

2017

Children with 5'-end NF1 gene mutations are more likely to have glioma

Corina Anastasaki

Washington University School of Medicine in St. Louis

Stephanie M. Morris

Washington University School of Medicine in St. Louis

Feng Gao

Washington University School of Medicine in St. Louis

David H. Gutmann

Washington University School of Medicine in St. Louis

Follow this and additional works at: https://digitalcommons.wustl.edu/open_access_pubs

Recommended Citation

Anastasaki, Corina; Morris, Stephanie M.; Gao, Feng; and Gutmann, David H., "Children with 5'-end NF1 gene mutations are more likely to have glioma." *Neurology Genetics*.3,5. e192. (2017).
https://digitalcommons.wustl.edu/open_access_pubs/6608

This Open Access Publication is brought to you for free and open access by Digital Commons@Becker. It has been accepted for inclusion in Open Access Publications by an authorized administrator of Digital Commons@Becker. For more information, please contact engeszer@wustl.edu.

Children with 5'-end *NF1* gene mutations are more likely to have glioma

OPEN

Corina Anastasaki, PhD
Stephanie M. Morris, MD
Feng Gao, MD, PhD
David H. Gutmann, MD,
PhD

Correspondence to
Dr. Gutmann:
gutmannnd@wustl.edu

ABSTRACT

Objective: To ascertain the relationship between the germline *NF1* gene mutation and glioma development in patients with neurofibromatosis type 1 (NF1).

Methods: The relationship between the type and location of the germline *NF1* mutation and the presence of a glioma was analyzed in 37 participants with NF1 from one institution (Washington University School of Medicine [WUSM]) with a clinical diagnosis of NF1. Odds ratios (ORs) were calculated using both unadjusted and weighted analyses of this data set in combination with 4 previously published data sets.

Results: While no statistical significance was observed between the location and type of the *NF1* mutation and glioma in the WUSM cohort, power calculations revealed that a sample size of 307 participants would be required to determine the predictive value of the position or type of the *NF1* gene mutation. Combining our data set with 4 previously published data sets ($n = 310$), children with glioma were found to be more likely to harbor 5'-end gene mutations (OR = 2; $p = 0.006$). Moreover, while not clinically predictive due to insufficient sensitivity and specificity, this association with glioma was stronger for participants with 5'-end truncating (OR = 2.32; $p = 0.005$) or 5'-end nonsense (OR = 3.93; $p = 0.005$) mutations relative to those without glioma.

Conclusions: Individuals with NF1 and glioma are more likely to harbor nonsense mutations in the 5' end of the *NF1* gene, suggesting that the *NF1* mutation may be one predictive factor for glioma in this at-risk population. *Neurol Genet* 2017;3:e192; doi: 10.1212/NXG.0000000000000192

GLOSSARY

CI = confidence interval; **NF1** = neurofibromatosis type 1; **OR** = odds ratio; **WUSM** = Washington University School of Medicine.

Neurofibromatosis type 1 (NF1; OMIM162200) is characterized by substantial clinical variability, with individuals prone to the development of numerous medical complications, ranging from neurofibromas and bone defects to autism and nervous system tumors. The wide phenotypic variation seen in this disorder creates a particular challenge for clinicians when counseling families. For this reason, there is a pressing need to identify potential predictive risk factors.

NF1 is caused by germline pathogenic variants in the *NF1* locus, containing a large gene with 57 exons.¹ While thousands of different *NF1* gene mutations have been identified, recent studies have suggested that genotype-phenotype correlations may exist. In particular, 2 types of *NF1* mutations have been described, in which patients do not develop neurofibromas (c.2970_72delAAT²; p.Arg1809³). However, several groups have evaluated the predictive value of the location of the *NF1* mutation as a potential risk factor for optic glioma with conflicting conclusions.⁴⁻⁷ An important limitation to these analyses is the small sample size of each cohort. This is particularly relevant to brain tumors, which arise in only ~20% of children with NF1.^{8,9} For this reason, we combined participants from our own institution (Washington University School of Medicine [WUSM] cohort) with the 4 previously published studies that used MRI scans to

Supplemental data
at Neurology.org/ng

From the Department of Neurology (C.A., S.M.M., D.H.G.) and Department of Surgery (F.G.), Washington University in St. Louis, MO. Funding information and disclosures are provided at the end of the article. Go to Neurology.org/ng for full disclosure forms. The Article Processing Charge was funded by the authors.

This is an open access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND), which permits downloading and sharing the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

determine the presence or absence of gliomas.⁴⁻⁷ As such, we performed adequately powered unadjusted and weighted analyses to determine the relationship between the type and location of the *NF1* mutation and glioma in over 300 individuals with NF1.

METHODS Study population. This retrospective analysis was performed using pre-existing clinical data in the electronic medical records under an approved Human Studies protocol at the WUSM. Only those participants with a known *NF1* gene mutation (performed at the University of Alabama, Birmingham Medical Genomics Laboratory) and brain MRI (performed at Barnes-Jewish Hospital/St. Louis Children's Hospital) were included in this study. A total of 37 participants met these criteria: 14 participants were identified with glioma (optic, cerebellar, brainstem, and temporal lobe glioma) using previously published radiographic criteria,⁸ while 23 did not (negative scan after age 10 years). Deidentified data analyzed included patient sex (male/female), family history of NF1 (yes/no), *NF1* gene mutation (DNA and predicted protein change), and glioma (presence/absence) (table e-1 at Neurology.org/ng).

Standard protocol approvals, registrations, and patient consents. This is a retrospective analysis performed using pre-existing clinical data in the electronic medical records under a WUSM-approved Human Studies protocol (IRB#201703143). As such, no patient consent forms were required.

Statistical analysis. All categorical variables were analyzed using χ^2 or Fisher exact tests (SPSS, v23). Odds ratios (ORs) were reported with 95% confidence intervals (CIs), estimated using 2×2 contingency tables to compare outcomes (presence of glioma) with the variant type and location within the *NF1* gene. Sensitivity analyses were performed by meta-analysis,^{4,6,7} excluding 1 data set that lacked a control group.⁵ Adjusted ORs and associated 95% CIs were calculated using weights, based on the inverse of the variance, while individual studies included in the meta-analysis were assumed to be random samples of the target population. Statistical significance was defined as a *p* value of <0.05 (2 sided).

RESULTS A total of 37 individuals with identified *NF1* gene mutations were included (4 splice, 3 missense, 18 nonsense, 9 frameshift deletion, and 3 frameshift insertion mutations; WUSM cohort; table e-1). Based on radiographic criteria,⁸ 14 participants had a glioma, while 23 participants did not. There was no association between patient sex and family history of NF1 and glioma, and 5'-end (exons 1–26') clustering of mutations in patients with glioma was not observed. In addition, no differences were observed when mutations were stratified by variant type (table e-1). While there was a larger proportion of participants with glioma who harbored mutations predicted to cause premature protein truncation (nonsense, frameshift insertions/deletions) compared with participants without glioma (64.3% vs 30.4%), no statistically significant difference between groups was observed (*p* = 0.09). Similarly, with one exception,⁶ no significant association between the mutation

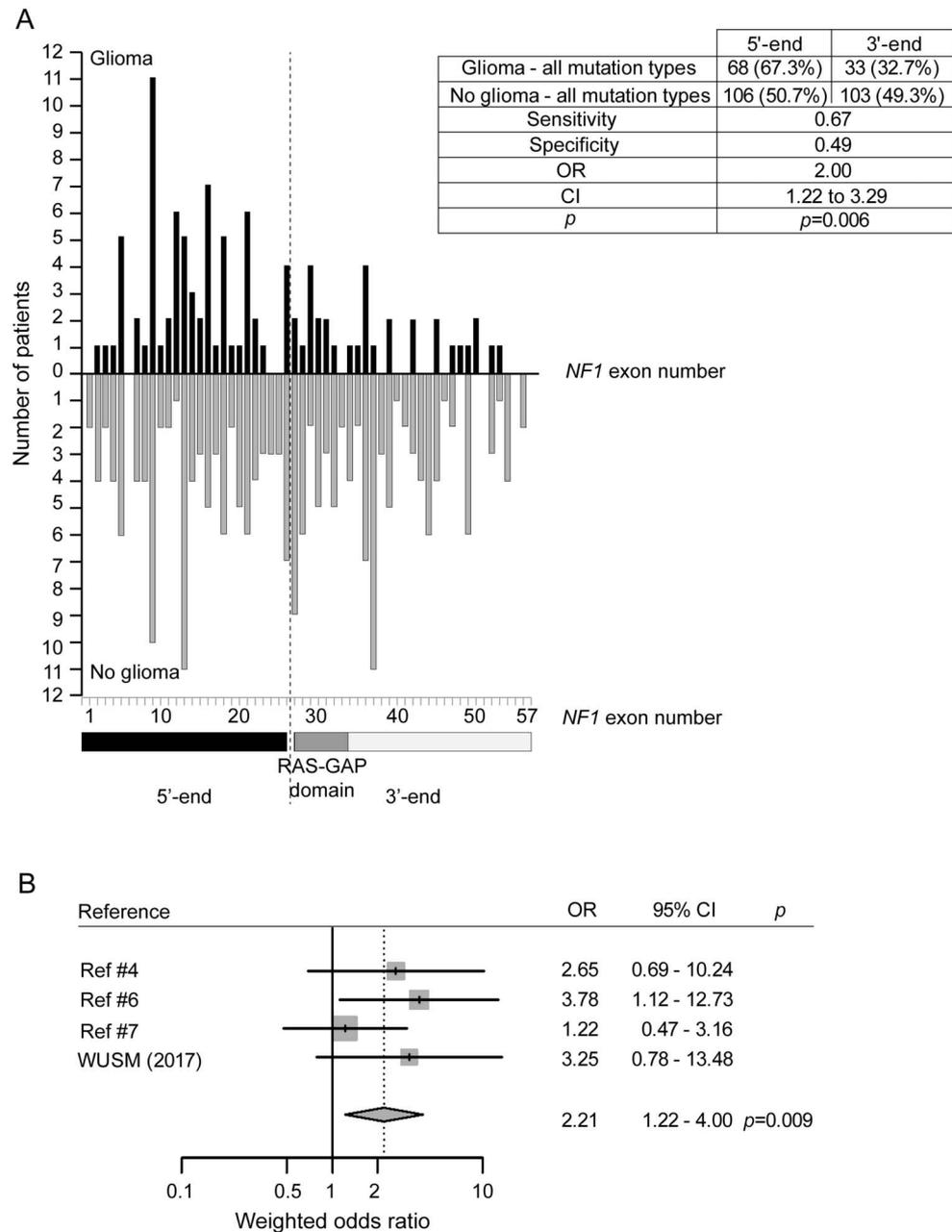
type or location and glioma diagnosis was found when each of the 3 previously published cohorts that contained participants with and without glioma were analyzed individually^{4,6,7} (table e-2).

A priori power analysis using the G*power 3.0.10 program (Universität Kiel, Germany) indicated that a total sample of 307 participants would be needed to detect an effect size of 0.16 with 80% power using a χ^2 test to detect differences in proportions with an alpha level of 0.05. Since each of the studies are limited by small participant numbers, we combined our data with the 4 previously published data sets, and after excluding participants with unknown mutations or genomic microdeletions, 101 participants with “glioma” and 209 controls with a “no glioma” diagnosis were available (table e-3). Using this combined data set, more 5'-end (exons 1–26) mutations were found in participants with glioma than in controls (67.3% vs 50.7%; OR = 2.00; 95% CI: 1.22–3.29; *p* = 0.006) (figure 1A). In addition, this significance persisted when the OR was adjusted for sample size and heterogeneity using a meta-analysis with control groups (*n* = 296) (OR = 2.21; 95% CI: 1.22–4.00; *p* = 0.009; figure 1B and table e-4A).

When mutations were stratified by type, the unadjusted OR for glioma was 3.93 (95% CI: 1.52–10.18; *p* = 0.005; figure 2A), and following meta-analysis, the weighted OR was 3.46 (95% CI: 1.19–10.10; *p* = 0.023; figure 2B and table e-4B) for participants with 5'-end nonsense mutations. Similarly, the unadjusted OR for glioma was 2.32 (95% CI: 1.23–4.18; *p* = 0.005; figure 2C), while the adjusted OR was 2.65 (95% CI: 1.12–6.24; *p* = 0.026; figure 2D and table e-4C) in participants with 5'-end truncation mutations relative to those with 3'-end mutations. When the 3 WUSM patients with cerebellar brainstem and temporal lobe glioma were excluded from the analyses, the significance of the findings was unchanged (table e-5). Of note, when we expanded the 5' end to include mutations within the RAS-GAP domain (exons 1–34), the significance observed in unadjusted ORs for glioma (table e-6) was lost when weighted analyses were performed (table e-6).

The utility of these findings in the clinic setting depends on their specificity and sensitivity in predicting the presence or absence of a glioma. Unfortunately, as stand-alone factors, the position and type of the *NF1* gene mutation lack sufficient specificity (0.49, 0.71, and 0.54) or sensitivity (0.67, 0.62, and 0.67 for all 5'-end, 5'-end nonsense, and 5'-end truncation mutations, respectively) to enable accurate risk assessment. This point is underscored by the finding that the same mutation can be found in participants with and without glioma (table e-7).

Figure 1 Distribution of *NF1* gene mutations in neurofibromatosis type 1 participants with and without glioma



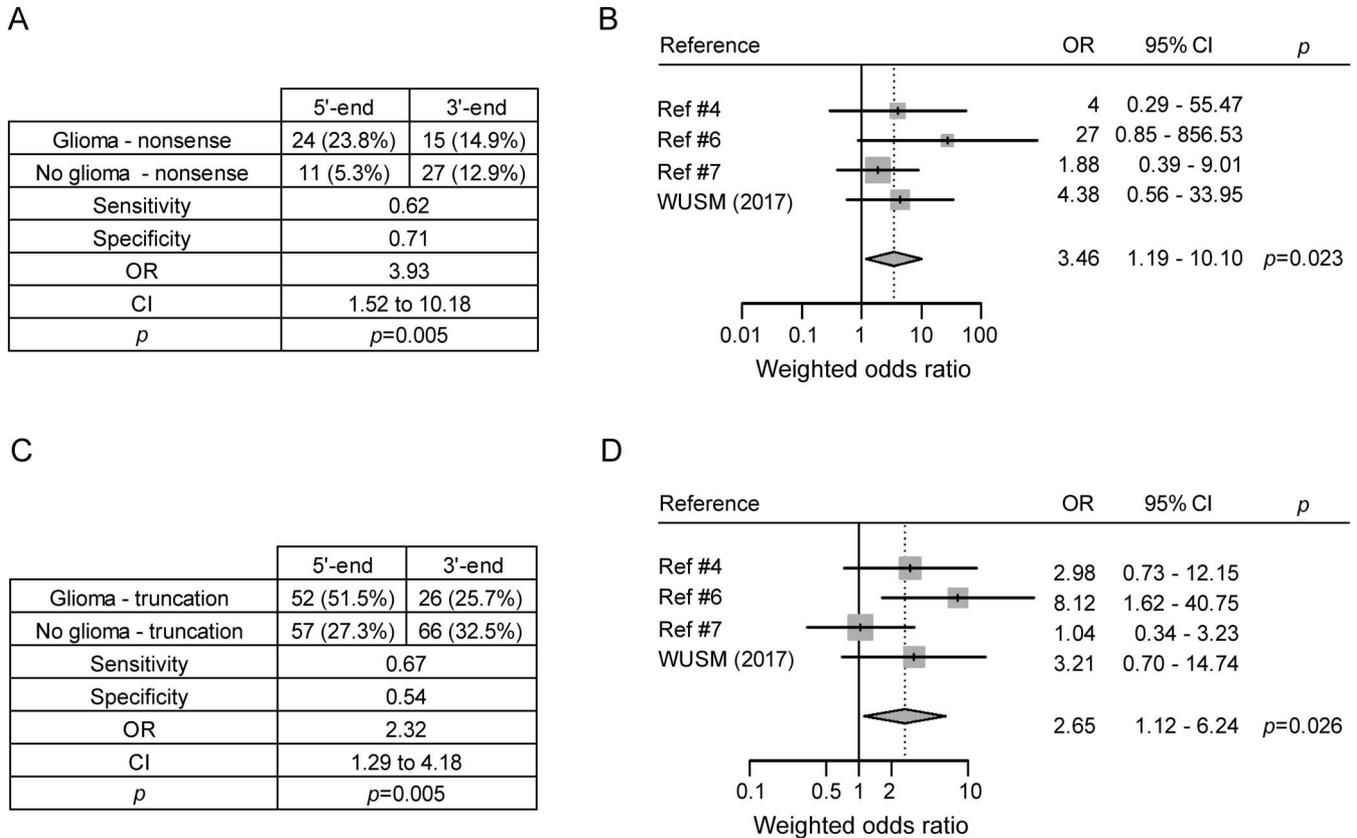
(A) Histogram indicating the location of *NF1* gene mutations identified in participants with glioma ($n = 101$; black bars) or without glioma ($n = 209$; gray bars). The different regions of the *NF1* gene are highlighted below the exons (5' end: exons 1–26 and 3' end: exons 27–57, including the RAS-GAP domain). The specificity, sensitivity, and unadjusted odds ratio (OR) for glioma in participants with any *NF1* gene mutation are shown in the table above the graph. (B) Summary of the combined weighted OR after sensitivity meta-analysis ($n = 296$ patients). CI = confidence interval; WUSM = Washington University School of Medicine.

DISCUSSION In the era of precision medicine, it becomes critical to identify risk factors that influence disease pathogenesis and clinical variability. This problem is illustrated by *NF1*, where no robust predictive factors have been identified. Based on insights derived from the use of *NF1*-patient-induced pluripotent stem cells¹⁰ coupled with emerging genotype-phenotype correlations,^{2,3} one potential factor important for conferring disease variability is the germline *NF1* mutation. Here,

we perform the largest genotype-phenotype analysis to determine whether the position or type of the *NF1* gene mutation is a risk factor for glioma.

First, *NF1* participants who harbor *NF1* mutations proximal to the RAS-GAP domain are more likely to develop gliomas, similar to two previously published reports.^{5,6} Of interest, *NF1* mutations predicted to produce premature stop codons were also associated with higher glioma risk.⁴ This mutational

Figure 2 Specificity, sensitivity, and odds ratios for glioma in participants with neurofibromatosis type 1 and nonsense or truncating *NF1* gene mutations



(A) Summary of the specificity, sensitivity, and unadjusted odds ratio (OR) for glioma in participants with nonsense mutations in the 5' end of the *NF1* gene (exons 1-26). (B) Summary of the combined weighted OR for glioma after sensitivity meta-analysis (*n* = 73 patients). (C) Summary of the specificity, sensitivity, and unadjusted OR for glioma in participants with truncating mutations in the 5' end of the *NF1* gene (exons 1-26). (D) Summary of the combined weighted OR for glioma after sensitivity meta-analysis (*n* = 193). CI = confidence interval; WUSM = Washington University School of Medicine.

specificity could reflect the impact of the mutation on protein expression¹⁰; however, this hypothesis will require further mechanistic exploration.

Second, each of the previous analyses used different statistical methods to look for genotype-phenotype correlations and included only modest numbers of participants. It is important that future studies incorporate power calculations to determine whether the analyses planned are sufficiently powered to detect differences. One limitation inherent in the combined analysis of multiple data sets is that the precise radiographic criteria used by each institution to diagnose gliomas could vary slightly. Moreover, it is critical to use clinically applicable analyses, such as OR calculations, as measures of risk factor significance.

Finally, while 5'-end mutations, and 5' nonsense or truncation mutations in particular, are associated with glioma, neither the location nor the mutation type is a highly specific marker of glioma (figure 1 and tables e-2 and e-4-e-7). We interpret this result to indicate that the germline mutation alone is not a sufficiently robust risk factor to provide prognostic

information to families. Future studies that incorporate multiple independently significant risk factors may yield useful predictive assessment profiles for children with NF1.

AUTHOR CONTRIBUTIONS

C.A. collated the data, performed the analyses, and wrote the manuscript. S.M.M. performed the statistical analyses and participated in the generation of the final manuscript. F.G., as the study biostatistician, performed the meta-analysis and reviewed all biostatistical analyses. D.H.G. collated the data from Washington University, provided the funding, and was responsible for generation of the final manuscript.

STUDY FUNDING

D.H.G. is supported by a Research Program Award from the NINDS (1-R35-NS097211-01), while S.M.M. is supported by the Neurological Sciences Academic Development Award (K12 NS001690).

DISCLOSURE

C. Anastasaki reports no disclosures. S.M. Morris has received research support from the NIH. F. Gao reports no disclosures. D. Gutmann has served on the American Association for Cancer Research grant review panel and the National Institute for Neurological Disorders and Stroke Advisory Council; has served on the editorial boards of *Glia* and *Familial Cancer*; holds the following patents: Identification of the NF1 gene and protein (NF1 Gene, 1999) Identification of NF1 protein as mTOR

regulator (mTOR, 2012); has received research support from US Army Department of Defense, NCI Tumor Microenvironment Network (TMEN) U01, the NIH, The Giorgio Foundation, the NF1-Dermal Neurofibroma Consortium, the Children's Tumor Foundation Synodos, and the Low-Grade Glioma Consortium; receives license fee payments for TSC1 knockout mouse; and receives royalty payments from the University of Michigan. Go to Neurology.org/ng for full disclosure forms.

Received June 16, 2017. Accepted in final form August 15, 2017.

REFERENCES

1. Anastasaki C, Le LQ, Kesterson RA, Gutmann DH. Updated nomenclature for human and mouse neurofibromatosis type 1 genes. *Neurol Genet* 2017;3:e169. doi: 10.1212/NXG.000000000000169.
2. Upadhyaya M, Huson SM, Davies M, et al. An absence of cutaneous neurofibromas associated with a 3-bp inframe deletion in exon 17 of the NF1 gene (c.2970-2972 delAAT): evidence of a clinically significant NF1 genotype-phenotype correlation. *Am J Hum Genet* 2007;80:140–151.
3. Rojnueangnit K, Xie J, Gomes A, et al. High incidence of noonan syndrome features including short stature and pulmonary stenosis in patients carrying NF1 missense mutations affecting p.Arg1809: genotype-phenotype correlation. *Hum Mutat* 2015;36:1052–1063.
4. Ars E, Kruyer H, Morell M, et al. Recurrent mutations in the NF1 gene are common among neurofibromatosis type 1 patients. *J Med Genet* 2003;40:e82.
5. Sharif S, Upadhyaya M, Ferner R, et al. A molecular analysis of individuals with neurofibromatosis type 1 (NF1) and optic pathway gliomas (OPGs), and an assessment of genotype-phenotype correlations. *J Med Genet* 2011;48:256–260.
6. Bolcekova A, Nemethova M, Zatkova A, et al. Clustering of mutations in the 5' tertile of the NF1 gene in Slovakia patients with optic pathway glioma. *Neoplasma* 2013;60:655–665.
7. Hutter S, Piro RM, Waszak SM, et al. No correlation between NF1 mutation position and risk of optic pathway glioma in 77 unrelated NF1 patients. *Hum Genet* 2016;135:469–475.
8. Mahdi J, Shah AC, Sato A, et al. A multi-institutional study of brainstem gliomas in children with neurofibromatosis type 1. *Neurology* 2017;88:1584–1589.
9. Listernick R, Ferner RE, Liu GT, Gutmann DH. Optic pathway gliomas in neurofibromatosis-1: controversies and recommendations. *Ann Neurol* 2007;61:189–198.
10. Anastasaki C, Woo AS, Messiaen LM, Gutmann DH. Elucidating the impact of neurofibromatosis-1 germline mutations on neurofibromin function and dopamine-based learning. *Hum Mol Genet* 2015;24:3518–3528.

Neurology[®] Genetics

Children with 5'-end *NF1* gene mutations are more likely to have glioma

Corina Anastasaki, Stephanie M. Morris, Feng Gao, et al.

Neurol Genet 2017;3;

DOI 10.1212/NXG.0000000000000192

This information is current as of September 22, 2017

Neurol Genet is an official journal of the American Academy of Neurology. Published since April 2015, it is an open-access, online-only, continuous publication journal. Copyright © 2017 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology. All rights reserved. Online ISSN: 2376-7839.



Updated Information & Services	including high resolution figures, can be found at: http://ng.neurology.org/content/3/5/e192.full.html
Supplementary Material	Supplementary material can be found at: http://ng.neurology.org/content/suppl/2017/09/22/3.5.e192.DC1
References	This article cites 10 articles, 3 of which you can access for free at: http://ng.neurology.org/content/3/5/e192.full.html##ref-list-1
Citations	This article has been cited by 2 HighWire-hosted articles: http://ng.neurology.org/content/3/5/e192.full.html##otherarticles
Subspecialty Collections	This article, along with others on similar topics, appears in the following collection(s): Case control studies http://ng.neurology.org/cgi/collection/case_control_studies Neurofibromatosis http://ng.neurology.org/cgi/collection/neurofibromatosis Primary brain tumor http://ng.neurology.org/cgi/collection/primary_brain_tumor Risk factors in epidemiology http://ng.neurology.org/cgi/collection/risk_factors_in_epidemiology
Permissions & Licensing	Information about reproducing this article in parts (figures,tables) or in its entirety can be found online at: http://ng.neurology.org/misc/about.xhtml#permissions
Reprints	Information about ordering reprints can be found online: http://ng.neurology.org/misc/addir.xhtml#reprintsus

Neurol Genet is an official journal of the American Academy of Neurology. Published since April 2015, it is an open-access, online-only, continuous publication journal. Copyright © 2017 The Author(s). Published by Wolters Kluwer Health, Inc. on behalf of the American Academy of Neurology.. All rights reserved. Online ISSN: 2376-7839.

