Alterations in Brain Morphology by MRI in Adults with NF1

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Search Terms: Neurofibromatosis Type 1, Brain Morphology, MRI, Neuropsychological assessment
ABSTRACT

Objective:

To explore and characterize alterations in brain morphology by MRI in adults with neurofibromatosis 1 (NF1).

Methods:

MRI measurements of 29 intracranial structures were obtained for 389 adults with NF1 and 112 age- and sex-matched unaffected control subjects. A subset of NF1 patients (n = 70) was also assessed for clinical severity of NF1 features and neurological problems and received psychometric testing for attention deficiencies and IQ. Brain morphological measurements were compared between NF1 and control subjects, and correlation analyses were performed between principal components of the intracranial measurements and clinical and psychometric features.

Results:

Four of nine corpus callosum measurements were significantly greater in adults with NF1 than in sex- and age-matched controls. All seven brainstem measurements were significantly greater in adults with NF1 than in controls. No robust correlations were observed between the size of these structures and clinical or neuropsychometric assessments.

Conclusions:

Our findings are consistent with the hypothesis that dysregulation of brain myelin production is an important manifestation of NF1 in adults.

INTRODUCTION

Neurofibromatosis 1 (NF1), an autosomal dominant disease caused by mutations of the NF1 gene, affects approximately 1 in 3000 live births\(^1\)-\(^3\). NF1 causes a wide range of clinical features: neurofibromas, café-au-lait macules, macrocephaly, learning disabilities, and attention deficits\(^4\)-\(^15\). Associated gliomas, malignant peripheral nerve sheath tumours, skeletal dysplasias, and cardiovascular disease may cause serious disability or death in affected children or young adults. The NF1 gene encodes neurofibromin, which is a negative regulator of the RAS cellular proliferation pathway\(^16\). Loss of NF1 function results in increased Schwann cell proliferation, a primary feature of NF1 pathology\(^16\).

Many studies have used magnetic resonance imaging (MRI) to study brain morphology in children and adults with NF1. The most frequent features found on MRI are increases in white matter volume, total brain volume, corpus callosum (CC) area, CC length, and optic nerve tortuosity (ONT)\(^6\),\(^7\),\(^9\),\(^10\),\(^17\)-\(^25\). However, the robustness of these findings is unclear, given the small number and young age of NF1 patients in most reported studies\(^10\).
Previous research has attempted to correlate brain morphological differences with cognitive or behavioural abnormalities in children with NF1, but inconsistent results have been observed\(^\text{10, 22, 26, 27}\). To date, there have been no large-scale MRI studies of brain morphological differences and their relationship to cognitive or behavioural abnormalities in adults with NF1.

We characterized brain morphological differences in adults with NF1 within three regions of interest – the CC, brainstem, and optic nerves and ocular globes – and examined associations of these changes with neuropsychometric findings among NF1 patients.

**METHODS**

**Participants**

Between 2003 and 2015, all patients seen in the NF Outpatient Department of the University Hospital Hamburg-Eppendorf in Hamburg, Germany, were offered brain MRI to monitor their intracranial tumour burden. Head MRI was obtained on 434 adults with NF1; each patient was imaged an average of 3.02 times. Participants were excluded if they had optic gliomas or brain tumors. Sex and age were recorded at the time of each examination. Unaffected control individuals, who had undergone head MRI for other reasons at Vancouver General Hospital, Vancouver, Canada, were age- (within 24 months) and sex-matched to 25% of randomly selected adults with NF1. Affected and unaffected participants were excluded if no image was available that permitted clearly defined morphological measurements. In total, 389 adults with NF1 and 112 unaffected controls were included in the morphological analysis.

A subset of adults with NF1 (n = 70) received clinical evaluations for NF1 severity, neurological severity, and ADHD severity, and neuropsychometric evaluations for attention deficiencies and IQ.

**Standard Protocol Approvals, Registrations, and Patient Consents**

The ethical committees of the Medical Chamber in Hamburg and the Research Ethics Board of the University of British Columbia approved the study. Written consent was obtained from all study participants before study begin. All data were de-identified before analysis.

**Morphological Measurements**

Head MRI exams were conducted in a 1.5 or 3.0 Tesla scanner. Multiple coronal, axial, and/or sagittal T1, T2, and FLAIR images were obtained. OsiriX Lite\(^\text{28}\) and IMPAX PACS (Agfa, Ridgefield Park, NJ) programs were used for NF1 and unaffected control MRI measurements respectively. Image slices were \(\leq 5.5\) mm thick for NF1 patients and \(\leq 6\) mm thick for unaffected populations. In total, 29 measurements were obtained for each MRI set for each participant. Eight measurements were obtained for the CC: area, length, height, genu width, anterior body width, mid-body width, posterior body width, and splenium width (Figure 1A). Seven
measurements were obtained for each of the ocular globes: anterior to posterior (AP) length, diameter, anterior to diameter (AD) length, anterior to interzygomatic line (AIZ) length, posterior to interzygomatic line (PIZ) length, optic nerve displacement (OND), and optic nerve path (ONP) (Figure 2A). Seven measurements were obtained for the brainstem: midbrain width, midbrain AP length, pons AP length, left and right middle cerebellar peduncle (MCP) lengths, medulla oblongata width, and medulla oblongata AP length (Figure 3A).

CC bulbosity was calculated by dividing the splenium width by the posterior body width. Ocular globe elongation was calculated by dividing AD length by AP length. Globe protrusion was calculated by dividing AIZ by the sum of AIZ and PIZ. Optic nerve tortuosity (ONT) was calculated by dividing ONP by OND.

**Clinical and Neuropsychometric Assessments**

**Clinical NF1 Severity**

Clinical NF1 severity for adults with NF1 (n = 70) was categorised into four grades by Dr. Victor Mautner using the Riccardi criteria\(^29,30\). Intellectual and psychological functioning were excluded from the NF1 clinical severity rating to avoid confounding between learning disability and medical severity.

**Clinical Neurological Severity**

Clinical neurological severity for adults with NF1 (n = 70) was categorised by Dr. Victor Mautner into one of four grades. Grade 1 encompasses no deficits. Grade 2 includes discrete neurological deficits only: muscle hypotonia, sensation deficits, balance problems, or speech problems. Grade 3 includes neurological dysfunction: paresis, ataxia, significant ocular motor deficits, or substantial pain. Grade 4 encompasses neurological deficits that seriously compromise health: intractable pain, paralysis, or drug-resistant seizures.

**Clinical Attention deficit hyperactivity disorder (ADHD) Severity**

Clinical ADHD severity for adults with NF1 (n = 50) was categorized into three grades using the established criteria in DSM-IV\(^31\). Grade 1 encompasses no clinical diagnosis. Grade 2 is subclinical attention deficit with no ADHD diagnosis. Grade 3 includes those with a diagnosis of either Attention Deficit Disorder (ADD) or ADHD.

**Intelligence quotient (IQ)**

Full-scale, verbal, and performance IQ were obtained for 61 adults with NF1 using the Wechsler Adult Intelligence Scale-Revised (WAIS-R)\(^32\). WAIS-R test scores were standardized according to age and sex.
Attention Deficiency Measurement

Attention deficiency measurements for adults with NF1 (n = 68) were obtained using the Visual Test of Variables of Attention, version 7.0.3 or 8.0. Measurements were obtained for variability of response time (consistency), response time, commission error (impulsivity), errors of omission (inattention), post-commission response times, and multiple and anticipatory responses. An Attention Comparison Score was calculated for each patient. All test scores were standardized according to age and sex.

Statistical Analysis

All data were analysed using R Studio 3.4.1. A False Discovery Rate (FDR) adjusted significance level of p < 0.05 was used throughout to account for multiple comparisons.

Demographic Analysis

Mean age was compared between adults with NF1 and unaffected controls using the Student-T test after demonstrating satisfactory dataset normality and variance using the Shapiro-Wilk test and Fisher’s F test, respectively. Sex ratios were compared between adults with NF1 and unaffected controls using the χ² test.

Morphological Analysis

Means of the 26 different brain morphological measurements were compared between adults with NF1 and unaffected controls. Student-T tests were used for normally distributed data. Non-normal distributions were compared using Mann-Whitney U tests. CC bulbosity means were compared between males and females for both adults with NF1 and unaffected adults using two-way ANOVA test. Dataset normality and variance were determined using the Shapiro-Wilk test and Fisher’s F test respectively. Significance (p-value) was adjusted using FDR to account for 26 comparisons.

Brain Morphology - Neuropsychometric Correlation Analysis

Brain morphology measurements for the CC, ocular globes, and brainstem were independently grouped using principal component analysis (PCA) to maximize the number of patients included in the PCA. PCs for the correlation analysis were selected if the eigenvalue was greater than one. Clinical NF1 severity, clinical neurological severity, and clinical ADHD diagnosis were analysed as ordinal data. Attention Comparison Score was analysed as a continuous measurement of attention deficiency. Total IQ scores from WAIS-R were analysed as a continuous measurement of intelligence. PCs from the three brain structures were independently compared to the neuropsychometric measurements using Pearson correlation for continuous data and Spearman’s Rank-Order correlation for ordinal data. No significance testing was conducted and p-values were not adjusted.
Data Availability Statement

Anonymous data is available for appropriate research purposes through V.F. Mautner, MD.

RESULTS

Demographics

This study included 494 participants: 389 adults with NF1 and 112 age- (within 24 months) and sex-matched unaffected controls. There were no statistically significant differences in age or sex ratio distribution between adults with NF1 and unaffected controls overall or in the subgroups in whom the three brain structures (CC, ocular globes, and brainstem) were measured by MRI (Table 1).

Brain Morphology Comparison

Four corpus callosum measurements (midsagittal length, height, anterior body width, mid body width) were significantly greater and one (genu width) was significantly shorter in adults with NF1 than in unaffected control participants (Figure 1B, C). CC length, posterior body width, splenium width, and CC bulbosity did not differ between the two groups (Figure e-1). The significance was determined after FDR adjustment.

Of the five measurements made for each ocular globe, only the AP lengths were significantly reduced in both eyes in adults with NF1 compared to unaffected controls (Figure 2B). Optic nerve tortuosity was greater among adults with NF1 than among unaffected individuals. This difference was small and reached statistical significance only on the left side.

The brainstems of individuals with NF1 were significantly larger than expected in all seven sites measured (midbrain width, midbrain AP length, pons AP length, left and right MCP lengths, medulla oblongata width, and medulla oblongata AP length) (Figure 3E). The averages, standard deviations, and number of individuals analysed are summarised in Table e-1.

Morphology - Neuropsychometric Correlations

Twenty-nine adults with NF1 had complete datasets for the clinical and neuropsychometric assessments as well as for the CC measurements analysed. The top three PCs were selected for the CC as they each had an eigenvalue greater than one (Figure e-2A). None of the CC PCs were strongly correlated with any of the five clinical assessments or neuropsychometric measurements (Figure 4A). Some stronger correlations were: increased clinical ADD/ADHD severity is correlated with increased neurological severity ($r_s = 0.55$, unadjusted $p = 0.032$) and with decreased clinical severity ($r_s = -0.49$, unadjusted $p = 0.031$). The correlation ($r$ and $r_s$) values, and unadjusted $p$-values are summarized in Table e-2.

Twenty-six adults with NF1 had complete datasets for the clinical and neuropsychometric assessments and brainstem measurements analysed. The top three PCs were selected for the brainstem as they had eigenvalues greater than one (Figure e-2C). PC1 was inversely correlated
with both neurological severity ($r_s = -0.51$, unadjusted $p = 0.019$) and clinical ADD/ADHD diagnosis ($r_s = -0.49$, unadjusted $p = 0.043$). The three largest weights for PC1 were the right MCP length (-0.42), left MCP length (-0.41), and the pons AP length (-0.40). Thus, a smaller PC1 value indicates an overall thicker middle brainstem. The relationship between smaller PC1 and both clinical and neurological severity indicates that a thicker middle brainstem is correlated with greater clinical and neurological severity. Clinical severity was inversely correlated with clinical ADD/ADHD diagnosis ($r_s = -0.56$, unadjusted $p = 0.018$), and neurological severity was inversely correlated with IQ ($r_s = -0.38$, unadjusted $p = 0.0068$) (Figure 4B). The correlation ($r$ and $r_s$) values, and unadjusted p-values are summarized in Table e-3.

Forty-four adults with NF1 had complete datasets for the clinical and neuropsychometric assessments and ocular globe measurements analysed. The PCs that had eigenvalues greater than one (top five) were selected for the ocular globes (Figure e-2B). None of the variables were strongly correlated (Figure e-3). The correlation ($r$ and $r_s$) values, and unadjusted p-values are summarized in Table e-4.

**DISCUSSION**

We conducted the first large-scale MRI study of brain morphological differences and their relationship to cognitive or behavioural abnormalities in adults with NF1. We found that adults with NF1 have apparent enlargement of the corpus callosum and brainstem in comparison to unaffected adults. We did not find any obvious correlation between the brain morphological changes we observed and the clinical or neuropsychometric assessments in these patients.

Previous brain MRI studies in people with NF1 have found evidence of increased white matter volume, increased brain volume, and megalencephaly.$^6, 7, 9, 10, 17-20$ We were not able to study any of these measurements in our study as our MRI scans were not dedicated volumetric scans.

We observed increased CC area, height, and anterior body and mid-body widths in this large group of adults with NF1. Our findings are consistent with those of previous studies in children and smaller groups of adults with NF1.$^{21, 23}$ Loss of function of $NF1$ causes dysregulation of proliferation in Schwann cells, which is responsible for the myelination of axons in the peripheral nervous system.$^{16}$ In the brain, white matter is mainly comprised of myelinated axons.$^{36}$ It is hypothesized that the increases in white matter volume, total brain volume, and megalencephaly that occur in NF1 are related to the dysregulation of oligodendrocytes, the myelin-producing cells in the central nervous system.$^{21, 37}$ If this interpretation is correct, the observed enlargement of the CC, a structure composed of white matter, might be expected in people with NF1. The decrease in genu width and increase in the anterior and mid-body widths raises the possibility that the CC shape is changed. Additionally, enlargement of the CC height and area without significant alteration of the length is consistent with a change in CC shape and volume.
The enlargement of the brainstem that we observed among adults with NF1 is a novel finding. The brainstem consists of both grey and white matter, with the MCP (which was clearly enlarged among NF1 patients in this study) comprised mostly of white matter. Thus, the brainstem enlargement we observed is also consistent with dysregulated myelin proliferation in people with NF1.

Both age and sex are known to affect CC size, ocular globe size and position, and brainstem size. We carefully matched unaffected individuals by age and sex to avoid confounding by these factors in our analysis. However, the adult NF1 group was recruited from Hamburg, Germany, while the unaffected comparison group was obtained in Vancouver, Canada. Different MR imaging procedures and measurement software were used for the NF1 group and the unaffected comparison control group. The different imaging procedures resulted in an inability to detect differences between the two groups smaller than 0.05 cm, but all statistically significant differences in brain morphological measurements observed between NF1 and control subjects were greater than 0.05 cm.

Another limitation of our study is the lack of dedicated orbital MRIs. The asymmetry in the ONT we observed probably does not represent a true anatomical difference, and may be a result of measurement error, as previous papers have not found ONT asymmetry. The current study is less accurate than Ji et al.’s (2013) as we only measured ONT in one axial plane. Meanwhile, Ji and associate used dedicated 3-dimensional magnetization-prepared rapid gradient echo sequences with 1 mm slices.

Our correlation analyses between structures measured on MRI and clinical/ neuropsychometric assessments are limited by relatively small sample sizes as only a subset of our NF1 patients had psychometric testing. Some previous studies have found that increased CC volume or CC index correlated with decreased academic achievement and IQ in children with NF1. In contrast, Kayl et al. (2000b) found that decreased total corpus callosum volume is correlated with increased attention problems as reported by teachers and parents. Furthermore, a more recent study using diffusion tensor imaging to examine myelination of white matter specifically failed to find a significant relationship between total CC area and IQ scores in children with NF1. We did not find any robust correlations between the brain morphology and neuropsychometric measurements in adults with NF1.

We conducted the largest study of brain morphology in adults with NF1 reported to date. The apparent enlargement of the CC and brainstem that we observed in adult NF1 patients lends support to the hypothesis that neurofibromin haploinsufficiency causes dysregulation of myelin production in the brain. The relationship of this overgrowth of myelinated brain structures to the frequent occurrence of central nervous system gliomas and of benign and malignant peripheral nerve sheath tumours in NF1 patients is unknown but clearly merits further study.
## APPENDIX:

| Name                     | Location                                                                 | Role     | Contribution                                                                 |
|--------------------------|---------------------------------------------------------------------------|----------|------------------------------------------------------------------------------|
| Su Wang                  | Department of Medical Genetics, University of British Columbia, Children's & Women’s Hospital, 4480 Oak Street, Vancouver, Canada. | Author   | Designed experiment, collected morphological data, analyzed all data, drafted and revised manuscript for intellectual content. |
| Victor-Felix Mautner, MD, PhD | Department of Neurology, University Hospital Hamburg-Eppendorf, Hamburg, Germany. | Co-author | Recruited patients. Major role in the acquisition of MRI and neuropsychometric data, revised manuscript for intellectual content. |
| Jan M. Friedman*, MD, PhD | Department of Medical Genetics, University of British Columbia, Children's & Women’s Hospital, 4480 Oak Street, Vancouver, Canada. | Senior author | Designed experiment, revised manuscript for intellectual content. |
| Manraj K. S. Heran*, MD   | Diagnostic and Therapeutic Neuroradiology, University of British Columbia, Vancouver, Canada. | Senior author | Designed experiment, guided morphological data collection, revised manuscript for intellectual content. |

*These authors contributed equally to the manuscript.

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FIGURE LEGEND

Table 1: Participant demographics for adults with NF1 and matched unaffected controls. The demographics are analysed in total and in measurement groups.

Figure 1: Corpus callosum measurements and averages for adults with NF1 and unaffected control participants. (A) Representative MRI image showing T1-weighted midline sagittal view of a 33-year-old male with NF1. (B) Average CC midsagittal area. (C) Average CC measurements. Coloured lines and area outlined in (A) show where structures indicated by the same colour in (B) or (C) are measured. Error bars indicating one standard deviation and asterisks indicating FDR-adjusted statistical significance are shown in (B) and (C). p < 0.001 = ***. Abbreviations used in figure: Genu = genu width, Ant-body = anterior body width, Mid-body = mid-body width, Post-body = posterior body width, and Splenium = splenium width.

Figure 2: Ocular globe measurements and averages for adults with NF1 and unaffected control participants. (A) Representative MRI image showing T2-weighted axial view of a 25-year-old male with NF1. (B) Select average ocular globe measurements. Unique measurements are shown on one side but were obtained on both left and right side. Coloured lines in (A) show where structures indicated by the same colour in (B) are measured. Error bars indicating one standard deviation and asterisks indicating FDR-adjusted statistical significance are shown in (B). p < 0.5 = *; p < 0.001 = ***. Abbreviations used in the figure: L = left, R = right, AP = anterior to posterior length, AD = anterior to diameter length, AIZ = anterior to interzygomatic line length, IZ = interzygomatic line, PIZ = posterior to interzygomatic line length, OND = optic nerve displacement, and ONP = optic nerve path.

Figure 3: Brainstem measurements and averages for adults with NF1 and unaffected control participants. (A-D) Representative MRI images showing T1-weighted midline sagittal and T2-weighted axial views of a 25-year-old male with NF1. (E) Average brainstem measurements. The labeled lines in (A) show position of the midbrain (B), the pons and the middle cerebellar peduncle (C), and the medulla oblongata (D). Coloured lines in (B-D) show where structures indicated by the same colour in (E) are measured. Error bars indicating one standard deviation and asterisks indicating FDR-adjusted statistical significance are shown in (E). p < 0.001 = ***. Abbreviations used in the figure: L = left, R = right, AP = anterior to posterior length, and MCP = middle cerebellar peduncle.

Figure 4: Correlation matrixes of brain structures with clinical and neuropsychometric assessments in adults with NF1. (A) Top three corpus callosum PCs, and (B) top three brainstem PCs are correlated to neuropsychometric measurements (clinical severity, clinical neurological severity, clinical ADD/ADHD diagnosis, IQ, and ACS). The correlation matrix combines the Pearson correlation and Spearman correlation results. Pearson correlations was conducted for comparisons between clinical severity, clinical neurological severity, clinical ADD/ADHD diagnosis, and the PCs. Spearman correlations was conducted for comparisons between IQ, ACS, and the PCs. The colour scale represents the degree of correlation from -1 (red) to 1 (blue). The size of the circles also represents the size of the correlation, with values closer to -1 or 1 larger than those that are closer to 0. Abbreviations used in the figure: Clin. Sev.
clinical NF1 severity, Neuro. Sev. = clinical neurological severity, ADD/ADHD = clinical Attention deficit hyperactivity disorder severity, IQ = intelligence quotient, ACS = Attention Comparison Score, and PC = principal component.
|                | ADULTS WITH NF1 | UNAFFECTED CONTROLS | DIFFERENCE |
|----------------|----------------|---------------------|------------|
| **ALL SUBJECTS** |                |                     |            |
| N               | 389            | 112                 |            |
| Mean Age (SD)   | 37.37 (13.50)  | 39.42 (13.07)       | not significant |
| Age Range       | 18.12 - 72.92  | 19.38 - 73.26       |            |
| Female: Male    | 218:171 (56.04%) | 63:49 (56.25%)     | not significant |
| (Female Percentage) |            |                     |            |
| **SUBJECTS WITH MRI MEASUREMENTS OF CORPUS CALLOSUM** | | | |
| N               | 226            | 57                  |            |
| Mean Age (SD)   | 37.15 (13.65)  | 38.03 (13.22)       | not significant |
| Age Range       | 18.15 - 72.92  | 19.38 - 64.71       |            |
| Female: Male    | 126:100 (55.75%) | 31:26 (54.39%)     | not significant |
| (Female Percentage) |            |                     |            |
| **SUBJECTS WITH MRI MEASUREMENTS OF OCULAR GLOBES** | | | |
| N               | 386            | 96                  |            |
| Mean Age (SD)   | 37.44 (13.51)  | 39.63 (13.06)       | not significant |
| Age Range       | 18.12 - 72.92  | 19.38 - 73.26       |            |
| Female: Male    | 214:172 (55.44%) | 56:40 (58.33%)     | not significant |
| (Female Percentage) |            |                     |            |
| **SUBJECTS WITH MRI MEASUREMENTS OF BRAIN STEM** | | | |
| N               | 375            | 96                  |            |
| Mean Age (SD)   | 37.24 (13.35)  | 39.50 (13.10)       | not significant |
| Age Range       | 18.11 - 72.92  | 19.38 - 73.26       |            |
| Female: Male    | 209:166 (55.73%) | 56:40 (58.33%)     | not significant |
| (Female Percentage) |            |                     |            |
Figure 1: Corpus callosum measurements and averages for adults with NF1 and unaffected control participants.
Figure 2: Ocular globe measurements and averages for adults with NF1 and unaffected control participants.
Figure 3: Brainstem measurements and averages for adults with NF1 and unaffected control participants.
Figure 4: Correlation matrixes of brain structures with clinical and neuropsychometric assessments in adults with NF1.