הקרן הלאומית למדע
المؤسّسة الإسرائيلية للعلوم
Israel Science Foundation

כ״ח בתמוז, תשפ״ב
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מספרנו : 171/22

לכבוד
פרופ׳ גיל עצמון
מדעי החיים
אוניברסיטת חיפה

לפרופ׳ עצמון שלום רב,

הנדון : בקשתך למענק מחקר בנושא :
**המבנה התלת מימדי של הגנום ותפקודו אצל אנשים וחיות מודל מאריכי חיים**

הצעת המחקר אשר הגשת לקרן הלאומית למדע לא נכללה, לצערנו, בין ההצעות אשר זכו במענקי
מחקר השנה.

מצ״ב עיקרי חוות הדעת.

לתשומת לבך, החלטות הנהלת הקרן מתקבלות על סמך סיקור עמיתים ודיונים בוועדות
מקצועיות. ראוי להדגיש כי הוועדה המקצועית אשר בחנה את הצעת המחקר התבססה על מכלול
ההערות והציונים שהתקבלו מסוקרים חיצוניים ומחברי הוועדה שהם מומחים בתחום, ונתנה
משקל, בדיוניה, רק לאותן ביקורות והערכות שהיו מקובלות עליה.

מאחר שכספי ההקצבה השנתית מחולקים עד תום, החלטות הנהלת הקרן הן סופיות ואינן ניתנות
לשינוי.

אנו מאחלים לך הצלחה בהמשך דרכך המדעית.

בכבוד רב,

ד״ר תמר יפה-מיטווך
מנכ״ל

העתק : רשות המחקר, אוניברסיטת חיפה


מוזמנים לבקר באתר קול המדע https://kolhamada.isf.org.il/ - מיזם של הקרן הלאומית
למדע, המגיש מידע מדעי מחזית המדע, לציבור ולקהילה המדעית בישראל.

# Reviewer No. 1

In this proposed research, Prof. Atzmon intends to explore the genomic architecture that supports healthy aging. To do so, he initiated a collaborative network to explore the three-dimensional organization of the genome, in mice and human aging, primarily utilizing Hi-C. The main focus of this proposal is to measure and analyze the chromatin interaction in exceptionally long-lived individuals, their children (which are expected to be healthier), and controls (65+). These experiments will be coupled with parallel comparisons in mouse aging cohorts. By comparing the 3D genome organization in all these conditions, key differences will be identified that could consequently point towards molecular mechanism that contribute to healthy aging.

The proposed research is innovative, brings together leaders in their respective fields (Dr. Noam Kaplan and Prof. Haim Cohen), ambitious, and could provide a novel point of view for exploring cellular and organismal aging. The methodologies are state of the art, and in-depth characterization of the aging 3D genome is indeed lacking. The combination of a large-scale and impressive human cohort, coupled with detailed mouse studies, provides a powerful comparative approach.

Several aspects of the proposal merit further consideration. The proposal at its current state is a high-risk-high-gain project, as it lacks strong preliminary data that supports the main hypothesis (beyond technical feasibility), which could have further allowed for more detailed predictions. Additional consideration should be taken in the experimental design, such as possible changes in cell composition, which could impact cell-type specific parameters. Although this might be beyond the scope of the current proposal, how this research could provide mechanistic insight beyond correlation should be better discussed.

Ultimately, this research will generate a large and comprehensive dataset, that will be an important resource for the community, and undoubtedly serve as the foundation for additional studies.

# Reviewer No. 2

Originality & innovation
Strength:
1. The use of ELLI cohort
2. Combined analysis of both human and mouse data

Weakness:
1. Although Hi-C is a state-of-the-art method to detect 3-D chromatin interactions, it will require additional methods and datasets to corroborate any findings that could be corelating with any physiological changes investigating, in this case, aging. The a few examples of drastic 3-D mapping alterations are caused by significant genetic defects/deletions. Any change associated with old age is likely subtle and unlikely to produce anything close to that level of change. Hence, Hi-C alone is unlikely to yield any significant findings in aging cohorts.

Project importance and contribution to scientific knowledge
Strength:
1. Study of 3-D chromatin features in the ELLI cohort
2. Data generated from this project will be useful for the aging research community

Weakness:
1. There is a lack of justification and evidence that Hi-C alone can yield any significant findings from this project.
2. The project appears to be entirely resources/data generating. It lacks testable hypotheses. When combined with the singular use of methodology, it is unlikely to yield any significant novel mechanism of aging regulation.

Adequacy of methods
Strength:
1. Recruitment of ELLI cohort
2. Collaboration with a lab with expertise in Hi-C analysis

Weakness:
1. The use of Hi-C as the sole experimental method in this project. No sufficient justification for why this method alone can yield enough insights and mechanisms for aging regulation. Hi-C often needs other methods, like ChIP-seq for CTCF and cohesin, RNA-seq, ATAC-seq, etc, to corroborate its findings.
2. The use of blood and neutrophils for Hi-C is not well justified.
3. The lack of preliminary data for detectible Hi-C contact changes in neutrophils associated with either aging or age-related diseases.

Suitability of investigators' scientific background to the project
Strength:
1. PI has ample experience in study epigenomic changes associated with human aging
2. One of the collaborators has experience in Hi-C data analysis
3. One of the collaborators has experience in mouse aging

Weakness:
1.      The lack of plans and arrangements for communication and data sharing among PI and collaborators

Summary (strengths / weaknesses of the proposal)
Strength:
1.      Study of ELLI cohort
2.      Combined analysis with mouse
3.      The use of Hi-C technology
4.      Expertise of PI and collaborators

Weakness:
1.      This project is heavy on data generation, but lack of a focus on testing hypothesis.
2.      The use of Hi-C methodology alone without sufficient justification
3.      The lack of justification and consideration for sample choice (neutrophils in blood)
4.      Overall, it is unlikely to yield any significant model/theory of aging

# Reviewer No. 3

1) Originality & innovation
The variability of long lifespan phenotype is an interesting question, which is not new and the mechanisms underlying healthy aging phenotype remain largely unknown. Several studies in the past highlighted the role of epigenetic alterations and chromatin conformations change occur during aging and senescence. Following on from previous projects by the candidate on the epigenomic effects of ageing in extremely long-lived individuals (ELLIs),  this project proposes to focus on the 3D genome organization.
In previous results they had described younger biological age (as measured by epigenetic clocks) in centenarians, despite the same number of mutations, creating a sort of buffer effect in ELLIs. It is therefore logical to question whether the key to understading this lies in 3D chomatin organization.

In this project, the candidate proposes to use current methods to map chromatin structure (Hi-C) to compare ELLIs to elderly and middle age individuals using an existing cohort for which the candidate has already produced methylation data and collected clinical and related data (from the diagram 200+200 individuals).

They then plan to move to mice to perform similar experiments on a population of 100 in which they will monitor chromatin structure and physiological phenotypes longitudinally.

Finally, they will perform follow-up of the offspring of the human cohorts for 5 years (0, 1, 3, 5 years) to ensure monitoring of the development of conditions and changes in the 3D chromatin organization either in the same individual or across groups.
The then formulate the following 3 hypotheses:
-Cohorts have in chromatin structure,
-Mice results reinforce evidence in human
-ELLI chromatin organization is inherited by their offspring

Originality:
Identify fundamental differences in 3D genome organization of the different groups (centenarian and middle age, and their offsprings) - hasn't been done
Combine phenotypic description in human and mice with 3D structure

Innovative:
This work will give us a picture of 3D genome organization in centenarian people and their offspring.
It will be useful for future research works. This work in healthy and exceptional long lived people (which reflect decelerating aging) could be used as reference study

2) Project importance & implications
The application of HiC in a very rare population of ELLI is a first and based on the availability of the cohort which is already being constructed, hence achievable. The planned HiC datasets would be a great resource for the community.

This project is ambitious and if it is successful, it could give us information about the specificities of the 3D structure in aging and longevity. It will be useful to study the relationship between 3D genome organization and physiological changes. The data produced could become a reference to study diseases associated with old age (Alzheimer, sarcopenia, some cancers, etc.) and could suggest new targets for epigenetic drug developments.

3) Adequacy of methods
The resarchers designed a cross sectional study in humans and two longitudinal studies in humans and mice. About the human cohorts, the physiological survey is well designed and explained and builds on the expertise of one of the collaborators. The choice to use Hi-C on neutrophils makes sense since blood is easily available and they are the most abundant cell type. It's important to purify them to not confuse epigenomic changes with changes in cell type proportion. The idea to follow up on specific selected loci by capture HiC is also good.
Analysing HiC data by looking for TADs is not a particularly novel approach and few details are given to understand whether the mentioned age x TAD interaction can be studied and how.
The proposal does not describe the details of the HiC performed in terms of expected sequencing depth per sample, even suggesting aggregation of the entire groups in each cohort. Whereas I see why this would help to increase resolution from single patient samples that would inevitably generate quite sparse data, I wonder if this wouldn't just confuse things and hide patterns. The authors should comment on which type of interactions they plan to observe or whether they would be happy with TAD definitions. The proposal does not contain details of computational and statistical methods that will be used to compare the different cohorts and conditions/time points and to link the physiological survey to the Hi-C result. This point is crucial for the success of the project.

The text has several typo/citation errors. Two figures have the same number (figure 4). The first citation about the figure 6 must show the Mirny cooler pipeline and shows a Hi-C interaction map cited later in the proposal. The figures from the previous grant are not very legible.

4) Suitability of investigators' scientific background to the project
Prof. Atzmon has worked in human aging and longevity since 2001. He has numerous publications on this research theme. He has been interested in aging and epigenomics aspects for several years. He won a previous grant on this subject (ISF grant No. 193/16), which is also quite related to this one, setting the stage for it. He mentions  a pre-existing collaboration with Noam Kaplan, a specialist of genome organization and Hi-C, which does not seem to have been published. For this project, he also has a collaboration with Haim Cohen who has a strong background in genetics and aging and mice models.

5) Summary (strengths / weaknesses of the proposal)

Strengths:
-The application is interesting and promising.
-Ambitious project, three objectives and their hypothesis fit well with the knowledge in the litterature.
-The topic is new and trending
-Potential to prove that centenarian people and their offspring are able to maintain their 3D genome organization differently to other individuals or have a special chromatin configuration.
-Good cohort (recruitment standard), already existing, plenty of complementary data available, large enough size for good statistics
-The project would create a great resource for other scientists
-The proposed methods (Hi-C/phenotype screening) are appropriate


Weaknesses:
-The hypotheses are not so specific
-They don't mention how they could integrate their results with the epigenetic clocks (DNA methylation, their previous works)
-No details about how to deal with possible human/mice discrepancies
-No estimation of sequence depth of libraries, key to understand usefulness of data
-Computational methods are poorly described or unspecific
-No mention about integration with epigenetic clock or their previous works
-The proposal accumulates several typo/citation errors and some figures (from the previous grant) are not very legible.
-If I understood well, budget is twice the average for these grants (from ISF webpage)

# Reviewer No. 4

1. The proposed research is novel and addresses an interesting question in genomics of aging – if there is a correlation between the structure of the genome organization and aging.

2. Aging is important topic to study taking into account the fast aging of human population and the relatively small amount of knowledge we have on this part of our physiology, comparing to the others. There are many factors that contribute to aging and it is quite possible that changes in the genome structure might be part of these changes or reflect on them. Understanding of this connection would be important for understanding of the determinants of aging in humans.

3.The research methods are appropriate to address the aims of the proposal in general. It is not clear, however, what are the individual differences in the HI-C between different individuals. It is possible that these signatures, especially in the blood cells, are dependent on many factors, like previous and current diseases, drug treatments etc, in much higher manner then they are dependent on aging. Therefore, comparing different individuals in a relatively small cohort might not be enough to find a statistically significant correlations. Therefore, the longitudinal experiment is the most promising one. I hope it will succeed. Of note, it is not clear at all if high level genome organization changes with age and how. I hope that interesting line of research described in this proposal will give some answers.

4. Prof. Atzmon has an extensive record in the genetics of aging, which is the main topic of this proposal. His expertize is a great fit and the proposal is based on cohorts under he studies. He has not performed Hi-C experiments before and will acquire the necessary expertize in Hi-C from Dr. Kaplan. Aim 2 describes mouse experiments. Prof. Atzmon does not have background in such experiments, and, according to the proposal, all these experiments will be performed at the lab of Prof. Cohen at Bar-Ilan University. Prof. Cohen is outstanding scientist who would be able to perform the proposed experiments. Significant amount of funds supposedly will be transferred to the lab of Prof. Cohen for this purpose.

Summary
This is interesting and novel proposal. Overall the proposed research might advance our understanding of the genetics of aging if the correlation will be found between the groups and the Hi-C signature. One caveat is that the individual differences in the HI-C signatures might mask the aging signature and therefore the identified specific signal will be week. In this respect proposed longitudinal studies are exciting and should be able to overcome the above concern. I would suggest to focus mainly on these studies.