Is *NF2* a Key Player of the Differentially Expressed Gene Between Spinal Cord Ependymoma and Intracranial Ependymoma?

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■ BACKGROUND: **Although intracranial and spinal ependymomas are histopathologically similar, the molecular landscape is heterogeneous. An urgent need exists to identify differences in the genomic profiles to tailor treatment strategies. In the present study, we delineated differential gene expression patterns between intracranial and spinal ependymomas.**

■ METHODS: **We searched the Gene Expression Omnibus database using the term "ependymoma" and analyzed the raw gene expression profiles of 292 ependymomas (31 spinal and 261 intracranial). The gene expression data were analyzed to find differentially expressed genes (DEGs) between 2 regions. The fold change (FC) and false discovery rate (FDR) were used to assess DEGs after gene integration ($|\log_2 FC|>2$; FDR $P < 0.01$). Enrichment and pathway analysis was also performed.**

■ RESULTS: **A total of 201 genes (105 upregulated and 96 downregulated) were significant DEGs in the data sets. The underexpression of *NF2* in spinal ependymomas was statistically significant (FDR $P = 7.91 \times 10^{-9}$). However, the FC of *NF2* did not exceed the cutoff value ($\log_2 FC$, -1.2). The top 5 ranked upregulated genes were *ARX*, *HOXC6*, *HOXA9*, *HOXA5*, and *HOXA3*, which indicated that spinal ependymomas frequently demonstrate overexpression of *HOX* family genes, which play fundamental roles in specifying anterior/posterior body patterning. Moreover, the gene ontology enrichment analysis specified "anterior/posterior pattern specification" and "neuron migration" in spinal and intracranial ependymomas, respectively.**

■ CONCLUSIONS: **The most substantial magnitude of DEGs in ependymoma might be *HOX* genes. However, whether the differential expression of these genes is the cause or consequence of the disease remains to be elucidated in a larger prospective study.**

INTRODUCTION

Ependymomas are glial tumors thought to arise from primitive ependymal or subependymal cells in the vicinity of the ventricles and remnants of the central spinal canal.[1-4] These tumors occur in both the brain and the spine in pediatric and adult populations.[5,6] Although ependymomas from different locations are histopathologically similar, their molecular landscape is quite heterogeneous. They show differences in DNA copy number alterations, messenger RNA expression profiles, genetic and epigenetic alterations, and diverse transcriptional programs.[5,7-11] Estimation of the clinical course is further complicated because ependymal tumors are heterogeneous with respect to morphology, the age at which the first clinical manifestation occurs, and the site-specific prognosis.[12,13] Ependymomas of the same histologic type often comprise clinically and molecularly distinct subgroups; however, the etiology of these

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- Differential expression
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- *HOX*
- Intracranial
- Microarray
- *NF2*
- Spinal

Abbreviations and Acronyms
DAVID: Database for Annotation, Visualization and Integration Discovery
DEGs: Differentially expressed genes
FC: Fold change
FDR: False discovery rate
GEO: Gene Expression Omnibus
GO: Gene ontology
GSEA: Gene set enrichment analysis
HOX: Homeobox

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subgroups is unknown.[8] Thus, an urgent need exists to identify differences in the genomic profiles of intracranial and spinal cord ependymomas to personalize the treatment strategies.[14,15]

Several previous genetic studies have investigated the occurrence of targeted gene aberrations; these genetic studies did not examine whole genomic variation but focused on targeted genes only. Recently, researchers have attempted to identify the underlying genomic reasons for differences between the various tumor types by conducting whole genomic sequencing and analysis. An increasing amount of gene expression profile data has become available, and the use of bioinformatics to analyze gene expression profile data has become a new research hotspot.[16] However, the results of whole genome expression studies are often not reproducible because of the large number of hypotheses tested and the relatively small sample size used. Compared with single-study analysis, gene expression meta-analysis can improve reproducibility by integrating data from multiple studies.[17]

The present study aimed to delineate differential gene expression patterns between histologically identical, but genomically distinct, intracranial and spinal cord ependymomas using bioinformatics methods to provide new insights on the pathogenesis of ependymoma.

METHODS

Gene Expression Profile Data

We searched the Gene Expression Omnibus (GEO) database (available at: http://www.ncbi.nlm.nih.gov/geo/) using the term "ependymoma" and found 43 data sets. We selected data sets containing both intracranial and spinal cord ependymomas in humans and using the same microarray chip set. Finally, 2 data sets, gene chips GSE64415 and GSE21687, which used the GPL570 Affymetrix Human Genome U133 Plus 2.0 chip (HG-U133_Plus_2; Affymetrix, Santa Clara, California), were selected. Expression data from these data sets were downloaded from GEO. For differential expression analysis, raw CEL files were downloaded and combined into a single meta-data set. This platform consisted of 292 samples, of which 261 samples were from intracranial ependymomas and 31 were from spinal cord ependymomas.

Raw Data Preprocessing and Screening and Integration of Differentially Expressed Genes

In combining multiple data sets of whole genome microarray analysis, normalization should help remove any systematic biases, such as a batch effect, and ensure that any comparisons between chips provide meaningful results.[18] The robust multi-array average method, a classic and powerful technique for preprocessing Affymetrix gene expression arrays, was used for quality control, standardization, and $\log_2$ conversion of the raw gene chip data (**Supplementary Figure 1**).[19] The empirical Bayes method[20] and multiple testing correction (Benjamini-Hochberg method)[21] were used for differentially expressed gene (DEG) analysis. The observed fold change (FC) in expression was used to assess the magnitude of the difference.[22] DEG analysis of microarray data was performed with R software (R Foundation for Statistical Computing, Vienna, Austria) and limma package[23] using a transcriptome analysis console (version 4.0). We screened the raw data of the 2 gene chips for DEGs with a threshold of $|\log_2 FC| > 2.0$ and a false discovery rate (FDR) $P < 0.01$.

Functional Enrichment Analysis of DEGs

Gene ontology (GO) enrichment analysis was performed out to identify biological processes and pathways that were differentially expressed. The Database for Annotation Visualization and Integration Discovery (DAVID) network software (National Institutes of Health, Bethesda, Maryland) contains nearly all major public bioinformatics resources and is designed to facilitate high-throughput gene functional analysis. DAVID enriches the biological information of individual genes and can be used to annotate gene-related biological mechanisms using standardized gene terminology. DAVID and the Cytoscape software (San Diego, California) "Bingo" plug-in unit were used for GO enrichment analysis.[24]

The Kyoto Encyclopedia of Genes and Genomes pathway database, a relatively common and comprehensive database that contains a variety of biochemical pathways, was used for further enrichment analysis of the DEGs identified by the microarray data analysis.[25] Kyoto Encyclopedia of Genes and Genomes pathway analysis and functional annotation of DEGs were also performed using the DAVID tool.

Gene Set Enrichment Analysis

Gene set enrichment analysis (GSEA) calculates an enrichment score reflecting the overrepresentation of a certain gene set at the top or bottom of a ranked list of genes from the expression data set of 2 classes.[26,27] This method applies the Kolmogorov-Smirnov test to identify deviations between 2 distributions. In brief, genes are ranked according to their signal-to-noise ratio. Using Kolmogorov-Smirnov statistics, predefined sets of genes are scored, and significance is tested by empirical permutation, followed by correction for multiple hypotheses.[27,28] The significance of the deregulation of the gene sets selected from the Molecular Signature Database (available at http://www.broadinstitute.org/gsea/msigdb/index.jsp) was measured using the GSEA tool with the GO biological process gene set. The GSEA software parameters were set to their default values. The statistical significance of the normalized enrichment score associated with each gene set was assessed through 1000 random permutations of the phenotypic labels. An FDR of <0.05 was used as the cutoff value for assessing the statistical significance of the estimates.

RESULTS

Screening of DEGs

We screened the normalized expression data from 2 independent studies using the same chip set for DEGs between intracranial and spinal cord ependymomas. A total of 201 DEGs were identified from the 2 gene data sets; 105 genes were significantly upregulated and 96 genes were significantly downregulated after gene integration ($|\log_2 FC| > 2$; FDR $P < 0.01$; **Figure 1**; **Supplementary Table 1**). Spinal cord ependymomas showed 105 overexpressed genes, and intracranial ependymomas showed 96 overexpressed genes ($P < 0.01$). The top 5 ranked upregulated (highly expressed in spinal ependymoma) genes were *ARX*, *HOXC6*, *HOXA9*, *HOXA5*, and *HOXA3*, and the top 5 downregulated genes

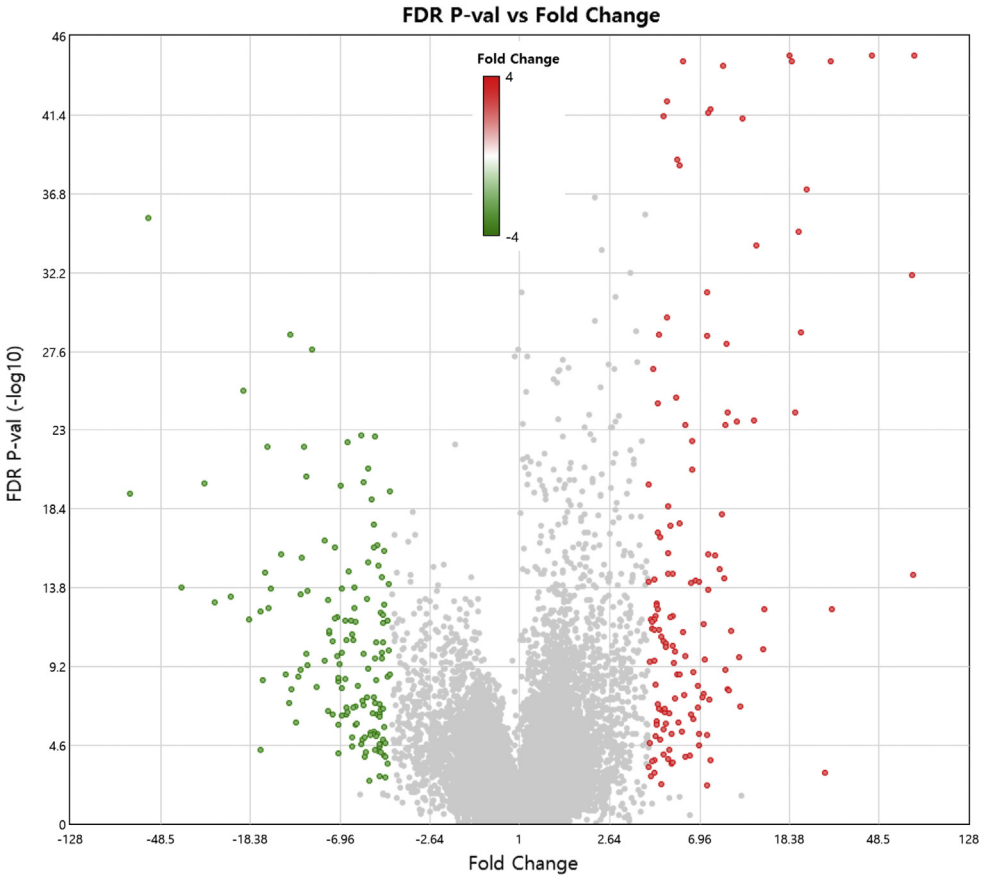

Figure 1. Volcano plot of differentially expressed genes. *Red dots* indicate spinal cord ependymoma-specific genes exhibiting a ≥2-fold change in expression. *Green dots* indicate highly expressed genes predominantly in intracranial ependymomas. FDR, false discovery rate; P-val, *P* value.

(highly expressed in intracranial ependymoma) were *ZIC2*, *IGF2*, *DACHI*, *ZIC1*, and *LPAR3* (Figure 2). Spinal ependymomas expressed multiple homeobox (*HOX*) family members. *HOX* genes are known to regulate the expression of targeted genes and direct the formation of many body structures during early embryonic development.[29] *HOX* genes might play a role in the maintenance of the cancer stem cell phenotype in spinal ependymomas because *HOX* family members, such as *HOXC6*, *HOXA9*, *HOXA5*, and *HOXA3*, are predominantly overexpressed in spinal ependymomas.[8]

Verification of Previously Reported DEGs

Several studies have reported genetic and genomic variations between intracranial and spinal cord ependymomas.[2,30] A meta-analysis of genetic studies identified 9 possible genes showing a different frequency of genetic aberrations.[30] The expression of these 9 genes is detailed in Table 1. *NF2*, *NEFL*, *HOXB13*, *PDGFRA*, and *EGFR* showed expression $|\log_2 FC| \geq 1$ and an FDR $P < 0.01$. Of these 5 genes, *NEFL* showed very a high FC ($|\log_2 FC| = 19.57$) between intracranial and spinal cord ependymomas.

Biological Pathways Closely Associated with Spinal Cord and Intracranial Ependymomas

After identification of the DEGs, the online tool DAVID was used to perform GO enrichment analysis of the 105 upregulated genes and 96 downregulated genes in the spinal cord and intracranial ependymomas, respectively. The DEGs were mainly enriched for "anterior/posterior pattern specification," "embryonic skeletal system morphogenesis," "neuronal action potential," and "axon guidance" in spinal cord ependymomas and for "neuron migration," "regulation of ion transmembrane transport," "Wnt signaling pathway," and "central nervous system development" in intracranial ependymomas (Table 2). The key Kyoto Encyclopedia of Genes and Genomes pathways were "ECM-receptor interaction" in spinal cord ependymomas and "dopaminergic synapses" and "retrograde endocannabinoid signaling" in intracranial ependymomas (Table 3).

Gene Set Enrichment Analysis

The list of DEGs was analyzed using the GSEA tool. The whole genome (54,613 probes) expression values were uploaded to the software and compared with catalog C5 GO gene sets in the Molecular Signature Database, which contains 670 GO biological

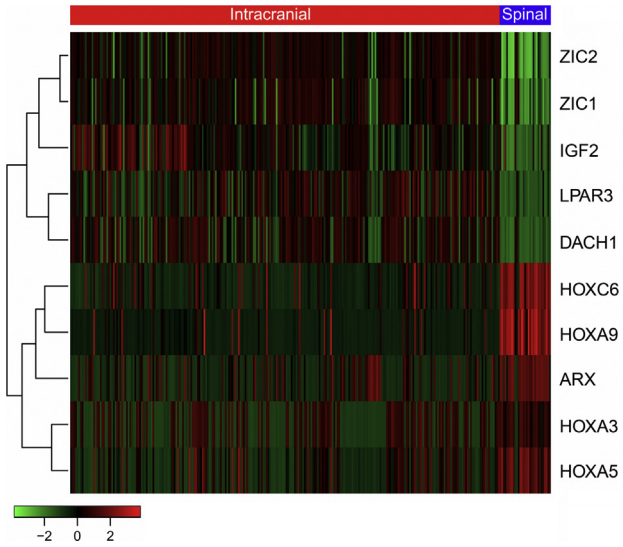

Figure 2. Heat map of the top 5 features for each phenotype and a plot showing the distribution of expression between the ranked genes and the phenotypes. Expression values in the heatmap are represented as colors, with the z-score range of colors (*green, black, red*) showing the degree of relative expression. *Red* represents high-expression genes, and *green* represents low-expression genes (the scale is provided on the left). The heatmap color bar represents the location of ependymomas. *Red* and *blue bars* represent the intracranial and spinal cord, respectively.

process gene sets. Of the 670 GO biological process gene sets, 320 and 350 gene sets were enriched in spinal cord ependymomas and intracranial ependymomas, respectively. None of the gene sets were significantly enriched (FDR <25% or nominal $P < 0.01$) in either intracranial or spinal cord ependymomas.

DISCUSSION

Previous studies examined only several putative target genes without screening of whole genomic variations.[30-32] Recently, investigators have identified critical genomic aberrations among the overall genomic variations. The present study synthesized and analyzed the whole exome expression data of each of the 292 individuals from 2 public data sets to identify DEGs in intracranial versus spinal cord ependymomas through differences in expression and enrichment analysis. Four of the top 5 upregulated DEGs were *HOX* family genes, which play fundamental roles in specifying anterior/posterior body patterning and development of the spine. GO enrichment analysis also specified anterior/posterior pattern specification in spinal cord ependymomas.

The NF2 gene mutation is regarded as a representative genetic aberration in spinal cord ependymomas and has been reported to be present in 30%–71% of sporadic (nonsyndromal) ependymomas.[2] In addition, loss of heterozygosity on chromosome 22 (the chromosome in which NF2 is located) and loss of merlin (the protein encoded by NF2) were frequently observed in spinal cord ependymomas compared with intracranial ependymomas.[2] The NF2 mutation might show a significant difference between spinal and intracranial ependymoma among a set of a few genes preselected as putative targets. However, our study demonstrated that several hundred genes displayed significant differential expression that was greater than NF2 between intracranial and spinal ependymomas. Although NF2 showed significant differential expression ($\log_2$FC, −1.2; FDR $P = 7.91 \times 10^{-9}$), the $|\log_2 FC|$ of NF2 did not exceed the cutoff value of 2.

Another DEG study also demonstrated that the NF2 gene was 1266th in order of magnitude of differential expression in the list of DEGs.[33] The differential expression of NF2 might not be the most substantial among numerous DEGs, and the clinical difference between spinal cord and intracranial ependymomas might not result from NF2 mutation and differential expression. Comparison with a well-curated normal sample could help analyze and interpret the DEG patterns. However, direct comparisons are practically impossible, although the expression data from healthy tissues from the Genotype-Tissue Expression resources can be used.[34] The Genotype-Tissue Expression is a comprehensive atlas and open database of gene expression and gene regulation across human tissues that provides a "normal"

Table 1. Previously Proposed Genes Showing Different Aberration Frequencies Between Intracranial and Spinal Cord Ependymoma

Gene Symbol	Spinal Average ($\log_2$)	Intracranial Average ($\log_2$)	Spinal SD	Intracranial SD	FC* (Linear)	*P* Value	FDR *P* Value
NF2	7.54	7.8	0.45	0.28	−1.20	1.97×10^{-9}	7.91×10^{-9}
NEFL	7.16	2.87	4.06	1.21	19.57	9.46×10^{-28}	9.57×10^{-25}
HOXB13	4.11	4.13	2.42	0.33	−1.01	1.22×10^{-31}	1.85×10^{-28}
PDGFRA	4.49	4.47	0.92	0.5	1.01	2.39×10^{-5}	0.0003
RASSF1	6.38	6.61	0.28	0.47	−1.17	0.0331	0.0982
RB1	5.08	4.88	0.39	0.39	1.14	0.0108	0.0418
SMARCB1	5.65	5.74	0.26	0.26	−1.06	0.023	0.0744
CDKN2A	6.45	6.17	0.69	0.88	1.21	0.5701	0.718
EGFR	8.06	8.54	1.11	1.05	−1.39	0.001	0.0063

SD, standard deviation; FC, fold change; FDR, false discovery rate.
*Calculated using the formula of $\log_2$FC.

Table 2. Gene Ontology Enrichment Analysis

Term	Count	*P* Value	Fold Enrichment
Upregulated genes (spinal cord ependymoma predominant)			
GO:0009952~anterior/posterior pattern specification	12	1.1×10^{-13}	31.10
GO:0048704~embryonic skeletal system morphogenesis	7	2.8×10^{-8}	37.21
GO:0019228~neuronal action potential	4	3.1×10^{-4}	29.62
GO:0007275~multicellular organism development	10	8.2×10^{-4}	3.98
GO:0030326~embryonic limb morphogenesis	4	9.1×10^{-4}	20.73
GO:0071320~cellular response to cyclic adenosine monophosphate	4	1.9×10^{-3}	15.95
GO:0008344~adult locomotor behavior	4	1.9×10^{-3}	15.95
GO:0000122~negative regulation of transcription from RNA polymerase II promoter	11	2.2×10^{-3}	3.17
GO:0009954~proximal/distal pattern formation	3	5.8×10^{-3}	25.91
GO:0030878~thyroid gland development	3	6.3×10^{-3}	24.88
GO:0007411~axon guidance	5	7.0×10^{-3}	6.52
GO:0008584~male gonad development	4	0.010	8.82
Downregulated genes (intracranial ependymoma predominant)			
GO:0001764~neuron migration	7	1.7×10^{-5}	12.72
GO:0034765~regulation of ion transmembrane transport	6	2.8×10^{-4}	10.31
GO:0007628~adult walking behavior	4	5.4×10^{-4}	24.62
GO:0001501~skeletal system development	6	7.3×10^{-4}	8.36
GO:0006813~potassium ion transport	5	8.6×10^{-4}	11.64
GO:0034220~ion transmembrane transport	6	4.7×10^{-3}	5.45
GO:0010107~potassium ion import	3	9.2×10^{-3}	20.44
GO:0016055~Wnt signaling pathway	5	0.016	5.10
GO:0070588~calcium ion transmembrane transport	4	0.024	6.41
GO:0007417~central nervous system development	4	0.024	6.36
GO:0007155~cell adhesion	7	0.032	2.91
GO:0045665~negative regulation of neuron differentiation	3	0.034	10.22
GO:1903779~regulation of cardiac conduction	3	0.034	10.22
GO:0006810~transport	6	0.035	3.29
GO:0007268~chemical synaptic transmission	5	0.036	3.98
GO:0007416~synapse assembly	3	0.040	9.38
GO:0051965~positive regulation of synapse assembly	3	0.041	9.23

Enrichment *P* values for gene ontology terms with biological pathways were calculated using the hypergeometric test; GO terms with nominal $P < 0.05$ were selected.

reference data set against which to compare tumor-based expression and regulation data. The expression of NF2 in the Genotype-Tissue Expression data was greater in the brain than in the spinal cord (Figure 3). Thus, the difference in the expression of

Table 3. Enrichment of Kyoto Encyclopedia of Genes and Genomes Pathways Among Significantly Upregulated Genes

Term	Count	*P* Value	Fold Enrichment
Upregulated genes (spinal cord ependymoma predominant)			
hsa04512: ECM-receptor interaction	3	0.064	7.01
Downregulated genes (intracranial ependymoma predominant)			
hsa04728: Dopaminergic synapse	6	2.3×10^{-4}	10.12
hsa04723: Retrograde endocannabinoid signaling	4	0.010	8.55
hsa05033: Nicotine addiction	3	0.014	16.20
hsa04261: Adrenergic signaling in cardiomyocytes	4	0.027	5.92
hsa04971: Gastric acid secretion	3	0.042	8.87
hsa04020: Calcium signaling pathway	4	0.045	4.83
hsa04024: Cyclic adenosine monophosphate signaling pathway	4	0.058	4.36
hsa05032: Morphine addiction	3	0.062	7.12
hsa04713: Circadian entrainment	3	0.067	6.82
hsa04725: Cholinergic synapse	3	0.088	5.84
hsa04724: Glutamatergic synapse	3	0.092	5.68

Enrichment *P* values for Kyoto Encyclopedia of Genes and Genomes pathways were calculated using the hypergeometric test; pathways with nominal $P < 0.1$ were selected.

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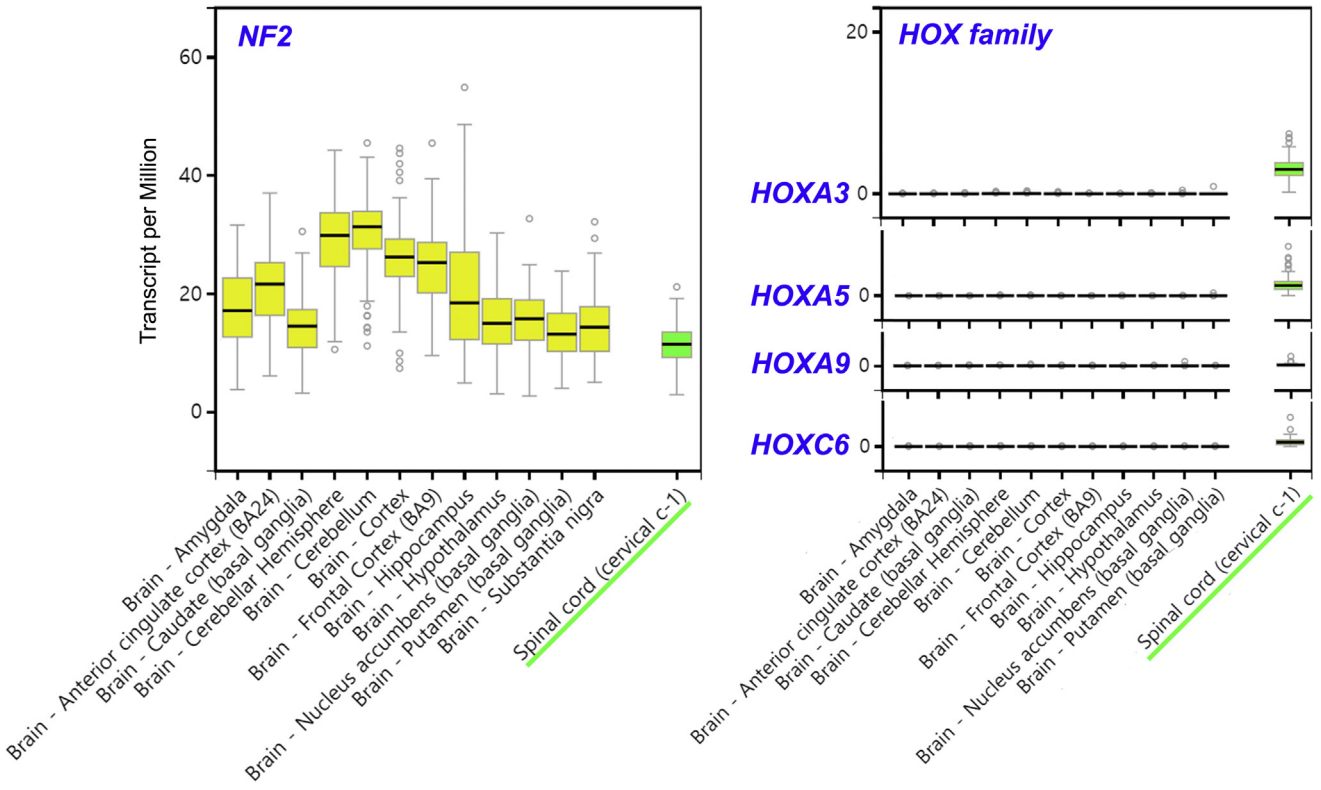

Figure 3. Expression level of normal tissue. *NF2* gene is expressed highly in the brain compared with the spinal cord. The differential expression of *NF2* in intracranial and spinal cord ependymomas is observed even in the normal brain. *HOX* family genes are rarely expressed in adults, and the differential expression of these genes is not substantial between healthy brain and spinal cord tissues. Modified from GTEx data.[34]

NF2 in intracranial and spinal cord ependymomas is present even in the normal brain and spinal cord, and it is difficult to determine whether the difference in expression levels is caused by the ependymoma. In contrast, *HOX* genes are rarely expressed in the brain and spinal cord of healthy adults, and increased differential expression can be considered to have been induced explicitly by the ependymoma.

In the present study, the most significant DEGs in spinal cord ependymoma were *HOX* genes. *HOX* genes encode a family of evolutionarily conserved transcription factors that have fundamental roles in specifying anterior/posterior body patterning and spine development. It has long been known that *HOX* genes are central players in patterning the vertebrate axial skeleton.[35] In vertebrates, Taylor et al.[8] reported that the caudal body regions show an increase in the amount and diversity of *HOX* expression. Also, deletion of either *HOXA9* or *HOX-C*, which are selectively expressed by human spinal ependymomas, causes spinal abnormalities in mice.[8] In humans, Palm et al.[36] reported overexpression of *HOX* genes in spinal ependymoma. Another expression study identified 4 of the top 5 DEGs between intracranial and spinal cord ependymomas and found that 22 of the 27 *HOX* genes annotated in the microarrays were significantly upregulated in spinal ependymomas.[33] Other microarray-based expression studies that have correlated molecular signatures with clinicohistologic characteristics demonstrated that the *HOX* genes were related to spinal cord ependymomas.[8,36,37] Thus, *HOX* family genes might have a pivotal role in the clinical differences between intracranial and spinal ependymomas, rather than NF2. Additional studies are needed to investigate the mechanism underlying the roles of *HOX* genes in the spinal cord ependymomas.

A previous DEG analysis identified *HOXB7*, *HOXC9*, *HOXB8*, *HOXB6*, and *CFTR* as the top 5 DEGs between intracranial and spinal cord ependymomas (in order of FDR *P* value).[33] If DEGs were sorted in order of the FDR *P* value in our study, the top 5 DEGs would be *HOXA9*, *HOXA10*, *HOXB7*, *ARHGEF28*, and *JPH2*. Both studies showed that HOX family genes are significant DEGs. However, the exact genes involved differed. The previous study was similar to our study in that it combined data sets from various microarray chip sets, which can result in critical misreading.[38] Batch effects can occur when combining data sets using different chip sets from different companies because measurements are affected by laboratory conditions, reagent lots, and personnel differences.[39] These differences become a significant problem when batch effects are correlated with an outcome of interest and lead to incorrect conclusions.[39]

The microarray quality control project resulted in the conclusion that the inter-platform comparability and reproducibility of microarray gene expression measurements is inadequate.[38] For these reasons, we included 2 GEO data sets performed using the same platform among 9 data sets.

Study Limitations

Our study had some limitations that should be considered when interpreting our results. First, DEG analysis describes only differences in expression. Upregulated genes such as *HOX* genes might be overexpressed in spinal ependymoma or underexpressed in intracranial ependymoma because of a lack of expression data for normal tissue, although the expression pattern of the genes in control tissues can show a similar difference to that in ependymomas. Further studies are needed to determine the critical gene responsible for the regional difference in ependymoma. Second, the present study was limited by its small sample size. Gene expression in ependymomas can be altered by many factors, and the small sample size failed to cover different races and regions, which can affect gene expression in ependymomas. Third, potential pitfalls were present, such as study design, genotyping error, and population stratification, that could have distorted the results regarding genomic study weakness and bias.[40] This bias of genetic and genomic studies is related to enrolled primary studies. Finally, we used a public database of microarray chips; however, the most reliable gene chip data were selected to avoid errors resulting from strict inclusion criteria. We matched the microarray chip set and data set platform to reduce the batch effect; however, experimental bias could still have occurred. Our future studies will focus on these probable genes and pathways. Because studies addressing ependymoma genomics and epigenomics are limited, more studies on these topics are needed to improve the diagnosis, treatment, and prognosis of ependymoma.

CONCLUSIONS

Differences in the pathogenesis between intracranial and spinal cord ependymoma might be related to the *HOX* family genes rather than *NF2*. However, because the level of evidence in the present study was limited, whether the differential expression of these genes is the cause or the consequence of the disease remains to be elucidated in a larger prospective study.

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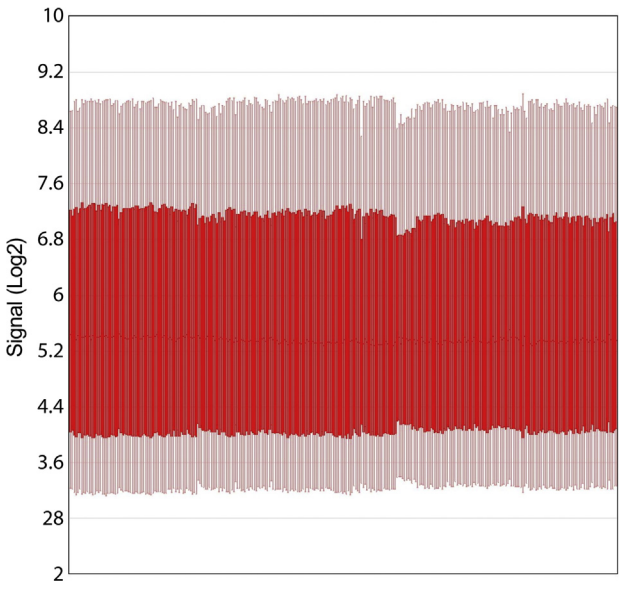
APPENDIX



Supplementary Figure 1. Box plot representation of chip-wise perfect match log intensity distributions. After normalization graph using the robust multi-array average method, all intensity distributions appeared similar. Because of the inconsistent distributions before normalization, the effect of the deviating chips should be carefully investigated with additional analysis steps.

Supplementary Table 1. Differentially Expressed Genes Between Intracranial and Spinal Cord Ependymoma

Gene Symbol	Spinal Average (log_2)	Intracranial Average (log_2)	FC (Linear)	*P* Value	FDR *P* Value
ARX	10.07	3.94	70.08	1.22E-17	2.88E-15
HOXC6	10.74	4.63	69.05	3.65E-36	8.67E-33
HOXA9	8.80	3.00	58.01	1.40E-45	1.40E-45
HOXA5	9.20	4.34	29.03	1.61E-15	2.71E-13
HOXA3	8.42	3.66	27.10	1.00E-04	0.001
HOXB-AS3	7.24	2.76	22.20	2.54E-41	9.25E-38
CFC1	8.19	3.85	20.22	9.46E-39	2.72E-35
NEFL	7.16	2.87	19.57	9.46E-28	9.57E-25
HOXA10	7.98	3.78	18.39	1.40E-45	1.40E-45
HOXB7	7.89	4.28	17.64	1.40E-45	2.80E-45
HOXA7	8.21	4.30	15.05	1.06E-32	1.93E-29
HOXB6	7.84	4.15	12.91	6.45E-38	1.76E-34
IGF1	8.09	4.53	11.87	1.60E-15	2.70E-13
CHEK2	8.79	5.32	11.09	1.40E-45	6.37E-42
FRAS1	7.00	3.63	10.35	3.26E-27	2.93E-24
SH3GL2	8.66	5.40	9.60	3.40E-10	1.66E-08
HOXD8	6.81	3.59	9.33	5.56E-32	8.93E-29
ANXA3	6.69	3.48	9.24	5.49E-27	4.76E-24
HOTAIRM1	9.27	6.00	9.15	3.57E-09	1.33E-07
KCNJ13	7.10	3.82	9.11	9.94E-17	2.01E-14
KCNJ16	10.04	6.34	9.06	3.51E-14	4.45E-12
JPH2	8.16	5.00	8.92	1.40E-45	5.61E-45
WT1	6.38	3.24	8.81	1.96E-21	7.81E-19
ME1	9.24	6.16	8.63	6.74E-19	1.97E-16
KCNE1	8.41	5.30	8.61	5.36E-18	1.39E-15
CPNE4	7.83	4.34	8.51	2.52E-28	2.60E-25
SKAP2	9.96	7.10	7.98	1.41E-11	9.66E-10
HOXA2	7.48	4.51	7.83	1.43E-05	2.00E-04
HOXB8	5.88	2.92	7.80	1.40E-45	1.86E-42
TRDN	6.09	3.14	7.72	1.34E-09	5.62E-08
MIR31HG	9.16	6.23	7.66	5.81E-19	1.72E-16
C9orf135	8.79	5.88	7.52	9.00E-04	0.0054
PLA2R1	6.97	4.06	7.50	1.91E-32	3.17E-29
XKR4	9.14	6.30	7.16	9.43E-10	4.17E-08
ANKRD29	7.35	4.55	7.09	3.13E-12	2.49E-10
HOXB3	9.94	7.14	6.96	2.45E-07	5.39E-06
SCN9A	6.30	3.51	6.92	1.43E-06	2.46E-05

Continues

Supplementary Table 1. Continued

Gene Symbol	Spinal Average (log_2)	Intracranial Average (log_2)	FC (Linear)	*P* Value	FDR *P* Value
SLC27A6	9.79	7.09	6.48	1.91E-11	1.27E-09
PRUNE2	6.19	3.51	6.41	3.62E-24	2.08E-21
INHBA	9.06	6.39	6.39	3.56E-17	7.78E-15
CXCL12	10.08	7.41	6.35	1.12E-08	3.64E-07
TTC6	6.68	4.02	6.30	6.78E-06	9.47E-05
LRRC2	9.06	6.42	6.28	9.35E-13	8.48E-11
CTTNBP2	7.20	4.56	6.26	4.17E-35	9.10E-32
ROBO2	7.87	5.16	6.22	1.00E-17	2.42E-15
NR4A3	8.85	6.14	6.18	7.76E-21	2.88E-18
ANK3	9.19	6.60	6.00	1.95E-12	1.63E-10
EFHB	8.75	6.17	6.00	7.96E-06	1.00E-04
C21orf62	9.33	6.80	5.91	9.90E-18	2.40E-15
SCN1A	8.03	5.47	5.87	5.84E-10	2.73E-08
ARHGEF28	6.88	4.27	5.78	1.40E-45	2.80E-45
EYA4	9.74	7.22	5.73	1.82E-07	4.14E-06
TMOD1	10.19	7.67	5.70	1.23E-28	1.29E-25
DIRAS2	7.79	5.32	5.58	2.77E-11	1.77E-09
DRD1	6.80	4.34	5.52	4.42E-08	1.20E-06
C8orf4	7.77	5.36	5.32	1.04E-09	4.52E-08
SHROOM3	9.47	7.09	5.24	4.90E-14	6.01E-12
SOCS2	8.79	6.42	5.20	3.80E-13	3.75E-11
POSTN	7.90	5.53	5.20	2.02E-05	2.00E-04
CADPS2	8.20	5.83	5.19	4.39E-15	6.75E-13
FAM65C	8.16	5.80	5.15	2.37E-07	5.25E-06
CRIP1	9.79	7.44	5.12	2.73E-05	3.00E-04
MAP3K7CL	6.68	4.33	5.10	1.01E-20	3.69E-18
COL4A3	5.58	3.24	5.10	5.02E-15	7.64E-13
XKR4	7.20	4.87	5.02	9.64E-09	3.19E-07
NRN1	8.54	6.22	5.01	2.99E-06	4.68E-05
DSP	8.35	6.04	4.97	1.25E-05	2.00E-04
EVA1C	9.05	6.75	4.94	4.71E-19	1.41E-16
HOXC10	6.32	4.02	4.91	1.35E-33	2.74E-30
CFTR	6.27	3.98	4.88	1.40E-45	6.08E-43
TENM1	6.90	4.63	4.81	5.28E-08	1.41E-06
SNX31	6.00	3.75	4.78	5.50E-09	1.95E-07
AQP1	10.33	8.07	4.77	8.20E-09	2.77E-07
MYH2	6.00	3.76	4.71	1.40E-45	4.89E-42
MIR21; VMP1	10.22	7.99	4.69	1.24E-07	2.97E-06

Continues

Supplementary Table 1. Continued

Gene Symbol	Spinal Average (log_2)	Intracranial Average (log_2)	FC (Linear)	*P* Value	FDR *P* Value
MAMDC2	7.06	4.83	4.69	6.19E-06	8.77E-05
HOXB-AS1	7.63	5.43	4.60	1.06E-13	1.20E-11
NT5E	7.67	5.48	4.59	6.40E-09	2.21E-07
CD36	9.13	6.94	4.58	7.00E-04	0.0048
SALL1	9.79	7.60	4.57	5.51E-20	1.86E-17
SGPP2	7.86	5.73	4.55	7.56E-15	1.11E-12
SHC4	7.41	5.24	4.51	1.56E-32	2.66E-29
FOXA1	6.01	3.84	4.51	3.58E-14	4.54E-12
SNCA	8.98	6.81	4.49	4.76E-09	1.72E-07
DAB1	7.11	4.95	4.45	1.77E-15	2.93E-13
DKK1	7.20	5.05	4.42	2.74E-09	1.05E-07
HAS2	7.27	5.13	4.40	7.60E-16	1.34E-13
DIRAS3	8.31	6.19	4.37	3.58E-08	9.95E-07
SCUBE2	9.02	6.89	4.37	5.75E-08	1.51E-06
PDLIM1	11.21	9.08	4.36	1.02E-15	1.78E-13
RARRES2	10.04	7.92	4.35	1.33E-10	7.14E-09
DLGAP1	5.82	3.70	4.34	3.39E-07	7.16E-06
NEFM	5.36	3.25	4.32	4.76E-15	7.26E-13
EVA1A	6.65	4.55	4.30	2.16E-17	4.87E-15
DOCK5	7.97	5.87	4.29	3.46E-12	2.73E-10
FNDC1	8.23	6.14	4.26	1.47E-05	2.00E-04
STOML3	7.93	5.84	4.26	1.00E-04	0.001
XK	7.40	5.33	4.18	3.22E-14	4.12E-12
CNKSR3	8.93	6.88	4.16	1.10E-14	1.52E-12
CLSTN2	10.17	8.11	4.16	1.86E-05	2.00E-04
PRSS35	6.12	4.08	4.11	2.00E-04	0.0015
MFSD6	7.82	5.78	4.09	8.23E-15	1.20E-12
SLC47A2	9.84	7.81	4.09	9.27E-07	1.70E-05
TLR4	7.28	5.25	4.07	4.59E-12	3.53E-10
CD47	10.89	8.87	4.04	2.97E-23	1.49E-20
ZIC2	5.11	10.90	−55.30	1.31E-39	3.96E-36
IGF2	5.50	11.06	−35.07	1.06E-22	4.83E-20
DACH1	3.67	8.41	−25.97	7.37E-17	1.52E-14
ZIC1	6.76	11.09	−24.84	4.76E-29	5.20E-26
LPAR3	3.93	8.14	−18.54	8.29E-15	1.20E-12
WIF1	2.93	6.97	−16.39	3.03E-06	4.72E-05
GDF10	3.71	7.71	−16.06	6.68E-11	3.84E-09
NCAN	5.31	9.27	−15.61	8.19E-18	2.04E-15

FC, fold change; FDR, false discovery rate.

Continues

Supplementary Table 1. Continued

Gene Symbol	Spinal Average (log_2)	Intracranial Average (log_2)	FC (Linear)	*P* Value	FDR *P* Value
NALCN	4.06	7.99	−15.29	1.29E-25	9.18E-23
KCNJ3	3.50	7.41	−15.03	1.34E-15	2.31E-13
ZIC1	4.43	8.31	−14.71	8.58E-17	1.75E-14
CALB1	3.40	7.00	−12.11	2.62E-11	1.69E-09
PCP4	5.49	9.07	−12.00	2.05E-09	8.18E-08
RELN	5.47	8.96	−11.21	4.40E-08	1.20E-06
DOK6	3.19	6.60	−10.63	2.10E-16	4.08E-14
RNF43	5.31	8.71	−10.52	8.97E-19	2.58E-16
MECOM	5.70	9.07	−10.45	7.16E-12	5.21E-10
FXYD6	6.56	9.93	−10.31	1.33E-25	9.31E-23
KCNJ10	5.76	9.09	−10.05	1.34E-12	1.16E-10
KCNA2	4.22	7.54	−10.01	9.48E-24	5.28E-21
CRB1	6.14	9.44	−9.87	1.27E-16	2.54E-14
GPM6A	7.97	11.32	−8.93	1.46E-32	2.57E-29
SCG3	4.44	7.40	−7.82	4.24E-14	5.28E-12
TOX	3.46	6.42	−7.81	5.85E-14	7.07E-12
LINGO1	5.08	8.00	−7.58	1.84E-13	1.96E-11
APLNR	7.02	9.94	−7.54	1.17E-08	3.78E-07
ASCL1	6.00	8.95	−7.52	7.17E-09	2.45E-07
TNFRSF11B	5.97	8.86	−7.43	3.48E-12	2.74E-10
PSD2	7.64	10.52	−7.38	2.17E-19	6.89E-17
MYO3A	4.01	6.88	−7.33	6.13E-15	9.24E-13
NTM	6.17	9.11	−7.32	8.75E-20	2.93E-17
RORB	3.95	6.86	−7.20	1.88E-10	9.72E-09
ABCA8	5.93	8.77	−7.18	1.94E-12	1.62E-10
STXBP5L	5.40	8.24	−7.17	5.81E-15	8.79E-13
NSG1	5.09	7.91	−7.08	6.05E-12	4.51E-10
FAM89A	4.64	7.44	−6.96	3.79E-23	1.87E-20
CLDN10	4.26	7.04	−6.87	1.39E-08	4.34E-07
ATP2B2	5.25	8.02	−6.83	2.05E-10	1.05E-08
APBA2	5.95	8.64	−6.45	6.80E-26	4.95E-23
PKIA	5.90	8.63	−6.41	7.50E-17	1.55E-14
PTN	8.41	11.07	−6.30	8.90E-15	1.27E-12
PRRX1	5.79	8.04	−6.17	4.66E-16	8.52E-14
GRIA4	3.16	5.78	−6.15	3.93E-07	8.11E-06
SLC6A13	5.42	8.03	−6.09	1.84E-06	3.07E-05
GPR37L1	5.93	8.52	−6.00	1.43E-15	2.44E-13
LSAMP	6.64	9.24	−5.96	1.65E-13	1.79E-11

Continues

Supplementary Table 1. Continued

Gene Symbol	Spinal Average (log_2)	Intracranial Average (log_2)	FC (Linear)	*P* Value	FDR *P* Value
ADGRL3	5.13	7.66	−5.95	8.94E-10	3.99E-08
EYA2	4.97	7.52	−5.88	6.30E-08	1.64E-06
NKAIN4	4.93	7.46	−5.82	4.67E-11	2.82E-09
RSPO2	2.82	5.35	−5.79	4.66E-08	1.26E-06
SERPINA3	7.76	10.28	−5.76	1.53E-10	8.12E-09
HAPLN1	3.72	6.10	−5.63	4.67E-06	6.87E-05
FIGN	5.39	7.88	−5.60	3.97E-09	1.46E-07
CACNA1A	4.44	7.10	−5.54	1.02E-12	9.18E-11
SLC35F1	6.11	8.55	−5.41	2.24E-23	1.15E-20
CDK1	5.55	7.98	−5.39	2.97E-09	1.13E-07
CDH10	4.37	6.78	−5.34	4.36E-07	8.85E-06
GABRG1	6.43	8.85	−5.33	8.65E-06	1.00E-04
HIF3A	3.76	6.15	−5.24	3.87E-06	5.86E-05
KIAA1549L	5.15	7.57	−5.22	4.09E-15	6.34E-13
IL17D	6.24	8.61	−5.17	1.11E-11	7.78E-10
SYNDIG1	6.25	8.62	−5.15	1.73E-18	4.82E-16
PMP2	7.33	9.68	−5.11	4.00E-04	0.0028
ATP1A2	8.95	11.24	−5.07	2.44E-07	5.39E-06
CAMK2B	4.50	6.77	−5.02	8.45E-15	1.22E-12
BTBD11	4.95	7.27	−5.02	2.09E-07	4.70E-06
TOP2A	5.03	7.38	−4.92	1.17E-06	2.07E-05
ASPM	3.43	5.73	−4.90	2.25E-07	5.02E-06
CPE	8.94	11.24	−4.88	3.11E-24	1.82E-21
BMPR1B	7.60	9.87	−4.87	3.05E-26	2.35E-23
SCG2	6.28	8.55	−4.84	1.70E-07	3.92E-06
GAS2L3	4.29	6.55	−4.80	2.00E-09	8.00E-08
PDZRN4	4.36	6.62	−4.78	3.15E-06	4.91E-05
DRAXIN	3.71	5.95	−4.72	7.13E-11	4.07E-09
CHI3L2	5.62	7.86	−4.71	3.59E-07	7.49E-06
F3	7.08	9.31	−4.70	2.39E-13	2.46E-11
GNAL	4.29	6.51	−4.69	1.28E-08	4.06E-07
AQP4	7.75	9.98	−4.60	2.55E-09	9.87E-08
FBLN1	6.41	8.58	−4.57	1.60E-17	3.74E-15
HCN1	2.91	5.10	−4.55	1.12E-06	1.99E-05
RHOBTB3	8.06	10.23	−4.54	2.22E-19	6.97E-17
LIN7A	5.69	7.88	−4.54	1.10E-08	3.59E-07
TOX3	2.83	5.00	−4.54	3.43E-06	5.27E-05
PPP2R2C	5.46	7.64	−4.53	1.82E-08	5.46E-07

Continues

Supplementary Table 1. Continued

Gene Symbol	Spinal Average (log_2)	Intracranial Average (log_2)	FC (Linear)	*P* Value	FDR *P* Value
ASPA	4.64	6.82	−4.52	1.61E-06	2.73E-05
EPHA7	3.89	6.07	−4.52	2.00E-04	0.0015
CDH4	4.72	6.88	−4.45	7.41E-23	3.46E-20
PAQR6	7.36	9.52	−4.45	2.38E-12	1.95E-10
SEZ6L	4.08	6.22	−4.43	2.40E-12	1.96E-10
MAPT	5.86	8.00	−4.42	8.62E-16	1.51E-13
LRRN1	7.10	9.22	−4.36	2.26E-13	2.34E-11
PLPP4	4.73	6.84	−4.33	7.92E-08	2.00E-06
GPR27	7.07	9.16	−4.27	2.00E-04	0.0017
PTX3	4.47	6.56	−4.25	8.62E-06	1.00E-04
DPYSL4	5.46	7.51	−4.15	3.82E-11	2.35E-09
NAV1	6.91	8.95	−4.10	7.69E-13	7.13E-11

FC, fold change; FDR, false discovery rate.