



Published in final edited form as:

Acta Neuropathol. 2020 April ; 139(4): 625–641. doi:10.1007/s00401-019-02002-2.

An update on the central nervous system manifestations of neurofibromatosis type 1

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Abstract

Neurofibromatosis 1 (NF1) is an autosomal dominant genetic disorder that presents with variable phenotypes as a result of mutations in the neurofibromatosis type 1 (*NF1*) gene and subsequently, abnormal function of the protein product, neurofibromin. Patients with NF1 are at increased risk for central nervous system (CNS) manifestations including structural, functional and neoplastic disease. The mechanisms underlying the varied manifestations of NF1 are incompletely understood, but the loss of functional neurofibromin, resulting in sustained activation of the oncoprotein RAS, is responsible for tumorigenesis throughout the body, including the CNS. Much of our understanding of NF1-related CNS manifestations is from a combination of data from animal models and natural history studies of people with NF1 and CNS disease. Data from animal models suggest the importance of both *Nf1* mutations and somatic genetic alterations, such as *Tp53* loss, for development of neoplasms, as well as the role of the timing of the acquisition of such alterations on the variability of CNS manifestations. A variety of non-neoplastic structural (macrocephaly, hydrocephalus, aqueductal stenosis, vasculopathy) and functional (epilepsy, impaired cognition, attention deficits and autism spectrum disorder) abnormalities occur with variable frequency in individuals with NF1. In addition, there is increasing evidence that similar appearing CNS neoplasms in people with and without the NF1 syndrome are due to distinct oncogenic pathways. Gliomas in people with NF1 show alterations in the RAS/MAPK pathway, generally in the absence of *BRAF* alterations (common to sporadic pilocytic astrocytomas) or IDH or histone H3 mutations (common to diffuse gliomas subsets). A subset of low grade astrocytomas in these patients remain difficult to classify using standard criteria, and occasionally demonstrate morphologic features resembling subependymal giant cell astrocytomas that afflict patients with tuberous sclerosis complex (“SEGA-like astrocytomas”). There is also emerging evidence that NF1-associated high grade astrocytomas have frequent co-existing alterations such as *ATRX* mutations and an alternative lengthening of telomeres (ALT) phenotype responsible for unique biologic properties. Ongoing efforts are seeking to improve diagnostic accuracy for CNS neoplasms in the setting of NF1 versus sporadic tumors. In addition, MEK inhibitors, which act on

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the RAS/MAPK pathway, continue to be studied as rational targets for the treatment of NF1-associated tumors, including CNS tumors.

Keywords

Neurofibromatosis; Neurofibromin; Glioma; Brain Tumor; vasculopathy; hydrocephalus; seizure

Introduction

Neurofibromatosis type 1 (NF1) is an autosomal dominant genetic disorder characterized by alterations in the *NF1* gene, resulting in phenotypically heterogeneous systemic manifestations. The prevalence of NF1 is estimated at approximately 1 in 3,000 worldwide [115,41], and therefore it represents the most common and well known neurocutaneous disorder. Approximately half of the patients develop sporadically without a known family history. NF1 is diagnosed clinically by two or more features including: the presence of > six café-au-lait macules, skinfold freckling, Lisch nodules, characteristic lesions of the bone, optic pathway gliomas, neurofibromas of the skin or deep nerve, and a first-degree relative with NF1 [73]. Central nervous system (CNS) manifestations of NF1 include neoplasms, learning disabilities, macrocephaly, hydrocephalus and seizures.

NF1 affects a variety of organs and tissues, in the form of neoplasms and non-neoplastic manifestations (Table 1). Neurofibromas are among the most common manifestations in these patients. They are composed predominantly of a neoplastic Schwann cell, but typically have a variety of soft tissue and nerve components, including perineurial cells, axons, mast cells and fibroblasts that likely contribute to tumor growth. They are predominantly of the cutaneous form, and they may be numerous. More worrisome are large plexiform neurofibromas, which have a propensity to transform to malignant peripheral nerve sheath tumors (MPNST), a significant cause of mortality in these patients. Ocular manifestations are also important for the clinical diagnosis of NF1. Lisch nodules (asymptomatic hamartomatous aggregates of melanin-containing cells on surface of iris) occur in almost all patients, and are evaluable through ophthalmologic exam. More recently, choroidal abnormalities representing hamartomatous thickening (“ganglioneuroma”) have been highlighted in the pediatric literature, and modern imaging techniques disclose abnormalities in greater than 80% of patients, which also has diagnostic implications [118].

The current review presents updates about CNS manifestations of NF1. An overview of the genetics, genomics and molecular pathways in NF1 is followed by discussion of current findings in non-neoplastic manifestations (neurocognitive function, vasculopathy, epilepsy) and CNS neoplasms with a focus on diagnostic and molecular pathology.

Genetics and Signaling Pathways

The *NF1* gene spans approximately 60 exons and is located on chromosome 17q11.2. It encodes neurofibromin, a GTPase-activating protein that is expressed in many cell types, including neurons, astrocytes, and oligodendrocytes. People with NF1 are born with one inactivated *NF1* allele and develop tumors when the second allele is lost [78].

Challenges in NF1 genetic screening in the clinical setting are compounded by the gene size, variety of pathogenic gene variants (including over 2600 reported) and lack of clear genotype phenotype correlations [41,113]. Sabbagh and colleagues analyzed 565 index cases from the NF-France Network and achieved high detection of *NF1* pathogenic gene variants [92]. Tsipi and colleagues have more recently reported 70 novel genetic alterations through a peripheral blood-based screening assay utilizing next generation sequencing (NGS) and multiplex ligation-dependent probe amplification (MLPA) [113]. However, only limited genotype-phenotype correlations have been extrapolated from these studies [92,113]. Patients with large *NF1* deletions may have more severe phenotypes and structural CNS anomalies [58], although the extent of this association is still being clarified.

Although the broad functions of neurofibromin remain to be defined, it is known that neurofibromin directly inhibits RAS activation by converting the active form of GTP-bound RAS to its inactive, GDP-bound state [41,23,113]. Inactivation of *NF1* leads predominantly to unchecked RAS signaling, its best studied function. GTP-bound RAS leads to activation of mitogen-activated protein kinases (MAPK), extracellular signal-regulated kinase 1 and 2 (ERK1 and ERK2). The end result of RAF/MAPK activation is stimulation of transcription and cell growth [40,54,24,29,30] (Figure 1). Unchecked RAS activation can also lead to cross-activation of another important pathway for cell proliferation and survival, the PI3K-mTOR pathway. For example, GTP-bound RAS can bind and activate PI3K leading to survival and proliferation effects through AKT and mTOR activity. ERK can also facilitate mTOR activation through phosphorylation of TSC2, which in turn drives cell growth and survival [70]. Thus, neurofibromin loss can lead to disease through multiple pathways.

Neurofibromin function has also been linked with altered activity in the cAMP signaling pathway. *Nf1*-deficient drosophila, zebrafish and mice exhibit reduced cAMP levels in the brain [126,49,13]. In the mammalian brain, neurofibromin may regulate cAMP through RAS-mediated activation of protein kinase C zeta (PKC ζ) outside of the traditional RAS/MEK signaling pathway. RAS is believed to activate PKC ζ , which in turn leads to inhibition of G α s and subsequently decreased cAMP production in the brain [1]. Warrington and colleagues also reported that reduced cAMP levels may lead to gliomagenesis in a genetically engineered mouse model of optic pathway gliomas [122]. As such, cAMP modulation in NF1 may have pharmacological utility to both neurocognitive and neoplastic treatment strategies.

Neurodevelopmental and cognitive abnormalities in NF1

A variety of non-neoplastic CNS manifestations are encountered in patients with NF1 through imaging studies, including macrocephaly (~50%), hyperintense T1/T2 lesions, ventricular dilatation [116], and cerebellar hypoplasia [109]. Individuals with NF1 frequently develop MR hyperintensities that are asymptomatic and should not be mistaken for neoplasms (“unidentified bright objects (UBOs)”) [116]. These UBOs histologically demonstrate myelin vacuolation with possible increased water content [110,27]. Neurocognitive deficits are common in people with NF1 and autism spectrum disorder has been also increasingly recognized in children [37,121]. Alterations in white matter tracts in the frontal lobes identified by diffusion tensor imaging may be a contributing factor [55]. At

the molecular level, cognitive abnormalities are thought to be related to alterations in neurofibromin production. Decreased cAMP levels in the brain are implicated in learning and memory deficits as well as alterations in neural and glial development [126,46,49,12]. Hyman and colleagues in an observational study of 81 Individuals with NF1 and 49 unaffected sibling controls found that children with NF1 suffered from deficits in reading, spelling, mathematics, attention, executive functioning, receptive and expressive language, and motor skills, compared to controls [51]. Although the causes of neurocognitive deficits are not fully explained, investigations using animal models have revealed possible mechanisms of such deficits.

Epilepsy in NF1

Epilepsy is another recognized neurologic complication in patients with NF1, with a prevalence estimated at 4–13% [59,8]. Observational studies suggest that epileptic seizures in individuals with NF1 are often associated with intracranial tumors or structural abnormalities, including hippocampal sclerosis and polymicrogyria[8]. However, in a subset of cases the etiology of seizures and/or an epileptic focus is not identified [36,50,77]. Although the brain in NF1 has been shown to be hyperexcitable due to alterations in GABA signaling and ion channel dysfunction in mice, this hyperexcitability has not been convincingly implicated as a direct contribution to epileptogenesis in NF1 [72,101].

Cerebrovascular Disorders in NF1

NF1 has been associated with CNS vasculopathy in both adult and pediatric populations. Multiple retrospective imaging studies have found a variety of vascular malformations including vessel ectasia, moyamoya, aneurysm, hypoplasia, and vessel narrowing, including severe stenosis [90,17,74] (Figure 2a–c). However, intracranial aneurysms were not shown to be associated with NF1 in a large Finnish population-based study aimed at assessing Individuals with NF1 with intracranial aneurysms or aneurysmal subarachnoid hemorrhage [60].

An important potential complication of vascular malformations associated with NF1 is stroke. This complication is compounded by the fact that individuals with NF1 frequently suffer from hypertension [35], a well-documented risk factor for stroke. Terry and colleagues, using a population-based, case-control study of the US Nationwide Inpatient Sample, demonstrated associations of younger mean age of patients suffering from stroke, increased likelihood of hypertension in pediatric patients, and greater likelihood of stroke diagnosis, particularly hemorrhagic strokes, in individuals with NF1 compared to the general population [107]. Causes of hypertension in these patients include renal artery stenosis, and on occasion an underlying pheochromocytoma.

The mechanism underlying vasculopathy in NF1 is poorly understood. At least some vasculopathy encountered, particularly moyamoya, may be attributable to radiotherapy effects [17,38,114]. However, vascular abnormalities in individuals with NF1 are observed in the absence of radiotherapy, suggesting an underlying intrinsic mechanism of vasculopathy. Bajaj et al. studied the effect of neurofibromin loss on endothelial cells.

Neurofibromin loss results in increased proliferation and cell cycle entry. Additionally, *NF1*-deficient cells demonstrated abnormal morphogenesis, did not branch normally in co-culture assays, resulting in few tubules and branches [4].

An early hypotheses for vasculopathy in NF1 focused on possible Schwann cell proliferation within arteries, which was proposed by Salyer and Salyer based on pure morphologic observations describing a putative resemblance to cellular areas of neurofibroma [93]. However, this hypothesis has lost support, with other cell components such as smooth muscle being more likely involved in the vasculopathy [45]. For instance, findings by Li and colleagues in vascular smooth muscle cell cultures from *Nf1* +/- mice and human individuals with NF1 suggest increased proliferation and migration of vascular smooth muscle cells as a result of a Ras-induced increase of platelet-derived growth factor (PDGF) secondary to neurofibromin loss [65]. As such, neurofibromin-induced vascular smooth muscle cell dysfunction may contribute to vasculopathy.

Additionally, vasculopathy in NF1 has been hypothesized to be a result of intimal proliferation secondary to macrophage dysfunction. *Nf1* +/- and wildtype mice results from Stansfield and colleagues showed Ras-Erk directed recruitment of macrophages, increased macrophage proliferation, migration, and adhesion, and neointimal formation following injury in neurofibromin-deficient mice compared to wild-type controls [103]. Bessler and colleagues further hypothesized that MCP-1, a chemokine anchored to endothelium as well as secreted, and CCR2 positive macrophages are important mediators of neointima formation in the context of NF1 [10].

Histological and ultrastructural assessment of vessels in NF1 have demonstrated thickened intima, smooth muscle nodules and proliferation, fibromuscular hyperplasia, Schwann cell hyperplasia, and neural proliferation in vessel walls [35,71]. Neointimal thickening is identifiable in small caliber vessels in spinal cord, leptomeningeal and choroid plexus vessels of individuals with NF1 [68](Figure 2d-i), and *Nf1* deficiency increases neointimal formation in mice after injury [102].

Central Nervous System Neoplasms in NF1

Individuals with NF1 are prone to developing neoplasms of the CNS and are particularly at risk for optic pathway gliomas [34]. Individuals with NF1 are additionally at an increased risk for other CNS tumors [31], including infiltrating gliomas [39]. Rare CNS tumors such as ganglioglioma, astrocytomas resembling SEGA, and tumors that are difficult to classify and grade using standard criteria developed for sporadic tumors also occur in individuals with NF1 [88]. Predilection for the optic pathways (Figure 3a) and multifocality (Figure 3b) are features that may be encountered in low grade gliomas of individuals with NF1. As mentioned above, asymptomatic MR hyperintensities are frequent in these individuals (Figure 3c).

Pilocytic Astrocytomas in NF1

Optic pathway gliomas are estimated to affect 15–20% of children with NF1. However, the clinical term, “optic glioma,” is not a pathologic term. Most gliomas of the optic nerve are

pilocytic astrocytomas [87] [84]. They are typically followed or treated clinically without pathological confirmation of tumor type or resection, as many tumors behave in an indolent fashion such that many regress without intervention or respond to first line chemotherapy. Anatomic location and the morbidity of the procedure also play a role in the rarity of these biopsies in this special population.

Specific *NF1* gene variants may also be important to varying optic glioma behavior and severity. Toonen and colleagues demonstrated distinct neurofibromin levels, proliferation rates, microglial content, and clinical behavior when comparing nonsense (R681X) and missense (G848R) *NF1* mutations in genetically engineered mouse models [111]. Of interest, studies of the tumor microenvironment in optic pathway gliomas have highlighted an important biologic role for microglia. Microglia have been found to constitute a significant constituent of glial tumors, possibly as a result of monocyte chemoattractant protein-1 (MCP-1) release by gliomas [123]. Within the tumor microenvironment, microglia can have immunosuppressive and tumor permissive effects through release of chemokines such as tumor growth factor beta (TGF β), interleukin 6 (IL-6), prostaglandins, and vascular endothelial growth factor (VEGF), aiding in glioma growth and survival [123]. Interestingly, higher microglia levels and varying axonal injury sensitivity have been observed in female mice compared to male counterparts, a difference thought to be mediated by estrogen effects on estrogen receptor β [112]. Additionally, some studies have shown clinical differences in optic glioma behavior based on sex, with females being more likely than males to have a poorer clinical course [25,112]. Experiments aimed at reducing microglial function and recruitment of microglia to gliomas have demonstrated attenuated optic glioma growth, raising the possibility of immunomodulation of microglia as a possible therapeutic strategy in such neoplasms [81,99,48,56].

A neuropathology-oriented study revealed that PA are the most common CNS tumors in individuals with NF1 [88]. PA are low grade, generally well-circumscribed glial neoplasms often exhibiting a biphasic histologic pattern of compact and loose areas (Figure 3). Immunohistochemically, PA are positive for glial markers such as glial fibrillary acid protein (GFAP) and oligodendrocyte transcription factor 2 (OLIG2) [75].

Within the context of NF1, PA tumorigenesis is thought to arise secondary to altered levels and or activity of neurofibromin, suggesting that NF1-associated PA arise through a distinct tumorigenic pathway versus their sporadic counterparts [54,40,61,34]. Loss of neurofibromin in astrocytes leads to increased RAS activity and subsequently downstream RAS effects hypothesized to result in PA tumorigenesis [43,61,34]. For example, MAPK/ERK activation has been shown across all astrocytoma types, including sporadic and NF1-associated PA; however, MAPK/ERK activation in NF1-associated PA is independent of *BRAF* alterations [85].

Whereas most PA behave in an indolent manner and are designated as World Health Organization (WHO) Grade I, a subset behave more aggressively and may demonstrate anaplastic features, such as necrosis, increased mitotic activity, and hypercellularity [86]. Recent studies into PA with anaplasia, including those in the context of NF1 syndrome, demonstrated alternative lengthening of telomeres (ALT) or features of *alpha thalassemia*/

mental retardation syndrome X-linked (*ATRX*) loss in a substantial portion of cases. Specifically, *ALT* was present in half (4 of 8) cases developing individuals with *NF1* and *ATRX* protein loss in 5 (of 8) [86], although *NF1* status was not independently associated with a poorer prognosis. Reinhardt et al. also reported on a group of anaplastic astrocytomas with piloid features, and reported *ATRX* loss or pathogenic gene variants in 45% of their cases. Of interest, *MAPK* pathway alterations were present in most tumors (75%) and variants in the *NF1* gene were the single most frequent alteration [83]. More recently, D'angelo et al. in a comprehensive genomic analysis of gliomas developing in individuals with *NF1* reported *ATRX* pathogenic gene variants in 38% of high grade gliomas compared to 3.1% of low grade gliomas [22].

Overall, *NF1*-associated PA have a better prognosis than sporadic counterparts in children. [85]. However, it is increasingly recognized that *NF1*-associated PA in adults may behave more aggressively [16,47,67,91,104].

Diffuse Gliomas

Although not as common as PA, patients with *NF1* suffer from an increased risk of diffuse and high grade gliomas, particularly in patients over 10 years of age [44,88,18](Figure 4). A retrospective pathologic study of *NF1*-associated gliomas found that diffuse astrocytomas accounted for 27% of such cases [88], and they usually develop outside of the optic pathways. Limited data is available on glioblastomas within the context of *NF1* syndrome. In a recent report of four *NF1*-associated glioblastomas, the tumors were well-circumscribed on imaging, had relatively long survival, and did not feature alterations in *ATRX*, *EGFR* amplification, *IDH1*, *BRAFV600E*, or *TERT* promoter [95]. All high grade glioma morphologic variants may develop in individuals with *NF1*, including giant cell glioblastoma, its close mimic anaplastic pleomorphic xanthoastrocytoma and gliosarcoma (Figure 4b–f). The latter is important to have in mind for pathologic diagnosis, given the difficulties in separating from MPNST. Our previous work has also demonstrated a high frequency of *ATRX* loss and *ALT* in *NF1* associated diffuse/high grade gliomas [89](Figure 4g,h,i), and as mentioned above, *ATRX* pathogenic variants are typical of high grade gliomas in individuals with *NF1*.

Indeterminate and Rare CNS Neoplasms in NF1

Although the most common CNS neoplasms in *NF1* are PA followed by diffuse gliomas, a number of indeterminate and rare neoplasms may occur in individuals with *NF1*. A diagnostic challenge in the context of *NF1* is the evaluation of low grade gliomas lacking definitive characteristics of PA, often with concerning features such as infiltrative growth or increased proliferation (Figure 3h). Nonetheless, such indeterminate low grade gliomas have been shown to follow a similar clinical course to classical PA [88].

Other rare CNS neoplasms observed in Individuals with *NF1* include a variety of glial and glioneuronal tumors, such as the pilomyxoid astrocytoma variant of PA, ganglioglioma, desmoplastic infantile ganglioglioma [88], pleomorphic xanthoastrocytoma [108,105], dysembryoplastic neuroepithelial tumor (DNT) [64], and rosette-forming glioneuronal tumor (RGNT) [98,57] (Figures 3–5). Regarding glioneuronal tumors in specific, it is unclear if

they are more common in individuals with NF1 since most papers describing them are limited to isolated case reports. In a prior study of 100 patients with NF1, two (2%) glioneuronal tumors were identified: one ganglioglioma and one desmoplastic infantile ganglioglioma [88]. The molecular alterations in these cases have not been adequately studied either.

Tumors with morphologic and immunophenotypic features resembling SEGA, a tumor typically developing in patients with tuberous sclerosis complex (TSC) are also known to develop in individuals with NF1 [76](Figure 6). These SEGA-like astrocytomas are predominantly low grade, and characterized histologically by the presence of large, plump cells with ample cytoplasm and macronuclei that stain for GFAP, OLIG2, and S100. They variably express neuronal markers, demonstrate mTOR pathway activation, and most contain *NF1* pathogenic gene variants. *IDH1*, *IDH2*, and *BRAF* alterations are not observed [76]. Of interest, *NF1* loss results in mTOR pathway activation, and mTOR pathway activation is a molecular basis for the pathogenesis of SEGA. More recently, primary intracranial sarcomas containing *DICER1* mutations have been reported predominantly in the sporadic setting but also in at least one individual with NF1 [63].

Animal Models of NF1 deficiency

Models of NF1 deficiency have been particularly useful in the study of a variety of non-neoplastic and neoplastic CNS manifestations of the disease. For example, experiments using heterozygous *Nf1*^{+/-} mice with and without corrective *Ras* mutations demonstrated that spatial learning deficits in *Nf1* heterozygous mice could be corrected with *Ras* null mutations. Such learning deficits are thought to result secondary to RAS mediated increases in GABA-mediated inhibition [21]. In addition, Zhu and colleagues in a Cre-LoxP *Nf1* model directed against neuronal populations in mice demonstrated abnormal cerebral cortex development and astrogliosis [130]. Other genetically engineered mice modeling heterozygous *Nf1* germline loss and somatic inactivation in neuroglial progenitor cells demonstrated abnormalities in early communicative behavior, relevant to autism spectrum disorders [69]. Heterozygous null *Nf1* mouse studies implicate neurofibromin-related Ras pathway alterations leading to GABA dysregulation in the brain as a cause of learning deficits and working memory impairment in individuals with NF1 [96,21]. Dopamine dysregulation has also been implicated as a cause of learning and attention deficits in individuals with NF1 [26,14]. Short term trials have found a possible beneficial effect of methylphenidate in individuals with NF1 and ADHD [66] and additional trials of methylphenidate and neurostimulants in these patients are ongoing (NCT02944032) [82]. *Nf1* mutant *Drosophila* flies have also been used to study memory deficits [15]. A new porcine model developed by White and colleagues recapitulates features of NF1, such as learning and memory deficits, in addition to physical manifestations such as café au lait macules [124]. Such a model may prove useful for future investigation into the mechanistic underpinnings of neurocognitive deficits in NF1.

Modeling NF1 in animal models has demonstrated several technical challenges. For instance, traditional *Nf1* knockout mouse model features heterozygous *Nf1* genes, as a homozygous knockout genotype is incompatible with life [42,52]. The heterozygous *Nf1*^{+/-}

mouse model described by Jacks and colleagues demonstrated increased tumorigenesis, including pheochromocytoma and myeloid leukemia, and these neoplasms demonstrated loss of wild type *Nf1*, a finding consistent with the “two-hit” hypothesis of tumorigenesis in individuals with NF1. However, the heterozygous mouse model described by Jacks et al. did not develop classical phenotypic characteristics of NF1, such as café-au-lait macules, Lisch nodules, and neurofibromas [52] for unknown reasons. Additional studies using the heterozygous *Nf1* model have demonstrated increased astrocyte proliferation and growth advantage of *Nf1*^{+/-} mice compared to wild type controls through increased activation of the p21-RAS pathway [43,5].

In order to produce peripheral nerve sheath tumorigenesis in mouse models, a chimeric approach has been used in some studies by injecting *Nf1*^{-/-} cells into a blastocyst. Cichowski and colleagues successfully used this approach to generate plexiform neurofibromas in such a chimeric mouse model [19]. In order to induce malignant peripheral nerve sheath tumors (MPNST), mice heterozygous for *Nf1* and *Tp53* are used, though when respective mutations are on opposite chromosomes (trans) the genetically engineered mice also developed aggressive sarcomas, in addition to MPNST [19].

The synergistic effects of *Nf1* and *Tp53* mutations are also utilized in mouse models to generate astrocytomas. Zhu and colleagues using Cre-LoxP recombination with a neural-specific *Nf1* mutation and varying *Tp53* genotype demonstrated that *Tp53* loss before or concurrent with *Nf1* loss was crucial for astrocytoma formation, including high grade astrocytoma. The authors concluded that early *Tp53* loss provides a selective advantage for astrocytes, which progress to astrocytoma through RAS-pathway activation secondary to *Nf1* loss [128]. In this manner, not only is the cooperation of *Nf1* and *Tp53* alterations important to astrocytoma development in mouse models but, also the timing of such alterations.

Pilocytic astrocytoma of the optic pathway, a common tumor encountered in NF1, is also studied using mouse models. Bajenaru and colleagues demonstrated in a Cre-LoxP *Nf1* mouse model that optic pathway glioma formation was dependent on astrocytic *Nf1* loss within a heterozygous *Nf1* background [6]. Zhu and colleagues demonstrated hyperplastic optic nerves with cellular disorganization using a Cre-LoxP *Nf1* mouse model and suggest that such hyperplasia and disorganization is consistent with early optic pathway gliomas [129]. Though optic pathway gliomas produced by the Bajenaru and Zhu groups show some histological features of pilocytic astrocytoma, both lack other common features of pilocytic astrocytoma such as Rosenthal fibers and eosinophilic granular bodies [129,6]. A recent study by Solga and colleagues using a conditional knockout mouse model found that both germline neural progenitor/stem cell *Nf1* knockouts and Olig2⁺ cells with somatic *Nf1* loss can give rise to low grade gliomas, though the temporal course of optic glioma tumorigenesis differed [100]. This study suggests the possibility of different progenitor origins contributing to differing tumor behavior among low grade gliomas in individuals with NF1.

Studies of *Nf1* effects in zebrafish have used *NF1* orthologue knockdown strategies such as morpholino oligonucleotides and zinc finger nucleases as well as targeting induced local

lesions in genomes (TILLING) strategies. Shin and colleagues developed lines in zebrafish demonstrating oligodendrocyte progenitor cell (OPC) hyperplasia and proliferation, Schwann cell hyperplasia, myelination abnormalities, upregulation of RAS pathway signaling, and, in combination with *Tp53* mutation, high grade glioma and MPNST development [97]. Lee and colleagues have also shown increased OPC proliferation and migration with *Nf1* loss using morpholino knockdown in zebrafish [62]. This abnormal accumulation of OPC has additionally been demonstrated in *Nf1*-mutant mouse models and cell cultures, and again tumorigenic qualities of *Nf1* mutants, such as survival and proliferation, were suggested [9]. Such abnormal accumulations of progenitor cells, a consequence of neurofibromin loss, may thus contribute to both neoplastic and neurostructural NF1 manifestations.

Therapeutic Aspects of CNS Manifestations in NF1

Clinical monitoring and treatment of CNS manifestations of NF1 are varied, and many strategies are still in the process of optimization. The most common CNS manifestation, optic pathway gliomas may become symptomatic, but when this happens it usually occurs in young children [11]. Hence, close surveillance of visual acuity as a manifestation of OPG is recommended in children with NF1, and specifically in the context of clinical trials [33]. MRI of the brain is not used as a screening tool for OPG, but patients with suspicious symptoms (deteriorating vision, endocrine issues, headaches, seizures, HC increase) should consider getting a MRI +/- contrast.

A major impetus for treatment in optic pathway gliomas is vision loss, which has spurred interest in the monitoring and assessment of vision deterioration in children affected with NF1 [11]. Fisher and colleagues recommend visual acuity assessment using Teller acuity cards as a primary endpoint with additional assessments of visual acuity depending on the child's age [33]. A novel approach to visual acuity monitoring is optic coherence tomography, which assesses the thickness of the retinal nerve fiber layer (RNFL), an approach used to assess visual function in the setting of multiple sclerosis [33,11,106]. As RNFL thinning is associated with clinically significant vision loss [106], optic coherence tomography may offer an objective strategy for vision monitoring, particularly in young children for which visual acuity assessment may be difficult [3,11,33].

When optic pathway glioma treatment is needed, chemotherapy is typically used. Symptomatic optic pathway gliomas have traditionally been treated with carboplatin in combination with vincristine [33]. Ongoing chemotherapy trials at the time of this writing include the use of lenalidomide (NCT01553149), selumetinib (NCT01089101), vinblastine +/- bevacizumab (NCT02840409), pomalidomide (NCT02415153), and pegylated interferon (NCT02343224) (clinicaltrials.gov).

Among novel NF1 neoplastic therapeutics, selumetinib, an oral MEK1 and MEK2 inhibitor, is of particular interest as there is ongoing investigation of its utility in NF1-related optic pathway gliomas and plexiform neurofibromas. Inhibition of MEK proteins works to mitigate the hyperactivation of RAS signaling secondary to neurofibromin loss in NF1 [53].

An ongoing selumetinib trial in NF1-related plexiform neurofibromas has shown decreased neurofibroma volume with treatment [28]. Selumetinib is also eliciting preliminary treatment responses in sporadic and NF1-associated PA [32,7]. Relatedly, MEK inhibition has shown promise in *NF1*-deficient glioblastoma cell lines [94], suggesting the applicability of selumetinib therapy to high grade gliomas within the context of NF1 syndrome.

CNS tumor surveillance other than optic pathway glioma surveillance in pediatric NF1-syndrome patients is less defined. For asymptomatic patients, Evans and colleagues recommend that clinicians be aware of the increased risk of low grade and high grade gliomas and that families are educated on clinical warning signs of such CNS disease [31]. For patients who develop high grade gliomas, genetic investigation for constitutional mismatch repair deficiency syndrome (CMMRD), a rare syndrome caused by mutations in mismatch repair genes, is recommended as patients with CMMRD are susceptible to high grade gliomas and show phenotypic overlap with NF1 syndrome. Any of the typical manifestations of NF1 may be present, including café au lait spots, freckling, dermal neurofibromas, plexiform neurofibromas, Lisch nodules and bone abnormalities. However, only a minority of patients satisfy established clinical criteria for NF1 [31,125].

Clinical trials, outcome assessment, and intervention for non-neoplastic CNS manifestations of NF1 is challenging given the multifaceted nature of neurocognitive function. The Neurocognitive Committee of the Response Evaluation in Neurofibromatosis and Schwannomatosis (REiNS) International Collaboration aims to review and provide recommendations for future NF1 trials in the realm of cognitive deficit evaluation and intervention [120].

Sporadic CNS Neoplasms with *NF1* Alterations

It is important to note that *NF1* alterations in tumorigenesis are not limited to tumors developing in individuals with NF1. Somatic *NF1* loss is implicated in a variety of human cancers including glioblastoma, sarcomas, desmoplastic melanoma, ovarian carcinoma, breast cancer, paragangliomas, and acute myeloid leukemia among many others. As such, both animal models and cell lines are employed to investigate sporadic *NF1* mutation effects in such neoplasms [80].

Sporadic PA are often characterized molecularly by alterations in the MAPK pathway, particularly by *BRAF* fusions including KIAA1549:*BRAF* and point mutations such as *BRAF*V600E. Other alterations found in PA include *FGFR1*, *FGFR-ITD*/fusion, *NTRK* fusions, and *NF1* gene variants [20]. *NF1* pathogenic gene variant may occur in PA in the absence of clinical evidence of NF1, where they are usually mutually exclusive with *BRAF* alterations. More recently, the molecular alterations responsible for anaplasia in PA with anaplasia (anaplastic astrocytoma with piloid features) have been studied. Alterations leading to MAPK activation are frequent, particularly *NF1* pathogenic gene variants [83,86] (Figure 7)

The role of *NF1* gene alterations has been studied in sporadic diffuse gliomas. *NF1* alterations are associated with the mesenchymal subtype of sporadic glioblastomas, a

molecular subtype based on a gene expression signature and associated with short survival [79,117,2,127].

Glioblastoma cell line studies knocking down neurofibromin levels have shown increased secretion of chitinase-3-like protein 1 (CHI3L1), a glycoprotein associated with increased invasion and survival, endoglin (ENG), a TGF β component implicated in mesenchymal differentiation, and interleukin-8 (IL-8), a cytokine with pro-angiogenic effects [127]. However, a recent review of The Cancer Genome Atlas (TCGA) data did not find a significantly worse prognosis in sporadic glioblastomas with *NF1* loss, although sporadic lower grade diffuse gliomas with *NF1* loss did demonstrate significantly shorter survival [119]. *NF1* pathogenic gene variants occurred in 21 (7%) of the lower grade glioma group. IDH mutations co-occurred with *NF1* alterations in this group in only 7 (38%). However, 3 (of 5) patients with follow up data died during the follow-up period, suggesting that the low frequency of IDH mutations alone does not explain the adverse outcome.

Given the increased availability of comprehensive platforms for molecular genetic testing, *NF1* sequence variants in brain tumor tissue are increasingly uncovered during routine molecular diagnostics. Criteria for the diagnosis of NF1 remains clinical. However, the identification of *NF1* pathogenic variant with associated loss of heterozygosity may provide a rationale to suggest comprehensive clinical evaluation and germline *NF1* testing.

Conclusion

Ongoing investigation into NF1-related CNS disease is increasingly geared toward early detection of disease and exploiting therapeutic targets. The complexity of the large *NF1* gene and its many known gene variants without clear genotype-phenotype correlation present a challenge for clinicians and researchers alike. However, new drugs such as those targeting the RAS/MAPK pathway show promise in treating NF1-related neoplasia. Efforts at standardizing outcome assessment in trials for non-neoplastic NF1 disease may add increasingly reliable data for investigation into the multifaceted nature of neurocognitive assessment and treatment. Finally, roles of the microenvironment as well as patient characteristics such as sex may further contribute to a precision medicine-based approach to combating CNS disease in NF1 syndrome.

Acknowledgments.

The authors would like to thank contributors to several figures Drs. Doris Lin (figure 2), Bette Kleinschmidt-Demasters (figure 2), Christopher Heaphy (Figure 4 and 6) and Liam Chen (figure 7),

The authors have no conflict of interest to report. All authors participated in the writing, reviewed, and approved the manuscript. The work was funded in part by This work was supported in part by Pilocytic/Pilomyxoid Fund, including Lauren's First and Goal, and the Stick it to Brain Tumors Annual Women's Ice Hockey Tournament (F.J.R.) and NIH grant P30 CA006973 to the Sidney Kimmel Comprehensive Cancer Center (PI: W. Nelson).

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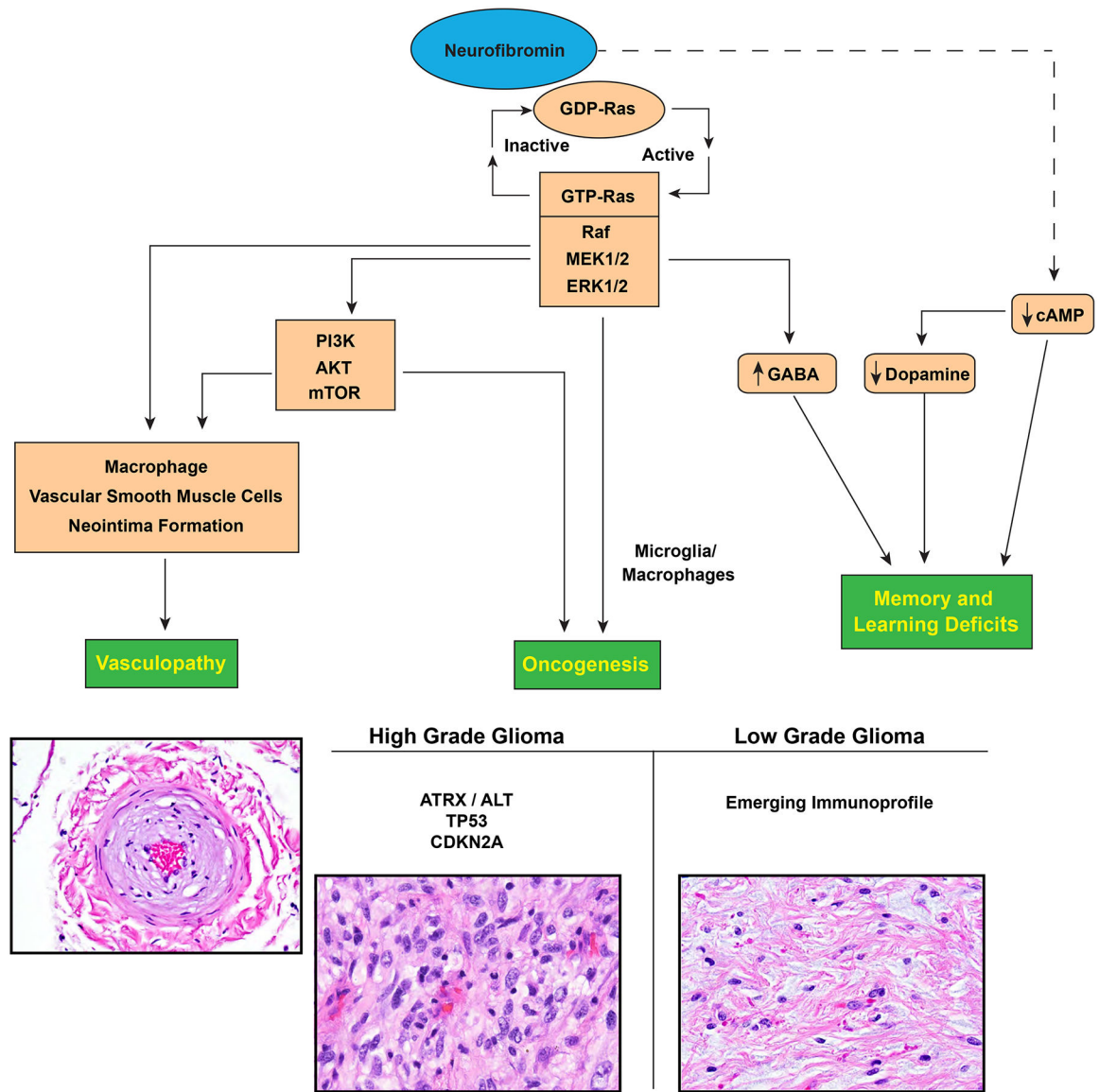


Figure 1. CNS manifestations resulting from neurofibromin loss and aberrant signaling pathways.

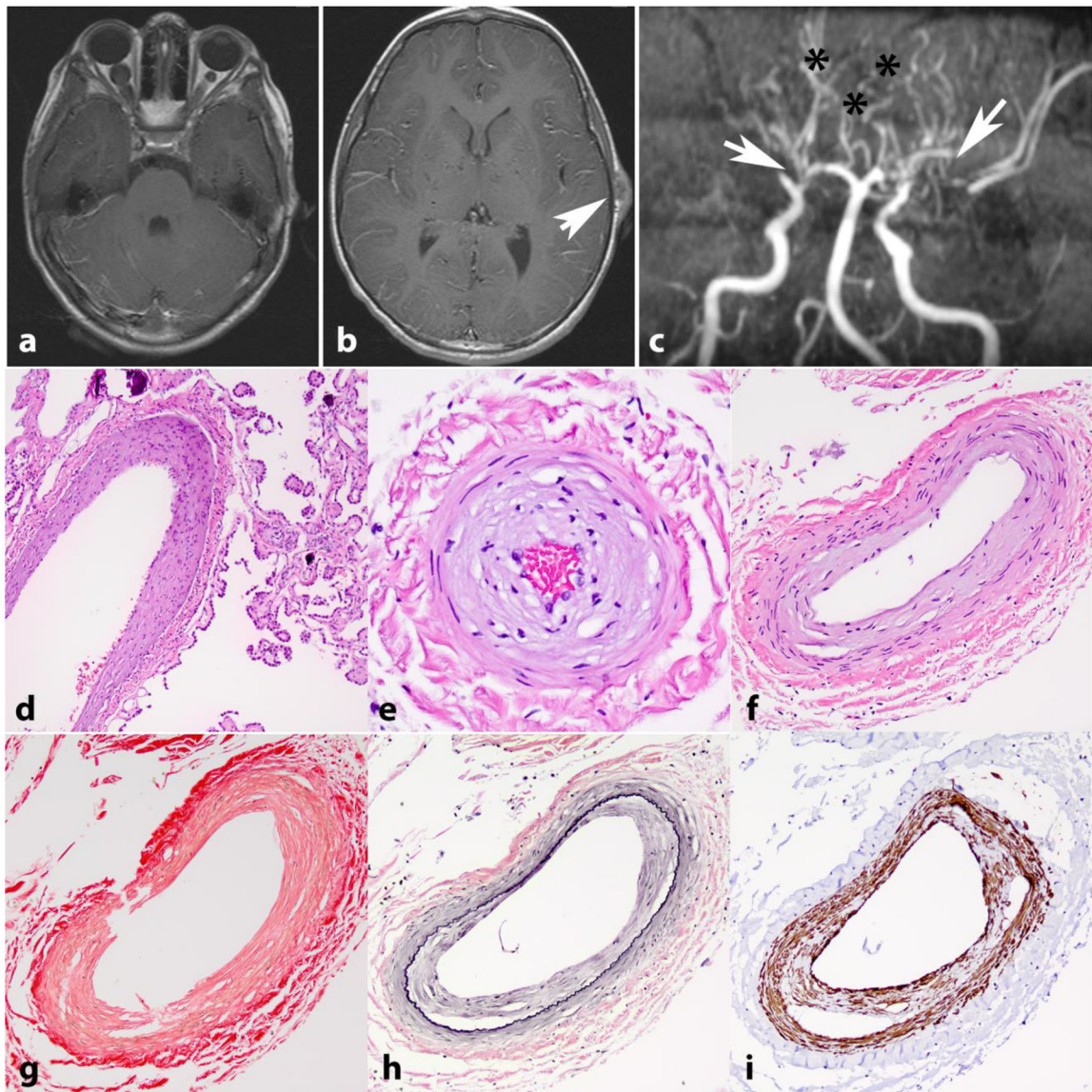


Figure 2. Intracranial vasculopathy in NF1.

Axial T1 weighted image with contrast in a 15-year-old boy with NF1 shows bilateral optic nerve gliomas, larger on the right (a). Axial T1 weighted image with contrast also shows a left frontoparietal scalp soft tissue lesion representing a cutaneous neurofibroma (arrow) (b). MR angiography of the circle of Willis shows stenosis of the distal internal carotid arteries, with no flow in the right middle cerebral artery and severe narrowing of the left middle cerebral artery (arrows). Many collateralized vessels in a moya-moya pattern are seen (asterisks) (c). Histologic sections from a 21 year-old patient with NF1 demonstrating intimal thickening involving vessels in the choroid plexus (d) and leptomeninges (e,f). Picrosirius red stain demonstrates normal mural collagen fiber architecture (g). VVG highlights intimal hyperplasia central to internal elastic lamina (black)(h) and SMA immunostain shows positivity in many of these cells (i).

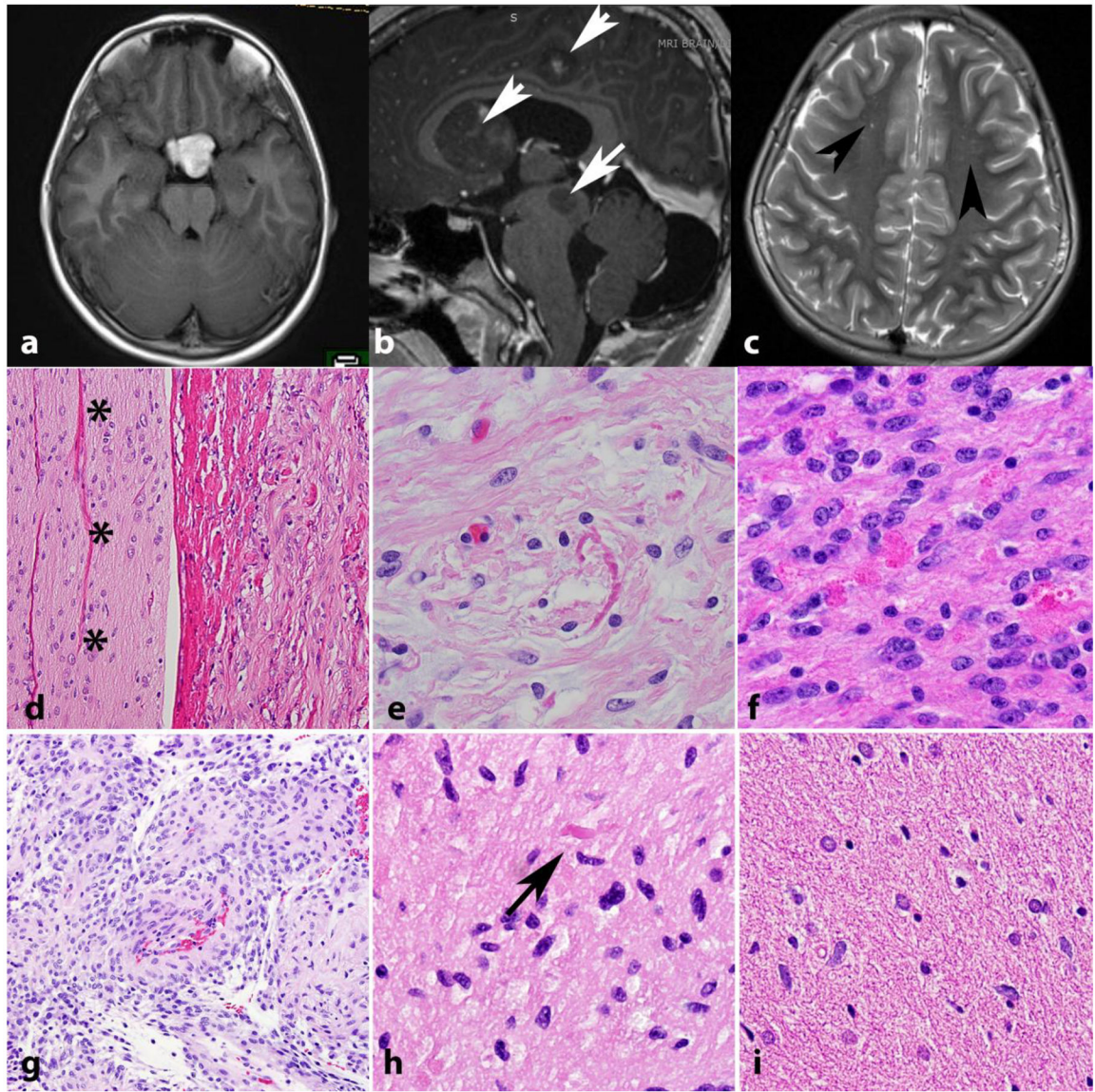


Figure 3. Low grade astrocytomas in NF1.

The optic pathways, including the optic chiasm are favored sites for low grade astrocytomas in NF1. Axial T1 weighted MR image in a 41-year-old woman with NF1 demonstrates a bulky mass involving the optic chiasm (a) that histologically proved to be a pilocytic astrocytoma. Multicentricity (arrows) of pilocytic astrocytomas is a feature of NF1 (arrows) (b). “Unidentified bright objects” represent hyperintensities on MRI that do not grow and frequently regress over time (arrowheads). They are frequent in individuals with NF1 and should not be misinterpreted as tumors (Axial T2-weighted image) (c). Pilocytic astrocytomas involving the optic nerve (asterisks) (optic nerve glioma) frequently extend into the subarachnoid space (d). Most gliomas in individuals with NF1 are pilocytic astrocytomas, characterized by frequent Rosenthal fibers (e) and eosinophilic granular bodies (f). The pilomyxoid variant of pilocytic astrocytoma also may develop in individuals with NF1 (g). Low grade astrocytomas that are difficult to classify, usually having infiltrative

features but with occasional piloid features such as rare Rosenthal fibers (arrow) are also relatively frequent (**h**). Diffuse astrocytomas also occur in individuals with NF1 and resemble sporadic diffuse astrocytomas, particularly single cell infiltration and lack of Rosenthal fibers(**i**).

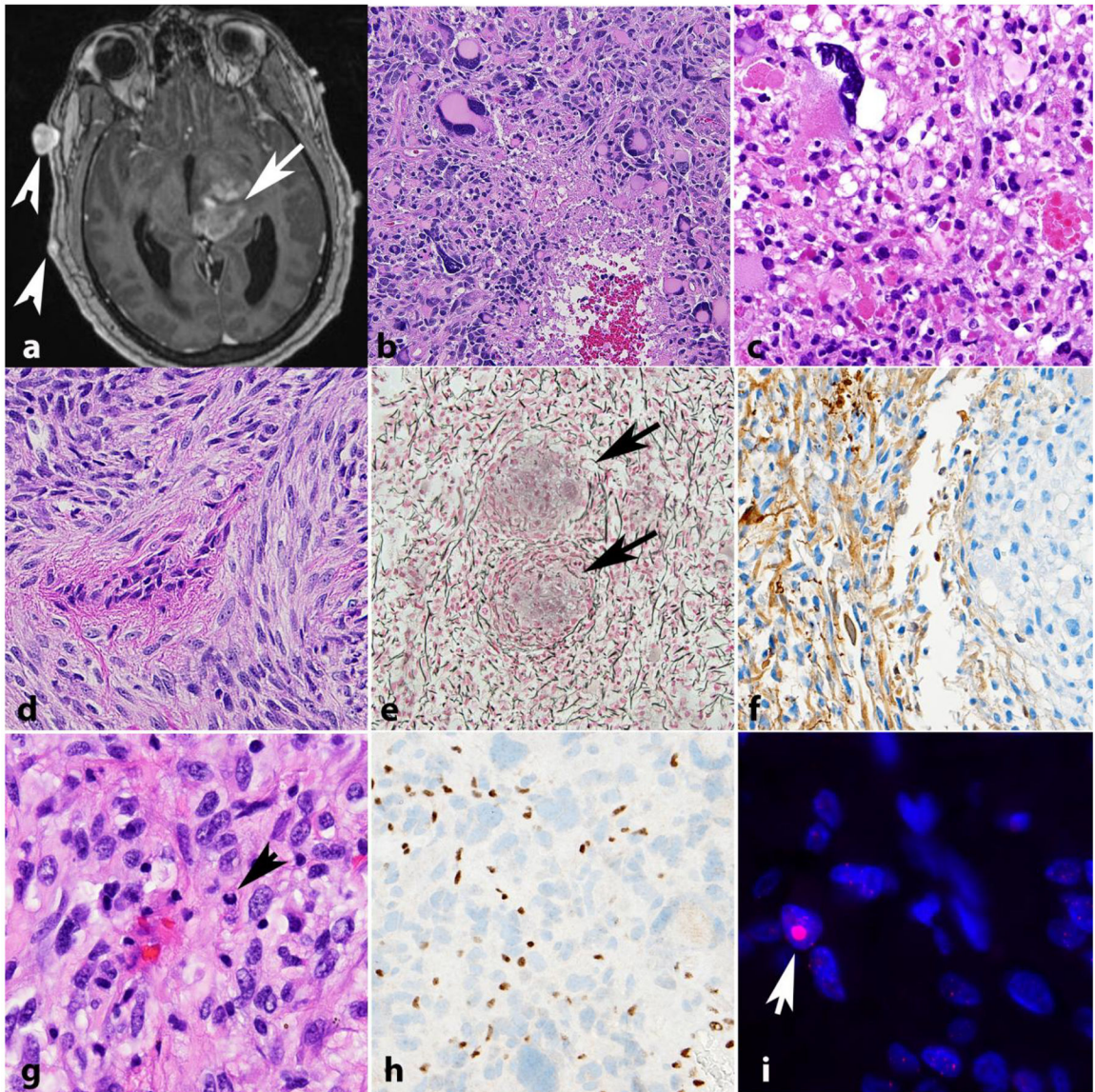


Figure 4. High grade astrocytomas in NF1.

High grade astrocytoma forming a deep contrast enhancing mass (arrow). Other manifestations of NF1 in this patient included neurofibromas of the scalp (arrowheads) (axial T1-weighted post-contrast image)(a). The histology of high grade astrocytomas in individuals with NF1 is also variable, and includes giant cell glioblastoma (b) and anaplastic pleomorphic xanthoastrocytoma (c). NF1 associated gliosarcoma (d) with biphasic components highlighted by reticulin special stain, including islands of reticulin poor glioma cells (arrows) surrounded by reticulin rich sarcomatous areas (e). GFAP immunoreactivity in this tumor is limited to glial areas (left) and is negative in the pleomorphic sarcomatous component (right)(f). Anaplastic astrocytoma in NF1 patient with mitotic activity (arrow)(g), ATRX expression loss by immunohistochemistry (h) and large foci in telomeric FISH (arrow) consistent with alternative lengthening of telomeres (i).

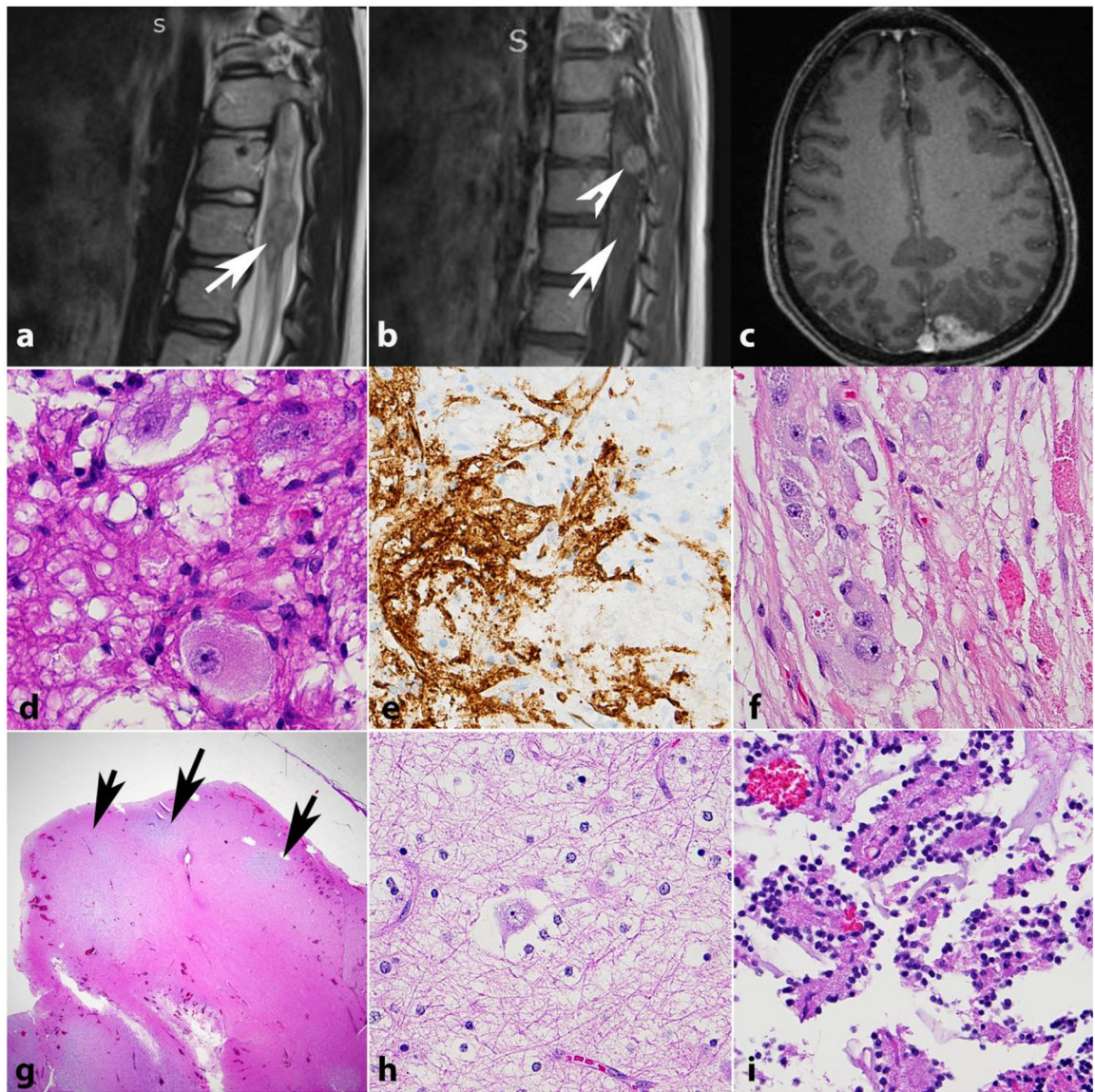


Figure 5. Glioneuronal tumors in NF1.

Gangliogliomas centered in the conus (**a,b,d,e**) and occipital cortex (**c,f**) in individuals with NF1. CD34 expression may be seen (**e**) as in sporadic gangliogliomas. Dysembryoplastic neuroepithelial tumor forming mucoid cortical nodules (arrows)(**g**) and containing floating neurons on high power (**h**). Rosette forming glioneuronal tumors also may occur in individuals with NF1, often outside the posterior fossa (**i**).

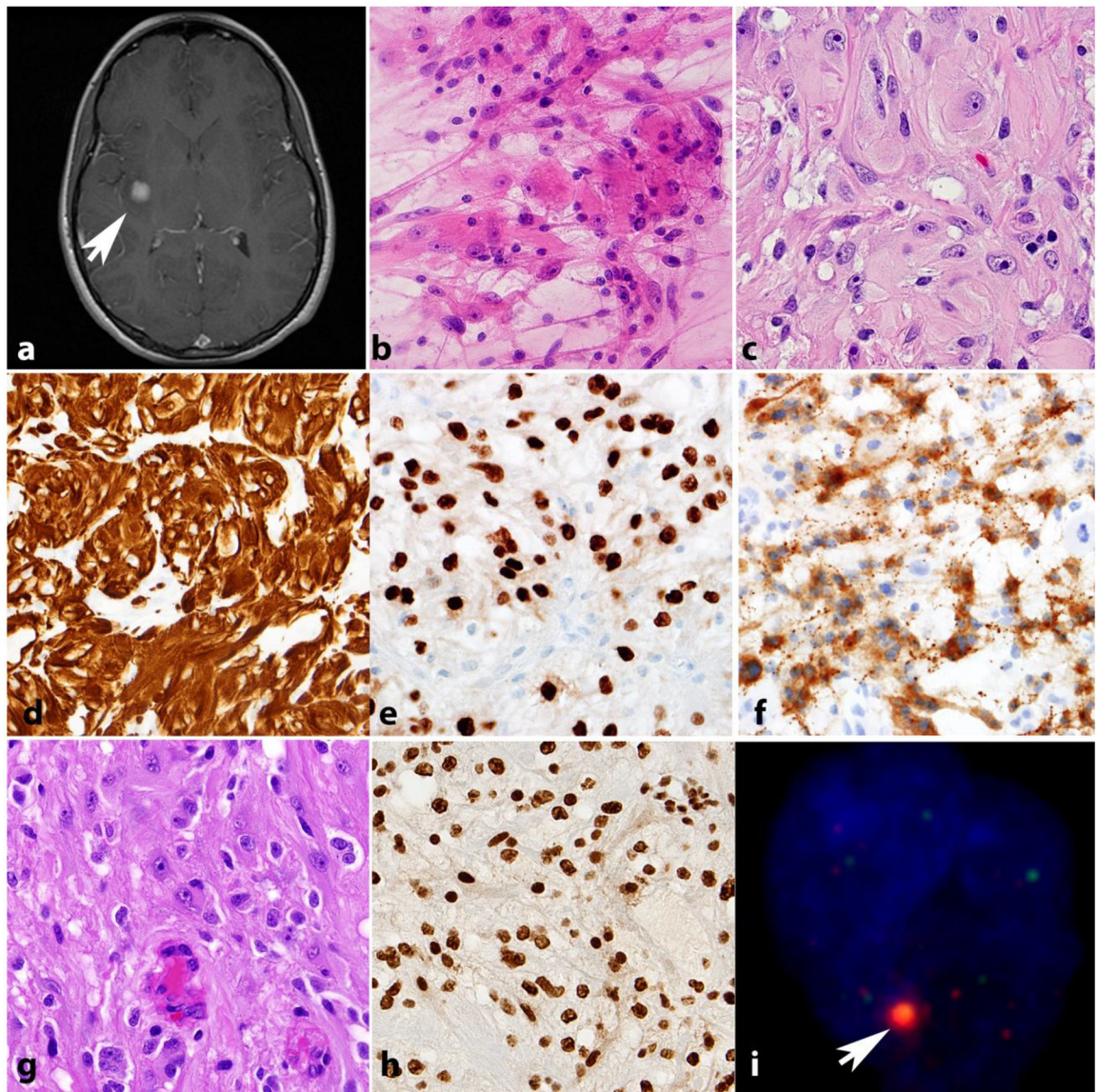


Figure 6. SEGA-like astrocytoma.

Neoplasms resembling subependymal giant cell astrocytomas (SEGA) typical of tuberous sclerosis occur in a subset of individuals with NF1, although they tend to be hemispheric (**a**, **b,c**). As SEGA, they express glial markers (GFAP, **d**; OLIG2, **e**) and neuronal markers (synaptophysin, **f**). SEGA-like astrocytoma in NF1 patient (**g**) with intact ATRX expression (**h**) but large telomere foci (red) colocalizing with Promyelocytic bodies (green)(arrow) consistent with alternative lengthening of telomeres (**i**).

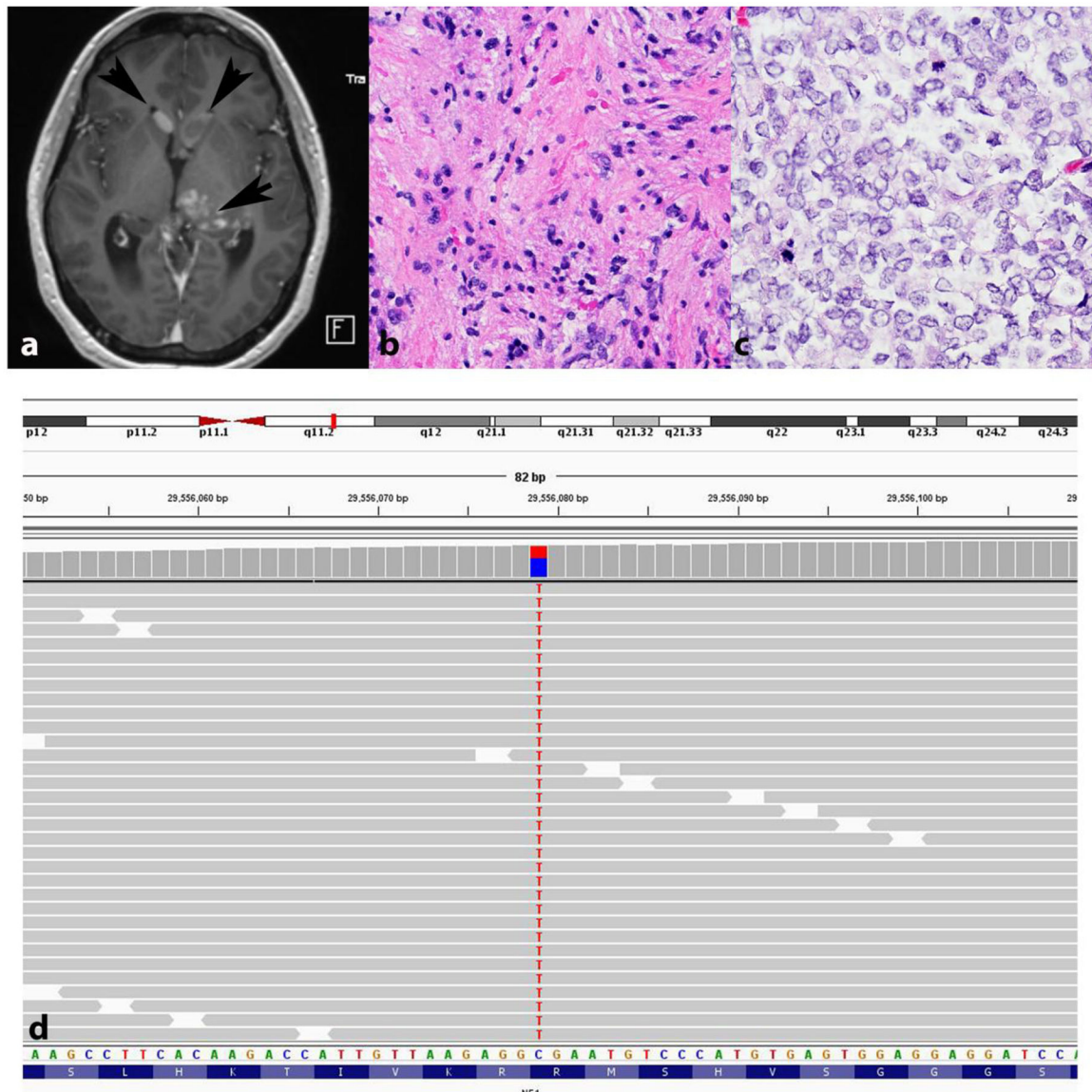


Figure 7. Sporadic pilocytic astrocytoma with anaplasia and *NFI* pathogenic gene variant. Axial T1-weighted MRI demonstrating a thalamic mass with heterogeneous enhancement (arrow), as well as intraventricular nodules consistent with subependymal spread (arrowheads) (a). First biopsy demonstrated a pilocytic astrocytoma (b). Recurrence in the absence of treatment showed a cellular glial neoplasm with brisk mitotic activity consistent with anaplastic progression (c). Next generation sequencing demonstrated a somatic truncating *NFI* p.R816* variant with a variant allele frequency (VAF) of 38.5 (c). No other significant gene sequencing variants were identified.

Table 1

Clinical manifestations and pathology of NF1

Non-Neoplastic Manifestations	
Ophthalmic	<ul style="list-style-type: none"> • Lisch nodules • Diffuse hamartomatous infiltration of the uveal tract
Central Nervous System	<ul style="list-style-type: none"> • Unknown bright objects (UBOs) • Macrocephaly • Learning disabilities (ADHD) • Epilepsy • Autism • Hydrocephalus • Aqueductal stenosis • Cerebral arteriopathy (Moyamoya disease)
Skin	<ul style="list-style-type: none"> • Café au lait spots • Axillary/inguinal (intertriginous skin) freckling
Musculoskeletal	<ul style="list-style-type: none"> • Sphenoid wing dysplasia/hypoplasia • Scoliosis • Pseudoarthrosis • Bowing of long bones
Cardiovascular	<ul style="list-style-type: none"> • Hypertension • Renal artery stenosis • Pulmonary artery stenosis
Central Nervous System Tumors	<ul style="list-style-type: none"> • Pilocytic astrocytoma • Diffuse astrocytomas (WHO grades II-IV) • SEGA-like astrocytomas • Glioneuronal Tumors • Indeterminate Gliomas
Peripheral Nervous System Tumors	<ul style="list-style-type: none"> • Cutaneous Neurofibroma • Diffuse Neurofibroma • Plexiform Neurofibroma • Massive Soft Tissue Neurofibroma • Malignant Peripheral Nerve Sheath Tumor
Other Tumors	<ul style="list-style-type: none"> • Gastrointestinal Schwannoma • Gastrointestinal Stromal Tumor • Intestinal Ganglioneuromatosis • Pheochromocytomas • Glomus Tumors

Non-Neoplastic Manifestations	
	<ul style="list-style-type: none">• Neuroendocrine Neoplasms• Juvenile Myelomonocytic Leukemia• Breast Carcinoma• Rhabdomyosarcoma

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