

Reviewer 1

Dear Professor,

Thank you for reviewing our manuscript.

I included both provided conclusions in the manuscript as following:

With appreciation

Parvin Mehdipour

In Abstract

CONCLUSION

We highlighted application of the Single Circulating Neural Cells (CNCs) and Correlated Ratio based between Brain channels by providing the 5xP personalized clinical management model for and early detection and therapy of the patients with AD and their targeted/predisposed relatives.

At the end of manuscript: page 31

CONCLUSION

ETS2 gene has multi-correlational capabilities with neurodegenerative phenotype encoding a transcription factor, regulating Bcl-XL intracellular β -amyloid and expression of P53. These characteristics of ETS2 were the highlighted reasons to explore this gene at functional level which is the end destination of the functional journey.

Brain is a complicated, systematic, with an interactive strategy, and a directive ability of the body through the disciplined manner. By referring to the available data on the neural organization, a

valid link between Neuro-Science and clinic is estimated. The Classified early detection of the nervous system, including the brain neoplasm and Alzheimer's disease is not available within the current clinical programme. Moreover, therapy is restricted due to the blood brain barrier.

Brain is a territory with the multi-channels, diverse and complicated expression of different related key proteins. Furthermore, EEG is applicable as a fundamental, non-invasive and valuable technique for the basic clinical diagnosis at any age.

Regarding the screening of the high enumeration of the Circulating Neural Cells , limited publications on the CNCs, diversity, evolutionary based classification , and early detection of the abnormal events in brain have not been considered.

The CNCs were explored in ten patients affected with AD and patients with Down's syndrome. Protein expression of ETS2 was assayed by Flow-cytometry (FC) which has been, previously, published ^[11] . PE of ETS2 has important pathogenic role in these two different diseases. There was statistically significant difference for ETS2 between Alzheimer patients with Control ($P = 0.006$), and DS with control ($P = 0.007$), and interestingly, expression of ETS2 protein was significantly higher in DS patients than in AD patients ($P= 0.044$).

By considering late systematic investigation of the brain, harmonization of the standard psychological atmosphere with the biological and the health of the individuals' body systems is required. Brain is rather an insecure- and inaccessible organ for sampling and therapy. Hence, a routine early detection is applicable by the Circulating Neural Cells (CNCs) at any age, with only 2-3 ml of the peripheral blood sample, or any other non-invasive sampling, including buccal mucosa or smear.

The majority of the publications have applied the machine-based analysis by focusing on the functional aspect at protein level, and regarding the Circulating Neural Cells (CNCs), enumeration of limited single cells is reported to be adequate.

With respect

Parvin Mehdipour

Running title:

List of common issues in revised manuscripts by authors and comments

No.	Location	Common issues and comments
1	<p>Title: Exploring Circulating Neural Cells and electroencephalography: Personalized-early detection-Model based in Alzheimer disease:</p> <p>Personalized early detection Model in Alzheimer disease</p>	
2	<p>Running title</p> <p>Exploring Circulating Neural Cells and electroencephalography</p>	
3	<p>Author List</p> <p>Mehdipour P^{1*}, Fathi N², Nosratabadi M²</p>	
4	<p>Author Contributions</p> <p>Mehdipour P designed the hypothetical and evolutionary based research, analytical strategy and contributed the data on the single cell based protein expression. Fathi N and Nosratabadi, M, and Mehdipour P contributed equally to this work and analyzed data of the brain</p>	

	channels. All authors have participated in editing process , have read, and approved the final manuscript.	
5	Supportive Foundations There was no supportive foundation	
6	Corresponding Author: Parvin Mehdipour Comments	
1	1. I suggest changing the title. In my opinion, in the present form it seems to be too wordy and not enough informative and appropriate. Also, as the reader may still be confused about what the acronyms relate to, I would suggest to not use abbreviations here.	Title has been, accordingly, changed. Regarding the Abbreviation, the comment is considered.
2	2. Abstract: In my opinion, Authors should consider rephrasing this section. According to the Journal's guidelines, the Abstract should contain most of the following kinds of information in brief form. Please, consider giving a synthetic (Fake, Artificial) overview of the paper's key points: I would suggest rephrasing the results and conclusion to make them clear for readers to understand. That said, I would like the authors to focus on proportionally presenting the background including the objectives, the short summary, and the conclusion without subheadings. The background should include the general background (one to two sentences), the specific background (two to three sentences), and current issue addressed to this study (one	The comments have been considered in the abstract

	<p>sentence), leading to the objectives. The short summary should close with one to two sentences which put the body of manuscript into a more general context. The conclusion should include one sentence describing the main message using such words like “Here we highlight”. The conclusion should write the potential and the advance this study has provided in the field and finally a broader perspective (two to three sentences) readily comprehensible to a scientist in any discipline.</p>	
3	<p>3. I would suggest adding a graphical abstract that will visually summarize the main findings of this article.</p>	Graphic abstract is included.
4	<p>Keywords: Please list as many keywords as allowed by the journal from Medical Subject Headings (MeSH) (https://meshb.nlm.nih.gov/) and use as many as possible in the title and in the first two sentences of the abstract. I would suggest adding ‘(5xP) GPT’ as a keyword.</p>	The comments have been considered in the related text.
5	<p>In general, I recommend the authors to include more evidence to back their claims, especially in the Introduction of the article, which I believe is currently lacking. Thus, I recommend the authors to focus on deepening the subject of their manuscript, as the bibliography (add more REFERENCES) is too concise: nonetheless, in my opinion, less than 50 articles for a research article are too low. Therefore, I suggest the authors to focus their efforts on researching relevant literature: I believe that adding more studies and reviews will help providing better and more accurate background to this study.</p>	<p>More references are included (total:46). The references at single cell level and based on high enumeration were unavailable.</p>

6	<p>Furthermore, I would like the authors to clarify the following points in the abstract and the body of manuscript</p> <p>: a) What is the significance of exploring circulating brain cells and electroencephalography in the management of AD?</p>	<p>1. a) : ETS2 has the heterogenic cellular behavior of CNCs in AD. Protein expression of ETS2/ATM/VEGF/EGF reflects the quadrat-angle-heterogeneity at genomics level in AD-patients. Such panel offers the chain of 5xP model, as the personalized-management, early detection and therapy.</p> <p>- Application of EEG, upon observation any sin of AZ disease in the target person, is required to be periodically, under the physician decision is helpful.By issuing the Periodic Brain ID card (<i>PB-ID</i>) at different stages of the predisposed individuals, the early detection, prognostic and early therapy would be possible.</p>
7	<p>b) How does the 5xP personalized model differ from other approaches to clinical management of AD?</p>	<p>2)planning for the multiple brain electroencephalography (EEG), as the Periodic Brain ID card (<i>PB-ID</i>) at different stages of the predisposed individuals, based on the pedigree information through different generations.</p>
8	<p>c) Are there any promising developments in the early detection and treatment of AD that are discussed in this file?</p>	<p>Yes, if the sequential managements are applied accordingly. This matter is discussed through the manuscript.</p>
9	<p>2. Introduction: I suggest the authors to reorganize the Introduction section, which seems <u>inhomogeneous and dispersive</u>, and specifically, <u>not enough informative</u> as an Introduction should be. I</p>	<p>-The valuable suggestions have been applied -through the Introduction.</p> <p>- The reference has been used as Ref 48.</p>

recommend that the authors **focus on presenting the following crucial elements of abstract including** the **introduction, methods, results, and conclusion**, with **several paragraphs consisting of up to 1000 words**, to **introduce the main constructs of this study**, which should **be understood to a reader in any discipline and make persuasive enough to put forward “the main purpose of current research the authors have conducted and the specific purpose the authors has intended by this study”**. I would like to encourage the authors to present the **introduction starting with the general background, proceeding to the specific background, and finally the current issue addressed to this study, leading to the objectives**. The Those **main structures should be organized in a logical and cohesive manner**. For this reason, I believe that a **general overview about incidence, prevalence and pathogenesis and biochemical hallmarks of AD**, for example

‘Dissecting Neurological and Neuropsychiatric Diseases:

	<p>Neurodegeneration and Neuroprotection (https://doi.org/10.3390/ijms23136991)', would be very useful.</p>	
10	<p>patients' memory and emotional behavior impairments: this information may provide a better understanding of prefrontal cortex's key role and how its disrupted function may contribute to irregular behavioral responses (doi:10.17219/acem/139572; DOI: 10.3390/biomedicines10122999).....etc.....</p>	<p>The brain channel-section have been revised by inserting the required information and the references in the manuscript.</p>
11	<p>3.Materials and methods: I recommend opening this section with a short introductory paragraph regarding the study design and methodology. Also, I suggest citing more references to ensure the reliability and the integrity of evidence in the study design the authors have built and the methodology the authors applied to this study.</p>	<p>The required text are inserted within the material and methods by the highlighted texts in yellow color.</p> <p>Regarding more citation: Unfortunately, Flow Cytometry is traditionally is performed.</p> <p>So, similar strategy was unavailable for citation. According to my experience, I have performed a research project , by comparing the results based on manual count with Flow cytometry ; and, in spite of high cell count, I found that the machine is unable to distinguish cellular heterogeneity, it provides positive or negative.</p> <p>But with manual analysis remarkable heterogeneity is detectable, including Low, medium and high-intensity.</p>

12	<p>4. Did the authors investigate relationships between the Spectral power ratio and cognitive functions in AD patients? In my opinion, that measure would have provided more information about how the specific ratio pattern could be specifically associated with cognitive dysfunctions in a domain- and diagnosis-specific manner of AD.</p>	<p>Thank you for providing an excellent point.</p> <p>It would be very useful, but it requires another project with high number of cases.</p>
13	<p>5. I would ask the authors to add a proper and defined ‘Results’ section, to adequately state statistical significance of findings. Thus, I believe that this section would benefit from a more detailed and precise rewriting, in order to ensure in-depth understanding of the findings.</p> <p>I recommend that the authors close the results section with a paragraph which put the results into a more general description.</p>	<p>Thank you for the valuable comment.</p> <p>As the number of patients are limited, and the achieved results based on the statistical analysis would be very descriptive.</p> <p>Furthermore, the achieved data is absolutely personalized, and even for 100 patients we could apply the achieved data for each individual. Unless we consider the spectrum for the data. Even within the same pedigree, we achieved heterogenic data.</p>
14	<p>6. Discussion: The authors need to present the independent discussion section with up to 1500 words and to focus on the following essential elements for discussion. Starting with an introductory paragraph, I would like the authors to present the summary of the previous</p>	<p>Thank you for the comments. By referring to our results, the major challenges are related to lack of previous data on single cell analysis. Besides, no images were available to discuss and compare our results with it. We tried our best to deliver the required messages, upon your valuable comments within the text.</p>

	<p>section and to develop discussion on the potential of this study complementing as the extension of the previous work, the implication of the findings of this study, **how this study could facilitate future research, the ultimate goal, the challenge, the knowledge and the technology necessary to achieve this goal, the statement about this field in general, and finally the importance of this line of research</p>	<p>Therefore, the results will include the information on the achieved data and the referral images.</p> <p>In discussion the highlighted points are delivered.</p> <p>**If the importance of single cell based analysis is appreciated, the provided strategy would lead to an early detection, within the frame of predictive, prognostic, and most importantly early therapy for te target relatives as well.</p>
15	<p>7. In my opinion, the ‘Article highlights’ section would benefit from some thoughtful as well as in-depth considerations by the authors, that should make their effort to explain the theoretical implication as well as the translational application of their research</p> <p>8. I think that a proper and defined ‘Conclusions’ paragraph would be useful to ultimately summarize key points of the article. In my opinion, this section would benefit from some thoughtful as well as in-depth considerations by the authors and try to explain the theoretical implication as well as the translational application of their research.</p>	<p>The achieved results of this research is characterized with the following items:</p> <ol style="list-style-type: none"> 1) Theoretically, Protein expression assay is the end point of the molecular event up to the end of functional assay; which have translational ability to the neurological clinics. 2)the results are trustable; 3) none-invasive process; 3) convincing costs; 3) is relatively a fast procedure; 4) with be applied for the AD-patients' relatives from childhood, even on the fetus by testing the maternal blood sample. Such strategy guarentee the real early detection for minding micro- and macro environmental hazards; and 5) Such strategy guarantee the real early detection for minding the micro- and macro environmental Hazards, and genetic predisposing factor.

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17	<p>9. In according to the previous comment, I would ask the authors to include a proper and defined ‘Limitations and future directions’ section before the end of the manuscript, in which authors can describe in detail and report all the technical issues brought to the surface.</p>	<p>Is included at the end of manuscript.</p>
18	<p>10. Regarding the Tables and Figures: According to the Journal’s guidelines, Authors should provide an <u>clarifying caption for each table within the text.</u> Overall, the manuscript contains ten figures, two tables and 25 references. I believe that this manuscript may carry important value in studying CBCs as biomarkers for early detection of AD. I hope that, after these careful revisions, this paper can meet the Journal’s high standards for publication. I am available for a new round of revision of this article. I declare no conflict of interest regarding this manuscript. Best regards, Reviewer</p>	<p>Has been provided through the text</p>
	<p>Dear respected reviewer , I do appreciate the time you have devoted to read and explore the lacking items, which is partially related to our busy research- and editorial schedule. I,</p>	

	<p>personally, owe to the translational science.</p> <p>With appreciation for devoting, you time to review our manuscript.</p> <p>Respectfully,</p> <p>Parvin Mehdipour</p>	

Respected Reviewer 2

1	<p>Authors are requested to send their revised manuscript to a professional English language editing company or a native English-speaking expert to polish the manuscript further. When the authors submit the subsequent polished manuscript to us, they must provide a new language certificate along with the manuscript.</p>	Will be provided.
	<p>5 ABBREVIATIONS</p> <p>In general, do not use non-standard abbreviations, unless they appear at least two times in the text preceding the first usage/definition. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC,</p>	The points are considered.

	RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, and mAb, do not need to be defined and can be used directly.	
	(1) Title: Abbreviations are not permitted. Please spell out any abbreviation in the title.	Is applied in the article.
	(2) Running title: Abbreviations are permitted. Also, please shorten the running title to no more than 6 words.	Is applied in the article.
	(3) Abstract: Abbreviations must be defined upon first appearance in the Abstract. Example 1: Hepatocellular carcinoma (HCC). Example 2: <i>Helicobacter pylori</i> (<i>H. pylori</i>).	Is applied in the article.
	(4) Key Words: Abbreviations must be defined upon first appearance in the Key Words.	Is applied in the article.
	(5) Core Tip: Abbreviations must be defined upon first appearance in the Core Tip. Example 1: Hepatocellular carcinoma (HCC). Example 2: <i>Helicobacter pylori</i> (<i>H. pylori</i>)	Is applied in the article.
	(6) Main Text: Abbreviations must be defined upon first appearance in the Main Text. Example 1: Hepatocellular carcinoma (HCC). Example 2: <i>Helicobacter pylori</i> (<i>H. pylori</i>)	Is applied in the article.

	<p>(7) Article Highlights: Abbreviations must be defined upon first appearance in the Article Highlights. Example 1: Hepatocellular carcinoma (HCC).</p>	<p>Is applied in the article.</p>
	<p>(8) Figures: <u>Abbreviations are not allowed in the Figure title</u>. For the <u>Figure Legend text</u>, abbreviations are allowed but must be defined upon first appearance in the text. Example 1: <u>A: Hepatocellular carcinoma (HCC) biopsy sample; B: HCC-adjacent tissue sample. For any abbreviation that appears in the Figure itself but is not included in the Figure Legend textual description, it will be defined (separated by semicolons) at the end of the figure legend. Example 2: BMI: Body mass index; US: Ultrasound.</u></p>	<p>If the entire name of gene and proteins appear in the figure title, the title will be too long.</p> <p>Thank you for your guide, I will apply it</p>
	<p>all abbreviations used in tables are defined (separated by semicolons) directly underneath the table. Example 1: BMI: Body mass index; US: Ultrasound</p>	<p>Sure, Is applied</p>
	<p>6 EDITORIAL OFFICE'S COMMENTS</p> <p>Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:</p>	<p>Manuscript has been revised three times. I hope that is convincing.</p>

	<p><i>(1) Science editor:</i></p> <p>The manuscript has been peer-reviewed, and it's ready for the first decision.</p>	
	<p><i>(2) Company editor-in-chief:</i></p> <p>I have reviewed the Peer-Review Report, full text of the manuscript, and the relevant ethics documents, all of which have met the basic publishing requirements of the World Journal of Experimental Medicine, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors. Please</p> <p>provide the original figure documents. Please prepare and arrange the figures using <u>PowerPoint</u> to ensure that all graphs or arrows or text portions can be reprocessed by the editor. In order to respect and protect the author's intellectual property rights and prevent others from misappropriating figures without the author's authorization or abusing figures without indicating the source, we will indicate the author's copyright for figures originally generated by the author, and if the author has used a figure published elsewhere or that is copyrighted, the author needs to be authorized by the previous publisher or the copyright holder and/or indicate the reference source and copyrights. Please check and confirm whether the figures are original (i.e.</p>	<p>The original figures and power point file will be provided.</p> <p>All the provided figures are original and are selected from my personal archive.</p>

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**If the picture is 'original', the author needs to add the following copyright information to the bottom right-hand side of the picture in PowerPoint (PPT):
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With respect and appreciation

Parvin Mehdipour