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

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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_72delAAT[2]; p.Arg1809[3]). 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**

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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[1]) 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
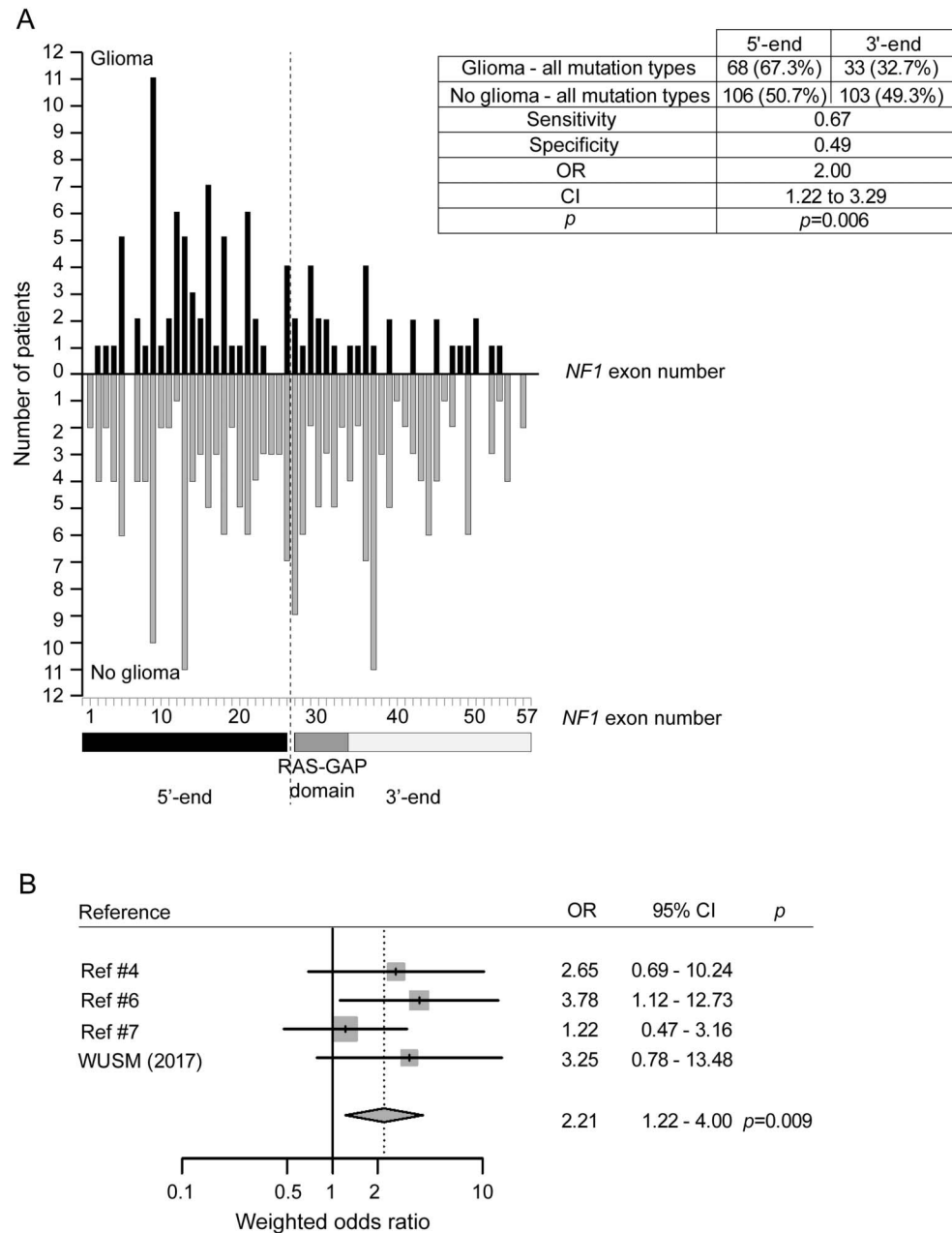
type or location and glioma diagnosis was found when each of the 3 previously published cohorts that contained participants with and without glioma were analyzed individually[4,6,7] (table e-2).

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

	5'-end	3'-end
Glioma - all mutation types	68 (67.3%)	33 (32.7%)
No glioma - all mutation types	106 (50.7%)	103 (49.3%)
Sensitivity	0.67	
Specificity	0.49	
OR	2.00	
CI	1.22 to 3.29	
p	*p*=0.006	

B

Reference		OR	95% CI	*p*
Ref #4		2.65	0.69 - 10.24	
Ref #6		3.78	1.12 - 12.73	
Ref #7		1.22	0.47 - 3.16	
WUSM (2017)		3.25	0.78 - 13.48	
		2.21	1.22 - 4.00	*p*=0.009

Weighted odds ratio

(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 cells[10] 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
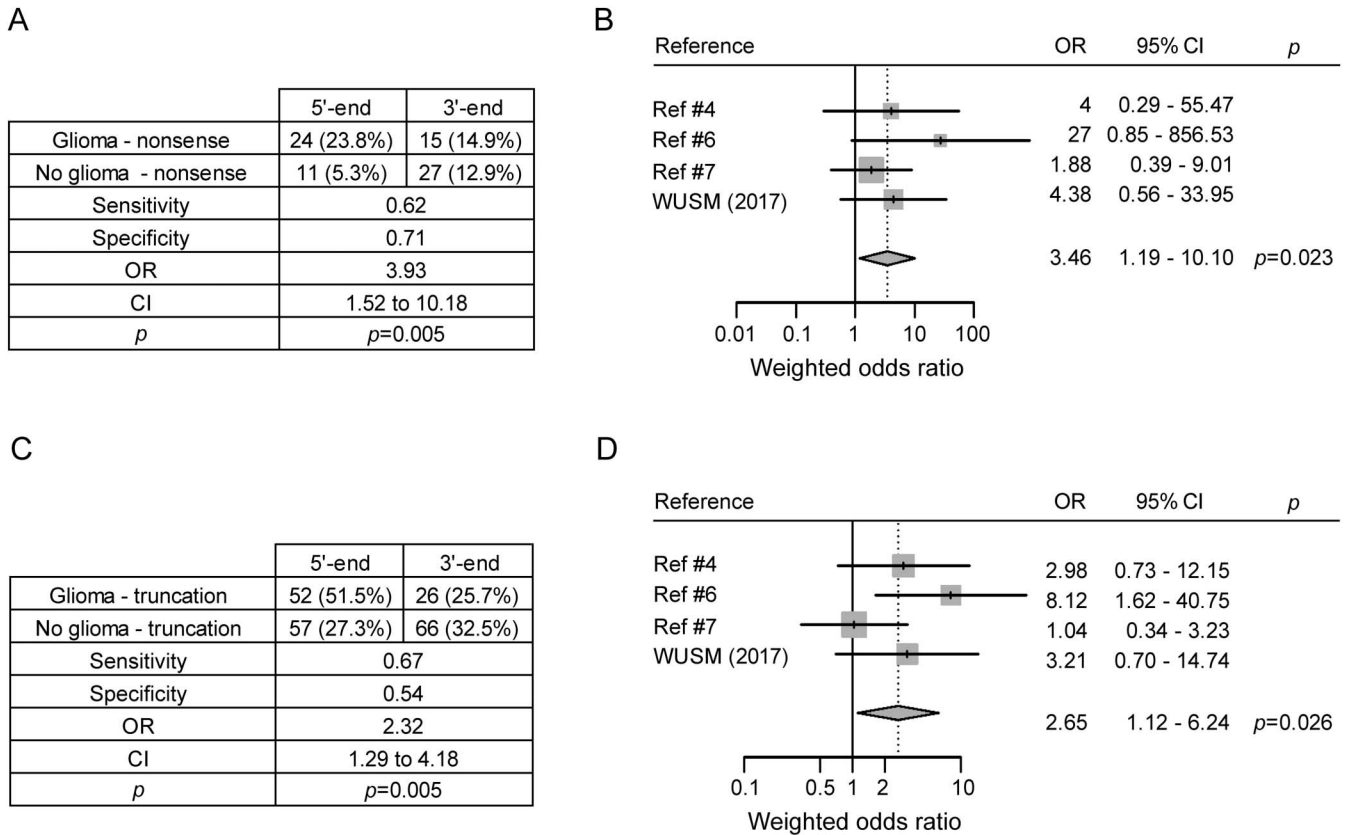
(A) Summary of the specificity, sensitivity, and unadjusted odds ratio (OR) for glioma in participants with nonsense mutations in the 5′ end of the *NF1* gene (exons 1–26). (B) Summary of the combined weighted OR for glioma after sensitivity meta-analysis (n = 73 patients). (C) Summary of the specificity, 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 expression[10]; 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.

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