UNIVERSITY OF CINCINNATI

I, ____________________________,
hereby submit this work as part of the requirements for the degree of:
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Medical Genetics

It is entitled:
Central Nervous System Associations in Neurofibromatosis Type 1

This work and its defense approved by:
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Central Nervous System Associations in Neurofibromatosis

Type 1

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Abstract

Neurofibromatosis type 1 (NF1) frequently involves the central nervous system (CNS), but there is extreme variability in the expression of CNS complications, even within families with the same causative mutation. It has been proposed, but not proven, that CNS complications tend to cluster within individuals with NF1. We conducted a retrospective medical record review to determine if CNS abnormalities occur more frequently in individuals with NF1 who have an optic pathway glioma (OPG). Seventy-two (72) subjects with OPG and 189 without OPG were screened for CNS complications. OPGs were found to be associated with a diagnosis of additional CNS tumors and T2 hyperintensities, supporting a difference in CNS complications manifested by patients who develop OPG versus those who do not. Additional understanding of the biology of brain lesions in NF1 is necessary to further elucidate why patients with OPG are more likely to develop additional CNS lesions.

Keywords: neurofibromatosis type 1 (NF1), optic pathway glioma (OPG), central nervous system (CNS)
Acknowledgements

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Introduction

Neurofibromatosis type 1 (NF1) is a dominantly inherited genetic condition that affects approximately 1 in 3500 individuals (Szudek et al 2000). It is characterized by an increased risk for vision loss, benign and malignant tumors, learning difficulties, and skeletal problems. The NF1 gene is a tumor suppressor gene that encodes for the protein neurofibromin, which is expressed in many cells of the central and peripheral nervous system, including neurons, oligodendrocytes, and Schwann cells (Daston et al 1992). Thus, mutations in the NF1 gene lead to abnormal cell growth and tissue differentiation of the nervous system (Gill et al 2006).

There are many findings associated with NF1; the clinical phenotype is highly variable (Friedman and Birch 1997b). A diagnosis of NF1 is made when a patient has 2 or more of the following: six or more café-au-lait spots; two or more neurofibromas of any type or 1 or more plexiform neurofibroma; freckling in the armpit or groin regions; tumor of the optic pathway; two or more lisch nodules; distinctive bony lesion such as dysplasia of the sphenoid bone or thinning of the cortex of the long bones; and a first-degree relative with NF1 (DeBella et al 2000). There is also extreme variability in the expression of central nervous system (CNS) complications in individuals with NF1, even within families with the same causative mutation.

A great deal of research has been conducted in the area of CNS abnormalities associated with NF1 (Creange et al 1999; Duffner et al 1989; Listernick et al 1989). Individuals with NF1 are at an increased risk for developing several types of CNS neoplasia, including optic pathway gliomas (OPGs) and brainstem gliomas (Gutmann et al 1997). OPGs are the most common of the CNS tumors, occurring in approximately
15% of children with NF1 (Pollack and Mulvihill 1996). They are defined as grade I pilocytic astrocytomas and can affect the prechiasmatic and chiasmatic portion of the optic nerve as well as the optic tract (Listernick et. al 1999). OPGs most often develop in the first decade of life, but rarely require intervention (Parsa et. al 2001). OPGs that affect the optic chiasm are more likely to affect vision and require treatment, often with symptoms showing before the age of 6 years (King et. al 2003). Though OPGs have been associated with significant morbidity in NF1, they have also been shown to spontaneously regress in some individuals (Parsa et. al 2001).

T2 hyperintensities (also known as unidentified bright objects or UBOs) are also associated with NF1. These brain lesions are areas of increased signal intensity on magnetic resonance imaging (MRI) found in 60-80% of children with NF1 (Pollack and Mulvihill 1996). They occur most commonly in the cerebellum, basal ganglia, and brainstem (Duffner et. al 1989). Their frequency decreases with age and they are rarely seen in the third decade of life or later (Gill et. al 2006). The underlying pathology of these lesions is not clear, but it has been proposed that they represent areas of fluid within the myelin resulting from dysplastic glial cell proliferation (DiPaolo et. al 1995). They are considered to be characteristic of NF1 (Raininko et. al 2001). Seizures are an additional CNS finding that have been reported in individuals with NF1. Studies have reported that approximately 4-6% of children with NF1 have one or more seizures, a prevalence two-three times greater than that of the general population (Ferguson et. al 2005; Korf et. al 1993; Kulkantrakorn and Geller 1998). Furthermore, nearly 25% of patients have macrocephaly, defined as a head circumference greater than or equal to two standard deviations above the general population average (Szudek et. al 2000).
The variability seen in the expression of complications can be difficult for families and health care professionals alike. Currently, there is no way to predict which complications an individual will manifest in his or her lifetime. Genotype-phenotype correlation studies have failed to suggest a way to predict outcome in NF1. It has been shown that patients with large deletions in the NF1 gene are more likely to have mental retardation, facial dysmorphism, malignant peripheral nerve sheath tumors (MPNSTs) and/or an increased frequency of cutaneous neurofibromas. However, not all patients with a deletion exhibit these phenotypic features and not all patients with these features have a gene deletion (Upadhyaya et al. 1998; Upadhyaya et al. 2006). Furthermore, the variability in phenotype seen among family members makes it unlikely that a particular gene mutation alone causes a specific phenotype.

It is not known, therefore, whether some individuals are more susceptible to certain complications of NF1 or if there is equal susceptibility in all individuals. It has been proposed, but not proven, that CNS complications tend to cluster within certain individuals with NF1. Friedman and Birch (1997a) reported an association between OPGs and other CNS neoplasms in patients with NF1. They did not find a similar association between OPGs and non-CNS neoplasms. Our study looked at CNS neoplasms as well as other CNS abnormalities including seizures, headaches, and macrocephaly. The purpose of this study was to determine if CNS abnormalities occur more frequently in children with OPG. Determination of a high-risk group would provide more specific anticipatory guidance to families and may help with management and treatment of patients with NF1.
Methods

Study Subjects

IRB approval was obtained from Cincinnati Children’s Hospital and University of Cincinnati for the review of medical records. A clinical neurofibromatosis (NF) database was used, which included patients of all ages with a diagnosis of NF seen in the NF clinic at Cincinnati Children’s Hospital since 1992. This NF clinic is a multi-disciplinary clinic that receives referrals for a wide variety of indications. Clinic protocol includes routine brain MRI at age of diagnosis or at 15 months old, whichever comes later. MRIs are only repeated if an abnormality is detected. Patients with OPG identified on brain MRI scans have repeat scans every 3-6 months until the lesion appears stable.

A total of 621 subjects in the database met the criteria of the National Institutes of Health for a diagnosis of NF1 (DeBella et. al 2000). Subjects included in the study were those who had at least one brain MRI scan between the ages of 15 months and 14 years, had been followed in NF clinic for at least two years, and had their first visit in NF clinic before the age of 15. These inclusion criteria were specified to ensure that T2 hyperintensities and other brain findings were adequately assessed. Subjects excluded from the study were those who did not have an MRI scan between the ages of 15 months and 14 years, had not been followed for at least two years in NF clinic, had been over 14 years old at the time of their initial visit, and/or those with incomplete information in the database or medical record regarding OPG, headaches, seizure disorder, macrocephaly, T2 hyperintensities, or brainstem gliomas.
**Medical Record Review**

A retrospective medical record review was conducted using the clinical database described above and NF Clinic patient charts. Medical records of NF clinic visits and magnetic resonance imaging (MRI) reports were reviewed for each subject who met the inclusion criteria. All MRIs were interpreted by a neuro-radiologist familiar with NF1. Subjects were divided into two groups, those with OPG and those without. Each subject was further screened for the presence of six additional CNS abnormalities, including: T2 hyperintensities, seizure disorder, headaches, macrocephaly, brainstem glioma, and “other” findings on brain MRI scan. Headaches were defined as more than one headache per month reported at any clinic visit. Macrocephaly was defined as a head circumference greater than two standard deviations above the mean (>98th percentile). “Other” MRI findings were defined as brain abnormalities reported in one or more written MRI report.

**Data Analysis**

To determine whether the two groups of patients were equivalent with respect to demographic variables, the frequencies of males and females, and Caucasians, African Americans, and “Others” were calculated and compared using chi-square analyses. The frequencies of CNS abnormalities and MRI findings were determined in 1) the full sample of subjects, 2) in the subset of subjects who have optic pathway gliomas and 3) the subset that do not have optic pathway gliomas. The frequency of each CNS abnormality and MRI finding was compared in the group of patients with OPG to the group without OPG using chi-square analyses. Mean ages at first and last MRI scan were also calculated for each group and compared using t-tests. A value of $p \leq 0.05$ was
considered to be statistically significant. Data analyses were performed with SAS® software, version 9.1 and EpiInfo™, version 6.

Results

A total of 621 subjects in the clinical database who met the criteria for a diagnosis of NF1 were screened for this study, 108 of whom had an OPG (17%). Two hundred sixty one (261) subjects from the total group met the inclusion criteria with complete records; 72 had an OPG and 189 did not. The frequency of OPG in the final study population of 261 subjects (27%) was different from the frequency of OPG in the total sample (17%), likely due to the fact that patients with OPG were more likely to have prolonged follow-up and more complete records. Of those subjects with an OPG, 37 (51.39%) had involvement of the optic chiasm and 18 (25%) had an OPG that required treatment.

Table I summarizes the demographics of the study population. There were more females in the OPG group compared to the group without OPG. This difference was statistically significant (p=0.0428). However, there were no significant differences in the frequencies of other CNS abnormalities when controlling for sex. Though the number of African Americans in the study was small (n=19), African Americans were less frequent in the OPG group, consistent with a previously reported study suggesting that fewer African Americans develop OPG (Saal et. al 1995).

In the sub-group with OPG, 11 subjects (15.28%) had a second CNS tumor, compared to 8 subjects (4.23%) without OPG having a CNS tumor. This difference was statistically significant (p= 0.0021) and suggests an association between the presence of
OPG and other CNS tumors. The CNS tumors detected in this study population are described in Table II. There were no significant differences in other specific findings on brain MRI, including orbital plexiform neurofibromas, Chiari malformations, tonsillar ectopia, arachnoid cysts, and vascular anomalies, as reported in Table III. However, when comparing all MRI findings combined, there were significantly more brain MRI findings in subjects with OPG than those without OPG (p= 0.0218).

The frequencies of CNS abnormalities in the total subject population and the sub-groups with and without OPG are reported in Table IV. Significant differences were found in the frequencies of T2 hyperintensities and headaches between the two groups. T2 hyperintensities were more common in subjects with OPG. The difference in frequency between the two groups could be due to the fact that subjects with OPG had an earlier mean age at first brain MRI scan (5.31 years) and later age at last brain MRI scan (9.26 years) than subjects without OPG (6.40 years and 7.72 years respectively). The difference in age at first MRI scan approached statistical significance (p=0.0517) while the difference in age at last MRI scan was statistically significant (p=0.0228). This, along with the fact that patients with OPG had more brain scans, could account for some of the difference seen.

Discussion

There are many potential CNS complications related to a diagnosis of NF1 and currently no predictors for which complications an individual will manifest in his or her life. Such uncertainty is understandably difficult for families; being able to define a high-risk or low-risk group would be useful for both families and health care providers alike.
OPGs are the most common CNS tumor associated with NF1 and have been shown to be associated with other CNS tumors in the past (Friedman and Birch 1997a; Kuenzle et. al 1994). Friedman and Birch found a strong association between both symptomatic and asymptomatic OPGs and other CNS tumors (gliomas) in 684 patients with NF1. Our study confirmed that individuals with OPG are more likely to have other CNS tumors than those without OPG. These tumors included cerebellar tumors and low-grade gliomas that enhanced on imaging studies. Furthermore, our results showed that subjects with OPG are more likely to have T2 hyperintensities on brain MRI scan as well as one or more additional MRI finding than subjects without OPG.

The significance of T2 hyperintensities is largely unknown, but they are the most common MRI abnormality detected in patients with NF1 (Pollack and Mulvihill 1996). Due to their high prevalence in our study population, we did not include them in the category of “1 or more MRI finding” because our frequencies would have reached nearly 100%. These lesions are generally benign and follow a stable course, often disappearing in young adulthood. It has been proposed that they represent areas of dysplastic glial cell proliferation leading to abnormal myelination (DiPaolo et. al 1995). The fact that subjects with OPG were more likely to have both T2 hyperintensities and additional CNS tumors, and the fact that OPG and T2 hyperintensities have both been shown to spontaneously regress, raises the question of whether there is a similar mechanism of formation or common precursor between these brain lesions.

The other CNS abnormalities studied, including seizure disorder, headache, and macrocephaly, were not increased in frequency in subjects with OPG. The frequency of seizure disorder in our study population was slightly higher than previously reported for
NF1, at 8% compared to previous studies reporting a 4-6% prevalence (Korf et. al 1993; Kulkarntrakorn and Geller 1998). Seizures were more frequent in those with OPG (11.11%) than those without (7.41%); however, this difference was not significant. The frequency of macrocephaly was greater in our study population (44%) than in a previously reported study (24%) by Szudek et al. (2000). Headaches have been previously reported to affect approximately 30% of patients with NF1, a prevalence similar to that of the general population (Clementi et. al 1996). In our sample, 44% of subjects reported more than one headache per month at a clinic visit. Interestingly, headaches were reported more frequently in subjects without OPG, a finding that has not been reported in previous studies to our knowledge.

Limitations of the study design include the retrospective nature of the study as well as the use of patients from a single NF clinic. However, use of one clinic reduces heterogeneity in diagnosis and management. Additionally, patients who have never had brain MRI imaging were excluded from the study and could represent individuals with a milder course of disease, thus biasing our sample towards individuals with more complications. However, nearly 80% of subjects in the clinical database had at least 1 MRI scan; thus, most exclusions from the study were due to lack of follow-up in clinic, being over the age of 14 at first clinic visit, and missing data in the medical record rather than lack of MRI scan. Our results may have additional bias in that patients with OPG generally have multiple MRI scans, making it more likely that asymptomatic lesions, such as other gliomas, would be detected in this group on follow-up scans. Many statistical tests were performed in the analysis; thus, it is possible that some false
positives may have been obtained by chance. Results should be interpreted with caution and need to be replicated in additional NF populations.

In our study population, patients with NF1 and OPG appear to be at a higher risk to be diagnosed with a second CNS tumor (particularly gliomas) and T2 hyperintensities on brain MRI scan, but do not appear to be at a higher risk for other CNS complications such as seizure disorder, headaches, and macrocephaly. These findings support the idea that there is a difference in CNS complications manifested by patients with NF1 who develop OPGs versus those who do not. Possible explanations could include a second hit event occurring in a CNS precursor cell responsible for the development of multiple tumor types, or modifier genes that have yet to be discovered. Additional investigation and understanding of the biology of brain lesions in NF1 is necessary to further elucidate why patients with OPG are more likely to develop additional CNS lesions.
Bibliography


Friedman JM, Birch P. 1997b. Type 1 neurofibromatosis: a descriptive analysis of the disorder in 1,728 patients. American Journal of Medical Genetics 70:138-143.


NF1 patients with malignant peripheral nerve sheath tumours (MPNSTs). Human Genetics 27(7):1-7.
Figure I. Exclusion criteria

621 Subjects with NF1

Excluded subjects who did not have a brain MRI between 15 mo and 14 yrs of age

487 Subjects with NF1

Excluded subjects not followed in NF clinic for 2 years or with missing information

261 Subjects with NF1

72 with OPG

189 without OPG
Table I. Subject demographics

<table>
<thead>
<tr>
<th></th>
<th>SUBJECTS WITH OPG N=72 (%)</th>
<th>SUBJECTS WITHOUT OPG N=189 (%)</th>
<th>TOTAL SUBJECTS N=261 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Males</td>
<td>28 (38.89)</td>
<td>100 (52.91)</td>
<td>128 (49.04)</td>
</tr>
<tr>
<td>Females</td>
<td>44 (61.11)</td>
<td>89 (47.09)</td>
<td>133 (50.96)</td>
</tr>
<tr>
<td><strong>Race</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>65 (90.27)</td>
<td>162 (85.71)</td>
<td>227 (86.97)</td>
</tr>
<tr>
<td>African American</td>
<td>3 (4.17)</td>
<td>16 (8.47)</td>
<td>19 (7.28)</td>
</tr>
<tr>
<td>Other</td>
<td>4 (5.56)</td>
<td>11 (5.82)</td>
<td>15 (5.75)</td>
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</table>

Table II. CNS tumors

<table>
<thead>
<tr>
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<th>SUBJECTS WITH OPG N=72</th>
<th>SUBJECTS WITHOUT OPG N=189</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brainstem glioma</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Cerebellar tumor</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Temporal lobe glioma</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Corpus callosum glioma</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Brachium pontis glioma</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Septum pellucidum glioma</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
**Table III. Brain findings on MRI**

<table>
<thead>
<tr>
<th>Subject Type</th>
<th>Subjects with OPG N=72 (%)</th>
<th>Subjects without OPG N=189 (%)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Orbital Plexiform</td>
<td>0 (0)</td>
<td>3 (1.59)</td>
<td></td>
</tr>
<tr>
<td>Chiari malformation</td>
<td>2 (2.77)</td>
<td>2 (1.05)</td>
<td>0.3121</td>
</tr>
<tr>
<td>Tonsillar ectopia</td>
<td>1 (1.39)</td>
<td>4 (2.12)</td>
<td>0.7015</td>
</tr>
<tr>
<td>Arachnoid cyst</td>
<td>2 (2.77)</td>
<td>3 (1.59)</td>
<td>0.5305</td>
</tr>
<tr>
<td>Vascular anomalies°</td>
<td>3 (4.17)</td>
<td>7 (3.70)</td>
<td>0.8617</td>
</tr>
<tr>
<td>Other CNS tumors^</td>
<td>11 (15.28)</td>
<td>8 (4.23)</td>
<td>0.0021*</td>
</tr>
<tr>
<td>1 or more MRI finding</td>
<td>19 (26.39)</td>
<td>27 (14.29)</td>
<td>0.0218*</td>
</tr>
</tbody>
</table>

* p <0.05  
° Carotid stenosis, moyamoya, carotid aneurysm, ischemia, venous angioma, encephalomalacia  
^ Brainsstem glioma, brachium pontis glioma, cerebellar tumor, septum pellucidum glioma, temporal lobe glioma

**Table IV. Frequencies of CNS abnormalities**

<table>
<thead>
<tr>
<th>Subject Type</th>
<th>Subjects with OPG N=72 (%)</th>
<th>Subjects without OPG N=189 (%)</th>
<th>Total Subjects N=261 (%)</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2 Hyperintensities</td>
<td>67 (93.06)</td>
<td>143 (75.66)</td>
<td>210 (80.46)</td>
<td>0.0015*</td>
</tr>
<tr>
<td>Seizure Disorder</td>
<td>8 (11.11)</td>
<td>14 (7.41)</td>
<td>22 (8.43)</td>
<td>0.3357</td>
</tr>
<tr>
<td>Headaches</td>
<td>19 (26.39)</td>
<td>89 (47.09)</td>
<td>108 (41.38)</td>
<td>0.0024*</td>
</tr>
<tr>
<td>Macrocephaly</td>
<td>34 (47.22)</td>
<td>82 (43.39)</td>
<td>116 (44.44)</td>
<td>0.5770</td>
</tr>
</tbody>
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* p <0.05