Inflammation and Immune System Activation in Aging: A Mathematical Approach

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Memory and learning declines are consequences of normal aging. Since those functions are associated with the hippocampus, I analyzed the global gene expression data from post-mortem hippocampal tissue of 25 old (age ≥ 60 yrs) and 15 young (age ≤ 45 yrs) cognitively intact human subjects. By employing a rigorous, multi-method bioinformatic approach, I identified 36 genes that were the most significant in terms of differential expression; and by employing mathematical modeling, I demonstrated that 7 of the 36 genes were able to discriminate between the old and young subjects with high accuracy. Remarkably, 90% of the known genes from those 36 most significant genes are associated with either inflammation or immune system activation. This suggests that chronic inflammation and immune system over-activity may underlie the aging process of the human brain, and that potential anti-inflammatory treatments targeting those genes may slow down this process and alleviate its symptoms.

In the absence of any neurodegenerative disease, the aging process of the human brain is inevitably and quintessentially characterized by memory and learning impairments. Unlike in the case of a neurodegenerative disease, normal aging has not been associated with neuronal loss. Rather, it has been observed that the impairments induced by normal aging are associated with synaptic remodeling, and that they are more likely to affect functions that are associated with the hippocampus, i.e. several areas of memory and learning.

In order to study the process of human normal aging, this study focuses on the most vulnerable target of that process, namely, the hippocampus. Given the long, gradual course of the normal aging process, I arbitrarily defined the boundaries of the two groups as follows: 1) Old subjects (O) with age ≥ 60 years and 2) Young subjects (Y) with age ≤ 45 years. This 15-yr age gap, I theorized, would accentuate the contrast between the two groups in connection with this otherwise continuous and overlapping process. I analyzed the global gene expression data from post-mortem hippocampal tissue (harvested from the body of the hippocampus at the level of the lateral geniculate nucleus) of 25 old and 15 young cognitively intact human subjects, posted at the Gene Expression Omnibus (GSE11882). Demographical information pertaining to all 40 subjects is shown in Supplementary Table 1.

Results

Having employed three different and independent methods of statistical significance, namely, ROC curve analysis, fold change, and P-value, I was able to identify 36 genes that were the most significant in terms of differential expression. Fig. 1b depicts the results of K-Means clustering analysis based on the expression of the top 36 most significant genes. All K-Means clustering analysis results (with respect to both the housekeeping genes and the 36 most significant genes) are shown in Supplementary Table 2. As can be seen in both Fig. 1b and Supplementary Table 2, there is a clear separation of the two groups. Fig. 2 depicts the heat map that resulted by plotting the expression of those 36 genes for all 40 subjects (15 young and 25 old). As can be seen by the relative intensities, all of the 36 most significant genes are over-expressed (red color) in the case of the old subjects as compared with the case of the young subjects (blue color). The direction of the differential expression of those 36 genes also appears in Table 1. Moreover, Fig. 3 provides a 3D representation of the differential expression of those 36 genes between the two groups in a surface-contour plot.

Mathematical modeling of aging. Given the aforementioned biovariability in connection with hippocampal gene expression, I wanted to explore whether, via mathematical modeling, I could generate a function that could
classify the 40 subjects with a high accuracy. Such a model would be valuable in future studies of global gene expression analysis of post-mortem hippocampal tissue investigating biological and chronological aging. To that end, I randomly selected approximately 70% of the subjects [11/15 young subjects and 18/25 old subjects] for the development of the function (hereinafter referred to as super variable), and I used the remaining subjects (4 young and 7 old) solely for the purpose of validating the super variable. Employing a general methodology that I have previously introduced, I was able to generate a super variable (function) that, based on the input of 7 genes from the 36 most significant genes, was able to identify/classify accurately all but one of the old subjects [subject # 33 [64 yrs (F)]] [sensitivity = (24/25) = 0.96] and all of the young subjects [specificity = (15/15) = 1.00]. Those overall results of the performance of the F1 super variable were obtained by combining the results from the development and the validation phases. According to the rank that appears in Table 1, the seven genes that provide the input to the F1 super variable are: C4A (C4B), ADORA3, MS4A7, BCL6, CD44, C3AR1, and HLA-DRB1. All of those seven genes are, in terms of biological function, either genes of inflammation or genes of immune system activation. Supplementary Fig. 1 shows the F1 super variable function in relation to its 7 input gene variables. Fig. 4 and Supplementary Table 3 show the overall results of the F1 super variable, i.e. the F1 scores of all 40 subjects used in this study, as well as their respective classification. Fig. 4 and Supplementary Table 3 were created by combining the results from the development phase (the F1 scores of all 29 subjects that were randomly selected and used exclusively for the development of the model) with the results from the validation phase (the F1 scores of all 11 subjects that were randomly selected and used exclusively for testing purposes). The results of the F1 super variable in the development phase are shown in Supplementary Fig. 2 and Supplementary Table 4, whereas the results in the validation phase are shown in Supplementary Fig. 3 and Supplementary Table 5.

It is interesting to note here that, assessing and comparing the performance of the F1 super variable (Supplementary Table 3) with that of the supervised K-Means clustering (Supplementary Table 2), one can see that the former yielded one misclassification as opposed to four yielded by the latter.

Finally, it should also be noted here that, owing to the constraints of this study, namely, the paucity of healthy, normal human brain tissue samples and respective available data, the F1 super variable needs to be further validated with a larger, independent cohort.

Biovariability of aging. It has long been observed empirically that aging is not a steady-state, uniformly continuous process; that it is characterized by a relatively wide biovariability; and that biological age may not necessarily coincide with chronological age. The results of my study corroborate those observations. Looking at the expression of the 36 most significant hippocampal genes of all 40 subjects [15 young (columns 1–15) and 25 old (columns 16–40)] in Fig. 2, one notices that four old subjects [# 22, 27, 33, and 35 [80 yrs (M), 83 yrs (M), 64 yrs (F), & 86 yrs (M), respectively]] displayed gene expression patterns that were distinctly closer to those of the young subjects than the patterns of the rest of the old subjects. Conversely, the same observation, albeit in the opposite direction, can be made for one of the young subjects [# 2 [45 yrs (F)]]. The results of K-Means clustering analysis supported numerically those observations (Supplementary Table 2). Moreover, the aforementioned observations about the biovariability of the aging process were also supported by the results of hierarchical clustering analysis performed on the F1 scores of all 40 subjects (Supplementary Fig. 4).
### Table 1: Top 36 most significantly differentially expressed genes

| Rank | Probe Set   | Gene Name         | Diff. Expr. (O) | ROC AUC | Fold Change | P Value   | Key Known Function/Process                                                                 |
|------|-------------|-------------------|----------------|---------|-------------|-----------|-------------------------------------------------------------------------------------------|
| 1    | 208451_s_at | C4A (C4B)         | 0.98400        | 1.22894 | 2.087E-10   |           | inflammatory response, complement activation, innate immune response, inflammatory response, positive regulation of leukocyte migration, histamine secretion by mast cell, signal transduction |
| 2    | 206171_at   | ADORA3            | 0.96800        | 1.22659 | 2.480E-09   |           | unique expression pattern among hematopoietic cells and nonlymphoid tissues, associated with mature cellular function in the monocytic lineage, and it may be a component of a receptor complex involved in signal transduction |
| 3    | 224358_s_at | MS4A7             | 0.96000        | 1.23646 | 7.397E-09   |           | regulation of inflammatory response, regulation of immune response, B cell differentiation, positive regulation of B cell proliferation, regulation of memory T cell differentiation, negative regulation of T-helper 2 cell differentiation, negative regulation of type 2 immune response |
| 4    | 215990_s_at | BCL6              | 0.96000        | 1.13544 | 3.401E-08   |           | inflammatory response, wound healing involved in inflammatory response, positive regulation of neutrophil apoptosis, macrophage fusion, neuron projection development |
| 5    | 228532_at   | C1orf162          | 0.95733        | 1.19777 | 1.617E-07   |           | protein coding, unknown function/process                                                    |
| 6    | 209443_at   | SERPINAS5         | 0.95733        | 1.10590 | 4.544E-08   |           | heparin binding, regulation of blood coagulation, serine protease inhibitor, glycosaminoglycan binding, platelet alpha granule, platelet dense tubular network |
| 7    | 213566_at   | RNASE6            | 0.94933        | 1.23400 | 7.506E-08   |           | RNA catabolic process, defense response, ribonuclease activity                               |
| 8    | 204489_s_at | CD44              | 0.94933        | 1.21095 | 1.042E-08   |           | inflammatory response, wound healing involved in inflammatory response, positive regulation of neutrophil apoptosis, macrophage fusion, neuron projection development |
| 9    | 232568_at   | MGC24103          | 0.94667        | 1.12816 | 4.835E-08   |           | unknown function/process                                                                  |
| 10   | 209906_at   | C3AR1             | 0.94667        | 1.12143 | 4.492E-07   |           | complement receptor mediated signaling pathway, inflammatory response, positive regulation of macrophage chemotaxis, positive regulation of neutrophil chemotaxis, elevation of cytosolic calcium ion concentration, signal transduction |
| 11   | 204912_at   | IL10RA            | 0.94667        | 1.11718 | 6.043E-07   |           | interleukin-10 receptor activity, response to lipopolysaccharide, signal transducer activity |
| 12   | 209612_s_at | ADH1B             | 0.94400        | 1.20914 | 2.449E-08   |           | metabolic process, ethanol oxidation, reactive oxygen species regulation, noradrenaline & adrenaline & serotonin degradation |
| 13   | 240578_at   | LOC100507531      | 0.94400        | 1.13432 | 1.558E-07   |           | regulated by peripheral blood monocytes, regulation of transcription, cell differentiation, hormone-mediated signaling pathway, oxidation reduction |
| 14   | 212689_s_at | KDM3A             | 0.94400        | 1.05485 | 3.431E-08   |           | complement activation, alternative pathway, negative regulation of interleukin-2 production, negative regulation of T cell proliferation, innate immune response |
| 15   | 204787_at   | VSIG4             | 0.94133        | 1.24063 | 2.204E-07   |           | antigen processing and presentation of peptide or polysaccharide antigen via MHC class II, immune response, signal transduction, MHC class II receptor activity |
| 16   | 208306_x_at | HLA-DRB1          | 0.94133        | 1.12707 | 3.108E-07   |           | protein self-association, SAM domain binding, negative regulation of transcription from RNA polymerase II promoter |
| 17   | 219666_at   | MS4A6A            | 0.93867        | 1.19913 | 4.561E-07   |           | unique expression pattern among hematopoietic cells and nonlymphoid tissues               |
| 18   | 218084_x_at | FXYD5             | 0.93600        | 1.08851 | 2.032E-07   |           | up-regulation of chemokine production, ion transport, actin binding, ion channel activity |
| 19   | 210184_at   | ITGAX             | 0.93600        | 1.05952 | 3.555E-07   |           | immune response, IL-8 signaling, integrin signaling, TREM1 signaling, leukocyte migration |
| 20   | 221698_s_at | CLEC7A            | 0.93333        | 1.17697 | 1.946E-07   |           | inflammatory response, T cell activation, innate immune response, positive regulation of phagocytosis, MHC protein binding |
| 21   | 1560477_a_at| SAMD11            | 0.93333        | 1.05869 | 2.586E-08   |           | protein self-association, SAM domain binding, negative regulation of transcription from RNA polymerase II promoter |
| 22   | 203561_at   | FCGR2A            | 0.93067        | 1.22043 | 2.682E-06   |           | IgG binding, receptor activity, protein binding                                           |
| 23   | 225353_s_at | C1QC              | 0.93067        | 1.20862 | 3.082E-07   |           | immune response, complement activation (classical pathway), negative regulation of granulocyte differentiation, innate immune response, negative regulation of macrophage differentiation |
| 24   | 229635_at   | UKNOWN GENE      | 0.92533        | 1.27241 | 5.179E-07   |           | unknown function/process                                                                  |
of the model (F1 super variable), and all of which are – in terms of function – inflammation or immune system activation genes (Table 1). This suggests that – to a large extent, and insofar as it pertains to the hippocampal area of the brain – the dual process of a chronic inflammation and the elicited chronic immune-system response and activity can differentiate between old and young brains with a high accuracy. This is further supported by the fact that the aforementioned seven genes employed by the F1 super variable, all of which are genes of inflammation or genes of immune system activation, can discriminate between old and young brains with almost a perfect accuracy [sensitivity = (24/25) = 0.96 and specificity = (15/15) = 1.00].

### Inflammation and immune system activation in aging

Remarkably, of the 30 known genes out of the 36 most significant genes, 27 were – in terms of function – either genes of inflammation or genes of immune system activation (Table 1). This suggests that – to a large extent, and insofar as it pertains to the hippocampal area of the brain – the dual process of a chronic inflammation and the elicited chronic immune-system response and activity can differentiate between old and young brains with a high accuracy. This is further supported by the fact that the aforementioned seven genes employed by the F1 super variable, all of which are genes of inflammation or genes of immune system activation, can discriminate between old and young brains with almost a perfect accuracy [sensitivity = (24/25) = 0.96 and specificity = (15/15) = 1.00].

### Discussion

The seven genes [C4A (C4B), ADORA3, MS4A7, BCL6, CD44, C3AR1, and HLA-DRB1], which are the constituent input variables of the model (F1 super variable), and all of which are – in terms of function – inflammation or immune system activation genes (Table 1), were all found to be over-expressed in the old subjects compared with the young subjects (Table 1). C4A (C4B) has been observed to be over-expressed in patients with Huntington disease. The final 36 most significantly differentially expressed genes between the old and the young subjects, ranked according to their ROC AUC value. The arrows indicate over-expression (↑) or under-expression (↓) of the old subjects (O) as compared with young subjects (Y).

![Figure 3](https://www.nature.com/scientificreports)
and Alzheimer disease, in mice with rheumatoid arthritis, etc. ADORA3 has been found to be over-expressed in the hippocampus of patients with Parkinson disease, in patients with astrocytomas, etc. MS4A7 has been observed to be over-expressed in mice with rheumatoid arthritis. BCL6 has been observed to be over-expressed in patients with Huntington disease, with ischemic stroke, with rheumatoid arthritis, with B-cell lymphoma, etc. CD44 has been found to be over-expressed in patients with systemic lupus erythematosus, with immune thrombocytopenia, with schwannomas, with Huntington disease, and numerous other diseases and conditions. Over-expression of C3AR1 has been observed in patients with severe acute respiratory syndrome, with asthma, etc., while over-expression of HLA-DRB1 has been observed in patients with multiple sclerosis, with rheumatoid arthritis, with Duchenne muscular dystrophy, etc.

Previous studies using animal models have observed associations between aging and inflammation in connection with the hippocampus, the neocortex, and the cerebellum. Using animal models or human subjects with early-stage neurodegenerative diseases, such as Alzheimer, other studies have observed a link between neuroinflammation and deficits in synaptic plasticity, especially long-term potentiation (LTP) in the hippocampus, which is associated with long-term memory consolidation. The fact that definitive causality cannot be established here notwithstanding – in other words, whether it is the normal aging process that induces inflammation/immune-system-overactivity, or whether the vice versa occurs, or whether another, hitherto unspecified, process engenders the normal aging process, which in turn induces inflammation/immune-system-overactivity, or whether that unspecified process engenders inflammation/immune-system-overactivity, which in turn induces the normal aging process – the results of my study support a direct causal link between the normal aging process and the process of inflammation/immune-system-overactivity. When considered collectively, therefore, the results of my study and all of the above observations from the other aforementioned studies point to a plausible theory on the normal aging process. At some point in time, chronic, low-level inflammation establishes itself and elicits a corresponding chronic immune response and activity. These two conjugate processes ultimately are responsible for a gradual loss of synaptic plasticity, particularly LTP in the hippocampus, accompanied with a minimal neuronal loss. It is this loss of synaptic plasticity – at least in the hippocampus part of the brain – that is associated with the phenotypical changes of normal aging.

The results of my study, in addition to providing evidence for this dual process of chronic, low-level neuroinflammation/immune-system-activation in connection with normal aging, suggest a means of a potential treatment. Regardless of the exact causal sequence of the events, administration of anti-inflammatory drugs/chemicals that can normalize the expression of the aforementioned 27 genes of inflammation/immune-system-activation may decelerate the onset of the aging process, as well as the aging process itself, and mitigate its symptoms by restoring synaptic plasticity throughout the hippocampus and possibly throughout the rest of the brain. Supplementary Table 6 lists all those 27 most significant genes as possible targets for the development of such an anti-inflammatory treatment, along with potential candidate drugs/chemicals that are known (via Ingenuity Pathway Analysis) to interact with those genes.

It is worth noting here that various anti-inflammatory drugs have been used in an effort to slow down the progression of neurodegenerative diseases, such as Alzheimer, with various degrees of success. The magnitude of the neuroinflammatory processes in the case of Alzheimer disease or other neurodegenerative diseases, however, cannot be compared to that of the neuroinflammation in the normal aging process; and by virtue of the same argument, the task of

Figure 4 Overall results of the F1 super variable (function). The F1 uses 7 of the 36 most significant genes as its input variables. Using the expression value of those 7 genes for a particular subject, the F1 yields the F1 score of that subject; and, based on the determined cut-off score of 53.450, the F1 classifies that subject as young if the F1 score is < 53.450 or as old if the F1 score is ≥ 53.450. As can be seen by the overall performance, the F1 classified correctly all subjects except one old one [sensitivity = (24/25) = 0.96 and specificity = (15/15) = 1.00]. The mean F1 score of the Y subjects was 45.028 (top of the blue bar) and their standard deviation (whiskers above or below the top of the blue bar) was 6.514. The significance level was set at α = 0.001 (two-tailed), and the probability of significance for the F1 was P = 4.18 × 10⁻¹² (independent t-Test with T-value = 9.927). The F1 is parametrically distributed with respect to both groups. The F1 scores of all 40 subjects are shown in Supplementary Table 3.
Incorporating the three aforementioned independent methods of statistical significance assessment, and in order to minimize the number of false negatives in the case of the third method, I set the overall significance criterion as follows: in order for any variable to be included in the final list of the most significant variables, it would have to meet the significance criteria of the first method (ROC AUC $\geq 0.920$) and those of at least one of the other two methods ($FC \geq 1.10$ (or $FC \leq 0.91$) and/or $P < 9.15 \times 10^{-7}$).

Mathematical modeling. Utilizing the final 36 most significant genes, I wanted to explore the possibility of developing – via mathematical modeling – a function that could identify as correctly as possible the age status (O or Y) of an unknown subject based on the expression of any combination of those 36 most significant genes. To that end, I randomly selected approximately 70% of the subjects [11/15 young (Y) and 18/25 old subjects (O)] that could be used only for the development phase of such function. In other words, a function could be developed only by the exclusive use of those 29 subjects. The remaining 11 subjects (4 young and 7 old ones) were designated unknown (test) subjects and were used solely for the purpose of validating any promising function generated in the development phase. This split into two fixed sets, whereby one is used only for training and the other for validation, represents the simplest implementation of K-fold cross validation.

A function was deemed promising in the development phase only if it exhibited a sensitivity $\geq 0.90$ and a specificity $\geq 0.90$ in connection with the 29 subjects of the development phase. Pertaining to the validation phase, and in connection with the 11 unknown subjects, a promising function would have to exhibit the same minimum classification accuracy (a sensitivity $\geq 0.90$ and a specificity $\geq 0.90$) in order to be accepted. I was able to generate one such function (F1 – henceforth also referred to as super variable) that fulfilled all of the aforementioned criteria. Supplementary Fig. 1 shows the equation of F1 as a function of 7 genes.

The cut-off score of the F1 was determined by taking into account the results of the following two analyses: 1) calculation of the optimal point on the ROC curve based on the 29 F1 scores of the 29 subjects used in the development phase (optimal point is defined as the point with the highest sensitivity and the lowest false positive rate (1-specificity)) and 2) calculation of the 99.99% confidence intervals for the mean F1 score of those 29 subjects (O and Y), along with their respective standard deviations. The 99.99% confidence intervals were calculated based on a bootstrap sample size of 100,000. Taking into account the aforementioned ROC optimal point, as well as the relative overlap of MO and MY ($[MO = LLO - SDO and MY = ULY + SDY]$) (LLO: the 99.99% confidence lower limit for the mean of the O group; SDO: standard deviation of the O group; ULY: the 99.99% confidence upper limit for the mean of the Y group; SDY: standard deviation of the Y group), the cut-off score of the F1 super variable was determined to be 53.450. If a subject’s F1 score is $< 53.450$, then that subject is classified as Y (young); otherwise, if the F1 score is $\geq 53.450$, then that subject is classified as O (old). It should be pointed out here that, based on the equation of the F1 (Supplementary Fig. 1), a given F1 score is just a numerical value and does not signify age or number of years.

In addition to the main validation method explained above, and in order to further assess the performance of the F1 super variable, I employed two other and different cross validation methods: 1) a 10-fold cross validation and 2) a leave-one-out cross validation. Both of those methods yielded a classification rate of 0.95 and a mean absolute error of 0.05 in connection with the 29 F1 super variable. The results of those methods, along with the confusion matrices generated by them, are shown in Supplementary Table 9. As can be seen in Supplementary Table 9, each one of those two and different validation methods resulted in a correct classification of all of the young subjects and in a correct classification of all but two of the old subjects.

Computer software. All analyses in this study were carried out with custom software written in MATLAB R2012b. All computer programs in connection with the model were also created using MATLAB R2012b.

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J.B.N. conceived, designed, and carried out all aspects of this study and wrote and edited the manuscript.

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