Host Genome Variation is Associated with Neurocognitive Outcome in Survivors of Pediatric Medulloblastoma

Abstract

Host genome analysis is a promising source of predictive information for long-term morbidity in cancer survivors. However, studies on genetic predictors of long-term outcome, particularly neurocognitive function following chemoradiation in pediatric oncology, are limited. Here, we evaluated variation in host genome of long-term survivors of medulloblastoma and its association with neurocognitive outcome. Whole-genome sequencing was conducted on peripheral blood of long-term survivors of pediatric medulloblastoma who also completed neuropsychological testing. Cognitively impaired and less impaired survivors did not differ in exposure to chemoradiation therapy or age at treatment. Unsupervised consensus clustering yielded two distinct variant clusters that were significantly associated with neurocognitive outcome. Interestingly, 34 of the 36 significant variants were found in noncoding DNA regions with unknown regulatory function. A separate unsupervised cluster analysis of variants within DNA repair genes identified discrete variant groups that were not associated with neurocognitive outcome, suggesting that variations in genes corresponding to a single functional group may be insufficient to predict long-term outcome alone. These findings are supportive of the presence of a genetic diathesis for treatment-related neurocognitive morbidity in medulloblastoma that may be driven by variation in noncoding regulatory elements.

Introduction

Advances in the treatment of medulloblastoma, the most common central nervous system malignancy in children, have led to a substantial increase in survival [1,2]. Despite this improvement, survivors are at a high risk of long-term neurocognitive impairment, largely driven by core cognitive abilities of processing speed, working memory, and attention [3–6]. There is a considerable degree of heterogeneity in neurocognitive outcome that cannot be entirely explained by molecular tumor subtype, cranial radiation dose, or age at treatment. A growing body of evidence suggests the presence of genetic determinants that predispose some brain tumor survivors to experience marked cognitive impairment following treatment, whereas others experience only mild deficits [7].
Materials and Methods

Study Participants and Data Acquisition

The study was approved by the Institutional Review Board of the Georgia State University/Georgia Tech Joint Center for Advanced Brain Imaging, Emory University, and Children’s Healthcare of Atlanta (CHOA). Informed consent was obtained from all participants or their legal guardians where appropriate. The study took place at the Center for Advanced Brain Imaging in accordance with the relevant guidelines and regulations. All participants were long-term survivors of childhood medulloblastoma. Long-term survivorship was defined as being at least 5 years from completion of therapy and without any clinical evidence of residual or recurrent tumor. Individuals were excluded from the study if they had a history of moderate to severe traumatic brain injury, major psychiatric disorders, neurofibromatosis, cancer predisposition syndromes, or recurrent or progressive medulloblastoma. Clinical and demographic information was obtained by review of participants’ electronic medical records. All medulloblastoma survivors treated at CHOA who met inclusion criteria were invited to participate in the study. Molecular subgroup classification was performed on tumor samples by NanoString assay according to established methods [26,27]. Genomic sequencing data are available in the European Genome-Phenome Archive under accession number EGAD00001004115.

Neuropsychological Measures and Cognitive Impairment Classification

Eighteen participants completed a neuropsychological evaluation. The battery consisted of widely used clinical tests with well-developed norms. Given the large number of cognitive performance measures available, we employed a composite neuropsychological score based on key cognitive components of neurodevelopmental models of long-term outcomes of childhood brain tumors [4–6]. The composite score computed the average z scores for the following performance measures: oral processing speed (Oral Symbol Digit Modality Test) [28], working memory (Auditory Consonant Trigram) [29,30], attention span (Digit Span Forward subtest from Wechsler Memory Scale) [31], Verbal IQ (Wechsler Abbreviated Scale of Intelligence Vocabulary & Similarities) [32], Performance IQ (WASI Block Design & Matrix Reasoning) [32], and reading academic achievement (Letter Word Identification subtest of the Woodcock Johnson Tests of Achievement, Third Edition) [31,33]. Consistent with the literature, clinically significant cognitive impairment was defined as an average z score of −1.5 or lower [34].

Genome Sequencing and Bioinformatics analysis

Whole genome DNA sequencing was performed on blood samples from 22 pediatric medulloblastoma survivors on the Illumina HiSeq X platform as described in Johnston et al. (2017) [35]. Following sequencing, all base calling was performed using standard Illumina software to generate the final FastQC files for each sample. The quality of raw reads generated from Illumina sequencing was assessed using FastQC [36]. Reads were filtered and trimmed using the Trimmomatic tool [37]. BWA aligner was used to map post-quality filtered reads against the human reference genome (hg19) [38]. The alignment quality was evaluated using SAMtools [39] and Picard-Tools (http://picard.sourceforge.net). The mean target coverage was 30×, and 95% of the targeted bases have a coverage of 10× or greater. Potential PCR duplicates were removed with Picard-Tools. Somatic variants (SNV and Indel) were called using SAMTools [39] with Varscan2 [40] and annotated using ANNOVAR [41]. Variants with low-quality read depth (<6×) were excluded from the analysis. A variant proportion was estimated for each gene variant for each sample. Here, variant proportion is defined as the reads supporting
the variants divided by the total number of reads supporting the variant and the reference allele, hence ranging from 0 to 1. A value of 0 means no reads supporting the variant have been identified, a value of 0.5 means half of the reads support variant and half support reference allele, and a value of 1 means all reads are supporting the variant allele. Genomewide variants were subsetted into variants identified to be associated with disease as reported in the COSMIC v81 and ClinVar2017 databases using a 10× coverage threshold. Variants were also subsetted into those associated with DNA repair function as reported in the REPAIRtoire (http://repairtoire.genesilico.pl), Human DNA Repair Genes (http://sciencepark.mdanderson.org/labs/wood/DNA_Repair_Genes.html), and repairGenes (http://www.repairgenes.org/) databases.

**Statistical Analysis**

To test the association between gene variant profile and cognitive outcome, two sets of unsupervised hierarchical agglomerative clustering analyses were performed: first on variants within all disease-associated genes and then on variants within DNA repair genes alone. To test the association of the disease-associated gene variants with the cognitive outcome, disease-associated gene variants were identified using the cosmic 81 and ClinVar 2017 databases. Among them, the top most variable gene variants that had an interquartile range of greater than or equal to 99.5th percentile were identified and used for downstream analysis. Variant proportions (p) were transformed using log2((1+p)/(1-p)), and a two-sided t test between the impaired and less impaired samples was performed to identify clinically significant gene variants. Fold-change was calculated as the difference in the mean transformed proportions between the two sample groups. Statistically significant variants (false discovery rate, FDR < 0.05) were defined as core gene variants and used to generate the heatmap. Unsupervised (within the context of samples) hierarchical agglomerative heatmap clustering using the original variant proportions was carried out using euclidean distance and ward.D clustering. Heatmap clustering analysis was conducted using NOJAH [23].

For the DNA repair gene analysis, we first identified the topmost variable gene variants in DNA repair genes identified from the REPAIRtoire, Human DNA Repair Genes, and repairGenes databases using an interquartile range of greater than 99th percentile. We then identified sample clusters using ConsensusClusterPlus R package with 1-Pearson correlation distance, ward.D agglomerative hierarchical clustering, 80% item resampling, 80% gene resampling, and 1000 resamplings [42]. To define a set of core gene variants, the topmost variable gene variant proportions were first transformed into binary values: variants with gene variant proportion ≤ 0.5 were coded to 0, and those with variant proportion > 0.5 were coded to 1. Core gene variants were defined as those that were significantly (P value < .05; FDR < 0.326) associated with the core sample clusters based on a Fisher’s exact test. Unsupervised (in the context of the samples) hierarchical agglomerative clustering was performed on the original variant proportion values for the core gene variants and core samples using a 1-Pearson correlation distance and ward.D clustering [43,44].

In order to assess whether the frequency of the disease-associated variants in the study sample differed from the general (nonstudy) population, allele frequencies (AFs) were calculated in aggregate from three online human genomic variation databases: 1000 genomes project (phase 3), the NHLBI GO Exome Sequencing Project, and the Genome Aggregation Database (gnomAD) [45–47]. Variants were referenced by their dbSNP identification number [48]. For each of the 36 variants identified as significantly different in variant proportion between the cognitively impaired and less impaired individuals, between 2667 and 131,771 control individuals were sequenced. AFs from these databases are herein referenced as “expected.” Observed AFs were derived for all study participants who completed neuropsychological assessment and for the less impaired subgroup. A minimum of two and a maximum of three alleles were identified at each variant site. The expected AFs were compared to each of the observed AFs by the χ² Yates-corrected method, where numbers of alleles in the study population permitted. Statistical comparisons between observed and expected alleles were not performed for the impaired participants because of low sample size or for the variants where the expected or observed AF was very low.

**Results**

**Clinical Characteristics**

Genome sequencing was conducted on 22 long-term medulloblastoma survivors. Of these, 18 completed the neuropsychological evaluation (Table 1). The composite neurocognitive score was calculated by averaging the standardized scores from the six cognitive evaluations.

| Table 1. Clinical Characteristics by Cognitive Impairment Group⁴,†,‡,§ |
|-------------------------|--------|--------|--------|
|                         | Impaired | Less Impaired | p*       |
| N                       | 4       | 14      |        |
| Age at diagnosis, years, M (SD) | 6.3 (3.7) | 8.8 (3.9) | .26     |
| Latency, years, M (SD)    | 18.3 (10.6) | 12.3 (6.5) | .17     |
| Molecular subtype        |        |        |        |
| WNT                     | 1       | 1       | .81     |
| SHH                     | 0       | 4       | .65     |
| Group 3                 | 1       | 2       | 1.00    |
| Group 4                 | 2       | 7       | 1.00    |
| CSI dose, Gy, n          | 18      | 0       | 1       | 1.00    |
| 23.4                    | 2       | 10      | .81     |
| 35.36                   | 2       | 3       | .60     |
| Total PF dose, Gy, n     | 30.6    | 0       | 1       | 1.00    |
| 37.8                    | 1       | 0       | .44     |
| 54-56                   | 3       | 13      | .81     |
| Chemotherapy regimen, n  |        |        |        |
| Average risk            |        |        |        |
| CCG 99601               | 1       | 4       | 1.00    |
| ACNS 0331               | 1       | 3       | 1.00    |
| CCG 9892                | 0       | 1       | 1.00    |
| CHP 693                 | 0       | 1       | 1.00    |
| High risk               |        |        |        |
| CCG 99701               | 0       | 1       | 1.00    |
| CCG 99703               | 0       | 1       | 1.00    |
| ACNS 0332               | 0       | 1       | 1.00    |
| Unknown                 | 2       | 2       | .39     |
| Neurologic complications |        |        |        |
| Hydrocephalus           | 3       | 11      | 1.00    |
| Cerebellar atrophy      | 1       | 2       | 1.00    |
| Radiation necrosis      | 0       | 1       | 1.00    |
| Secondary tumor§        | 1       | 1       | .81     |
| Endocrine dysfunction, n|        |        |        |
| GHD                     | 4       | 10      | .65     |
| Hypopituitarism         | 4       | 10      | .65     |
| AI                      | 1       | 0       | .44     |
| HPG                     | 2       | 5       | 1.00    |

CSI, craniocerebral irradiation; PF, posterior fossa; GHD, growth hormone deficiency; AI, adrenal insufficiency; HPG, hypothalamic-pituitary-gonadal dysfunction (e.g., primary ovarian insufficiency, precocious puberty).  
Impaired defined as composite cognitive score of less than –1.5 z.

* P value by Student t test for continuous variables or Fisher’s exact test for categorical variables. 
† Latency between treatment completion and neuropsychological evaluation.  
§ Secondary tumor was meningioma in both cases.
measures. The composite scores ranged from -2.64 to 0.22 $z$ (mean = -0.91, SD 0.72). Four participants had scores less than -1.5 and were categorized as impaired (range = -2.64 to -1.54). The less impaired group ranged from an average $z$ score of 0.22 to -1.4.

The age range at time of medulloblastoma diagnosis was 2 to 16 years (mean 8.2, SD 3.9 years). One participant in each cognitive group was diagnosed and received radiation treatment before age 5 years. Molecular subgroup distribution ascertained by NanoString assay was 2 WNT, 4 SHH, 3 group 3, and 9 group 4, which is representative of the medulloblastoma population treated at CHOA. All participants were at least 5 years from completion of therapy at the time of study enrollment (mean time since treatment 13.7, SD 7.6 years). All participants were treated with surgery, chemotherapy, and radiation. Chemotherapy protocols included CCG 9961 ($n=5$), CCG 9892 ($n=1$), CCG 99703 ($n=1$), CCG 99701 ($n=1$), CHOP 693 ($n=1$), ACNS 0331 ($n=4$), ACNS 0332 ($n=1$), and unknown ($n=4$). All but two participants received a total posterior fossa radiation dose of between 54 and 56 Gy. Two participants (one in each cognitive group) received a reduced posterior fossa dose (30.6 and 37.8 Gy), one because of shunt infection and one for an unknown reason. Three participants had postoperative cerebellar mutism, two had radiation necrosis, and two had secondary meningiomas. Endocrine dysfunction was highly prevalent, with all but one participant diagnosed with an endocrine disorder. Growth hormone deficiency and hypothyroidism were most common, followed by hypothalamic-pituitary-gonadal dysfunction and adrenal insufficiency. Clinical characteristics were not significantly different between the cognitively impaired and less impaired groups.

**Figure 1.** Disease-associated gene variants unsupervised clusters and association with cognitive impairment. Heatmap derived from hierarchical clustering analysis of relative variant expression between impaired vs. less impaired survivors. Each column is a single participant, and each row is a single nucleotide variant. The variant proportion is represented by scale on the top left, where dark orange signifies a higher proportion relative to the reference and yellow signifies a lower proportion. In this heatmap, cognitively impaired survivors exhibit a higher proportion of gene variants in cluster 1 and a lower proportion of gene variants in cluster 2 relative to less-impaired survivors.
Variant Cluster Analysis of Disease-Associated Genes

A total of 1,172,762 disease-associated gene variants were identified using the cosmic 81 and Clinvar 2017 databases. Of these, 6540 topmost variable variants were identified, 36 of which were found to be significantly different in prevalence between the impaired and less impaired survivors using a FDR-adjusted P value of .05 (Figure 1). Among the significant variants, 2 were exonic and 34 were located in noncoding regions: 10 intronic, 1 untranslated region, 3, and 23 intergenic (Table 2).

Of additional interest was whether the variants included in the cluster analysis were found in or near genes that have been previously reported to be associated with neurocognitive function. Thirty-three genes of interest were identified post hoc from the literature, including genes associated with cognitive outcome in survivors of brain tumors (APOE4, BDNF, COMT, IRS1, ERCC4, ABCC1, IL16, PPPARD, NOS1, POLE, MSR1, SLC22, GSTT1, GSTM1, SOD2, and DTNBPI) [16–20,49], leukemia (MS, MTHFR, GSTP1, MAOA, NOS3, SLC22A1, HFE, TSER, and CBS) [50–52], and traumatic brain injury (GAD1, ADORA1, APOE, ACE, ANKK1, WWCI, DBH, and GRIN2A) [53]. Of these genes, nine contained variants that met filtering criteria for inclusion in the unsupervised hierarchical agglomerative cluster analysis (Supplemental Table 1).

None of the variants were significantly different in variant proportion between the impaired and less impaired groups, although variants near two genes (ACE and ERCC4) had fold-change values of less than −4.0 and FDR-adjusted P values < .10.

Variant Cluster Analysis of DNA Repair Genes

A total of 158,754 variants were identified within the 409 genes associated with DNA repair pathways. Within the 1583 topmost variable gene variants, 242 variants among 21 genes were identified as core gene variants. Additionally, 17 core samples were identified among the 22 patient samples. Unsupervised consensus clustering using the two identified patient groups with distinct variant profiles is depicted in the heatmap in Figure 2. Fisher’s exact tests (results not shown) were performed to assess whether there were differences between the patient groups in prevalence of endocrine disorders, sleep impairment, cerebellar mutism, radiation necrosis, or secondary tumors. Similarly, Student t tests (results not shown) were performed to assess differences in age at diagnosis, radiation dose, and cognitive function. The table below shows the frequency of mutated samples among cognitively impaired and less impaired survivors.

| SNP     | Gene/Flanking Gene | Allele | Region | Hom Impaired | Het Impaired | NC Impaired | Hom Less Impaired | Het Less Impaired | NC Less Impaired |
|---------|--------------------|--------|--------|--------------|--------------|-------------|------------------|------------------|-----------------|
| rs227368 MANBA | C/T | EX | 4 | 0 | 0 | 4 | 4 | 6 |
| rs3740199 ADAM12 | C/G | EX | 4 | 0 | 0 | 3 | 3 | 8 |
| rs1273918 LINC0121, NRSA2 | C/G | IG | 0 | 1 | 3 | 9 | 2 | 3 |
| rs1509038 LINC01492, LOC101928523 | C/T | IG | 0 | 1 | 3 | 9 | 2 | 3 |
| rs9487870 QKI, MEAT6 | T/C | IG | 0 | 1 | 3 | 10 | 1 | 3 |
| rs2662780 LINC01492, LOC101928523 | C/A | IG | 0 | 1 | 3 | 9 | 1 | 4 |
| rs364888 LINC01492, LOC101928523 | G/C | IG | 0 | 1 | 3 | 9 | 0 | 5 |
| rs372046 LINC01492, LOC101928523 | G/T | IG | 4 | 0 | 0 | 4 | 4 | 6 |
| rs378466 LINC01492, LOC101928523 | T/C | IG | 4 | 0 | 0 | 5 | 3 | 6 |
| rs418119 LINC01492, LOC101928523 | A/G | IG | 4 | 0 | 0 | 4 | 4 | 6 |
| rs13161948 FLT4, OR2Y1 | C/T | IG | 4 | 0 | 0 | 4 | 4 | 6 |
| rs2507304 ANKRD20A3, MIR4477A | A/C | IG | 4 | 0 | 0 | 6 | 2 | 6 |
| rs400549 LINC01492, LOC101928523 | G/A | IG | 4 | 0 | 0 | 3 | 5 | 6 |
| rs412741 LINC01492, LOC101928523 | A/G | IG | 4 | 0 | 0 | 3 | 5 | 6 |
| rs419472 LINC01492, LOC101928523 | A/T | IG | 4 | 0 | 0 | 4 | 4 | 6 |
| rs4585689 PODXL, LOC101928782 | G/T | IG | 4 | 0 | 0 | 4 | 3 | 7 |
| rs7861436 LOC103988605, FAM27C | T/C | IG | 4 | 0 | 0 | 3 | 4 | 7 |
| rs9273206 HLA-DQA1, HLA-DQB1 | T/C | IG | 4 | 0 | 0 | 3 | 4 | 7 |
| rs10148510 LOC101927620, MIR5580 | G/C | IG | 4 | 0 | 0 | 7 | 0 | 7 |
| rs10005153 CLNK, MIR572 | T/G | IG | 4 | 0 | 0 | 2 | 5 | 7 |
| rs10279849 PMS2P9, CCDC146 | A/C | IG | 4 | 0 | 0 | 4 | 3 | 7 |
| rs1326623 ARL4A, ETV1 | T/A | IG | 4 | 0 | 0 | 4 | 3 | 7 |
| rs2803191 LCA5, LCA5 | T/C | IG | 4 | 0 | 0 | 5 | 1 | 8 |
| rs659494 FAM35A, NUTM2A | T/A | IG | 4 | 0 | 0 | 4 | 2 | 8 |
| rs7938520 ALX4, CD82 | C/A | IG | 4 | 0 | 0 | 3 | 3 | 8 |
| rs1482089 ARHGAP24 | C/T | IN | 0 | 0 | 4 | 9 | 2 | 3 |
| rs2062100 ARHGAP24 | T/A | IN | 0 | 1 | 3 | 10 | 0 | 4 |
| rs2086429 NFIA | C/T | IN | 0 | 0 | 4 | 10 | 0 | 4 |
| rs370593786 CEFAP4 | C/A | IN | 0 | 1 | 3 | 9 | 0 | 5 |
| rs4693720 ARHGAP24 | C/T | IN | 4 | 0 | 0 | 5 | 2 | 7 |
| rs10748323 CLEC1I | C/T | IN | 4 | 0 | 0 | 4 | 3 | 7 |
| rs4908277 COL11A1 | C/T | IN | 4 | 0 | 0 | 2 | 5 | 7 |
| rs10422502 ZNF71 | A/C | IN | 4 | 0 | 0 | 4 | 3 | 7 |
| rs2326797 LAMA2 | A/G | IN | 4 | 0 | 0 | 4 | 2 | 8 |
| rs13806877 LINC00836 | G/A | IN | 4 | 0 | 0 | 3 | 3 | 8 |
| rs30886 PDE6A | C/T | UTR3 | 4 | 0 | 0 | 4 | 3 | 8 |

For each variant, homozygosity was defined as AF ≥ .70, heterozygosity as AF ≥ .20, and no call as AF = 0. Hom, homozygous; Het, heterozygous; NC, no call; IG, intergenic; IN, intronic; EX, exonic; UTR, untranslated region.
assessment scores. There were no significant differences between patient groups along any of these parameters.

**Allelic Frequency Analysis**

For each of the 36 disease-associated variants identified in the cluster analysis, observed AFs in the study sample were compared to expected AFs derived from the general population (Supplemental Table 2). Seven variants, all of which were in noncoding DNA, had significant differences between the expected and observed AF. Of these, four variants (rs2326797, rs13236623, rs138306877, and rs9347870) were more prevalent in the study sample than the general population, and three variants (rs7861436, rs659494, and rs9273206) were less prevalent. For rs7861436 and rs659494, this difference was driven by low AFs in the impaired participants, while for rs9273206, it was driven by low AF in the less impaired participants. Additionally, within the less impaired subgroup, rs364288 had a significantly lower AF and rs2507304 had a significantly higher AF compared to the general population.

**Discussion**

In the present study, we conducted whole-genome sequencing of host blood in a cohort of long-term medulloblastoma survivors to identify genomic variants associated with neurocognitive morbidity. We found that cognitively impaired survivors did not differ from less impaired survivors in terms of exposure to chemoradiation or age at diagnosis but did have differences in host genome profile. Unsupervised analysis of all genome-wide disease-associated variants demonstrated that the cognitive groups have distinct variant profiles. The survivors also segregated into a separate set of two groups with distinct DNA repair gene profiles by unsupervised consensus clustering. These DNA repair profiles were not associated with cognitive outcome, suggesting that variation in genes corresponding to a single functional group may be insufficient to predict long-term cognitive outcomes alone.

In recent years, efforts have been made to deescalate radiotherapy with a goal of reducing long-term impairment in medulloblastoma patients. However, deescalation may not be a viable option in most
cases. The most recent Children’s Oncology Group clinical study for average-risk medulloblastoma (ACNS 0331) attempted to reduce the craniospinal dose from 24 to 18 Gy but found that patients receiving the lower radiation dose had an unacceptable rate of tumor relapse and overall survival [54]. Findings from this phase III randomized trial indicate that late toxicity from craniospinal irradiation will continue to be a major clinical problem for the majority of future medulloblastoma survivors. As a result, identifying clinically predictive genetic profiles to provide individualized prognostic information is an important area of research.

Prior genetic studies in survivors of childhood CNS tumors have assessed the effect of specific candidate SNPs on neurocognitive outcome, identified a priori or by pathway-oriented methods [16,17]. In contrast, the approach taken by the present study is novel in three ways. First, it incorporated host whole-genome sequencing data, allowing for detection of clinically meaningful but rare variants in both coding (genic) and noncoding (intergenic and intronic) DNA regions. Second, it employed a hierarchical cluster analysis of variant proportion data, a technique that identifies coherent subpopulations within an immense amount of sequencing data and has previously been applied in other populations but not in cancer survivors [25,55]. Third, the allelic frequencies of the identified variants in the study sample were compared to those of the general population using an aggregate of three large, well-validated human genome sequencing databases. This allowed for an assessment of whether the identified variants are specific to individuals with medulloblastoma and are therefore more likely to be clinically meaningful.

Notably, 94% of the variants identified in the disease-associated analysis are located in the noncoding DNA regions. Noncoding DNA makes up 98.8% of the entire human genome and has been previously dismissed as “junk DNA” with no function [56]. However, more recent studies indicate that noncoding DNA is responsible for gene regulation and facilitating complex temporal and spatial gene expression through combinatorial interactions with other gene regulatory elements, with the major regions involved in gene regulation being the 5′ and 3′ untranslated regions and introns [57]. These potentially important regulatory DNA sequences would be missed in the absence of whole genome analysis.

The precise function of the noncoding DNA regions identified in this cluster analysis is not known [58]. However, allelic frequency analysis demonstrated several highly statistically significant differences between the study population and the general population, as well as specific differences between the less impaired and impaired participants. Taken together, these differences suggest that the identified variants may be involved both in tumor development in patients without a known cancer predisposition syndrome and in vulnerability to neurocognitive radioxicity. They also provide a key starting point for mechanistic studies employing combinatorial in silico and experimental methods to examine cause-and-effect relationships between noncoding DNA variation and patient outcomes.

This study has several strengths. First, the neurocognitive data were obtained by standardized performance measures rather than by self-report and examined using a composite neuropsychological score based on key components of empirically and theoretically derived neurodevelopmental models of long-term outcomes of childhood brain tumors [4–6]. Second, the participants all had medulloblastoma and had a distribution of molecular subgroups that is representative of the larger medulloblastoma population at our institution, thus reducing the likelihood of potential confounds that may occur when examining cognition in survivors with diverse tumor sites and characteristics. Participants in the cognitively impaired and less impaired groups were also found to be similar in terms of clinical features and comorbidities known to influence cognition, including age at diagnosis, radiation dose, and cerebellar mutism [59]. In sum, the homogeneity in tumor characteristics and even distribution of plausible confounds between cognitive groups made differences in long-term cognitive outcome more easily attributable to differences in host response to treatment rather than to differences in clinical presentation, course, or the treatment itself.

The current study must be considered within the context of a limited sample size and relatively small number of impaired survivors relative to less impaired survivors, which restricted the ability to detect statistically significant differences in variant proportion between the cognitively impaired and less impaired groups among genes previously identified to be involved in neurocognitive outcome or between DNA repair gene profiles. Variants within these genes could be identified as associated with cognitive impairment in a larger sample.

The current study contributes to the identification of genetic influences on outcome in medulloblastoma by employing complementary genome-wide and pathway-specific approaches coupled with the innovative technique of hierarchical clustering. The robust segregation of our cohort into genetically distinct clusters suggests that these methods represent a previously untapped avenue for identifying genetic risk factors for cognitive impairment many years following the complex chemoradiation treatment. Likewise, similar methods could be used to identify SNPs associated with resiliency or only mild cognitive difficulties following the same treatment. Future multisite studies using a longitudinal case-control genetic association method (genome-wide and candidate gene) with functional validation in independent cohorts are needed to confirm these findings and translate them to clinical practice. This study establishes an evidence base to justify such larger scale investigations and provides a blueprint for independent replication.

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**Appendix A. Supplementary data**

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tranon.2019.03.004.
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