**Research program**

**Scientific background**

Parkinson's disease (PD) is caused by the accumulation of neurodegenerative damage and other changes in the brain that occur throughout life as a result of changing factors such as age, environment, and genetics (1). The most common neurodegenerative disease after Alzheimer's disease, PD affects approximately 1% of the adult population over the age of 60, and 5% of the population over the age of 85 (2). While most research on PD focuses on surgical and pharmaceutical treatments aimed at alleviating and reducing symptoms, the proposed research project **focuses on delaying or preventing the disease** by elucidating some of the mechanisms of activation of the genes responsible for its development.

The complexity of PD and the multitude of systems, both genetic and physiological, that affect its onset are the main barriers to drug development (ref). The effect of genetics is limited (only about 20%), while environmental impact is significant (about 80%) (ref). Active PD is believed to develop from the association of specific genes with a trigger – a certain set of environmental stimuli (which varies from one person to another, depending on their genetic characteristics) (ref). Epigenetic changes that mediate between environmental stimuli and DNA and are unique to PD accumulate in the bloodstream and reflect key biological events that occur in the body (ref). These chemical changes, of which the addition of a methyl group (CH3) to DNA is one of the most prevalent, may indicate the beginning of the chain of events leading to PD and could be instrumental in predicting disease onset at its very early stage (ref).

Analysis of environmental components and their effect on the biology of the disease may enable early prediction. The proposed research focuses on examining the chain of events leading to changes in gene expression (prevention/activation), through the identification and monitoring of methylation patterns, which play a critical role in controlling gene expression.

Based on 18F–FDOPA PET-CT brain mapping (ref) characteristics (will be done by neurologist experts), four groups will be defined and compared PD patients, patients diagnosed in the pre-disease phase (prodromal), prodromal patients that become PD (trans-PD), and age- and gender-matched control groups. We will evaluate epigenomic methylation (of all genes) using an epigenomic novel platform that allows estimation of the rate of methylation over about 920,000 CpG sites on the DNA surface.

Analysis of epigenomic methylation among the four research groups (classified by brain mapping) will enable identification of methylation pattern differences. By examining the association between those DNA sites that demonstrate differential methylation changes and the onset of PD and its symptoms within the four groups, we will advance our understanding of the association between individual epigenomic profiles and the development of PD.

The research will trace the gene activation regulation and expression that lead to the development of PD in people predisposed to developing the disease. By identifying, isolating, and blocking the assembly of elements that triggers the genes' activation, we hope to prevent or at least delay disease onset.

One of our strategies is based on the epigenome of centenarians (super control). Epigenetic variation is highly plastic, responding to an individual’s environment and life experiences. In our previous project (ISF 196/16), we hypothesized that: ***i) aging is associated with epigenetic changes in humans, and ii) centenarians have a distinct pattern of methylation that protects them from age-related diseases and therefore increases their healthy lifespan.*** These hypotheses were examined and demonstrated the oft-neglected effect of the environment on longevity and the potential to reach exceptional age (3-7). We propose that epigenetic changes are a central mechanism by which aging predisposes to many age-related diseases. We successfully tested this hypothesis (preliminary results and paper by Gutman*.*(7)) in our unique Israeli Multi-Ethnic Centenarian Study (IMECS) cohort comprising a minority of individuals with exceptional lifespan (i.e., centenarians)(7). Here, we take a different approach (i.e., decelerating the Parkinson's process and, in some cases, preventing or delaying its occurrence), hypothesizing that elderly patients with a family history of longevity might have epigenetic factors that contribute to a long and, in the current context, PD free lifespan. Mouse and human studies will be conducted in parallel (physiology) and based on knowledge gained from human study (epigenetic) to develop a partial mechanistic view of the longevity-buffering effect(8).

As rodents are widely used as animal models of human biology, the data established herein will be relevant to humans. Comparison of the PD cohort with animal model data will enable us to define markers of PD progression that can be used to further investigate possible therapeutic avenues aimed at achieving homeostasis and slowing the progression of PD.

The research interfaces with modern personalized medicine, as the resulting database will lay the groundwork for the future integration of artificial intelligence, to be followed by a 'tailor-made’ set of medical instructions for each prospective patient, for the purpose of delaying or preventing disease outbreak.

To achieve our experimental objectives, we propose the following:

**Objective #1:** **To establish candidate epiloci prioritization (for Parkinson's progression) for further investigation based on the Parkinson’s Progression Markers Initiative (PPMI) (preliminary results).**

1a. We obtained a systematic cross-sectional and longitudinal epigenome profiling reference (9) for participants in the PPMI cohort (10), and conducted epigenomic analyses. This multi-center, longitudinal, observational natural history study examined the progression of clinical characteristics and outcomes of PD from prodromal to intermediate disease. More than 3700 individuals were divided into four groups: PD patients, trans-PD, prodromal subjects and controls. This dataset is available through the PPMI organization and provides us with baseline and longitudinal epigenomic profiling.

1b. We compiled the physiological characteristics of these individuals including known disease states, health conditions, and family health history. This dataset will be supplemented with various anthropomorphic measurements and lab tests to aid in the assessment and classification of disease-related conditions based on data already collected.

1c. We established a link between the epigenomic profile of the PPMI cohort and their physiological functioning (preliminary results). We analyzed epigenomic profiling between patient groups with different phenotypes with the goal of generating large amounts of data and also accurately interpreting and quantifying the nature of the epigenetic changes observed across the measured loci concerning physiological changes, disease occurrence, anthropomorphic measurements, and laboratory test results.

1d. We will prioritize epiloci (Table 1) based on statistical analysis, functionality (based on PPMI RNAseq data), sequence annotation, region size, and other important features to target differentially methylated candidate loci between the groups at baseline and longitudinal epigenomic profiling.

**This work will result in a list of candidate epiloci that will be pursued in the next objective.**

**Objective #2:** **To validate and prioritize candidate epiloci (for Parkinson's progression) from Objective #1 for further investigation based on the PPMI cohort (preliminary results).**

2a. We will obtain a systematic cross-sectional and longitudinal epigenomic profiling reference for the participants in the trans-PD in the PPMI cohort and develop a comprehensive cross-sectional epigenomic profiling reference using the EPIC array (which contains about 920,000 CpG sites for methylation assessment) for the new recruit to the trans-PD group (70 individual), comprising 30 individuals (from the PPMI group). This dataset will grow throughout this project and will provide us with baseline and longitudinal epigenome profiling.

2b. As in Objective #1, we will compile (new recruit and PPMI trans-PD) the physiological characteristics of these individuals, including known disease states, health conditions, and family health history. This dataset will be supplemented with various anthropomorphic measurements and lab tests that will aid in the assessment and classification of disease-related conditions based on data already collected.

2c. We will establish a link between the epigenomic profile of the trans-PD group and their physiological functioning. We will analyze epigenomic profiling among this group with different phenotypes with the goal not only of generating large amounts of data but also accurately interpreting and quantifying the nature of the epigenetic changes observed across the measured loci concerning physiological changes, disease occurrence, anthropomorphic measurements, and laboratory test results.

2d. We will prioritize (Table 1) epiloci based on statistical analysis, sequence annotation, region size, and other important features to target differentially methylated candidate loci between the groups at baseline and longitudinal. We will then obtain a list of new candidate epiloci based on the new subject and the new platform (EPIC 920k). We will compare the two lists and use the overlapped epiloci to identify physiological changes, disease occurrence, anthropomorphic measurements, and laboratory test results.

**Prioritized list of candidates:** Our prioritization scheme among epiloci will be based on statistical analysis, sequence annotation, region size, and other important features for targeting differentially methylated candidate loci listed in Table 1 and proposed by Fazzari (11). We are likely to include several epiloci per kb in our catalog for epigenetic prioritization. Some epiloci are likely to be novel, and among these, some will be more common. We will almost certainly need to prioritize these epiloci for follow-up with epigenetic studies and Sequenom validation. Variants will be ranked according to Table 2 and the prioritization of differentially methylated loci will be based on significant methylation differences between the initial screened groups and the priority in Table 2.

If a candidate epilocus overlaps with more than one category listed in Table 2, it will be ranked higher in the list for further examination. Table 2 provides our current working approach to differentially methylated loci ranking, with category 1 being a priority. This priority list will help us to choose our candidate regions for further validation in an independent cohort (future direction). We are also interested in, but will not directly pursue in this application, differentially methylated loci at nonsynonymous variants predicted to be deleterious, or methylated loci predicted by any of a set of programs (i.e., PolyPhen, SIFT).

|  |  |
| --- | --- |
| Table 1. Prioritization categories for differentially methylated loci | |
| Priority | Annotation |
| 1 | Differentially methylated loci at validated regulatory elements: non-coding RNAs (RNAdb(12)), enhancers (VISTA Enhancer Browser(13)), ORegAnno elements(14), and microRNA target sites(15). |
| 2 | Differentially methylated loci overlap with variants predicted by PWMScan (16) to cause allele specific binding affinity at TF binding sites within a first 1kb sequence upstream and downstream of a known transcription start site (TSS). |
| 3 | As in 2, but 5kb to 1kb upstream of known TSS. |
| 4 | Differentially methylated loci occurring at variants within 1kb of promoter regions, 3’ and 5’ UTRs, conserved RNA secondary structures (EvoFold(17)), predicted cis- regulatory modules or regions of accelerated substitution along human lineage. |
| 5 | Differentially methylated loci overlap with variants within DNase hypersensitive sites and histones with recognition pattern of modification and transcription factor (TF). |
| 6 | Differentially methylated loci occurring at variants within a multi-species conserved non- coding sequence. |
| 7 | Differentially methylated loci overlap with variants predicted by TargetScanS(18) or PicTar(19) to have allele-specific binding affinity to microRNAs. |
| 8 | Differentially methylated loci occurring at gene bodies |

**This work will result in a refined list of candidate epiloci that will be pursued in the next objectives.**

**Objective #3: To establish candidate longevity epiloci that buffer the prior selected candidate Parkinson’s epiloci (for Parkinson's progression).**

3a. This interplay of two fields of research (buffering epiloci in centenarians (8) as a model of successful aging [ELLI] and epiloci associated with Parkinson's progression) will further our understanding of the complex aspects of a healthy lifespan by identifying loci which, when altered epigenetically, may have important ramifications for the transition from illness to healthy lifespan.

3b. Using the IMEC centenarians’ epigenome results from our prior ISF-funded project, we will compare the overlapped epiloci candidate list of Objectives #1 and #2 to the identified longevity epiloci that demonstrate oppose functionality to the hyper/hypo methylated epiloci associated with Parkinson progression.

**Objective #4: To establish an unprecedented cross-sectional view of the list of candidate epiloci (for Parkinson's progression) for functional studies in rodents.**

We will develop a comprehensive cross-sectional epigenetic reference using a Parkinson animal model (PD patients, TransPD, pre-PD subjects, and controls). We will sacrifice 20 animals in each group and subject them to physiological, biological, and molecular analyses. We will establish a link between the epigenetic profile of each group and their physiology. Comparison of these groups is expected to highlight changes in interactions and epigenome modifications associated with Parkinson's.

**Research design & methods**

The decline in actual performance brought on by PD progression causes people to become secluded, severely impacting their health. Thus, a deeper understanding of each person's unique epigenetic traits and functional capacities is crucial. In order to highlight the interactions among these variables and provide a predictive tool, we propose creating individualized epigenetic profiles of trans-PD subjects, and pre-PD and correlating them with their 18F–FDOPA PET-CT brain mapping classification.

This will theoretically enable us to pinpoint the crucial elements of both of our two primary research pillars—PD and epigenetics—and to create a model of the interrelationships between them. In practice, this model will help policymakers to better understand the characteristics and needs of those afflicted with this neurodegenerative disease. At the individual level, early detection of change and provision of novel preventive and ameliorative non-medication interventions provide individuals with strategies, compensation, and adaptation methods to avoid physical and emotional health consequences (20).

**Methods**

**Study sample**

Subjects will include 200 men and women from the resources of the PPMI cohort (10). They will be required to be cognitively competent to provide written informed consent for inclusion in the study. The 200 subjects recruited, will be 100 TransPD (This group will be defined by a neurologist based on early symptoms such as loss of smell, tremor, movements, stiffness, coordination and balance, family medical history, and brain mapping classification by 18F–FDOPA PET-CT), and 100 will be age- and gender-matched controls.

Subjects will be excluded if they have a history of traumatic brain injury, meningitis, encephalitis, significant motor dysfunction due to stroke, active malignancy, substance abuse, current manic episode or psychotic disorders, chest pathology incompatible with pressure changes (including active asthma), inner ear disease, claustrophobia, or an inability to provide informed consent.

**Epigenetic studies in a PD mouse model**

C57BL/6-Tg(Thy1-SNCA\*E35K\*E46K\*E61K)3798Nuber/J mice(21) will be used to assess epigenetic changes in the blood and brain. This work will be done through collaboration with Prof. Dan Frenkel, who has vast experience working with neurodegenerative mouse models, in particular this PD mouse model. At approx. 6 months of age, homozygous transgenic mice exhibit subtle motor impairment, such as abnormal gait, increased time to climb down a pole, and reduced 4-limb wire test endurance. The advantage of this mouse model lies in the different behavioral activities tested. Based on lab experience and the literature, mice exhibit motor deficiency already at 3 months. We will assess pathological changes that reflect epigenetic changes (Candidate homologous to human genes resulted from aims 1 and 2 will be assessed by Sequenom) in the blood and brain at different time points of the disease: prior to clinical appearance (2 months), early pathological changes (3 months), and at 6 months when profound pathology can be identified. Ten mice per group will be compared to non-Tg mice.

**Epigenetic assays**

**EPIC array.** We will characterize methylation patterns using the genome-wide methylation array (EPIC) which examines ~920,000 epiloci and assess methylation changes in the trans-PD group of from the PPMI cohort. An epigenome reference index will be constructed using DNA methylation data from their leukocytes. DNA from PPMI will be sent to Prof. Gil Atzmon's lab at the University of Haifa on ice. The prepared DNA will be transfer to the Technion Genome Center (Haifa, Israel), which is equipped with next-generation sequencing (NGS) technology. Bisulfite-converted DNA will be hybridized to the EPIC array following the Illumina protocol. BeadChips will be washed and scanned using the Illumina HiScan SQ scanner, and intensities will be extracted from GenomeStudio (v.2011.1) and Methylation Module (1.9.0) software, which normalizes within-chip data. Following a strict QC pipeline (Bioinformatics Service Unit, University of Haifa), the microarray data will be subjected to analysis in the Atzmon lab.

**Sequenom.** The second phase of analysis involves accurate quantification of cytosine methylation at the loci identified to be the most discordantly methylated and with the greatest number of expressed methylation sites among PD patients, trans-PD, pre-PD subjects, and controls. A number of platforms to measure the relative frequencies of a nucleotide at a specific genomic position were developed for SNP quantification. However, the same platforms can be applied to bisulfite-converted DNA to measure the relative amount of C compared with T at a CG dinucleotide, thus examining whether the cytosine at that position was originally methylated (remaining as a C) or unmethylated (converted to a T). Prof. Atzmon’s lab shares a MassArray Epityper from Sequenom that is used for this purpose.

The assay is performed by PCR amplification of bisulfite-converted DNA, incorporating a T7 promoter for one of the primers. A pipeline for design, analysis, and visualization of MassArray data (22) allows us to test whether the use of the T7 sequence on the forward or reverse primer and rCTP or rTTP cleavage maximizes the proportion of CGs that can be tested in the target sequence of interest. We have previously used direct sequencing and pyrosequencing of bisulfite-converted DNA for the same purpose, and have found the Sequenom system to be substantially better in terms of ease of use, robustness of performance, and size of regions testable. Our bioinformatics pipeline for bisulfite MassArray analysis (22) will be used to design PCR primers and analyze the resulting validation data from the MassArray output. We will measure variability for individual CG dinucleotides at all of the loci tested in order to identify significant changes between different DNA samples. In this manner, we will confirm the results of the EPIC array and define the loci at which epigenetic dysregulation occurs during PD progression.

**Epigenetic statistical analysis and interpretation of results**

Applying the EPIC array, we will compare locus-specific methylation patterns as well as global methylation summaries across the three time points using linear or Poisson regression models. The goal of this analysis is not only to collect large amounts of methylation data, but also to interpret and quantify the nature of the methylation changes observed across the ~920k epiloci measured. To accomplish this goal in an efficient and meaningful way, we will have a multi-stage approach to identify the most important set of loci. We will estimate the PD progression effect in unadjusted (univariate) models. Raw p-values will be generated for each locus using the appropriate model for the comparison of interest. We will then annotate each locus with information about genomic context. On the basis of this additional genomic and positional information, we will adjust the raw p-values using the novel prioritization scheme described above (Table 1).

This will refine our list to be more consistent with expected biologically relevant groups, reducing the number of uninterpretable epiloci identified in the top candidate list. Epiloci highlighted as candidates for epigenetic effects on cognition will be examined further in our third objective for validation.

**Power calculation**

With an estimated power of 0.8, we are powered to detect genome significance difference in ~920k loci in the mean population difference, with a sigma value of 5 in a sample size of 200 individuals.

Potential problems and solutions

**Epigenetic assays.** One question that frequently arises is whether the cytosine methylation assay proposed is sufficiently robust to provide insight into the epigenomic dysregulation in question. We will test promoter sequences at greater resolution than other genomic contexts, but all genomic contexts will be explored, including gene-rich regions (exomes) and intergenic regions, conserved or repetitive sequences, CpG islands, or our alternative definition of CG clusters (23) and other annotated contexts. The method of choice is unlike affinity-based approaches which test cytosine methylation that are strongly influenced by CG dinucleotide density, requiring sophisticated analytical approaches to extract useful information from the CG-depleted majority of the genome (22). We, therefore, believe that the array provides sufficient genomic coverage, with a high likelihood of reporting the loci at which changes are occurring, and from the comparable MSCC assay (24), we should have sufficient quantitative ability to identify even moderate changes in cytosine methylation between samples.

**Preliminary results**

**Epigenetics.** Germane to this proposal, methylation levels at specific sites appear to change with aging mostly through hypermethylation (25). The pattern of methylation discovered in extremely old subjects points to deceleration of the aging process and even maintenance of aging homeostasis. The objectives of the current proposal are to advance this observation of younger biological age (determined by methylation profiling) in extremely healthy elderly subjects compared to either PD patients, pre-PD or trans-PD subjects and thus identify epiloci that are associated with the pathology of the disease.

We will compare the expression of the candidate epiloci in healthy subjects to expression of the identical epiloci in PD patients, pre-PD and trans-PD subjects to identify the basic mechanism by which PD develops. Conversely, opposite expression of this same pattern of methylation acquired with disease progression may be a major lead mechanism predisposing the elderly to many age-related diseases and affecting healthy lifespan. In my ISF-funded project, I utilized my IMECS, a cohort of centenarians (frequency of 1 in 3000 in the population) and controls (offspring of parents with normal longevity), and studied their phenotypes and epitypes (candidate epigenetic loci and EWAS approaches). Published data showed a difference between patterns of methylation in centenarians compared to controls (26). We used state-of-the-art technology to obtain significant information on age-related changes in methylation and assessed the patterns inherited between centenarians and their offspring.

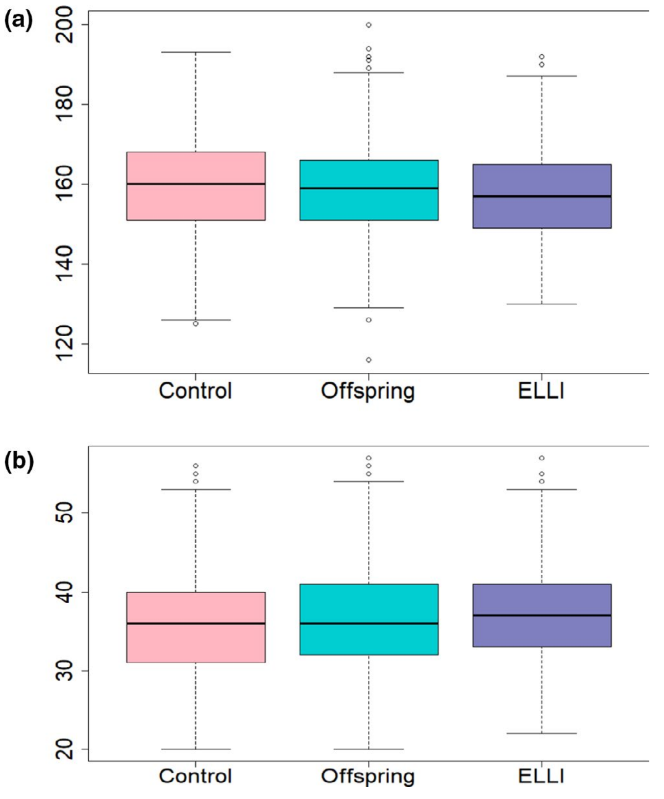


**Figure 1:** Actual vs. DNAm PhenoAge age in IMECS participants. Paired t-test p<0.01 for all inner-group comparisons.

**Studies and results**

Using the EPIC array on subjects’ leukocyte DNA, we have demonstrated the ability to perform this epigenome-wide assay for cytosine methylation in humans. The results indicate that an adequately powered study and the analysis described can reveal the subset of loci that characterize the centenarian methylation profile, as well as provide insight into their exceptional longevity. We have also investigated the ability to highlight epigenetic changes between the two selected groups.

**Figure 2:** Comparison of age-associated disease variants (Y-axis) in the 3 groups. Variants in the respective distribution. (a) Heterozygous age-associated disease variants per individual by group. (b) Homozygous age-associated disease variants per individual by group.



We applied the epigenetic clock developed by Prof. Steve Horvath with adaptation to the 860k epiloci from the **EPIC-1** array to calculate the DNAm PhenoAge (phenotypic age predicted by DNA methylation) vs. chronological age within and between groups. As seen in **Fig. 1**, all centenarians demonstrate younger DNAm PhenoAge age (7). The resilience of the centenarians to aging has previously been established (3, 8, 27-39), and we recently demonstrated this phenomenon, using exome sequencing to test the burden of pathogenic coding variants in extremely long-lived individuals and individuals without exceptional longevity (26). We proved that the burden of pathogenic variants did not differ between the groups (**Fig. 2**), suggesting a buffering mechanism that is expressed in centenarians but not in controls (26).

In summary, my results demonstrate the power and advantage of utilizing whole epigenome association studies to study exceptional longevity. We have shown that centenarians have slightly higher global methylation changes, supporting the hypothesis that subjects with exceptional longevity may exhibit different levels of DNA methylation compared to younger controls. We also showed decelerated aging in this unique group, suggesting a decelerated aging buffering mechanism that will be explored with this proposal as well. These preliminary results indicate that methylation loci demonstrate changes with aging as well as with Parkinson progression. Defining the role of epigenomics in specific mechanisms related to a healthy lifespan may lead to potential therapeutic options.

**Epigenetic profiling in Parkinson**

Epigenetic profiling primary analysis was conducted on 580 subjects of the Parkinson’s Progression Markers Initiative (PPMI)(40). Epigenomic data were downloaded from this initiative site (10), and analysis was performed in the Atzmon lab at the University of Haifa. The array (containing ~450k epiloci) results were analyzed using the *ChAMP* pipeline and methylation profiles were used to generate a heat map (**Fig. 3**) that presents unsupervised clustering indicating relationships between the different groups. The left panel in Fig. 3 shows three groups (healthy controls, pre-PD subjects, and PD patients), with some similarity demonstrated between the control and the PD patients, while the pre-PD subjects differ somewhat.

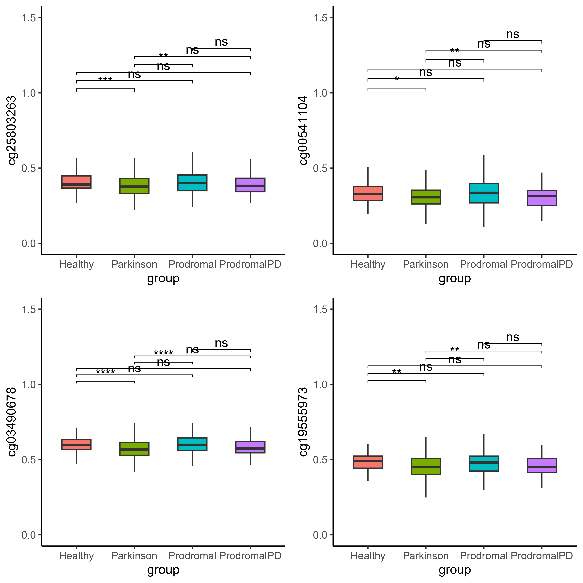
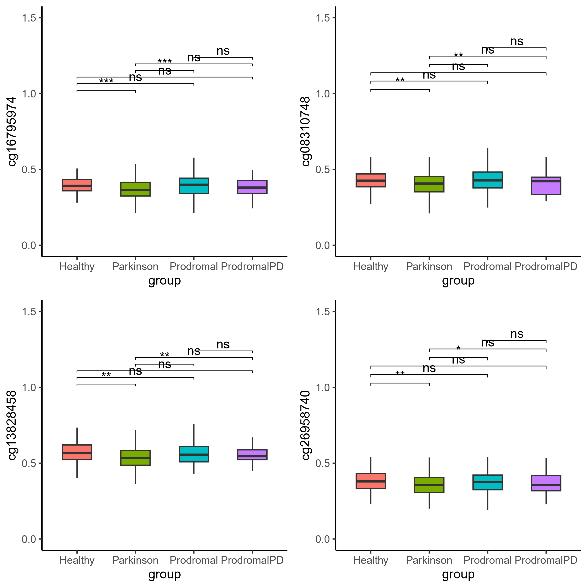


**Figure 3.** Heat map of the top differential methylation positions in control subjects, pro-PD (subjects prone to develop PD) and PD (subjects with PD) (left panel), and control subjects, pro-PD, trans-PD (subjects of the pro-PD who developed PD), and PD (right panel).

The difference in methylation pattern increases when the prodromal group is split into pre-PD subjects who did not develop PD and those who did (longitudinal follow-up in which pre-PD subjects developed PD (trans-PD) in the right panel suggests that the main transition reflects the epigenome profile during the progression of the disease). We then followed the differentially methylated positions (DMPs) between control and PD patients, with 8,346 DMPs (p<0.05) identified using the Wilcoxon signed-rank test; of these DMPs, 4147 are located within the genes and 1262 are located within CpG islands.

The eight epiloci (cg25803263 (DCAF7), cg00541104 (PTPRN2), cg03490678 (ASB6), cg19555973 (open sea), cg16795974 (LOC105376360), cg08310748 (SPTLC2), cg13828458 (USP7), cg26958740 (LINC01091)) with the greatest change in methylation were chosen for further analysis. In a pairwise comparison of each gene group, it appears that all of the tested genes have statistically significant methylation differences between PD patients and control, (DCAF7 [Δ=0.03, p=0.01]), PTPRN2 [Δ=0.03, p=0.05], ASB6 [Δ=0.03, p=0.0004], cg19555973 an open see CpG site [Δ=0.03, p=0.01], LOC105376360 [Δ=0.03, p=0.002], SPTLC2 [Δ=0.03, p=0.02] USP7 [Δ=0.03, p=0.02], LINC01091 [Δ=0.03, p=0.03]). All of which showed a pattern of hypomethylation following PD progression (**Fig. 4**, **Table 2**). Moreover, six of the selected gene were located in the gene body, one in the 5’UTR and one in the open sea (**Table 2**).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Table 2. Means of methylation difference and the significant DMPs in the two test groups.** | | | | | | | |
| Sites | CHR | Pos. | Hypo/Hyper | Mean diff. | Pval PD vs. C | Gene | Gene Loc. |
| cg25803263 | 17 | 61649698 | Hypo | 0.03 | 0.01 | DCAF7 | Body |
| cg00541104 | 7 | 158359935 | Hypo | 0.03 | 0.05 | PTPRN2 | Body |
| cg03490678 | 9 | 132402646 | Hypo | 0.03 | 0.0004 | ASB6 | Body |
| cg19555973 | 20 | 31866225 | Hypo | 0.03 | 0.01 | Open sea | Open sea |
| cg16795974 | 10 | 3507176 | Hypo | 0.03 | 0.002 | LOC105376360 | Body |
| cg08310748 | 14 | 77974814 | Hypo | 0.03 | 0.02 | SPTLC2 | 3'UTR |
| cg13828458 | 16 | 8991166 | Hypo | 0.03 | 0.01 | USP7 | Body |
| cg26958740 | 4 | 124845769 | Hypo | 0.03 | 0.03 | LINC01091 | Body |



A

B

**Figure 4, panels A and B**. Differential methylation in eight CpG sites of the genes; *DCAF7, PTPRN2, ASB6*LOC105376360, *SPTLC2, USP7*, *LINC01091* and, cg19555973 an open see CpG site, among healthy controls, PD, pre-PD, and trans-PD groups.

Here again, we demonstrate the power and advantage and our ability to utilize whole epigenome association studies in studying the effect of PD progression. Defining the role of epigenomics in specific mechanisms related to PD or resulting in a healthy lifespan may lead to potential therapeutic options.

**The strengths of this project include:**

* The close interaction between the PI and collaborators, each representing a complementary field of expertise, covering epigenetic technologies (Prof. Atzmon); enrolling PD, pre-PD, trans-PD and healthy control subjects to the study (PPMI cohort); animal studies (prof. Frenkel (letter attached)
* The research infrastructure for this project is already in place as a result of the ongoing collaborations among the PI and collaborators as well as with genomic scientists, physicians, and statistical geneticists through multiple programs at the University of Haifa and Tel Aviv University.
* The use of state-of-the-art high-throughput genetic technology and bioinformatics analyses.
* The use of state-of-the-art brain classification including 18F–FDOPA PET-CT done by physicians.
* The high probability of unbiased discovery of epimarkers to investigate the leading mechanistic view of healthy aging.

This project is expected to lead to future studies that will help define the role of epigenetics in specific leading mechanisms related to PD and perhaps ultimately lead to potential therapeutic options. We believe that longitudinal follow-up is crucial for understanding the biology of PD. We therefore plan to pursue substantially longer longitudinal studies in the future. We also plan to further explore the mechanistic view of the targeted candidate loci resulting from this study, employing cell and tissue cultures in *in vivo* studies.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 2030 | | | 2029 | | | | 2028 | | | | 2027 | | | | 2026 | | | | \* | **Task** | **Obj.** |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | Task a. Analysis of acquired PPMI epigenome profile *(Prof. Atzmon)*  Task b. Establish candidate loci *(Prof. Atzmon)* | **1** |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | Task a. *Subject classification (PPMI)* | **2** |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | Task b. *Assemble epigenomic profile of 200 trans-PD (Prof. Atzmon)* |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | Task c. *Conduct EWAS on 200 subjects**(Prof. Atzmon)* |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | Task d. *Validation of candidate epiloci (Prof. Atzmon)* |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | *Establish candidate longevity-buffering epiloci that (Prof. Atzmon)* | **3** |
|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  | *Animal studies (Prof. Frenkel)* | **4** |

\*2025 last quarter

First, we plan to establish a PD epilocus candidate on a PD cohort of PPMI with EWAS assessments completed (ages 40-85). These subjects are enrolled in the **PPMI** cohort, which aims to longitudinally and cross-sectionally identify epigenetic variants associated with PD; this task will be done in the first year. In parallel, we will perform the second round of tasks (sample collection, blood drawing, and epigenomic profiling will be performed in parallel to brain mapping and subject classification), resulting in validated candidate loci), which involve a tremendous amount of work, limiting us to 200 samples (this Task will be done by collaboration with PPMI) and will therefore last for the entire project. Thus, Objectives #3 and #4 (collaboration with Prof. Frenkel (letter attached)) will run in parallel for the last years of the project as the candidate epiloci evolve.

**Bibliography**

1. Mhyre TR, Boyd JT, Hamill RW, Maguire-Zeiss KA. Parkinson's disease.Subcell Biochem.2012;**65**:389-455.

2. Reeve A, Simcox E, Turnbull D. Ageing and Parkinson's disease: why is advancing age the biggest risk factor?Ageing Res Rev.2014;**14**:19-30.

3. Rajpathak SN, Liu Y, Ben-David O, Reddy S, Atzmon G, Crandall J*, et al.* Lifestyle factors of people with exceptional longevity.J Am Geriatr Soc.2011;**59**:1509-1512.

4. Kato K, Zweig R, Schechter CB, Barzilai N, Atzmon G. Positive attitude toward life, emotional expression, self-rated health, and depressive symptoms among centenarians and near-centenarians.Aging Ment Health.2016;**20**:930-939.

5. Kato K, Zweig R, Schechter CB, Verghese J, Barzilai N, Atzmon G. Personality, self-rated health, and cognition in centenarians: do personality and self-rated health relate to cognitive function in advanced age?Aging (Albany NY).2013;**5**:183-191.

6. Kato K, Zweig R, Barzilai N, Atzmon G. Positive attitude towards life and emotional expression as personality phenotypes for centenarians.Aging (Albany NY).2012;**4**:359-367.

7. Gutman D, Rivkin E, Fadida A, Sharvit L, Hermush V, Rubin E*, et al.* Exceptionally Long-Lived Individuals (ELLI) Demonstrate Slower Aging Rate Calculated by DNA Methylation Clocks as Possible Modulators for Healthy Longevity.Int J Mol Sci.2020;**21**.

8. Bergman A, Atzmon G, Ye K, MacCarthy T, Barzilai N. Buffering mechanisms in aging: a systems approach toward uncovering the genetic component of aging.PLoS Comput Biol.2007;**3**:e170.

9. PPMI database. PPMI database. <https://ida.loni.usc.edu/>,.

10. PPMI. PPMI <https://www.ppmi-info.org/>.

11. Fazzari MJ, Greally JM. Introduction to epigenomics and epigenome-wide analysis.Methods Mol Biol.2010;**620**:243-265.

12. Pang KC, Stephen S, Dinger ME, Engstrom PG, Lenhard B, Mattick JS. RNAdb 2.0--an expanded database of mammalian non-coding RNAs.Nucleic Acids Res.2007;**35**:D178-182.

13. Visel A, Minovitsky S, Dubchak I, Pennacchio LA. VISTA Enhancer Browser--a database of tissue-specific human enhancers.Nucleic Acids Res.2007;**35**:D88-92.

14. Griffith OL, Montgomery SB, Bernier B, Chu B, Kasaian K, Aerts S*, et al.* ORegAnno: an open-access community-driven resource for regulatory annotation.Nucleic Acids Res.2008;**36**:D107-113.

15. Papadopoulos GL, Reczko M, Simossis VA, Sethupathy P, Hatzigeorgiou AG. The database of experimentally supported targets: a functional update of TarBase.Nucleic Acids Res.2009;**37**:D155-158.

16. Levy S, Hannenhalli S. Identification of transcription factor binding sites in the human genome sequence.Mamm Genome.2002;**13**:510-514.

17. Pedersen JS, Bejerano G, Siepel A, Rosenbloom K, Lindblad-Toh K, Lander ES*, et al.* Identification and classification of conserved RNA secondary structures in the human genome.PLoS Comput Biol.2006;**2**:e33.

18. Lewis BP, Burge CB, Bartel DP. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets.Cell.2005;**120**:15-20.

19. Krek A, Grun D, Poy MN, Wolf R, Rosenberg L, Epstein EJ*, et al.* Combinatorial microRNA target predictions.Nat Genet.2005;**37**:495-500.

20. Hertzog C, Kramer AF, Wilson RS, Lindenberger U. Enrichment Effects on Adult Cognitive Development: Can the Functional Capacity of Older Adults Be Preserved and Enhanced?Psychol Sci Public Interest.2008;**9**:1-65.

21. Nuber S, Rajsombath M, Minakaki G, Winkler J, Muller CP, Ericsson M*, et al.* Abrogating Native alpha-Synuclein Tetramers in Mice Causes a L-DOPA-Responsive Motor Syndrome Closely Resembling Parkinson's Disease.Neuron.2018;**100**:75-90 e75.

22. Thompson RF, Suzuki M, Lau KW, Greally JM. A pipeline for the quantitative analysis of CG dinucleotide methylation using mass spectrometry.Bioinformatics.2009;**25**:2164-2170.

23. Illumina Support Illumina Support <http://support.illumina.com>.

24. Ball MP, Li JB, Gao Y, Lee JH, LeProust EM, Park IH*, et al.* Targeted and genome-scale strategies reveal gene-body methylation signatures in human cells.Nat Biotechnol.2009;**27**:361-368.

25. Hannum G, Guinney J, Zhao L, Zhang L, Hughes G, Sadda S*, et al.* Genome-wide methylation profiles reveal quantitative views of human aging rates.Mol Cell.2013;**49**:359-367.

26. Gutman D, Lidzbarsky G, Milman S, Gao T, Sin-Chan P, Gonzaga-Jauregui C*, et al.* Similar burden of pathogenic coding variants in exceptionally long-lived individuals and individuals without exceptional longevity.Aging Cell.2020;**19**:e13216.

27. Freudenberg-Hua Y, Li W, Abhyankar A, Vacic V, Cortes V, Ben-Avraham D*, et al.* Differential burden of rare protein truncating variants in Alzheimer's disease patients compared to centenarians.Hum Mol Genet.2016;**25**:3096-3105.

28. Milman S, Barzilai N. Dissecting the Mechanisms Underlying Unusually Successful Human Health Span and Life Span.Cold Spring Harb Perspect Med.2015;**6**:a025098.

29. Milman S, Atzmon G, Huffman DM, Wan J, Crandall JP, Cohen P*, et al.* Low insulin-like growth factor-1 level predicts survival in humans with exceptional longevity.Aging Cell.2014;**13**:769-771.

30. Milman S, Atzmon G, Crandall J, Barzilai N. Phenotypes and genotypes of high density lipoprotein cholesterol in exceptional longevity.Curr Vasc Pharmacol.2014;**12**:690-697.

31. Lai JY, Atzmon G, Melamed ML, Hostetter TH, Crandall JP, Barzilai N*, et al.* Family history of exceptional longevity is associated with lower serum uric acid levels in Ashkenazi Jews.J Am Geriatr Soc.2012;**60**:745-750.

32. Andersen SL, Sebastiani P, Dworkis DA, Feldman L, Perls TT. Health span approximates life span among many supercentenarians: compression of morbidity at the approximate limit of life span.J Gerontol A Biol Sci Med Sci.2012;**67**:395-405.

33. Atzmon G, Cho M, Cawthon RM, Budagov T, Katz M, Yang X*, et al.* Evolution in health and medicine Sackler colloquium: Genetic variation in human telomerase is associated with telomere length in Ashkenazi centenarians.Proc Natl Acad Sci U S A.2010;**107 Suppl 1**:1710-1717.

34. Motta M, Bennati E, Vacante M, Stanta G, Cardillo E, Malaguarnera M*, et al.* Autopsy reports in extreme longevity.Arch Gerontol Geriatr.2010;**50**:48-50.

35. Selim AJ, Fincke G, Berlowitz DR, Miller DR, Qian SX, Lee A*, et al.* Comprehensive health status assessment of centenarians: results from the 1999 large health survey of veteran enrollees.J Gerontol A Biol Sci Med Sci.2005;**60**:515-519.

36. Bernstein AM, Willcox BJ, Tamaki H, Kunishima N, Suzuki M, Willcox DC*, et al.* First autopsy study of an Okinawan centenarian: absence of many age-related diseases.J Gerontol A Biol Sci Med Sci.2004;**59**:1195-1199.

37. Atzmon G, Schechter C, Greiner W, Davidson D, Rennert G, Barzilai N. Clinical phenotype of families with longevity.J Am Geriatr Soc.2004;**52**:274-277.

38. Barzilai N, Atzmon G, Schechter C, Schaefer EJ, Cupples AL, Lipton R*, et al.* Unique lipoprotein phenotype and genotype associated with exceptional longevity.JAMA.2003;**290**:2030-2040.

39. Baggio G, Donazzan S, Monti D, Mari D, Martini S, Gabelli C*, et al.* Lipoprotein(a) and lipoprotein profile in healthy centenarians: a reappraisal of vascular risk factors.FASEB J.1998;**12**:433-437.

40. Gerraty RT, Provost A, Li L, Wagner E, Haas M, Lancashire L. Machine learning within the Parkinson's progression markers initiative: Review of the current state of affairs.Front Aging Neurosci.2023;**15**:1076657.