Comprehensive neurological evaluation of a cohort of patients with neurofibromatosis type 1 from a single institution

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1. Introduction

Neurofibromatosis type 1 (NF1; OMIM 613113) is a phenotypically heterogenous multisystem cancer predisposition syndrome manifesting in childhood and adolescents. The prevalence is approximately 1 in 3000 individuals worldwide [1]. About half of the conditions are inherited with a known family history [2]. Yet, even with an identical pathogenic *NF1* variant in inherited cases, clinical presentation can be highly variable. NF1 is diagnosed clinically by the National Institutes of Health (NIH) diagnostic criteria (established in 1987, confirmed in 1997) [1,3] or the revised diagnostic criteria from 2021 [4].

NF1 presents with various central nervous system (CNS) manifestations including structural, neurodevelopmental, and neoplastic disease.

Non-neoplastic structural manifestations of NF1 include

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macrocephaly, stenosis of the aqueduct, vasculopathy, and focal areas of signal intensity (FASI) [5]. Vasculopathy includes stenosis or occlusion of the (arterial) vessels, aneurysms, arteriovenous fistulae, and Moyamoya syndrome. It typically affects children and adolescents. Moyamoya syndrome increases the risk of both ischemic and haemorrhagic strokes. FASI are usually located in the cerebellar white matter, medial temporal lobe, thalamus, basal ganglia, and brain stem, and are more likely to be bilateral.

Neurodevelopmental abnormalities include problems such as motor delay, lowering of intellectual abilities, deficits in executive functioning, impaired visuospatial processing, and attention deficit hyperactivity disorder (ADHD) [6–8]. Movement difficulties comprise manual dexterity, ball skills, and balance. Specific learning disorders encompass lower performance in reading, mathematics, and/or writing. Deficits in executive function include both organization and regulation, specifically with respect to working memory, abstraction, initiation of mental rules or task sets, cognitive flexibility, planning, and problems solving, emotional regulation, and attentional control [9,10]. Moreover, children with NF1 also harbour difficulties in social functioning across multiple domains including autism spectrum disorder (ASD) and other psychiatric conditions (mood and anxiety disorders). Males and females appear to be similarly affected by ASD [11].

Neoplastic CNS manifestations during childhood and adolescence are mostly low-grade gliomas (LGGs), predominantly pilocytic astrocytoma WHO grade I [12–15]. These tumours arise with a predilection for the optic pathway/hypothalamus (66–75%), followed by the brain stem (10–15%). Many optic pathway gliomas (OPGs) are asymptomatic, but they can be associated with visual loss, and – if encroaching the hypothalamus - precocious puberty.

To better predict phenotypic expression and to improve clinical management in patients with NF1, several studies aimed at establishing genotype-phenotype correlations. However, more than 3000 different *NF1* pathogenic variants have been identified [2]. As a consequence, to date, only 4 genotype-phenotype correlations (*NF1* p.Met992del; *NF1* p.Arg1809; *NF1* microdeletions type 1–4; missense mutations in one of the codons 844–848) were confirmed in larger data sets [16–19]. In addition, neuroimaging studies aim to establish a link between cognitive deficits and the presence, number, and/or localization of FASI and, thus, to explain the cognitive phenotype in patients with NF1 [20–22].

We aimed to (1) characterize the spectrum of CNS manifestations of NF1 in a paediatric population, (2) explore radiological features of NF1 in the CNS by image analyses, and (3) correlate genotype with phenotypic expression for those with a genetic diagnosis.

2. Patients and methods

We performed a database search in the hospital information system (ORBIS® v. 08.043.302.11210 DACHL, Agfa Health Care N.V., Belgium) of the University Children's Hospital Augsburg with International Classification of Disease-10 code Q85.0 (phakomatoses, not elsewhere classified). We checked results for plausibility reviewing text entries (NF1, Legius syndrome, Café-au-lait macule (CALM), freckling, optic pathway glioma, neurofibroma, Lisch nodule). Patients aged <18 years at first contact between January 1, 2017 and December 31, 2020 were included. Patients who did not fulfil NIH diagnostic criteria for NF1 at last follow-up were excluded from this analysis unless genetic testing identified a pathogenic variant in *NF1*. The study was approved by the responsible ethics committee of the Ludwig Maximilian University of Munich (approval number, 21–1103), Germany.

The University Children's Hospital is a tertiary care children's hospital caring for children and adolescents aged <18 years in the catchment area of Swabian Bavaria. Patients are referred from primary and secondary care paediatricians as well as other children's hospitals. There is a strong expertise in paediatric (neuro-) oncology and a focus on neuropaediatrics. Until December 31, 2018, the national reference centre for children and adolescents with LGG was situated at the University Children's Hospital.

We evaluated neurological manifestations through retrospective chart review according to those clinical signs documented at the first and subsequent visits until last follow-up. Data were recorded by general paediatricians, neuropaediatricians, and/or paediatric oncologists during regular and/or unscheduled visits for signs and symptoms including data from the "yellow booklet" (documenting preventive medical checkups) and medical history provided by parents. All patients have been evaluated at least once by a neuropaediatrician or paediatric oncologist with strong expertise in neuro-oncology (AKG, MCF). A sign was considered not present for an individual if no information was provided in the patient's chart and/or if magnetic resonance imaging (MRI) and/or ophthalmologic evaluation was not performed. Follow-up ended on June 30th, 2020.

Macrocephaly was defined as a head circumference >97th percentile according to the German Health Interview and Examination Survey for Children and Adolescents (KIGGS) study and the WHO scale, respectively.

Neurologic development was evaluated based on the early childhood development milestones. Electroencephalography was conducted if clinically indicated. Epilepsy was diagnosed based on the 2014 International League Against Epilepsy definition. Attention deficit hyperactivity disorder (ADHD) and autism spectrum disorder (ASD) were diagnosed through neuropsychological assessment (conducted in other institutions). Further behavioural problems, social interaction disturbances, and learning difficulties, were assessed based on parental report.

Patients registered with the German LGG study received standardized neuropsychological after-care. Criteria for neuropsychological evaluation of the German LGG studies for patients with LGG are specified in Traunwieser et al. [23] Neuropsychological functions were assessed with the German "Neuropsychological Basic Diagnostic" screening tool (NBD). The NBD analyses cognitive dimensions in several major domains with age-appropriate tests. These cognitive domains include fluid intelligence (FI), crystallized intelligence (CI), verbal short-term memory (STM), visual processing (VP), psychomotor speed of the dominant (PMS-DH), non-dominant (PMS-NDH), and coordination of both hands (PMS-BH), as well as cognitive processing speed (PS). NBD results were compared with age-corrected normative data and are displayed as standard scores (SS, mean $\bar{x} = 100$ and standard deviation [SD] = 15). Due to small sample size, we performed the Mann–Whitney *U* test to compare the data with the expected population score. Cohen's d was compiled to analyse effect sizes, with $d \geq 0.8$ interpreted as a highly relevant effect. Results were exploratory and no significance level was fixed. $p < .01$ was regarded as statistically noticeable to adjust for multiple testing, whereas values between $p > .01$ and $p < .05$ were interpreted as marginally statistically noticeable.

Magnetic resonance imaging (MRI) of the brain with or without contrast was performed for symptoms [e.g., ophthalmological findings, neurological symptoms, macrocephaly] or screening with or without spinal MRI. In the case of suspected low-grade glioma, reference evaluation was performed by the national reference radiologist for paediatric brain tumours (B.B.). OPG were retrospectively classified according to the PLAN criteria of the modified Dodge Classification (MDC) [24].

For image analyses, patients were included if they had been scanned at the University Children's Hospital Augsburg on a 3 T MRI system (Ingenia, Philips) with an identical protocol including both a coronal T2 FLAIR and a 1 mm 3D T1-weighted scan after intravenous contrast administration. MRI series were exported anonymously, subsequently defaced using the open-source brain extraction tool HD-BET [25] and transferred to a dedicated workstation for lesion segmentation. All cerebral lesions that were identified and reported by experienced paediatric radiologists were subsequently segmented using the open-source software 3D Slicer (https://www.slicer.org/). Finally, the number of all lesions were assessed, and volumes of all lesions were measured for each scan.

3. Results

A total of 59 children and adolescents with NF1 were identified at the University Children's Hospital Augsburg within the 4 year period. Thirty-one (of 59; 52.5%) patients were female. Details on demographic data and CNS manifestations at last follow-up are given in Table 1. As of June 30, 2021, one patient had died of progressive plexiform neurofibroma and pilocytic astrocytoma of the medulla oblongata due to central respiratory dysfunction and obstruction of the nasopharynx.

As reported [26], 44 (of 59; 74.6%) patients met the NIH criteria at first visit (median age 4.1 years; range, 0.0–13.6) and 55 (of 59; 93.2%) patients at last follow-up (median age 10.6 years; range, 1.1–22.6). In 26 (of 29; 89.7%) patients, diagnosis of NF1 was genetically confirmed, 30 (of 59; 50.8%) patients did not receive genetic testing.

A total of 49 (of 59; 83.0%) patients presented with a wide spectrum of neurological manifestations including 28 patients with structural and neurodevelopmental findings, 16 patients with neurodevelopmental, and 5 patients with structural findings only.

3.1. Non-neoplastic structural CNS manifestations

Macrocephaly was present in 12 (of 59; 20.3%) patients, an additional number of n = 5 (of 59; 8.5%) demonstrated disproportional head growth.

Brain MRI with and without contrast was performed in 39 (of 59; 66.1%) patients [for ophthalmological findings (n = 11) such as deteriorating vision or vision loss, nystagmus, strabismus, papilledema, papillary atrophy, proptosis, swelling of the eyelid; macrocephaly and/or neurological symptoms (n = 7) such as headache, developmental delay, suspicion of seizures, behavioural problems; suspicion of NF1 (n = 12); other or unknown reasons (n = 9)] at a median age of 5.3 years (range, 0.2–17.7).

Stenosis of the aqueduct was diagnosed in 4 (of 39; 10.3%) patients with brain stem gliomas (n = 3) and idiopathic (n = 1), respectively. Subsequent hydrocephalus was treated with ventriculoperitoneal shunt in 3 of these.

Moyamoya syndrome was identified in 4 (of 39; 10.3%) patients, in of those hypoplasia of the posterior cerebral artery was diagnosed. Two of the patients with Moyamoya syndrome suffered from a stroke following occlusion of the middle cerebral artery.

FASI were identified in 29 (of 39; 74.4%) patients in various localizations throughout the brain.

3.1.1. Image analyses

MRI scans of 23 (of 39; 59.0%) patients (median age of 7.6 years; range, 1.1–13.8) were analysed, 16 (of 39; 51.3%) patients were excluded from image analyses due to different imaging protocols (n = 11), missing post-contrast series (n = 3), and movement artifacts (n = 2). T2 hyperintense lesions were identified in 22 patients (of 23; 95,6%; mean number: 4.0 ± 2.2; range, 1–7) with a total mean volume of 6.6 ± 6.8 cm^3 (range, 0.4–34.6). Four (of 23; 17.4%) patients had lesions (mean number: 1.5 ± 0.9) with enhancement on T1 post-contrast scans and a mean volume of 1.6 ± 0.8 cm^3 (Table 2).

MRI revealed T2 hyperintense lesions in the cerebellum (n = 11), basal ganglia (n = 19), brain stem (n = 12), corpus callosum (n = 6), subcortical (n = 4) and periventricular (n = 4) regions (Fig. 1).

3.2. Neurodevelopmental CNS manifestations

A history of neurodevelopmental delay was reported in 27 (of 59; 45.8%) patients, in 20 (of 59; 33.9%) motor development delay was diagnosed, in 17 (of 59; 28.8%) speech delay, and in 11 (of 59; 18.6%) cognitive delay. Six (of 59; 10.2%) patients were identified with global developmental disorder.

Reduced muscle strength was present in 7 (of 59, 11.9%) patients and motor performance deficits (mostly in fine motor skills) in 19 (of 59; 32.3%) patients. In 13 (of 19; 68.4%) patients with motor performance deficits, delayed motor development in early childhood was reported. Motor coordination difficulties were reported in 7 (of 59; 11.9%) patients. Of those, 2 patients presented with hemiparesis [following tumour resection (n = 1) and stroke (n = 1)] and 1 patient with severe visual impairment due to an OPG. Four (of 59; 6.8%) patients were diagnosed with sensory deficits due to spinal plexiform neurofibroma in 3 of those. Of those, 1 patient additionally presented with paraplegic syndrome due to a giant thoracolumbar neurofibroma.

Learning difficulties including dyslexia were reported in 19 (of 59; 32.2%) patients, concentration difficulties in 12 (of 59; 20.3%) patients, and (suspicion of) ADHD in 4 (of 59; 6.8%) patients.

Behavioural anomalies were reported in 5 (of 59; 8.5%) patients including aggressiveness towards themselves or others, disturbances in social interaction, and low frustration tolerance. ASD was diagnosed in 1 (of 59; 1.7%) patient.

Epilepsy was diagnosed in 3 (of 59; 5.1%) patients with central nervous system tumours (n = 2) and following stroke (n = 1). Three (of 59; 5.1%) other patients had epileptic potentials without clinical correlation.

3.2.1. Neuropsychological function tested by the German `Neuropsychological Basic Diagnostic' screening tool

Neuropsychological data were available for 10 (of 25; 40.0%) patients with LGG (demographic details in Supplemental Table 1). Of those, 8 patients had a radiologically diagnosed OPG. Six patients had received chemotherapy prior to the NBD screening including one patient with prior surgery.

The median age at diagnosis was 4.6 years, with a slightly higher female ratio (7 of 10). The cohort showed statistically noticeable impairments compared to the expected population score in visual processing, psychomotor speed (PMS) of the non-dominant hand (NDH), as well as of both hands ($p = .008$; $d = 3.88–3.89$). Marginally statistically noticeable results were detected for the PMS of the dominant hand (DH) as well as cognitive processing speed ($p = .011$-$.034$; $d = 2.67–3.21$). Median scores were within the lower normal range for fluid intelligence and visual processing, as well as below the lower normal range for PMS-DH, PMS-NDH, PMS-BH and PS (Table 3).

3.3. Neoplastic CNS manifestations

OPGs were diagnosed radiologically in 18 (of 59; 30.5%) patients at a median age of 3.9 years (range, 0.7–7.9 years) being symptomatic in 5 (of 18; 27.8%; median age 2.5 years) at the time of diagnosis. According to the PLAN-criteria, optic nerves were affected unilaterally (MDC 1a) or bilaterally (MDC 1b) in 2 (of 18; 11.1%) patients each, and in the prechiasmatic (junctional) section (MDC 1c) in 10 (55.5%) patients. The chiasm was involved centrally (MDC 2a) in 6 (33.3%) and asymmetrically (MDC 2b) in another 6 (33.3%). Optic tract involvement (MDC 3) was identified in 6 (33.3%) patients, diffuse involvement of the posterior tracts (MDC 4) concerned 2 (11.1%), and of the hypothalamus (MDC H+) another 5 (27.8%) patients (Fig. 2). Nine (of 18; 50.0%) patients received vincristine/carboplatin chemotherapy according to the effective German multicentre studies, indicated by clinical/visual deterioration and/or radiological progress, just 2 had additional resection (indication ill-defined, treated abroad previously) or biopsy (n = 1; for diagnostic workup in metastatic disease).

Thirteen (of 59; 22.0%) patients, including 6 with OPG, had LGGs outside the visual pathways (Fig. 1). Five (of 13; 38.5%) tumours were histologically classified as pilocytic astrocytoma, while biopsy was not performed in 8 (of 13; 61.5%) patients. Three patients (of 13; 23.1%) with brain stem (n = 2) and temporo-mesial (n = 1) LGG were treated with chemotherapy for radiological progress and/or severe symptoms.

Table 1
Demographic details and neurological manifestations in 59 children and adolescents with neurofibromatosis type 1 at last follow-up.

Pat-ID	Age at last follow-up	Sex	NIH criteria fulfilled	Heterozygous pathogenic *NF1* variant	FASI	OPG	LGG other than OPG	Stenosis of aqueduct	Vascular anomalies	Macro cephaly	Motor development delay	Speech delay	Cognitive delay	Global developmental disorder	Reduced muscle strength	Motor performance deficits/ coordination difficulties	Sensorimotoric deficits	Learning difficulties	Behavioural anomalies	ADHD	ASD	Epilepsy
1	18,3	m	+	n.d.	+	+	+	–	–	–	+	+	+	+	–	+	–	–	+	–	–	–
2	16,9	f	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
3	1,8	f	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–	–	–	–	–	–	–	–	–	–	–	–	–
4	17,4	m	+	n.d.	+	+	+	–	–	+	–	–	–	–	+	–	–	+	–	–	–	–
5	19,8	f	+	n.d.	+	+	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
6	16,3	m	+	n.d.	+	+	+	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–
7	17,4	m	+	n.d.	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
8	17,8	m	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
9	2,6	f	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	–	–	–	–	–	–	–	–	–	–	–	–	–
10	20,0	f	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	–	–	–	–	–	–	–	–	+	–	–	–	–
11	5,8	m	+	+	–	–	–	–	–	+	+	+	–	–	–	–	–	–	–	–	–	–
12	12,2	m	+	n.d.	–	–	–	–	–	–	–	+	–	–	–	–	–	+	+	–	–	–
13	8,2	f	+	+	+	+	–	–	–	–	+	–	+	–	–	+	–	+	–	–	–	–
14	4,3	m	+	n.d.	+	+	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–
15	11,2	f	+	+	+	+	–	–	–	–	+	–	–	–	–	+	–	–	–	–	–	–
16	16,8	f	+	n.d.	+	–	–	+	–	–	–	+	–	–	–	–	–	+	–	–	–	–
17	4,8	m	–	+	n.d.	n.d.	n.d.	n.d.	n.d.	–	+	–	–	–	–	+	–	–	–	–	–	–
18	17,3	f	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	+	–	+	–	–	+	–	–	–	–	–	–	–
19	5,7	f	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–	–	–	–	–	–	–	–	–	–	–	–	–
20	16,3	f	+	n.d.	+	–	–	–	–	–	+	–	+	–	+	+	–	+	–	–	–	–
21	1,1	f	+	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
22	11,2	f	+	–	+	+	+	–	–	–	+	+	+	+	–	+	–	+	–	–	–	–
23	12,6	m	+	n.d.	+	–	+	–	–	–	–	–	–	–	–	+	–	+	+	+	+	+
24	2,4	f	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	–	–	–	–	–	–	–	–	–	–	–	–	–
25	6,7	f	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–	–	–	–	–	–	–	–	–	–	–	–	–
26	9,3	m	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	–	–	–	–	–	–	–	–	–	–	–	–
27	1,6	m	–	+	n.d.	n.d.	n.d.	n.d.	n.d.	–	–	–	–	–	–	–	–	–	–	–	–	–
28	12,0	f	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	–	–	–	–	–	–	–	+	–	–	–	–
29	15,5	f	+	+	+	–	–	–	+	–	–	–	–	–	–	–	–	+	–	+	–	–
30	8,2	m	+	+	+	–	–	–	–	+	+	–	+	–	+	–	–	–	–	–	–	–
31	9,6	f	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	–	–	–	–	–	–	–	+	–	–	–	–
32	12,2	f	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	+	–	–	–	–	–	–	–	–	–	–	–	–
33	6,1	f	+	+	+	–	+	–	+	–	–	–	–	–	–	–	+	–	–	–	–	–
34	15,3	m	+	n.d.	+	+	–	–	–	–	+	+	+	+	–	+	–	+	–	–	–	–
35	12,9	f	+	n.d.	–	–	+	–	+	+	+	+	–	–	+	+	–	–	–	–	–	+
36	7,9	m	–	+	n.d.	n.d.	n.d.	n.d.	n.d.	–	+	–	–	–	–	–	–	–	+	–	–	–
37	4,2	f	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	–	–	+	–	–	–	–	–	–	–	–	–	–
38	7,2	m	+	+	–	+	+	–	–	–	+	+	+	+	–	–	–	–	–	–	–	–
39	12,2	m	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	–	–	–	–	–	–	–	–	+	–	+	–	–
40	11,1	f	+	n.d.	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
41	11,4	f	+	n.d.	+	+	–	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–
42	13,1	m	+	n.d.	+	–	+	–	–	–	+	+	+	+	–	–	–	+	–	–	–	–
43	19,4	f	+	n.d.	+	–	+	+	+	–	–	–	–	–	–	–	+	–	–	–	–	–
44	6,5	m	+	+	+	–	–	–	–	+	+	+	–	–	–	–	–	–	+	–	–	–
45	22,6	m	+	n.d.	–	–	–	–	–	+	+	–	–	–	–	+	+	–	–	–	–	–
46	13,0	m	+	n.d.	+	–	–	–	–	–	+	–	+	–	+	–	–	+	–	–	–	–
47	15,9	f	+	n.d.	+	–	+	–	–	–	–	–	–	–	–	+	–	+	–	–	–	–
48	12,3	f	+	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	–	–	–	–	–	–	–	–	–	–	–	–	–
49	13,6	f	+	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
50	17,6	m	+	–	n.d.	n.d.	n.d.	n.d.	n.d.	–	–	+	+	–	–	–	+	+	–	–	–	–

(*continued on next page*)

Table 1 (*continued*)

Pat-ID	Age at last follow-up	Sex	NIH criteria fulfilled	Heterozygous pathogenic *NF1* variant	FASI	OPG	LGG other than OPG	Stenosis of aqueduct	Vascular anomalies	Macro cephaly	Motor development delay	Speech delay	Cognitive delay	Global developmental disorder	Reduced muscle strength	Motor performance deficits/ coordination difficulties	Sensorimotoric deficits	Learning difficulties	Behavioural anomalies	ADHD	ASD	Epilepsy
51	15,5	f	+	n.d.	+	+	+	+	–	–	–	–	–	–	–	+	–	–	–	–	–	–
52	4,2	m	+	+	–	–	–	–	–	–	–	+	–	–	–	–	–	+	–	+	–	–
53	1,4	m	+	+	+	+	–	–	–	–	+	–	–	–	+	+	–	–	–	–	–	–
54	5,4	m	–	+	+	–	+	+	–	–	+	+	+	+	–	+	–	–	–	–	–	+
55	12,2	m	+	n.d.	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
56	7,3	m	+	+	+	+	–	–	–	–	+	+	–	–	–	+	–	–	–	–	–	–
57	1,7	f	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	+	–	–	–	–	–	–	–	–	–	–	–	–
58	1,1	m	+	+	n.d.	n.d.	n.d.	n.d.	n.d.	–	–	–	–	–	–	–	–	–	–	–	–	–
59	3,6	f	+	n.d.	+	+	–	–	–	–	–	+	–	–	–	–	–	–	–	–	–	–

Legend: n.d., MRI not done.

3.4. Correlation of neuroradiological manifestations with neurological symptoms

Volume of lesions and phenotype: A single lesion with a total volume of 34.6 cm^3 involving the brain stem, thalamus, basal ganglia, corpus callosum, and periventricular and cerebral subcortical regions was identified in one patient with global developmental disorder and epilepsy. A single lesion of 0.4 cm^3 in the corpus callosum was identified in one patient with learning difficulties and ADHD. Single lesions of 0.8 cm^3 (involving the brain stem and thalamus one each) and 12.3 cm^3 (involving the cerebellum, brain stem, basal ganglia, and thalamus) were identified in three neurologically unimpaired patients. Beside those 3 clinically asymptomatic patients, 19 (of 22; 86.4%) patients with evidence of lesions in detailed image analyses presented with a various spectrum of neurological manifestations.

Number of lesions and phenotype: Seven lesions were identified in 3 patients, 2 of those were diagnosed with development delay, motor coordination and learning difficulties. A single lesion was diagnosed in 6 patients, 3 of those each presented with motor difficulties and epilepsy, 2 each with development delay, ADHD, and learning difficulties, and one patient each presented with behavioural anomalies and ASD. In the three clinically unaffected patients, one (n = 2) and six lesions were identified in image analyses.

Location and phenotype: In 19 patients, T2 hyperintense lesions involved the basal ganglia. Of those, 11 patients presented with development delay, 12 patients with motor and/or coordination difficulties, and 7 patients with learning difficulties. The brain stem was involved in 12 patients presenting with development delay (n = 5), motor and/or coordination difficulties (n = 7), and learning difficulties (n = 5). In 11 patients, lesions involved the cerebellum (development delay n = 7, motor and/or coordination difficulties n = 7, learning difficulties n = 6). Of 11 patients with lesions affecting the thalamus, 7 patients each presented with development delay and motor/coordination difficulties, 4 patients with learning difficulties. In the three clinically unaffected patients, lesions involved the thalamus, basal ganglia, brain stem, and cerebellum (Fig. 3).

3.5. Genotype-phenotype correlation

Details on *NF1* gene variants were available in 21 of 26 patients with genetically confirmed diagnosis of NF1 (Fig. 4). Deletions were identified in 8 (of 21; 38.1%) patients, nonsense variants in 5 (of 21; 23.8%) patients, missense and intron variants in 3 (of 21; 14.3%) patients each, and splice site variants and insertions in 1 (of 21; 4.8%) patient each. Of the variants, 6 (of 21; 28.6%) were located in the cysteine serine rich domain, 2 (of 21; 9.5%) variants in the C-terminal domain, and 1 (of 21; 4.8%) variant each in the tubulin binding domain, GTPase activating protein related domain, and SEC14p homology domain.

A 5.15 Mb microdeletion 17q11.2q12(29,296,310–34,450,651)x1 (GRCh37/hg19) encompassing the *NF1* gene was identified in 1 (of 21; 4.8%) patient with facial dysmorphism, multiple CALM, reduced muscle strength, motor development delay, cognitive deficits, and propensity to infections. No other variant with an established genotype-phenotype correlation was identified.

4. Discussion

Neurofibromatosis type 1 may be associated with a spectrum of CNS manifestations manifesting in childhood. Our data mirror this association; at least 83.0% of patients presented with neurological symptoms and/or neuroradiological findings.

Cerebrovascular disease is a rare but important manifestation of NF1 primarily affecting the arterial blood vessels. Moyamoya syndrome was diagnosed in 4 (6.8%) patients, 2 of those suffered from a stroke. These numbers are within the reported range (2.5–6%) [27–29]. The mechanism underlying vasculopathy in NF1 is poorly understood, Particularly,

Table 2
Details on image analyses in 23 patients with MRI data and focal areas of signal intensity.

		FLAIR		T1 post contrast		Localization						
Pat. ID	Age at MRI	Number of lesions	Volume cm^3	Number of lesions	Volume cm^3	Cerebellum	Brain stem	Thalamus	Basal ganglia	Corpus callosum	Periventricular	Cerebral subcortical
1	11.6	5	6,8	3	2,8	+	+	+	+	–	–	–
4	10.4	n.a.	n.a.	n.a.	n.a.	+	+	–	+	–	–	–
5	13.8	6	5,4			–	+	–	+	+	+	–
6	13.3	5	10,1			–	–	–	–	+	–	–
12	10.2	0		0		–	–	–	–	–	–	–
13	3.7	7	9,4			–	–	+	+	–	–	–
14	2.7	5	4,8			+	–	–	+	–	–	–
15	4.8	n.a.	n.a.	n.a.	n.a.	–	–	+	+	–	–	–
21[a]	1.1	1	0,8			–	–	+	–	–	–	–
22	4.6	6	12,3			+	+	+	+	–	–	–
23	8.1	1	1,3	1	1,1	–	–	–	–	–	–	+
29	13.4	1	0,4			–	–	–	–	+	–	–
30	5.9	7	6,5			+	–	+	+	–	–	–
33	3.3	4	4,4			–	–	–	+	–	–	+
34	8.9	6	4,9			+	–	–	+	–	–	–
35	11.5	1	3,8			+	–	–	–	–	–	–
40[a]	6.7	6	5,7			+	–	–	+	–	–	–
41	8.1	7	8,8			+	+	+	+	–	–	–
42	10.8	4	2,1			+	+	–	+	–	–	–
43	13.3	4	5,6	1	1,6	–	+	+	+	+	–	–
47	8.8	6	4,8	1	0,7	+	+	+	+	+	+	–
49[a]	7.7	1	0,8			–	+	–	–	–	–	–
51	9.5	n.a.	n.a.	n.a.	n.a.	–	+	–	+	–	–	+
53	1.4	2	4,2			–	–	–	+	–	+	–
54	3.6	1	34,6			–	+	+	thco	+	+	+
59	3.0	3	7,2			–	+	+	+	–	–	–

[a] Clinically unaffected by neurological manifestations.

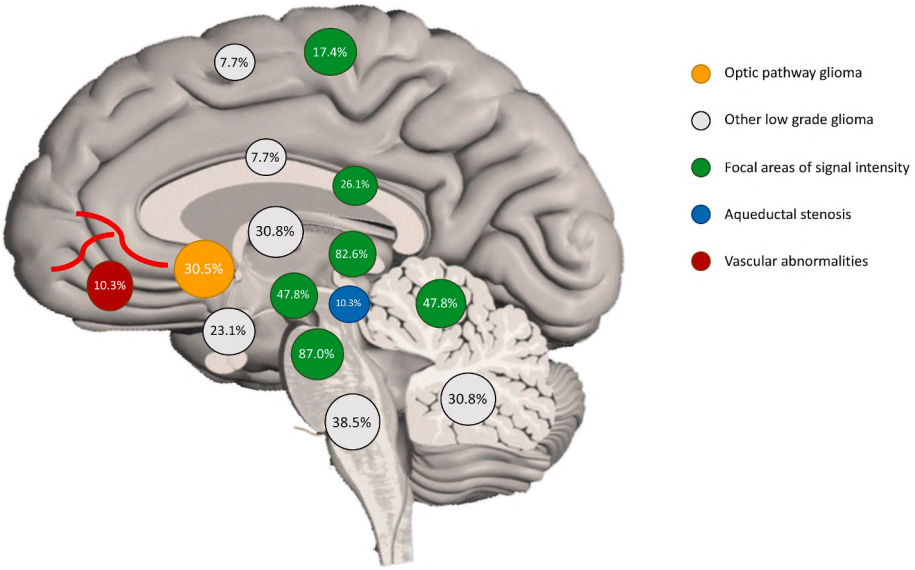

Fig. 1. Percentage of patients with optic pathway gliomas, stenosis of the aqueduct, and vascular anomalies in 43 patients with MRI. Percentage of low-grade glioma (LGG) and focal areas of signal intensity (FASI) refer to the number of patients with LGG (n = 13) and FASI in MRI image analyses (n = 23), respectively.

Moyamoya syndrome may be attributable to radiotherapy effects [30–32]. However, none of the patients diagnosed with Moyamoya syndrome underwent radiotherapy.

Motor problems were reported in approximately one third to one half of children with NF1 [33,34]. In line with this, 33.9% of the patients in our cohort were diagnosed with motor development delay, 32.3% with motor performance deficits, and 11.9% of patients with motor coordination difficulties.

Neurocognitive deficits including mental learning and concentration difficulties, intellectual disability, attention deficits including ADHD, and ASD are increasingly recognized in children with NF1 [35–37].

Specific learning disorders and thus, lower performance in a particular subject, such as reading, mathematics, or writing, were observed in approximately 20% of NF1 patients and, thus, double that in the general paediatric population [38]. This is typically not attributable to lower general cognitive ability or comorbid medical conditions. 75% of children with NF1 will require some type of school accommodation or remedial teaching [39]. Learning and concentration difficulties were documented in 32.3% and 20.3% of the patients in our cohort. The general reduction in cognitive functioning observed among children with NF1 requires prompt recognition through comprehensive neuropsychological evaluation, and the early initiation of educational support

Table 3
Results of the Neuropsychological Basic Diagnostic (NBD) screening (cognitive domain) compared to the expected population score in 10 patients with NF1 and low-grade glioma.

Cognitive domain	Results of the NBD screening compared to the expected population score (IQ-scores; p-values, Cohen's d; *p < .05, **p < .01)
Fluid intelligence (FI)	$p = .169$, $d = 0.97$
Crystallized Intelligence (CI)	$p = .611$, $d = 0.33$
Verbal short-term memory (STM)	$p = .057$, $d = 1.51$
Visual processing (VP)	$\mathbf{p = .008^{**}, d = 3.89}$
Psychomotor speed dominant hand (PMS-DH)	$p = .011^{*}$, $d = 3.21$
Psychomotor speed non-dominant hand (PMS-NDH)	$\mathbf{p = .008^{**}, d = 3.88}$
Psychomotor speed both hands (PMS-BH)	$\mathbf{p = .008^{**}, d = 3.88}$
Cognitive processing speed (PS)	$p = .034^{*}$, $d = 2.67$

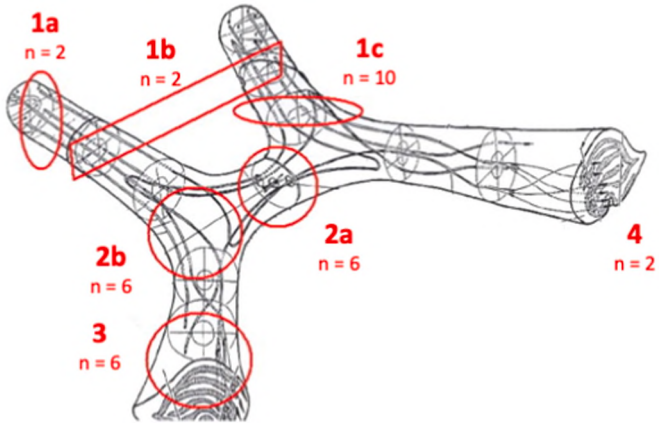

Fig. 2. Sites of optic pathway gliomas in 18 patients illustrated by the modified Dodge classification (Figure modified from Tyler et al., British Journal of Radiology, 2008).

mechanisms. When directly tested, children with NF1 tend to show deficits in more global measures of executive function, such as problem solving, planning, and task switching [40,41]. Similarly, visuospatial processing is specifically impaired in children with NF1 [40]. In line with this, visual processing, psychomotor speed, and cognitive processing speed were impaired in our cohort. However, these data need to be interpreted with caution as the number of children was small. In addition, only individuals with NF1 and LGG were tested [23]. This may have had an impact on neuropsychological function [42]. However, *NF1*-knockout mice have provided evidence that *NF1* gene alterations are sufficient to cause specific learning deficits [43]. Thus, the presence of a LGG might add to neuropsychological malfunctioning but certainly is not the main cause.

Children with NF1 have specific deficits in attention, particularly in sustained attention. As many as 60% of children with NF1 meet diagnostic criteria for ADHD, most likely inattentive-type, or combined-type ADHD [44]. In our cohort, only 6.8% of patients were diagnosed with ADHD. Neuropsychological assessment was only performed sporadically, which may have contributed to the low number of affected patients. In addition, documentation may be incomplete. Nevertheless, increased awareness is necessary to identify and bring potentially affected children to neuropsychological assessment.

An overall decline in social functioning across multiple domains as well as an increase in the incidence of clinical ASD (approximately 25–40%) was demonstrated in individuals with NF1 [35,36]. In addition, the spectrum of ASD traits is shifted towards the more symptomatic end of the spectrum [36]. In our cohort, the number of patients diagnosed with ASD was remarkably low; we identified only one (1.7%) patient with the diagnosis of ASD. However, behavioural anomalies were reported by parents in an additional 8.5% of patients.

Considering the far-reaching consequences of neurocognitive deficits, ADHD, and ASD, we advocate for introducing neuropsychological assessment in the care of each child with NF1. The use of physiotherapy, occupational and psychological therapy as well as remedial teaching as appropriate should be considered especially at young age to prevent further developmental deficits.

The prevalence of epilepsy in patients with NF1 is estimated at 4–13% [45–47] In line with this, 5.1% of patients each were diagnosed with epilepsy and epilepsy action potentials without clinical correlation. Of note, all patients with epilepsy had intracranial tumours or structural anomalies. This was previously reported from an observational study [45]. In contrast, the aetiology of seizures and/or epileptic potentials cannot be identified in a subset of patients with NF1 [48–50].

MR hyperintensities/FASI are found in 70% to almost all individuals with NF1. FASI are thought to regress spontaneously during adolescence and puberty. It should not be mistaken for neoplasms [51]. The significance of FASI for cognitive (dys)function in individuals with NF1 is controversially discussed. Recent studies found a favourable

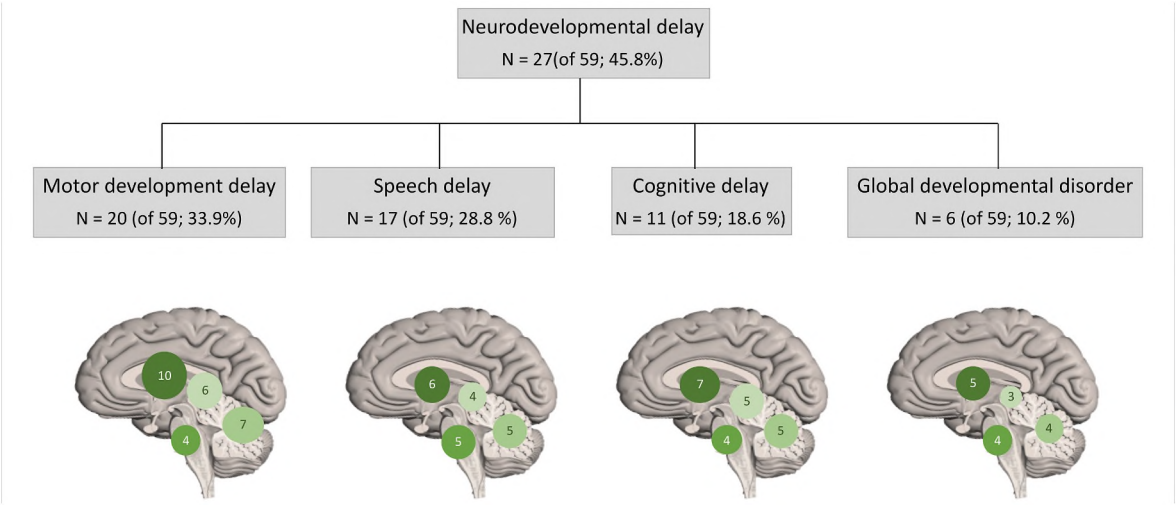

Fig. 3. Details on neurodevelopmental delay in 31 patients with neurofibromatosis type 1. Numbers given in the brain images refer to 12 patients with focal areas of signal intensity in the detailed MRI analysis.

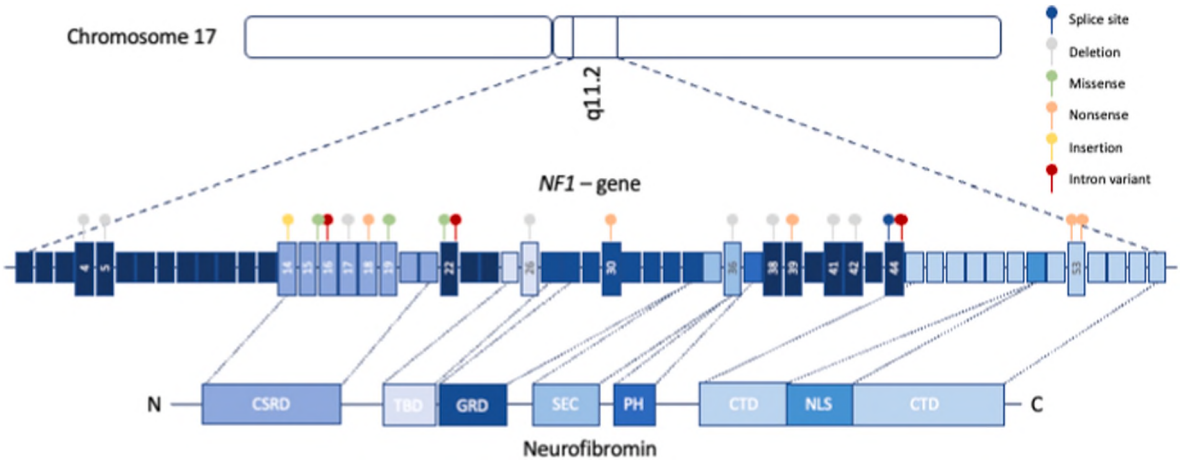

Fig. 4. Type of gene variants in 21 patients with genetically confirmed NF1. Details were not available in 5 patients.
Legend: CSRD, Cysteine Serine Rich Domain; CTD, C-Terminal Domain; GRD, GTPase Activating Protein Related Domain; NLS, Nuclear Localization Site; PH, Pleckstrin Homology Domain; SEC, SEC14p Homology Domain; TBD, Tubulin Binding Domain.

relationship with the presence, number, and location of FASI areas [52,53]. Thalamic lesions seem to be strongly associated with cognitive impairment [54]. However, an association between location of FASI and cognitive deficits was not confirmed by others [55,56]. In our cohort, FASI were identified in 74.4% of patients. In 23 patients, MRI data were sufficient for more detailed image analysis. FASI occurred in the basal ganglia, brain stem, corpus callosum, subcortical and periventricular regions. However, we could not establish a relationship between the presence, number, and location of FASI areas and cognitive deficits. Moreover, there may be a major bias. In our cohort, MR imaging was mainly performed for symptoms. Thus, we may have missed a substantial number of FASI in children without neurological manifestations. Recent studies in individuals with NF1 suggest that diffusion tensor imaging (DTI) identifies microstructural alterations which may be related to the cognitive phenotype [57]. In addition, it may help to assess optic pathway integrity. These developments may help parental counselling regarding their child's prognosis in the future.

LGGs are the most common childhood brain tumour in the general population [58] and in individuals with the NF1 cancer predisposition syndrome [12,13]. The majority of LGG arises in the optic pathways [12,13,59], and our NF1 cohort demonstrated the variable, but often extensive involvement of all segments [24,60]. Though asymptomatic in a portion of patients, we confirmed a high prevalence of brain tumours in our cohort [61]. The large number of OPGs and other LGGs following radiologic investigation in a subgroup of patients only may also reflect the recruitment bias of a university based paediatric oncology centre [59]. There is debate concerning the indication for routine diagnostic imaging in asymptomatic patients in this at-risk population of children [59,62], but during follow-up more than half of our patients required treatment for visual deterioration and/or radiologic progression emphasizing the need for appropriate oncologic surveillance [59–61]. However, it should not go unmentioned that baseline MRI without follow-up is of minimal value, as a negative baseline MRI does not exclude future development of a LGG [63,64]. Moreover, Blanchard and colleagues did not find a clear benefit of systematic MRI in children with NF1 [65]. In line with this, in the recently published tumour surveillance guidelines for individuals with neurofibromatosis type 1 by the European Reference Network on Genetic Tumour Risk Syndromes (ERN GENTURIS) NF1 tumour management guideline group routine MR imaging in asymptomatic children is not recommended [66].

To date, only 4 clinically confirmed genotype-phenotype correlations in patients with NF1 were reported, relevant to 10–15% of the NF1 population [67]. In our cohort, a 5.15 Mb microdeletion 17q11.2q12 encompassing the *NF1* gene was identified in one severely affected patient. No other variants with an established genotype-phenotype correlation were identified. Yet, no association of genotype and neurological severity was established.

Our study has several limitations.

- Our approach was based on retrospective chart review. Important information may not have been fully documented. In particular, we considered a sign not present if no information was provided. In so doing, chances are that we missed neurological manifestations and, thus, underestimated CNS manifestations in children and adolescents with NF1.
- Routine MRI for surveillance in children and adolescents is still controversially discussed. Thus, indication for brain MRI varied. In addition, MRI is not standardized across different institutions leading to failures in image analyses.
- Only patients with LGG underwent neuropsychological testing. Thus, data on neuropsychological testing is not representative for the entire NF1 cohort.
- Genetic information was only available in 21 of 26 patients with genetically confirmed diagnosis of NF1.

5. Conclusion

Neurofibromatosis type 1 was associated with a spectrum of CNS manifestations in at least 83.0% children and adolescents. About a quarter of patients were affected by neurocognitive deficits. This highlights the need for regular neuropsychological assessment complementing frequent clinical and ophthalmologic testing for OPG in the care of each child with NF1 within multidisciplinary management programs. Besides the established *NF1* microdeletion, neither genotype nor FASI number, volume, and location were associated with the neurological phenotype in our cohort.

Ethics declaration

The study was approved by the ethics committee of Ludwig Maximilian University Munich, Germany (approval number, 21–1103). Due to the retrospective manner of the study written informed consent was not obtained.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported by the PLGA Fund at the Pediatric Brain Tumor Foundation of the United States, Inc.. The research of MK on hereditary cancer predisposition is supported by research funding of the Medical Faculty, University Augsburg, Germany. This work was partly done within the Zentrum für Seltene Erkrankungen of the University Hospital Düsseldorf (ZSED). One of the authors of this publication (D. W.) is member of the European Reference Network on Rare Congenital Malformations and Rare Intellectual Disability ERN-ITHACA [EU Framework Partnership Agreement ID: 3HP-HP-FPA ERN-01-2016/739516].

Parts of this work were presented as a poster presentation at the 20th International Symposium on Pediatric Neuro-Oncology, Hamburg, Germany, 12 – 15 June 2022 and as an oral presentation at the Deutsche Gesellschaft für Kinder- und Jugendmedizin e.V. annual meeting, Düsseldorf, Germany, 7 – 10 September 2022.

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