UNIVERSITA' DEGLI STUDI DI NAPOLI "FEDERICO II"

DIPARTIMENTO DI SCIENZE BIOMEDICHE AVANZATE

DOTTORATO DI RICERCA

IN SCIENZE BIOMORFOLOGICHE E CHIRURGICHE

(XXXV CICLO)

Coordinatore: Prof Alberto Cuocolo

TESI DI DOTTORATO

"THE ROLE OF UBOs IN THE NEUROCOGNITIVE PROFILE OF NEUROFIBROMATOSIS TYPE 1 CHILDREN: A VOLUMETRIC MRI ANALYSIS"

Relatore **Candidato**

Prof. Andrea Elefante Dr.ssa Martina Di Stasi

INTRODUCTION

Neurofibromatosis type 1 (NF1, OMIM #162200) is the most common neurocutaneous disorder, affecting 1/2700 live births worldwide [1] with a complete penetrance and without a known gender or ethnicity predilection [2]. It is caused by a germline heterozygous mutation in the *NF1* gene, encoding for the tumor-suppressor protein neurofibromin, with a pattern of autosomal dominant inheritance or de novo mutations in 42% of individuals [1].

This condition is characterized by a wide range of clinical manifestations, as highlighted in the recently published NIH Revised Diagnostic Criteria [3], with the most frequent being the presence of café-au-lait macules (CALMs), freckling in the axillary or inguinal region, neurofibromas of any type or plexiform neurofibromas, iris Lisch nodules, optic pathway glioma, and bone lesions (e.g. sphenoid dysplasia, bowing of the tibia, pseudarthrosis of long bone). With reference to Central Nervous System (CNS) involvement, cognitive dysfunction represents the most significant complication in NF1 children, with about 80% of patients showing moderate to severe impairment in at least one area of cognitive functioning [4]. Indeed, previous studies demonstrated that the mean IQ score, as measured by means of the Wechsler Intelligence Scales for Children-Revised (WISC-R), tends to reach lower ranges in NF1 patients, falling within one standard deviation of the general population [5,6]. Furthermore, deficit in specific skills, including but not limited to visuospatial ability, executive function, expressive and receptive language, have also been reported in literature [4,7,8,9] , with a relatively wide proportion of patients (from 38 to 50%) that might meet diagnostic criteria for ADHD[10].

From a neuroimaging standpoint, the main brain parenchymal alteration in NF1 is the presence of focal areas of T2-weighted hyperintensity defined Unidentified Bright Objects (UBOs). These alterations, further with decrease in cognition and behavioral skills[11], have been extensively indagated, achieving conflicting results [12]. In particular, while some studies suggested a relation

between the presence of UBOs in thalamus and striatum and impairment on calculation and behavioral performances, respectively [13], other failed to prove such correlation [14]. Similarly, with reference to intellectual performances, some studies showed that thalamic [15,16] and cerebellar [17] UBOs were associated with lower IQ scores, although these correlations are not consistent between the different studies published [14].

Given that cognitive dysfunctions, especially related to the language and social behavior domains, have a serious impact on NF1 patients' quality of life [11], further data are required not only to understand the causal pathophysiological mechanisms behind the development of these changes, but also and especially in the identification of potential diagnostic biomarkers of cognitive involvement in NF1. Furthermore, no recent information about the possible correlation between UBOs lesion volume, and not their absence/presence or localization, and cognitive or language deficit is available in literature. Given this background, in the current thesis we tried to investigate the possible relation between UBOs' volume, cognitive impairment and language disability in NF1 patients.

MATERIALS AND METHODS

Participants

This retrospective single center study has been performed at the University of Naples "Federico II" in compliance with the Helsinki Declaration, with all patients (or legal guardians in case of subjects with less than 18 years) that provided a written consent to execution of the imaging exams and collection of clinical data for research purposes.

A flow-diagram for the selection of the included subjects is available in Figure 1. Briefly, inclusion criteria were the following: fulfillment of the revised diagnostic NF1 criteria [3], ability to undergo a neuropsychological examination, availability of brain MRI data acquired on the same scanner and with the same acquisition protocol. On the other hand, patients with significant artifacts on neuroradiological examination, concurrent neurologic disorders beyond the spectrum of NF1 as continuing seizures, serious psychiatric illness and previous neurosurgery or coexisting brain neoplasm (except optic pathway glioma – OPG – or small pilocytic astrocytoma) were excluded from the study.

Clinical data

For all subjects, general clinical informations were collected by a board-certified pediatric clinical geneticist (IS, with more than 10 years of expertise).

To assess general intellectual functioning and the presence of language deficit the Leiter R scale [18] was administered to all subjects of the study, while the Wechsler Intelligence Scale for Children IV (WISC-IV) [19] was administered to all patients who were able to take the test.

Patients were stratified in two groups (affected/unaffected) according to the presence or absence respectively of cognitive impairment (defined present for IQ scores < 70), language deficit, and optic pathway glioma (absence/presence).

MRI data acquisition

All MRI data were acquired on the same 1.5T scanner (Gyroscan Intera, Philips Medical System, Best, Netherlands) with a standard 16 channels head coil. The acquisition protocol included, along

with other clinically routine acquired sequences (e.g. diffusion weighted imaging, sagittal and/or coronal T2-weighted sequences, MR-angiography sequences for the study of the intracranial vasculature system in clinical suspect of Moya Moya syndrome, etc.) an axial Fluid Attenuated Inversion Recovery (FLAIR) sequence (TE=100ms; TR=10805ms; slice thickness=4mm; no gap) and an axial Turbo Spin Echo (TSE) T2-weighted sequence (TE=98ms; TR=6500ms; slice thickness 2mm; no gap) for the evaluation of the UBOs, and a coronal fat-saturated TSE T2-weighted sequence (TE=104ms; TR=9530ms; slice thickness=3mm; no gap) for the identification of OPGs of the optic-diencephalic region.

MRI data analysis

All MRI data were evaluated in consensus by two readers (MDS and SC, board-certified neuroradiologist both with more than 6 years of expertise in neuroimaging).

Both axial TSE T2-weighted and FLAIR images were used, independently, for the UBOs segmentation, with the additional aim of probing if significant differences between the two sequences were present in the identification of these lesions. The readers evaluated randomly FLAIR and T2-weighted images, and after a washout period of 30 days the other sequence was segmented. The segmentation procedure was carried out using a semiautomatic approach (Jim 8, Xinapse Systems, Northants, UK), and total UBOs' volume, expressed in milliliters, was obtained for each subject (Figure 2.A). For normalization purposes, biparietal diameters on T2w images were also recorded to normalize for head size (Figure 2.B), and UBOs volumes were divided for this value.

Finally, the presence and extent of OPG was determined according to the modified Dodge classification (mDC) [20], which, briefly, proposes an MRI-based method to categorize tumors in greater detail also considering functional visual risk. The new classification introduces three subcategories of optic nerves involvement (unilateral, bilateral, and chiasmatic junction), two categories of chiasma site (central or asymmetric), three categories of optic tracts extension (symmetric or asymmetric and diffuse posterior tracts) and considers the neoplastic involvement of hypothalamic region and leptomeningeal spread.

Statistical analysis

Possible differences in terms of UBOs volumes (normalized for biparietal diameters) between cognitively affected and preserved patients, as well as differences between subjects with or without language impairment were tested via General Linear Model analyses, corrected for age and sex. Similarly, given that previous data suggested that individuals with OPG had significantly more UBOs than individuals without OPG [21], we investigated if a similar feature was present in our group. Finally, possible differences in terms of volumes between measurement evaluated on FLAIR or T2-weighted images were tested via paired t test.

All analyses were performed using the Statistical Package for Social Science (SPSSv25.0, IBM corp.) with a significance level set for $\alpha=0.05$.

RESULTS

Following inclusion and exclusion criteria and review of MR data, a final number of 21 NF1 patients (M/F=12/9; mean age 10.1 ± 6.X, range 5-18) referred to our Clinical Genetic Unit were included in this study. From a clinical perspective, with the complete list of findings that is available in Table 1., we found that 13 out of 21 subjects (61.9%) showed a cognitive deficit, while in 9 patients (42.9%) an impairment of the language domain was present.

On MRI, 11 patients out of 21 (52.4%) proved to have an OPG, with more than half of these subjects (6/11) that showed an mDC grade equal to Ia, with the remaining patients that scored either a grade 2b (4/11) or 4b (1/11). On the other hand, all patients (21/21, 100.0%) proved to have at least one UBO.

A complete list of the volumetric analyses results is available in Table 2. When evaluating UBOs volumes, we found a significantly higher lesional volumes when segmentation was obtained on FLAIR images compared to the T2-weighted images (8.4 ± 9.1 ml vs 7.2 ± 7.8 ml, p=0.01). Nevertheless, when probing possible differences between cognitively affected and preserved patients, we failed to find significant differences between the two groups neither using FLAIR (normalized UBOs volume: 0.08 ± 0.07 vs 0.03 ± 0.02, p=0.30) nor T2-weighted (0.07 ± 0.06 vs 0.03 ± 0.02, p=0.35) sequences. Similarly, no differences emerged between patients with or without language impairment for the two sequences (0.08 ± 0.09 vs 0.05 ± 0.04, p=0.40, and 0.07 ± 0.07 vs 0.04 ± 0.05, p=0.47, for FLAIR and T2-weighted respectively). Finally, no differences between patients with and without OPG emerged neither using FLAIR (0.08 ± 0.07 vs 0.04 ± 0.06, p=0.20) nor T2-weighted (0.07 ± 0.06 vs 0.03 ± 0.05 ml, p=0.16) sequences.

DISCUSSION

Being present in approximately 70% of NF1 subjects UBOs represent the most common intracranial finding in these patients, with the first frequent location in the globus pallidus, thalamus and internal capsule, and second one the diencephalic and medial cerebellar regions [22]. They tend to vary in number and size over time and in respect of different localization, showing a non-linear trend: during the childhood, the number of affected brain regions is relatively high, but this tends to decrease in ages between 5 and 13 years, increasing again afterwards [23].

The exact nature of UBOs is not yet completely understood, mostly due to the relative paucity of histopathological data. An autoptic examination of the brain regions corresponding to T2-weighted hyperintensity seen at MRI examination in 3 NF1 patients found showed spongiotic changes with fluid-filled vacuoles of 5 -100 μm in the myelin sheath, in absence of demyelination or axonal loss[24]. In absence of large post-mortem datasets, several advanced MRI techniques have been used to investigate, from a neuroimaging perspective, macro- and microstructural alterations behind UBOs development and brain changes occurring beyond these features. In particular, affected children are known to show an increased brain volume, often associated with macrocephaly, although no clear correlation with the cognitive changes have been found [25]. Furthermore, the volume of the corpus callosum (CC), the thalamus and the striatum seem to be increased in NF1 children, correlating with lower scores in academic achievement and visual-spatial and motor-skills [26]. In addition, positive correlations were found between cognitive abilities, social skills, and the volume of subcortical structures (i.e. hippocampus, thalami, striatum, amygdala and accumbens nucleus) [27], although these results have not been replicated in a different, more recent, article [14]. Brain involvement is clearly not related only to gray matter (GM) in NF1, extending also to the white matter (WM) compartment. Indeed, it has been shown that NF1 patients encounter widespread microstructural WM changes, either in terms of increased apparent diffusivity coefficient (ADC) and decreased fractional anisotropy (FA) values, along with alterations in axial (AD) and radial diffusivity (RD), indicative of looser fiber packaging rather than demyelination [28]. Interestingly, these alterations seem to occur

independently from the presence of the pathognomonic parenchymal UBOs. With reference these latter features, one study aimed to characterize their nature by combining results from advanced white matter imaging such as Multi-Exponential T2 relaxation (MET2) and Neurite Orientation Dispersion and Density Imaging (NODDI), showing the presence of intracellular water molecules-pool with extracellular-like properties, endorsing the hypothesis of intramyelinic vacuolization [29].

Independently from their pathophysiology, the relation between UBOs and cognition in NF1 patients has been extensively indagated in literature, nevertheless leading to somehow conflicting results. In particular, while some studies showed no statistical difference in IQ scores or learning disabilities in patients with and without of UBOs [30–32], other found that although presence, number, and locations of UBOs seem not to influence the general cognitive status, thalamic and striatal localization seems to respectively impact calculation and behavioral performances [13]. In contrast with this result, a more recent study showed that thalamic UBOs seemed to not have a significant impact on cognitive functioning, in absence of correlations between thalamic or other subcortical structure volumes and specific cognitive scores [14]. With specific reference to intellectual performances, several papers reported that UBOs can affect this feature, with one study showing that the number of UBOs might predict sibling-referenced lowering IQ [33], while other Authors reported a relation between basal ganglia UBOs volume and brain volume ratio and siblings-pairwise Judgement Line Orientation deficit [34].Furthermore, thalamic lesions were associated with lower intellectual function in two separate studies [15] [16], while cerebellar UBOs have been associated with worse scores on verbal IQ, full-scale IQ and visuospatial tests [17]. Nevertheless, these findings have been recently rediscussed, given that have not been recently replicated [14].

In this study, we analyzed UBOs using two different conventional imaging techniques (TSE T2w and FLAIR sequences) by means of semiautomatic segmentation method, to further investigate the possible relation between UBOs' volume, cognitive impairment and learning disability in NF1 patients. Our first result is that, compared to T2-weighted sequence, FLAIR sequence is able to detect a higher and more reliable lesional volume. This result is not unexpected, given that FLAIR sequences

are known to have higher sensitivity in the detection of myelin alterations, especially in lesions close to cerebrospinal fluid and adjacent to grey matter [35] [36]. In line with these considerations, in a condition different form a pathophysiological standpoint but characterized by the presence of white matter lesions such as Multiple Sclerosis, lesion volume are known to be higher when FLAIR sequences are evaluated compared to T2w images [35]. When possible differences between affected and unaffected patients were probed in terms of UBOs volumes, we failed to find any significant differences, independently from how patients were stratified and which MRI feature was evaluated. Although disappointing, the lack of differences is not unexpected, given the available literature. In particular, it is possible to hypothesize that the occurrence cognitive impairment and language deficit might be more related to widespread loss of normal appearing white matter microstructural integrity and abnormal neuronal connectivity as already reported in NF1 patients [28]. This speculation is also supported by findings in different conditions, such as Tuberous Sclerosis Complex (TSC), another neuro-phacomatosis characterized by a similar decreased neurological outcomes in intellectual skills and learning abilities, and in which a similar pattern of brain changes has been reported [37]. In particular, analogously to UBOs in NF1, neither tubers load nor their localizations seem to show strong correlation with cognitive outcome in TSC patients, and it has been suggested that TSC symptoms may be contingent on abnormal connections independent from local alterations evident at conventional imaging. This possible explanation is supported by the finding that NODDI parameters, (which reflect white matter microstructure [38]) in TSC cases show altered scores compared with controls even in brain tissue without tubers, and such results in long association fiber tracts related to higher cortical functions have a slight relation with mental retardation [39].

On the other hand, cognitive deficit (lower IQ evaluated with WISC IV) related to modifications in white matter microstructure (reduced FA and RD and increased MD and AD in many white matter tracts including associative and motor ones) has been reported in conditions as congenital hypothyroidism, which does not present pathognomonic local brain lesions at MR conventional imaging [40].

These observations are also applicable for language and other academic achievements, in which the more pronounced involvement of frontal lobes' white matter integrity in the setting of diffuse alterations has been reported as a fundamental determinants to the development of specific neurocognitive profile in NF1 patients [28].

Although all these considerations are plausible from a pathophysiological standpoint, we cannot exclude that the lack of difference here observed might be related to the low numerosity of our sample, which is the main expected limitation of this study. Indeed, as often happens when dealing with rare disorders, only 21 NF1 patients have been included in this study, obviously limiting our statistical power in possibly observing small difference between the different groups. Nevertheless, it has to be stressed that all the subjects here included underwent the same standardized MRI protocol and clinical evaluation, in order to minimize possible differences in terms of acquisition parameters that might influence semiautomatic measurements as the ones here produced. Nevertheless, future studies, conducted using a larger sample size, are warranted to further validate the hypothesis, here corroborated, of the absence of a significant effect of UBOs in the development of cognitive impairment and learning disability in NF1 patients.

FIGURES

FIGURE 1.

Flow diagram showing the patients' selection procedure.

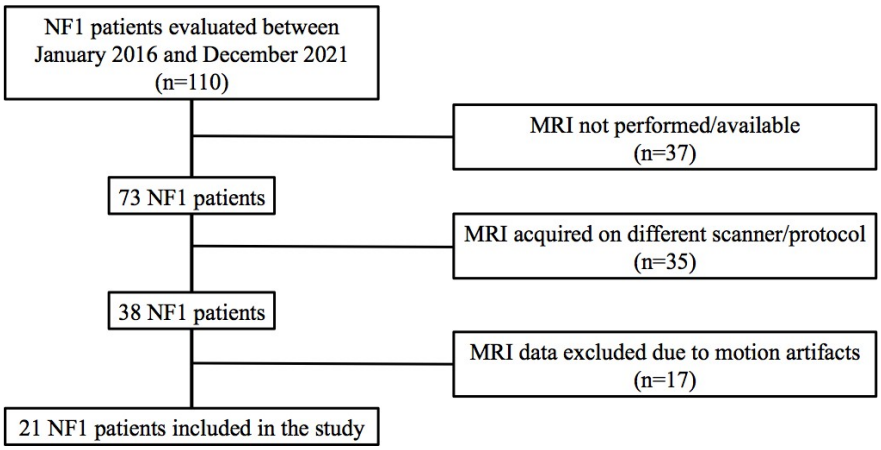
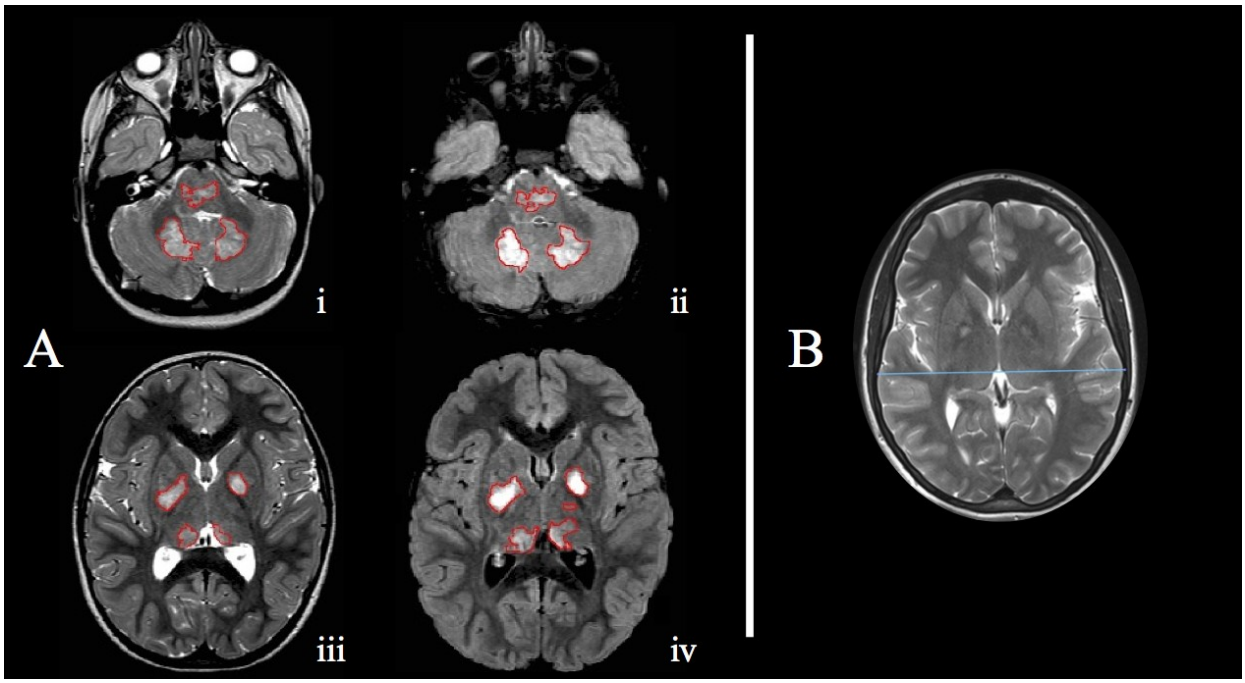


FIGURE 2.

In A, presence of infra- (i; ii) and supratentorial (iii; iv) UBOs segmentation masks on T2-weighted and FLAIR images, respectively, of a 6 years old NF1 patient.

In B, an example of the biparietal diameter (blue line) used for normalization purposes, measured at the level of Monro foramen in a 16 years old NF1 patient.



TABLES

TABLE 1.

	NF1 (all patients, n=21)	NF1 patients with cognitive impairment (n=14)	NF1 patients with language deficit (n=9)	NF1 patients with OPG (n=11)
Age (mean ± SD)	10.1 ± 4.5	9.4 ± 4.1	10.4 ± 5.4	9.7 ± 4.2
Sex (M/F)	12/9	9/5	7/2	6/7
Macrocrania	7/21	7/14	4/9	2/11
Plexiform neurofibromas	3/21	1/14	1/9	2/9
Bone anomalies	3/21	2/14	2/9	1/11

Clinical and demographic information of the enrolled patients. Age is expressed in year.

NF1: Neurofibromatosis Type 1; SD: Standard Deviation.

TABLE 2.

	NF1 (all patients)	NF1 patients with cognitive impairment	NF1 patients without cognitive impairment	NF1 patients with language deficit	NF1 patients without language deficit	NF1 patients with OPG	NF1 patients without OPG
UBOs volume (T2-weighted)	7.2 ± 7.8	9.1 ± 8.9	3.4 ± 3.1	9.5 ± 9.6	5.9 ± 6.2	9.6 ± 8.5	4.6 ± 6.5
UBOs volume (FLAIR)	8.4 ± 9.1	10.8 ± 10.2	3.7 ± 3.2	11.6 ± 11.9	6.6 ± 5.7	10.9 ± 9.4	5.7 ± 8.4

Results of the volumetric analyses, divided per groups. Volumes are expressed in milliliters (mean ± standard deviation).

REFERENCES

1. Evans DG, Howard E, Giblin C, et al. Birth incidence and prevalence of tumor-prone syndromes: estimates from a UK family genetic register service. Am J Med Genet A 2010;152A(2):327–32.

2. Friedman JM. Epidemiology of neurofibromatosis type 1. Am J Med Genet 1999;89(1):1–6.

3. Legius E, Messiaen L, Wolkenstein P, et al. Revised diagnostic criteria for neurofibromatosis type 1 and Legius syndrome: an international consensus recommendation. Genet Med 2021;23(8):1506–13.

4. Hyman SL, Shores A, North KN. The nature and frequency of cognitive deficits in children with neurofibromatosis type 1. Neurology 2005;65(7):1037–44.

5. North K, Joy P, Yuille D, et al. Specific learning disability in children with neurofibromatosis type 1: significance of MRI abnormalities. Neurology 1994;44(5):878–83.

6. Krab LC, Aarsen FK, de Goede-Bolder A, et al. Impact of neurofibromatosis type 1 on school performance. J Child Neurol 2008;23(9):1002–10.

7. Chaix Y, Lauwers-Cancès V, Faure-Marie N, et al. Deficit in phonological processes: a characteristic of the neuropsychological profile of children with NF1. Child Neuropsychol 2018;24(4):558–74.

8. Schwetye KE, Gutmann DH. Cognitive and behavioral problems in children with neurofibromatosis type 1: challenges and future directions. Expert Rev Neurother 2014;14(10):1139–52.

9. Ozonoff S. Cognitive impairment in neurofibromatosis type 1. Am J Med Genet 1999;89(1):45–52.

10. Mautner V-F, Kluwe L, Thakker SD, Leark RA. Treatment of ADHD in neurofibromatosis type 1. Dev Med Child Neurol 2002;44(3):164–70.

11. Noll RB, Reiter-Purtill J, Moore BD, et al. Social, emotional, and behavioral functioning of children with NF1. Am J Med Genet A 2007;143A(19):2261–73.

12. North K. Neurofibromatosis type 1. Am J Med Genet 2000;97(2):119–27.

13. Parmeggiani A, Boiani F, Capponi S, et al. Neuropsychological profile in Italian children with neurofibromatosis type 1 (NF1) and their relationships with neuroradiological data: Preliminary results. Eur J Paediatr Neurol 2018;22(5):822–30.

14. Baudou E, Nemmi F, Biotteau M, et al. Are morphological and structural MRI characteristics related to specific cognitive impairments in neurofibromatosis type 1 (NF1) children? Eur J Paediatr Neurol 2020;28:89–100.

15. Goh WHS, Khong P-L, Leung CSY, Wong VCN. T2-weighted hyperintensities (unidentified bright objects) in children with neurofibromatosis 1: their impact on cognitive function. J Child Neurol 2004;19(11):853–8.

16. Chabernaud C, Sirinelli D, Barbier C, et al. Thalamo-striatal T2-weighted hyperintensities (unidentified bright objects) correlate with cognitive impairments in neurofibromatosis type 1 during childhood. Dev Neuropsychol 2009;34(6):736–48.

17. Piscitelli O, Digilio MC, Capolino R, Longo D, Di Ciommo V. Neurofibromatosis type 1 and cerebellar T2-hyperintensities: the relationship to cognitive functioning. Dev Med Child Neurol 2012;54(1):49–51.

18. Leiter-3 Leiter International Scale- Thirth Edition (di Roid, Miller, Pomplun, Koch). Adattamento italiano. Manuale [Internet]. [cited 2022 Oct 18];Available from: https://ora.uniurb.it/handle/11576/2632882

19. Grizzle R. Wechsler intelligence scale for children, fourth edition. In: Goldstein S, Naglieri JA, editors. Encyclopedia of child behavior and development. Boston, MA: Springer US; 2011. p. 1553–5.

20. Taylor T, Jaspan T, Milano G, et al. Radiological classification of optic pathway gliomas: experience of a modified functional classification system. Br J Radiol 2008;81(970):761–6.

21. Griffith JL, Morris SM, Mahdi J, Goyal MS, Hershey T, Gutmann DH. Increased prevalence of brain tumors classified as T2 hyperintensities in neurofibromatosis 1. Neurol Clin Pract 2018;8(4):283–91.

22. Lopes Ferraz Filho JR, Munis MP, Soares Souza A, Sanches RA, Goloni-Bertollo EM, Pavarino-Bertelli EC. Unidentified bright objects on brain MRI in children as a diagnostic criterion for neurofibromatosis type 1. Pediatr Radiol 2008;38(3):305–10.

23. Kraut MA, Gerring JP, Cooper KL, Thompson RE, Denckla MB, Kaufmann WE. Longitudinal evolution of unidentified bright objects in children with neurofibromatosis-1. Am J Med Genet A 2004;129A(2):113–9.

24. DiPaolo DP, Zimmerman RA, Rorke LB, Zackai EH, Bilaniuk LT, Yachnis AT. Neurofibromatosis type 1: pathologic substrate of high-signal-intensity foci in the brain. Radiology 1995;195(3):721–4.

25. Steen RG, Taylor JS, Langston JW, et al. Prospective evaluation of the brain in asymptomatic children with neurofibromatosis type 1: relationship of macrocephaly to T1 relaxation changes and structural brain abnormalities. AJNR Am J Neuroradiol 2001;22(5):810–7.

26. Moore BD, Slopis JM, Jackson EF, De Winter AE, Leeds NE. Brain volume in children with neurofibromatosis type 1: relation to neuropsychological status. Neurology 2000;54(4):914–20.

27. Huijbregts SC, Loitfelder M, Rombouts SA, et al. Cerebral volumetric abnormalities in Neurofibromatosis type 1: associations with parent ratings of social and attention problems, executive dysfunction, and autistic mannerisms. J Neurodev Disord 2015;7:32.

28. Karlsgodt KH, Rosser T, Lutkenhoff ES, Cannon TD, Silva A, Bearden CE. Alterations in white matter microstructure in neurofibromatosis-1. PLoS ONE 2012;7(10):e47854.

29. Billiet T, Mädler B, D'Arco F, et al. Characterizing the microstructural basis of "unidentified bright objects" in neurofibromatosis type 1: A combined in vivo multicomponent T2 relaxation and multi-shell diffusion MRI analysis. Neuroimage Clin 2014;4:649–58.

30. Moore BD. NF1, cognition, and MRI. Neurology 1995;45(5):1029–30.

31. Moore BD, Slopis JM, Schomer D, Jackson EF, Levy BM. Neuropsychological significance of areas of high signal intensity on brain MRIs of children with neurofibromatosis. Neurology 1996;46(6):1660–8.

32. Legius E, Descheemaeker MJ, Steyaert J, et al. Neurofibromatosis type 1 in childhood: correlation of MRI findings with intelligence. J Neurol Neurosurg Psychiatr 1995;59(6):638–40.

33. Denckla MB, Hofman K, Mazzocco MM, et al. Relationship between T2-weighted hyperintensities (unidentified bright objects) and lower IQs in children with neurofibromatosis-1. Am J Med Genet 1996;67(1):98–102.

34. Mott SH, Skryja PA, Baumgardner TL, Reiss AL, Hofman K, Denckla MB. Neurofibromatosis type I: Association between volumes of T2-weighted high-intensity signals on MRI and impaired judgment of line orientation. Pediatr Neurol 1994;11(2):88.

35. Haacke EM, Bernitsas E, Subramanian K, et al. A comparison of magnetic resonance imaging methods to assess multiple sclerosis lesions: implications for patient characterization and clinical trial design. Diagnostics (Basel) 2021;12(1).

36. Simão RS, Alves RF de A, Boschi SRM da S, et al. Multiple Sclerosis: Comparison of the Conventional Spin-Echo T2-Weighted and Flair Techniques Through Image Processing. J Med Imaging Hlth Inform 2018;8(3):425–30.

37. Peters JM, Sahin M, Vogel-Farley VK, et al. Loss of white matter microstructural integrity is associated with adverse neurological outcome in tuberous sclerosis complex. Acad Radiol 2012;19(1):17–25.

38. Zhang H, Schneider T, Wheeler-Kingshott CA, Alexander DC. NODDI: practical in vivo neurite orientation dispersion and density imaging of the human brain. Neuroimage 2012;61(4):1000–16.

39. Taoka T, Aida N, Fujii Y, et al. White matter microstructural changes in tuberous sclerosis: Evaluation by neurite orientation dispersion and density imaging (NODDI) and diffusion tensor images. Sci Rep 2020;10(1):436.

40. Perri K, De Mori L, Tortora D, et al. Cognitive and white matter microstructure development in congenital hypothyroidism and familial thyroid disorders. J Clin Endocrinol Metab 2021;106(10):e3990–4006.