(1) The hypothesis of the proposal is that Parkinson Disease-specific epigenetic markers in the bloodstream may represent important biological processes, which may reflect systemic changes that reflect brain specific degeneration. DNA methylation at CpG sites may be able to reveal these molecular changes that eventually result in PD. Eventually, identifying such methylation changes may serve as markers for detecting disease onset at an early stage.

(2) The strength is a unique PPMI cohort on which methylation will (or already was) be conducted. Standard methylation EPIC platform been applied. Dividing subjects into PD patients, pre-PD subjects, and controls can reveal progressive changes in methylation markers.

(3) Findings may provide biomarkers.

(4) Project may lead to large data generation that will be useful in further analyses.

We humbly grateful for the reviewers’ strength points’ notifications. Especially the recognition that the project is important as identification of PD early biomarkers is of relevance for public health decisions. In this version, we attempted to build on these comments to develop a stronger and more cohesive proposal.

(1) Although it is mentioned in the significance and hypothesis of the project that study methylation will provide insights into gene expression changes that have pathogenic consequence, the study is not designed to reveal this, p=or at least it is not clearly described how these analyses will be performed.

(2) Mouse studies are described but with few details on analyses of molecular mechanisms.

We acknowledge our minimal and perhaps misguiding analysis structure and interpretation. We believe that our resubmission provides better interpretation of the analysis scheme.

1. We already started analyzing the PPMI dataset of limited Trans-PD subjects (only 30 people).
2. This analysis resulted with couple of suggestive loci (p<5x10-6) which were further subjected to *In vitro* studies. These results are included in the preliminary results.
3. Exciting from our preliminary results, we establish a collaboration with Tel Aviv Sourasky Medical Center and are in a midst of recruiting more Trans-PD subjects to achieve the 100 subjects goal.
4. We refine the analysis plan to reflect our aimed objective. For example; based on 18F–FDOPA PET-CT brain mapping characteristics, 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. Thus, the PET scan will assist us with group classification (will be done by neurologist experts) and the EWAS (epigenome wide association study) will be conduct on the groups and not on the mapping results.

Overall there is a lack of details in methods which makes it difficult to evaluate what type of data is already generated, what will be generated, and how mechanistic insights will be sought.

(1) Mapping methylation patterns in blood amongst the groups is unlikely to help find mechanistic links to development of PD.

(2) Fig. 3 is indicated to be a heatmap of unsupervised clustering of methylation but legend indicates that the heat map is of differentially methylated sites among the groups compared. This is confusing to understand the degree of differences in the groups. Additionally, are these analyses from all 580 subjects of the Parkinson’s Progression Markers Initiative (PPMI) or only 3/4 individuals in the figure-2 panels? Further, it is not clear what other co-variates were considered/adjusted for the DMP analyses.

(3) How the mouse model will be studied for pathological changes that reflect epigenetic changes in the blood and brain is unclear. Further, how these studies will be integrated with the findings from the human studies is unclear. Are the DMPs that is expected to be altered in human leucocytes is also expected to be similarly altered in mouse leucocytes/brain? It is stated that a link between the epigenetic profile of each mouse group and their physiology will be established. If there are no direct relations between epigenetic changes in the human and mouse models, what alternative approaches of analyzing the mouse models to identify epigenetic role in PD should be considered.

(4) Based on the description, it is assumed by this reviewer that the epigenome profiling reference from 3700 participants in the PPMI cohort is available. Also PPMI RNAseq data is available. So it is not clear what DMP sites are discovered from these 3700 participants, which is a large dataset.

Only 400 are available of which only 30 are Trans-PD.

(5) In terms of project execution, are patient samples already available from 200 men and women from the PPMI cohort after considering the exclusion criteria. Are these 200 patients a subset of the 3700 patients subcategorized by subjects who are pre-PD that progress into PD.

As listed only 30 Trans-PD are available from the PPMI study this is why we are aiming to recruit more.

(6) The study mentions that it will trace the gene activation mechanisms and expression that lead to the development of PD in people predisposed to developing the disease. Since data is collected from blood, it is unlikely that such gene expression mechanistic insights can be directly linked to PD. Further, methylation relation with gene expression needs to be established for these samples.

This link will be done in the mouse model

Reviewer No. 2

Originality & innovation

The proposed research project aims to employ epigenetic analyses to elucidate mechanisms of gene activation leading to the development of Parkinson’s disease in order to delay or prevent the disease. To this end, DNA methylation patterns will be compared between people diagnosed with the disease and healthy controls. Several previous studies have already investigated DNA methylation between PD patients and control subjects (e.g., Young et al, 2019, https://doi.org/10.1212/NXG.000000000000034, Henderson et al., 2021, https://doi.org/10.3389/fgene.2021.640266, also reviewed in Miranda-Morales et al 2017, 10.3389/fnmol.2017.00225). The innovative aspect of this proposal is thus mostly in including a group of subjects diagnosed before the onset of the disease as well as centenarians that might allow the identification of risk epiloci and protective epiloci, respectively.

Thanks for the kind words

Parkinson’s disease is a debilitating neurodegenerative disease that tremendously reduces the quality of life of affected patients as well as posing a substantial burden on the health care system worldwide. As there are no curative therapies, prevention and delay of onset of the disease are particularly important. In this context, identifying epigenomic risk loci and their interaction with environmental risk factors, as outlined in the proposal, have the potential to provide novel insights into the disease mechanisms. The Parkinson’s disease risk epiloci catalogue envisioned by the authors can provide an important fundament for follow-up analyses and validation experiments in animal models. Identifying epiloci associated with healthy aging and longevity can offer novel insight into factors promoting healthy aging and longevity and offsetting the effect of risk loci for developing Parkinson’s disease.

Thanks for the kind words

In my opinion, the description of the methods that will be used for data analysis is often rather sparse and not sufficiently detailed. For instance, only generic tests such as linear and Poisson regression for epigenetic analysis as well as t-test and chi-square test for PET CT data are mentioned. However, there is no clear description of how the applicant plans to integrate different data sources together. In particular, the researchers do not sufficiently describe how they plan to relate differentially methylated risk loci to the PET CT brain mapping data. It is also not sufficiently clear how physiological characteristics and environmental stimuli data will be integrated to achieve the ultimate goal of designing a prevention strategy to reduce the risk of developing Parkinson’s disease by avoiding identified risk factors. The sample size estimation is also not clearly described. Certainly, it is not possible to perform the calculation for all 920K loci in the epigenomic assay and I could not understand what is meant by the mean population difference in this case. The targeted value of this population effect is also not mentioned.

We are not planning to analyze the PET results rather to use it to define the groups. Further,

1. We already started analyzing the PPMI dataset of limited Trans-PD subjects (only 30 people).
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3. Exciting from our preliminary results, we establish a collaboration with Tel Aviv Sourasky Medical Center and are in a midst of recruiting more Trans-PD subjects to achieve the 100 subjects goal.
4. We refine the analysis plan to reflect our aimed objective. For example; based on 18F–FDOPA PET-CT brain mapping characteristics, 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. Thus, the PET scan will assist us with group classification (will be done by neurologist experts) and the EWAS (epigenome wide association study) will be conduct on the groups and not on the mapping results.

The investigator has a proven scientific background related to the research questions presented in this project with a strong publication record in international peer-reviewed journals. Prof. Atzmons’s previous research has focused on understanding the genetic and epigenetic features of healthy aging and longevity, neurodegenerative and psychiatric diseases, which fits well with the outlined research aims. Furthermore, the applicant has relevant experience in generating and analyzing data sets from human cohorts. Therefore, there is, in principle, no doubt that the investigator can perform the research activities outlined in the proposal. The application is also strengthened by the collaboration with Prof. Dan Frenkel, who brings complementary expertise on mouse models of Parkinson’s disease.

Thanks for the kind words

Summary (strengths/weaknesses of the proposal)

Overall, this is an ambitious and interesting research project dealing with a medical need concerning the broader population. The strengths of the proposal include the complementary expertise of the applicant and the main collaborators allowing analyses in both humans and animals. Including the additional group of pre-PD patients is particularly interesting for identifying novel risk epiloci and the comparison with centenarians offers the opportunity to detect methylation patterns associated with longevity. However, the description of the methods, particularly concerning the analysis of the data, should have been more detailed and specific.

We acknowledge our minimal and perhaps misguiding analysis structure and interpretation. We believe that our resubmission provides better interpretation of the analysis scheme.