Author's response to reviews

Title: Allele-specific polymerase chain reaction for the detection of Alzheimer's disease-related single nucleotide polymorphisms.

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Author's response to reviews: see over
Response to Reviewers

Title of manuscript:
Allele-specific polymerase chain reaction for the detection of Alzheimer’s disease-related single nucleotide polymorphisms.

Dear Editor,
Thank you for considering our manuscript for publication in your journal. We have revised the manuscript according to the comments of the reviewers and are grateful for your kind invitation to resubmit the paper after making the appropriate revisions.

Response to Reviewer 1 (1634607708848732_comment):

The authors should provide the ethnicity of the population and compare the genotypes frequency of the selected SNPs to that of the same population from NCBI.
The ethnicity of the population (Malaysian Chinese 60%, Malaysian Indian 20%, Malaysian Malay 20%) was not presented in detail as the focus of the manuscript was on the development and validation of a cost effective technique to determine selected SNPs associated with late onset Alzheimer’s disease. The frequency of genotypes presented in table 2 has also been removed as suggested by reviewer 2 (see point 2 from reviewer 2).

Response to Reviewer 2 (3242187228511171_comment):

1) The inclusion of the most important AD risk factor, APOE, within the genes analyzed by this methodology will surely increase the interest and applicability of this manuscript.
The AS-PCR for APOE genotyping was successfully developed to differentiate the ε2/ε3/ε4 genotype by Wenham and colleagues in 1991. Donohoe and colleagues further improved this method in 1999. The last paragraph of the background section has been expanded slightly to clarify this (page 5 second line).
2) Table 2 describing the frequency of genotypes from 100 human DNA samples is not relevant, as the focused of the paper is different.
Agree. Table 2 removed.

3) Figure 3 depicting an example of direct DNA sequencing results is also not relevant for the manuscript.
Figure 3 was included to show how the result could be interpreted in order to differentiate between homozygous and heterozygous genotypes. However, we agree that removing it will not affect the clarity of the manuscript. Figure 3 removed.

4) Opposite, the different bands originated from each of the PCR allele specific protocols should be described in detail in the “results” section or included in a Table.
A new table was amended in the revised manuscript to describe the different bands originated from each AS-PCR protocol for every genotype (page 16).

5) The description of commonly used terms such as DNA, APOE, ApoE should be avoided.
Agree. Descriptions removed.

6) Please check Table 1 for inconsistencies. It seems that some primers are incorrectly underlined at the 3’-end. It would be also convenient to follow the same order to describe the primers for the different genes.
The inconsistencies in Table 1 have been corrected and the primers rearranged in the same order.

7) Figure 2 is difficult to follow. It would be advisable to check for clarity.
Figure 2 illustrates the interaction of each primer used in table 1 to perform the PCR. The arrows indicate the size of each amplicon and the direction of the primers during the amplification process, which started at 3’ of primers. All SNP points were labeled with “X” (white). The respective SNP IDs, and each DNA template were labelled with the
respective accession numbers. This illustration was created in high resolution for publication purposes (1825 x 2020 pixels). The figure was changed to a colored image and each item in this illustration has been labeled clearly and accurately to make paragraph 3 of page 9 of the manuscript easier to follow. We hope Figure 2 will be deemed acceptable.

8) Some paragraphs in the “results” section should be rewritten for improve readability.
   Sentences in the paragraph rephrased as suggested.

9) Quality of written English: Needs some language corrections before being published.
   The manuscript was rechecked for grammatical and typographical errors. Some sentences were rephrased.

Response to Reviewer 3 (1151648008571773_comment):

1) I think that this manuscript did not provide any improvement in the knowledge of Alzheimer’s disease. Indeed, several studies have highlighted that these genetic variants are significantly associated with AD but they did not have any clinical relevance as diagnostic tool.

The aim of this manuscript was to share a method of carrying out genetic polymorphism studies for genetic risk factors associated with AD without the need for expensive instrumentation and reagents. The focus of this study was not to identify new genetic risks for the disease. We do agree with the reviewer that at present, genetic studies do not play a diagnostic role in AD. However, recent recommendations from the National Institute on Aging and the Alzheimer’s Association workgroup on diagnostic guidelines for Alzheimer’s disease have incorporated biomarkers previously and still under research. It is not inconceivable that in the future, as understanding of this disease continues to
improve, that genetic markers may also be part of the diagnostic criteria (Jack et al., 2011).

2) Furthermore these methodologies are based on old techniques (allele-specific PCR). To date technological developments are continuously increasing and genetic analyses tend to analyze large portions of human genome. Therefore the proposed approaches are obsolete.

We agree with the reviewer that the method outlined in this manuscript is based on an old technique. However, we disagree that it is obsolete. Developing countries have the fastest growing elderly populations and therefore research into age related conditions such as AD is absolutely critical particularly as most of these countries have very different populations to those which have been extensively studied (Caucasian high income populations). Such countries may not have the resources or the expertise available to develop high tech genetic research. A cost effective, relatively straightforward method accessible to minimally equipped laboratories, as described in this manuscript, is therefore an important option.

3) Quality of written English: Not suitable for publication unless extensively edited
Reviewer 1 and reviewer 2 did not have any major issues with the quality of the written English. We have checked the manuscript again for typographical and grammatical mistakes. Some sentences have been changed to improve readability.

4) Statistical review: Yes, but I do not feel adequately qualified to assess the statistics
We would agree with reviewer 1 and reviewer 2 that a statistical review is not required as this manuscript deals with the development of a cost effective method for determining SNPs of \textit{BIN1}, \textit{CLU}, \textit{ABCA7}, \textit{CRI} and \textit{PICALM}. 
References:

