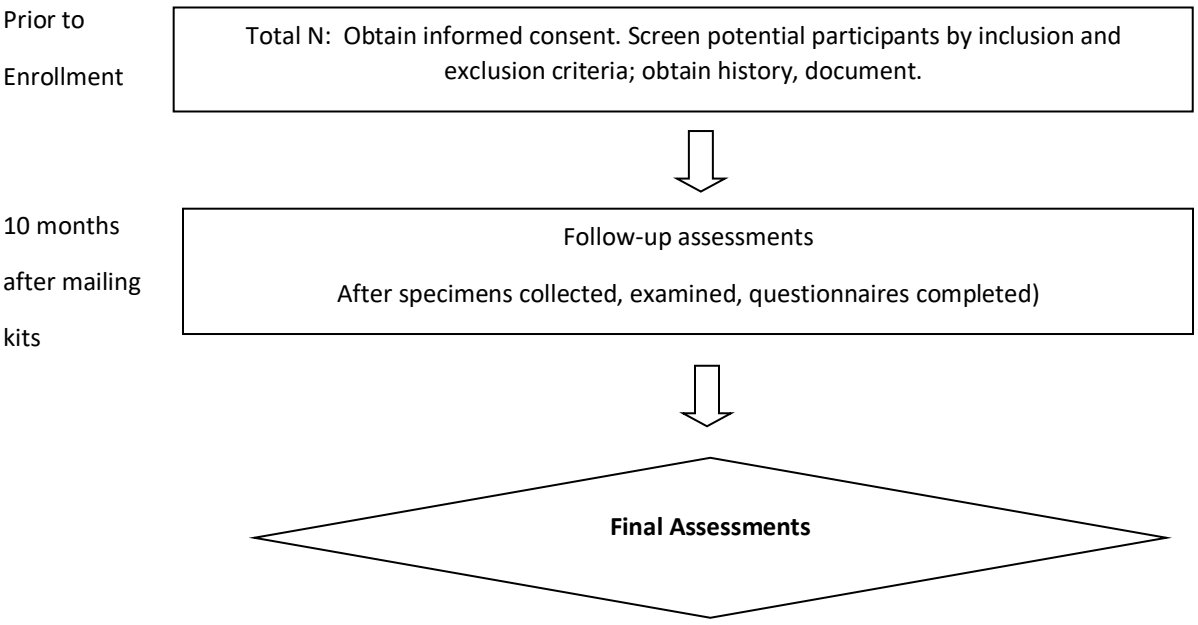
STUDY DESIGN

Study Type: Observational

Proposed Enrollment: 50

Allocation: Non-Randomized

Primary Purpose: Diagnostics



RECRUITMENT

Patients will be recruited from TMAU testing program, other previous studies and non-interventional surveys. In addition, the study will be advertised on blogs, social media patient support groups and on Clinicaltrials.gov.

Inclusion Criteria:

- 18 years or older
- unpredictable and uncontrollable episodes of malodor
- willing and able to ship a urine sample (in the kit provided) by an overnight courier to Edmonton, Alberta, Canada
- good general health

Exclusion Criteria:

- serious medical conditions that require treatment
- conditions that, in the opinion of the investigator, would prevent participation
- under the age of 18
- elect not to participate in the study

STATISTICAL ANALYSIS

STUDY HYPOTHESIS

The hypothesis of this study is that, in spite of genetic and environmental heterogeneity, the pathology involves common patterns in the urine metabolome.

SAMPLE SIZE

No formal sample size calculation was performed. Obviously, larger sample numbers allow for higher accuracy. Our exploratory study aimed at recruiting the largest possible number of volunteers suffering from the above-mentioned rare conditions and residing in Canada.

FINAL ANALYSIS PLAN

Study coordinator will assign each human subject a de-identified unique identifier code. It will be used to link kit IDs with medical histories.

The data will then be examined for errors (e.g., outliers - out of range values), consistency, missing and spurious values. Missing data will not be imputed unless specifically noted. Any apparently spurious data will be verified. Non verified data will be excluded from summaries or analyses. Initial data exploration will be undertaken using inspection of frequency distributions and plots (i.e., graphical methods such as histograms and box plots), estimation of z-scores, estimation of skewness and kurtosis, and test for normality (i.e., analytic methods). If necessary, data will be transformed and centered for minimizing problems.

Standard summary statistics including means, standard deviations, proportions, and 95% confidence intervals will be produced for all measures. The focus of the data exploration will be on exploring the shape of the curves, identifying peaks and troughs. For the Primary Study Endpoint, we will identify population-wide signatures

Descriptive statistics will be calculated and compared across all groups. Inter-group differences will be computed using statistical tests.

If the assumptions of the t-test cannot be met, yet the observations are independent, the Mann–Whitney test will be used for two group comparisons, if the data are at least ordinal in nature. Wilcoxon matched pairs test will be used for samples of the same subjects before and after improvement of symptoms.

Statistical analyses will be separately defined for each endpoint. There will be no adjustment of p-values for multiplicity unless specifically noted. P-values generated for this study are not intended to be conclusive, but provided for guidance only.

Appropriate algorithms of clustering or statistical models will be used to define subgroups of MEBO/PATM subjects with symptoms of different severity based on the variation in specific metabolic, inflammatory or dietary markers.

ETHICS/PROTECTION OF HUMAN SUBJECTS

This study is to be conducted in accordance with applicable US government regulations and international standards of Good Clinical Practice, and applicable institutional research policies and procedures. This protocol and any amendments are submitted to a properly constituted independent Institutional Review Board (IRB).

Potential Risks and Discomforts

The primary risk to participants is loss of privacy. To mitigate such risk, we link all data to coded identifiers for the participants during the collection and transmission process. Coded data will be only accessible to trained researchers.

Withdrawal of Subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. They can also request study coordinators to have their personal data permanently deleted from our databases.

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