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REVIEW ARTICLE

# Genetic differences on intracranial versus spinal cord ependymal tumors: a meta-analysis of genetic researches

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**Abstract**
*Purpose* Although ependymomas occur in both the brain and the spine, the prognosis is quite varied by tumor location. Spinal ependymomas usually follow a relatively benign course with more favorable prognosis than that of the intracranial ependymomas. The aim of this study is to evaluate the genetic differences between spinal ependymomas and their intracranial counterparts using a meta-analysis.
*Methods* We searched PubMed, Embase, Web of Science, and the Cochrane library. Comparative or single arm genetic studies that enrolled patients with both intracranial and spinal ependymoma were included. The frequency of genetic aberration was calculated in each group. We calculated the odds ratio (OR) with 95 % confidence intervals (CIs) for direct comparative studies and the logit event rate (LER) and 95 % CI for single arm studies.
*Results* Twenty-five studies comprising of 380 spinal ependymomas and 964 intracranial ependymomas were compared to determine the association of the genetic differences of ependymomas at different locations. There were 25 comparable genetic aberrations between spinal and intracranial ependymomas. Among the genes, the *NF2* mutation was significantly associated with the spinal ependymomas rather than with the intracranial ependymomas (spinal tumor: LER −0.750, 95 % CI −1.233 to −0.266, intracranial tumor: LER −3.080, 95 % CI −3.983 to −2.177). Intracranial ependymomas were found to be significantly associated with *EPB41L3* deletion (OR 0.34; 95 % CI 0.14–0.80) and *HIC1* methylation (OR 0.12; 95 % CI 0.02–0.68).
*Conclusion* The genetic aberrations of spinal ependymomas are quite different from those of intracranial ependymomas. The difference in prognosis of ependymoma by location may be associated with genetic difference. A more detailed understanding of them may enable the development of targeted therapy and the estimation of prognosis.

**Keywords** Ependymoma · Gene · Spinal · Intracranial · Meta-analysis

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## Introduction

Ependymomas occur in both the brain and the spine in pediatric and adult populations [1, 2]. Although ependymomas from different locations are histopathologically similar, their molecular landscape is very heterogeneous; they show differences in DNA copy number alterations, mRNA expression profiles, genetic and epigenetic alterations, and diverse transcriptional programs [1, 3–7]. The genetic landscape of ependymoma is also quite heterogeneous; these tumors mostly show complex aberration patterns with frequent deletions or gains of chromosomes,

with the deletions occurring primarily on chromosomes 1p, 4q, 6q, 9, 10, 13q, 16, 17, 19q, 20q, and 22q [8–13].

The greater difficulty in estimating the clinical course is that ependymal tumors are heterogeneous with respect to morphology, the age at which the first clinical manifestation occurs, and site-specific prognosis [14]. In children, 90 % of the ependymomas develop in the intracranial region and are associated with frequent recurrences [1, 15–17]. In adults, 60 % of the ependymomas develop in the spinal cord with rare recurrences [15, 16]. It has been previously reported that age ≥18 years, spinal localisation, and complete resection were positive prognosticator for the progression free survival in ependymoma [18]. These observations support the hypothesis that the histological entity "ependymoma" in fact is comprised of a group of related diseases that are likely to require different treatment approaches [4, 13, 19]. However, treatment is mainly based on surgery with or without radiation therapy, which has not been significantly changed in the last 20 years [18]. There is an urgent need for prognostic markers to tailor the treatment strategy.

The meta-analysis described here resolves the genetic differences between spinal ependymomas and their intracranial counterparts, which is essential in guiding therapeutic strategies and estimating prognosis.

## Materials and methods

### Literature search

We used the Embase, PubMed, Web of science, and Cochrane Library databases to undertake a comprehensive systematic literature review of all the gene studies published until March 15, 2016, with the objective to evaluate the relation of intracranial and spinal ependymomas. The following search terms were used: ependymoma, intracranial, spinal, and gene. The data were independently extracted by two reviewers who had expertise in spinal diseases and bioinformatics. In case of a discrepancy, a third author participated in the discussion until a consensus was reached. Articles in the reference lists of the selected studies were also searched manually. The search was not limited to any specific language.

### Study eligibility criteria

We systematically reviewed and selected the published studies that met the following criteria: (1) case–control or cohort studies that included data regarding genetic aberrations in the patients with either intracranial or spinal intramedullary ependymoma, or both, (2) studies that provided the number of genetic aberrations and total inspected patients, (3) studies that clearly described the DNA genotyping methods used and the sources. We excluded case reports, narrative reviews, letters, editorials, comments, biomechanical, and cadaveric studies. Studies that did not meet the eligibility criteria listed above and duplicate publications were excluded.

### Data synthesis and analysis

The retrieved data included the following items: (1) study profile (author's name, publication date, and journal), (2) study type and region, (3) participants' demographics (such as mean age, sample size, and gender), and (4) counts of genetic aberrations in intracranial and spinal ependymomas. If the data were insufficient or unclear in a study, we attempted to contact the authors for further details.

### Statistical analysis

All of the data in the direct comparative studies were dichotomous data, expressed as odds ratio (OR) of event rate with 95 % confidence interval (CI), to assess the association between genetic abnormalities and ependymomas. To analyze the relation with regard to genetic alterations, we calculated the OR with 95 % CI for comparative studies. For the single arm study, the effect size was calculated event rate as same as pooled frequency and compared with each other using the logit event rate (LER) of the standard normal ($Z$) distribution analysis of variance. The studies were weighted in the meta-analysis by the inverse of the variance, which included both within and between-study errors.

Between-study heterogeneity was evaluated using the inconsistency index ($I^2$) statistic and Chi square ($\chi^2$), with the statistical significance set at $P < 0.10$. When the heterogeneity was not statistically significant and the value of $I^2$ was less than 50 %, the fixed-effects model was used to estimate the pooled OR or LER. The funnel plot and Egger's test were performed to assess publication bias. The overall effect was tested by Fisher's $z$ transformation (statistical significance set at $P < 0.05$). Sensitivity analysis was performed by varying assumptions used in the meta-analysis and by single elimination of the studies. If there was no mutation in all groups (i.e., a "zero cell" in the 2 by 2 table), 0.5 was added to each cell so that the estimated values would not be 0 or infinity and so that the standard error could be calculated. Statistical analyses were performed using Review Manager (RevMan) Version 5.3. (The Nordic Cochrane Centre, the Cochrane Collaboration, Copenhagen, Denmark), comprehensive meta-analysis, version 3.0 (Biostat, Englewood, NJ, USA), and R software (version 3.2.0, The R Foundation for Statistical Computing, Vienna, Austria).

## Results

Based on the selection strategy described in the methods section, a total of 79 articles were identified (PubMed, 21; EMBASE, 34; Web of Science, 24; Cochrane library, 0). After screening and reviewing for eligibility, a total of 25 studies, including 380 spinal and 964 intracranial ependymomas, were retrieved for this meta-analysis. The detailed selection process is shown in Fig. 1. We sent seven corresponding authors e-mail to request their data twice, none answered the request.

The baseline characteristics of the studies included in this meta-analysis are presented in Table 1. Of the 25 articles we identified, the study/studies conducted in the USA [15, 20–25], Europe [6, 14, 26–39], and Asia [40, 41] were 7, 16, and 2, respectively. In all these studies, the patients with intracranial ependymoma were much younger than those with spinal ependymoma in Table 1. Intracranial ependymomas usually showed male predominance, consistent with a previous epidemiologic report [42]; however, spinal ependymomas did not demonstrated such clear sex-predominance.

Six papers investigated the association of *NF2* gene with ependymoma susceptibility. The pooled frequency of *NF2* mutations in spinal and intracranial ependymoma was 32.1 and 4.4 % in Table 2, respectively. Comparison performing using the LER of forest plots were significantly different in mutation frequency between the two groups as shown in Fig. 2. According to $\chi^2$ statistic (intracranial, $P = 0.63$; spinal, $P = 0.21$) and $I^2$ statistic (intracranial, $I^2 = 0$ %; spinal, $I^2 = 31.36$ %), heterogeneity was assessed and found not to be significant, therefore, a fixed-effect approach was used to determine pooled frequency of *NF2* mutation of spinal versus intracranial ependymomas.

*EPB41L3* deletion accounted for 46.6 % of intracranial ependymoma and 21.7 % of spinal ependymoma (OR 0.34; 95 % CI 0.14–0.80; $P = 0.01$) and the *EPB41L3* deletion is substantially associated with intracranial ependymoma compared with spinal ependymoma in Fig. 3. The pooled frequency of *HIC1* hypermethylation was 93.8 % of intracranial ependymoma and 65.0 % of spinal ependymoma, which displayed the close association between intracranial ependymoma and *HIC1* hypermethylation (OR 0.12; 95 % CI 0.02–0.68; $P = 0.02$).

The other gene mutations in the following genes did not show a predominant association with tumors at any location: *NEFL*, *CDKN2A*, *EGFR*, *HOXB13*, *PDGFRA*, *RASSF1*, *RB*, *SMARCB1*, *CDKN2B*, *TNC*, *CASP8*, *MOS*, *MEN1*, *NOTCH1*, *SHC3* and *S1PR3*, *THBS1*, *PTEN*, *TP73*, *MGMT*, *TIMP3*, *ERBB2*, *and MDM2* genes as shown in Table 2 and Fig. 3. The pooled frequency of *NEFL* overexpression in spinal and intracranial ependymoma was 59.1 % (95 % CI 42.9–73.6 %) and 38.3 % (95 % CI 27.8–50.0 %) in Table 2, respectively. The gene did not show a substantial association with the spinal ependymomas in Fig. 4. Among 22 myxopapillary ependymomas in the spinal cord tumor, *NEFL* overexpression accounted for 69.0 % (95 % CI 44.1–86.3 %) of the tumor, which demonstrated no substantial difference in frequency of *NEFL* mutation between myxopapillary ependymoma and the other ependymomas.

Ten studies investigated *CDKN2A* mutations in intracranial and/or spinal ependymoma. *CDKN2A* mutations were observed in 20.6 % in intracranial ependymoma and 21.9 % of spinal ependymoma in Table 2. LER of intracranial and spinal ependymoma was −1.347 and −1.121, respectively, in Fig. 5. The pooled frequency of

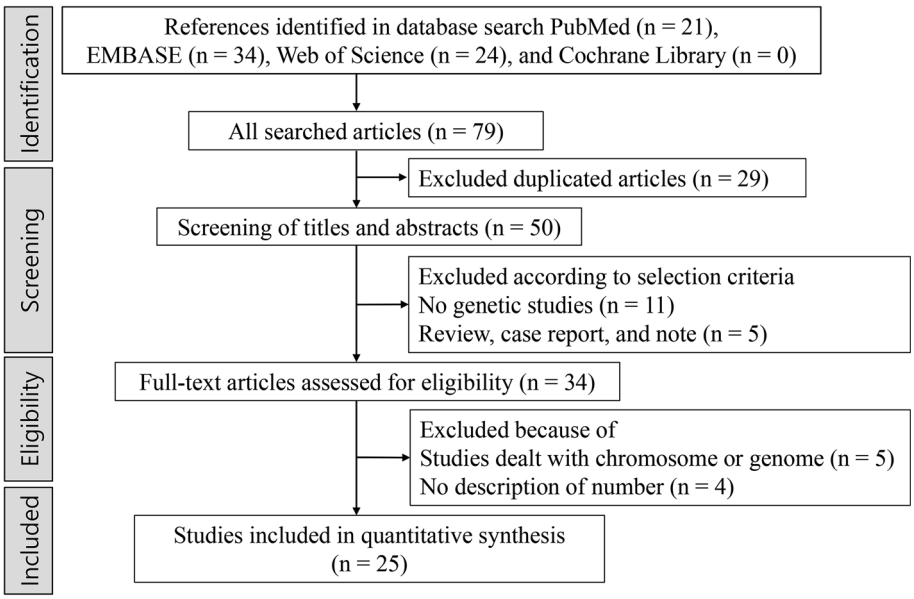


**Fig. 1** A flow chart showing the process of study identification and exclusion

**Table 1** The baseline characteristics of the included studies

| References | Study design | Study region | Number | | Mean age | | Male (%) | |
|---|---|---|---|---|---|---|---|---|
| | | | IC | Sp | IC | Sp | IC | Sp |
| Ebert et al. [14] | Retro | Germany | 39 | 23 | 24 | 41 | 51 | 65 |
| Suzuki et al. [41] | Retro | Japan | 16 | 4 | 23 | 37 | 75 | 100 |
| Lamszus et al. [33] | Retro | Germany | 20 | 32 | 21 | 39 | 86 | 58 |
| Bortolotto et al. [29] | Retro | Italy | 16 | 0 | N/D | N/D | N/D | N/D |
| Kraus et al. [32] | Retro | Germany | 31 | 17 | 26 | 40 | N/D | N/D |
| Singh et al. [15] | Retro | USA | 12 | 15 | 17 | 39 | 86 | 29 |
| Gilberston et al. [30] | Retro | UK | 120 | 1 | N/D | N/D | N/D | N/D |
| Rousseau et al. [38] | Retro | Belgium | 60 | 43 | N/D | N/D | N/D | N/D |
| Athanasiou et al. [28] | Retro | UK | 29 | 5 | N/D | N/D | N/D | N/D |
| Rajaram et al. [20] | Retro | USA | 50 | 51 | 16 | 41 | N/D | N/D |
| Alonso et al. [26] | Retro | Spain | 2 | 25 | 18 | 31 | 56 | 50 |
| Waha et al. [39] | Retro | Germany | 35 | 20 | 16 | 39 | 38 | 48 |
| Hamilton et al. [31] | Retro | UK | 9 | 11 | N/D | N/D | N/D | N/D |
| Rajaram et al. [25] | Retro | USA | 51 | 33 | 7[a] | 41[a] | N/D | N/D |
| Mendrzyk et al. [35] | Retro | Germany | 49 | 19 | N/D | N/D | N/D | N/D |
| Michalowski et al. [36] | Retro | France | 27 | 0 | N/D | N/D | N/D | N/D |
| Andreiuolo et al. [18] | Retro | France | 66 | 0 | N/D | N/D | 45 | N/D |
| Barton et al. [22] | Retro | USA | 23 | 5 | 3[a] | 13[a] | 65 | 25 |
| Magrassi et al. [34] | Retro | Italy | 16 | 19 | 15 | 44 | 69 | 47 |
| Korshunov et al. [6] | Retro | Germany | 122 | 0 | N/D | N/D | 48 | N/D |
| Modena et al. [37] | Retro | Italy | 60 | 0 | 3 | N/D | 57 | N/D |
| Stephen et al. [23] | Retro | USA | 0 | 19 | N/D | 13 | N/D | 63 |
| Bettegowda et al. [21] | Retro | USA | 40 | 19 | N/D | N/D | N/D | N/D |
| Karakoula et al. [24] | Retro | USA | 40 | 0 | N/D | N/D | N/D | N/D |
| Gupta et al. [40] | Retro | India | 31 | 19 | 20 | 32 | 71 | 68 |

*IC* intracranial ependymoma, *Sp* spinal ependymoma, *Retro* retrospective study, *N/D* not described

[a] Median age

**Table 2** Pooled frequency (event rate) of genetic mutations in ependymoma of single group studies

| Locus | Gene | Intracranial ependymoma | | Spinal ependymoma | |
|---|---|---|---|---|---|
| | | Event rate (%) | 95 % CI (%) | Event rate (%) | 95 % CI (%) |
| 22q12 | *NF2* mutation | 4.4 | 1.8–10.2 | 32.1 | 22.6–43.4 |
| 8p21 | *NEFL* overexpression | 38.3 | 27.8–50.0 | 59.1 | 42.9–73.6 |
| 9p21 | *CDKN2A* deletion | 20.6 | 16.4–25.6 | 21.9 | 15.9–29.4 |
| 7p11 | *EGFR* overexpression | 52.1 | 38.5–65.3 | 29.8 | 13.0–54.5 |
| 17q21 | *HOXB13* overexpression | 22.2 | 5.6–57.9 | 59.0 | 39.5–76.0 |
| 4q12 | *PDGFRA* overexpression | 84.6 | 54.9–96.1 | 83.8 | 68.3–92.5 |
| 3p21 | *RASSF1* methylation | 64.5 | 47.7–78.4 | 90.0 | 67.6–97.5 |
| 13q14 | *RB* deletion | 15.9 | 9.2–26.2 | 23.3 | 14.0–36.2 |
| 22q11 | *SMARCB1* deletion | 15.3 | 6.7–31.2 | 21.4 | 7.1–49.4 |

Event rate showed significant difference between two groups

*CDKN2A* mutations was slightly higher in the spinal ependymoma than that in the intracranial counterpart, which is not substantially different.

*TNC* mutation was frequently observed in infratentorial ependymoma, in comparison to spinal and supratentorial ependymomas. The expression frequency of *TNC* mutation in infratentorial ependymoma was reported to be 50.0 %, whereas it was 18.8 and 31.2 % in spinal and supratentorial ependymomas, respectively [40]. *NOTCH1* mutation in supratentorial tumors was significantly higher (73 %)

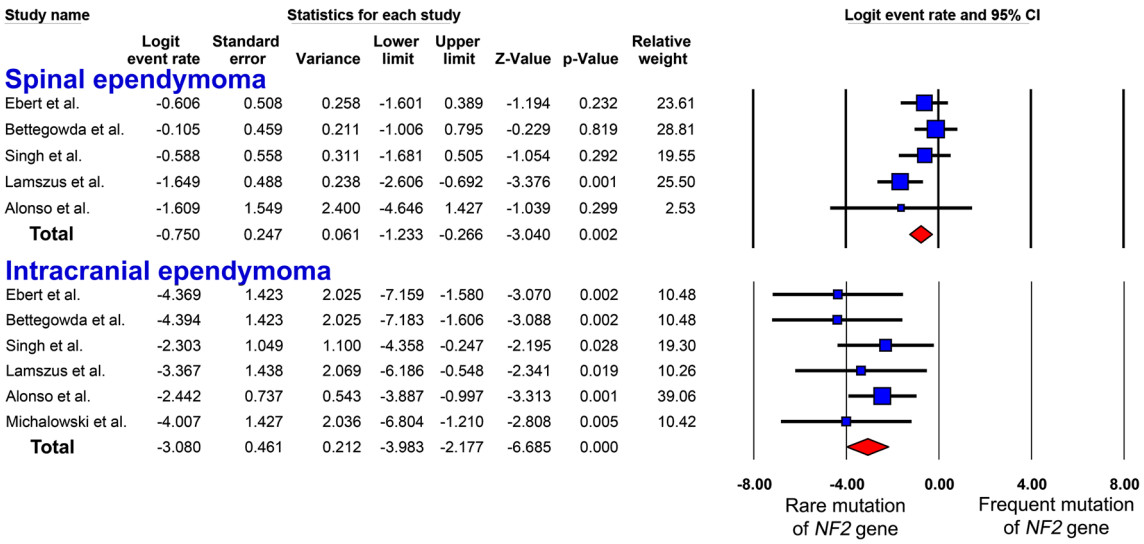


**Fig. 2** Forest plot of the logit event rate of *NF2* mutation by study and subgroup in the spinal and intracranial ependymoma group. Spinal ependymoma shows substantially frequent mutation of *NF2* gene compared with the intracranial ependymoma

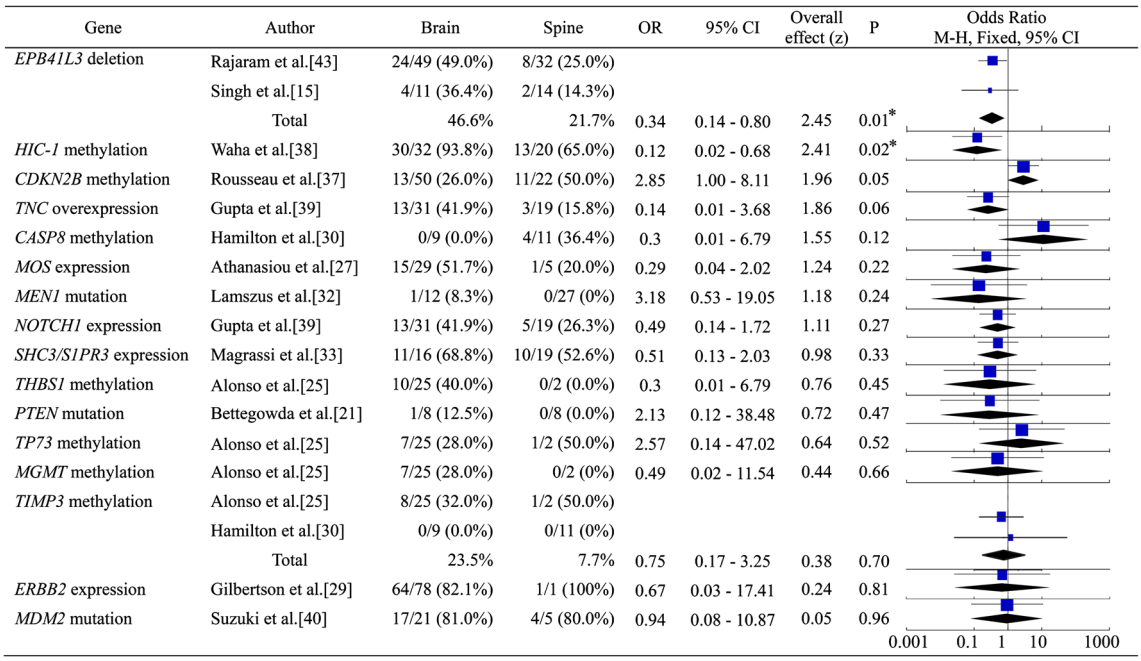


**Fig. 3** Pooled frequency of genetic mutations in ependymoma of direct comparative studies. *NEPB41L3* and *HIC1* aberrations show substantially different association between intracranial and spinal ependymoma. *Odds ratio shows significant difference in the pooled frequency of mutation between two groups

than in infratentorial (19 %; $P = 0.001$) and spinal (26 %; $P = 0.01$) tumors [40]. *MEN1* and *PTEN* mutations have been rarely observed in ependymomas [14, 21, 33]. Comparisons of more than three studies demonstrated usually minimal to moderate heterogeneity in Table 3. Funnel plots and the Egger test showed a little risk of publication bias except intracranial *RB* mutation. It calculated as $-2.27$ ($P = 0.04$), which may indicate an underpowered analysis.

## Discussion

Although pathological findings of spinal and intracranial ependymomas are similar to each other, their clinical course and commonly affected age are quite different. Genetic difference can be a possible reason of this discrepancy and this meta-analysis displayed that spinal ependymoma is associated with *NF2* whereas intracranial ependymoma is associated with *EPB41L3* and *HIC1*. The

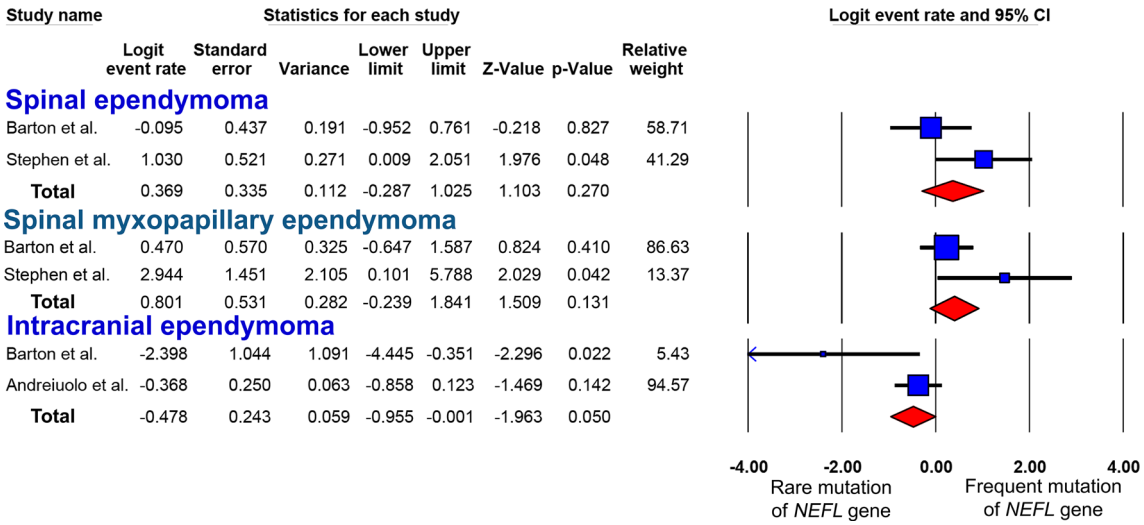


**Fig. 4** Forest plot of the logit event rate of *NEFL* mutation by study and subgroup in the spinal and intracranial ependymoma group. *NEFL* mutation is frequent in myxopapillary ependymoma. However, there is no significant difference in frequency of mutation. *CI* confidence interval

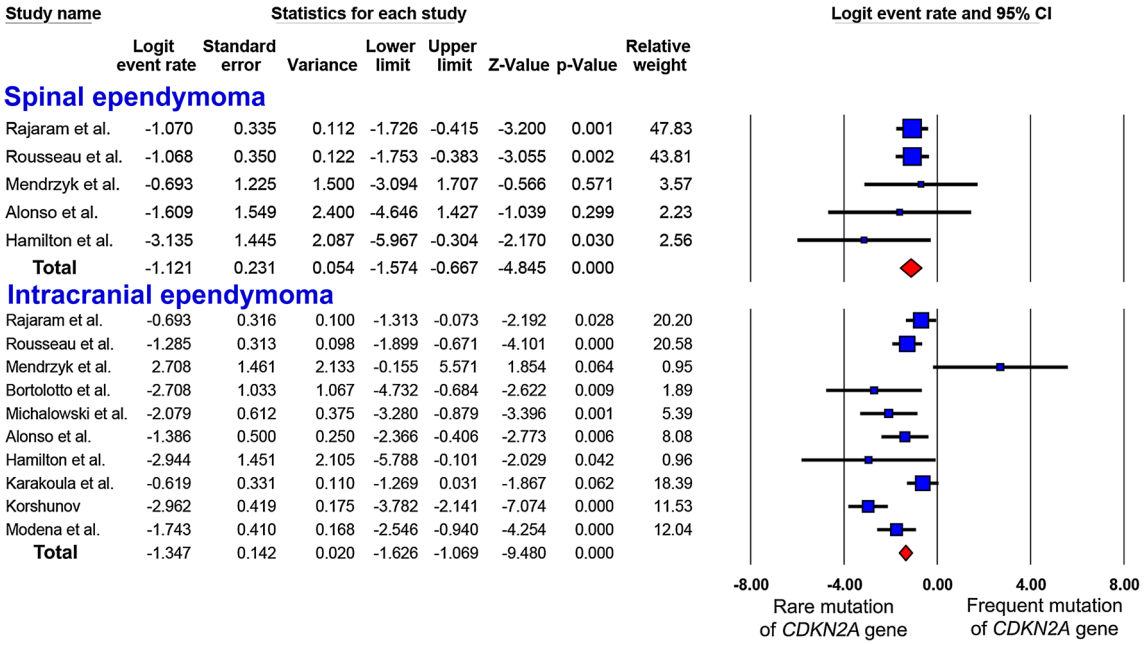


**Fig. 5** Forest plot of the logit event rate of *CDKN2A* mutation by study and subgroup in the spinal and intracranial ependymoma group. Both intracranial and spinal ependymoma shows low to moderate frequency of *CDKN2A* mutation. *CI* confidence interval

**Table 3** Statistical tests for heterogeneity and publication bias

| Gene | Location | $\chi^2$ (*P* value) | $I^2$ | $\tau^2$ | Egger (*P* value) |
|---|---|---|---|---|---|
| *NF2* mutation | Intracranial E | 3.43 (0.63) | 0.0 | 0.0 | −2.36 (0.23) |
| | Spinal E | 5.83 (0.21) | 31.36 | 0.15 | −0.90 (0.68) |
| *CDKN2A* deletion | Intracranial E | 31.68 (0.00) | 74.74 | 0.73 | −0.31 (0.85) |
| | Spinal E | 2.17 (0.54) | 0.0 | 0.0 | −0.69 (0.44) |
| *RB* deletion | Intracranial E | 7.00 (0.07) | 57.09 | 1.20 | −2.27 (0.04) |
| | Spinal E | 2.02 (0.37) | 0.72 | 0.01 | −1.13 (0.37) |

other 22 genes did not show substantial difference in frequency of mutation by tumor location.

Although a variety of numerical and structural chromosomal abnormalities have been shown to be associated with ependymomas, inactivation of the *NF2* gene as well as the sporadic mutations in *NF2* have been well documented in spinal ependymomas [7, 14, 15, 43]. The importance of the *NF2* gene in spinal ependymoma pathogenesis is further emphasized by the observation that *NF2* mutations and the loss of expression of Merlin (the protein encoded by the *NF2* gene) are found in 30–71 % of sporadic (non-syndromic) ependymomas [15, 44]. Among six enrolled studies on *NF2* included in our meta-analysis, four studies reported that *NF2* mutation of intracranial ependymoma was not observed at all among 120 patients [14, 21, 33, 36]. Another study reported that *NF2* loss was only a trend at the DNA level, whereas it was highly clearly associated with spinal ependymomas at the protein (Merlin) level [15, 45].

Although *NF2* loss was frequently observed in spinal ependymoma, pooled frequency of *NF2* mutation in spinal ependymoma was 32.1 %. The low frequency of genetic mutation may be explained by dilution of high and low frequency. A previous studies addressed that *NF2* mutations were observed in a high percentage of spinal ependymoma (WHO grade II), but in low percentage of intracranial WHO grade II ependymomas as well as all WHO grade I myxopapillary ependymoma, WHO grade I subependymoma, and WHO grade III ependymoma studied [14]. Because of insufficient data, this study could not perform subgroup analysis by WHO grade. Further clinical study is needed to determine the relation between specific genetic mutation and ependymoma.

This analysis revealed that intracranial ependymomas clearly demonstrated *EPB41L3* and *HIC1* mutations, in comparison to their spinal counterparts [15, 25, 39]. Two studies included a total of 106 patients investigated *EPB41L3* genes in ependymomas and one study of 52 patients dealt with *HIC1* mutation. Although the difference in frequency of these genes mutation was statistically significant, further evaluation and large study are needed because only a few studies investigated *HIC1* and *EPB41L3* genes.

Myxopapillary ependymomas may be considered molecularly different from intracranial ependymomas as well as other spinal ependymomas [22]. Previous studies reported that they showed high expression of some genes, including *NEFL*, *HOXB5*, *PLA2G5*, and *ITIH2* [22]. We performed subgroup analysis of myxopapillary ependymoma for all reported genes. However, no unique genetic mutation of myxopapillary ependymoma was observed. Among evaluated genes, we showed the event rate of *NEFL* aberration because *NEFL* is known to the common genetic mutation of myxopapillary ependymoma. This meta-analysis showed that *NEFL* overexpression was 69.0 % in spinal myxopapillary ependymomas, 53.8 % in other spinal ependymomas (except myxopapillary ependymoma), and 38.3 % in intracranial ependymoma. Myxopapillary ependymoma showed a tendency of high incidence of *NEFL* mutation, but it did not show statistical differences in frequency.

Some review papers dealt with genetics of ependymomas [45–47]. The review papers simply described possible genes, proteins, and chromosomal aberrations in spinal, intracranial ependymoma, or both. They reported the differences between spinal and intracranial ependymoma in aspect of gene, protein, and chromosome. They addressed the frequency of genetic aberrations of each comparative study between brain and spine region. In contrast, this meta-analysis included both the comparative studies and single region studies in brain or spinal cord lesion, and summarized pooled effect size of genetic mutation. It may provide more precise perspective in aspect of gene than the review papers.

Currently, the only effective treatment for clinically symptomatic ependymoma is surgery, with or without radiotherapy [44]. Such surgical procedures, however, pose substantial risks, such as worsening of neurological deficits, paralysis, and death [16]. Despite active genetic researches, targeted therapeutics for ependymomas is in very early stage of its development. The currently used targeted therapeutic approaches depend on the similarities of ependymomas with other glial tumors, rather than influencing pathways specific to ependymomas. Merlin regulates the growth of the spinal cord neural progenitor cells in a Rac1- and ErbB2-dependent manner, which firmly establishes a central growth control target for *NF2*-associated spinal ependymoma [44, 48]. *NF2*-associated spinal ependymomas exhibit increased ErbB2 activation, resulting from Rac1-mediated ErbB2 retention on the plasma membrane [44]. Lapatinib, a selective ErbB1 and ErbB2 inhibitor prolonged disease stabilization in patients with ependymoma, in the phase I study [49]. Based on these encouraging data, a phase II study of bevacizumab and lapatinib in children with recurrent or refractory ependymomas was conducted [50]. Unfortunately, the combination therapy proved ineffective in treating recurrent ependymomas. Although this meta-analysis cannot demonstrate the reliable treatment of ependymoma based on the genetic perspective, it may help understanding ependymoma and be a cornerstone of ependymoma treatment.

## Limitations of our study

Our study has some limitations that must be considered when interpreting our results. Firstly, there are potential pitfalls which could distort the results in weakness and

biases of genetic studies, such as study design, genotyping error, and population stratification [51]. This bias of genetic studies is related with enrolled primary studies. It is regarded to be corrected by replication studies. The bias of this meta-analysis may be low because the meta-analysis enrolled all relative studies and produce the summary of estimate. Each study verified genetic aberrations in gene or protein level using different tools, such as comparative genomic hybridization (CGH), fluorescence in situ hybridization (FISH), and immunohistochemistry (IHC). Heterogeneity in the design and clinical characteristics of the studied populations might also have contributed to slightly attenuate the power of our study to detect additional significant associations. Secondly, publication bias is another potentially important limitation of this study. Although neither Egger's test nor Begg's test found evidence for publication bias in this meta-analysis, negative studies are less likely to be published indeed, which may affect the validity of analysis [52]. Besides, few papers dealt with *EPB41L3* or *HIC1* genes. So, considering the potential limitations of studies included in current meta-analysis, our results should be interpreted with caution.

## Conclusion

The genetic landscape of spinal ependymomas is quite different from that of intracranial ependymomas; the former seems to be closely associated with *NF2* mutation, whereas the latter seems to be associated with *EPB41L3* deletion and *HIC1* methylation. A more detailed understanding of these various genetic aberrations may enable the identification of more specific prognostic markers, as well as the development of customized and targeted therapy.

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**Compliance with ethical standards**

**Conflicts of interest** None of the authors has any financial interest in the subject under discussion in this paper.

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