INTRACRANIAL MANIFESTATIONS IN NEUROFIBROMATOSIS 1

by

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B.Sc., Ludwig Maximilians University, 2014

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE AND POSTGRADUATE STUDIES

(Medical Genetics)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

April 2017

Abstract

Introduction: Gliomas in the brain and the optic pathway affect up to 20% of all children with neurofibromatosis 1 (NF1); however, their frequency and natural history in adults is poorly described. Our objectives were to characterize the frequency and natural history of gliomas seen in NF1 patients by serial head magnetic resonance imaging (MRI) and to investigate associations between combined annotation-dependent depletion (CADD) scores and the presence of MRI features of NF1.

Methods: 1775 head and whole-body MRI scans of 562 unselected NF1 patients were collected at the University Hospital Hamburg-Eppendorf in Hamburg, Germany. All scans were analyzed, and the frequency and natural history of gliomas was determined. In addition, the constitutional disease-causing variants of the *NF1* gene in 283 patients were annotated with CADD scores, and genotype-phenotype correlations were performed.

Results: Between 1 and 12 MRI scans were collected for each patient; the median length of follow-up was 3.7 years. We found the prevalence of non-optic gliomas to be 4.3%, with a median age at glioma diagnosis at 21.2 years. The prevalence of optic pathway gliomas (OPGs) was 9.3%, with a median age at diagnosis at 12.1 years. We determined the rates of appearance, progression and regression of both of these tumour types. We found that individual CADD scores were associated with the presence of plexiform neurofibromas (but not with the presence of UBOs or optic gliomas) in NF1 patients in whom the pathogenic mutation had been identified.

Conclusion: The frequencies of gliomas in the brain and optic pathway is higher in adults with NF1 than previously reported. NF1 patients with constitutional mutations associated with high CADD scores appear to be at higher risk to develop plexiform neurofibromas than other NF1 patients.

Preface

The research for this project was conducted at the University of British Columbia at the BC Children's Hospital and at the Vancouver General Hospital. All projects were approved by the Children's and Women's Research Ethics Board of the University of British Columbia (certificates number H15-00068).

I contributed to identification and design of the research program. I translated all MRI reports from German to English and selected patients with gliomas for evaluation by the study neuroradiologists. I performed all data analysis and wrote all publications.

Dr. Marco Marangoni and Dr. Manraj Heran performed evaluation of patient head and whole-body MRIs at the Vancouver General Hospital.

A version of chapter 3.2 has been published. Laura Sellmer, S. Farschtschi, M. Marangoni, M. K. S. Heran, P. Birch, R. Wenzel, J. M. Friedman, V.-F. Mautner (2017) Non-optic gliomas in adults and children with neurofibromatosis 1. Orphanet Journal of Rare Diseases. 12(1):34. I translated all reports for this manuscript and selected patients with non-optic gliomas. I also performed the data analysis and wrote the manuscript.

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List of Abbreviations

ADCY8	Adenylyl cyclase type 8
BRAF	Rapidly Accelerated Fibrosarcoma homolog B
CADD	Combined annotation-dependent depletion
cAMP	Cyclic adenosine monophosphate
DNET	Dysembryoplastic neuroepithelial tumour
ESP	Exome Sequencing Project
ExAC	Exome Aggregation Consortium
FGFR1	Fibroblast growth factor receptor 1
FISH	Fluorescence in-situ hybridization
GDP	Guanosine diphosphate
GTP	Guanosine triphosphate
logMAR	Logarithm of the minimum angle of resolution
MAPK	Mitogen-activated protein kinase
MLPA	Multiplex ligation-dependent probe amplification
MPNST	Malignant peripheral nerve sheath tumour
MRI	Magnetic resonance imaging

mTOR	Mammalian target of rapamycin
NF1	Neurofibromatosis 1
NTRK2	Neurotrophic tyrosine kinase receptor type 2
OPG	Optic pathway glioma
PA	Pilocytic astrocytoma
PET	Positron emission tomography
PTPN11	Tyrosine-protein phosphatase non-receptor type 11
RAS	Rat sarcoma
WHO	World Health Organization

Acknowledgements

I would like to acknowledge Prof. Jan Friedman, who has been my mentor during this thesis. He has helped me with infinite wisdom and patience and showed me how to be a scientist. His questions made me think more deeply about NF1. Without his guidance, I would not have been able to get to this point.

I would also thank Prof. Victor-Felix Mautner, who provided all of the data used in this thesis and funded this work. He engaged me in many helpful discussions and gave me the possibility to see my research from a clinical perspective.

My great thanks go to the two other members of my Master's committee, Dr. Manraj Heran and Dr. Millan Patel. They made me see my work from different angles and think critically about my results.

I would also like to acknowledge Patricia Birch, who had the patience to answer my endless statistics questions, helped me out with using databases and always brought food.

I would also like to thank the rest of the Friedman lab. You have made work fun every day and always brought food when Patricia did not.

Chapter 1: Introduction

1.1 Diagnosis of NF1

Neurofibromatosis 1 (NF1) is a neurocutaneous disorder that was first described by the German pathologist Friedrich von Recklinghausen in 1882 [1]. It is an autosomal dominant disorder with an estimated incidence of 1 in 3000 live births [2] and is caused by mutations in the *NF1* gene, which lead to a lack of suppression of the rat sarcoma (RAS) pathway.

Diagnosis of an individual with NF1 can be based on clinical symptoms and supported by genetic analysis. The National Institutes of Health developed consensus criteria in 1987 that are still used today to diagnose NF1 in a clinical setting [3]. A diagnosis of NF1 can be made if 2 or more of the following 7 features are present:

- Six or more café-au-lait spots larger than 5 mm in prepubertal individuals and larger than 15 mm in postpubertal individuals
- Two or more neurofibromas of any type or at least one plexiform neurofibroma
- Axillary or inguinal freckling
- Optic glioma
- Two or more iris hamartomas (Lisch nodules)
- A distinctive bony lesion typical for NF1, such as sphenoid wing dysplasia or tibial pseudarthrosis

- A first-degree relative with an NF1 diagnosis according to the above-listed criteria

Pigmentary abnormalities (café-au-lait spots and axillary freckling) occur in up to 90% of affected individuals. Café-au-lait spots are typically between 1 and 3 cm in diameter and slightly darker than the individual's skin. Freckles in NF1 patients are no different than those in unaffected individuals; the only difference is their location, as they occur in areas that are not sun-exposed [4].

NF1-associated neurofibromas are present in the vast majority of adult patients; plexiform neurofibromas, however, are rare in children and only develop during late childhood or adolescence [5,6]. There are 3 types of neurofibromas: cutaneous, subcutaneous and plexiform neurofibromas. Cutaneous neurofibromas can be found in almost 100% of all NF1 patients [7], whereas plexiform neurofibromas affect only 50% of all adult patients [8]. Cutaneous and subcutaneous neurofibromas are focal lesions detectable by external examination. Even though they are benign, they may cause itching, tingling, and be in inconvenient locations (such as the belt line) [7]. NF1 patients with cutaneous or subcutaneous neurofibromas are at an increased risk of also developing internal plexiform neurofibromas [9]. Plexiform neurofibromas can be superficial enough to be found during an external exam or be entirely internal so that they can only be detected by using imaging techniques, such as magnetic resonance imaging (MRI). Even though these lesions are typically benign, they can cause pain, neurological dysfunction, obstruction of hollow organs, tissue erosion, disfigurement and orthopedic problems [7]. In addition, plexiform neurofibromas have the potential to transform into malignant peripheral nerve sheath tumours (MPNSTs) [9].

Optic gliomas occur in 15-20% of children with NF1 [10]. Their prevalence in adults is unknown, but seems to be much lower. While optic gliomas are relatively common in children with NF1, they are rare in children without NF1, and the possibility of a child presenting with optic glioma having NF1 should always be investigated. Optic gliomas can affect any part of the optic pathway but are usually located in the optic nerves. Fortunately, optic gliomas remain asymptomatic in most NF1 patients and may even spontaneously regress [11].

Another diagnostic criterion for NF1 is the presence of Lisch nodules. Lisch nodules can be detected during an ophthalmological exam using a slit lamp. They are comprised of aggregated melanocytes in the iris and do not cause symptoms. Lisch nodules are not present at birth and develop during childhood and adolescence. By age 16 years, they are present in virtually all affected individuals [12].

Certain congenital bone lesions, such as tibial pseudarthrosis, scoliosis, and sphenoid wing dysplasia, are uncommon, but typical for NF1. These defects result from altered bone mineral metabolism and associated changes in bone structure [13]. Pseudarthrosis usually involves the lower extremities [14] and may lead to fractures. Bracing of tibial pseudarthrosis and scoliosis may help to prevent fracture; however, this conclusion is equivocal [15]. Sphenoid wing dysplasia can cause exophthalmos or strabismus and is often associated with an adjacent plexiform neurofibroma [16].

Approximately 50% of children with NF1 inherit their constitutive mutation from one of their parents; the other 50% of mutations arise *de novo*. Almost all children with an inherited NF1 mutation can be diagnosed within the first year of life, as having a first-degree relative with NF1

already fulfills one of the diagnostic criteria, with café-au-lait spots starting to develop in infancy in the majority of patients [17]. Children without a family history may be diagnosed later in life, but almost all children meet the diagnostic criteria by 8 years of age [18]. In adults, the diagnostic criteria have very high specificity and sensitivity [19].

1.2 Genetics and pathogenesis of NF1

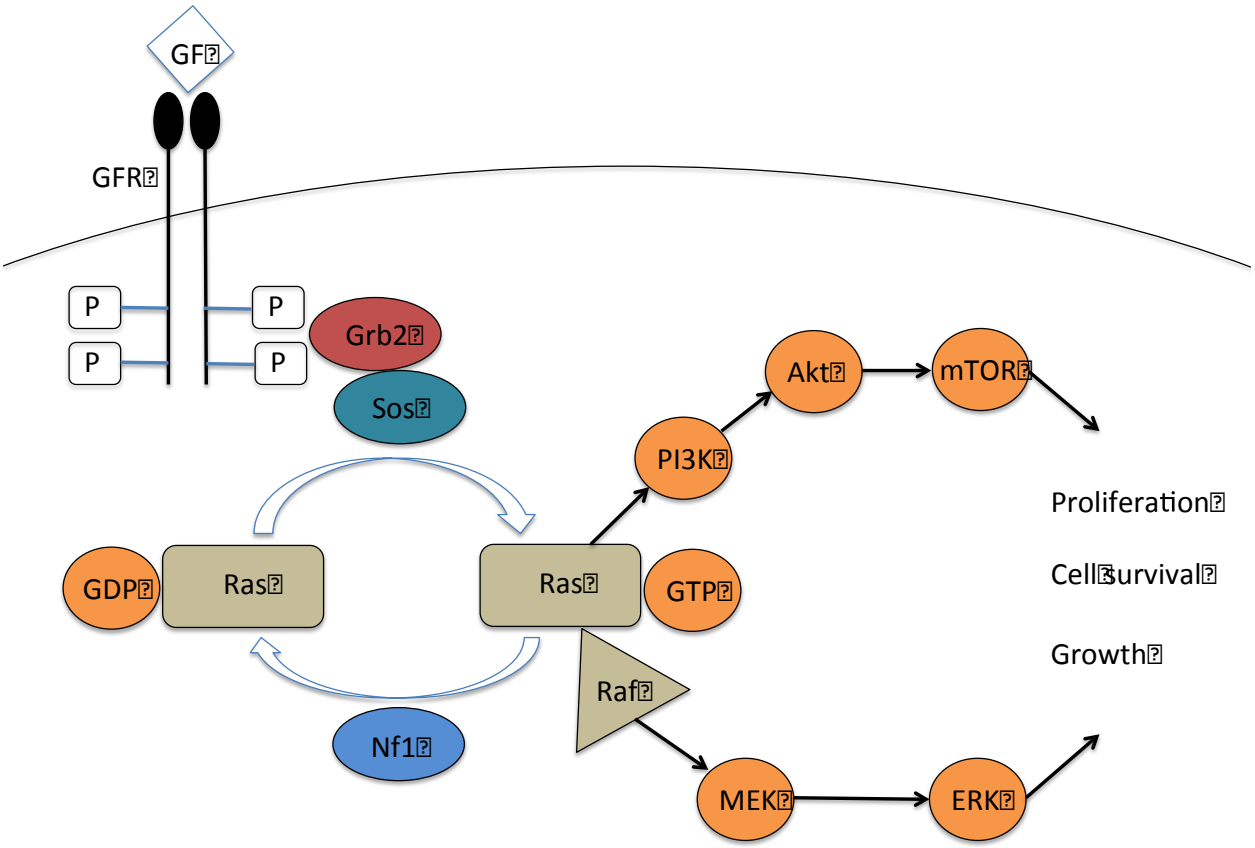
By analyzing the genetic material of patients with NF1 caused by large deletions and translocations, the causative gene (subsequently termed the *NF1* gene) was successfully mapped to chromosome 17q11.2 in 1990 by two independent groups [20,21]. This gene spans over 350 kb of genomic DNA and is one of the largest genes in the human genome. NF1 follows an autosomal dominant inheritance pattern, and affected individuals therefore have a 50% chance to pass on the *NF1* disease allele to their offspring. While biallelic *NF1* inactivation is believed to be lethal in the embryo, individuals with one inactive *NF1* germline allele can acquire loss of the second allele in somatic tissue, consistent with Knudson's two hit hypothesis [22], through a variety of mechanisms (such as deletion or a second inactivating mutation).

NF1 possesses a GTPase (guanosine triphosphatase) and a RAS-binding domain [23]. RAS requires GTP binding to phosphorylate and thereby activate its downstream targets, such as the MAPK (Mitogen-activated protein kinase) and the mTOR (mammalian target of rapamycin) pathways [24]. This phosphorylation step causes GTP to be converted to GDP (guanosine diphosphate), causing RAS to no longer be able to activate its downstream targets. Since *NF1* contains a GTPase domain, it can convert GTP to GDP, thereby limiting the amount of GTP

available for RAS activation [23]. Loss of *NF1* expression leads to overactive RAS signaling and subsequently increased MAPK and mTOR activation. Upregulation of these pathways leads to increased cell proliferation, migration, growth and survival and has been implicated in a variety of different kinds of human cancer [25].

Independent from RAS regulation, the NF1 protein (neurofibromin) is also a modulator of cAMP (cyclic adenosine monophosphate) levels. The role of neurofibromin in cAMP signaling is less well studied than its role in the RAS pathway. It is clear, however, that loss of *NF1* expression leads to decreased activity of the cAMP pathway and increased cell proliferation [26]. It was shown in *Drosophila* that cAMP null mutants have reduced body size [26], a feature that may be similar to the short statue often seen in NF1 patients [27].

Figure 1: Overview of regulation of Ras kinase and its downstream targets through neurofibromin.



NF1 is ubiquitously expressed and plays an essential role during development. In adults, it shows tissue-specific expression of its two differently spliced isoforms [28], with predominance of isoform 1 in most tissues. In the brain, isoform 1 is the dominant isoform in neurons, while isoform 2 is the most commonly expressed one in cells of glial origin [28].

1.3 Other clinical features of NF1

There are many clinical features that are more common in NF1 patients than in the general population; however, not all of these are used for diagnostic purposes. Firstly, there are a variety of neurological complications. NF1 patients have, on average, a normal IQ; however, learning difficulties affect 50-70% of children with NF1 [29]. Specifically, children with NF1 show deficits in memory, language, visuospatial abilities, and executive, motor and social function [30,31]. Over 30% of children with an NF1 diagnosis suffer from attention deficit-hyperactivity disorder [29], and the same percentage of children present with mild manifestations of autism spectrum disorder [32]. Data on neurological deficits in adults with NF1 is sparse, but many of these problems persist into adulthood [33,34].

NF1 patients also have an increased risk for cardiac and vascular complications. These include, but are not limited to, congenital heart defects, hypertension, stenosis of the renal arteries, aneurysms and moya-moya (formation of small telangiectatic blood vessels in the brain) [35–37]. While uncommon in unaffected children, hypertension affects ~15% of those with NF1 [36]. Interestingly, NF1-associated hypertension is not normally caused by a high body-mass index (as it is in individuals without NF1). Instead, the cause of hypertension is often unknown, but renal artery stenosis or pheochromocytoma (a tumour of the medulla of the adrenal glands producing catecholamines) are much more common in NF1 patients [38]. Vasculopathy and associated cardiac defects can be found in 2-5% of NF1 patients and are one of the major reasons for premature death [39].

Another feature typical of NF1 is the increased risk of various types of tumours. The most common causes of death in people with NF1 are malignant peripheral nerve sheath tumours (MPNSTs) and gliomas [39]. All of these tumours have different molecular underpinnings than their sporadic counterparts, as they often carry two inactivated *NF1* alleles and lack some of the mutations typical for non-NF1 associated tumours [40–42].

MPNSTs arise within plexiform neurofibromas and occur in 10-15% of NF1 patients [43]. Compared to the general population, NF1 patients have a 3000- to 7000-fold increased risk to develop an MPNST. [39] MPNSTs in people with NF1 develop at a significantly younger age [43] and have a poorer 5-year survival rate than in the general population[43]. MPNSTs in NF1 patients are highly aggressive and difficult to treat, as they often metastasize, and do not respond well to chemotherapy [44], leaving surgical removal with wide negative margins as the only viable treatment option in most cases.

Gliomas are the second most common cause of death in NF1 patients, and are the most common cause in young patients (under 20 years of age at time of death) [39]. Gliomas are separated into 2 different groups based on their location: optic pathway gliomas residing anywhere in the optic pathways and non-optic gliomas, which can be found anywhere else in the brain. NF1 patients have a 7- to 30-fold increased risk of dying from a glioma compared to unaffected individuals; however, most NF1-associated gliomas are indolent and do not require treatment.

1.4 Gliomas in NF1 patients

Gliomas affect approximately 20% of NF1 patients during their lifetime. As mentioned, these tumours are often divided into optic pathway gliomas (OPGs), and gliomas outside the optic pathway (so called non-optic gliomas). In addition to being in different locations, they differ in the following aspects:

- Frequency: Optic gliomas affect ~15% of children [10], and non-optic gliomas affect only ~5% of children with NF1. [45]
- Prognosis: Optic gliomas almost never cause death, whereas non-optic gliomas, depending on their size and location, can lead to death.
- Patient age: Optic gliomas almost always appear in children under 6 years of age; non-optic gliomas are rarely diagnosed until later in childhood or adulthood.

1.4.1 Glioma formation and tumour microenvironment

For the last 20 years, animal models have been employed to study the effects of the loss of one *NF1* allele on various tissues as models of tumour formation in NF1. Considerable efforts have been focused on improving the understanding of glioma development, and mice have been genetically engineered to carry either constitutive or conditional knock-out *NF1* alleles.

Twenty years ago, it was known that the Ras kinase pathway is overactive in sporadic astrocytomas [46] and that neurofibromin negatively regulates the Ras pathway. Since a loss of *NF1* would be expected to lead to a loss of suppression of the Ras pathway, these observations triggered the hypothesis that astrocytomas (the type of glioma that usually occurs) in NF1

patients are caused by complete inactivation of the *NF1* gene product. Astrocytes isolated from mice heterozygous for an *Nf1* knockout (KO) allele showed a growth advantage, with an increase of up to 40% in astrocyte numbers isolated from brains of $Nf1^{+/-}$ mice compared to wild type litter mates [47]. Interestingly, when these cells were cultured *in vitro*, they lost their growth advantage [47], indicating that their cellular environment is important for increased proliferation.

While homozygous inactivation of *Nf1* is an embryonic lethal in mice, the use of conditional knockout technology allows inactivation after the critical period for *Nf1* function during embryogenesis has passed. Gutmann et al. knocked out both *Nf1* alleles to investigate if biallelic loss is sufficient to promote brain tumour formation in accordance with Knudson's two-hit hypothesis [48]. While they did observe increased astrocyte proliferation in $Nf1^{-/-}$ astrocytes in $Nf1^{+/+}$ mice, the astrocytes lacked tumourigenic properties [48]. As the next step, the same group created $Nf1^{-/+}$ mice that were lacking *Nf1* expression only in astrocytes and showed that a homozygous *Nf1* loss in astrocytes on the background of a heterozygous *Nf1* loss in all other brain tissues caused mice to develop optic gliomas [49]. It was later demonstrated by histologic examination of mice optic glioma samples that these tumours show increased invasion by microglia [50], immune cells of the central nervous system able to secrete a variety of chemo- and cytokines. Through extensive RNA-sequencing of microglia, the chemokine Ccl5 was identified to be overexpressed in mouse optic gliomas compared to normal mouse optic nerves [51]. In addition, antibodies against Ccl5 slowed down glioma progression and improved retinal dysfunction [51].

More recently, attention has been focused on the effects of an individual's *NF1* mutation on glioma formation. Several groups showed that different human-derived constitutional *NF1*

mutations introduced into one mouse Nf1 allele lead to different levels of residual *NF1* expression and different phenotypes, thereby resembling the phenotypic variability seen in humans with NF1 [52,53].

1.4.2 Non-optic gliomas

1.4.2.1 Non-optic glioma pathology and treatment

Gliomas outside the optic pathway affect approximately 5% of children with NF1 [54]. The prevalence in adults in unknown, but non-optic gliomas are a common cause of premature death in NF1 patients. [39,55] Non-optic gliomas can also cause hydrocephalus, headaches, seizures and other symptoms [56]. Compared to the general population, however, non-optic gliomas in NF1 patients usually have a more benign course and a better overall and recurrence-free 5-year survival [57]. Pilocytic astrocytomas (PAs), which are WHO (World Health Organization) Grade 1 (low-grade) gliomas, are the most common type of glioma in the pediatric population, with or without NF1 [57,58]. WHO Grade 2, 3 and 4 tumours (other low-grade gliomas, anaplastic astrocytomas and glioblastoma multiforme, respectively) also occur in NF1 patients [59] but are much rarer than WHO Grade 1 PAs.

Most low-grade non-optic gliomas in NF1 patients remain indolent and never require treatment. If a tumour does cause symptoms or exhibit rapid radiological progression, several treatment regimens are available. These options include surgery and/or chemotherapy using carboplatin, vincristine and temozolomide [60], with other next-generation agents being in clinical trials. Radiotherapy is not recommended for treatment of non-optic gliomas in NF1 patients, as the risk

for secondary malignancies is drastically elevated in NF1 patients after childhood radiation treatment [61].

1.4.2.2 **Differences between non-optic glioma in children and adults with NF1**

Non-optic gliomas occur at a frequency of ~5% in children with NF1 [4]. The prevalence in adults is most likely lower; however, no large studies exist that evaluated the prevalence of non-optic gliomas in adults with NF1.

Non-optic gliomas in children with NF1 are often located in the cerebellum or brain stem [60,62] but they occasionally occur in other locations, such as the corpus callosum [63]. In adults with NF1, these tumours are more likely to be found in locations that are considered atypical in children with NF1, such as the parietal or frontal lobe [64]. However, adults were only included in studies if they were symptomatic, and it is not known where asymptomatic non-optic gliomas in adults occur.

It is thought that gliomas in children with NF1 generally are less aggressive, are of a lower WHO Grade, have a better outcome, and allow for longer survival than their counterparts in adults [57,65,66]; however, this is mostly based on studies of small numbers of selected patients. In addition, spontaneous regression of non-optic gliomas has occasionally been described in children with NF1 [67–69], but it is not known if non-optic gliomas in adults can spontaneously regress.

1.4.3 Optic gliomas

1.4.3.1 Optic glioma pathology and treatment

In comparison to non-optic gliomas, OPGs occur much more frequently, and are studied more often in people with NF1 [10]. They are most common (with a prevalence of ~15%) in children under 6 years of age [70] but are known to occur in older children and adults as well [71,72]. It is unknown if the OPGs in older NF1 patients arose later in life or if they were present since early childhood.

Optic gliomas can involve any part of the optic pathway but are most often found in the optic nerves and only rarely involve post-chiasmatic structures [73]. Optic gliomas are often indolent and do not progress in the majority of NF1 patients [10], and, in contrast to non-optic gliomas, rarely cause death. If OPGs do become symptomatic, they can cause vision loss, precocious puberty in children, and proptosis [10]. Current guidelines do not recommend systematic MRI screening for optic pathway gliomas in NF1 patients of any age [70] because there is no evidence that early detection and treatment improves outcome [73].

Only symptomatic OPGs are treated in NF1 patients. Treatment options for children with OPG include a combination of vincristine and carboplatin or surgery, although surgery is usually reserved for cases with radiological progression when vision has already completely deteriorated. Treatment for adults is less standardized and is usually based on clinical judgement of the physician. It is not known why some patients' OPGs become symptomatic while others do not, or if tumours can become symptomatic after childhood. It is also currently unknown what happens to symptomatic or asymptomatic gliomas of children when they become adults.

There are many case reports of spontaneously regression of optic gliomas in children with NF1 [11,74,75]. It is unknown which factors influence spontaneous regression, if both symptomatic and asymptomatic OPGs can spontaneously regress and if spontaneous regression occurs in adults with NF1.

1.4.3.2 **Vision testing**

Vision outcomes can be examined using various methods, depending on the age of the individual. Teller acuity cards can be used in very young children, as they employ preferential looking instead of symbol recognition [76]. In older preschool children, Lea symbols or HOTV charts (charts showing the letters H, O, T and V used to assess visual acuity in preliterate and literate children) can be used to evaluate if children can see and name symbols [76]. In literate children and adults, Snellen charts with letters are used to determine visual acuity [76]. In all of these tests, the symbols/letters become increasingly closer to each other (Teller acuity cards) or increasingly smaller (Lea symbols/HOTV and Snellen charts), making them harder to distinguish. Visual acuity measurements can be converted into the logarithm of the minimum angle of resolution (logMAR), allowing for a standardized comparison of measurements. LogMAR is calculated as logMAR=1/(visual acuity decimal notation, e.g. 20/200). A difference of 0.2 in logMAR was recommended to be considered a significant decline in vision [76].

A method aiming to evaluate structural intactness of the optic pathway is optic coherence tomography. This technique determines the thickness of the axons of the retinal ganglion cells, and was shown to be associated with and predict vision loss in OPGs [77].

Visual field testing is another frequently performed examination in NF1 patients with OPGs. Visual field testing examines central and peripheral vision by fixing the patient's gaze and presenting objects in different spots of the visual field. Often, defects in the visual field are associated with deficits in visual acuity [76] and treatment is only rarely initiated if they are the only symptom. Visual field testing requires a cooperative patient and takes several minutes, so it is important to evaluate if the patient can focus on the test long enough to complete the examination. This can be a problem particularly in the young NF1 population, as attention deficit disorders are common [78].

Other methods, such as visual evoked potentials and measurements of optic disk pallor and optic disk swelling, are not currently recommended for the evaluation of visual acuity [76].

1.4.3.3 **OPG outcome in NF1 patients**

Most OPGs in NF1 patients never become symptomatic [73]. This is better established in children than in adults, as no large studies exist that investigate OPGs in adults with NF1. In children, less than 20% of patients experience symptoms attributable to their glioma, and, hence, most OPGs are managed conservatively [73]. OPGs that do become symptomatic may cause visual field defects, strabismus, decline in visual acuity, optic nerve atrophy, or proptosis [79]. Many studies have attempted to identify factors predicting which children will experience vision loss [80–85]; however, these studies have yielded mixed results.

Optic tumour location was proposed to be associated with visual outcome. One study found tumours in the optic radiations to be a significant predictor of worse visual outcome [80], while

another study found this association to apply to chiasmatic as well as post-chiasmatic structures [85].

In addition to tumour location, Fisher et al. found very young (<2 years at diagnosis) and older children (>5 years at diagnosis) to have worse visual outcomes from their OPGs [80].

Most recently, patient sex was identified as an important factor for visual decline in children with NF1. While girls are just as likely to develop OPGs as boys, they are 3 times more likely to experience visual decline [82]. This was demonstrated in an animal model to be a result of an interaction between patient sex and polymorphisms in the *Adcy8* (Adenylyl cyclase type 8) gene, leading to reduced cAMP levels in female mice, causing downstream growth promoting effects [82,86].

Very mixed visual outcomes have been reported after treatment of symptomatic OPGs in children with NF1. Of children who were treated with standard chemotherapy regimens, 32% had improved vision, 40% showed stable vision and 28% had a decline in vision [80]. One analysis of multiple studies concluded that there is insufficient evidence to determine if chemotherapy improves vision [87].

Interestingly, radiological progression is not usually associated with visual decline in children with NF1 and OPGs [80], and radiological progression alone usually is not sufficient reason for treatment.

1.5 Mutations analysis and mutation types found in NF1 patients

1.5.1 Genetic analysis

In addition to clinical examination, genetic analysis is sometimes used to make or corroborate an NF1 diagnosis, for example if NF1 is suspected but the number of diagnostic criteria present is insufficient and a diagnosis would change management of the patient (e.g. in a child presenting with OPG). A comprehensive protocol including cytogenetics, FISH (fluorescence in-situ hybridization), cDNA sequencing and MLPA (multiplex ligation-dependent probe amplification) can detect disease-causing variants in up to 95% of patients [88,89].

Whole-gene deletions affecting *NF1* and neighboring genes (so-called "microdeletions") can be found in ~5% of patients, and various single- or multi-exon deletions affect another 2-3% [89]. Other pathogenic mutations are single nucleotide variants or small deletions/insertions: 30-40% are nonsense variants, 20-30% are small deletions/insertions causing frameshifts, 20-30% of variants affect splicing and 5-10% are missense mutations [89,90]. A small minority of patients have translocations or other structural chromosomal rearrangements [89]. No single mutation, other than microdeletions, accounts for more than 1% of NF1 patients.

1.5.2 Genotype-phenotype correlations in NF1

Genotype-phenotype correlation in NF1 is generally poor; however, particular mutations have been found to be related to the presence or absence of certain symptoms.

Patients with NF1 microdeletions generally have a more severe phenotype with distinctive facial features, higher risk for development of plexiform neurofibromas and MPNSTs, somatic overgrowth, and cognitive impairment [91–93]. While some of these features are believed to be due to deletion of the entire *NF1* gene, others may be caused by the concurrent deletion of other genes [94]. Based on a comparison between deletion and non-deletion NF1 patients and gene expression analysis, Venturin et al. proposed that *OMG* and/or *CDK5R1* may be responsible for learning disabilities and that *SUZ12* and/or *CENTA2* are involved in cardiovascular malformations [94].

A small in-frame deletion (c.2970-2972 delAAT) in exon 17 of the *NF1* gene was found to be linked to the absence of surface plexiform and dermal neurofibromas [95]. This deletion is associated with an overall milder phenotype.

In 2012, a study of 149 NF1 patients found that patients with mutations affecting splicing are at an increased risk of developing gliomas and MPNSTs [96].

More recently, multiple missense mutations affecting p.Arg1809 in exon 37 of the *NF1* gene have been shown to be associated with a high incidence of pulmonic stenosis and short statue [97], also known as Noonan-like features.

Other proposed genotype-phenotype correlations, such as patients with a mutation in the 5' tertile of the *NF1* gene being more susceptible to optic pathway gliomas [98], could not be replicated by other groups [99].

Other authors failed to find any genotype-phenotype correlations in groups of 77 or 78 patients, respectively [99,100].

1.6 Hypothesis

Non-optic gliomas and OPGs in NF1 patients differ from their sporadic counterparts in pathogenesis, outcome, location, age of onset and associated symptoms. While considerable efforts have been focused on sporadic gliomas, the natural history of non-optic gliomas and OPGs in NF1 patients is incompletely understood, especially in adults. Based on the reviewed literature, I hypothesize that:

- Non-optic gliomas and OPGs are more frequent in adults with NF1 than previously thought, as previous studies only included symptomatic patients;
- The natural history, including rate of progression and spontaneous regression, of non-optic gliomas and optic gliomas differs between children and adults with NF1;
- There is an association between the presence of optic gliomas and other clinical features of NF1;
- Certain kinds of *NF1* mutations predispose to the development of optic gliomas and/or other tumours.

1.7 Objectives

Children with NF1 are often seen in pediatric care centers after their diagnosis; however, as they grow older they are usually lost to follow-up. Therefore, most studies only include children, and, if they do include adults, these adult patients typically are being seen because of their symptoms, thereby biasing the study population. We collected a unique patient cohort and followed them

longitudinally for over a decade. In order to investigate the natural history of gliomas in NF1 patients of all ages, I undertook to:

- Determine the prevalence of non-optic and optic gliomas in unselected children and adults with NF1;
- Describe the rates of appearance, progression and spontaneous regression of non-optic and optic gliomas in NF1 patients;
- Identify clinical features that predispose to OPG development; and
- Perform regression analyses to find genotype-phenotype correlations in NF1.

Chapter 2: Non-optic gliomas

2.1 Summary

In this chapter, I present an examination of the natural history of non-optic gliomas in children and adults with NF1. We investigated the prevalence per age group as well as rates of appearance, progression and regression of non-optic gliomas in NF1 patients. We found non-optic gliomas to be twice as common in NF1 patients as previously thought. This is most likely due to a higher prevalence in older adults as well as an increased detection frequency of non-optic gliomas when routine MRIs are performed.

2.2 Methods

2.2.1 Patient recruitment

All patients that were seen in the NF outpatient department of the University Hospital Hamburg-Eppendorf in Hamburg, Germany, between 2003 and 2015 were offered brain MRIs to monitor their intracranial tumour burden. Brain MRIs were offered to all patients and were therefore not biased towards patients with symptomatic tumours. All patients were diagnosed with NF1 according to the clinical criteria described previously. Patients were referred to the NF1 outpatient department from physicians all over Germany. Written informed consent was obtained from all study participants. The ethical committee of the Medical Chamber in Hamburg, Germany, and the Research Ethics Board of the University of British Columbia in Vancouver, Canada, approved this study.

2.2.2 MRI imaging

All MRIs were obtained at the MRI Institute Altona in Hamburg, Germany. Up until 2013, a 1.5 Tesla Siemens Avanto scanner was using for image acquisition; after that, a 3.0 Tesla scanner was used. Multiple coronal, axial and/or sagittal images with T1-, T2- or FLAIR (fluid attenuation inversion recovery)-weighting were obtained, and additional sequences, such as pre- and post-gadolinium T1-weighted scans, were obtained if clinically indicated. All scans were performed with a slice thickness of 5.5 mm or less. Head MRIs were obtained using a cranial protocol.

MRIs were analyzed by two neuroradiologists (Dr. Marco Marangoni and Dr. Manraj K.S. Heran) for intracranial anomalies (see Table 1).

Table 1: Items extracted from MRI reports for the purpose of this study.

Sex	Size of non-optic glioma
Age at scan	Evidence for surgical resection of glioma
Presence of optic glioma	Presence of other type of intracranial tumour
Location of optic glioma	White/grey matter anomalies
Size of optic glioma	Hydrocephalus
Optic nerve tortuosity	Unidentified bright objects
Optic nerve thickness	Corpus callosum anomalies

Optic chiasm/radiation involvement	Blood brain barrier disruption
Presence of non-optic glioma	Presence of internal plexiform neurofibroma in whole-body MRI
Location of non-optic glioma	Presence of MPNST of whole-body MRI

Non-optic gliomas were identified using the following criteria: localization of a lesion, size of a lesion, enhancement, presence of mass effect, signal hyperintensity on T2-weighted images, signal hypointensity on T1-weighted images, and lesion evolution over time. Lesions that demonstrated signal hyperintensity on T2-weighted scans, no mass effect and no enhancement were classified as definite unidentified bright objects (UBOs). Lesions that demonstrated signal hyperintensity on T2-weighted scans, no mass effect but did show enhancement were provisionally classified as UBOs, and a diagnosis was made based on the evolution of the lesion.

A shortlist of patients clinically diagnosed with non-optic or optic gliomas was generated from the 1775 German MRI reports. All head MRIs from these patients were then re-evaluated by the two study neuroradiologists (Dr. Marco Marangoni and Dr. Manraj K.S. Heran), and a glioma diagnosis was established for each patient based on the diagnostic criteria described above.

2.2.3 Statistical analysis

In order to determine the prevalence of non-optic pathway gliomas, we divided patients into 10-year age groups. Every glioma patient was counted once per age group, independent of how many times the patient was scanned in this age group. Patients could appear in multiple age groups if they had scan with gliomas present in multiple age groups. If a patient with glioma did

not have the glioma present in an earlier scan in the same age group, he or she was counted as having glioma in that age group. 95% confidence intervals of non-optic glioma prevalence were calculated as ± 1.96 standard deviations of a Poisson distribution.

To determine the percentage of NF1 patients who have a non-optic glioma present at first scan per age group, we divided patients into 10-year age groups. Every glioma patient was counted only once in the age group that had they first scan in. 95% confidence intervals of non-optic glioma prevalence were calculated as ± 1.96 standard deviations of a Poisson distribution.

Tumour volume of non-optic gliomas was calculated using a box model.

We divided non-optic glioma patients in two groups: One including patients diagnosed before the median age of diagnosis (21.2. years of age), and one including patients diagnosed after the median age of diagnosis. Tumour volumes of patients in these groups were non-normally distributed and were therefore compared with a Mann-Whitney U test. For patients who underwent treatment, the non-optic glioma volume on the last scan before treatment was used. For progressing non-optic gliomas, the volume at which the tumour stayed stable in size was used.

Wilson's method with continuity correction was used to calculate 95% confidence intervals for appearing and progressing tumours.

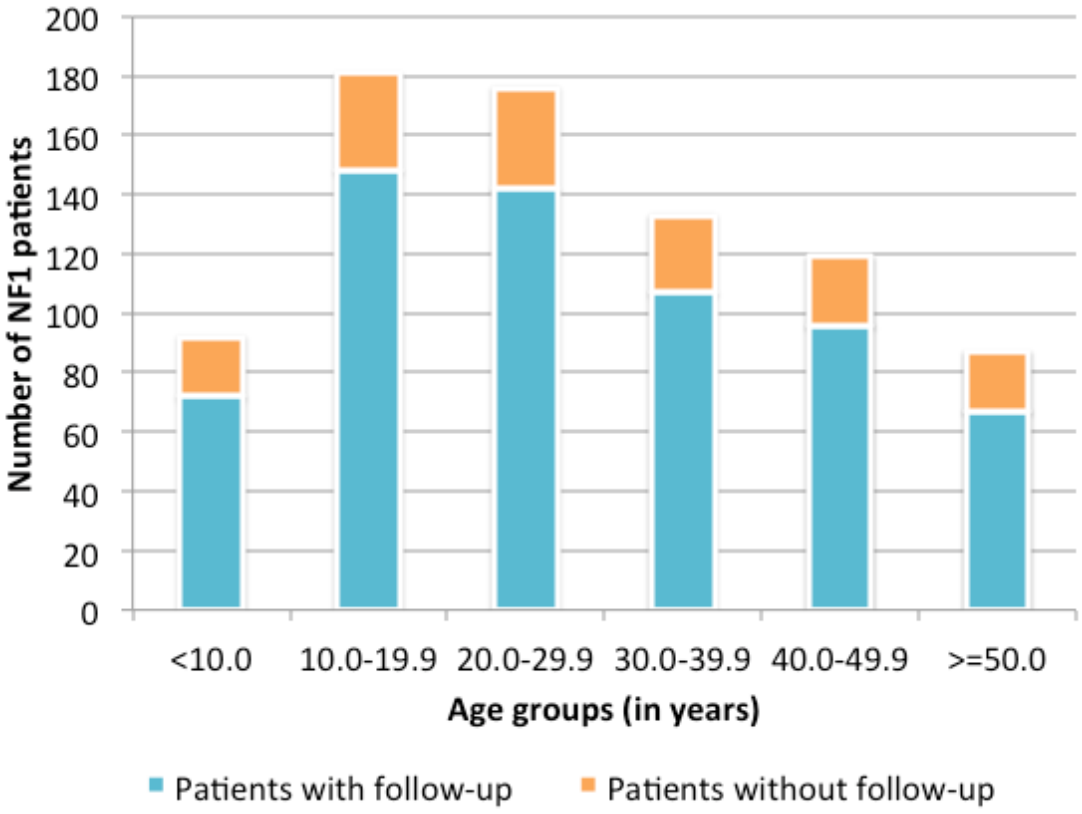
Results were considered significant if $p \leq 0.05$.

2.3 Results

2.3.1 Patient demographics

A total of 1755 MRI of 562 NF1 patients were collected between 2003 and 2015 at the NF outpatient clinic of the University Hospital Hamburg-Eppendorf in Hamburg, Germany. The median time of follow-up was 3.7 years (range: 0 to 13.0 years), with a median number of 3 scans per person (range: 1 to 13 scans). At the time of MRI, patient ages ranged from 0.4 to 72.8 years of age. An overview of all patients with follow-up (patient had more than one MRI scan) and without follow-up (patient had only one MRI scan) can be found in Figure 2. Our cohort consisted of 264 males and 298 females (0.89:1.0 ratio).

Figure 2: Overview of all NF1 patients with and without follow-up per age group included in this study.



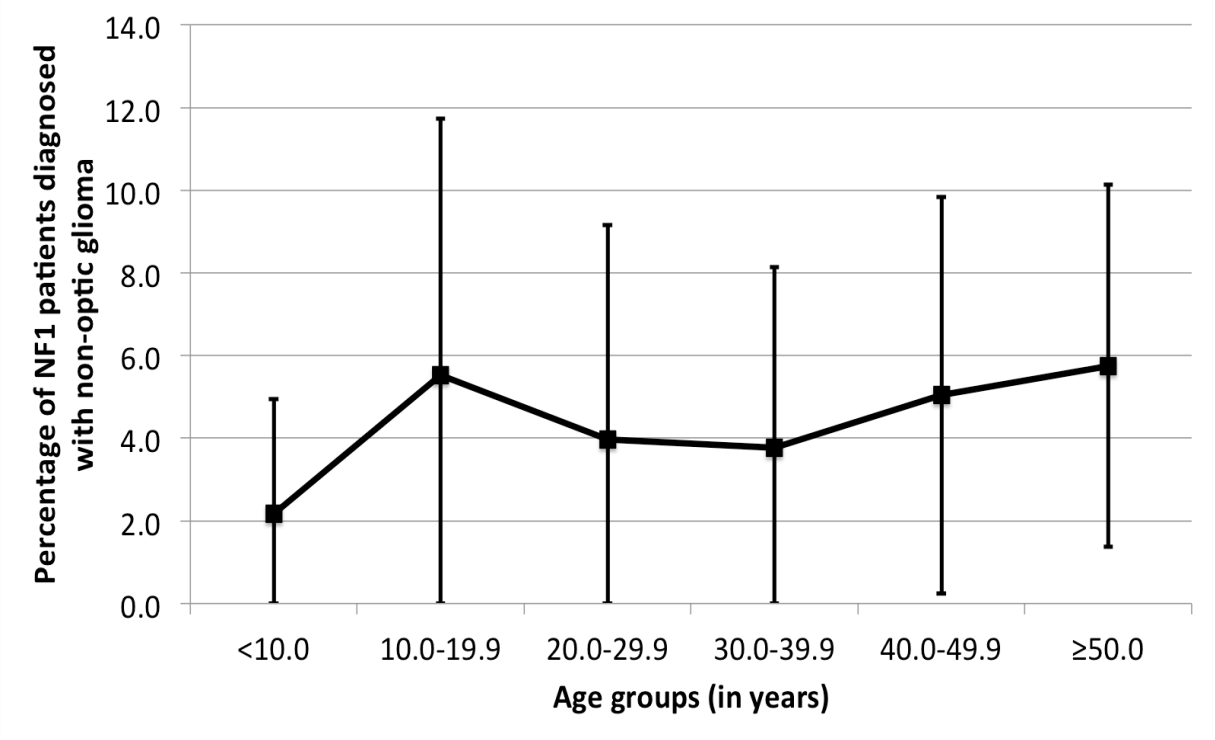
2.3.2 Prevalence of non-optic glioma and age at first scan with non-optic glioma present

27 NF1 patients had a diagnosis of non-optic glioma made by the clinical neuroradiologists based on clinical readings. In 3 of these patients, the study neuroradiologists could not confirm the non-optic glioma using the diagnostic criteria described above. Only the 24 NF1 patients with concurrent diagnoses were included in the analysis. The median follow-up time of the 24

non-optic glioma patients was 5.0 years (range: 0 to 11.8 years), with a total of 133.8 years of follow-up.

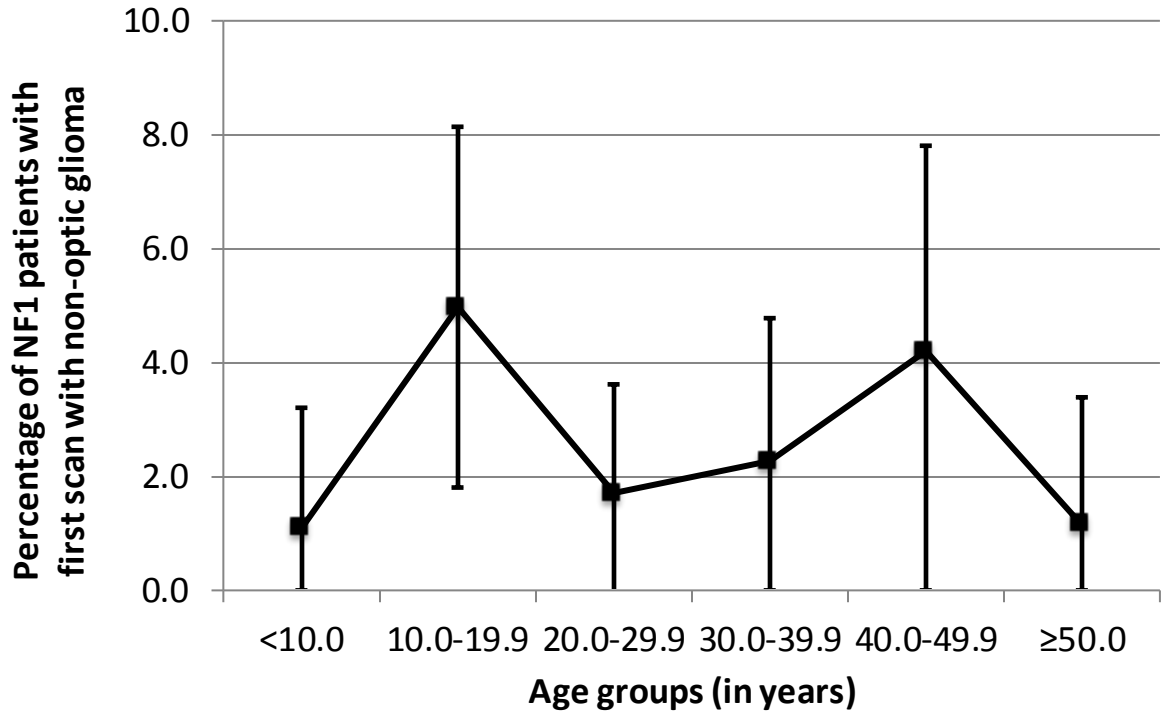
Thus, 24 of the 562 NF1 patients (4.3%) were diagnosed with non-optic glioma. The prevalence of non-optic glioma per 10-year age group is shown in Figure 3. The lowest prevalence is found in children below 10 years of age; the highest prevalence is found in adults over 50 years of age. The prevalence increases with increasing age; however, it remains stable in all patients above 10 years of age.

Figure 3: Prevalence of non-optic glioma in NF1 patients. Errors are 95% confidence intervals of a Poisson distribution.



In order to investigate in which age groups non-optic gliomas may appear in NF1 patients, we plotted the percentage of NF1 patients who had a non-optic glioma present on their first scan in different age groups (see Figure 4). There are no significant differences between the percentages of NF1 patients with non-optic glioma present on their first scan in different age groups, and non-optic gliomas seem to appear in every age group.

Figure 4: Percentages of NF1 patients with non-optic glioma present on their first scan per 10-year age group. Error bars are 95% confidence intervals of a Poisson distribution.



2.3.3 Clinical description of non-optic gliomas

An overview of all non-optic glioma cases is provided in Table 2. We found a total of 32 non-optic gliomas in 24 NF1 patients. Of these, 19 patients had one non-optic glioma and 5 had multiple non-optic gliomas (2 patients had 2 gliomas and 3 patients had 3 gliomas). Only 7 of 24 glioma patients were symptomatic.

Table 2: Clinical features of non-optic gliomas in NF1 patients.

Patient number	Sex	Age at first scan with glioma (in years)	Total follow-up (in years)	Enhance-ment	Symptoms	Histology	Location	Status	Treatment
1	M	20.9	4.5	Yes - Diffuse	Headache	PA Grade 1	Frontal	Decreased size	Surgery
2	F	41.6	10.1	Yes - Diffuse	No		Temporal	Decreased size	Chemotherapy (breast cancer)
3	F	48.6	2.3	No	No		Brainstem	Stable	
4	F	50.8	0.0	Yes - Diffuse	No		Temporal	No follow up	
5	F	23.4	0.6	Yes - Diffuse	Seizures		Brainstem	Stable	
6	F	10.0	8.8	No	Seizures, headache		Cerebellum	Stable	
7	M	31.3	2.5	No	No		Brainstem	Stable	
8	M	13.1	6.5	No	No		Frontal	Stable	
9a	M	44.6	9.7	Yes	Ataxia		Cerebellum	Stable	

Patient number	Sex	Age at first scan with glioma (in years)	Total follow-up (in years)	Enhance-ment	Symptoms	Histology	Location	Status	Treatment
9b	M	44.6	9.7	Yes	Ataxia		Cerebellum	Stable	
9c*	M	50.5	9.7	Yes	Ataxia	PA Grade 1	Cerebellum	Decreased size	Surgery
10	M	6.5	4.0	Yes - Uneven	No	PA Grade 1	Brainstem	Increased size & enhancement	Chemotherapy, biopsy
11*	F	34.9	11.8	No	No		Brainstem	No follow up	
12a	F	16.4	10.2	Yes - Uneven	No	PA Grade 1	Corpus Callosum	Increased enhancement	Incomplete resection before study begin
12b*	F	25.3	10.2	No	No		Brainstem	Stable	
13	F	38.5	2.8	Yes	No		Cerebellum	Stable	
14	M	17.2	0.2	Yes - Uneven	Seizures, headache	PA Grade 1	Cerebellum	Stable	Surgery
15	M	21.3	11.0	Yes	No	PA Grade 1	Corpus Callosum	Decreased size & enhancement	Surgery

Patient number	Sex	Age at first scan with glioma (in years)	Total follow-up (in years)	Enhance-ment	Symptoms	Histology	Location	Status	Treatment
16	M	42.5	2.4	Yes	No		Temporal	Stable	
17	M	42.7	11.2	No	Seizures	DNET	Cerebellum	Stable	Incomplete resection before study begin
18a	F	14.8	0.0	Yes	No	PA Grade 1	Corpus Callosum	No follow up	Surgery
18b	F	14.8	0.0	Yes - Uneven	No		Thalamus	No follow up	
19*	F	18.3	7.6	Yes - Uneven	No		Brainstem	Increased in size	
20	F	16.0	3.0	Yes - Uneven	No		Internal capsule	Decreased enhancement	
21	F	39.8	5.5	Yes - Uneven	No		Cerebellum	Stable	

Patient number	Sex	Age at first scan with glioma (in years)	Total follow-up (in years)	Enhance-ment	Symptoms	Histology	Location	Status	Treatment
22a	F	10.9	3.4	Yes - Uneven	No		Cerebellum	Decreased enhancement	
22b	F	10.9	3.4	Yes - Uneven	No		Cerebellum	Increased then decreased in size	
22c	F	10.9	3.4	Yes - Uneven	No		Cerebellum	Stable	
23	M	17.4	10.7	Yes - Uneven	No		Brainstem	Increased size & enhancement	
24a	M	18.1	5.0	Unknown	Double vision, nystagmus	PA Grade 1	Brainstem	Decreased size	Surgery
24b	M	18.1	5.0	Yes - Uneven	Double vision, nystagmus		Corpus callosum	Increased in size	
24c	M	18.1	5.0	Yes - Uneven	Double vision, nystagmus		Cerebellum	Stable	

Abbreviations: PA: Pilocytic astrocytoma, M: Male, F: Female, DNET: Dysembryoplastic neuroepithelial tumour, * denotes patients with newly-appearing tumours, ^ denotes patients with concurrent optic glioma

Of the 32 non-optic gliomas, 12 were located in the cerebellum, 9 in the brain stem, 4 in the corpus callosum, 3 in the temporal lobe, 2 in the frontal lobe, 1 in the thalamus and 1 in the internal capsule. The median volume of an individual tumour was 1.6 cm^3 (range: 0.04 to 98.4 cm^3).

Tissue enhancement signifies a disruption of the blood-brain-barrier and may be due to tumour invasion or inflammation. Tumour enhancement was evaluated in all non-optic glioma patients; however, Patient 24a in Table 2 had his only MRI exam before surgery performed without the use of contrast. We were, however, able to assess enhancement of his other two tumours on later images. 25 of the 32 non-optic gliomas enhanced after gadolinium administration: 8 enhanced avidly, 4 enhanced diffusely, and 13 showed only patchy or peripheral enhancement. Of the 25 enhancing tumours, 3 increased in enhancement and 3 decreased in enhancement over time. This change was not associated with changes in clinical or radiological status.

9 patients underwent treatment for their non-optic gliomas: 8 patients received respective surgery and 1 patient received vincristine and carboplatin following a surgical biopsy (see Table 2). Patient 2 was treated with tamoxifen for coexisting breast cancer during the study. Her glioma minimally regressed in size during breast cancer treatment; however, it regressed less than 30% per year.

Histology was available for 9 tumours and demonstrated 8 pilocytic astrocytomas (WHO Grade 1) and 1 dysembryoplastic neuroepithelial tumour. DNETs are glioneuronal tumours and classified as WHO Grade 1 tumours. There were no higher-grade tumours (WHO Grade 2, 3 or 4).

2.3.4 Newly-appearing non-optic gliomas

There were 4 NF1 patients with newly-appearing non-optic gliomas (Patients 9c, 11, 12b and 19 in Table 2).

Patient 9 had two gliomas in the cerebellum and was 50.5 years old when an additional third tumour was diagnosed in his right cerebellar hemisphere. Before that, he had had 5 scans spanning 5.9 years during which his right cerebellar hemisphere was glioma-free. The tumour grew from being undetectable to a volume of 4.3 cm^3. After detection, the tumour was partially removed and histology showed a pilocytic astrocytoma Grade 1. All 3 gliomas remained stable in volume in the 5 years of follow-up after surgery. In addition to the cerebellar gliomas, he also had an optic glioma in the intraorbital segment of the left optic nerve causing a loss of visual acuity. It was treated with radiation 5.6 years before the glioma in the right cerebellar hemisphere appeared.

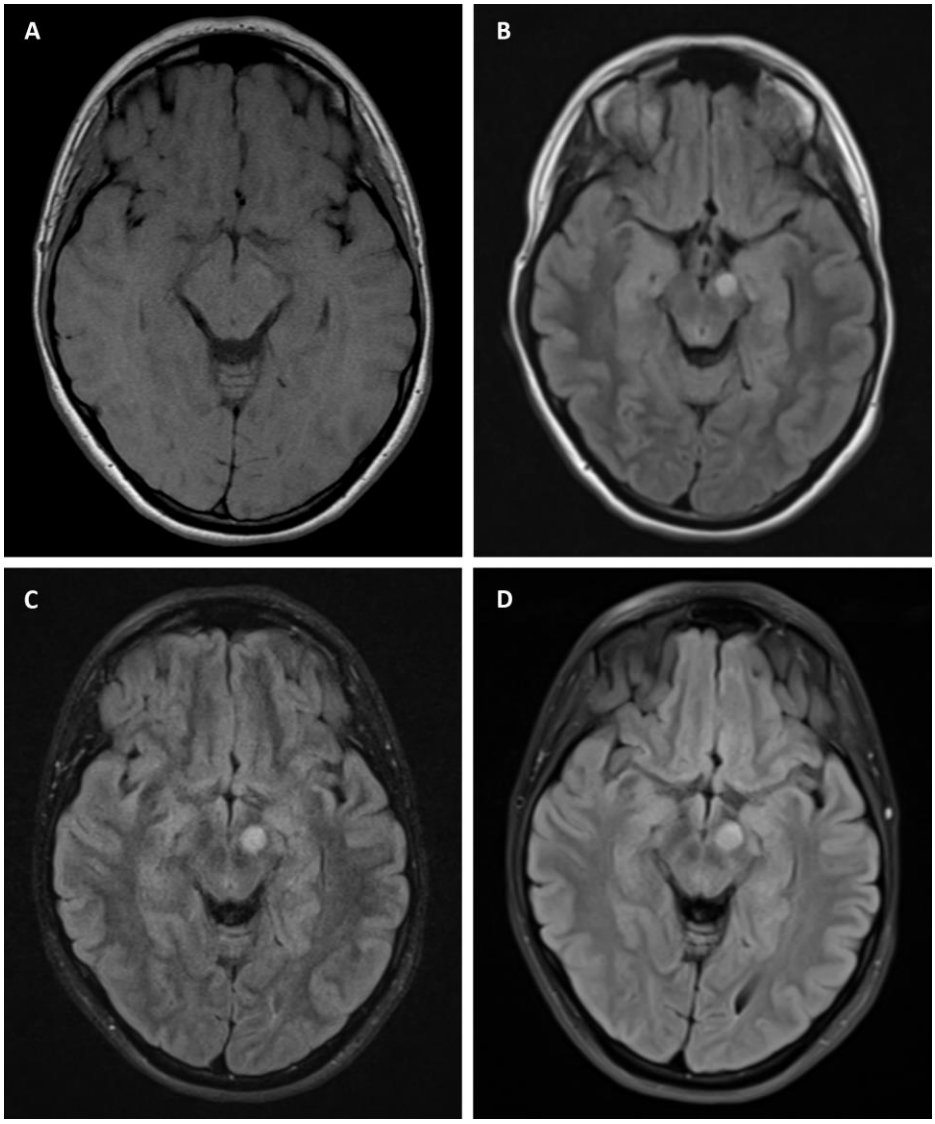
Patient 11 was 34.9 years of age when she was diagnosed with a non-optic glioma in her right cerebellar peduncle. It had grown from being undetectable to a volume of 0.2 cm^3 in 6.1 years. Before diagnosis, she had had six glioma-free scans spanning 11.8 years. No treatment or biopsy was performed and she did not have any follow-up scans after diagnosis. She also had an asymptomatic optic glioma in the intraorbital segment of her left optic nerve.

Patient 12 had a non-optic glioma in the corpus callosum and was 22.3 years old when an additional glioma in the pons was diagnosed. Prior to this, her pons had been glioma-free for 7 scans spanning 4.7 years. The pontine glioma had grown to 0.04 cm^3 in 2.0 years and remained stable in volume during the 4.3 years of follow-up. She did not undergo biopsy or treatment.

Patient 19 was 18.3 years old when a non-optic glioma was diagnosed in her left cerebellar peduncle. Before diagnosis, she had had 1 scan 4.0 years earlier. The glioma increased in volume from being undetectable to measuring 0.5 cm^3. During follow-up, the glioma grew to a volume of 0.9 cm^3 1.0 years later and further grew to 1.3 cm^3 3.0 years after the initial diagnosis. She did not undergo any treatment or biopsy. The appearance of her glioma is shown in Figure 5.

We calculated the rate of appearance of non-optic gliomas in NF1 patients over 10.0 years of age (since glioma prevalence remains stable in patients aged older than 10.0 years). 4 non-optic gliomas appeared in 13.0 patient years during the 2110.5 patient years of follow-up of patients older than 10 years of age, resulting in a rate of 0.62% (95% confidence interval 0.35% to 1.1%) of new non-optic glioma development. There were no newly-appearing non-optic gliomas in children under 10 years of age.

Figure 5: Newly-appearing glioma in the left cerebral peduncle in Patient 19. (5A) There is no visible glioma on the patient's first scan. (5B) 4 years later, an enhancing glioma appeared in the left cerebral peduncle measuring 0.5 cm^3. (5C) 5 years after the initial scan, the glioma increased to a volume of 0.8 cm^3. (5D) 7 years after the initial scan, the glioma measured 1.3 cm^3. The patient remained asymptomatic during follow-up and also has an optic glioma (not shown on these images).



2.3.5 Progressing non-optic gliomas

There were 4 individuals with progressing non-optic gliomas in our study (Patients 10, 19, 23 and 24b in Table 2). The volume evolution of the progressing gliomas is shown in Figure 5.

Patient 10 was 6.5 years of age when a glioma measuring 18.6 cm^3 was diagnosed in his left cerebral peduncle and thalamus. On his next scan 2.3 years later, the tumour volume had increased to 40.6 cm^3 and biopsy showed a PA Grade 1. He was treated with carboplatin and vincristine, leading to stabilization of the tumour in the two years of follow-up after treatment. The progression of his tumour is shown in Figure 6.

Patient 19 was diagnosed with a newly-appearing non-optic glioma in the left cerebral peduncle measuring 0.5 cm^3 at age 18.3. The next scan was performed 1.0 years later and showed an increase in volume to 0.8 cm^3. A scan performed 0.9 years later showed no progression; however, a scan performed 3.0 years after the initial MRI demonstrated further volume progression to 1.3 cm^3. The tumour remained stable on the last scan 0.5 years later. No treatment or biopsy was performed. The evolution of his tumour is shown in Figure 5.

Patient 23 was diagnosed with a pontine glioma with a volume of 1.4 cm^3 at the age of 17.4 years. On his next scan 2.0 years later, the tumour volume had increased to 3.3 cm^3. Another scan performed 1.7 years later showed no progression of the tumour. 2.9 years after the initial progression, the tumour increased in volume again to 4.9 cm^3. The volume remained stable in the following 5.8 years of follow-up. No treatment or histology was performed.

Patient 24 was diagnosed with a non-optic glioma in the corpus callosum at the age of 18.1 years. On a scan performed 2.2 years later, the tumour had increased in volume from 0.5 cm^3 to

2.0 cm^3. The tumour remained stable in volume for the 2.8 years of follow-up after progression. Patient 24 also had a glioma in the cerebellar vermis (Patient 24c in Table 2) of unknown histology, and a pontine glioma (Patient 24a in Table 2), which was shown to be a PA Grade 1.

All tumours progressed in the first 5 years after diagnosis. Therefore, we calculated the rate of progressing non-optic gliomas only in the first 5 years after diagnosis. We observed progression spanning 11.7 patient years of follow-up during a total of 85.1 patient years of follow-up in the first 5 years after diagnosis. The rate of progression was 14% (95% confidence interval 8% to 24%). Four of 21 non-optic gliomas (19%, 95% confidence interval 6-43%) progressed after the initial diagnosis (3 patients did not have follow-up MRI after diagnosis).

Figure 6: Progressing glioma in Patient 10. (5A) First scan with glioma present in the left cerebral peduncle and left thalamus on T2 sequence. (5B) Another scan performed 2 years after the previous one (again T2 sequence). The glioma has markedly increased in size and was treated with chemotherapy one month after this scan was performed.

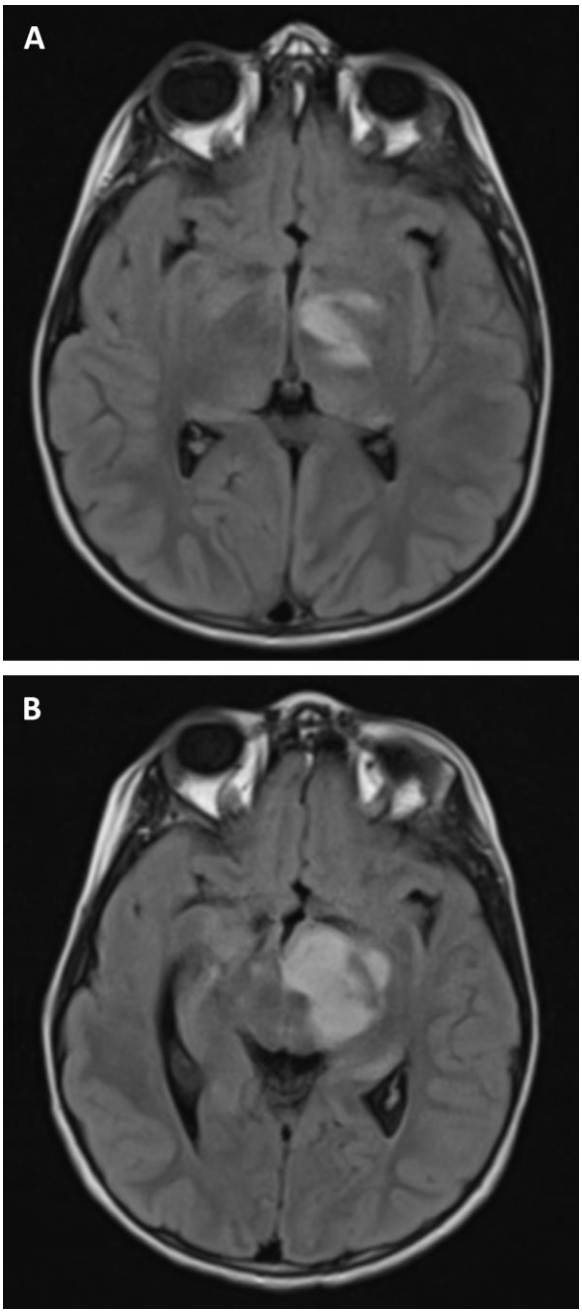
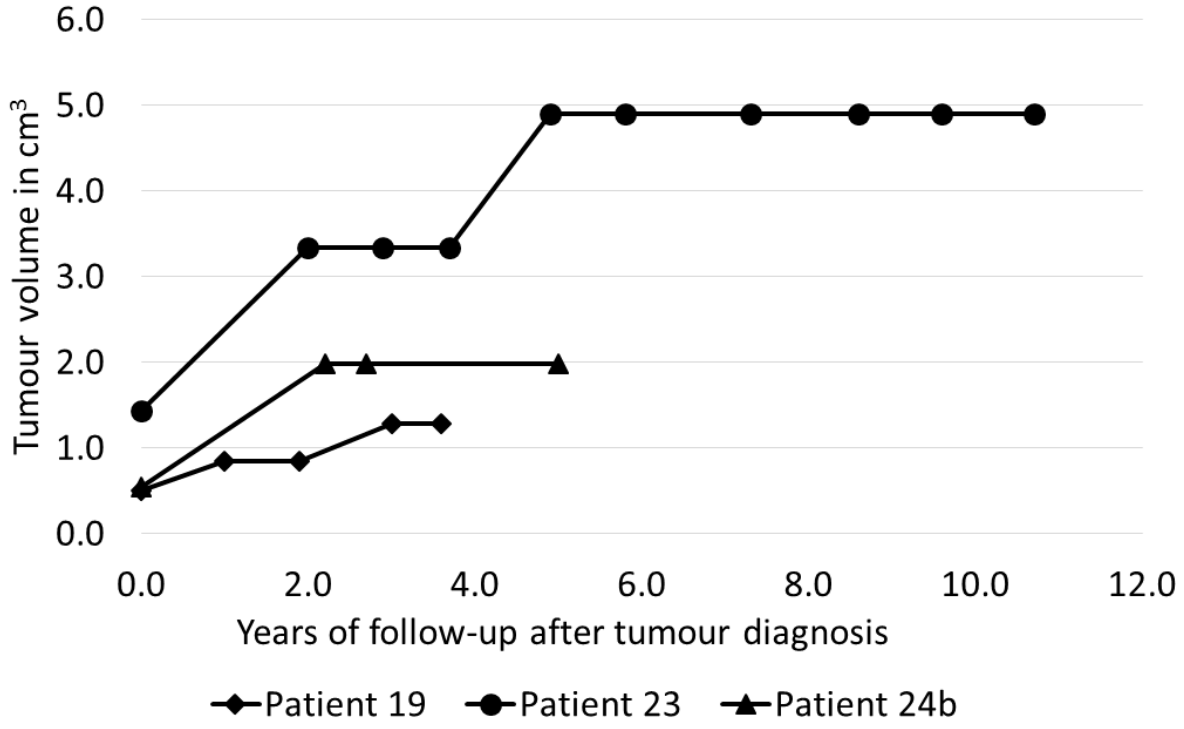


Figure 7: Volume evolution of progressing non-optic gliomas in Patients 19, 23 and 24b.



2.3.6 Spontaneously regressing non-optic gliomas

There were no cases of spontaneously regressing gliomas in our study. However, one patient (Patient 22b in Table 2) developed a cystic tumour component, which later regressed in size. On the first scan, a glioma was present in her right cerebellar hemisphere. On the next scan 0.5 years later, the lesion had developed a cystic component measuring 1.0 cm^3. The cystic component had regressed to a volume of 0.7 cm^3 on the next scan performed 1.8 years later. The volume of the non-cystic component of her tumour remained constant and the patient remained asymptomatic throughout follow-up.

2.3.7 Correlation of presence of non-optic gliomas and presence of UBOs

A previous study indicated that non-optic gliomas may arise from unidentified bright objects in children with NF1 [101]. We reviewed the scans of the 4 patients with appearing non-optic gliomas to check if there was a UBO present in the location where the tumour later appeared.

None of the 4 patients with newly-appearing non-optic gliomas had UBOs present in the location of their tumour before it appeared.

2.4 Discussion

24 of 562 NF1 patients (4.3%) in this study were diagnosed with non-optic gliomas. This prevalence is higher than estimated by most other authors in prospective and retrospective studies (see Table 3).

Table 3: Prevalence of non-optic brain tumours found in published prospective and retrospective studies.

Authors	Study type	Cohort size	Age range of cohorts (in years)	Median age at diagnosis	Number of tumour patients	Rates of tumours	How were tumour patients identified?
Cnossen et al. [102]	P	150	0-18	Unknown	4	2.70%	Symptomatic patients
Zöller et al. [103]	P	70	20-81	Unknown	1	1.43%	Cross-referencing with cancer registry
Friedman and Birch [54]	R	684	0-73	< 6 years	25	3.65%	MRI/CT*
McGaughran et al. [104]	R	523	0-74	Unknown	12	2.29%	Symptomatic patients
Menor et al. [105]	R	72	0.8-14	Unknown	8	11%	MRI*
Seminog and Goldacre [45]	R	6739	0-80+	Unknown	322	4.78%	Hospital records
Varan et al. [56]	R	473	0-33	8 years	11	2.32%	Symptomatic patients

*Cases in this study were selected based on availability of cranial MRI/CT scans.

Abbreviations: P: Prospective, R: Retrospective

The major caveat of these studies is them being limited to only including symptomatic patients. Most NF1 glioma patients, however, are asymptomatic (only 7 of 24 non-optic glioma patients in this study were symptomatic). An unbiased estimate of the true prevalence of non-optic brain tumours can only be achieved by offering MRI scans to all patients, as was done in our study.

We found the prevalence of non-optic gliomas in adults to be higher than in children. This may be due to multiple reasons: Firstly, tumours may develop throughout a patient's lifetime, leading to an increase in tumour prevalence with increasing age. Secondly, this study used patients' ages at first scan with non-optic glioma present rather than age at diagnosis to calculate age-dependent prevalence. Therefore, glioma prevalence in younger age groups may be underrepresented in this study. Previous studies underestimated the prevalence of non-optic gliomas in adults, as the majority of adult NF1 glioma patients are asymptomatic. In addition, some symptoms of brain tumours in adults (e.g. headaches) are also common in NF1 patients without brain tumours. Even though it has long been known that NF1 patients have an increased risk for development of gliomas as well as other tumours, head or whole-body MRIs are not recommended as part of their routine clinical care [7,106,107].

The median age at non-optic glioma diagnosis in this study is 21.2 years of age; much higher than been previously reported (see Table 3). Gutmann et al. reported the largest cohort of adult non-optic glioma patients thus far [64]. Most of the patients described by this group had higher-grade tumours (WHO Grade 2, 3 and 4), whereas all of our patients had WHO Grade 1 tumours. This is most likely due to Gutmann et al. only including patients with symptomatic tumours, which are more likely to be higher grade than low-grade tumours. Another study described 3 adult cases, 2 of which were symptomatic [72].

Gliomas in NF1 patients (especially lower grade gliomas) are associated with a better outcome than those diagnosed in a non-NF1 setting [57,108]. 8 of the 9 biopsied tumours in this study were pilocytic astrocytomas (one was a dysembryoplastic neuroepthelial tumour), which is the most common intracranial tumour diagnosed in people with or without NF1 [109,110]. The 5-year and 10-year survival for NF1 patients with pilocytic astrocytomas was shown to be 85% and 68%, respectively [57]. This is most likely an underestimate, since the patients investigated had to undergo surgery for their tumours, therefore biasing the study towards more severe cases. The true overall 5-year and 10-year survival of NF1 patients with pilocytic astrocytomas is most likely higher than 85% and 68%, respectively; in accordance, no patients with biopsy-proven pilocytic astrocytoma died during a total of 44.6 patient years of follow-up. All non-optic glioma patients combined had a cumulative 133.8 patient years of follow-up with no deaths.

There were 5 patients with multiple non-optic gliomas in this study: Two patients with two gliomas and three patients with three gliomas. The chance of developing one non-optic glioma according to our study is 4.3% Therefore, we expected to see one patient with two independent non-optic gliomas and zero patients with three independent non-optic gliomas in a study this size. Even though these numbers are small, they do support the possibility of an increased risk of development of additional gliomas if the patient has already developed one. An increased risk for the development of non-optic gliomas in patients with optic gliomas was proposed 20 years ago [54]; it was, however, not known if this correlation also applies to the development of additional non-optic gliomas.

Only two of the five NF1 patients with multiple non-optic gliomas were symptomatic. There are case reports of NF1 patients with multiple non-optic gliomas, and all these patients were

symptomatic [111,112]. Our results show that patients with multiple non-optic gliomas can clearly remain asymptomatic. A publication by Guillamo et al. assessed risk factors for death in NF1 patients; they did not find having multiple brain tumours to be a factor associated with premature death [65]. However, they did not distinguish between optic and non-optic brain tumours.

We identified 4 patients with newly-appearing non-optic gliomas in patients older than 10 years of age (there were no newly-appearing non-optic gliomas in patients under 10 years of age). These tumour appeared in patients between 18.3 years and 50.5 years of age, highlighting that non-optic gliomas may appear in younger as well as older patients. This stands in contrast to the age of appearance of OPGs, which almost solely occur in childhood [113].

There were four patients with radiological progression. Progressing gliomas were only identified in patients who were diagnosed before 21.2 years, the median age at diagnosis. In addition, no glioma progressed past the patient age of 22.3 years. Gliomas only progressed until 4.9 years after the initial diagnosis, and all but one patient remained asymptomatic despite radiological progression. This suggests that non-optic gliomas are most active during childhood and young adulthood, and are stable in older adult patients. Tumours in adult patients are usually considered to have a worse prognosis than those identified in children [65,66]; however, this is likely not true and merely a reflection of the fact that adults were only included in previous studies if they were symptomatic.

None of the 32 non-optic gliomas identified in 24 patients in this study spontaneously regressed in size by more than 30% per year. Although spontaneous regression of non-optic gliomas has been described in case reports [68,114], it seems to be a very rare phenomenon.

We compared the volume of non-optic gliomas in patients diagnosed before, and patients diagnosed after the median age at diagnosis (21.2 years). Patients diagnosed before 21.2 years of age had significantly greater tumour volume than patients diagnosed later than the median age at diagnosis ($p=0.02$). This is counterintuitive if most tumours arise in childhood and grow in childhood and young adulthood. However, it parallels an observation made in plexiform neurofibromas in NF1 patients. Tumours grow most rapidly during childhood and tumour volume is inversely correlated with age [5,115]. One explanation for this finding is that large and rapidly growing tumours in childhood may be treated or have fatal consequences, leaving only small and less aggressive tumours to be identified in adults.

This study contains the first estimates of rates of appearance and progression of non-optic gliomas in NF1 patients ever published. While these estimates are imprecise and are not clinically informative about the risk of progression per year in individual patients, they do provide information on the proportion of time within the first five years after diagnosis that non-optic gliomas are growing in this series. Additional studies that include many more patients and longer longitudinal follow-up are needed in order to establish reliable annual rates of non-optic glioma appearance and progression.

Our study has multiple limitations. Patients with mild disease manifestations are less likely to be referred to the NF outpatient department than severely affected patients, and this study is likely underestimating the prevalence of asymptomatic patients. Additionally, patients with severe disease manifestations are more likely to get follow-up imaging than patients less severely affected. Even though all patients were offered whole-body MRI, parents of asymptomatic young children may be more likely to refuse than parents of symptomatic children, as MRI on young

children requires sedation. Another limitation is that we cannot exclude the possibility that the 3 cases which had discordant calls between German and Canadian radiologists are in fact non-optic tumours. As histology is not available for these tumours, it is impossible to determine the true nature of those lesions. Of note, there were patients with higher-grade tumours seen in the NF outpatient department who did not undergo whole-body MRI.

This is the largest study of unselected head MRIs of NF1 patients published to date. It is also the first study to investigate the natural history of non-optic gliomas in an unselected cohort with an age range this wide. Our data should be considered when treating NF1 patients with or without gliomas.

Chapter 3: Optic gliomas

3.1 Summary

In this chapter, I present an examination of the natural history of optic gliomas in children and adults with NF1. We investigated the prevalence per age group as well as rates of appearance, progression, and regression of optic gliomas in NF1 patients. In addition, I analyzed clinical features correlating with optic glioma presence and volume. We found the presence of UBOs and optic gliomas to be strongly associated, suggesting that, like UBOs, asymptomatic optic gliomas may not be true neoplasms.

3.2 Methods

3.2.1 Patient recruitment

Details on patient recruitment are described in Chapter 2.2.1.

We were able to collect 20 additional MRI scans from patients already included in the study, thereby increasing the total available number of MRI reports and scans to 1775.

3.2.2 MRI imaging

All MR images were obtained at the MRI Institute Hamburg Altona, Germany. Before 2013, a 1.5 T scanner (Siemens Avanto) was used; in 2013, the scanner was upgraded to a 3.0 T machine (Siemens Skyra). Coronal, axial and sagittal sequences were obtained with a slice thickness of

5.5 mm or less. Pre- and post-gadolinium sequences were obtained if clinically indicated. Head MRIs were performed using a cranial protocol.

We defined four locations to evaluate the extent of OPGs: intraorbital optic nerves, prechiasmatic optic nerves, chiasm and optic tracts/radiations. An OPG diagnosis in the optic nerves was made if the lesion was larger than 4mm in diameter and demonstrated hyperintensity on T2-weighted images, or if it enhanced after contrast injection. The chiasm was defined as involved by glioma if it was larger than 18mm x 3.5mm (transverse x craniocaudal diameter), if the normal architecture was distorted, or if it enhanced after contrast injection. A glioma in the optic radiations/optic tracts was diagnosed if they demonstrated hyperintensity on T2-weighted images, or if it enhanced after contrast injection.

A list of patients with a clinical diagnosis of OPG was generated based on the 1775 clinical MRI reports. All head MRI of patients with a clinical OPG diagnosis were re-evaluated by two study neuroradiologists and the presence of OPG was established by consensus based on the criteria detailed above.

3.2.3 Features extracted from MRI and clinical reports

The following features were collected for all 562 patients: Age at scan, sex, presence or absence of OPG, presence or absence of non-optic gliomas, presence or absence of UBOs and presence or absence of plexiform neurofibromas on whole-body MRI. In OPG patients, we also recorded OPG location (right or left intraorbital optic nerve, right or left prechiasmatic optic nerve, chiasm, and right or left optic radiations), enhancement, tortuosity, length, right and left optic

nerve diameter and chiasm diameter on craniocaudal and transverse images. Details on OPG treatment and symptoms were extracted from clinical records.

3.2.4 Statistical analysis

In order to determine the prevalence of optic pathway gliomas, we divided patients into 10-year age groups. Every glioma patient was counted once per age group, independent of how many times the patient was scanned in this age group. Patients could appear in multiple age groups if they had scan with gliomas present in multiple age groups. If a patient with glioma present on one scan did not have the glioma present in an earlier scan in the same age group, he or she was counted as having glioma in that age group. 95% confidence intervals of optic pathway glioma prevalence were calculated as ± 1.96 standard deviations of a binomial distribution.

To determine the percentage of NF1 patients who have an optic pathway glioma present at first scan per age group, we divided patients into 10-year age groups. Every glioma patient was counted only once in the age group they were first scanned in. 95% confidence intervals of optic pathway glioma prevalence per age group was calculated as ± 1.96 standard deviations of a binomial distribution.

Tumour volume of chiasmatic optic gliomas was calculated as ellipsoids, and volumes of optic nerve gliomas were calculated as cylinders. If tumours affected the chiasm and the optic nerves, the volumes were added to determine the total optic glioma volume. Volumes of postchiasmatic tumours could not be calculated due to their diffuse nature.

We used multiple logistic and linear regression to determine clinical features predicting OPG presence and volume, respectively. Natural log transformation was applied to total OPG volume to achieve normal distribution for linear regression. Independent variables for multiple logistic regression were age at first scan with OPG and presence or absence of non-optic gliomas, presence or absence of UBOs and presence or absence of plexiform neurofibromas. Predictor variables for OPG volume were age at first scan with OPG present, presence or absence of non-optic gliomas, presence or absence of UBOs, presence or absence of plexiform neurofibromas and presence or absence of subcutaneous neurofibromas.

The rate of progression/regression of OPG were calculated as the sum of years passed between a scan with progression/regression and the previous normal scan divided by the total observation time for all OPG patients. Wilson's method with continuity correction was used to calculate 95% confidence intervals for progressing and regressing tumours. Differences between patients under 20 years of age and patients over 20 years of age were calculated using a χ^2-test.

UBO prevalence in patients with OPG and without OPG per age group was compared using χ^2-tests. The age groups including patients between 40.0 to 49.9 years of age and patients $\geq$50.0 years of age were combined because of the low prevalence of OPGs in these age groups.

All calculations were performed using SPSS version 23. Results were considered significant if $p \leq 0.05$, and confidence intervals were considered significant if they excluded 1.0.

3.3 Results

3.3.1 Demographics

562 NF1 patients (264 males and 298 females) who attended the University Hospital Hamburg-Eppendorf NF outpatient department were included in this study. A total of 1775 whole body and head MRI examinations were performed on these patients between 2003 and 2015, with a median follow-up time of 3.7 years (range 0 to 13.0 years) and a median number of 3 scans per person (range 1 to 13 scans). At the time of the MRI scan, patient ages ranged from 0.4 to 72.8 years.

3.3.2 Prevalence of OPG per age group

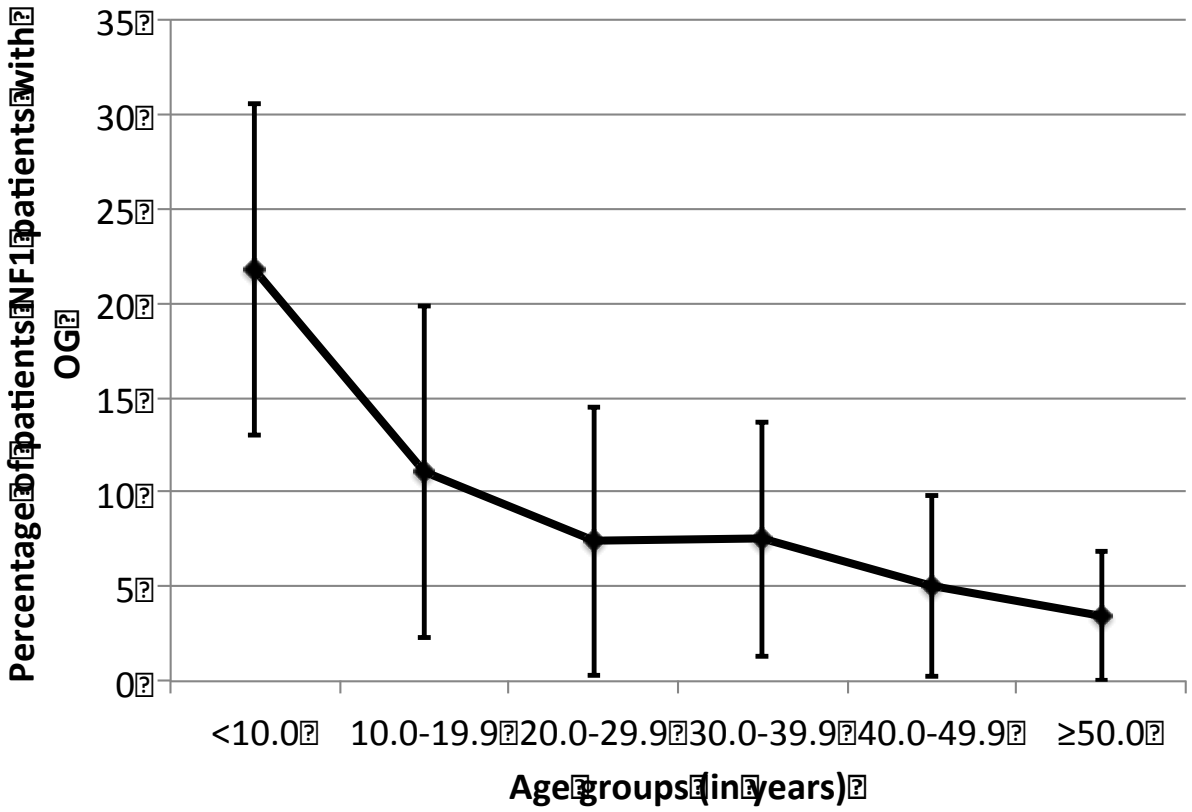
A diagnosis of OPG was made by the clinical neuroradiologists in 56 NF1 patients based on clinical reading of the patients' MRI scans. In 52 of these patients, the study neuroradiologists were able to confirm the clinical diagnosis using the diagnostic study criteria. In 4 patients, our neuroradiologists were unable to confirm the diagnosis, and these patients were subsequently excluded from the analysis.

A total of 52 of 562 NF1 patients (9.3%) were diagnosed with OPG. The prevalence is the highest in children aged younger than 10 years of age (20 of 91 children, or 22%), and declined steadily with increasing age to 3.4% in patients 50 years and older (4 of 92 adults) (see Figure 8). The median follow-up time for OPG patients was 5.2 years (range: 0.0-13.0 years), with a

combined total of 283.2 years of follow-up. Patients had a median of 4 MRI scans (range: 0-12 scans).

17 of the 52 OPG patients were symptomatic. All symptomatic patients experienced vision loss and 13 showed visual field defects. One patient had been blind in both eyes since childhood, so visual fields could not be investigated more closely. Seven symptomatic patients underwent treatment for their OPG: 5 received chemotherapy with carboplatin and vincristine, 1 underwent surgery, and 1 underwent surgery and radiation.

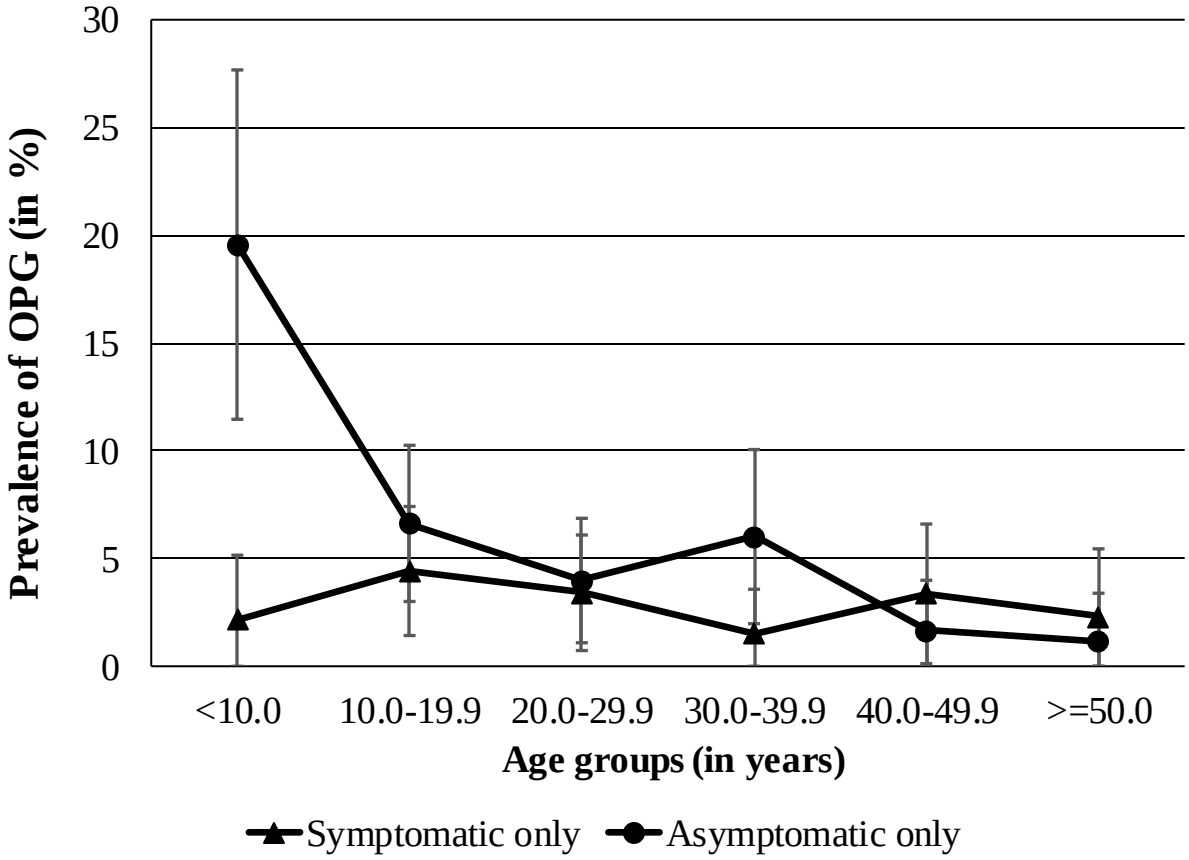
Figure 8: Prevalence of OPG in NF1 patients per 10-year age group. Error bars are 95% confidence intervals of a binomial distribution.



In addition, we separated symptomatic and asymptomatic tumours and plotted their prevalence by age group (see Figure 9). The prevalence of symptomatic tumours is stable throughout all age groups, whereas the decline in prevalence with increasing age seen in Figure 8 is driven by the decline of prevalence of asymptomatic tumours with increasing age.

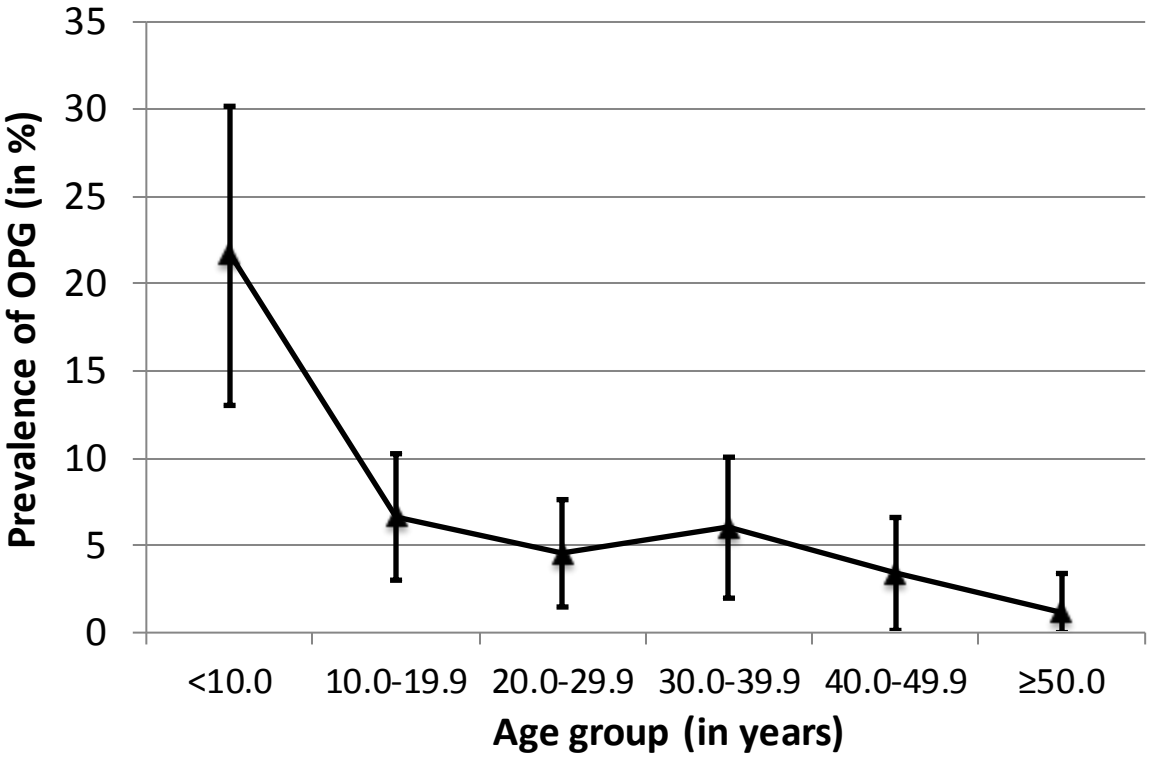
Figure 9: Prevalence of symptomatic and asymptomatic tumours per age group. Errors of the prevalence of asymptomatic tumours are 95% confidence intervals of a binomial

distribution, errors of the prevalence of symptomatic tumours are 95% confidence intervals of a Poisson distribution.



In order to investigate in which age groups optic gliomas appear in NF1 patients, we calculated the percentage of NF1 patients who had an OPG present on their first scan in different age groups (see Figure 9). There is a significant difference between the percentage of children under 10 who had an OPG present on their first scan and the percentage of all patients above 10 years of age who had an OPG present on their first scan (p=0.0001).

Figure 10: Percentages of NF1 patients with OPG present on their first scan per 10-year age group. Error bars are 95% confidence intervals of a binomial distribution.



3.3.3 Clinical description of OPGs

Of the 52 OPG patients in this study, two patients had seven segments of their optic pathway affected (both intraorbital optic nerves, both prechiasmatic optic nerves, the optic chiasm and both optic radiations), one patient had six segments affected, two patients had five segments affected, four patients had four segments affected, six patients had three segments affected, 14 patients had two segments affected, and 23 patients had one segment affected. The intraorbital

optic nerves were affected in 29 individuals, the prechiasmatic optic nerves were affected in 30 individuals, the chiasm was affected in 18 individuals, and postchiasmatic structures were affected in 12 individuals. The OPG was limited to the chiasm and/or the radiations in only four individuals. When comparing symptomatic and asymptomatic patients, the post-chiasmatic structures were the only structures to be significantly more often affected in symptomatic patients ($p=0.04$). Of the 48 gliomas that affected the optic nerves, 29 were unilateral and 19 were bilateral. 13 of 52 OPG patients had multifocal gliomas (multiple aspects of the same tumours seemingly unconnected on MRI).

Total tumour volumes ranged from 95.4 mm^3 and 9260 mm^3, with a median tumour volume of 879 mm^3.

Seven patients received all their MRI scans without the use of contrast matter; therefore, enhancement could be evaluated in 45 OPG patients. 34 patients showed no enhancement, two patients showed diffuse enhancement, two patients showed mild enhancement, and six patients showed avid enhancement of their tumours. Seven of the 15 (47%) patients with symptomatic tumours showed enhancement; this was only seen in 4 of 30 patients with asymptomatic optic gliomas $\left(X_c^2=4.3,\ p=0.04\right)$.

The presence or absence of the following clinical features was extracted from MRI reports for all 562 NF1 patients: UBO were present in 246 patients (44%), plexiform neurofibromas were present in 321 patients (57%). Among the 52 OPG patients, 34 patients had UBOs (65%) and 32 patients had plexiform neurofibromas (62%) Subcutaneous neurofibromas were only evaluated in the 52 OPG patients and were seen in 22 patients (42%).

Non-optic gliomas were seen in 34 of the 562 NF1 patients included in this study: Nine (17%) of the 52 OPG patients but only 25 (5.2%) of 485 patients without OPG had non-optic glioma (X_c^2=10.7, p=0.001). Of the nine patients with OPG and concurrent non-optic glioma, three had two non-optic gliomas and six had one non-optic glioma. These 12 non-optic gliomas were located as follows: four in the corpus callosum, two in the cerebellum, two in the anterior commissure, one in the thalamus, one in the cerebellar peduncle, one in the fornix and one in the brainstem. Four of the nine non-optic glioma patients were treated for clinically or radiologically progressive OPGs.

Table 4: Features of OPG patients. OPG location indicates tumour extent after shrinkage in patients with spontaneous partial regression.

Patient number	Sex	Age at first scan	OPG location	Enhancement	Changes during follow-up	Symptoms	Treatment	Changes after treatment
1	M	32.1	Left prechiasmatic ON	No	Stable	-	-	-
2	M	5.2	Chiasm, right radiations	No	Stable	-	-	-
3	M	6.3	Left prechiasmatic ON	No contrast used	Decreased in size	-	-	-
4	F	19.7	Left intraorbital ON	No	Stable	Reduced vision in left eye	-	-
5	F	10.8	Chiasm	Avid	Stable	Reduced vision and visual field defects in both eyes	Vincristin, carboplatine	Tumour shrinkage and improvement of vision
6	F	23.1	Left prechiasmatic ON	No	Stable	-	-	-

Patient number	Sex	Age at first scan	OPG location	Enhancement	Changes during follow-up	Symptoms	Treatment	Changes after treatment
7	F	5.1	Right and left prechiasmatic ON	No	Stable	-	-	-
8	M	1.3	Left prechiasmatic ON	Avid	Newly-appearing	-	-	-
9	F	54.1	Right and left intraorbital ON	No	Increased in size	-	-	-
10	M	2.7	Right and left intraorbital ON	No contrast used	Stable	-	-	-
11	F	10.8	Right intraorbital ON	No	Stable	-	-	-
12	M	10.2	Left prechiasmatic ON	No	Stable	-	-	-
13	M	6.2	Right prechiasmatic ON, chiasm, right radiations	Avid	Stable	Reduced vision in both eyes, visual field defect in right eye	Vincristin, carboplatine	Improved vision
14	M	25	Right and left intraorbital ON	No	Stable	Reduced vision in left eye, visual field defect in left eye	-	-

Patient number	Sex	Age at first scan	OPG location	Enhancement	Changes during follow-up	Symptoms	Treatment	Changes after treatment
15	M	11	Right and left intraorbital ON	No	Stable	-	-	-
16	M	2.4	Left intraorbital ON, right prechiasmatic ON	No	Stable	-	-	-
17	F	8.7	Right and left intraorbital ON	No	Stable	Reduced vision in both eyes	Vincristin, carboplatine	Improved vision
18	M	30.3	Right prechiasmatic ON, left prechiasmatic ON, chiasm, right radiations, left radiations	Diffuse	Stable	Reduced vision in right and left eye, visual field defects in both eyes	-	-
19	M	8.3	Right and left intraorbital ON	No contrast used	Stable	-	-	-
20	M	26.6	Left intraorbital ON	No	Stable	-	-	-
21	F	32.7	Right prechiasmatic ON, left prechiasmatic ON, chiasm,	No	Stable	-	-	-

Patient number	Sex	Age at first scan	OPG location	Enhancement	Changes during follow-up	Symptoms	Treatment	Changes after treatment
			right radiations, left radiations					
22	F	3.2	Right intraorbital ON, left intraorbital ON, right prechiasmatic ON, left prechiasmatic ON, chiasm, right radiations, left radiations	Mild	Stable	-	-	-
23	M	15	Left intraorbital ON	No	Stable	-	-	-
24	M	8.8	Left prechiasmatic ON, chiasm	No	Decreased in size	-	-	-
25	M	9.7	Left intraorbital ON	No contrast used	Stable	-	-	-
26	M	2.5	Right intraorbital ON	No	Stable	-	-	-

Patient number	Sex	Age at first scan	OPG location	Enhancement	Changes during follow-up	Symptoms	Treatment	Changes after treatment
27	F	13.1	Right radiations	Avid	Decreased in size	-	-	-
28	F	29.9	Right intraorbital ON, left radiation	No	Stable	-	-	-
29	M	18.1	Left prechiasmatic ON, chiasm, right radiations, left radiations	Mild	Stable	Blind in left eye, reduced vision and visuals field defect in right eye	-	-
30	F	15.3	Right intraorbital ON, left intraorbital ON, right prechiasmatic ON, left prechiasmatic ON, chiasm, right radiations, left radiations	Diffuse	Stable	Reduced vision in both eyes, visual field defect in left eye	Vincristin, carboplatine	Decreased vision
31	M	48.9	Right intraorbital ON, left intraorbital ON, right prechiasmatic	No contrast used	Stable	Blind in both eyes	-	-

Patient number	Sex	Age at first scan	OPG location	Enhancement	Changes during follow-up	Symptoms	Treatment	Changes after treatment
			ON, left prechiasmatic ON					
32	F	8.3	Left prechiasmatic ON	No	Stable	-	-	-
33	F	34.6	Right prechiasmatic ON, chiasm	No	Stable	Reduced vision in right eye	-	-
34	M	9.8	Chiasm	No	Stable	-	-	-
35	F	25.4	Right intraorbital ON, right prechiasmatic ON, chiasm, right radiations	Mild	Stable	Blind in right eye, reduced vision and visual field defect in left eye	-	-
36	F	23.7	Right intraorbital ON	No	Stable	-	-	-
37	F	4	Left intraorbital ON, left prechiasmatic ON, chiasm	Avid	Stable	-	-	-

Patient number	Sex	Age at first scan	OPG location	Enhancement	Changes during follow-up	Symptoms	Treatment	Changes after treatment
38	M	3.8	Right prechiasmatic ON, left prechiasmatic ON	No contrast used	Stable	-	-	-
39	F	15.1	Left prechiasmatic ON, chiasm, left radiations	No contrast used	Stable	Blind in left eye, reduced vision and visual field defect in right eye	Radiation and surgery	Decreased vision
40	M	22.3	Right prechiasmatic ON	No	Stable	-	-	-
41	F	34.5	Left prechiasmatic ON	No	Stable	Reduced vision in both eyes, visual field defects in both eyes	-	-
42	F	35.9	Left prechiasmatic ON	No	Stable	-	-	-
43	M	12.3	Right intraorbital ON, left intraorbital ON, right prechiasmatic	No	Stable	Reduced vision in right and left eye, visual field defect in right eye	Vincristin, carboplatine	Stable vision

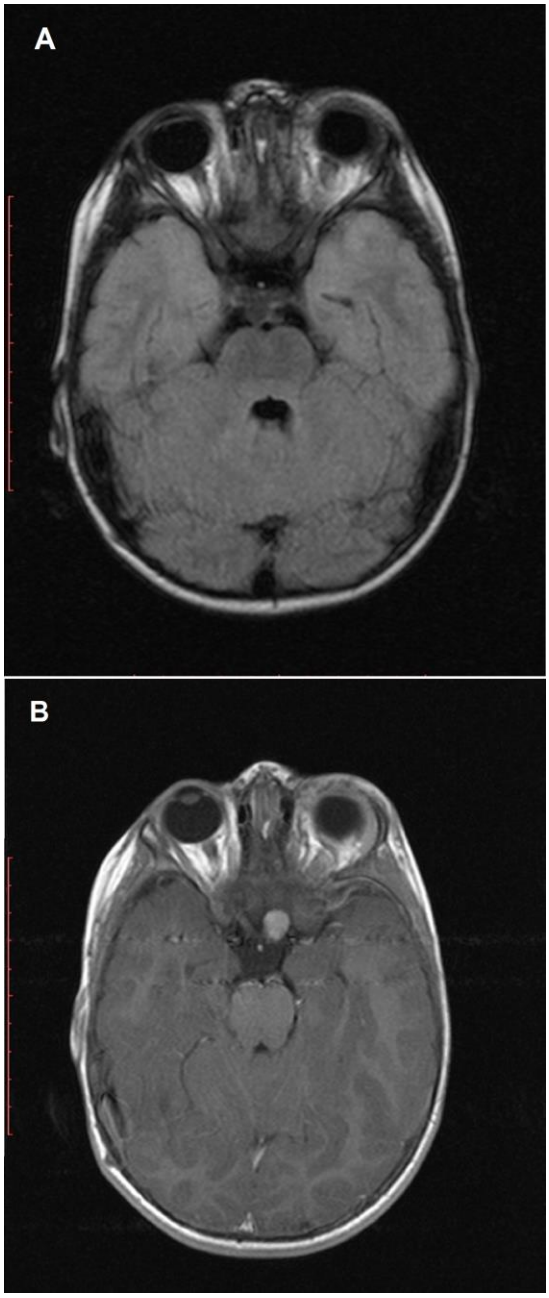
Patient number	Sex	Age at first scan	OPG location	Enhancement	Changes during follow-up	Symptoms	Treatment	Changes after treatment
			ON, left prechiasmatic ON, chiasm, right radiations					
44	M	10.5	Right prechiasmatic ON, left prechiasmatic ON, chiasm	No	Stable	-	-	-
45	M	46.1	Right intraorbital ON, right prechiasmatic ON, chiasm	No	Stable	Reduced vision in both eyes, visual field defects in both eyes	-	-
46	F	4.6	Left intraorbital ON, left prechiasmatic ON	No	Stable	-	-	-
47	F	32.9	Right intraorbital ON, left intraorbital ON, left	No	Stable	-	-	-

Patient number	Sex	Age at first scan	OPG location	Enhancement	Changes during follow-up	Symptoms	Treatment	Changes after treatment
			prechiasmatic ON					
48	F	2.5	Right intraorbital ON, left intraorbital ON, left prechiasmatic ON	No	Decreased in size	-	-	-
49	M	44.6	Left intraorbital ON	Avid	Stable	Blind in left eye	Surgery	Decreased vision
50	F	25.3	Right prechiasmatic ON	No	Stable	Reduced vision in right eye	-	-
51	M	5.2	Right prechiasmatic ON, left prechiasmatic ON, chiasm, right radiations, left radiations	No	Stable	-	-	-
52	F	30.3	Right prechiasmatic ON	No	Stable	-	-	-

3.3.4 Newly-appearing OPGs

The only patient (Patient 8 in Table 4) with a newly-appearing OPG was 1.3 years of age at his first scan, and no glioma was apparent on MRI. At 2.0 years of age, the patient had developed an avidly enhancing glioma in the left prechiasmatic optic nerve measuring 1820 mm^3 (see Figure 10). The OPG remained stable in volume and enhancement throughout the next 2 scans spanning 1.1 years. On a scan at 3.0 years of age, the enhancement decreased from avid to diffuse. The glioma remained unchanged during 6.9 years of follow-up, and the patient remained asymptomatic.

Figure 11: Newly-appearing OPG in the left prechiasmatic optic nerve of Patient 8. (10A) Glioma is not present on a scan perfomed when the patient was 1.3 years of age. (10B) Glioma has appeared in the left prechiasmatic optic nerve when the patient was 2.0 years of age.

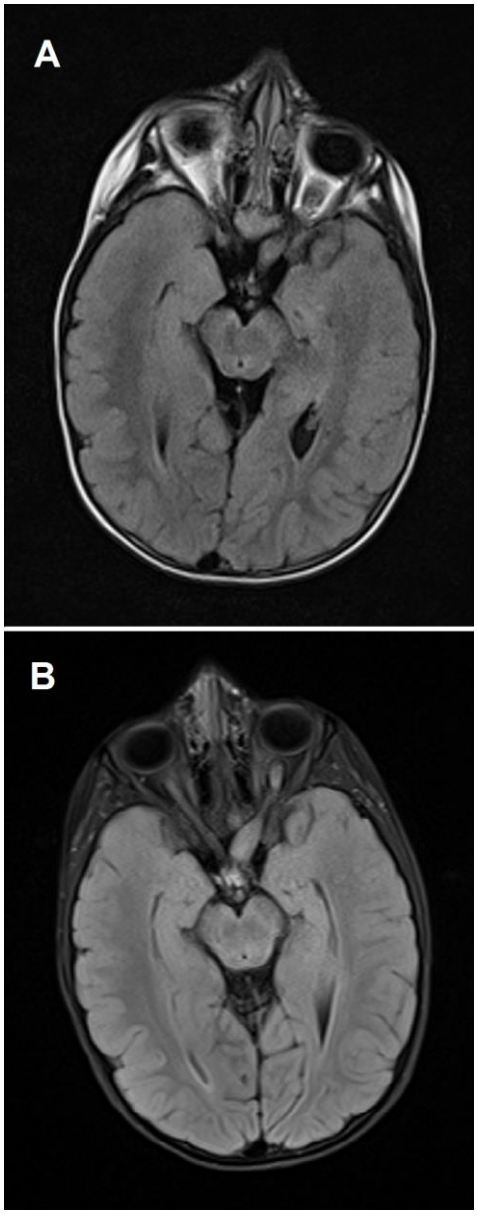


3.3.5 Progressing OPGs

The only patient with an OPG that increased in volume during observation in this study was Patient 37 (see Table 4). She was aged 4.0 years when she was diagnosed with a mildly enhancing OPG measuring 1862 mm^3 involving her left intraorbital and prechiasmatic optic nerve. On her next scan 1.0 years later, her glioma had increased in volume to 2636 mm^3 and showed avid enhancement. It now involved the left intraorbital optic nerve, the left prechiasmatic optic nerve and the optic chiasm. The tumour volume remained stable on a follow-up scan performed 0.9 years later. The patient remained asymptomatic throughout the entire follow-up.

We observed one OPG progression spanning 1.0 years of follow-up during 283.2 patient years of follow-up of OPG patients. This equates to a rate of progression of 0.35% (95% confidence interval: 0.02% to 2.3%). One of 52 OPGs (2%, 95% confidence interval 0-12%) progressed after the initial diagnosis.

Figure 12: Progressing glioma in Patient 37. (11A) On the first scan performed at 4.0 years of age, an OPG with a volume of 1862 mm^3 is present in the left intraorbital and prechiasmatic optic nerve. (11B) On the next scan performed 1.0 years later, the glioma has increased in volume to 2636 mm^3 and now involves the left intraorbital optic nerve, the prechiasmatic optic nerve, and the optic chiasm.



3.3.6 Spontaneously regressing OPGs

Four spontaneously regressing OPGs were identified during the study period.

Patient 3 had an asymptomatic non-enhancing OPG measuring 516 mm^3 of the left prechiasmatic optic nerve at 6.3 years of age (see Figure 12). The glioma was stable on 2 subsequent scans spanning 2.2 years, and then decreased in volume to 462 mm^3 0.8 years later. The tumour was stable on a scan performed 0.9 years later and then further regressed to 436 mm^3 when the patient was 11.2 years of age. The tumour volume remained stable on the last scan performed 0.5 years later. Contrast was not administered after the first scan.

Patient 24 had an avidly enhancing OPG measuring 721 mm^3 of the left prechiasmatic optic nerve and optic chiasm diagnosed on his first scan at 8.8 years. On the following scan 0.8 years later, the OPG was non-enhancing and had regressed to 598 mm^3. It remained stable for the following 5 scans spanning 6.4 years.

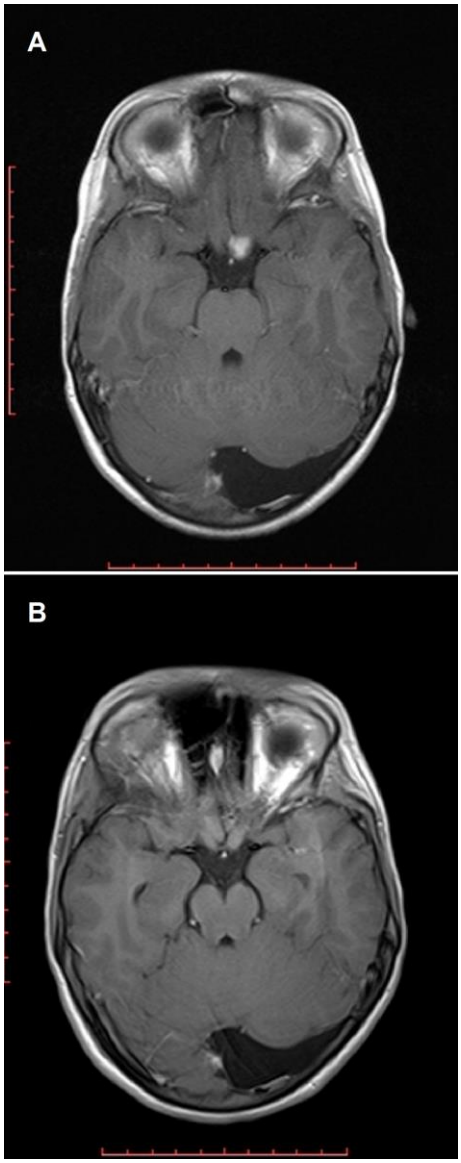
Patient 27 was first scanned at 13.1 years of age when an avidly enhancing OPG was diagnosed in her right optic radiations. Due to its diffuse nature, the tumour volume could not be determined, but its length was 25 mm. On the following scan 3.6 years later, the OPG was only mildly enhancing and the length had decreased to 21 mm. The length of the tumour was unchanged at the last follow-up 2.3 years later. The patient remained asymptomatic throughout the follow-up.

Patient 48 had a multifocal OPG diagnosed when she was first scanned at age 2.5 years. It affected the right intraorbital optic nerve, the left intraorbital optic nerve, the left prechiasmatic optic nerve and the optic chiasm. It was diffusely enhancing and measured 6219 mm^3. On a scan

performed 5.0 years later, the OPG was not enhancing and no longer involved the chiasm. In addition, the volume had decreased to 2936 mm^3. The tumour volume remained stable for the following 3 scans spanning 6.2 years. The patient remained asymptomatic throughout the follow-up.

We observed five instances of spontaneous regression during 11.2 patient years in four (8%, 95% confidence interval: 2-19%) of 52 NF1 patients with OPGs who were followed for a total of 283.2 patient years. The rate of OPG regression was 4% (95% confidence interval: 2% to 7%). All instances of OPG regression occurred in patients under the age of 20 years although only 54% of the patient-years of observation took place in this age group (p=0.002). In three of the four patients (Patients 24, 27 and 48) in whom regression occurred, the OPG showed enhancement prior to its shrinkage (no contrast enhanced studies were performed in Patient 3 after his first scan). Enhancement was significantly associated with tumour regression in our patient population (p=0.04). Enhancing tumours in patients without regression were (except for Patient 22) always symptomatic or in the process of appearing or increasing in size.

Figure 13: Spontaneous regression of an OPG in Patient 3. (12A) A glioma is present in the left prechiasmatic optic nerve measuring 516 mm^3 and was diagnosed at patient age 6.3 years. It stayed stable in volume during the following 2.2 years. (12B) 3.0 years after the initial diagnosis, the glioma volume decreased to 462 mm^3 and then to 436 mm^3 when the patient was 11.2 years old. The tumour remained stable in volume on the last scan performed 0.5 years later.



3.3.7 Association of OPG presence and volume with other clinical features

Multiple regression analysis was performed to identify factors contributing to OPG presence or volume in individual patients.

All factors that were investigated to influence OPG presence are summarized in Table 5.

None of the factors investigated in this study significantly contributed to final OPG volume.

Table 5: Age-adjusted associations of clinical features typical for NF1 with the presence of OPG.

Independent variable	Odds ratio	95% confidence interval	Significance (p-value)
UBOs	2.4	1.2 to 4.8	0.01
Age at first scan (in years)	0.96	0.94 to 0.99	0.003
Non-optic glioma	4.8	2.0 to 12	0.0004
Plexiform neurofibromas	1.2	0.61 to 2.2	0.65

3.3.8 Association of UBOs and asymptomatic OPGs in NF1 patients

Because both the prevalence of OPGs and UBOs decreases with increasing age [116], we investigated the relationship between these clinical features. There is a strong overall association between the presence of OPGs and UBOs ($X_c^2 = 11.6$, p=0.0007). As most OPGs are asymptomatic, but other become symptomatic and cause vision loss, we wondered if the association with UBOs was driven by asymptomatic or symptomatic OPGs. The prevalence of

asymptomatic OPGs decreases sharply with age, whereas the prevalence of symptomatic OPGs remains stable throughout all age groups (see Figure 9). Figure 14 shows that the prevalence of UBOs and asymptomatic OPGs decreases in a similar fashion, although the prevalence of UBOs is far greater in every age group. We found no association between the presence of UBOs and symptomatic OPGs, but the presence of UBOs and asymptomatic OPGs was associated in every age group over 10 years of age (see Table 6).

Figure 14: Percentage of NF1 patients with OPGs and UBOs per 10-year age group. Error bars are 95% confidence intervals of a binomial distribution.

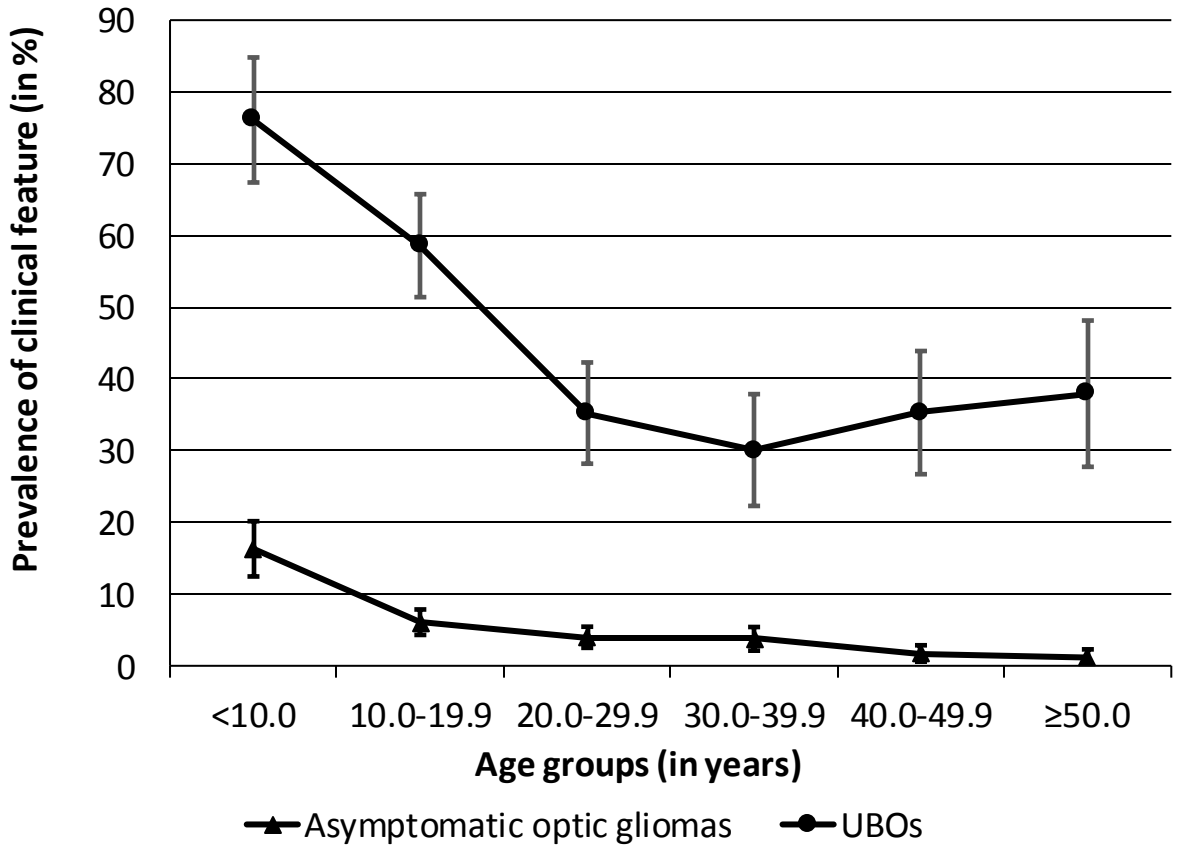


Table 6: Correlations between the presence of UBOs and the presence of asymptomatic OPG per 10-year age group.

Age groups	Chi-squared statistic	Significance (p-value)
0 to 9.9 years	0.65	0.42
10.0 to 19.9 years	3.9	0.05
20.0 to 29.9 years	13	0.0003
30.0 to 39.9 years	6.0	0.01
40.0 years and above	6.6	0.01

3.4 Discussion

In this study, we present the largest series of head MRIs to date in unselected NF1 patients. Our study is especially informative, since it includes a wide age range and is longitudinal, with an average of over 3 MRI scans per patient. This is also the first study to report symptomatic and asymptomatic plexiform neurofibromas diagnosed by routine whole-body MRI, as well as other typical NF1 features diagnosed by routine head MRI. Whole-body MRI revealed plexiform neurofibromas in approximately half of NF1 patients [6], even though most of these tumours are asymptomatic and do not appear on physical examination.

Most previous studies were performed retrospectively and have used convenience samples to estimate the frequency of OPG in children with NF1 [73,117]. However, this approach is inherently biased since patients are selected based on clinical symptoms that required them to undergo imaging. In our study, every patient seen at the NF outpatient department was offered whole-body MRI, so they are an unbiased representation of the NF1 patient seen in the clinic.

Recently, a French group performed a retrospective head MRI study including 306 children under the age of 6 years and found the prevalence of OPG to be 14.7% (95% confidence interval: 11.0% to 19.3%), with 80% of patients being asymptomatic [73]. Other authors found slightly higher prevalences ranging from 15% to 18% [117–119]. We found the prevalence of OPG in children to be 22% in our study. Since most OPGs are asymptomatic and histological samples are rarely obtained, it is difficult to determine the true OPG occurrence rate.

The prevalence of OPG in older children and adults has only rarely been evaluated, but is likely lower than in young children. A retrospective study of 138 adult NF1 patients found the

prevalence of OPG to be 5.8% (95% confidence interval: 2.7% to 11%). In concordance with this group, we found the prevalence in NF1 patients older than 19.9 years of age to be 4.9% (95% confidence interval: 3.3% to 7.2%).

There are several explanations to explain the decline in prevalence of OPG from childhood to adulthood. Firstly, some OPGs might be treated successfully in childhood, leaving fewer adults with OPGs to be identified. This is, however, unlikely, since most OPGs in childhood are asymptomatic and never require treatment [85,120]. Secondly, there might be increased mortality in young OPG patients, so that children with OPG are less likely than children without OPG to survive to adulthood. A study assessing risk factors for mortality in NF1 did not find that having an OPG is a risk factor for death in NF1 patients [65], and that OPG is rarely a cause of death in NF1, if ever [39]. On the other hand, OPGs were found to predispose to the development of non-optic gliomas [54], which are, in turn, are associated with increased mortality [65]. We found a strong association between the presence of OPGs and non-optic gliomas in this study. Thirdly, OPGs might regress spontaneously, a phenomenon that has been described many times in children with NF1 (see Table 7). It is currently unknown how frequently OPGs regress and which factors influence regression. Table 7 suggests that chiasmatic tumours are especially likely to regress, but that OPGs in other locations may regress as well. Nine of the 13 cases of spontaneous regression reported in the literature were symptomatic. In this study, all symptomatic tumours were treated, so a reduction in tumour size would likely be due to treatment response. We saw 4 cases of spontaneous partial regression in asymptomatic patients. Three of our 4 cases (except Patient 3 who had all examinations but his first one performed without contrast) and all 4 cases

of spontaneous regression described in the literature were younger than 20 years of age and showed avid regression before and mild to no enhancement after regression.

Our results indicate that the majority of OPGs are stable over time and only a few progress or regress. In this study, we only observed progression and new appearance of an OPG in 1 patient, respectively.

This work contains the first estimates of progression and regression of OPGs in NF1 patients ever published. These estimates provide information on OPG activity but they cannot be used to provide annual risks of progression for individual patients. Additional studies that include more patients with longer longitudinal follow-up are needed to establish reliable per patient rates of progression or regression.

There has been considerable controversy over the true biological nature of OPGs. Histologic analysis of OPGs showed them to be usually low-grade (WHO Grade 1) or occasionally higher-grade (WHO Grade 2 or 3) tumours [57]. These samples, however, are always obtained from clinically or radiologically progressive tumours and may not be representative of asymptomatic OPGs in NF1 patients.

Table 7: Published cases of spontaneous regression of symptomatic and asymptomatic OPG in NF1 patients.

Authors	Year	Sex	Age at diagnosis	Age at regression	OPG location	Enhancement	Symptomatic?	Extent of regression	Imaging
Shofty et al. [121]	2014	Unknown	11	Unknown	Unknown	Unknown	No	Unknown	MRI
Shofty et al. [121]	2014	Unknown	14	Unknown	Unknown	Unknown	No	Unknown	MRI
Piccirilli et al. [75]	2006	M	2	2.9	Chiasm	Avid, no enhancement after regression	Yes	Complete	MRI
Piccirilli et al. [75]	2006	F	1	4.9	Chiasm	Avid, no enhancement after regression	Yes	Complete	MRI
Piccirilli et al. [75]	2006	F	2	5	Chiasm	Avid, no enhancement after regression	Yes	Complete	MRI
Parsa et al. [11]	2001	M	7	11	Chiasm and right optic nerve	Avid, decreased enhancement after regression	Yes	Partial	MRI

Authors	Year	Sex	Age at diagnosis	Age at regression	OPG location	Enhancement	Symptomatic?	Extent of regression	Imaging
Parsa et al. [11]	2001	F	11	17	Chiasm and radiations	Avid, decreased enhancement after regression	Yes	Partial	CT/MRI
Parsa et al. [11]	2001	M	3	15	Chiasm	Unknown	Yes	Partial	MRI
Schmandt et al. [122]	2000	M	3.6	4.1	Chiasm, hypothalamus, optic nerves	Avid, decreased enhancement after regression	Yes	Partial	MRI
Gottschalk et al. [123]	1999	M	1.9	2.9	Chiasm and hypothalamus	Avid, no enhancement after regression	No	Partial	MRI
Perilongo et al. [124]	1999	F	2.5	3	Chiasm, right optic radiation	Avid, no enhancement after regression	Yes	Partial	MRI
Parazzini et al. [125]	1995	M	1.9	3.3	Chiasm and radiations	Avid, no enhancement after regression	Yes	Complete	MRI

Authors	Year	Sex	Age at diagnosis	Age at regression	OPG location	Enhancement	Symptomatic?	Extent of regression	Imaging
Parazzini et al. [125]	1995	M	2.1	3.3	Chiasm and hypothalamus	Avid, no enhancement after regression	No	Complete	MRI
Parazzini et al. [125]	1995	F	1.8	3.2	Chiasm and midbrain	Avid, no enhancement after regression	No	Partial	MRI
Parazzini et al. [125]	1995	F	13	13.7	Chiasm and radiations	Avid, no enhancement after regression	No	Partial	MRI

Sex has been identified as a determinant of which OPGs become symptomatic in NF1 patients. It does not seem to influence OPG location or frequency [73]; however, girls with OPG were reported to undergo imaging for visual symptoms significantly more often than boys, and girls are three times more likely to receive treatment for visual decline [82]. NF1 OPG mouse models indicate that this difference may be caused by reduced levels of cAMP and subsequent retinal ganglion cell loss in females, leading to vision loss [82]. Studies in human OPG patients with NF1 showed that this decline in cAMP levels may be a consequence of ADCY8 polymorphisms and patient sex [86]. In this study, we did not see a difference in tumour location, symptom status, and frequency of treatment between males and females. This disagreement between studies may be caused by females being more likely to report symptoms and undergo imaging and treatment, instead of a true increased symptom prevalence in females with NF1.

We observed a strong overall correlation between the presence of asymptomatic OPGs and the presence of UBOs even after age-adjustment. There was no correlation in children less than 10 years of age; this may be explained by the high abundance of UBOs in this age group. 76% of children less than 10 years of age have UBOs, whereas only 20% have asymptomatic OPGs, resulting in most children with UBOs not having concurrent OPG even if they are associated. All correlations in children ages over 10 years of age were statistically significant (see Table 6). The association between the presence of asymptomatic OPGs and the presence of UBOs made us wonder if the two share a common pathological basis. UBOs are areas of immature myelinization or intramyelinic edema [116,126,127]. The histology of asymptomatic OPGs in patients with NF1 is unknown, since biopsy or surgical removal is only performed on symptomatic tumours. Is has been assumed that the pathology and pathogenesis of asymptomatic and

symptomatic OPGs is the same in NF1 patients, even though there is little evidence supporting this assumption.

There are several intriguing parallels between UBOs and asymptomatic OPGs: their glial origin, benign course, spontaneous involution, development in early childhood, and decreasing frequency with increasing age. The decrease in prevalence of UBOs with increasing age is commonly attributed to the maturation of immature myelin structures [116], suggesting that spontaneous regression of OPGs might also represent tissue maturation. If asymptomatic OPGs are truly areas of immature myelin instead of neoplasms, it will alter our understanding of NF1 pathology. Asymptomatic OPGs are more prevalent in NF1 patients under 40 years of age than symptomatic OPGs, whereas in patients over 40 years of age, symptomatic OPGs become more prevalent. If asymptomatic OPGs are truly areas of immature myelinization, this change is most likely due to tissue maturation with increasing age instead of a conversion of asymptomatic OPGs to symptomatic OPGs. In accordance, we suggest that asymptomatic OPGs are haploinsufficient rather than exhibiting two independent hits in the *NF1* locus.

Enhancement, typically regarded as a characteristic of neoplasms, is also seen in a subset of regressing asymptomatic OPGs. This enhancing subset mostly consists of regressing OPGs, and may be caused by a disruption of the blood-brain barrier due to tissue inflammation in response to glial tissue maturation. Indeed, enhancement markedly decreases after regression in our study population as well as in those reported in the literature. There are no imaging techniques available to distinguish enhancement due to inflammation from enhancement due to neoplastic changes, apart from perfusion imaging or positron emission tomography (PET), both of which are rarely used in the screening setting. If tissue from asymptomatic OPGs was obtained in the

future (e.g. postmortem), it would be possible to check if the histological changes in asymptomatic OPGs are similar to the spongiotic or edematic changes found in UBOs [126,127].

Recently, it was proposed that at least some pediatric neoplasms (including gliomas) are neurodevelopmental disorders. Pediatric gliomas are distinct from their adult counterparts: they differ in location (posterior fossa and optic pathway in children versus supratentorial compartment in adults), WHO Grade (pediatric gliomas tend to be low-grade,whereas adult gliomas are often high-grade), histological subtype (pilocytic astrocytoma in children, compared to glioblastoma in adults) and their potential for malignant transformation (low in children versus high in adults) [128]. *NF1* is a tumour suppressor gene, and all cells in NF1 patients carry at least one defective *NF1* allele. A second hit on the functional allele in a glial progenitor cell might lead to a growth advantage over other progenitors, subsequent clonal expansion, and result in tumour formation. OPGs in NF1 are usually diagnosed in young children and only a few arise in adults. This stands in contrast to non-optic gliomas in NF1 patients, which do frequently arise in adults [64]. Further research is needed to investigate the differences between these two histologically similar glioma types.

There are currently no guidelines that recommend routine MRI screening for NF1 patients. We offered head magnetic resonance imaging to all NF1 patients at the time of their first evaluation in our clinic and follow-up imaging every 24 months to patients who did not have optic pathway (or other) gliomas. Follow-up imaging was offered every 12 months to asymptomatic patients found to have OPGs and every 6 months to patients with symptomatic OPGs in this study. OPGs rarely (if ever) become symptomatic after 11 years of age in people with NF1. Our oldest patient who became symptomatic under observation was 11 years old, and previous studies have found

younger ages after which OPGs became symptomatic in NF1 patients [113,117]. We are not aware of any report of an asymptomatic OPG first becoming symptomatic later in life in a patient with NF1.

Chemotherapy was the treatment of choice for symptomatic patients in our series, and surgery and/or radiation was only performed for progressive tumours if vision was already severely decreased. Chemotherapy led to stabilization or improvement of vision in 4 of 5 patients.

We observed spontaneous regression only in asymptomatic OPG patients; however, cases of regression of symptomatic OPG have been reported in the literature (see Table 7). Asymptomatic OPGs are underrepresented in the literature (as they are only identified through routine screening), and their potential for regression is therefore not well described. We observed spontaneous regression of OPGs in NF1 patients only in the first two decades of life, and this may be much less frequent in adulthood. The majority of regressing OPGs seems to involve the chiasm, sometimes in association with adjacent structures. Is it unclear what causes this pattern, but microenvironment may play a role [51,129].

Based on these observations, we draw the following conclusions: symptomatic OPGs only very rarely occur in patients older than 11 years of age, and asymptomatic OPGs rarely, if ever, become symptomatic later in life. Regression is possible in symptomatic and asymptomatic OPGs, and mostly occurs in chiasmatic OPGs in patients younger than 20 years of age.

The present study has several limitations. Even though our study population is representative of the NF1 patient population seen in the clinic, we cannot rule out referral bias. In addition, symptomatic patients may be more likely to consent to participate in the study and receive more

frequent follow-up examinations and scans. There are no diagnostic guidelines for OPGs in NF1 patients, and diagnosis is usually based on clinical expertise. We attempted to address this limitation by only including patients who were diagnosed with OPG by the clinical and study neuroradiologists; however, the true nature of these lesions is indeterminable without histology.

This is the largest prospective series of head and whole-body MRIs ever performed in NF1 patients of all ages. It is also the first study quantifying the rate of OPG progression and regression in NF1. Our data are important for scientists working to understand the pathogenesis of NF1-associated OPG as well as for clinicians assessing NF1 patients who have OPG.

Chapter 4: Genotype-phenotype correlation

4.1 Summary

In this chapter, I present an evaluation of genotype-phenotype correlations in NF1 patients. I grouped all 283 mutations based on their mutation class and annotated all 255 NF1 variants with CADD scores and grouped them into quartiles. I investigated associations of mutation classes as well as CADD quantiles with the presence or absence of optic gliomas, UBOs, plexiform neurofibromas, and MPNSTs. I found a significant association of whole-gene deletions with the presence of plexiform neurofibromas, and an association between the highest CADD score quartile with the presence of plexiform neurofibromas. This may influence management of NF1 patients with more deleterious variants.

4.2 Methods

4.2.1 Genotyping

DNA extracted from patient lymphocytes was used for whole-gene deletion analysis and genotyping. First, six intragenic microsatellite markers were investigated in order to identify patients possibly harbouring large gene deletions [130]. Patients homozygous (hemizygous) for all six markers were then investigated by MLPA (SALSA MLPA P122 NF1-area probe mix, MRC Holland), FISH and breakpoint-spanning PCRs as described previously [131–133] in order to confirm the deletions, identify their size and type, and somatic mosaicism with normal cells. In all

patients without large *NF1* deletions, the 57 constitutive *NF1* exons were amplified from genomic DNA using primers located in flanking introns and analyzed by Sanger sequencing. Small deletions or insertions were analyzed by MLPA (SALSA MLPA probe mix P081-C1/P082-C1 NF1,MRC Holland), and all variants under 20 bp were characterized by Sanger sequence analysis using an ABI 3730 analyzer (Applied Biosystems, Warrington, Cheshire, UK) [134]. All variants were annotated using ANNOVAR [135].

Annotation included variant function, frequency in reference databases, and CADD scores (when available). CADD scores for indels were obtained by means of the web-based CADD annotation tool (URL: http://cadd.gs.washington.edu/score).

For the purpose of this analysis, all mutations were grouped into one of the following five categories: Whole gene deletions, Nonsense (including small frameshift and stopgain) variants, Missense (including nonsynonymous single nucleotide variants and small in-frame deletion) variants, Splice variants, and Other variants. Splice variants were defined according to ANNOVAR as including 2 bp up- or down-stream of an exon-intron border. The Other category consisted of other types of variants, such as single exon deletions or duplications, each present in only a few patients. This category was very heterogeneous and was, therefore, excluded from the analysis.

4.2.2 Magnetic resonance imaging (MRI)

All magnetic resonance imaging was performed at the MRI Institute Hamburg Altona, Germany. Whole-body MRI scans were obtained on a 1.5T scanner before 2013; after that, a

3.0T scanner was used. T1-weighted, T2-weighted and FLAIR images were obtained with a slice thickness of 5 mm or less. Head MRI scans were performed using a cranial protocol. Pre- and post-gadolinium scans were obtained if clinically indicated. Whole-body MRIs were analyzed for presence of plexiform neurofibromas, and head MRIs were analyzed for presence of optic gliomas and unidentified bright objects (UBOs). Based on the clinical MRI reports, a list of patients was generated who had been diagnosed with UBOs, optic gliomas, or plexiform neurofibromas. For this study, the presence or absence of a feature was determined at the last scan during the study period. All findings were recorded in an encrypted database.

4.2.3 Statistical analysis

Logistic regression was performed to investigate associations between variant classes (as nominal variables) and the presence or absence of optic gliomas, unidentified bright objects (UBOs), or plexiform neurofibromas (as binary variables). Missense mutations were used as the reference category.

Three patients had a CADD score lower than 10 and were excluded from the analysis, since a variant with a CADD score under 10 is most likely not pathogenic [136]. Logistic regression was used for association between CADD scores (as an ordinal variable) and the presence or absence of optic gliomas, unidentified bright objects, or plexiform neurofibromas. All regression models were adjusted for age (as a continuous variable and were performed in SPSS version 23.

Results were considered significant if the 95% confidence interval excluded of the odds ratio excluded 1.0.

4.3 Results

4.3.1 Patient demographics

Our study included 404 unrelated NF1 patients. A causal pathogenic variant could not be identified in 91 of these patients. 25 of the remaining patients carried a constitutive variant that did not fall into the category of nonsense variant, missense variant, splice variant, or whole-gene deletion and were therefore excluded from the analysis, leaving 288 NF1 patients (71.2%) with a mutation in one of these four categories. 134 of the patients were male and 154 patients were female. Patient ages at the time of their last MRI scan ranged between 1.2 years and 68.8 years of age.

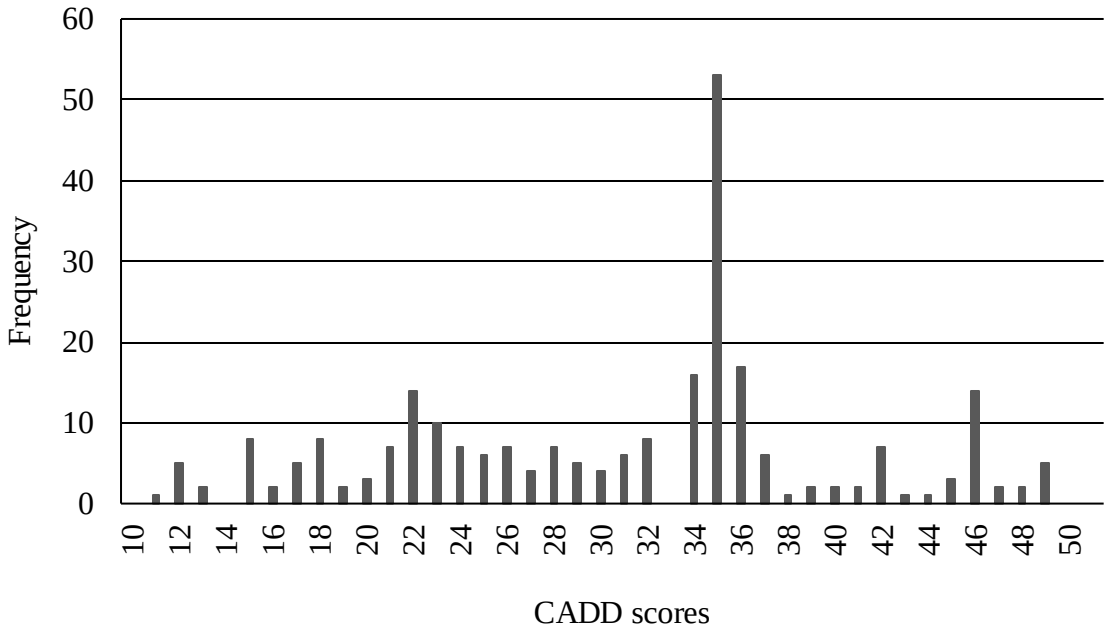
4.3.2 Genotypes and CADD scores

Of the 288 patients, 53 had missense variants, 172 had nonsense variants, 30 had splice variants, and 33 had non-mosaic whole-gene deletions (Table 8). Of these 33 whole-gene deletion patients, 28 patients had Type 1 deletions, 1 patient had a Type 2 deletion, and 4 patients had atypical deletions. Only the 28 Type 1 deletion patients were included in the analysis. This led to a total of 283 NF1 patients included in this study.

CADD scores can be assigned to single nucleotide variants and small indels but not to whole-gene to deletions [136]. We were able to annotate 255 variants with CADD scores.

The distribution of CADD scores in these 255 patients appears to be trimodal (Figure 15), so the CADD scores were divided into three bins for analysis: Low: 10 to 30, Intermediate: 30.1 to 38, and High: >38.

Figure 15: Histogram of CADD scores ranging from 10.0 to 49.0 in 255 NF1 patients.



4.3.3 Genotype-phenotype correlations

We performed multiple logistic regression to investigate associations between *NF1* variant classes (nonsense variant, missense variant, splice variant, or whole gene deletion) and the presence or absence of optic gliomas, UBOs, or plexiform neurofibromas on MRI while controlling for age. The frequency of these disease features per variant class is shown in Table 8.

The presence of a whole-gene deletion was associated with the presence of plexiform neurofibromas on MRI (OR=2.9, 95% confidence interval: 1.1 to 7.8) (see Table 9).

Table 8: MRI findings in 283 genotyped NF1 patients.

Mutation Class	Total Patients	Patients with UBOs	Patients with Optic Gliomas	Patients with Plexiform Neurofibromas
Missense	53	24 (45%)	5 (9.4%)	27 (51%)
Nonsense	172	71 (41%)	18 (10%)	113 (66%)
Splice	30	10 (33%)	1 (3.3%)	17 (57%)
Whole gene deletion	28	7 (25%)	2 (7.1%)	21 (75%)

Table 9: Age-adjusted odds ratios and (95% confidence intervals) of *NF1* mutation classes with the presence of UBOs, optic gliomas or plexiform neurofibromas in 283 NF1 patients. Asterisks indicate statistically significant results.

Mutation Class	Patients with UBOs	Patients with Optic Gliomas	Patients with Plexiform Neurofibromas
Missense	1.0 (reference)	1.0 (reference)	1.0 (reference)
Nonsense	0.91 (0.48-1.7)	1.2 (0.43-3.6)	1.9 (0.99-3.5)
Splice	0.64 (0.25-1.7)	0.36 (0.04-3.3)	1.3 (0.52-3.2)
Whole gene deletion	0.48 (0.19-1.2)	0.59 (0.11-3.3)	2.9 (1.1-7.8)*

In addition, we performed ordinal logistic regression to investigate associations between CADD scores (classified into Low, Intermediate or High bins) and the presence of optic gliomas, UBOs, or plexiform neurofibromas. The frequency of each disease feature per CADD score bin is shown in Table 10.

Table 10: Number of patients exhibiting the clinical features analyzed. The mutations of these 255 patients were assorted to bins based on their CADD scores.

CADD Scores	Total Patients	Patients with UBOs	Patients with Optic Gliomas	Patients with Plexiform Neurofibromas
Low (10-30)	107	41 (38%)	7 (6.5%)	51 (48%)
Intermediate (30.1.-38)	106	44 (42%)	13 (12%)	60 (57%)
High (>38)	42	20 (48%)	4 (9.5%)	29 (69%)

We found a significant association between High CADD scores and the presence of plexiform neurofibromas when compared to Low CADD scores (OR=2.4, 95% confidence interval: 1.1 to 5.1). None of the other features were significantly associated with CADD scores. The findings are summarized in Table 11.

Table 11: Age-adjusted odds ratios and (95% confidence intervals) of *NF1* mutation CADD scores with the presence of UBOs, optic gliomas, or plexiform neurofibromas in 255 NF1 patients. Asterisks indicate statistically significant results.

CADD Scores	UBOs	Optic Gliomas	Plexiform Neurofibromas
Low (10-30)	1.0 (reference)	1.0 (reference)	1.0 (reference)
Intermediate (30.1.-38)	1.2 (0.66-2.0)	2.0 (0.77-5.4)	1.4 (0.80-2.4)
High (>38)	1.4 (0.67-2.9)	1.4 (0.40-5.3)	2.4 (1.1-5.1)*

4.4 Discussion

Easton et al. [137], Szudek et al. [138] and Sabbagh et al. [139] showed that genetic factors contribute to the highly variable phenotype in NF1 patients. By analyzing the effects of familial relationships on the occurrence of clinical features in large groups of patients, these investigators found a contribution of the pathogenic *NF1* allele, which was assumed to be identical in affected relatives, to the phenotype. Ever since then, studies have attempted to correlate individual *NF1* variant alleles or variant classes with clinical phenotypes.

Only a few different pathogenic variants have been associated with specific phenotypes by observing similarities among unrelated patients with the same or similar *NF1* mutations [92,95,97,140,141]. This contrasts with the experience in some other dominantly-inherited tumour

predisposition syndromes, such as neurofibromatosis 2 [142], Li-Fraumeni syndrome [143], and familial adenomatous polyposis [144], in which allele-phenotype correlations are well established.

In this study, we present one of the largest *NF1* genotype-phenotype correlation analyses performed to date. As this analysis is based on MRI investigations and genotyping of an unselected cohort of patients, it has the unique strength of including symptomatic as well as asymptomatic tumours. Most NF1 patients do not undergo MRI if they are asymptomatic. However, most gliomas and plexiform neurofibromas in NF1 patients are asymptomatic [73], and, therefore, were not considered during previously performed genotype-phenotype association studies. This phenotypic misclassification is likely to have made previous genotype-phenotype analyses less sensitive.

A subset of the *NF1* whole-gene deletion patients included in this study were also analyzed in a previous study that compared the clinical features of *NF1* whole-gene deletion patients to those of NF1 patients without whole-gene deletions [92]. In concordance with the previous study, we found an association of whole-gene deletions and the presence of plexiform neurofibromas (OR=2.9, 95% confidence interval: 1.1 to 7.8).

CADD aggregates multiple genetic and genomic factors related to the pathogenicity of genomic variants, thereby providing scores for different mutations that predict functional consequences [136]. By using CADD scores, we associated predicted deleteriousness with the occurrence of plexiform neurofibromas, a characteristic feature of NF1 that is present in about half of all patients [6].

While NF1 is caused by the constitutional loss of one functional *NF1* allele, NF1-associated tumours sometimes exhibit complete loss of neurofibromin activity [41,47]. It is not known how much residual neurofibromin function, if any, most pathogenic *NF1* mutations possess. Recently, however, it was shown in mice that different patient-derived pathogenic *NF1* variants lead to different amounts of residual neurofibromin function [52]. Residual NF1 protein function probably varies within mutation classes, but CADD is likely to be more sensitive than mutation class as a measure of functionality.

We found a 2.4-fold increase in the risk of having plexiform neurofibromas among NF1 patients with mutations in the High CADD score bin in our study. Lower CADD scores indicate lower deleteriousness of a variant, and less deleterious variants may have more residual neurofibromin function and therefore lead to lower plexiform neurofibroma frequency.

It is worth noting that our CADD score analysis excluded patients whose constitutional mutation was a whole-gene deletion because CADD scores cannot be calculated for this class of mutations. However, patients with whole gene deletions are predicted to be completely haploinsufficient for neurofibromin, and such patients are known to have higher risk of developing plexiform neurofibromas than NF1 patients with other kinds of mutations (Table 11 and [92]). It seems likely that if whole gene deletions could have been included in our analysis, the risk of plexiform neurofibromas among patients with *NF1* mutations predicted to be most deleterious would have been even higher.

Although CADD scoring of mutations appears to be a useful way to analyze genotype-phenotype associations in NF1, our study has several limitations. Some clinical features, such as the

presence of UBOs, change over time in NF1 patients. Since we evaluated the presence or absence of a feature at the patient's most recent MRI examination, we cannot rule out the possibility that some features that were not present at that time were present earlier or might appear later in life. In addition, our study only identified protein sequence variants in *NF1* genomic DNA. There is a variety of other pathogenic variants within the *NF1* gene that influence splicing or transcription [89], and these variants are not included in our study.

Assessing the CADD score of an NF1-causing variant of a patient with neurofibromatosis 1 may be important with regard to clinical care because patients with more deleterious *NF1* variants may be at higher risk for plexiform neurofibromas (and consequently also malignant peripheral nerve sheath tumours) [92] than patients with less deleterious *NF1* variants. Genotype-phenotype correlation studies that include a more diverse range of clinical phenotypes and that compare CADD scores in other large groups of NF1 patients are needed to determine the clinical value of this approach.

Chapter 5: General discussion

In this Master's thesis, I have presented an evaluation of the natural history of non-optic and optic gliomas, including the rate of appearance, progression and regression, and a genotype-phenotype correlation study exploring the effects of individual variants on the presence of several typical features of NF1.

Non-optic and optic gliomas affect 5% and up to 18% of children with NF1 [54,73,105], respectively. Children with NF1 often undergo head MRI after their diagnosis; adults, however, only rarely have routine imaging performed in the absence of symptoms. Before this study, the lack of systematic screening of adults NF1 patients prevented an accurate estimation of the prevalence of non-optic or optic gliomas in adults with NF1. We performed the largest routine head MRI study to date, including NF1 patients of all ages. We found the overall prevalence of non-optic gliomas to be 4.3%, with a stable (albeit slightly lower) prevalence in adults. The overall prevalence of optic gliomas was 9.3% with a steady decline in prevalence from childhood (22% in children aged under 10 years) to older adults (3.4% in patients aged over 50 years). A comparison of several features of non-optic and optic gliomas in NF1 patients investigated in this study is presented in Table 12.

Table 12: Comparison of natural history of non-optic and optic gliomas according to this study.

Features	Non-optic gliomas	Optic gliomas
Overall prevalence	4.3%	9.3%
Prevalence in adults	Stable at ~5%	Declines to 3.4% with increasing age
Rate of appearance	0.19% (95% confidence interval 0.06% to 0.52%)	Not applicable
Age at appearance	Late childhood or adulthood	Early childhood
Rate of progression*	4.7% (95% confidence interval 1.5% to 12%)	0.35% (95% confidence intervals: 0.02% to 2.3%)
Age at progression*	< 25 years of age	< 20 years of age
Rate of regression*	Unknown	4.0% (95% confidence interval: 2.1% to 7.1%)
Age at regression*	Unknown	< 20 years of age
Association with other features typical for NF1	Optic gliomas	UBOs, non-optic gliomas

*Regression as well as progression are defined here as any increase or decrease in size

The majority of OPGs are asymptomatic and never require treatment. One of the big clinical challenges is to identify patients who will become symptomatic so that treatment can be offered early and vision can be preserved or even restored. It is not known why the majority of OPGs are asymptomatic or why symptomatic OPGs and asymptomatic OPGs behave differently.

In this work, I showed that asymptomatic OPG do not behave like most neoplasms, and I also found a strong association between the presence of UBOs and asymptomatic OPGs. I suggest that these findings may indicate that asymptomatic OPGs are not true neoplasms, but rather areas of immature myelin, just like UBOs [145]. If this were true, it would change our understanding of

OPG pathology in NF1 patients and greatly help clinicians in managing these lesions. More scientific as well as clinical research needs to be done in order to explain the similarities as well as the differences between optic and non-optic gliomas in people with NF1. These natural history investigations provide novel findings, and they will be important for both scientists and clinicians working with NF1 patients.

One possible explanation of the differences between non-optic glioma and optic pathway glioma formation and behaviour in NF1 is differences in the origin of the neural stem cells that give rise to these tumours. The cell of origin influences tumour development and clinical behaviour of non-NF1 gliomas [146–148]. In a murine NF1 model, it was shown that only neural stem cells isolated from the brain stem (but not those isolated from the neocortex) have increased proliferative potential after *Nf1* inactivation [149], explaining why non-optic gliomas predominantly arise in this location. In addition, Nf1 inactivation in mouse neural stem cells extracted from the third ventricular zone formed OPGs, while Nf1 inactivation in lateral ventricle-derived neural stem cells did not lead to OPG formation [149]. These findings highlight the importance of neural stem cell heterogeneity in glioma development, but their application to OPG development in humans with NF1 is uncertain.

I also present the largest genotype-phenotype correlation study performed to date in NF1 patients. Most studies were unable to identify correlations between variant classes and phenotypes; this may be due to the genetic and clinical heterogeneity of NF1 [89,150]. Only few genotype-phenotype correlations have been established in NF1, and not all of them could be replicated by other groups [95–99,140]. CADD scores, which provide a way to assess deleteriousness of a variant, have been employed in many studies to investigate associations between the

pathogenicity of candidate variants and specific phenotypes [151,152]. Here, we performed a novel analysis by investigating associations between the CADD scores of presumed pathogenic *NF1* variants and the presence of clinical features identified on routine whole-body and head MRI. We found an association between CADD scores and the presence of plexiform neurofibromas. *NF1* whole-gene deletions, for which CADD scores cannot be calculated, cause a severe NF1 phenotype, and are also characterized by an increased frequency of plexiform neurofibromas [92], thereby corroborating that deleterious variants are associated with an increased prevalence of this tumour type. If my observations are corroborated in other studies, patients with NF1 mutations associated with high CADD scores might be offered more frequent clinical monitoring and counselled differently regarding their risk of plexiform neurofibromas than NF1 patients with less deleterious variants.

One limitation of this study is that the NF1 patients we included may not be representative of the NF1 population as a whole because of referral patterns to the University Hospital Hamburg-Eppendorf NF clinic. Patients from further away may be less likely to travel to have follow-up imaging performed. Patients with more severe disease manifestations are more likely to be referred to this tertiary centre and get follow-up imaging than less severely affected patients. Even though all patients seen in the clinic were offered MRI, patients with symptoms suggestive of tumours (non-optic gliomas, OPGs, plexiform neurofibromas, or other tumours) may have been more likely to participate than asymptomatic patients. In addition, whole-body MRI requires sedation in young children, and parents of symptomatic children may have been more likely to consent to their child being sedated for the imaging studies than parents of asymptomatic children. Another limitation is that, while most patients did have two or three

follow-up imaging examinations, few patients had long-term follow-up so that much of the data are essentially cross-sectional. Larger cohorts that are followed longitudinally from early childhood well into mid-adulthood (or later) are needed to verify our findings and determine if they apply to the general NF population.

None of the statistical analyses reported in this study were adjusted for multiple comparisons, and therefore all of our analyses should be considered exploratory.

More research needs to be performed to understand the different behavior of non-optic and optic gliomas in children and adults with NF1 and to improve therapeutic options for patients with these tumours. Additionally, future research should investigate the molecular consequences of variants with higher CADD score to elucidate how plexiform neurofibromas development might be prevented or slowed.

Taken together, my research provides new insights into the natural history of non-optic and optic gliomas in NF1 as well as the pathogenesis of plexiform neurofibromas in NF1 patients. This may aid clinicians in counselling the families of young patients who have gliomas.

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