Alzheimer’s disease susceptibility genes in low-grade glioma

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Abstract

Background and Aim: Cognitive deficits in low-grade glioma are well documented. Patients with glioma who are carriers of the apolipoprotein E (APOE) e4 allele may have increased memory problems and executive dysfunction. While APOE is ranked the number one Alzheimer’s disease (AD) susceptibility gene, many other susceptibility genes have been identified in genome-wide association studies. This study aimed to analyze the expression of APOE and the next 23 ranked AD susceptibility genes in malignant gliomas to identify significantly co-occurrence genes. Materials and Methods: To identify the most important AD susceptibility genes, the AlzGene database (http://www.alzgene.org/) was consulted, which displays this information and regularly updates it. To analyze AD susceptibility genes in glioma, The Cancer Genome Atlas, a project begun in 2005, was used to catalog genetic mutations responsible for cancer, employing genome sequencing and bioinformatics. The cBioPortal for cancer genomics and the UCSC Xena browser were used to analyze the data in The Cancer Genome Atlas. Results: APOE and CD33 were the only significantly co-occurrence genes in 514 low-grade glioma tumor samples. APOE was altered in 1.8% of cases and CD33 in 3%. Heatmap indicates that the two genes tend to coexpress. The most common alteration was deep deletion. The cBioPortal and the Xena browser cannot distinguish or identify the alleles of APOE or CD33. Conclusions: AD patients with one or more APOE e4 alleles, having one or more copies of the CD33 risk allele (rs386544 C), are at increased risk of cognitive decline compared to APOE e4 carriers, no doubt reflected by the co-occurrence of APOE and CD33 alterations in the gliomas. Better understanding of the interaction of genes and cognition in glioma patients may lead to new opportunities to personalize cancer therapy.

Keywords: Alzheimer’s disease, apolipoprotein E, cBioPortal, CD33, co-occurrence, dementia, glioma, The Cancer Genome Atlas

INTRODUCTION

Cognitive deficits in low-grade glioma (LGG) are well documented.[1] In LGG, memory functioning and executive functioning are impaired, but might improve after surgery.[2,3] Cognitive function deteriorates over time, although some deterioration may be ascribed to antiepileptic drugs.[4] Polymorphisms in inflammation, DNA repair, and metabolic pathways affect cognition in patients with glioma, as well as other clinical outcomes.[5]

Patients with glioma who are carriers of the apolipoprotein E (APOE) e4 allele may have increased memory problems and executive dysfunction. Single-nuleotide polymorphisms in the APOE gene may also worsen cognition.[6] Patients with glioma often have progressive deterioration in attention and executive functions several years after treatment with radiation therapy and chemotherapy, possibly worsened by APOE e4.[7]

While APOE is ranked the number one Alzheimer’s disease (AD) susceptibility gene, many other susceptibility genes have been identified in genome-wide association studies.[8] We previously reported a significant positive correlation between AD mortality rate and malignant brain tumor mortality rate in 1999–2016 in individuals aged 65 years and older.[9] In the present study, we analyzed APOE and the next 23 ranked AD susceptibility genes in malignant gliomas and determined if the expression of these 24 genes within a glioma might be related to AD, as well as to tumor pathology.

MATERIALS AND METHODS

To identify the most important AD susceptibility genes, we consulted the AlzGene database (http://www.alzgene.org/), which displays this information and regularly updates it.[10]
The 695 genes in the database were identified in 1395 studies. As expected, APOE was number one [Table 1]. To analyze AD susceptibility genes in glioma, we used The Cancer Genome Atlas (TCGA). TCGA is a project, begun in 2005, to catalog genetic mutations responsible for cancer, employing genome sequencing and bioinformatics. We used cBioPortal for cancer genomics (https://www.cbioportal.org/) to analyze the data in TCGA. cBioPortal provides visualization, analysis, and download of large-scale cancer genomics datasets. cBioPortal can analyze APOE in gliomas, but cannot distinguish its three alleles: e2, e3, and e4. We used the Genomic Identification of Significant Targets in Cancer module in the cBioPortal to identify the regions of genome that are significantly amplified or deleted across a set of samples. cBioPortal also generated an Oncoprint and expression heatmap. Gene expression is quantitated as fragments per kilobase of transcript per million mapped reads upper quartile (fpkm-uq), which is a normalization method for analyzing RNA-seq data.

### Table 1: Highest ranking Alzheimer’s disease genes from the AlzGene database (http://www.alzgene.org/)

| Rank | Gene   |
|------|--------|
| 1    | APOE   |
| 2    | BIN1   |
| 3    | CLU    |
| 4    | ABCA7  |
| 5    | CR1    |
| 6    | PICALM |
| 7    | MS4A6A |
| 8    | CD33   |
| 9    | MS4A4E |
| 10   | CD2AP  |
| 11   | EPHA1  |
| 12   | HLA-DRB1 |
| 13   | PTK2B  |
| 14   | SORL1  |
| 15   | SLC24A4|
| 16   | RIN3   |
| 17   | DSG2   |
| 18   | INPP5D |
| 19   | MEF2C  |
| 20   | NMES   |
| 21   | ZCWPW1 |
| 22   | CELF1  |
| 23   | FERMT2 |
| 24   | CASS4  |

**Table 2: Apolipoprotein E (APOE) and CD33 alterations are significantly co-occurrence in 514 low-grade glioma tumor samples**

| A   | B   | Neither | A not B | B not A | Both | Log2 OR | P     | Q     |
|-----|-----|---------|---------|---------|------|---------|-------|-------|
| APOE| CD33|         | 490     | 2       | 8    | >3      | <0.001| <0.001|

P values were Bonferroni adjusted. Q value was derived from the Benjamini-Hochberg false discovery rate correction procedure for multiple comparisons. OR: Odds ratio

### Statistical analysis

Simple statistics were calculated to identify the patterns of mutual exclusivity or co-occurrence. For each pair of query genes (e.g., APOE and CD33), an odds ratio (OR) was calculated (equation 1) that indicates the likelihood that the events in the two genes are mutually exclusive or co-occurrence across the selected cases.

\[
OR = \frac{(A \times D)}{(B \times C)}
\]

Where, A = number of cases altered in both genes; B = number of cases altered in APOE but not CD33; C = number of cases altered in CD33 but not APOE; and D = number of cases altered in neither gene. Each pair was then assigned to one of the three categories, indicative of a tendency toward mutual exclusivity, of a tendency toward co-occurrence, or of no association. To determine whether the identified relationship is significant for a gene pair, Fisher’s exact test was performed. P values were Bonferroni adjusted. Q value was derived from the Benjamini–Hochberg false discovery rate correction procedure for multiple comparisons.

We used the UCSC Xena browser (https://xenabrowser.net) to separate LGG into prognostic groups. UCSC Xena allows users to explore functional genomic datasets for correlations between genomic and/or phenotypic variables.

### Results

#### Demographics (from cBioPortal)

The mean age of 514 patients was 43 ± 14 (mean ± standard deviation) years. Fifty-five percent of patients were male and 45% were female. Ninety-two percent of patients were Caucasian, 4% were African-American, 1% were Asian, and 3% were American Indian and other. Thirty-eight percent of tumors were classified as astrocytoma, 37% were oligodendroglioma, and 25% were oligoastrocytoma not otherwise specified. Forty-eight percent of tumors were Grade 2, 51% were Grade 3, and 1% were unclassified.

#### Co-occurrence and alterations

APOE and CD33 were the only significantly co-occurrence genes in 514 LGG tumor samples [Table 2]. APOE and CD33 mRNA expressions were highest in diploid tumors [Figure 1]. APOE and CD33 had two missense mutations in two tumor samples, one in each sample [Figure 2]. As was mentioned, the cBioPortal cannot distinguish or identify the alleles of APOE or CD33. Oncoprint and expression heatmap of APOE and CD33 are shown in Figure 3. APOE was altered in 1.8% of cases
and CD33 in 3%. Heatmap indicates that the two genes tend to coexpress. The most common alteration was deep deletion [Figure 4].

APOE and network of neighboring genes in LGG are shown in Figure 5. As indicated in Figure 5, serum albumin affects the APOE gene. Low serum albumin levels are associated with worse cognition.14

**Prognostic groups and methylation**

LGG can be separated into a poorer prognostic group, characterized by TP53 and ATRX mutations, and a better prognostic group, characterized by chromosome 1p/19q codeletions.15 The tumors with reduced copy numbers of APOE and CD33 fall into the better prognostic group. The methylation cg10129493 probe for chromosome 19q13.3 [column I, third interior column, Figure 6] shows increased CD33 transcript DNA methylation (lower vertical red stripe) of the better prognosis group.

**Discussion**

Cancer mortality and AD mortality increase with age, but some studies have shown an inverse relationship between the two diseases, that is, older individuals with cancer have a reduced
risk of AD and vice versa.\textsuperscript{[16]} However, other analyses suggest that AD and brain tumor might be positively correlated.\textsuperscript{[9]}

In one study, a comprehensive bioinformatics analysis on clinical microarray datasets of 1091 glioblastoma multiforme and 524 AD cohorts was performed. Significant genes and pathways were identified, in particular, extracellular signal-regulated kinase/mitogen-activated protein kinase signaling, upregulated in glioblastoma multiforme and angiopoietin signaling pathway, reciprocally upregulated in AD. Suppression of glioblastoma multiforme growth in an AD background was mediated by the extracellular signal-regulated kinase-AKT-p21-cell cycle pathway and antiangiogenesis pathway.\textsuperscript{[16]}

We previously reported a significant positive correlation between AD mortality rate and malignant brain tumor mortality rate in 1999–2016 in individuals aged 65 years and older in (a) 1101 US counties ($P < 0.001$) and (b) 50 US states ($P < 0.001$).\textsuperscript{[9]} Moreover, glial cells may play a role in the genesis of AD.\textsuperscript{[17]}

Desikan et al.\textsuperscript{[18]} have used APOE allele and other genotype information for genetic assessment of age-associated AD risk and development and validation of a polygenic hazard score. The polygenic hazard score combines the effects of more than two dozen genetic variants, most associated with only a small risk of AD. The polygenic score is better at predicting which cognitively normal older adults will go on to develop Alzheimer’s dementia than APOE e4 alone.

Cancer gene alterations usually do not occur at random. Alterations of certain cancer genes tend to co-occur, indicating that they may work in tandem to drive tumor formation and development.\textsuperscript{[10]} This may be the case with the co-occurring alterations in Table 2 of APOE and CD33.

Molecular relationships between AD and glioblastoma have already been established.\textsuperscript{[19]} AD patients with one or more APOE e4 alleles, having one or more copies of the CD33 risk allele (rs3865444 C), are at increased risk of cognitive decline compared with APOE e4 carriers, no doubt reflected by the co-occurrence of APOE and CD33 alterations in gliomas. AD pathology is also more severe in neuroimaging studies when both APOE e4 and the CD33 risk allele are involved.\textsuperscript{[20–22]}

Abnormal DNA methylation patterns have previously been demonstrated in AD.\textsuperscript{[23]} The methylation cg10129493 probe for chromosome 19q13.3 that shows CD33 differential DNA methylation [Figure 6] also has demonstrated differential DNA methylation in schizophrenia (https://bioinfo.uth.edu/SZGR/displayGenePage.do?geneid=27036).

It would be important to know which APOE allele was co-occurent with which CD33 alleles and whether co-occurrence in the tumor predicted increased risk of AD. This information could help in the identification of specific risk factors for glioma-related cognitive decline, which has at least two important implications for oncology care.

- The oncologist could better adjust cancer treatment for maximal effect while simultaneously minimizing risk of cognitive decline
- The oncologist might ameliorate cognitive problems in high-risk patients using modified radiation schedules or chemotherapy regimens. In addition, patients could be given medication to forestall impairment. At the same time, patients at low risk could receive intensified therapy for increased tumor control without additional toxicity.

However, therapy of LGG is still debated. Cognitive decline could be a delayed finding linked to radiotherapy, not a sign that drives correct management.
In summary, better understanding of the interaction of genes and cognition in glioma patients may lead to new opportunities to personalize cancer therapy.

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Conflicts of interest
There are no conflicts of interest.

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