1. Detailed Research Proposal Next Generation Sleep Analytics

## Specific aims

Sleep disorders and sleep dysregulation impact over 100 million Americans, causing enormous medical consequences including cardiovascular (arrhythmia, hypertension, stroke), metabolic (diabetes, obesity) and psychiatric (depression, irritability, addictive behaviors), to name only a few. Sleepiness costs millions due to workforce errors and accidents. Although the global market for just hypnotic products will reach $76.7 billion by 2019, our fundamental understanding of sleep remains a biological "black box". This curtails our ability to identify etiologies and thus treat sleep disorders effectively.

Sleep is genetically controlled and therefore one of the most powerful approaches to revealing its underlying biological basis is genetics. By scanning the entire genome of individuals, genetic studies such as genome-wide association studies (GWAS) do not require any a priori knowledge of underlying mechanisms. GWAS has been conducted on over 1,500 traits and diseases but with the exception of narcolepsy and restless legs syndrome (RLS), there has been no characterization of the genetic basis of sleep and sleep disorders. This critical gap is due to a lack of large samples where individuals have been characterized objectively using nocturnal sleep polysomnography (PSG) and subjectively using standardized and comprehensive questionnaires such as the Alliance Sleep Questionnaire (ASQ).

To address this unmet need, we propose to conduct large-scale genomics studies, sleep phenotyping and automated PSG data analysis. The information will be crucial for our understanding of the genetic architecture of sleep and to improve detection, treatment and prevention of sleep disorders. To maximize research potential, all tools and data will be made available to the research community.

Specific Aim 1: Collect and make available PSG, ASQ, neuropsychological testing data, biological samples and DNA on 30,000 adult/adolescent patients evaluated for sleep and sleep disorders at five centers, creating a unique reference database for the field of sleep medicine.

Specific Aim 2: Develop and make available software that will streamline PSG analysis, extract meaningful sleep phenotypes, and standardize analysis in large samples. Validate this software in smaller, existing cohorts and use software in the large 30,000 sample in conjunction with GWAS to answer critical questions related to sleep and sleep disorders.

Specific Aim 3: Use GWAS in conjunction with machine learning and phenotype analysis in the 30,000 sample to discover genetic modifiers for sleep and sleep disorders focusing on genetic variants that control EEG traits and hypersomnia phenotypes.

The proposed aims will develop the critical infrastructure needed for sleep and sleep disorder research and will provide essential tools and data for sleep research projects not currently available. We aim at nothing less than being a catalyst for changing the sleep field.

## Background and Preliminary Data

Over three-quarters of adults suffer from ~ 90 different sleep disorders during their life[1]. The most frequent sleep disorders are summarized in Table 1.

Sleep dysregulation and deprivation are common, and have effects on many aspects of the endocrine system, notably glucose regulation, stress axis, and appetite hormone regulation[1]. Sleep deprivation impairs performance, judgment, mood, and is a preventable contributor to accidents[1]. Despite this, the field of sleep receives limited funding, most of it disease-oriented and segregated by discipline so that a comprehensive picture of sleep is currently not possible.

*Sleep is a biological "black box".*

Sleep is a necessity, yet remains a biological mystery[2-5]. The most important question may be not why we sleep, but how we sleep, i.e., what are the main factors at the molecular, cellular, and circuitry levels that coordinate and generate sleep. Progress has been made toward describing sleep circuitry controlling brain activity changes[6,7], as well as molecular or structural correlates of sleep and sleep deprivation[8-11], but there is little functional data, so it is difficult to tell what is causative versus simply correlative.

In contrast, circadian control of sleep has made tremendous progress in the last 30 years thanks to genetic studies that first started in Drosophila[12] and were extended to mammals[13]. What has been revealed in this area is that specific genetic variants within a molecular clock greatly alter the timing of sleep, also creating pathological "early birds", "night owls", or an inability to synchronize with the light-dark cycle in humans[14].

*Genetic approaches are effective at opening biological black boxes, especially for traits that are objectively measured such as sleep.*

Sleep disorders and the EEG have high heritability consistent with genetics modulating these traits[15-27]. Genetic approaches are hypothesis-free and can lead to transformative insights as exemplified by circadian biology research. In our laboratory, the cloning of the canine narcolepsy gene advanced understanding of narcolepsy[28], linking the disorder to orexin/hypocretin, a new sleep/wake regulator[29,30]. Novel insights into RLS pathophysiology beyond the role of iron deficiency and dopamine involvement[31], and of human narcolepsy as an autoimmune disease, also occurred thanks to GWAS[32-35]. More than 1,500 traits have now been studied with GWAS (http://www.ebi.ac.uk/gwas/), but not sleep.

It is customary to distinguish the single-gene-high-penetrance and complex-disease-GWAS study approaches to human genetics[36]. In the field of circadian research, Fu and Ptacek reported single gene variants in circadian genes with strong effects on circadian rhythmicity and, in one case associated with short sleep in multiplex families[14,37-44]. Although this approach can be extended (see Specific Aim 3), it is limited by the possibility of phenocopies in small pedigrees. In our group, using exome sequencing, we identified DNA methyltransferase 1 (DNMT1) mutations as the cause of autosomal dominant cerebellar ataxia, deafness, narcolepsy (ADCA-DN) a disorder evolving into dementia[45]. This approach is most powerful when phenotypes are discrete and have familial clustering. It can also be useful to detect *de novo* mutations when disorders are phenotypically identical but etiologically heterogeneous (for example autism).

Table 1. Most frequent sleep disorders[1]

| Disorder | Population Affected | Clinical Relevance | Therapies | Unmet Clinical Needs |
|---|---|---|---|---|
| Insomnia | 42 million (14%) | Strong predictor of depression later in life. Insomnia alone has been associated with higher risk of heart disease. | Sleep restriction, light therapy, better sleep habits, and cognitive therapy bring 80% satisfaction in most cases. | Therapies are insufficiently applied; it is unknown whether treating insomnia early prevents mental health complications. |
| Obstructive sleep apnea | 30 million (10%) | Causes low oxygenation and sleep deprivation. Accepted as the leading treatable cause of hypertension. Strong predictor of stroke and heart disease. Difficulty with memory, intimacy, and attention. | Positive airway pressure (PAP) is the gold-standard therapy. Mandibular advancement devices, surgery, and various devices are alternative therapies, but provide variable benefit and incomplete cure. | Even with optimal correction of obstructive sleep apnea using PAP the symptomatic and clinical sequelae have a stochastic course of resolution. Better characterization of OSA sub-phenotypes will help target therapies and counseling. |
| Central sleep apnea (CSA) | Several millions | Causes hypoxia and sleep deprivation. ~50% of people with heart failure have central sleep apnea. Central sleep apnea is also associated with opioid use. | PAP therapy and adaptive servo-ventilation are the mainstay therapies. Adjunctive measures include medications (e.g. acetazolamide) or dead space-increasing measures. | Mixed results from trials of various PAP and servo-ventilation therapies in patients with heart failure. A limited understanding of the pathophysiology and treatment strategies exists for non-heart-failure-related CSA. |
| Restless legs syndrome (RLS) | 12 million (4%) | Seriously disrupts sleep and causes pain. Associated with depression, anxiety, heart disease and periodic leg movements (PLMs) during sleep. | Therapies range from behavioral (e.g. vibrating pads) to pharmacologic, with variable efficacy. Dopamine agonists, gabapentin, and narcotics are the main options, but are often limited by side effects, notably augmentation with dopamine agonists. | Substantial genetic effects for RLS have been discovered. Placebos have strong effects in all trials and choice of medications is done in an empiric, rather than, evidence-based fashion. Targeted therapies based on a better understanding of disease etiology are needed. |
| Narcolepsy and idiopathic hypersomnia | 200,000 (0.067%) | An autoimmune disease targeting hypocretin/orexin neurons causes type-1 narcolepsy. Symptoms include cataplexy and sleepiness. Cause of type-2 narcolepsy and hypersomnia (without cataplexy) is unknown. Relationship to depression and other mental disorders is frequent. | For type-1 narcolepsy, lifelong treatment with stimulants, antidepressants or powerful sedatives is generally required. Stimulant treatments are generally needed for type-2 narcolepsy and idiopathic hypersomnia. | For type-1 narcolepsy, treatments bring only partial relief and do not target the cause; hypocretin agonists and immune based-therapies are needed. For type-2 narcolepsy and hypersomnia, stimulants are often addictive or ineffective. We need to understand the cause and find new treatments. |

An advantage of this approach is the detection of functional mutations with large effect sizes, and the possibility of creating animal models as a result. GWAS is most effective for heritable complex traits resulting from small common additive effects[36]. It needs large samples, but it is more robust than family studies when etiological heterogeneity is present. GWAS is more effective for phenotypes that are objectively, rather than subjectively defined. For example, smaller sample sizes (in the thousands) were sufficient to find genes regulating measurable features of the electrocardiogram (ECG) or cholesterol levels, whereas tens of thousands are needed for body mass index (BMI), a more complex phenotype and even more for neuropsychiatric disorders, complex phenotypes that are subjectively defined [46-50].

*A wealth of data is collected daily by sleep clinics, but it is not stored and exploited digitally. We have preliminary data on new technologies for monitoring and analyzing sleep.*

Currently, sleep clinics evaluate patients using the gold standard PSG, comprised of multiple digital signals (EEG, ECG, EOG, chin and leg EMG, breathing effort, and airflow) recorded over the night (Table 2). PSGs are currently scored by humans who page through a night in 30-second segments to extract simple features such as sleep/REM sleep latency, sleep stage proportions, number of sleep apnea events (Apnea-Hypopnea Index, AHI), and number of periodic leg movements (PLMI). This is time consuming and variable based on individual scorers[51,52]. In some centers, semi-automatic programs now assist scoring, but these programs try to mimic humans and do not go beyond what is accepted by a consensus of sleep experts[51,52].

Another critical factor in establishing new techniques for automatic scoring and for discovering diseases biomarkers is validation in large clinical and population based samples. One of our gold standards is the Wisconsin Sleep Cohort (WSC), a 30-year longitudinal study of 1,300 volunteers, aged 30-60, that includes PSG evaluation every 4 years[53,54]. This cohort has been deeply phenotyped, including with the Multiple Sleep Latency Test (MSLT), an objective test to measure sleepiness[55]. Because of our close association with this group, we accessed data, conducted GWAS, and are now administering the ASQ (see letter of support). In addition, we manage the Stanford Sleep Cohort (SSC), a cohort of 11,000 baseline sleep studies with diagnostic information from the Stanford Sleep Clinic with ~900 sleep disorder patients that have already been GWASed (subset SSC)[54]. The data generated from the proposed study can be validated and longitudinally evaluated in these and other NIH funded cohorts of similarly small sample sizes (see: https://sleepgenetics.org/cohorts/). Appendix 1 reports on preliminary sleep analytic data generated by our center in the area of automatic scoring and biomarkers.

*Large-scale studies of sleep variation with available genetic data are lacking. Current genetic studies of sleep are few, underpowered, and inadequately designed.*

GWAS have tried studying the subjective experience of sleep (sleep duration) but findings have been borderline and difficult to replicate[56-62]. This is not surprising because these measures depend upon subjective metrics (e.g. bed partner, questionnaires), and are modified by environmental factors and sleep disorders. In contrast, the sleep EEG is measurable and has a high heritability.

Table 2. Signals gathered from the PSG and clinical utility.

| | |
|---|---|
| Electroencephalogram (EEG) | Detects sleep onset, all sleep stages, and by how much sleep is disturbed by other factors. For example, it can measure brief (4 sec) arousal reactions secondary to sleep apnea or PLMs. |
| Electrooculogram (EOG) | Detects REMs and slow rolling eye movements during REM sleep and light sleep, respectively. |
| Chin electromyogram (EMG) | Detects atonia and phasic events during REM sleep. |
| Electrocradiogram (ECG) | Detects arrhythmias, also provides surrogate measure of autonomic tone. |
| Right or/and left leg EMG | These can be merged into a single signal or replaced by piezoelectric detection. Detects motor activity in sleep-related movement disorders such as Periodic Leg Movements (PLM) and parasomnias. |
| Snore sensor or microphone | Detects snoring and help confirm sleep apnea events. |
| Nasal and oral airlflow | Detect sleep apneas and hypopneas. Can distinguish between mouth breathing and apneas. |
| Inductance plethysmography (belts) | Detects abdominal and thoracic movement as a measure of respiratory effort. Effort differentiates between central and obstructive sleep apnea. |
| Blood oxygen saturation ($SpO_2$) | Detects drops of at least 3-4% to score hypopnea. In some cases, measures of end tidal $CO_2$ or transcutaneous $CO_2$ can be added to capture hypoventilation or other issues. |
| Body position | Detects position-dependent (supine, left, right, belly) sleep disturbances, commonly observed in obstructive sleep apnea. |

Although NIH has funded studies where sleep has been recorded using PSGs (Sleep Heart Study, Mr. Os, Framingham Heart Study, etc.) and genetic data is available, EEG findings have been disparate and no clear genetic association has emerged[63,64]. One issue is that the combined sample size is only 8,000 subjects. Further, the entire data (with linked clinical information) is not available without original investigator approval, which is difficult. These studies used variable recording technologies, subjective measures, and ascertainment schemes, resulting in difficulty analyzing data between and within studies. Finally, most studies have been designed to study sleep apnea and AHI. As discussed in the preliminary data section (Appendix 1), the AHI is a consensus-driven and somewhat artificial metric that varies with BMI and craniofacial structure.

One of the ways to estimate power for GWAS is to estimate heritability using GTCA[65]. This has not been done for sleep and sleep disorder traits, as it typically needs a sample size of over 5,000 subjects. A preliminary GTCA estimation of heritability we performed for $\sigma$ power, AHI using GCTA[65] in 980 subjects

found high values ranging of 0.77±0.42 and 0.70±0.40 respectively, consistent with genetic epidemiology data. Based on power calculations, the complexity of sleep phenotypes and heritability estimates, sample sizes of 30,000-40,000 are likely required to find multiple significant markers using a GWAS design.

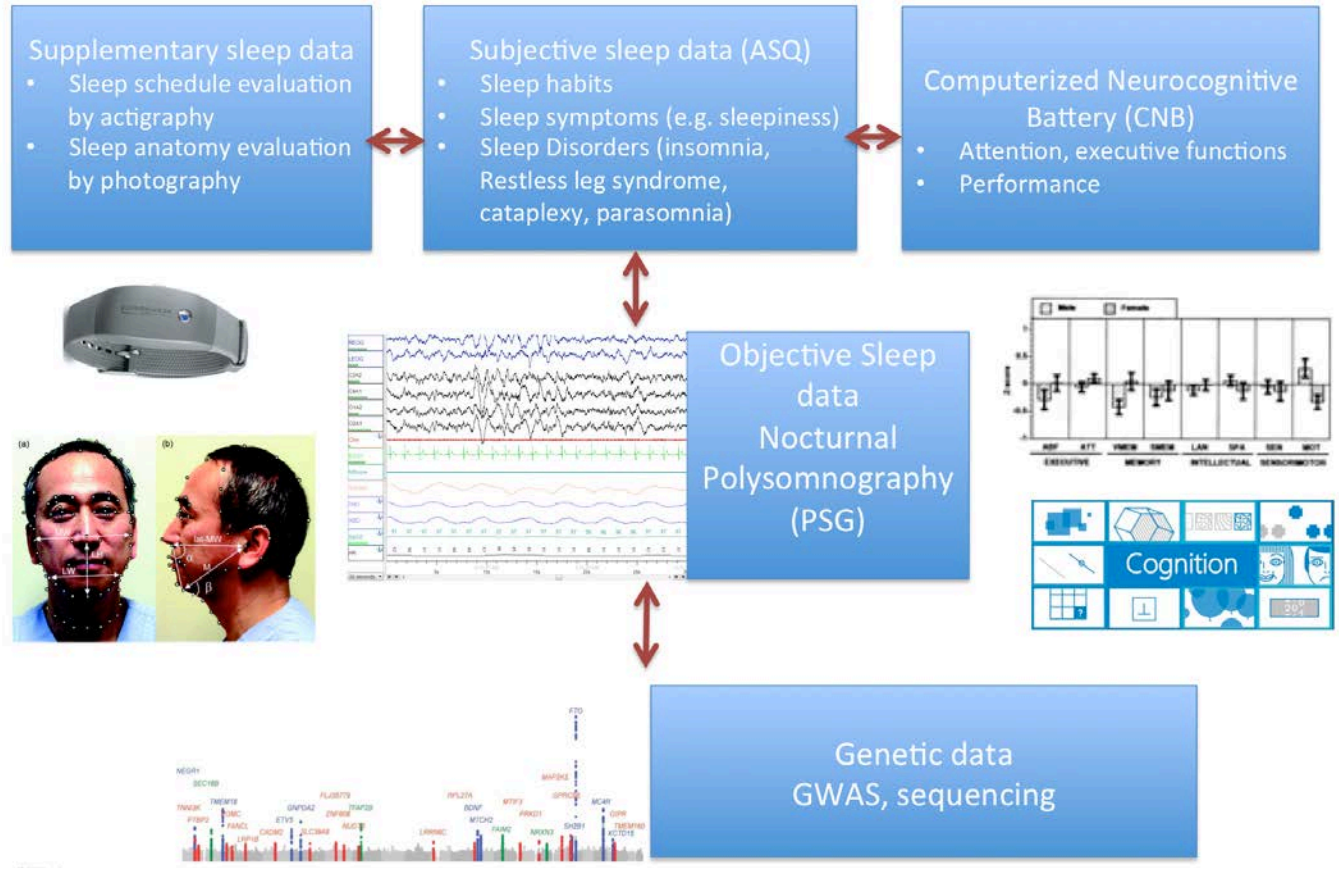
## Research design and methods

Specific Aim 1: Collect and make available PSG, ASQ, neuropsychological testing data, biological samples and DNA on 30,000 adult/adolescent patients evaluated for sleep and sleep disorders at five centers, creating a unique reference database for the field of sleep medicine.

### Rationale and Preliminary Data:

We aim to create a database of 30,000 PSG studies (age 14-100) (Figure 1) linked to genetic and phenotypic data that will be accessible to any investigator (see data sharing plan).

Figure 1. Overarching design of the study

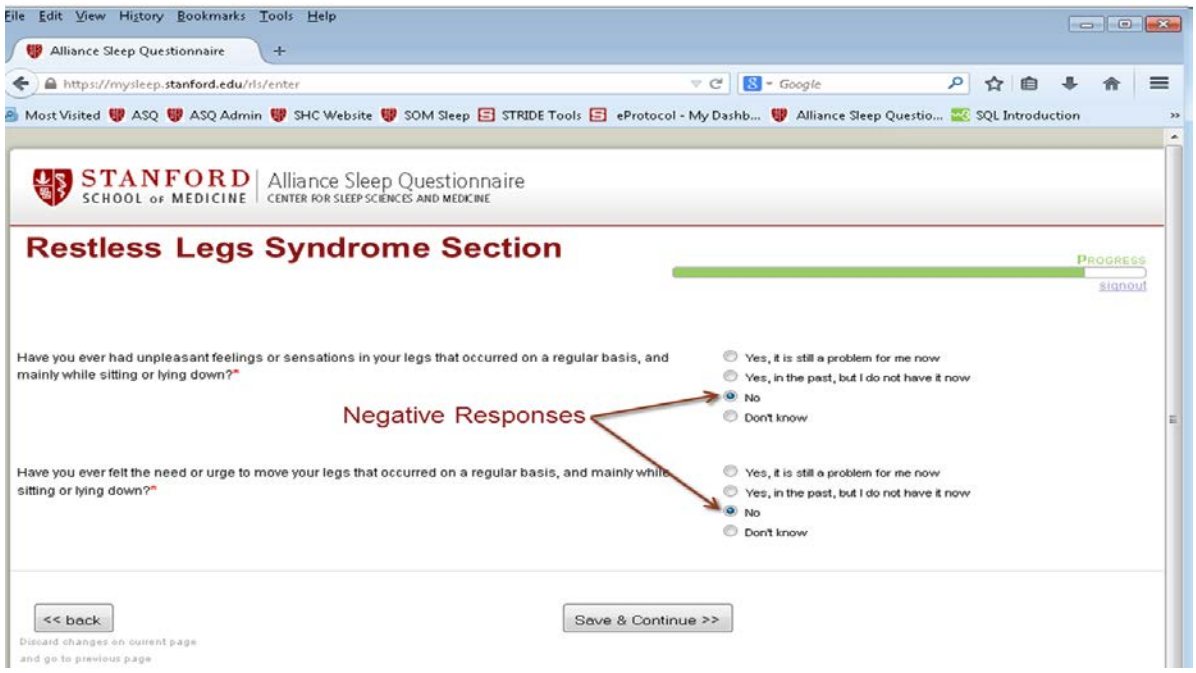



*Subject identification and blood sampling:* At each site, subjects will be assigned a database ID based on ASQ entry. This ID will be used for data and samples in de-identified datasets. Blood samples will be drawn,

centrifuged, separated into bar-coded aliquots and frozen in local clinics. Frozen aliquots will be sent every 3 months to RUCDR (http://www.rucdr.org/) and Stanford (for extra aliquots) where they will be logged and kept at -80ºC. Each site will keep a log of correspondence between PHI, bar codes, and ASQ number information, which links to the PSG file and other data.

*Alliance Sleep Questionnaire (ASQ):* The ASQ is a comprehensive, on-line questionnaire developed in collaboration with Harvard University, U Penn, U Madison-Wisconsin and St Louis[66]. It uses branching logic, so that questions or severity scales expand only if positive answers are given (see Figure 2). It takes a median of 35 minutes to complete for sleep disorder patients.

Figure 2. Example of branching logic in the Alliance Sleep Questionnaire




The ASQ is comprised of validated measures and novel questions designed to collect sleep history plus presence and severity of sleep symptoms, as listed in Table 3 below. It is now standard of care at the Stanford Sleep Clinic so that all new patients log in prior to their first clinic visit. The system generates a "physician summary report" that makes the visit more effective, reducing the risk of overlooking comorbid disorders such as RLS or insomnia, which are often overlooked when sleep apnea is present. Unlike other instruments that are disease specific, the ASQ reviews sleep habits and symptoms of all sleep disorders. Other general instruments have been designed but are either less complete or proprietary[67-69].

Validation of the ASQ against medical diagnoses has been performed for RLS, narcolepsy, and obstructive sleep apnea (OSA)[66,70-72]. ASQ and example report can be accessed at https://oursleep.stanford.edu/demo. We have over 5,000 completed baseline surveys and are working on tracking changes/outcomes. Before starting the ASQ, individuals fill an IRB-approved consent form requesting permission to use their de-identified data. 92% of individuals who log on complete the questionnaire and 85% agreed to have their data

included in our research database. Load analyses have shown that we can accommodate 100 subjects logged on simultaneously, which will be sufficient for this project.

Table 3. Sections of the ASQ

| | | |
|---|---|---|
| • Personal health information (PHI) | • Social history | • Parasomnia |
| • Demographics | • Apnea (multivariate apnea predictor or MAP[73]) | • Quality of life (functional outcome sleep questionnaire or FOSQ[74,75]) |
| • Medical history | • Sleepiness (Epworth sleepiness scale or ESS[76] and novel questions) | • Depression (personal health questionnaire or PHQ-9[74,77]) |
| • Medications | • Insomnia (insomnia severity index or ISI[78] and insomnia symptom questionnaire or ISQ[35]) | • Anxiety (general anxiety disorder or GAD-7[79,80]) |
| • Family history | • Restless leg syndrome (RLS) | • Fatigue (fatigue severity scale or FSS[81,82]) |
| • Bedtime habits | • Circadian rhythms (reduced morningness eveningness questionnaire or r-MEQ[83]) | • Satisfaction survey |
| • Schedule | • Narcolepsy | |

Methods:

*Improving ASQ:* During the first six months, we will add a few questions requested by participating sites and incorporate the ASQ into clinical workflows of all sites. Finally, a PHI-restricted module that allows users to take their picture (face and profile) with any smartphone will be created. Pictures are next subjected to a software extracting and storing craniofacial metrics predictive of sleep apnea; these are especially important to increase positive predictive value for non-obese individuals[84].

*PSG data:* To limit inter-site variability, specific channels and filter settings will be required from all sites so that raw data is minimally transformed. Site coordinators will export data into folders named by subject's ID. These will contain European Data Format (EDF) and event-scored text files. These folders will be ftp to our server and verified by the Stanford team using a specially designed program to ensure (1) consistent channel labeling, and (2) absence of PHI. Stanford will visit every site during the first 6 months to ensure all is done according to specifications.

*Locomotor activity:* Actigraph devices will be used to assess physical activity and the timing of sleep. The device utilizes a 3-axis accelerometer sensor to measure user's movements and positions. It is water resistant and requires periodic charging depending on device. Data are transferred onto smartphone or iPad via BTLE, emitting less than 1/10,000th the amount of electromagnetic energy of a cell phone. Devices (Figure 3) are sold worldwide, including at the Apple store, and the mobile app supports multiple foreign languages.

An independent groups have been evaluating the accuracy of different consumer activity trackers [85]. Test-retest revealed high reliability and mean absolute percentage errors in laboratory and free-living conditions of 0.2% and 1.1%. Algorithms have been validated for the measure of various types of physical activity, and

are sufficient to objectively measure total sleep time. Patients will be asked to wear these free devices during PSGs and until their follow up visit a few weeks later.

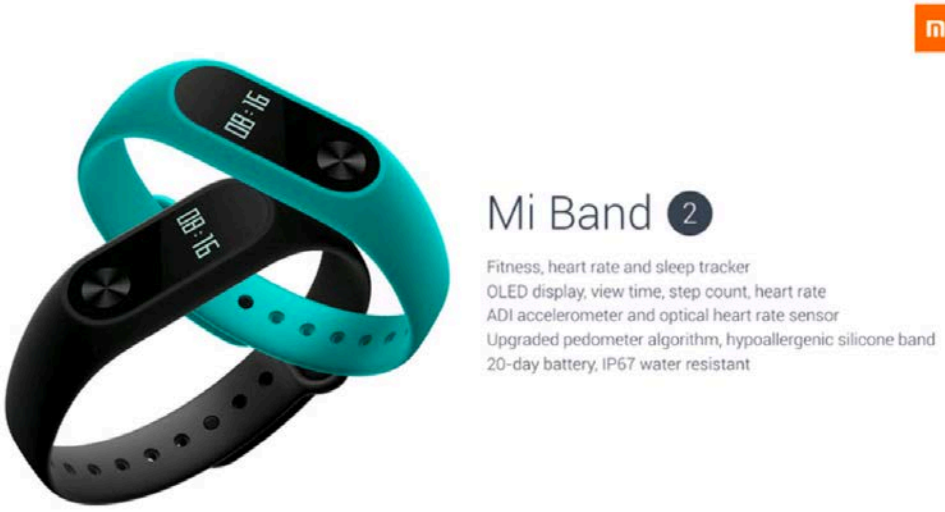



Figure 3: The Mi Band 2 device

*Neuropsychological testing:* A shortened version of a self-administered Computer-based Neurocognitive Test Battery (CNB, tested in over 100,000 individuals) designed by U. Pennsylvania will used [86-92]. It will probe domains most affected by sleep disturbances (Table 4).

Table 4 Neuropsychological testing units

| Test | Neurobehavioral function | Domain | Duration |
|---|---|---|---|
| Penn Matrix Analysis Test (PMRT) | Complex Cognition | Nonverbal reasoning | 6 min |
| Short Letter-N-Back (LNB) | Executive Control | Working Memory | 8 min |
| Penn Continuous Performance Test (PCPT) | Executive Control | Attention | 6 min |
| Psychomotor Vigilance Task (PVT) | Executive Control | Attention/Reaction Time | 10 min |
| | | | Total: 30 min |

Data will be scored using a program written in Perl and sent in CSV format to Stanford. A link to the CNB will be presented at the end of the ASQ to connect CNB and ASQ IDs. Responses (accuracy, speed) will be uploaded and stored in a U-Penn database with quality assurance procedures.

*Database and Data processing:* Details of the database (Figure 4) are reported in Appendix 3. High performance computing clusters administered by the Stanford Research Computing Center will also be used to analyze and run processes on de-identified datasets. One example is Sherlock, which we use for machine learning on PSG signals. It has 127 compute servers and associated storage and is available to run researchers' computational codes and programs, with resources managed through a fair-share algorithm using SLURM as the resource manager/job scheduler. Another example is the Stanford Genetics SCG3 cluster that we use for genome wide analyses. For this project, we plan to use the pipelines of the Psychiatry Genetic Consortium (PGC) and of Neil Risch (UCSF) (see letter of support).

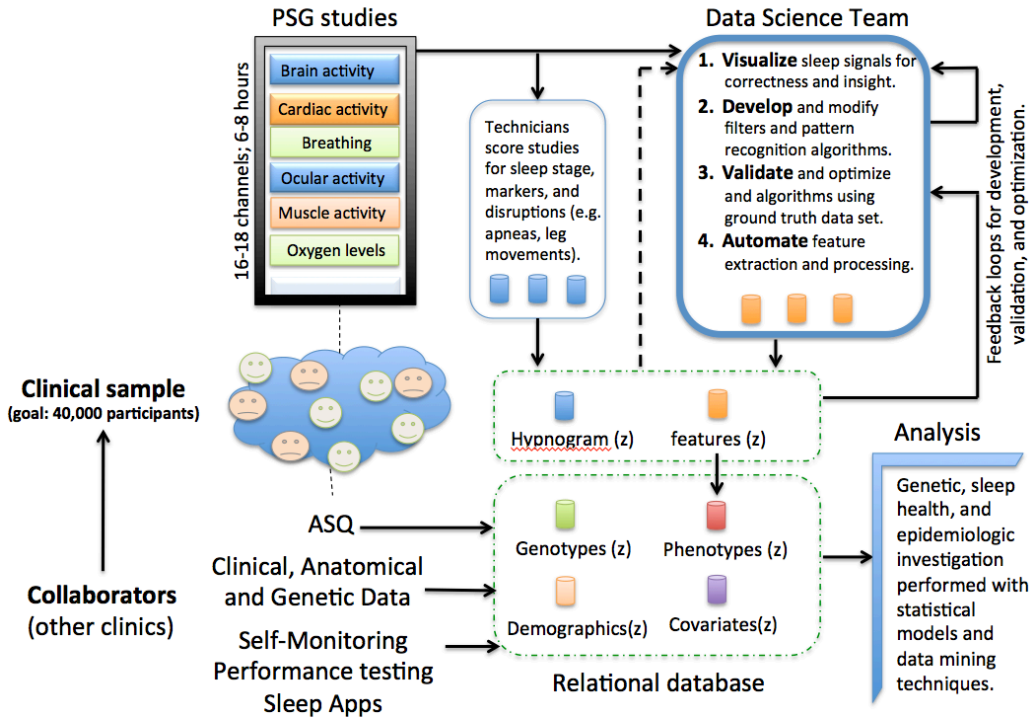



Figure 4. Architecture of the data to be used in this study

Specific Aim 2: Develop and make available software that will streamline PSG analysis, extract meaningful sleep phenotypes, and standardize analysis in large samples. Validate this software in smaller, existing cohorts and use in the large 30,000 sample in conjunction with GWAS to answer critical questions related to sleep and sleep disorders.

Rationale and Preliminary Data: We will use signal analysis to discover novel PSG biomarkers that correlate with clinical endpoints data (e.g. ASQ). These will also be endophenotypes for genetic analysis. We will streamline and automate PSG analysis for routines that are already used clinically (see Table 2 above). The resulting well-annotated software programs will be made available for (1) clinical and research sleep analytics and (2) use in the large 30,000 sample. What we develop is established in MATLAB or R, focusing on functional performance rather than robustness (which would be needed for subsequent commercial development). Our automatic PLM detector (now routinely used in our sleep clinic)[93] and other programs have already been made available (see Appendix 2).

Methods: Two trained sleep engineers are already working in our lab (Oscar Carillo and Hyatt Moore), and as shown by our letters of support, strong collaborations with engineering groups spanning the globe have been established. We have notably developed ties with the Danish Technical University (DTU) in Copenhagen, so that 1-2 Electrical Engineers (EE) master students rotate every six months through our laboratory. Table 5 below outlines PSG biomarkers that will be explored as part of this specific aim, with clinical applications (variables reported by classic PSG reports such as PSG sleep latency, Sleep Efficiency, although of interest are summarized as “sleep architecture”).

Table 5. Tentative PSG Biomarker list that will be developed, by topic/disorder.

| | |
|---|---|
| Insomnia, hypersomnia/narcolepsy, and psychiatric comorbidity | • Sleep architecture<br>• EEG power spectra features per sleep stage*<br>• Delta power during wake, Slow Wave Sleep amounts during sleep corrected for habitual sleep amounts<br>• Hilbert Huang Transform analysis of the EEG (see letter of support of Dr. Huang) †<br>• Sleep stage transition analysis*<br>• Sleep stage space analysis and automatic sleep/wake scoring by 10 sec epochs*<br>• Slow wave/delta power dynamics across the night and sleep cycle analysis<br>• Coherence analysis (inter- and intra- hemispheric, per sleep stage)<br>• Microarchitecture analysis (spindles*, K-complex, saw tooth waves detection)<br>• REM sleep features (REM density, REM sleep atonia)*<br>• Circadian-derived measures using actigraphy or night EEG<br>• Arousal detections out of various sleep stages†<br>• Measures of circadian phase through analysis of all signals in correlation with body temperature† |
| RLS and PLMs (may predispose to depression and cardiovascular disease) | • PLMI, periodicity index, dynamics across the night and by sleep stage, with and without arousal (frequency of PLMs)*<br>• Time locked analysis of EEG, EMG, ECG locked on PLM (impact of PLMs)* |
| Sleep disordered breathing (SDB)<br><br>(predisposes to cardiovascular disease and sleepiness) | • Apnea Hypopnea Index and other derivatives (by sleep stage, with various definitions with and without oxygen desaturation and arousal, central or obstructive) (frequency of respiratory events)*<br>• Time locked analysis of Oxygen saturation, EEG, EMG, ECG locked on SDB events per sleep stage (severity of respiratory events)*<br>• Oxygen saturation at baseline and end of the night, per sleep stage, time spent at various levels of Oxygen saturation*<br>• Breathing frequency, inspiration and expiration time per sleep stage†<br>• Respiratory cycle-related EEG changes |
| Parkinson's disease early biomarkers | • REM sleep without atonia*<br>• Sleep fragmentation, loss of spindles*<br>• ECG-power spectra changes across sleep stages* |
| Alzheimer's disease early biomarkers | • Power spectra in REM sleep*<br>• Coherence analysis |
| Seizure activity during sleep | • Automatic detection of events |
| ECG | • Analysis of shape, PR, QT, automatic detection of arrhythmia, and conduction defects across sleep stages |

* Already implemented, or close to completion, † next goals

Dimitri Perrin in Australia has pledged to help us develop an ASQ sleep application. We will extend collaboration with the Stanford Big Data initiative and the newly formed Department of Biomedical Data Sciences program with which we are both affiliated. We will also involve Pietro Perona (Caltech) and Fei-Fei (head of Artificial Intelligence labs at Stanford) for machine learning supervision (see letters of support). Biomarkers will be correlated with patient diagnosis, other chart measures, and subjective data extracted from the ASQ and CNB as outlined in Table 6 below.

Table 6. Subjective and indirect measures of disorders to be correlated with PSG biomarkers.

| | |
|---|---|
| Insomnia, hypersomnia/narcolepsy, psychiatric comorbidities | • Long and short habitual sleep duration or need (ASQ and actigraphy)<br>• Measures of sleep debt such as habitual sleep during workday versus weekend<br>• Daytime sleepiness (Epworth Sleepiness Scale-ESS, Fatigue Severity Scale, other sleepiness questions implemented in ASQ)<br>• Sleep paralysis, nightmares, hypnagogic hallucinations, dream enacting, sleep walking, sleep talking, night terrors (ASQ)<br>• Insomnia, depression and anxiety ratings (ASQ)<br>• Computerized Neurocognitive Battery (CNB) |
| Periodic Leg movements (PLMs) and Restless Legs Syndrome (RLS)<br>(may predispose to depression and cardiovascular disease) | • RLS questionnaire and severity (ASQ)<br>• Blood Ferritin, C-Reactive Protein† |
| Sleep Disordered Breathing (SDB)<br><br>(predispose to cardiovascular disease and sleepiness) | • Blood bicarbonate levels†<br>• Craniofacial features with impact on sleep apnea (via the ASQ photography app)<br>• PVT and Computerized Neurocognitive Battery (CNB)<br>• Baseline blood pressure, ferritin, lipid and glucose† |

*Already implemented, or close to completion, †next goals

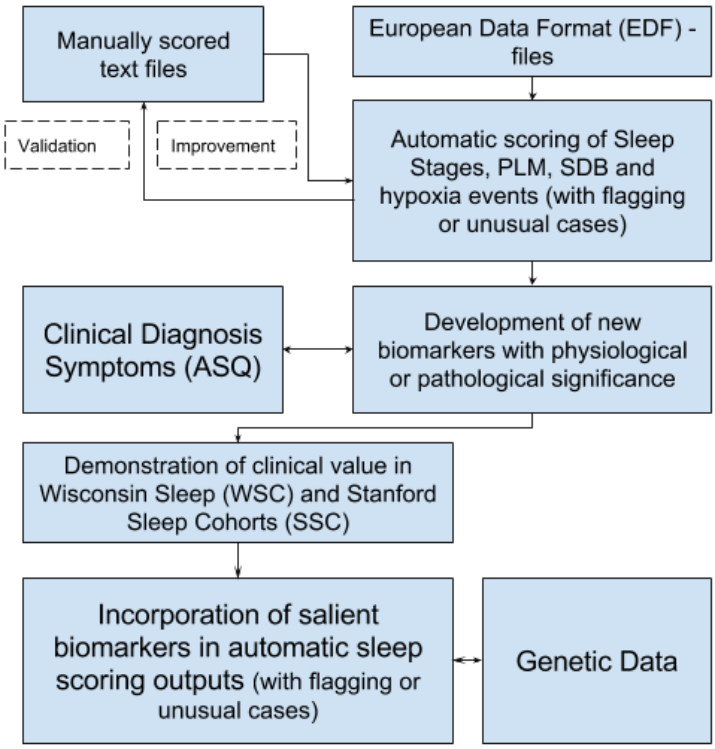
Validation and Analysis Plans:

Biomarkers will be first validated in the WSC and SSC cohorts, pending completion of the 30,000 sample. To increase reliability, we will design routines that can be used to streamline basic analysis of PSG data (automatic scoring of PMLI, AHI and sleep stages, see Figure 5). These will involve implementation of data cleaning procedures (for example adaptive filtering of ECG signal in the EMG, artifact detection/removal, etc[93]) and a combination of traditional rule-based event scoring techniques, such as those described in Appendix 2 for our PLM and SDB detector, plus machine learning techniques (both traditional and deep, see Appendix 2). Once these will have been established, the work on finding and implementing additional biomarkers as outlined in Table 5 will start.

Of particular potential is the development of deep machine learning routines. Traditionally, engineers had to spend hours scrutinizing datasets, and to couple it with prior knowledge about relevant pathologies, to design meaningful features for quantifying or diagnosing pathologies. Recent advances has led to powerful new

models often referred to as convolutional neural networks[94], in which such practices have become obsolete. These models receive raw (or slightly modified) data as input, and through an intricate network of filters coupled with a general purpose learning algorithm, feature vectors, which can accurately explain the differences in a data-set, are learned/developed automatically. Models like these have become the gold standard in many artificial intelligence disciplines, and have beaten human performance in areas such as visual recognition and speech analysis[95-97].

Figure 5. PSG analytical pipeline to be used in this study



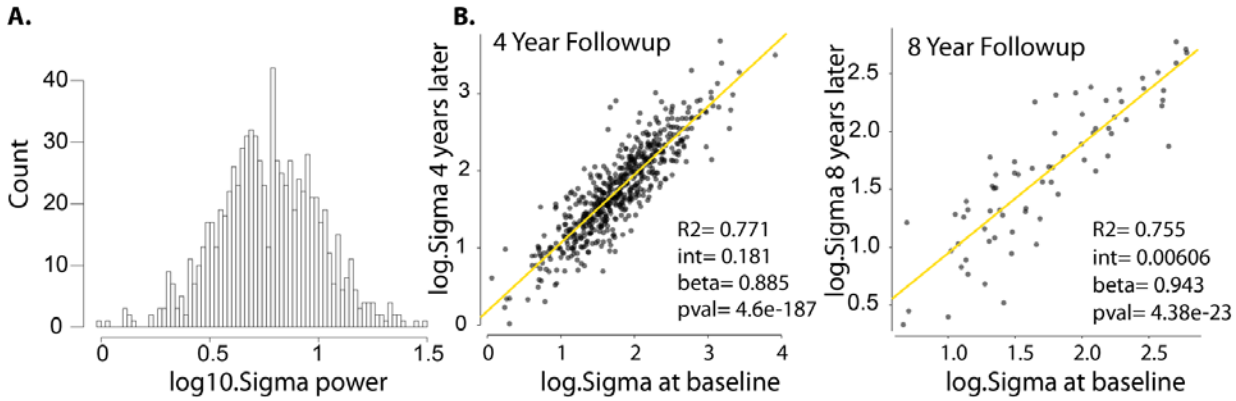

As shown in Appendix 2, a deep learning sleep-scoring algorithm is being developed. It is being validated on our data and on data from the AASM Inter-Scorer Reliability program, which has been assessed by hundreds of scorers and therefore provides an accurate true label. We believe deep-learning routines will eventually replace the incumbent golden standards for sleep scoring.

Importantly, anomalies may exist in the data either because of a bad recording, or because a patient has a "new" condition that is being analyzed. Blindly using deep learning methods where decision are made on the basis of rules created through an opaque algorithm may result in such interesting cases being unnoticed. One way to protect against this is quality control procedures and to use a combination of tools for analysis so that divergent results can be flagged and analyzed (for example discordant sleep staging results using manual, hierarchical machine learning or deep convolutional neural network analysis). In addition, deep learning also provides tools for automatically dealing with this problem called autoencoders[98]. These are trained to reproduce the input (sometimes a corrupted version to keep the network from learning the identity) through a network of hidden units. Features, which have been learned on known data, will yield a poor reconstruction of new data[99]. Such models may prove useful for detecting abnormalities in data quality or even new pathologies.

Several principles are used when undergoing validation of any biomarker. First, we start with cross-sectional analysis and divide samples into exploratory and confirmation samples. The exploratory sample is used to derive the ideal biomarker, but because of the exploratory nature of this first analysis, it is subject to over-fitting. Covariates and confounders are identified at this stage. For this experiment, we will use half of the SSC (5,500 subjects) then replicate in the other half. Once a biomarker is confirmed, it can then be assessed in the WSC for confirmatory cross-sectional association and, if expected to be predictive of complications

within the 16-year follow-up period of the cohort, longitudinal studies. In other cases, longitudinal studies are helpful to determine stability of a biomarker, which indirectly indicate rest-retest reliability. As an example, Figure 6 shows stability of power in the σ band across 4-8 years of follow up, a proxy of sleep spindles that has been shown to be highly heritable.

Figure 6. Stability of σ power in longitudinal studies of the Wisconsin Sleep Cohort




Plan will vary depending on each case, and may involve additional specialized cohorts (for example narcolepsy or specific pathologies). The fact the WSC cohort continues to age (current mean age 68) is especially valuable for discovering early markers for diseases associated with aging, such as stroke, neurodegenerative diseases (Parkinson's and Alzheimer's), depression, RLS, and insomnia.

Specific Aim 3: Use GWAS in conjunction with machine learning and phenotype analysis in the 30,000 sample to discover genetic modifiers for sleep and sleep disorders focusing on genetic variants that control EEG traits and hypersomnia phenotypes.

Rationale:

EEG power spectrum phenotypes have been selected because of stability over time and high heritability, with 95 percent of variation in EEG traits explained by genetic effects[100]. Hypersomnia phenotypes have been selected because of the special expertise of the PI. Indeed, a large number of studies have already been performed by the PI and his collaborators, Paul Peppard and Terry Young in the WSC[54], on hypersomnia phenotypes so that investigators are already familiar with confounders and covariates.

*Phenotype selection:* Phenotypes will include EEG profiles over the entire night (total and relative power) and per sleep stage. Hypersomnia phenotypes will include ESS, sleep paralysis, cataplexy, automatic behavior, napping frequency, short nocturnal PSG sleep latency, short PSG REM sleep latency (≤15 min) and any PSG-EEG measures that will be found to correlate with MSLT SL in the WSC. For each of these parameters, we will first identify relevant covariates in the WSC and the SSC. These covariates are then included or not included in the final genetic model of the 30,000 samples depending on phenotypic relevance. Analysis may include linear or logistic regression with meaningful clinical cutoffs. Once covariates are established, they will be repeated or added if significantly associated in the larger 30,000 sample, and a final model decided upon for GWAS analysis.

One of the most interesting phenotypes may be δ-power or % slow wave sleep, two classic markers of sleep homeostasis[101,102] and unexplained sleepiness. δ-power increases with sleep deprivation, but has interindividual variability at baseline and after sleep deprivation[90-92,101,102]. Sleep deprivation and sleepiness is also associated with shorter sleep onset latency (for example using the MSLT), and decreased performance measures as reported using the PVT[90-92]. We will explore relationships between these traits and performance measures after controlling for known confounding factors such as age, sex, or indirect measures of sleep debt (δ-power, habitual sleep time by actigraphy, or difference in sleep amounts reported during work days vs. non-work days). Once this is done, we plan to identify a composite multivariable that best reflect sleepiness independent of past sleep history.

*Power and sample size:* Common variant associations with OR=1.1 and MAF=0.2 have less than 1% power for detecting genome-wide significant variants in 5,000 cases versus 5,000 controls. With 30,000 subjects, we have ~80% power to detect such variants with GWAS significance.

*Genotyping and GWAS analysis:* We plan to pilot genotype 10,000 subjects in year 3 as a pilot so that we can start designing analysis early. We will perform imputation on top of genome-wide genotyping to increase coverage and to detect additional functional coding and regulatory variants that may contribute to phenotypes. The 30,000 samples will be imputed year 4.5 using 64,976 haplotypes and 39,235,157 SNPs from over 32,000 individuals (or more in a few years) of the Haplotype Reference Consortium including individuals from 1000 genomes[103]. Imputation would be done using Shapeit, Impute2, MACH, and software provided by the Haplotype Reference Consortium portal. Although challenging, using a large reference panel should enable imputation of rare variants with low minor allele frequency of up 0.005. Neil Risch (UCSF) and Carlos Bustamante (Stanford) will be extensively involved. We will also establish a strong collaboration with the Psychiatric Genetic Consortium (PGC) (see letter of support of Pat Sullivan) to explore comorbid psychiatric issues. Neil Risch has performed extensive analyses that derive from the genotyping of 100,000 individuals drawn from the Kaiser Genetic Epidemiology Research on Adult Health and Aging (GERA) Cohort[104]. Similarly, the PGC has a very effective pipeline, the Ripelli pipeline [105]. These include QC of the genotype data, analyses of population structure, imputation, and GWAS of various clinical phenotypes (see letters of support). We also have extensive prior expertise in GWAS[32-34].

Analytical Plan: Potential population differences and stratification will be controlled using the first 10 principal components from identity by descent (IBD) calculations based on the genotypes. GWAS analyses will be run using linear regression, adjusting for covariates and principal components using PLINK, GCTA, and/or SNPtest software. One difficulty in this study is that we will have multiple phenotypes that are partially correlated. To address this issue, the following approaches will be used:
Because multiple PSG and behavioral traits are correlated, we will use multivariate phenotype analysis, combining multiple phenotypes or global scores whenever possible[106-108]. This approach increases power substantially for detecting genetic variants affecting phenotypes by reducing multiple testing. For example, ESS, MSLT MSL, and PSG SL all correlate with r~0.31-0.35 and we hope to define a multivariate phenotype for sleepiness independent of sleep debt. Whenever possible, these "composite" phenotypes will be constructed after having conducted shared heritability estimates based on GWAS data of correlated traits using CGAT[65], LD Score regression (LDSR), and multi-phenotype Mendelian randomization (MMR) methods[109,110] (https://github.com/bulik/ldsc).

Because we anticipate studying ~50 phenotypes, we plan to report on genome-wide hits that reach significance $<1*10^{-9}$ if not replicated in other cohorts. In cases where the association can be replicated in other existing cohorts, $p<5x10^{-8}$ in the 30,000 sample plus significant replication appropriately controlled for multiple testing will be considered sufficient. Independent replication with similar effect sizes and appropriate p-values are the best guarantee against false positives.

We finally plan to conduct functional and pathway analyses for associations obtained with these phenotypes. Tissue specific function of GWAS significant variants will be assessed using data from the ENCODE roadmap and GTEx projects[111]. Any finding will lead to further characterization in functional models including mouse models in future studies.

## Timetable and Milestones

The Milestones are discussed in more detail in Appendix 3 which includes a list of key staff involved in each milestone. Table 7 below provides a high level overview of the entire project.

**Table 7: Timeline of Study Phases for Five Years of the Study**

| Year | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Month | 0-6 | 7-12 | 13-18 | 19-24 | 25-30 | 31-36 | 37-42 | 43-48 | 49-54 | 55-60 |
| **Phase 1:** | | | | | | | | | | |
| Planning & Protocols | | | | | | | | | | |
| Building IT Platform | | | | | | | | | | |
| **Phase 2:** | | | | | | | | | | |
| Recruitment | | 0.2K | 2.8K | 8K | 13.2K | 18.4K | 23.6K | 28.8K | 30K | |
| PSG Analytics | | | | P | | P | | P | | P |
| **Phase 3:** | | | | | | | | | | |
| Genotyping & Data Posted to dbGAP/ NIH | | | | | | 10K | | | | |
| Genetic Analysis (Hypersomnia) | | | | | | | | P | | |
| Data Sharing Portal | | | | | | | | | | |
| **Phase 4:** | | | | | | | | | | |
| Genotyping & Data Posted to dbGAP/ NIH | | | | | | | | | | 30K |
| All PSG Analytics Posted | | | | | | | | | | |
| Further Genetic Analysis | | | | | | | | | | P |

P=Expected publication point including code when applicable

Table 8 below is a detailed timeline for the milestones for the first two years of the project. The first 9 months of this study will be dedicated to creating detailed manuals to guide data collection at the sites. In addition, we will set up a stable and secure infrastructure for receiving and verifying the integrity of all data files, logging the information and providing feedback to the study committees. Site personnel will also receive reports to facilitate day-to-day operations of the study. Finally, we will set the foundation for a data storage/dissemination system that will provide convenient access to the scientific community once the data is publicly available.

Table 9 summarizes the provisional milestones for the remainder of the study. Emphasis will be on recruitment milestones, PSG data analytics and posting the data to the scientific community in two batches. These milestones and timeline will be adjusted slightly at the two-year mark with approval from the Steering Committee, External Review Committee and the Klarman Family Foundation.

Table 8: Timeline of Milestones for First Two Years of the Study

| Milestone | Anticipated Completion | Year 1 | | | | | | | | | | | | Year 2 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| 1. Secure Data Storage | Month 3 | | | | | | | | | | | | | | | | | | | | | | | | |
| 2. Finalize Data Elements | Month 6 | | | | | | | | | | | | | | | | | | | | | | | | |
| 3. Resource Sharing Plan Finalized w/NIH | Month 6 | | | | | | | | | | | | | | | | | | | | | | | | |
| 4. Informed Consent and IRB Approved | Month 9 | | | | | | | | | | | | | | | | | | | | | | | | |
| 5. Launch Data Management Portal | Month 9 | | | | | | | | | | | | | | | | | | | | | | | | |
| 6. Start Recruitment | Month 10 | | | | | | | | | | | | .2K | | | | | | | | | | | | |
| 7. Enroll 2,800 Participants | Month 18 | | | | | | | | | | | | | | | | | 2.8K | | | | | | | |
| 8. PSG Data Analytics (Version 1) | Month 24 | | | | | | | | | | | | | | | | | | | | | | | | |
| 9. Enroll 8,000 Participants | Month 24 | | | | | | | | | | | | | | | | | | | | | | | | 8K |

Table 9: Timeline of Provisional Milestones for Years Three through Five of the Study

| Milestone | Anticipated Completion | Year 3 | | Year 4 | | Year 5 | |
|---|---|---|---|---|---|---|---|
| | | 25-30 | 31-36 | 37-42 | 43-48 | 49-54 | 55-60 |
| 10. Enroll 15,000 Participants | Month 32 | | 15K | | | | |
| 11. PSG Data Analytics (Version 2) | Month 36 | | | | | | |
| 12. Data Posted to NDA and dbGaP | Month 36 | | 10k | | | | |
| 13. Enroll 25,000 Participants | Month 44 | | | | 25K | | |
| 14. PSG Data Analytics (Version 3) | Month 48 | | | | | | |
| 15. Complete Recruitment (30K) | Month 54 | | | | | 30K | |
| 16. PSG Data Analytics (Version 4) | Month 60 | | | | | | |
| 17. All Data Posted to NDA and dbGap | Month 60 | | | | | | |

## Potential limitations, risks and solutions

These are discussed in Appendix 4.

## Potential impact

This is discussed in Appendix 5.

References


1 Kryger, M. H., Roth, T. & Dement, W. C. *Principles and practice of sleep medicine*. 5th edn, (Elsevier/Saunders, 2011).

2 Mignot, E. Why we sleep: the temporal organization of recovery. *PLoS biology* **6**, e106, doi:10.1371/journal.pbio.0060106 (2008).

3 Tononi, G. & Cirelli, C. Sleep and the price of plasticity: from synaptic and cellular homeostasis to memory consolidation and integration. *Neuron* **81**, 12-34, doi:10.1016/j.neuron.2013.12.025 (2014).

4 Mackiewicz, M., Naidoo, N., Zimmerman, J. E. & Pack, A. I. Molecular mechanisms of sleep and wakefulness. *Ann N Y Acad Sci* **1129**, 335-349, doi:10.1196/annals.1417.030 (2008).

5 Krueger, J. M., Frank, M. G., Wisor, J. P. & Roy, S. Sleep function: Toward elucidating an enigma. *Sleep Med Rev* **28**, 42-50, doi:10.1016/j.smrv.2015.08.005 (2015).

6 Saper, C. B. The neurobiology of sleep. *Continuum (Minneap Minn)* **19**, 19-31, doi:10.1212/01.CON.0000427215.07715.73 (2013).

7 Luppi, P. H., Clement, O. & Fort, P. Paradoxical (REM) sleep genesis by the brainstem is under hypothalamic control. *Current opinion in neurobiology* **23**, 786-792, doi:10.1016/j.conb.2013.02.006 (2013).

8 Mackiewicz, M., Zimmerman, J. E., Shockley, K. R., Churchill, G. A. & Pack, A. I. What are microarrays teaching us about sleep? *Trends Mol Med* **15**, 79-87, doi:10.1016/j.molmed.2008.12.002 (2009).

9 Bellesi, M., de Vivo, L., Tononi, G. & Cirelli, C. Effects of sleep and wake on astrocytes: clues from molecular and ultrastructural studies. *BMC Biol* **13**, 66, doi:10.1186/s12915-015-0176-7 (2015).

10 Cirelli, C. & Tononi, G. Molecular neurobiology of sleep. *Handb Clin Neurol* **98**, 191-203, doi:10.1016/B978-0-444-52006-7.00012-5 (2011).

11 Aho, V. *et al.* Partial sleep restriction activates immune response-related gene expression pathways: experimental and epidemiological studies in humans. *PloS one* **8**, e77184, doi:10.1371/journal.pone.0077184 (2013).

12 Zehring, W. A. *et al.* P-element transformation with period locus DNA restores rhythmicity to mutant, arrhythmic Drosophila melanogaster. *Cell* **39**, 369-376 (1984).

13 Takahashi, J. S. Molecular components of the circadian clock in mammals. *Diabetes Obes Metab* **17 Suppl 1**, 6-11, doi:10.1111/dom.12514 (2015).

14 Hsu, P. K., Ptacek, L. J. & Fu, Y. H. Genetics of human sleep behavioral phenotypes. *Methods Enzymol* **552**, 309-324, doi:10.1016/bs.mie.2014.10.046 (2015).

15 Malone, S. M. *et al.* Heritability and molecular-genetic basis of resting EEG activity: a genome-wide association study. *Psychophysiology* **51**, 1225-1245, doi:10.1111/psyp.12344 (2014).

16 Caylak, E. The genetics of sleep disorders in humans: narcolepsy, restless legs syndrome, and obstructive sleep apnea syndrome. *Am J Med Genet A* **149A**, 2612-2626, doi:10.1002/ajmg.a.33087 (2009).

17 Dudley, K. A. & Patel, S. R. Disparities and genetic risk factors in obstructive sleep apnea. *Sleep Med*, doi:10.1016/j.sleep.2015.01.015 (2015).
18 Sehgal, A. & Mignot, E. Genetics of sleep and sleep disorders. *Cell* **146**, 194-207, doi:10.1016/j.cell.2011.07.004 (2011).
19 Tafti, M. Genetic aspects of normal and disturbed sleep. *Sleep Med* **10 Suppl 1**, S17-21, doi:10.1016/j.sleep.2009.07.002 (2009).
20 Barclay, N. L. & Ellis, J. G. Genetic influences contribute to neurobehavioral response to acute sleep deprivation. *Sleep* **35**, 1191-1192, doi:10.5665/sleep.2060 (2012).
21 Kuna, S. T. *et al.* Heritability of performance deficit accumulation during acute sleep deprivation in twins. *Sleep* **35**, 1223-1233, doi:10.5665/sleep.2074 (2012).
22 Riemann, D. *et al.* The neurobiology, investigation, and treatment of chronic insomnia. *Lancet Neurol* **14**, 547-558, doi:10.1016/S1474-4422(15)00021-6 (2015).
23 Barclay, N. L. & Gregory, A. M. Quantitative genetic research on sleep: a review of normal sleep, sleep disturbances and associated emotional, behavioural, and health-related difficulties. *Sleep Med Rev* **17**, 29-40, doi:10.1016/j.smrv.2012.01.008 (2013).
24 Adamczyk, M. *et al.* Genetics of rapid eye movement sleep in humans. *Transl Psychiatry* **5**, e598, doi:10.1038/tp.2015.85 (2015).
25 Parish, J. M. Genetic and immunologic aspects of sleep and sleep disorders. *Chest* **143**, 1489-1499, doi:10.1378/chest.12-1219 (2013).
26 Dauvilliers, Y. & Winkelmann, J. Restless legs syndrome: update on pathogenesis. *Curr Opin Pulm Med* **19**, 594-600, doi:10.1097/MCP.0b013e328365ab07 (2013).
27 Landolt, H. P. Genetic determination of sleep EEG profiles in healthy humans. *Prog Brain Res* **193**, 51-61, doi:10.1016/B978-0-444-53839-0.00004-1 (2011).
28 Lin, L. *et al.* The sleep disorder canine narcolepsy is caused by a mutation in the hypocretin (orexin) receptor 2 gene. *Cell* **98**, 365-376 (1999).
29 Peyron, C. *et al.* A mutation in a case of early onset narcolepsy and a generalized absence of hypocretin peptides in human narcoleptic brains. *Nat Med* **6**, 991-997, doi:10.1038/79690 (2000).
30 Nishino, S., Ripley, B., Overeem, S., Lammers, G. J. & Mignot, E. Hypocretin (orexin) deficiency in human narcolepsy. *Lancet* **355**, 39-40, doi:10.1016/S0140-6736(99)05582-8 (2000).
31 Winkelmann, J. *et al.* Genome-wide association study identifies novel restless legs syndrome susceptibility loci on 2p14 and 16q12.1. *PLoS Genet* **7**, e1002171, doi:10.1371/journal.pgen.1002171 (2011).
32 Han, F. *et al.* Genome wide analysis of narcolepsy in China implicates novel immune loci and reveals changes in association prior to versus after the 2009 H1N1 influenza pandemic. *PLoS genetics* **9**, e1003880, doi:10.1371/journal.pgen.1003880 (2013).
33 Kornum, B. R. *et al.* Common variants in P2RY11 are associated with narcolepsy. *Nat Genet* **43**, 66-71, doi:10.1038/ng.734 (2011).

34 Faraco, J. *et al.* ImmunoChip study implicates antigen presentation to T cells in narcolepsy. *PLoS Genet* **9**, e1003270, doi:10.1371/journal.pgen.1003270 (2013).
35 Hallmayer, J. *et al.* Narcolepsy is strongly associated with the T-cell receptor alpha locus. *Nat Genet* **41**, 708-711, doi:10.1038/ng.372 (2009).
36 Altshuler, D., Daly, M. J. & Lander, E. S. Genetic mapping in human disease. *Science* **322**, 881-888, doi:10.1126/science.1156409 (2008).
37 Toh, K. L. *et al.* An hPer2 phosphorylation site mutation in familial advanced sleep phase syndrome. *Science* **291**, 1040-1043 (2001).
38 Xu, Y. *et al.* Functional consequences of a CKIdelta mutation causing familial advanced sleep phase syndrome. *Nature* **434**, 640-644, doi:10.1038/nature03453 (2005).
39 Ptacek, L. J., Jones, C. R. & Fu, Y. H. Novel insights from genetic and molecular characterization of the human clock. *Cold Spring Harb Symp Quant Biol* **72**, 273-277, doi:10.1101/sqb.2007.72.017 (2007).
40 Xu, Y. *et al.* Modeling of a human circadian mutation yields insights into clock regulation by PER2. *Cell* **128**, 59-70, doi:10.1016/j.cell.2006.11.043 (2007).
41 Chong, S. Y., Ptacek, L. J. & Fu, Y. H. Genetic insights on sleep schedules: this time, it's PERsonal. *Trends Genet* **28**, 598-605, doi:10.1016/j.tig.2012.08.002 (2012).
42 Brennan, K. C. *et al.* Casein kinase idelta mutations in familial migraine and advanced sleep phase. *Sci Transl Med* **5**, 183ra156, 181-111, doi:10.1126/scitranslmed.3005784 (2013).
43 Hallows, W. C., Ptacek, L. J. & Fu, Y. H. Solving the mystery of human sleep schedules one mutation at a time. *Crit Rev Biochem Mol Biol* **48**, 465-475, doi:10.3109/10409238.2013.831395 (2013).
44 Jones, C. R., Huang, A. L., Ptacek, L. J. & Fu, Y. H. Genetic basis of human circadian rhythm disorders. *Exp Neurol* **243**, 28-33, doi:10.1016/j.expneurol.2012.07.012 (2013).
45 Winkelmann, J. *et al.* Mutations in DNMT1 cause autosomal dominant cerebellar ataxia, deafness and narcolepsy. *Human molecular genetics* **21**, 2205-2210, doi:10.1093/hmg/dds035 (2012).
46 McCarthy, M. I. *et al.* Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nat Rev Genet* **9**, 356-369, doi:10.1038/nrg2344 (2008).
47 Robinson, M. R., Wray, N. R. & Visscher, P. M. Explaining additional genetic variation in complex traits. *Trends Genet* **30**, 124-132, doi:10.1016/j.tig.2014.02.003 (2014).
48 Zeng, P. *et al.* Statistical analysis for genome-wide association study. *J Biomed Res* **29**, 285-297, doi:10.7555/JBR.29.20140007 (2015).
49 Schizophrenia Working Group of the Psychiatric Genomics, C. Biological insights from 108 schizophrenia-associated genetic loci. *Nature* **511**, 421-427, doi:10.1038/nature13595 (2014).
50 Frazer, K. A., Murray, S. S., Schork, N. J. & Topol, E. J. Human genetic variation and its contribution to complex traits. *Nat Rev Genet* **10**, 241-251, doi:10.1038/nrg2554 (2009).

51 Warby, S. C. *et al.* Sleep-spindle detection: crowdsourcing and evaluating performance of experts, non-experts and automated methods. *Nat Methods* **11**, 385-392, doi:10.1038/nmeth.2855 (2014).

52 Wendt, S. L. *et al.* Inter-expert and intra-expert reliability in sleep spindle scoring. *Clin Neurophysiol* **126**, 1548-1556, doi:10.1016/j.clinph.2014.10.158 (2015).

53 Young, T., Peppard, P. E. & Gottlieb, D. J. Epidemiology of obstructive sleep apnea: a population health perspective. *American journal of respiratory and critical care medicine* **165**, 1217-1239 (2002).

54 Andlauer, O. *et al.* Nocturnal rapid eye movement sleep latency for identifying patients with narcolepsy/hypocretin deficiency. *JAMA Neurol* **70**, 891-902, doi:10.1001/jamaneurol.2013.1589 (2013).

55 Goldbart, A. *et al.* Narcolepsy and predictors of positive MSLTs in the Wisconsin Sleep Cohort. *Sleep* **37**, 1043-1051, doi:10.5665/sleep.3758 (2014).

56 Cade, B. E. *et al.* Common Variants in DRD2 are Associated with Sleep Duration: The CARe Consortium. *Hum Mol Genet*, doi:10.1093/hmg/ddv434 (2015).

57 Allebrandt, K. V. *et al.* A K(ATP) channel gene effect on sleep duration: from genome-wide association studies to function in Drosophila. *Mol Psychiatry* **18**, 122-132, doi:10.1038/mp.2011.142 (2013).

58 Parsons, M. J. *et al.* Replication of Genome-Wide Association Studies (GWAS) loci for sleep in the British G1219 cohort. *Am J Med Genet B Neuropsychiatr Genet* **162B**, 431-438, doi:10.1002/ajmg.b.32106 (2013).

59 Gottlieb, D. J. *et al.* Novel loci associated with usual sleep duration: the CHARGE Consortium Genome-Wide Association Study. *Mol Psychiatry* **20**, 1232-1239, doi:10.1038/mp.2014.133 (2015).

60 Scheinfeldt, L. B. *et al.* Using the Coriell Personalized Medicine Collaborative Data to conduct a genome-wide association study of sleep duration. *Am J Med Genet B Neuropsychiatr Genet*, doi:10.1002/ajmg.b.32362 (2015).

61 Ollila, H. M. *et al.* Genome-wide association study of sleep duration in the Finnish population. *Journal of sleep research* **23**, 609-618, doi:10.1111/jsr.12175 (2014).

62 Utge, S. *et al.* Shared genetic background for regulation of mood and sleep: association of GRIA3 with sleep duration in healthy Finnish women. *Sleep* **34**, 1309-1316, doi:10.5665/SLEEP.1268 (2011).

63 Evans, D. S. *et al.* Common genetic variants in ARNTL and NPAS2 and at chromosome 12p13 are associated with objectively measured sleep traits in the elderly. *Sleep* **36**, 431-446, doi:10.5665/sleep.2466 (2013).

64 Musunuru, K. *et al.* Candidate gene association resource (CARe): design, methods, and proof of concept. *Circ Cardiovasc Genet* **3**, 267-275, doi:10.1161/CIRCGENETICS.109.882696 (2010).

65 Yang, J., Lee, S. H., Goddard, M. E. & Visscher, P. M. GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet* **88**, 76-82, doi:10.1016/j.ajhg.2010.11.011 (2011).

66 Leary, E. B. *et al.* Alliance Sleep Questionnaire (ASQ): a collaborative on line sleep assessment questionnaire. *Sleep* **Abstract suppl**, 432 (2012).

67 Ohayon, M. M. Improving decisionmaking processes with the fuzzy logic approach in the epidemiology of sleep disorders. *Journal of psychosomatic research* **47**, 297-311 (1999).
68 Yu, L. *et al.* Development of short forms from the PROMIS sleep disturbance and Sleep-Related Impairment item banks. *Behavioral sleep medicine* **10**, 6-24, doi:10.1080/15402002.2012.636266 (2011).
69 Buysse, D. J., Reynolds, C. F., 3rd, Monk, T. H., Berman, S. R. & Kupfer, D. J. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. *Psychiatry Res* **28**, 193-213 (1989).
70 Leary, E. B. *et al.* Validation of the Alliance Sleep Questionnaire (ASQ) Narcolepsy Module in Sleep Disordered Patients. *Sleep* **Abstract suppl.**, 1026 (2014).
71 Leary, E. B., Malunjkar, S., Ruoff, C., Walsh, J. K. & Mignot, E. Validation of the ASQ restless legs module in sleep disordered patients. *Sleep* **Abstarct Suppl**, 1219 (2015).
72 Leary EB, M. S., Cheung J, Schneider L, Walsh JK, Mignot E. Validation of the Alliance Sleep Questionnaire (ASQ) Obstructive Sleep Apnea (OSA) Module in Sleep Disordered Patients. *Sleep* **39** (2016).
73 Maislin, G. *et al.* A survey screen for prediction of apnea. *Sleep* **18**, 158-166 (1995).
74 Chasens, E. R., Ratcliffe, S. J. & Weaver, T. E. Development of the FOSQ-10: a short version of the Functional Outcomes of Sleep Questionnaire. *Sleep* **32**, 915-919 (2009).
75 Weaver, T. E., Chasens, E. R. & Arora, S. Modafinil improves functional outcomes in patients with residual excessive sleepiness associated with CPAP treatment. *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine* **5**, 499-505 (2009).
76 Johns, M. W. Reliability and factor analysis of the Epworth Sleepiness Scale. *Sleep* **15**, 376-381 (1992).
77 Kroenke, K., Spitzer, R. L. & Williams, J. B. The PHQ-9: validity of a brief depression severity measure. *J Gen Intern Med* **16**, 606-613 (2001).
78 Bastien, C. H., Vallieres, A. & Morin, C. M. Validation of the Insomnia Severity Index as an outcome measure for insomnia research. *Sleep Med* **2**, 297-307 (2001).
79 Spitzer, R. L., Kroenke, K., Williams, J. B. & Lowe, B. A brief measure for assessing generalized anxiety disorder: the GAD-7. *Arch Intern Med* **166**, 1092-1097, doi:10.1001/archinte.166.10.1092 (2006).
80 Wittchen, H. U., Zhao, S., Kessler, R. C. & Eaton, W. W. DSM-III-R generalized anxiety disorder in the National Comorbidity Survey. *Arch Gen Psychiatry* **51**, 355-364 (1994).
81 Krupp, L. B., LaRocca, N. G., Muir-Nash, J. & Steinberg, A. D. The fatigue severity scale. Application to patients with multiple sclerosis and systemic lupus erythematosus. *Arch Neurol* **46**, 1121-1123 (1989).
82 Valko, P. O., Bassetti, C. L., Bloch, K. E., Held, U. & Baumann, C. R. Validation of the fatigue severity scale in a Swiss cohort. *Sleep* **31**, 1601-1607 (2008).

83 Adan, A. & Almirall, H. The influence of age, work schedule and personality on morningness dimension. *Int J Psychophysiol* **12**, 95-99 (1992).
84 Sutherland, K. *et al.* Facial phenotyping by quantitative photography reflects craniofacial morphology measured on magnetic resonance imaging in Icelandic sleep apnea patients. *Sleep* **37**, 959-968, doi:10.5665/sleep.3670 (2014).
85 Kooiman, T. J. *et al.* Reliability and validity of ten consumer activity trackers. *BMC sports science, medicine and rehabilitation* **7**, 24, doi:10.1186/s13102-015-0018-5 (2015).
86 Gur, R. C. *et al.* Neurocognitive performance in family-based and case-control studies of schizophrenia. *Schizophr Res* **163**, 17-23, doi:10.1016/j.schres.2014.10.049 (2015).
87 Gur, R. E. *et al.* Negative symptoms in youths with psychosis spectrum features: complementary scales in relation to neurocognitive performance and function. *Schizophr Res* **166**, 322-327, doi:10.1016/j.schres.2015.05.037 (2015).
88 Ibrahim, I. *et al.* Practice effects distort translational validity estimates for a Neurocognitive Battery. *J Clin Exp Neuropsychol* **37**, 530-537, doi:10.1080/13803395.2015.1037253 (2015).
89 Moore, T. M., Reise, S. P., Gur, R. E., Hakonarson, H. & Gur, R. C. Psychometric properties of the Penn Computerized Neurocognitive Battery. *Neuropsychology* **29**, 235-246, doi:10.1037/neu0000093 (2015).
90 Basner, M., McGuire, S., Goel, N., Rao, H. & Dinges, D. F. A new likelihood ratio metric for the psychomotor vigilance test and its sensitivity to sleep loss. *Journal of sleep research*, doi:10.1111/jsr.12322 (2015).
91 Basner, M. & Dinges, D. F. An adaptive-duration version of the PVT accurately tracks changes in psychomotor vigilance induced by sleep restriction. *Sleep* **35**, 193-202, doi:10.5665/sleep.1620 (2012).
92 Basner, M., Rao, H., Goel, N. & Dinges, D. F. Sleep deprivation and neurobehavioral dynamics. *Current opinion in neurobiology* **23**, 854-863, doi:10.1016/j.conb.2013.02.008 (2013).
93 Moore, H. t. *et al.* Design and validation of a periodic leg movement detector. *PloS one* **9**, e114565, doi:10.1371/journal.pone.0114565 (2014).
94 LeCun, Y., Bengio, Y. & Hinton, G. Deep learning. *Nature* **521**, 436-444, doi:10.1038/nature14539 (2015).
95 He, K., Zhang, X., Ren, S. & Sun, J. Delving Deep into Rectifiers: Surpassing Human-Level Performance on ImageNet Classification. *CoRR, Computer Vision and Pattern Recognition (cs.CV)* (2015).
96 Loffe, S. & Szegedy, C. Batch Normalization: Accelerating Deep Network Training by Reducing Internal Covariate Shift. *Learning (cs.LG) CoRR 2015, arXiv:1502.03167*
(2015).
97 Hinton, G. *et al.* Deep neural networks for acoustic modeling in speech recognition: The shared views of four research groups. *Signal Processing Magazine, IEEE* **29**, 82-97 (2012).

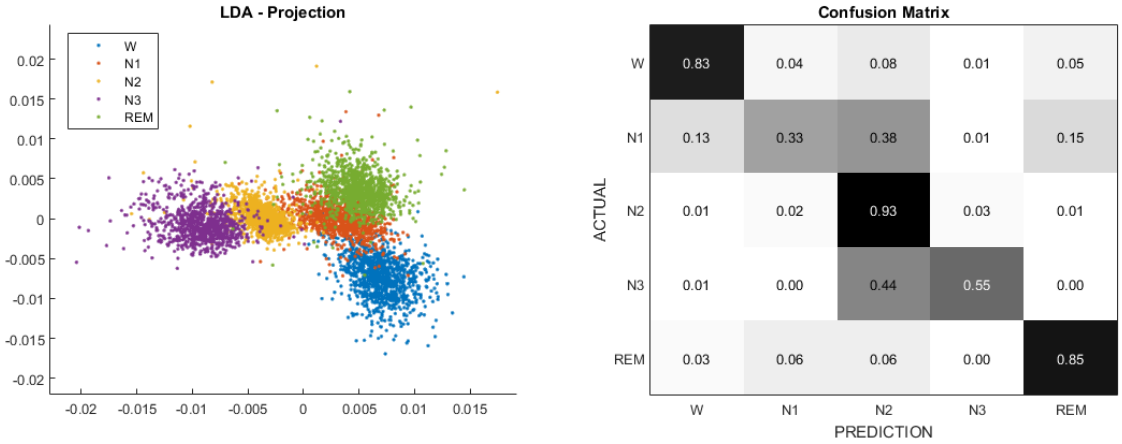
98 Hinton, G. E. & Salakhutdinov, R. R. Reducing the dimensionality of data with neural networks. *Science* **313**, 504-507, doi:10.1126/science.1127647 (2006).
99 Wu, C., Guo, Y. & Ma, Y. in *ThinkMind // COGNITIVE 2015, The Seventh International Conference on Advanced Cognitive Technologies and Applications /.* (ed IARIA) 181-186.
100 De Gennaro, L. *et al.* The electroencephalographic fingerprint of sleep is genetically determined: a twin study. *Ann Neurol* **64**, 455-460, doi:10.1002/ana.21434 (2008).
101 Borbely, A. A. From slow waves to sleep homeostasis: new perspectives. *Archives italiennes de biologie* **139**, 53-61 (2001).
102 Davis, C. J., Clinton, J. M., Jewett, K. A., Zielinski, M. R. & Krueger, J. M. Delta wave power: an independent sleep phenotype or epiphenomenon? *Journal of clinical sleep medicine : JCSM : official publication of the American Academy of Sleep Medicine* **7**, S16-18, doi:10.5664/JCSM.1346 (2011).
103 Genomes Project, C. *et al.* An integrated map of genetic variation from 1,092 human genomes. *Nature* **491**, 56-65, doi:10.1038/nature11632 (2012).
104 Kvale, M. N. *et al.* Genotyping Informatics and Quality Control for 100,000 Subjects in the Genetic Epidemiology Research on Adult Health and Aging (GERA) Cohort. *Genetics* **200**, 1051-1060, doi:10.1534/genetics.115.178905 (2015).
105 Sullivan, P. F. The psychiatric GWAS consortium: big science comes to psychiatry. *Neuron* **68**, 182-186, doi:10.1016/j.neuron.2010.10.003 (2010).
106 Ripatti, S. *et al.* A multilocus genetic risk score for coronary heart disease: case-control and prospective cohort analyses. *Lancet* **376**, 1393-1400, doi:10.1016/S0140-6736(10)61267-6 (2010).
107 Stephens, M. A unified framework for association analysis with multiple related phenotypes. *PloS one* **8**, e65245, doi:10.1371/journal.pone.0065245 (2013).
108 Van der Sluis, S. *et al.* MGAS: a powerful tool for multivariate gene-based genome-wide association analysis. *Bioinformatics* **31**, 1007-1015, doi:10.1093/bioinformatics/btu783 (2015).
109 Bulik-Sullivan, B. *et al.* An atlas of genetic correlations across human diseases and traits. *Nat Genet*, doi:10.1038/ng.3406 (2015).
110 Bulik-Sullivan, B. K. *et al.* LD Score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat Genet* **47**, 291-295, doi:10.1038/ng.3211 (2015).
111 Consortium, G. T. Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. *Science* **348**, 648-660, doi:10.1126/science.1262110 (2015).

## 1. Detailed Research Proposal Appendices:

### Appendix 1: Preliminary data on sleep analytics

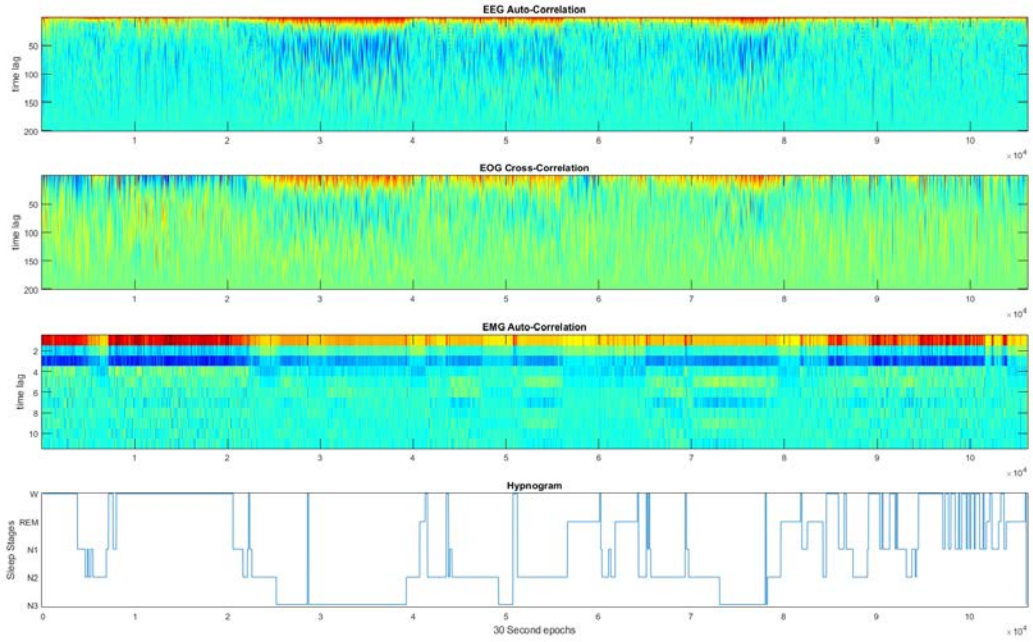
Sleep Stage Scoring: Using feature extraction and hierarchical clustering machine learning methods based on classical EEG (overlapping spectra bands), EOG and EMG features and, we successfully developed programs that can score all sleep stages with excellent accuracy (~85%) in large PSG datasets. We are now undergoing validation in multiple manually scored datasets, including the American Association of Sleep Medicine (AASM) standard set, which has been scored by a hundred of technicians. For these validations, our philosophy is to perform equally well as the gold standard (PSG technicians), but with better reproducibility, and to use a minimum of several thousand PSGs. Figure 1 illustrates sleep-scoring clusters using LDA projections as derived from EEG, EOG, and EMG data in the preliminary subset SSC and WSC cohorts together with a concordance table of our overall program performance.

Figure 1. Linear Discriminant Analysis (LDA) projection of hierarchical clustering components predicting sleep stages (left), together with a concordance table with manually scored epochs (right). Note highest confusion in N1, N2 and N2, N3, not surprisingly since N1 is a transitional stage and cut off SWS of N2 vs. N3 is artificially set.




As mentioned in the research plan, novel, deep learning [1] machine learning algorithms that do not use preselected features (EEG bands etc.) but raw or minimally transformed data are increasingly used. These have the advantage to continue learning with increasingly complex and large datasets (Figure 2).

Figure 2. Example of data structure obtained with deep learning using convolution networks. As can be seen very promising features are observed in parallel with the hynogram. These methods are likely to complement more traditional methods.



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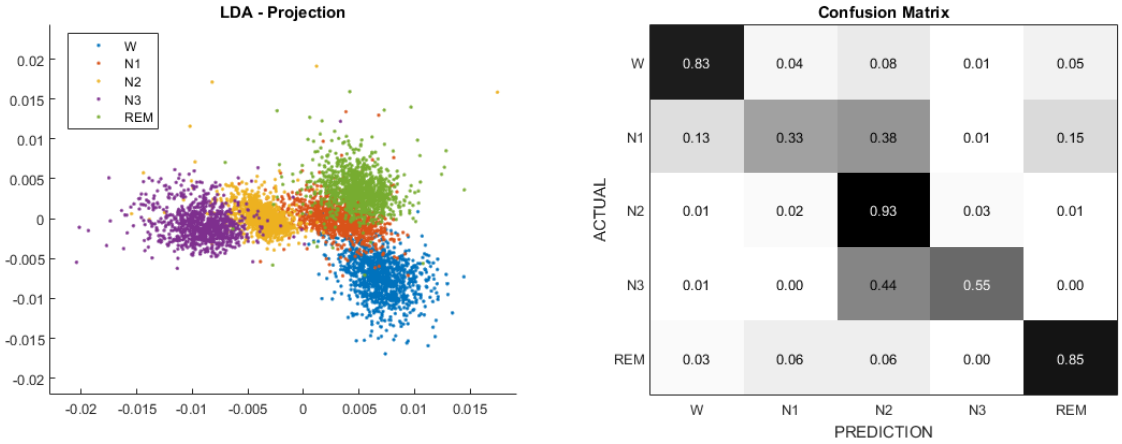


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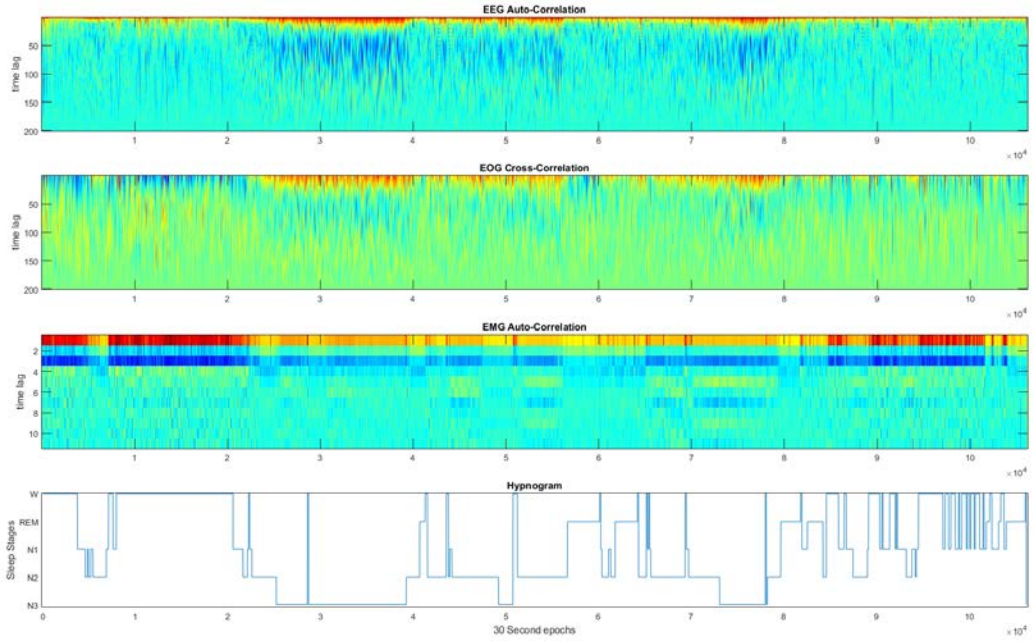
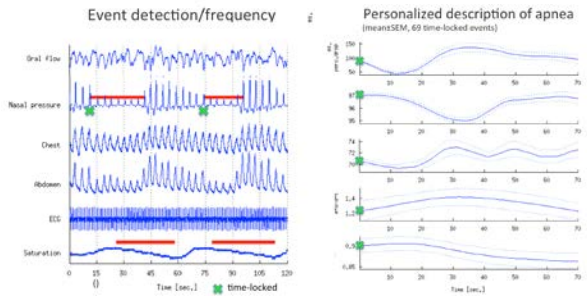


Periodic Leg Movement Scoring: Similarly, we recently established an automatic method that can score PLMS in exclusion of sleep apnea (PLMS can occur due to sleep apnea events), also finding strong genetic associations with known RLS polymorphisms. This program is highly accurate and has been validated and published using the WSC and the SSC, and the detailed code is freely available [2,3]. It can also be used to study physiological changes in association to individual time-locked events. Thanks to this study, the American Academy of Sleep Medicine (AASM) changed criteria for exclusion of LM secondary to breathing events from 0.3 sec to 5 sec. The study illustrates the importance of looking at all sleep disorders when considering sleep analytics.

Sleep-Disordered Breathing Scoring: Although unpublished and still under development, we designed a program that detects breathing pauses (Figure 3). With independent routine that detects desaturations of ≥2%, it is possible to "time lock" the two signals, creating a metric for breathing events with and without desaturations. We used this program to evaluate frequency of events and mean severity of abnormal breathing events. Using an objective measure of sleepiness (MSLT) performed in most of the Wisconsin Sleep Cohort (WSC), we confirmed clinical relevance of our findings. Using our new metric, we found that breathing pauses with (MSLT SL β=-0.34, p=0.04) and without (MSLT SL β=-0.67, p=0.0042) hypoxia are associated with sleepiness (a new finding) after adjusting for confounders, while only events with hypoxia correlate with high blood pressure (a known finding). Overall effects on sleepiness of all events combined were more visible objectively (MSLT SL β=-0.97, p=0.0001) than subjectively (EPW β=0.56, p=0.0001). No additional effects of mean severity of events were found on HBP, although analysis is still ongoing.

Figure 3. Automated detection of breathing pauses. (A) Breathing pauses were defined as >30% drops of nasal flow, for >10 seconds, without detectable increase in oral flow and with respiratory effort maintained. (B) Demonstration of time locking of breathing events to nasal pressure reduction flow events.

A. Nasal pressure detection algorithm. The nasal pressure signal is blue and the classified drop in pressure is red. The algorithm also detects periods of stable breathing, which can be used to measure respiratory cycle-related EEG changes.




B. Detected events (left) from one signal can be time locked to corroborate activity in another (right) so that for each patient a frequency (AHI) and mean severity of events (flow, Sa02, HR, EEG) is obtained.

Narcolepsy Biomarker identification: Novel biomarkers of diseases and physiology can also be developed. As an example, the gold standard for diagnosing narcolepsy is the MSLT, first developed at Stanford. A mean sleep latency (MSL) <8 min and ≥2 Sleep Onset REM sleep Periods (SOREMPs) during the test is approximately 95% specific and 95% sensitive for narcolepsy/hypocretin deficiency, but the test necessitates daytime recording, and is often overused, leading to an over-diagnosis of narcolepsy. Using nocturnal PSG analytics, we found new biomarkers that are highly specific, such as abnormal nighttime sleep transitions and evidence for non-differentiation of N1, wake, and REM sleep stages using clustering methods that integrate multiple signals [4,5]. We believe these new biomarkers of narcolepsy will be usable as alternative and cheaper methods for diagnosing patients.

In many cases, sleep biomarkers are already known, but "validation" has only been performed in small samples and in a narrow context (cases versus healthy controls). These are low-hanging fruits that demand proper programming, further validation in large, ecological patient samples (such as the one proposed in this study) and population based samples (such as the WSC), and adequate dissemination. As mentioned above, many more methods of analysis can be applied to extract novel objective markers of physiological or pathophysiological conditions that may also be amendable to genetic analysis. Table 5 in specific aim 2 illustrates these possible biomarkers.

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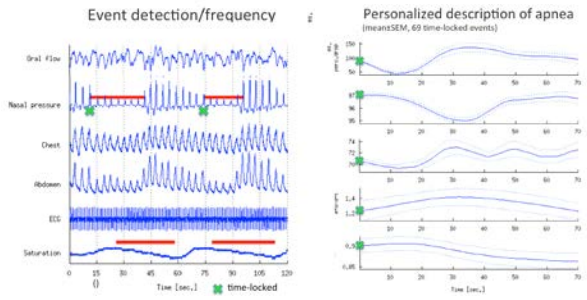


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Appendix 2: Description of the database

This project will produce six types of data: PSG, blood sample IDs, ASQ, CNB, genotypes and actigraphy. The bulk of the data will be housed on the mignot-sleep server, which is managed by Stanford Center for Clinical Informatics (SCCI) and hosted on Stanford's inner bastion (same level of security as electronic medical record). The server is split into several virtual machines (VMs), each with a different function. PHI information is segregated into a separate table in a database or section of the VM and is only available to critical data center and SCCI staff members using a conservative security protocol involving 2-step authentication. We are in the process of developing a MySQL database an efficient method for auditing and cross referencing these data to act as a central operator or link between all of these domain specific databases. This master database will serve as a codebook for matching each individual's data regardless of the source. It will be periodically checked for duplicate entries using automated scripts. Possible duplicates will be flagged for examination. Non-duplicate flagged entries will be added to a non-duplicate log file to prevent future flags being raised for the offsetting entries. A web-based interface is being developed to assist with merging or deleting data flagged for duplication, inconsistency, or other errors encountered with processing. Only key personnel will have access to the password protected master database, which will be accessed through 2-step authentication (SUNet ID and Duo Mobile).

The ASQ is run from three mignot-sleep virtual machines, one for the web portal, one for the MySQL production database and one for development. At our request, the ASQ system routinely undergoes security reviews performed by an independent Stanford Information Security Officer, the most recent having occurred 07/15. PSG files are de-identified (with the exception of date of service), converted to EDF and uploaded along with the scoring and event files to another virtual on the same server for processing. Prior to being stored, the PSG files go through a quality control procedure and are normalized before being logged into a MySQL database until they are linked with the ASQ and assigned a Universal ID. Unlike data we have stored from other cohorts, the channel naming convention and filter settings have been pre agreed with the investigators so that it should be uniform. To verify this during the normalization process, a routine program that we developed will flag any unusual labels to be replaced by our agreed nomenclature. The program also identifies any content in text file that is not conventional and could be inadvertent PHI information inadequately included in the scoring mask. If such information is found it is deleted or changed to accepted nomenclature. Once this cleaning procedure is completed, all PSG-ID-named folders will contain EDF and text files corresponding to every named channel. If extra channels are present, they will be left as additional channels with a description. Of note, we already have PSG files stored as EDFs and scoring mask text files for over 20,000 PSGs, and over 5,000 ASQ in our database, and have already worked with GWAS data extracted from over 20,000 subjects, so feasibility is not in question. What remains to be achieved is better organization, use of systematic routines to ensure homogeneity of data labeling and full proof procedures for de-identification.

Participants will use a one-time token generated by the ASQ database to link to the CNB data with a profile ID. The CNB is accessed via a web portal hosted by a firewalled University of Pennsylvania Web Server. No PHI is entered into the CNB interface. Software running on the Web Server stores the responses in a MySQL relational database instance then runs various scripts to generate scores and reports on the data using an automated scoring procedure developed in 2003. Encrypted data files will be sent quarterly to Stanford through a secure HTTPS Protocol.

The data collection site records Actigraph device serial number before watches are provided to individuals. In addition, the watches are set up with a coded user name and password to eliminate will eliminate PHI from the dataset. Most participants will self-sync the device to the Misfit app using Bluetooth Low Energy (BLE) on a compatible Apple (iphone, ipad, ipods) or Android device with Bluetooth 4.0 capability. Alternatively, a research staff member will sync the device on the participant's behalf. The de-identified data will first upload to a secure cloud based database. Using a secure API protocol or alternative secure transfer system, Stanford will transfer the raw actigraph data on a quarterly basis to a MySQL database on the mignot-sleep server. The coded user name and/or serial number associated with the data will be compared to the master database to ensure data is linked to the Universal ID.

Biological sample location will be at Rutgers and extra samples at Stanford (to limit costs). The ID will be linked to a bar code used at Stanford and Rutgers. Any investigator will be able to ask for a specific ID and obtain any given samples after an NIH review of the request. Once individual GWAS or exome sequencing data is available, it will be

downloaded to dbGAP a maximum of 6 months after generation of the data with a description containing the de-identified IDs. The phenotype and genotype data will be available through NIH by request and reviewed by the NIH data/sample access committee, which only includes NIH officials (none of the investigators involved in data collection will be involved).

High performance computing (HPC) clusters administered by the Stanford Research Computing Center will be used to analyze and run processes on de-identified data sets because they are more powerful than the mignot-sleep server. One example of a Stanford HPC is Sherlock, which we use for machine learning on PSG signals, and has 127 compute servers and associated storage and is available to run researchers' computational codes and programs, with resources managed through a fair-share algorithm using SLURM as the resource manager/job scheduler (http://sherlock.stanford.edu/mediawiki/index.php/Main_Page). Another example is the Stanford Genetics SCG3 cluster (https://doresearch.stanford.edu/research-scholarship/shared-facilities/genetics-bioinformatics-service-center-gbsc) that we use for associations/meta-analysis. In the past, we also used outside services such as DNAnexus for haplotype estimation and imputation, although for this project our tentative plan is to use both the pipeline of Neil Risch, our UCSF collaborator (see letter of support) and the Ricopili pipeline of the Psychiatry Genetic Consortium (PGC) for the secondary aim of consistency with psychiatric phenotypes (see letter of support of Pat Sullivan).

## Appendix 3: Project Tasks, Milestones, Timeline, and Payment Schedule

A project of this size requires infrastructure and a strong organization team to be successful. As we plan for the study to begin, we have been connecting with other group to foster integration and synergy between projects. For example, we will present the project at the upcoming Sleep Research Network Meeting. We have already started discussions with Dr. Susan Redline to ensure we can easily integrate with the National Sleep Research Resource (NSRR). We have contacted Kathy Hudson of the Precision Medicine Initiative in an effort to take advantage of any tools or resources developed for that project. Finally, we are in contact with Kathleen Merikangas and will include as many questions as possible that overlap with other more general surveys, such as NHANES or the British Biobank sample.

During the first phase of the project, our focus will be to set up protocols for data collection and build an IT platform that ensure the highest level of data integrity. We will detail specifics related to what constitutes a complete data set, including variables for each data element (i.e. questionnaire, polysomnography, etc.), procedures for standardization across locations, and mechanisms for data transfer/storage. We will create in interactive IT platform to manage the data and provide feedback to the sites regarding recruitment, data integrity, and performance. Once the protocols and platform are finalized and functional, we will address logistics related to data processing, analytics and sharing.

To facilitate project management, we have clustered these initial tasks into the categories below.

### Sub-contracts/Budget:

We have worked closely with the sites to develop budget estimates for this project that are equitable across sites. Every effort has been made to standardize costs for supplies and equipment or handle costs centrally at Stanford. Although every institution has different contract requirements, the process of finalizing the budgets and working with each site's contracts office should be straightforward. Currently each site is revising its submission to Stanford to reflect a reduction from 40K to 30K subjects and to make sure the requests are final. We will subsequently start processing subcontracts.

Sample Tasks:

- Finalize overall budgets, including changes mandated by the reviewers
- Finalize per patient costs at each data collection site
- Work with sites to finalize comparable subcontracts
- Ensure payments are structured consistently across sites to pay in arrears for participants enrolled
- Resolve any site specific obstacles

### Data Elements:

This project will produce five main types of data; raw polysomnography files (PSG) as EDF and scoring text files, blood sample IDs (serum, plasma, buffy coat, DNA), Alliance Sleep Questionnaire (ASQ) responses, Computerized Neurocognitive Battery (CNB) results and actigraphy. Each type of data is comprised of numerous variables/elements which need to be thought through and documented. Standard Operating Procedures manuals (SOPs) will be created covering each category of data to ensure the procedures and equipment are standardized.

We have already started working on this project. For the ASQ, we have met with Kathleen Merikangas for her thoughts regarding additional content to provide better psychiatric history and lifetime diagnoses using NHANES questions as a model. We will add the questions she proposes to the ASQ.

We will add a short, on-lie form to be filled out by the physician or study staff member which will include data from the electronic medical record. The content of the form will be designed by our team and approved by the Steering Committee and will at minimum include final diagnosis and medications at time of overnight PSG.

To explore the feasibility of using a consumer-grade wearable technology as an alternative as a clinical actigraphy watch, we have met with several experts in sleep actigraphy including Dr. Jamie Zeitzer at Stanford, Ken Hu at Harvard, Doug Kirsch at Carolinas HealthCare System Sleep Medicine-SouthPark and Kathleen Merikangas at the NIMH. Based on these discussions, we have designed a protocol to compare consumer wearables (for example, the Miband) against clinical actigraphy (for example, the Philips Actiwatch Spectrum Plus). We have submitted an IRB protocol to conduct

such a study. The study will collect wearable and actigraphy data on volunteer subjects (at least N = 10 subjects) who will wear both devices at the same time on their non-dominant wrist for 3 nights and days (72 hours). Data resolution at minute-by-minute activity will be obtained from both devices. Cole-Kripke sleep algorithm will be applied to the raw minute-by-minute data to achieve sleep and wake scoring for the main sleep period at night. Per minute epoch results will be compared between Philips Actiwatch Spectrum Plus and Miband side by side to examine for agreement. In parallel, we will send Kathleen Merikangas several Miband devices to run through her protocol of evaluation.

While it is likely that any company providing equipment or services (i.e., discounted actigraph device, data storage or computerized neurocognitive test battery) for this project will benefit by being associated with such a groundbreaking project, commercial profit is not a consideration when making decisions regarding devices or services to use. Instead we will focus on the company's ability to provide quality equipment or services at a competitive cost.

Sample Tasks:

- Review and evaluate existing ontologies related to sleep research and sleep medicine
- Create a global data dictionary documenting details for every variable
- Alliance Sleep Questionnaire
    - Update content, specifically addition of psychiatric history and additional comorbidities questions. Notably after consultation of protocols for other big data initiative in other areas such as NHANES
    - Add content changes to on-line version of survey
    - Resolve any bugs/issues (if needed)
    - Migrate software to cloud (if needed)
    - Complete load testing (ongoing)
    - Create custom URL for each site
    - Set up sites with admin access
    - Customize clinical report for each site (if needed)
    - Set up Business Associates Agreement (BAA) and data sharing agreements (as needed)
- Polysomnography (PSG)
    - Query sites for details on current hardware equipment and practices
        - Data acquisition system, montages, make/model of equipment
        - Hook up procedures (video may be helpful)
        - Scoring protocols, notably hypopnea definition and scoring of PLM
        - Decision trees regarding use of split night or emergency PAP protocol
    - Determine minimum criteria for PSG to be eligible
        - Channels and settings (filters)
        - Duration of recording
    - Identify equipment for standardization
        - Cannula
        - Oximeter
    - Confirm all sites meet required filter settings for all signals
    - Confirm all sites can export data to EDF
        - Obtain exemplar recordings of every montage used at each site (EDFs and scoring file)
    - Continue discussions with National Sleep Research Resource (NSRR) regarding strategies used to facilitate data sharing
- Blood
    - Distribute blood collection protocol and update if needed, depending of local equipment available
- Actigraphy
    - Contact vendors to get specification and example output files
    - Conduct pilot study to compare consumer devices to clinical devices
    - Have Kathleen Merikangas perform parallel study comparing devices
    - Finalize make and model of devices to be used

        - Reach out to large cohorts using that device to strategize about data integration (i.e., if GeneActive is selected, work with NHANES)
    - Negotiate with vendor to ensure devices won't have software/firmware changes mid-study
    - Determine ideal duration and timing of data collection with respect to clinic visits and distribution of devices
    - Define protocol for data transmission
- Computerized Neurocognitive Battery (CNB)
    - Work with University of Pennsylvania to finalize content of test battery
    - Confirm with external advisors that the selected test battery is suitable (i.e. David Dinges regarding PVT portion)
    - Develop system to link data to a Universal ID
    - Determine timeframe for CNB
- Electronic Medical Record (EMR)
    - Query sites regarding current Electronic Medical Records (EMR) system and possibilities for extraction
    - Develop list of key variables needed
    - Consult specialist to identify hurdles related to EMR extraction at each site
    - Determine best mechanism to obtain information (additional form requested vs EMR extraction, or both)

Informed Consent and IRB Approval:

We will send each of the sites a consent form template for this project that will mimic the NIMH Repository and Genomic Resource-Compliant Example Consent Form received from the NIMH. Our current Stanford ASQ biobank IRB consent form (protocol 15430) is based on the NIMH template, so we do not anticipate any issues related to language.

The consent template will cover standard topics including, but not limited to:

- The purpose of the research, which will include sleep and relationship to cognitive performance, work performance, well-being, physical and mental health
- Duration of involvement
- Detailed procedures
- Logistics of tissue sampling and genetic testing
    - Including question regarding whether the individual would like to receive results that may impact their health or their family's health
    - Including a clear statement that DNA and associated medical and research information will be uploaded to dbGaP and shared with the scientific community
- How to withdrawal from the study
- Possible risks and potential benefits
- Alternatives to participation
- Financial considerations
- Contact information
- The experimental subjects bill of rights
- Detailed information regarding the Health Information Portability and Accountability Act (HIPAA)
    - Including a clear statement that medical information related to sleep, mental health and general health from the electronic medical records as well as data collected specifically for this project will become part of the cohort
- Permission to re-contact the individual for future research

The Data Collection/Standardization/Quality Assurance and Control section has been updated to include our plan to follow current NIH guidelines regarding informing participants of findings. We will perform an annual review of the policy to ensure we are incorporating any changes to the ELSI program of the NGRI.

Sample Tasks:

- Create a template for the IRB Protocol and Informed Consent to ensure appropriate permissions are obtained from participants for data use, data sharing and the possibility of re-contacting participants after the end of the study. The consent will incorporate language from the NIMH template to data and samples can be used to answer diverse scientific questions.
    - Explore liability issues related to consent and providing genetic results to patients if requested, as this is now standard in the field
    - Ensure permissions to integrate the clinical aspects of this study with the research, including mental health
    - Explore and mimic current NIH guidelines regarding informing participants of findings. A mechanism will be developed to review the policy annually to ensure we are following current policy of the Ethical, Legal and Social Implications (ELSI) Research Program of the National Human Genome Research Institute (NGRI)

Data Collection/Standardization/Quality Assurance and Control:

We created a Steering Committee, which is scheduled to meet bi-monthly to provide oversight on the scientific integrity of the project. An Operations Team will also meet bi-monthly to discuss details associated with implementation and to resolve any operational issues that arise once data collection is underway. Finally, key team member(s) will travel to each site to ensure standardization of the protocol.

Regarding PSGs, we are exploring the possibility of gathering data using the same sensors or hardware for key measures across all sites. As an example, we are working with a company to see if they are willing to provide a dual nasal oral cannulas (pureflow) at very low cost that would be used in all sites.

Sample Tasks:

- Gather detailed information on current practices at each site and adjust to need
    - Patient flow through each clinic with respect to initial evaluation, nocturnal PSG, return visits (timing, duration, location, etc.)
    - Polysomnogram (PSGs) timing and practices
    - Electronic Medical Records (EMR)
- Create a template for the IRB Protocol and Informed Consent to ensure appropriate permissions are obtained from participants for data use, data sharing and the possibility of re-contacting participants after the end of the study. The consent will incorporate language from the NIMH template to data and samples can be used to answer diverse scientific questions related to sleep and outside of sleep medicine.
    - Explore liability issues related to consent and providing genetic results to patients if requested, as this is now standard in the field
    - Ensure permissions to integrate the clinical aspects of this study with the research, including mental health
    - Explore and mimic current NIH guidelines regarding informing participants of findings. A mechanism will be developed to review the policy annually to ensure we are following current policy of the Ethical, Legal and Social Implications (ELSI) Research Program of the National Human Genome Research Institute (NGRI)
- Based on information above, create detailed Standard Operating Procedures for each data type (PSG, Blood, ASQ, CNB, Actigraphy)
    - Timing, procedure and location for each data and sample collection decided at each site
    - Detailed manual of operations, with protocols for trouble shooting issues
    - Distribute list of supplies for blood collection (vacutainer tubes, etc.)
    - Ensure all sites are trained on barcode system for processing blood
    - Distribute a supply list for equipment/supplies to be standardized

    - Data and sample storage at each locality
- Develop a checklist to guide the site audits
- Develop a multisite protocol study that will gather hard data on final diagnoses independent of EMR for a subset of patients at each site (to be used for further ASQ validation)

Data Management:
Given the volume of data being collected at each location, it is essential we have a stable data pipeline for receiving data and allowing the sites to easily see the status of each patient's data in real time. A multisite data management system will be developed, likely using the Comparative Outcomes Management with Electronic Data Technology (COMET) platform. The COMET platform was developed by Dr. Clete Kushida and his team in the context of another grant to facilitate multicenter electronic clinical research. (http://www.ncbi.nlm.nih.gov/pubmed/25848590). Key members of the COMET team will be involved in this project to develop the platform for this study.

Sample Tasks:
- Explore the use of the COMET platform to monitor recruitment and data transfer
- Develop a quantitative dashboard to display status of each data point by patient
- Develop alert mechanisms to notify coordinators when data is missing or corrupt
- Develop database to link different user ids and assign a universal subject id
- Build visualization tools to identify areas for improvement in patient flow

Data De-identification and Transfer:
Participant confidentiality is imperative, so we will work closely with the Stanford Center for Clinical Informatics (SCCI) and Information Security Services (ISS) to negotiate the security concerns involved with building a multi-site research data repository containing various data files that must be linked together. Secure FTP or similar techniques will be used to transfer data to a centralized system managed by the Stanford Team. To minimize privacy risk, names of subjects and other PHI will only be available to critical staff members such as the Database Administrators and key site personnel (i.e., Site Director and Coordinator).

We already have a strong working relationship with SCCI and ISS who have managed and assessed the security of the Alliance Sleep Questionnaire and our research servers.

Sample Tasks:
- Develop Universal ID to ensure data can be linked
- Develop system to link Universal ID to each data type
- For each sleep acquisition system, write script to:
    - Replace PHI with coded information
    - Create local log containing PHI changes
    - Export PSG to EDF
    - Extract sleep staging and event files
    - Transfer files securely to storage repository
    - Log data transfer locally
- Explore existing data pipelines that can be customized for this project. Features would include secure data upload, checking data integrity, logging files, automated emails to sites, etc.
- Explore Bluetooth low energy (BLE) for transferring actigraph data

Data Storage and Resource Sharing Plan:
A secure, cost effective, and convenient solution to data storage and access is the core of this study given the volume of data that will be collected. We will explore how other groups, both research and commercial, have handled this task and determine which solution is best for our goals. We have already begun conversations regarding data storage with the Stanford Information Resources & Technology (IRT) group as well as with Microsoft.

An important part of this project is that all data will be made publicly available for analysis by any interested researcher, providing a request is submitted to the National Institutes of Health (NIH) and approved. All data will be submitted to dbGaP, who will assign a unique identifier to link the genetic and phenotypic data (PSG, ASQ, Computerized Neurocognitive Battery (CNB), actigraphy, sample organization, final diagnosis). Clinical data will be uploaded to the NIMH Data Archive (NDA) and will include the individual's identifier indexed through dbGaP to allow the data to be linked. Biological samples (serum, plasma and buffycoat) will be stored at Rutgers and will also be linked using the dbGaP ID. Extra aliquots of plasma and sera are collected as part of the primary need for DNA. We feel it would be a waste to throw away these extra aliquots as a new need may arise. However, these extra aliquots would be costly to store at Rutgers and are not likely to be used. Therefore, surplus buffy coat, serum and plasma samples will be sent to Stanford for more cost effective storage and as a geographic back up. We are very willing to work with Rutgers to replenish samples in their repository if they are used.

When the Steering Committee finalizes all of the data elements, we will submit an updated Resource Sharing Plan. The original plan submitted last October was based on the NIH's template. The updated version will incorporate changes to the project made during the last year and will include more detail regarding storage and linkage of data housed with NDA and dbGaP. The NIH will be responsible for determining the application process for receiving data. Stanford and the project research team have intentionally removed themselves from the application process to ensure there is no bias.

Sample Tasks:

- Explore other groups doing large scale data repositories such as PhysioNet /National Sleep Research Resource (NSRR), Precision Medicine Initiative, Integrating Data for Analysis, Anonymization, and Sharing (iDASH), Neuroscience Information Framework (NIF), Biologic Specimen and Data Repository Information Coordinating Center (BioLINCC), and Biospecimen Repository Access and Data Sharing (BRADS), UK Biobank, 23&me and Danish Genome registry to foster integration.
- Explore cloud storage such as Stanford Box and Microsoft, Amazon, or Google Cloud.
- Expand current draft Resource Sharing Plan
    - Incorporate changes made to project that impact data sharing
    - Continue discussions with National Institute of Mental Health Data Archive (NDA) and dbGaP to finalize protocol for linking phenotypic and genetic data
    - Continue discussions with NSRR to integrate data in their network

Recruitment Monitoring:

This project hinges on the data collection sites enrolling at the anticipated rate. From the very start of the project, it will be critical that we monitor enrollment rates as well as statistics on retention. Specifically, whether a complete data set is being collected on every individual. If a site consistently underperforms, the Steering Committee must decide whether the volume of participants warrants keeping the site and how to make up the difference. For example, a new site may be added, or an existing site may have capacity to over-recruit.

We are very excited to have such a diverse group of sites participate in the project as we believe combining for profit and non-profit sleep centers will result in a diverse dataset which will enhance its utility. None of the data collection sites will benefit from the project over above the benefit of being associated with a cohort that will be widely recognized by the sleep community, there will not a direct commercial benefit as recruitment is pulling from regularly scheduled patients.

Sample Tasks:

- Finalize recruitment matrix with start dates and recruitment goals for each location
- Create Enrollment Report by site to be reviewed at each Steering Committee and Operations Meeting
- Create Retention Report by site to be reviewed at each Steering Committee and Operations Meeting
- Develop and deploy strategies to optimize participation rates
- Query individuals who decline to identify and resolve perceived concerns related to enrollment

- Develop flow chart with countermeasures for handling sites that underperform, including the possibility of adding new sites

PSG Analytics:

The Stanford Center for Sleep Sciences and Medicine and the Danish Technical University have collaborated for many years on PSG analytics, a collaboration that was recently formalized into a Transatlantic Medicine and Technology Research Program. As part of this program, Masters and PhD students will bring to Stanford their advanced skills related to biomedical signal processing and machine learning algorithms with applications in medical diagnostics. This collaboration has proven to be a very effective way to create novel analytic pipelines for PSG analysis. Although the use of students may seem counterintuitive, most computer savvy companies compete for hiring similar talents, as the field is moving so rapidly that training becomes rapidly obsolete and good, senior engineers few and unaffordable to research.

The goal is to streamline PSG analysis, extract meaningful sleep phenotypes, and standardize analysis in large samples. The PSG Analytics will be done in four phases, with each phase building on the previous work. Because each phase is dependent on previous work, the tasks are dynamic and may change as the project progresses. Below is our current plan.

Version One will focus mainly on rule based detections to identify sleep stages, periodic leg movements and sleep disordered breathing events and will result in version one of our automated analysis pipeline. It will simply incorporate currently working algorithms.

Version Two will use machine learning to detect microarousals and integrate the new feature into the software developed during Phase One to create version two of our automated analysis pipeline. Although it will have additional features, it will update the version 1 pipeline to make it consistent with current scoring guidelines of the American Sleep Medicine Association for sleep disordered breathing events.

Version Three will start once we have the preliminary GWAS analysis of genetic variants that control hypersomnia phenotypes. It will use machine learning algorithms breathing and leg movement signals to score subtypes of breathing events and PLMs in concordance with rule based algorithms. Machine learning algorithms will be used on sound/vibration signal and respiratory channels to differentiate sub phenotypes of sleep apnea. These algorithms will be integrated into version two of our automatic analysis pipeline. Outliers between rule-based scored results and machine learning scored results will be flagged for inspection and process improvement.

Version Four will use more advanced machine learning and dimensional reduction applied to the combined data set to identify novel hypersomnia/sleepiness phenotypes, or define better the existing phenotypes. Machine learning detection of microarchitecture features such as spindles, K complexes, saw tooth waves, slope of delta waves will also be developed and integrated into the software. The result will be version four of the automatic analysis pipeline, which will deliver a large number of possible phenotypes to customers using the software.

The final tools will be used on the data from this study. We expect to publish peer-reviewed papers after each phase is completed and all code and software will be made available.

Sample Tasks for Version One:

- Projects for version one development
    - Automatic Sleep Stage analysis using machine learning
    - Automatic rule-based detection of periodic leg movements
    - Rule-based detection of sleep disordered breathing events with or without hypoxia
- Finalize tools to clean, normalize and parse PSG studies
- Document details regarding signal quality and scoring definitions (when appropriate)
- Create version one of our analytical pipeline that integrate the features above

Milestones for Years 1 and 2

0-6 Months

1. Secure Data Storage

   Where data will be stored is critical for this project. It will impact day-to-day operations at the sites as they upload files as well as accessibility once the project is completed. The Stanford Team has already started discussions with Stanford's Information Resources & Technology (IRT) team and the Site Directors. In addition, we will investigate methods used by other large scale data repositories to determine the best approach to manage, store, and share data for this project.

   To compare systems, we are evaluating security, speed, ease of uploading/sharing, and cost. We already have experience with and could use a secure local server at Stanford and are now exploring Google Cloud, the cloud storage system recommended by Stanford IRT.

   To complete this milestone, we will make a decision regarding cloud data storage versus local server storage and secure adequate space to start the study with room to scale up as data is collected.

   Individuals working on this milestone include Eileen Leary, Sanjay Malunjkar, Hyatt Moore and Ric Miller. Drs. Mignot and Kushida will make the final decision. In addition to personnel, there will be storage costs.

2. Finalize Data Elements

   Although we have already decided on the key types of data to be collected, each of the five data types are complex and contain multiple sub-variables. We will carefully evaluate each measure and create a detailed data dictionary. When possible we will use existing ontologies. Both raw (for example EDF and scoring files) and partially processed (for example summary statistics) data will be created and shared.

   Obtaining either an example file or a comprehensive file requirement description for each of the five data types will complete this milestone.

   Costs associated with this milestone are primarily personnel. Individuals working on this task include Emmanuel Mignot, Clete Kushida, Eileen Leary, Oscar Carrillo, Hyatt Moore and Sanjay Malunjkar.

3. Resource Sharing Plan Finalized with NIH

   Once the data elements are set, we will finalize the Resource Sharing Plan which will include detail regarding storage and linkage of data housed with dbGaP, the NIMH Data Archive (NDA) and Rutgers. The NIH will be responsible for determining the application process for receiving data.

   This milestone will be met when the final plan, approved by the NIH is submitted to the Klarman Family Foundation.

   Costs associated with this milestone are primarily personnel. Individuals working on this task include Emmanuel Mignot, Clete Kushida, and Eileen Leary.

6-12 Months

4. Informed Consent and IRB Approved

   Before starting recruitment, all sites must have an IRB approved protocol and consent form. Stanford will circulate a template and check list for the IRB Protocol and Informed Consent to ensure appropriate permissions are obtained from participants for data use, data sharing and the possibility of re-contacting participants after the end of the study. The consent will incorporate language from the NIMH template to data and samples can be used to answer diverse scientific questions. It will also include a clear statement that medical information related to sleep, mental health and general health from the electronic medical records as well as data collected specifically for this project will become part of the cohort. Each center will submit an IRB protocol and consent form using their institutions required language. Stanford will review the consent forms before submission to confirm the correct language is included.

This milestone will be met when all sites have an IRB approved protocol and consent form.

Costs associated with this milestone are primarily personnel. Individuals working on this task include Emmanuel Mignot and Eileen Leary.

5. Launch Data Management Portal

The data management portal will be set up to receive an heterogeneous set of data files from all sites, to verify the integrity of each file (i.e., no corrupt files), log the file, send automatic emails and reports to the sites with alerts regarding missing or corrupt data files and will eventually be used to share datasets with the scientific community. During the study, this site will be accessed daily by the sites to monitor recruitment and participant status, with information presented as a dashboard for each site. It will also serve as a document management site where current copies of key manuals and instructions can be located.

This milestone will be met once the data collection sites have access to a functioning data management portal.

Individuals working on this milestone include Emmanuel Mignot, Clete Kushida, Eileen Leary, Ric Miller, Deborah Nichols, Oscar Carrillo, Hyatt Moore and Sanjay Malunjkar.

6. Start Recruitment

All systems must be in place before starting recruitment. Detailed SOPs will have been created and distributed to the sites, describing what data will be collected as well as how it will be standardized, transferred, organized and stored. We will also verify that all of the data collection locations fully understand the SOPS, have the appropriate equipment and resources to collect the data in a standardized fashion. Therefore, each data collection location must pass a site audit to be cleared to start recruitment. We will launch the study at Stanford first to identify any areas where the workflow can be improved. After collecting data for one month at Stanford, the other four collaborators will begin data collection. Centers with multiple locations will start with just one site year one before expanding out to the additional sites starting in Year 2. We expect to enroll approximately 200 participants during this phase.

Enrollment of a first participant will achieve this milestone.

Emmanuel Mignot, Clete Kushida and Eileen Leary will work closely with the data collection sites to ensure each location is ready to start enrolling. This milestone will require travel for site audits. Stanford will purchase a Revco freezer and a plate bed scanner for processing and storing incoming samples. Stanford will also purchase select equipment and supplies for the sites (e.g. actigraphy devices, hand held bar code readers and aliquot tubes) and Carlos Perez will set up accounts for Rutgers for sample storage and University of Pennsylvania for the CNB. Eileen and Sanjay Malunjkar will ensure the Alliance Sleep Questionnaire (ASQ) is ready at each data collection location. The sites will be responsible for securing staff and equipment/supplies not provided by Stanford.

<u>12-18 Months</u>

7. Enroll a total of 2,800 participants study-wide

Based on our current recruitment matrix, we expect to enroll approximately 10,400 individuals every year, which works out to approximately 217 participants enrolled per week study-wide when enrolling at full capacity. We have built in a ramping period which includes starting enrollment at the Stanford sites first to identify and resolve any unforeseen issues before recruitment starts project-wide. Additionally, sites with multiple data collection sites will be encouraged to phase recruitment.

This milestone will be met once we have enrolled a total of 2,800 individuals study-wide.
Emmanuel Mignot, Clete Kushida and Eileen Leary will work closely with the sites to ensure each location is meeting enrollment targets and that all data is received for each individual, as monitored by the recruitment dashboard and incoming files. Biosamples will be shipped via FedEx in batches (using Stanford account) to Rutgers and to Stanford. Dr. Ling Lin and Jing Zhang will process and store residual samples at Stanford. Eileen and Sanjay Malunjkar will be the database administrators for the ASQ and will provide support to sites and users. Ric Miller and Deborah Nichols will manage the data portal with support from Eileen and Hyatt Moore. Oscar

Carrillo and Hyatt Moore will manage the PSGs as they are received. Carlos Perez will process invoices from Rutgers and University of Pennsylvania. The sites will be responsible for staff and equipment/supplies not provided by Stanford.

18-24 Months

8. PSG Data Analytics (Version One)

   While we are collecting data, the Transatlantic Medicine and Technology Research Program (TMTRP) students will work on Phase One of the PSG analytics project. They will develop software to streamline PSG analysis, extract meaningful sleep phenotypes, and standardize analysis in existing cohorts (Stanford Sleep Cohort and Wisconsin Sleep Cohorts).

   This milestone will be met once we have published on version 1 of our automated analysis pipeline, including the code.

   The team involved in this project includes Emmanuel Mignot, Eileen Leary, Oscar Carrillo, Hyatt Moore, and the TMTRP students.

9. Enroll a total of 8,000 participants study-wide (5,200 new participants)

   This milestone will be met once we have enrolled a total of 8,000 individuals study wide. Sites will continue recruiting at this rate until the full 30,000 participants have been enrolled.

   Emmanuel Mignot, Clete Kushida and Eileen Leary will work closely with the sites to ensure each location is meeting enrollment targets and that all data is received for each individual. Biosamples will be shipped via FedEx in batches (using Stanford account) to Rutgers and to Stanford. Dr. Ling Lin and Jing Zhang will process and store samples at Stanford. Another Revco will need to be ordered during this period to store samples at Stanford. Eileen and Sanjay Malunjkar are the database administrators for the ASQ and will be providing support to the sites and users. Carlos Perez will process invoices from Rutgers and University of Pennsylvania. The sites will be responsible for staff and equipment/supplies not provided by Stanford.

Provisional Milestones for Years 3-5

These milestones may be slightly revised at the 2-year review with approval from the Steering Committee, the External Review Committee, and the Klarman Family Foundation.

25-36 Months

10. Enroll a total of 15,000 participants study-wide (7,000 new participants)

    This milestone will be met once we have enrolled a total of 15,000 individuals study wide. Sites will continue recruiting at this rate until the full 30,000 participants have been enrolled. The same resources listed for Milestone 7 will be used.

11. PSG Data Analytics (Version Two)

    Phase Two of the PSG data analytics project will use machine learning to detect microarousals and integrate the new feature into the software developed during Phase One to create version two of our automated analysis pipeline. Although it will have additional features, it will update the version 1 pipeline to make it consistent with current scoring guidelines of the American Sleep Medicine Association for sleep disordered breathing events.
    This milestone will be met once we have published on version 2 of our automated analysis pipeline, including the code. The team involved in this project includes Emmanuel Mignot, Eileen Leary, Oscar Carrillo, Hyatt Moore, and the TMTRP students.

27-38 Months

12. Post data to NDA and dbGaP (on 10,000 participants)

    We will work with closely with National Institute of Mental Health Data Archive (NDA), dbGaP, and Rutgers to post phenotypic and genetic data linked by a GU ID. This milestone will be met once the data is posted on the first 10,000 participants. Several teams will work on this milestone to finalize the datasets and associated files. The group working on genetic data and biological samples will include Emmanuel Mignot, Ling Lin, Jing Zhang, and Rutgers. The group working on PSG data will include Emmanuel Mignot, Eileen Leary, Oscar Carrillo, Hyatt Moore, Deborah Nichols and Ric Miler. The ASQ team includes Eileen Leary, Sanjay Malunjkar, Deborah Nichols, and Ric Miller. The team assembling and linking the CNB data will include Eileen Leary, Sanjay Malunjkar, Deborah Nichols, Ric Miller and the University of Pennsylvania. The actigraphy team is comprised of Emmanuel Mignot, Eileen Leary, Hyatt Moore, Deborah Nichols, and Ric Miller. The team handling the EMR data includes Emmanuel Mignot, Clete Kushida, Eileen Leary, Deborah Nichols, and Ric Miller.

13. Enroll a total of 25,000 participants study-wide (10,000 new participants)

    This milestone will be met once we have enrolled a total of 25,000 individuals study wide. The same resources listed for Milestone 7 will be used.

14. PSG Data Analytics (Version Three)

    Phase Three of the PSG Data Analytics project will start once we have the preliminary GWAS analysis of genetic variants that control hypersomnia phenotypes. It will use machine learning algorithms breathing and leg movement signals to score subtypes of breathing events and PLMs in concordance with rule based algorithms. Machine learning algorithms will be used on sound/vibration signal and respiratory channels to differentiate sub phenotypes of sleep apnea. These algorithms will be integrated into version two of our automatic analysis pipeline. Outliers between rule-based scored results and machine learning scored results will be flagged for inspection and process improvement.

    This milestone will be met once we have published on version 3 of our automated analysis pipeline, including the code. The team involved in this project is the same as Milestone 8.

15. Complete recruitment with a total of 30,000 participants study-wide (5,000 new participants)

    This milestone will be met once we have enrolled a total of 8,000 individuals study wide. The same resources listed for Milestone 7 will be used.

16. PSG Data Analytics (Version Four)

    Version Four will use more advanced machine learning and dimensional reduction applied to the combined data set to identify novel hypersomnia/sleepiness phenotypes, or define better the existing phenotypes. Machine learning detection of microarchitecture features such as spindles, K complexes, saw tooth waves, slope of delta waves will also be developed and integrated into the software. The result will be version four of the automatic analysis pipeline, which will deliver a large number of possible phenotypes to customers using the software.

    This milestone will be met once we have published on version 4 of our automated analysis pipeline, including the code. The team involved in this project is the same as Milestone 8.

17. Post all data to NDA and dbGaP (on 30,000 participants)

    We will work with closely with National Institute of Mental Health Data Archive (NDA), dbGaP, and Rutgers to post the remaining phenotypic and genetic data linked by a GU ID. This milestone will be met once data is posted on all 30,000 participants as described in our data-sharing plan. Resources used are the same as Milestone 10.

Summary of Milestone Timeline

Below are three timelines and a list of key staff involved in each milestone. Table 1 is a detailed timeline for the milestones for the first two years of the project. The first 9 months of this study will be dedicated to creating detailed manuals to guide data collection at the sites. In addition, we will set up a stable and secure infrastructure for receiving and verifying the integrity of all data files, logging the information and providing feedback to the study committees. Site personnel will also receive reports to facilitate day-to-day operations of the study. Finally, we will set the foundation for a data storage/dissemination system that will provide convenient access to the scientific community once the data is publicly available.

Table 2 summarizes the provisional milestones for the remainder of the study. Emphasis will be on recruitment milestones, PSG data analytics and posting the data to the scientific community in two batches. *These milestones and timeline will be adjusted slightly at the two-year mark* with approval from the Steering Committee, External Review Committee and the Klarman Family Foundation.

Table 3 provides a high level view of the entire five-year project. Table 4 provides a breakdown of key personnel/groups involved in each milestone. Table 5 is a payment schedule.

**Table 1: Timeline of Milestones for First Two Years of the Study**

| Milestone | Anticipated Completion | Year 1 | | | | | | | | | | | | Year 2 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| 1. Secure Data Storage | Month 3 | | | | | | | | | | | | | | | | | | | | | | | | |
| 2. Finalize Data Elements | Month 6 | | | | | | | | | | | | | | | | | | | | | | | | |
| 3. Resource Sharing Plan Finalized w/NIH | Month 6 | | | | | | | | | | | | | | | | | | | | | | | | |
| 4. Informed Consent and IRB Approved | Month 9 | | | | | | | | | | | | | | | | | | | | | | | | |
| 5. Launch Data Management Portal | Month 9 | | | | | | | | | | | | | | | | | | | | | | | | |
| 6. Start Recruitment | Month 10 | | | | | | | | | | | | .2K | | | | | | | | | | | | |
| 7. Enroll 2,800 Participants | Month 18 | | | | | | | | | | | | | | | | | | 2.8K | | | | | | |
| 8. PSG Data Analytics (Version 1) | Month 24 | | | | | | | | | | | | | | | | | | | | | | | | |
| 9. Enroll 8,000 Participants | Month 24 | | | | | | | | | | | | | | | | | | | | | | | | 8K |

**Table 2: Timeline of Provisional Milestones for Years Three through Five of the Study**

| Milestone | Anticipated Completion | Year 3 | | Year 4 | | Year 5 | |
|---|---|---|---|---|---|---|---|
| | | 25-30 | 31-36 | 37-42 | 43-48 | 49-54 | 55-60 |
| 10. Enroll 15,000 Participants | Month 32 | | 15K | | | | |
| 11. PSG Data Analytics (Version 2) | Month 36 | | | | | | |
| 12. Data Posted to NDA and dbGaP | Month 36 | | 10k | | | | |
| 13. Enroll 25,000 Participants | Month 44 | | | | 25K | | |
| 14. PSG Data Analytics (Version 3) | Month 48 | | | | | | |
| 15. Complete Recruitment (30K) | Month 54 | | | | | 30K | |
| 16. PSG Data Analytics (Version 4) | Month 60 | | | | | | |
| 17. All Data Posted to NDA and dbGap | Month 60 | | | | | | |

## Table 3: Timeline of Study Phases for Five Years of the Study

| Year | 1 | | 2 | | 3 | | 4 | | 5 | |
|---|---|---|---|---|---|---|---|---|---|---|
| Month | 0-6 | 7-12 | 13-18 | 19-24 | 25-30 | 31-36 | 37-42 | 43-48 | 49-54 | 55-60 |
| **Phase 1:** | | | | | | | | | | |
| Planning & Protocols | | | | | | | | | | |
| Building IT Platform | | | | | | | | | | |
| **Phase 2:** | | | | | | | | | | |
| Recruitment | | 0.2K | 2.8K | 8K | 13.2K | 18.4K | 23.6K | 28.8K | 30K | |
| PSG Analytics | | | | P | | P | | P | | P |
| **Phase 3:** | | | | | | | | | | |
| Genotyping & Data Posted to dbGAP/ NIH | | | | | | 10K | | | | |
| Genetic Analysis (Hypersomnia) | | | | | | | | P | | |
| Data Sharing Portal | | | | | | | | | | |
| **Phase 4:** | | | | | | | | | | |
| Genotyping & Data Posted to dbGAP/ NIH | | | | | | | | | | 30K |
| All PSG Analytics Posted | | | | | | | | | | |
| Further Genetic Analysis | | | | | | | | | | P |

P=Expected publication point including code when applicable

## Table 4: Breakdown of Key Personnel/Groups Involvement in Each Milestone

| | Milestone | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Year 1 | | | | | | Year 2 | | | Year 3 | | | Year 4 | | Year 5 | | |
| **Involvement** | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 |
| Mignot | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Kushida | | X | X | | X | X | X | | X | X | | X | X | | X | | X |
| Leary | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Malunjkar | X | X | | | X | X | X | | X | X | | X | X | | X | | X |
| Lin | | | | | | X | X | | X | X | | X | X | | X | | X |
| Zhang | | | | | | X | X | | X | X | | X | X | | X | | X |
| Perez | | | | | | X | X | | X | X | | | X | | X | | |
| Carrillo* | | X | | | X | | X | X | X | X | X | X | X | X | X | X | X |
| Miller* | X | | | | X | | X | | X | X | | X | X | | X | | X |
| Moore* | X | X | | | X | | X | X | X | X | X | X | X | X | X | X | X |
| Nichols* | | | | | X | | X | | X | X | | X | X | | X | | X |
| TMTRP students | | | | | | | | X | | | X | | | X | | X | |
| Data Collection Sites | | | | | | X | X | | X | X | | | X | | X | | |
| Rutgers** | | | | | | | X | | X | X | | X | X | | X | | X |
| University of Penn** | | | | | | | X | | X | X | | X | X | | X | | X |

TMTRP = Transatlantic Medicine and Technology Research Program

* Consultants

**Subawards

**Milestone Legend**

1. Secure Data Storage
2. Data Sharing Plan with NIH
3. Finalize Data Elements
4. Informed Consent and IRB Approved
5. Launch Data Management Portal
6. Start Recruitment
7. Enroll 2,600 participants
8. PSG Data Analytics (Version 1)
9. Enroll 7,800 participants
10. Enroll 15,000 Participants
11. PSG Data Analytics (Version 2)
12. Data Posted to NDA and dbGaP
13. Enroll 25,000 Participants
14. PSG Data Analytics (Version 3)
15. Complete Recruitment (30K)
16. PSG Data Analytics (Version 4)
17. All Data Posted to NDA / dbGap

**Payment Schedule**

Table 5 summarizes our proposed schedule which includes 8 payments based on completion of agreed upon milestones.

**Table 5: Proposed Payment Schedule by Milestone**

| Payment Number | Year | Quarter | Month | Milestone Timeline | Recruitment Milestones |
|---|---|---|---|---|---|
| 1 | 1 | n/a | 0 | Grant Agreement Signed (20%) | n/a |
| 2 | 1 | Q1 | 3 | Completion of Milestone 1 | n/a |
| 3 | 1 | Q2 | 6 | Completion of Milestones 2, and 3 | n/a |
| 4 | 1 | Q3 | 9 | Completion of Milestones 4 and 5 | n/a |
| 5 | 1 | Q4 | 12 | Recruitment Report | 200 |
| 6 | 2 | Q1 | 15 | Recruitment Report | 750 |
| 7 | 2 | Q2 | 18 | Completion of Milestones 6 and 7 | 2,800 |
| 8 | 2 | Q3 | 21 | Recruitment Report | 5,400 |
| 9 | 2 | Q4 | 24 | Completion of Milestones 8 and 9 | 8,000 |
| 10 | 3 | Q1 | 27 | Recruitment Report | 10,600 |
| 11 | 3 | Q2 | 30 | Recruitment Report | 13,200 |
| 12 | 3 | Q3 | 33 | Recruitment Report | 15,800 |
| 13 | 3 | Q4 | 36 | Completion of Milestones 10, 11, and 12 | 18,400 |
| 14 | 4 | Q1 | 39 | Recruitment Report | 20,000 |
| 15 | 4 | Q2 | 42 | Recruitment Report | 23,600 |
| 16 | 4 | Q3 | 45 | Recruitment Report | 26,200 |
| 17 | 4 | Q4 | 48 | Completion of Milestones 13 and 14 | 28,800 |
| 18 | 5 | Q1 | 51 | Recruitment Report | 30,000 |
| 19 | 5 | Q2 | 54 | Resource Sharing Report | n/a |
| 20 | 5 | Q3 | 57 | Resource Sharing Report | n/a |
| 21 | 5 | Q4 | 60 | Completion of Milestones 15, 16, and 17 | n/a |

**Milestone Legend**

1. Secure Data Storage
2. Data Sharing Plan with NIH
3. Finalize Data Elements
4. Informed Consent and IRB Approved
5. Launch Data Management Portal
6. Start Recruitment
7. Enroll 2,600 participants
8. PSG Data Analytics (Version 1)
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15. Complete Recruitment (30K)
16. PSG Data Analytics (Version 4)
17. All Data Posted to NDA / dbGap

## Appendix 4: Potential limitations, risks and solutions

The sample is not representative of a general population as it is enriched in sleep disorders

The sample is not population-based, but composed of patients visiting sleep clinics. The main justification for this is cost, as sampling from the general population would add ~ $750/subject for sleep studies, thus $30M. Additionally, sampling from the general population would also lead to including sleep disorder patients, as ~ 20% of the population has sleep disordered breathing[6] and ~10% chronic insomnia[7,8]. In the WSC, 40% of subjects of age 60 had PLMI≥15/hr[9]. Sampling from healthy subjects without sleep disorders could be proposed, but this would add prescreening cost and would not address genetics of sleep disorders. In sum, having a bigger sample of subjects from sleep clinics, heterogeneous for sleep complaints, is more advantageous. Finally, additional population-based studies (~8,000 subjects) funded by NIH will be available to us for replication and it is possible to compare SNP frequency in our cohort versus other such as the GERA cohort (Kaiser study)[10]. If there is no effect, it is unlikely a genetic factor has been missed because of ascertainment bias.

Clinical significance of any marker will be doubtful

A critique of GWAS is that small effects are discovered so that translation is valid at the population, but not individual levels. Specific aim 3 addresses this by identifying rare phenotypes with stronger genetic effects. Further, as illustrated by the recent study of 23andMe on morningness-eveningness[11] and many other studies, for example those relevant to ECG electrophysiology[12], overlapping sets of genes are often found using these designs: in one case regulatory polymorphisms, and in the other penetrant coding mutations. It is also notable that the goal of the study is not only to unravel the genetic basis of sleep and sleep disorders but also to develop a registry and tools that will have widespread clinical applicability.

As outlined by our work on narcolepsy, the field of sleep disorders needs to go beyond subjective descriptions and clinical endpoints. Once biological insights are known, it becomes possible to develop human (for ex. iPSC) or animal (for ex. mouse) models that can be used as targets for drug development. In narcolepsy, iPSC models of hypocretin neurons are being used to develop autologous autoimmune models of hypocretin cell loss, and knowledge of hypocretin and immunology is leading to new therapeutic approaches, from hypocretin/orexin agonists to immune interventions.

## Appendix 5: Potential Impact

A large database linking subjective and objective sleep data with genetic information and biological samples will be made available worldwide. We anticipate that once available, more centers will continue contributing ASQ, PSG and blood samples. This will transform the sleep field, not unlike the Alzheimer's disease Neuroimaging Initiative (ADNI) and the PGC for neuropsychiatric disorders.

This project will also deliver software that will standardize the study of sleep. Researchers will be able to discover and validate novel biomarkers for cardiovascular and brain health. This will be important to guide the development of wearables or to further development of in-home PSG recordings, which will include lower fidelity physiological recordings, thereby relying upon newly discovered biomarkers.

Other deliverables for this project will include genetic variants regulating sleep and sleep disorders. Gene pathways that will be discovered will unveil the mysteries of the molecular basis of sleep regulation, and will also shed new light on new dimensions for sleep disorders.

We envision that this project will evolve into establishing a web portal that will help integrate sleep data, educational materials, and recommended apps based on ASQ response patterns, in order to provide a personalized sleep profile, geared toward guiding people to better health.

## References


1 Shi, J. *et al.* Multi-channel EEG-based sleep stage classification with joint collaborative representation and multiple kernel learning. *J Neurosci Methods* **254**, 94-101, doi:10.1016/j.jneumeth.2015.07.006 (2015).

2 Moore, H. t. *et al.* Design and validation of a periodic leg movement detector. *PloS one* **9**, e114565, doi:10.1371/journal.pone.0114565 (2014).

3 Moore, H. t. *et al.* Periodic leg movements during sleep are associated with polymorphisms in BTBD9, TOX3/BC034767, MEIS1, MAP2K5/SKOR1, and PTPRD. *Sleep* **37**, 1535-1542, doi:10.5665/sleep.4006 (2014).

4 Stephens, M. A unified framework for association analysis with multiple related phenotypes. *PloS one* **8**, e65245, doi:10.1371/journal.pone.0065245 (2013).

5 van der Sluis, S., Posthuma, D. & Dolan, C. V. TATES: efficient multivariate genotype-phenotype analysis for genome-wide association studies. *PLoS Genet* **9**, e1003235, doi:10.1371/journal.pgen.1003235 (2013).

6 Young, T., Peppard, P. E. & Gottlieb, D. J. Epidemiology of obstructive sleep apnea: a population health perspective. *Am J Respir Crit Care Med* **165**, 1217-1239 (2002).

7 Bastien, C. H., Vallieres, A. & Morin, C. M. Validation of the Insomnia Severity Index as an outcome measure for insomnia research. *Sleep Med* **2**, 297-307 (2001).

8 Riemann, D. *et al.* The neurobiology, investigation, and treatment of chronic insomnia. *Lancet Neurol* **14**, 547-558, doi:10.1016/S1474-4422(15)00021-6 (2015).

9 Moore, H. E. t., Andlauer, O., Simon, N. & Mignot, E. Exploring medical diagnostic performance using interactive, multi-parameter sourced receiver operating characteristic scatter plots. *Comput Biol Med* **47**, 120-129, doi:10.1016/j.compbiomed.2014.01.012 (2014).

10 Kvale, M. N. *et al.* Genotyping Informatics and Quality Control for 100,000 Subjects in the Genetic Epidemiology Research on Adult Health and Aging (GERA) Cohort. *Genetics* **200**, 1051-1060, doi:10.1534/genetics.115.178905 (2015).

11 Hu, Y. *et al.* GWAS of 89,203 individuals identifies genetic variants associated with being a morning person. *ASHG meeting abstracts* (2104).

12 Kolder, I. C., Tanck, M. W. & Bezzina, C. R. Common genetic variation modulating cardiac ECG parameters and susceptibility to sudden cardiac death. *Journal of molecular and cellular cardiology* **52**, 620-629, doi:10.1016/j.yjmcc.2011.12.014 (2012).