

## Answers to Reviewer 02950171

**The revisions have been highlighted in yellow.**

**Reviewer:** The authors review studies showing common (epi)genomic (with a focus on genomic) background for the strongly age-related diseases T2D and neurodegenerative diseases.

The reviewer finds the review rather speculative and without any strong, well-established common molecular factors to T2D and AD/PD. This points to that the focus of the review, which is indeed interesting, is at its infancy and that no strong relationships yet exists. If the authors agree that this is the case, this should be emphasized throughout the review.

**Authors response:** Thank you for your comment. As suggested, the genomic and molecular relationship between neurodegenerative diseases and T2D has been implemented throughout the manuscript. We better clarify that the shared genomic and epigenomic background still needs to be investigated and understood.

**Reviewer:** Title. Because the authors do not present any epigenetic data that are shared between T2D, AD and PD, I suggest that “(epi)” is removed from the title. Alternatively, the authors should add more data on epigenetic alterations that are shared between T2D and neurodegenerative diseases (if they exist), and introduce the focus on epigenetics in the abstract and introduction.

**Authors response:** Thank you for your comment, we modified the title according to your suggestion.

**Reviewer:** Abstract. Please specify/summarize the content of the review at the end of the abstract so that the reader gets an idea of where we are today: how well-established is the shared genetic background, that is not related to age per se, between T2D and neurodegenerative diseases?

**Authors response:** Thank you for your comment. As suggested, we modified the abstract.

**Reviewer:** Manuscript. Introduction, page 3: Brain insulin resistance is unknown to many. What is it, and how does it link T2D with AD/PD? Are insulin levels, the insulin receptors, the transport across the blood-brain barrier increased or decreased? Please explain the mechanisms behind it, and how it is related to neuroinflammation, which is mentioned frequently in this review.

**Authors response:** Thank you for your comment. We modified this section by expanding the description of this process, according to your suggestions, providing an additional reference (21). In particular, brain insulin levels, which are normally low in the brain compared to the systemic circulation, do not show differences between T2D and AD. However, the insulin receptors' levels decrease in the context of T2D, leading to the alteration of brain glucose

metabolism due to the insulin resistance. This mechanism may in turn perturbate the normal biological processes in the brain and contributing to neurodegeneration. To our knowledge, differences in the transport across the blood brain barrier need to be evaluated.

**Reviewer:** When introducing beta amyloid and Tau protein in the text, please explain the importance of these molecules for the development of AD, and include add a reference.

**Authors response:** Thank you for your suggestion, we explained their importance and provide an additional reference (28).

**Reviewer:** Page 4: Please remove the sentence “As previously mentioned, insulin resistance dramatically affects brain functions and neuronal activity.” This is neither well-explained in the review nor well-established in the scientific community.

**Authors response:** Thank you for your comment, we modified this sentence according to the literature data (7, 21).

**Reviewer:** What is the difference in 927 and 395 risk variants? Are these findings from the same study?

**Authors response:** The cited data are included in the same study (36). The 395 risk variants share the same risk allele between AD and T2D.

**Reviewer:** Please reformulate the sentence “These SNPs are involved in immunity/inflammation-related pathways, cell-cell communication and neuronal plasticity, and their dysregulation may lead to increase in the neuroinflammation typically occurring in T2D and AD.” SNPs can not be “dysregulated” and include reference(s) with information of how common neuroinflammation is in T2D and AD.

**Authors response:** Thank you for your comment. We modified the sentence according to your suggestion. Moreover, references on the brain inflammation occurring in AD and T2D are provided (7, 37, 38).

**Reviewer:** Page 5: Add a reference and specify the epigenetic modification of chromatin to the following sentence: “*KANSL1* has been found to be associated with AD, suggesting that the encoded protein, that is mainly involved in the epigenetic regulation of chromatin, may also take part in neuronal development.”

**Authors response:** According to your suggestion, we specified the molecular function of *KANSL1*, providing a reference (54).

**Reviewer:** “Therefore, the knowledge of shared genetic factors and gene expression profiles may help to further dissect the molecular network characterizing and linking T2D, AD and PD (Figure 1).” How does this sentence relate to the figure? What do you mean by “dissect the molecular network”?

**Authors response:** This sentence was intended to highlight the need to study the molecular networks that can connect the mentioned diseases, as shown in the figure. According to your comment, we explain this concept in the figure footnote. The analysis of the precise molecular interactions among AD, PD, T2D-associated genes, which altogether build the molecular networks underlying the pathogeneses represents the way to “dissect the molecular network”.

**Reviewer:** Epigenetics section, page 5-6: This section is very speculative and can be shortened. The scientific studies presented are either related to T2D or AD/PD. If there currently are no studies showing epigenetic data that is related to both T2D and AD or PD, this should be explained.

**Authors response:** Thank you for your comment. We agree that this section is speculative. We better clarify in the manuscript that the existence of a common epigenetic background needs to be evaluated and explored, because data concerning epigenetic factors which are shared between T2D and AD/PD are not present. Therefore, we agree that there are no studies concerning this topic to date. For this reason, we wanted to write a paragraph on the potential shared epigenetic background, since we believe that the discussion of insights into potential shared epigenetic contributors can be interesting.

**Reviewer:** Also, please reformulate the text about epigenetic modifications from “...epigenetic modifications, including DNA methylation and histones’ modification (by direct modulation at the transcription level), and noncoding (nc)RNAs (which mediate the gene expression at the post-transcriptional level)<sup>[55]</sup>.” to e.g. ““...epigenetic modifications, including DNA methylation and histone modifications (which might affect gene transcription), and noncoding (nc)RNAs (which might change gene expression at the post-transcriptional level)<sup>[55]</sup>.”

**Authors response:** We modified the sentence as you suggested.

**Reviewer:** Conclusion: The conclusion would improve by being less speculative and more humble in regards to what we know today and what is not yet known. Also, references should be added to all biological findings in this section.

**Authors response:** Thank you for your comment. We better clarify that the findings concerning shared genetic and epigenetic factors among the diseases are few and need more research. Moreover, we added the references for the biological findings also in this section.

**Reviewer:** Section 2: “On this subject, the enhancement of social and cognitive activities in the high-income countries”. Why only in “high-income countries”?

**Authors response:** A stable incidence of dementia has been reported in the high-income countries, compared to low- or middle-income ones for which the incidence is raised.

According to epidemiological studies (69, 70) this data could be due to the generally higher levels of education, social activities and wellness that characterizes high-income countries. Therefore, this environmental variable, that is not population- or ethnicity-specific, can positively influence the cognitive reserve against dementia. We wanted to stress the importance of education and cognitive activities, because it can be an useful measure to counteract dementia worldwide.

**Reviewer:** Section 3: What do you mean by “treat these conditions through a network medicine approach”? Please specify or reformulate.

**Authors response:** Thank you for your comment. We better specified this concept in the manuscript. The network medicine approach can refer to different diseases that show phenotypic similarities, such as T2D and neurodegenerative diseases. As a matter of fact, according to this approach, a multifactorial disease results from perturbed molecular networks, built by genes and proteins, but also by non-coding RNAs and metabolites, that altogether form a “disease module.” These disease-specific modules can overlap, thus triggering perturbations that can affect other disease modules. Thus, these perturbations may underlie pathological phenotypes which are shared by different diseases. Therefore, an integrated approach aimed to investigate the interactions among genomes, proteomes, metabolomes and environment can lead to the understanding of molecular perturbations, identifying novel pathogenic factors (candidate genes, epigenetic elements and environmental variabilities) that are related to the diseases and that can provide useful information for accurate diagnosis and treatment (73).

**Reviewer:** Figure. The figure is not very informative; it is not specified if the genes presented are based on SNP, epigenetic or transcriptional data and to what specific disease(s) they have been linked. Moreover, it is not indicated how the genes have been selected. Please add information to this figure or remove it from the review.

**Authors response:** The figure summarized genes which have been selected from the genetic studies discussed in the manuscript. We modified the figure footnote according to your suggestions and we decide not to include figure 1A. The figure 1B, now referred as figure 1, shows the known interactions among the selected genes, highlighting the need of their further investigation to identify the networks contributing to the shared pathogenesis.

**Reviewer:** Table. How have the genes included in Table 1 been selected? Please include information in e.g. the footnote of the table. Why are information on “Potential associated disease” not included for all SNPs? Potential association to T2D and PD and/or AD should be a criteria for all SNPs in the table? If possible, please include references to studies where these SNPs have been found associated with T2D, AD and PD, to the table.

**Authors response:** thank you for your comment. The genes reported in the Table 1 have been discussed in the section “Shared Genetic Make-Up and Functional Pathways Among T2D, AD and PD”. For each SNP, within these genes, the potential associated disease has

been added. We fixed the format of the table. Moreover, we highlighted the references for the associated SNPs in the table footnote (36, 39, 41–43, 49, 50).

#### **Answers to Reviewer 02904354**

##### **The revisions have been highlighted in green.**

The paper is clearly written. I recommend its potential publication in this journal. There are several comments.

**Reviewer:** The authors collected and described the evidence in this topic, but they seemed to be scattered. How to avoid the bias in reporting the evidence is important. So I suggest to do a systematic review of literature.

**Authors response:** Thank you for your comment. We reviewed the literature data on this topic and we were able to find more information concerning potential shared genetic factors, compared to shared epigenetic background. However, we agree that it is difficult to structurally describe the evidences because, as we now better state throughout the manuscript, overall the findings in both shared genomic and epigenomic factors are still exiguous and deserve to be further characterized. We aimed to report the most relevant evidence and to give insights concerning this topic.

**Reviewer:** The table is interesting. Give a reference for each biological function.

**Authors response:** Thank you for your suggestion. We include references for the biological functions in the table footnote (literature data [7, 22, 39, 49, 51–56] together with the interrogation of GeneCards.org).

**Reviewer:** From my side, the figure 1a is not clear. Lots of lines are difficult to be seen or understood.

**Authors response:** Thank you for this comment. We decided not to include figure 1A.