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STUDIO DELLE AREE VISIVE IN PAZIENTI CON NEUROFIBROMATOSI DI TIPO 1 E GLIOMA DELLE VIE OTTICHE MEDIANTE RISONANZA MAGNETICA FUNZIONALE

VISUAL NETWORK ANALYSIS IN PATIENTS WITH NEUROFIBROMATOSIS TYPE 1 AND OPTIC PATHWAY GLIOMA USING FUNCTIONAL MAGNETIC RISONANCE IMAGING

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Abstract

Neurofibromatosis 1 (NF1) is an autosomal dominant condition characterized by neuro-cutaneous involvement and a predisposition to tumour development. The most common NF1-associated central nervous system tumour is optic pathway glioma (OPG), affecting about 15% of NF1 patients and characterized by an unpredictable evolution with no clear prognostic factors identified so far.

Resting-state fMRI has recently emerged as a powerful tool for functional brain analysis, allowing the examination of brain functional networks.

The aim of our study was to analyze through resting-state fMRI the possible functional modifications of the visual network in patients affected by NF1 and OPG.

We enrolled 57 patients with NF1 (31 females and 16 males; mean age at brain MRI scan 13.31 ± 6.07). Of them 35 presented OPG: in 15 (42.8%) patients it involved only the optic nerves, in 20 (57.2%) also the chiasmatic area; of the latter, 5 (25%) patients also the posterior optic pathways. Eleven (19.3%) of our patients with NF1 presented altered visual acuity. All of them underwent resting-state brain fMRI to analyze the visual network. Nineteen subjects non-affected by NF1 were used as controls

Our data revealed a reduced connectivity in patients with NF1 and OPG limited to the optic nerves in the medial visual network in the area of paramedian cuneus bilaterally in the occipital lobe. No other significant difference were found in visual network connectivity between patients with larger OPG vs control or between patients with altered visual acuity vs. control.

In our study we analyzed the impact of OPG on the visual network in patients with NF1; we expected to find more significant abnormalities in patients affected with OPG involving largely the optic pathways, yet we detected a significant reduction of the network connectivity only in patients with OPG limited in the optic nerves. These findings may be secondary to the relatively small number of patients enrolled and to the indolent evolution of the OPG in our cohort of subjects. A follow-up study with a larger number of enrolled patients may help us clarify the possible predictive role of visual network connectivity in the OPG prognosis.

Riassunto

La Neurofibromatosi di tipo 1 (NF1), è una malattia neurocutanea monogenica caratterizzata dalla predisposizione allo sviluppo di tumori del sistema nervoso, sia benigni che maligni. Il glioma delle vie ottiche (OPG) è il tumore più comune in questi pazienti, con una prevalenza del 15% e un'evoluzione spesso imprevedibile; a tutt'oggi non sono stati individuati sicuri fattori prognostici.

L'obbiettivo del nostro studio è stato l'indagine tramite Risonanza Magnetica funzionale (fMRI) dell'impatto di OPG sulle reti neurali visive dei pazienti con NF1.

Sono stati selezionati 46 pazienti affetti da NF1 seguiti presso il nostro Dipartimento e 11 pazienti affetti da NF1 seguiti presso l'Ospedale Pediatrico di Genova (31 femmine e 16 maschi; età media alla fMRI 13.31 ± 6.07); 19 soggetti sani sono stati arruolati come controlli. I soggetti stati tutti sottoposti a Risonanza Magnetica con acquisizione di sequenze per lo studio funzionale e a valutazione oculistica con particolare attenzione all'acuità visiva.

Dei pazienti con NF1 35 presentavano OPG: in 15 (42.8%) coinvolgeva solo i nervi ottici, in 20 (57.2%) anche il chiasma e le vie retro-chiasmatiche; tra questi, in 5 (25%) casi erano coinvolti anche i tratti posteriori. Undici (19.3%) dei pazienti con NF1 presentavano acuità visiva alterata.

E' stata confrontata con fMRI la connettività della rete neurale visiva in pazienti con NF1 e OPG con diversa estensione e nei controlli.

Si è rilevata una riduzione della connettività della rete neurale visiva statisticamente significativa tra i pazienti con NF1 e glioma delle vie ottiche limitato ai nervi ottici e controlli, nell'area corrispondente al cuneo paramediano bilaterale. Non sono emerse differenze significative tra gli altri gruppi.

La mancanza di chiari fattori prognostici noti per quanto riguarda l'OPG ci ha spinto a valutare le differenze funzionali delle reti neurali visive in pazienti affetti. I risultati ottenuti dimostrano inaspettatamente una differente connettività solo in coloro affetti da OPG limitato ai nervi ottici; uno studio di follow-up, effettuato su una popolazione di numerosità maggiore ci potrà aiutare a chiarire questi dati.

1. BACKGROUND

1.1 NEUROFIBROMATOSIS TYPE 1

Neurofibromatosis type 1 (NF1) is a monogenic hereditary disease caused by mutations in the onco-suppressor gene *NF1*. It has an incidence of 1: 2500-3500 and a prevalence of 1:4000-5000 (Ferner, 2010); it is transmitted in an autosomal dominant manner, yet half of the patients present a *de novo* mutation.

NF1 is a neurocutaneous condition whose hallmark is the predisposition to the development of both benign and malignant tumours involving peripheral and central nervous system.

Penetrance is age-dependent and usually is completed by the 8 years of age; diagnosis may be achieved in the 95% of the patients at the age of 6 accordingly to the international diagnostic criteria established at NIH consensus conference 1987 (NIH Consensus Dev Conference, 1988).

Patients affected by NF1 show a wide range of clinical phenotypes with high intra- and inter-familiar variability.

Main clinical features have been classified as major and minor clinical signs and other clinical features (Huson, 2008):

major signs
- café-au-lait spot (CAL)
- atypical freckling
- Lisch nodules
- neurofibromas

minor signs
- macrocephaly
- short stature
- pectus excavtus

other frequent clinical features
- learning disabilities
- optic pathway glioma
- malignant tumours

- bones abnormalities

- cardiovascular manifestations

1.1.1 *Diagnostic Criteria*

The international diagnostic criteria for NF1were defined in 1987 by the *National Institute of Health*, showing high sensitivity and specificity in adult subjects (Table I). On the other hand, due to the age-dependent onset of NF1 signs, they result less sensitive in children with a clinical suspicion of NF1. A modification to these criteria were suggested in 2008 by Huson with the insertion of the identification of a pathogenic mutation to improve the criteria sensitivity when clinical signs are not yet recognizable (Table 1).

More recently also other modifications to established criteria have been suggested, including molecular analysis of *NF1*gene and less frequent cutaneous and extra-cutaneous signs, in the attempt to hasten NF1 diagnosis (Tadini *et al.*, 2014).

Table I *National Institute of Health 1987 NF1 criteria**

2 or more of the following:

6 or more CALs or hyperpigmented maculae ≥5 mm in diameter in prepubertal children and 15 mm postpubertal
axillary or inguinal freckles (>2 freckles)
2 or more typical neurofibromas or one plexiform neurofibroma
optic pathway glioma
2 or more iris hamartomas (Lisch nodules)
sphenoid dysplasia or typical long-bone abnormalities such as pseudarthrosis
first-degree relative (eg, mother, father) with NF1 **

* criteria must be satisfy with widespread body involvement to exclude a segmental form of NF1 (Huson, 2008)

** two affected siblings with clinically unaffected parents might be affected by mismatch repair deficiency syndrome (Huson, 2008)

1.1.2 Neurofibromatosis type 1: segmental form

Segmental form of NF1 is characterized by the presence of typical cutaneous signs, such as CALs, atypical freckling and neurofibromas, confined to one or more defined body areas (Huson, 2008)

This condition is secondary to somatic mosaicism due to a post-zigotic mutation of the *NF1gene*. In these patients molecular analyses performed on blood samples frequently result negative for NF1 mutations while tests on DNA extracted from samples of skin cells taken from CALs or Schwann cells taken from neurofibromas show biallelic inactivation of the gene (Maertens *et al.*, 2007).

Patients affected with segmental NF1 usually present with a smaller number of clinical manifestations and a lower risk of transmitting the mutation to the offspring, although this may be possible in case of gonadic mosaicism.

Rare cases of pure gonadal mosaicism have also been reported, in which patients without any clinical signs and NF1 mutation limited to gonadic cells have transmitted the condition to the offspring (Trevisson *et al.*, 2014).

1.1.3 Differential diagnosis

Diseases presenting with skins alterations or tumours that might be misidentified as CALs or neurofibromas must be clinically excluded in diagnosing NF1 (Table II, Ferner *et al.*, 2007).

Table II *NF1 Differential diagnosis*

Condition	Clinical features
Other neurofibromatosis	
Neurofibromatosis type II	bilateral vestibular Schwannoma, other cranial and peripheral nerves Schwannomas; cutaneous Schwannomas; Meningiomas; juvenile posterior subcapsular cataract

Schwannomatosis	multiple cranial, spinal and peripheral nerves Schwannomas without vestibular, cutaneous or ocular manifestations
Conditions with CALs or similar cutaneous manifestations	
McCune-Albright syndrome	large and irregular CALs, bone fibrous dysplasia, precocious puberty and other endocrinopathies
phenotype with multiple CALs	condition inherited with autosomal dominant transmission without other NF1 manifestations
mismatch repair deficiency syndrome	multiple CALs, axillary freckling, cutaneous neurofibromas, paediatric tumours, brain tumours, malignant haematological disorders, early-onset colorectal cancer
LEOPARD syndrome	freckling, hypertelorism, sensorineural hearing loss, pulmonary stenosis, cardiac arrhythmias, growth delay
Peutz Jegers syndrome	perioral, conjunctival and genital mucosae freckling, intestinal hamartomatous polyps, cancer involving gastro-intestinal tract, pancreas, breast, ovaries and uterus
Legius syndrome	condition inherited with autosomal dominant transmission, multiple CALs, atypical freckling, macrocephalia without Lisch nodules, neurofibromas and brain tumours
Piebald trait	altered pigmentation areas, white forelock
Conditions presenting with neurofibromas-like tumours	
Bannayan- Riley-Ruvalcaba syndrome	multiple lipomas and haemangiomas, macrocephalia, glandis pigmented spot, growth delay
Familial multiple lipomatosis	multiple cutaneous lipomas involving trunk and limbs
Juvenile hyaline fibromatosis	multiple cutaneous tumours, gingival hyperplasia
Multiple endocrine neoplasia type 2B	mucosae and conjunctival neuromas, pheochromocytoma, thyroid medullary carcinoma, ganglioneuromatosis of the gastrointestinal tract,

	marfanoid habitus
Conditions with localized overgrowth	
Klippel-Trenaunay-Weber syndrome	cutaneous haemangiomas, arteriovenous fistulas and emihypertrophy
Proteous syndrome	hamartomatous overgrowth of multiple tissues, epidermal and connectival nevi, hyperostosis
Congenital generalized fibromatosis	multiple tumours of cutaneous and subcutaneous layers, of skeletal muscles, bones and viscera

The main disorders that must be considered as differential diagnoses in evaluating a patient with possible NF1 are Legius syndrome, McCune-Albright syndrome and LEOPARD syndrome.

Legius syndrome is caused by mutations of the *SPRED1* gene (15q13.2) and is clinically characterized by multiple CALs, atypical freckling and facial dysmorphic features such as macrocephalia and hypertelorism. Although sharing these clinical features with NF1, Legius syndrome phenotype does not include Lisch nodule, optic pathway gliomas, neurofibromas or higher risk of tumour development.

Also patients with **McCune-Albright syndrome** present CALs but these are generally larger and irregular than those in NF1, and they are associated with fibrous dysplasia and endocrinopathies.

Finally, **LEOPARD syndrome** must be considered as a possible differential diagnosis since it is characterized by widespread freckling, hypertelorism, facial dysmorphism, genital abnormalities, pulmonary stenosis, cardiac arrhythmias and growth delay.

1.1.4 Clinical severity

Clinical severity in NF1 may be classified according to Riccardi scale (Riccardi, 1992) in minimal, mild, moderate and severe (Table III). NF1 severity is defined considering the presence of typical clinical signs, complications, life quality and expectancy reduction.

Table III *NF1 severity classification according to Riccardi*

I grade (minimal)	presence of signs without symptoms or clinical relevance such as CALs, freckling and Lisch nodules
II grade (mild)	asymptomatic lesions associated with lesions with aesthetic impairment such as facial neurofibromas
III grade (moderate)	symptomatic but treatable lesions without reduction of life expectancy
IV grade (severe)	presence of life-threatening lesion with reduction of life expectancy

Around 75% of patients present a phenotype with mostly cutaneous involvement while the remaining 25% of subjects develop at least one complications.

NF1 phenotype is characterized by a marked inter- and intra-familiar variability. This variability might be secondary to the involvement of contiguous genes, the role of modifying genes, somatic mutations, epigenetic factors such as methylation and ambient factors. Allelic heterogeneity seems on the other hand not to contribute since there is little correlation between genotype and phenotype in NF1 and NF1 mutations are usually inactivating.

Stochastic factors, particularly the somatic mutations of the second *NF1*allele, known also as "second hit", may explain the unpredictable natural history of the disease.

Life expectancy of patients with NF1 results shorter than in non-affected population with around 20 years of difference if patients of all age are considered; the difference results to be of only 10 years if only patients over 40 years of age are included, showing how NF1 reduces life expectancy especially in younger subjects. First causes of death seem to be the development of both peripheral and central nervous system malignant tumours (Masocco *et al*., 2011; Rasmussen *et al*., 2001).

1.2 The *NF1* gene

The *NF1* gene maps on 17q11.2 (Viskochil *et al*., 1990; Wallace *et al*., 1990); it is composed of 350 kb of basis and 61 exons that encode a transcript of 11-13 kb. In this a Open Reading Frame (ORF) of 8457 bp is included, which is entirely translate in neurofibromin, a protein composed of 2818 amino acids, with a molecular weight of 327 kDa (Viskochil *et al*., 1993).

Three small codifying genes EVI2A, EVI2B e OMGP (Upadhyaya *et al*., 1994). are located in intron 27b. EVI2A e EVI2B (ecotropic viral integration site) are expressed, respectively, in brain, bone marrow and peripheral blood and in bone marrow and peripheral blood; OMGP (oligodendrocyte myelin glycoprotein) is expressed in Schwann cells and oligodendrocytes during myelination.

EVI2B and OMGP seem to have both oncosoppressor roles (Pasmant *et al*., 2011).

1.2.2 Neurofibromin functions

Neurofibromin is a protein ubiquitously expressed in neurons, Schwann cells, oligodendrocytes, astrocytes, medullar layer of suprarenal gland and leukocytes. (Shen *et al*., 1996).

It is a negative regulator of the *ras* signal transduction pathway.

A central dominion, analogous to catalytic domain of GTPase activating protein, presents a *ras-GAP* activity *in vitro* and *in vivo* and it is called GAP-related domain (GRD) (Hattori *et al*., 1992).

Different isoforms of neurofibromin develop from alternative splicing and they are differently expressed according to tissues and age of development.

The neurofibromin GRD domain reduces the activity of protein p21ras by inhibiting cellular proliferation by enzymatic conversion to inactive form and by binding membrane protein Cav-1 that is involved in p21ras and growth factors receptors regulation (Boyanapalli *et al*., 2006).

Another distinct domain, called cysteine-serine rich domain (CSRD) (Izawa *et al*., 1996) seems to be involved in the interaction between neurofibromin and microtubules (Fahsold *et al*., 2000) that contributes to the regulation of cellular

proliferation after stimulation by growth factors (Mangoura *et al*., 2005).

Therefore the *NF1* gene is considered an onco-suppressor gene; the loss of neurofibromin function is associated with higher levels of the activated form of p21ras and mutations that activate *ras* are found in more than 30 % of human tumours (Viskochil *et al*., 1993).

Many mutations in the GRD region of *NF1* are present in different malignant tumours associated to NF1 (Garicochea *et al.*, 1998)

According to the "*two hits*" theory (Knudson, 1971), the inactivation of both alleles of an onco-suppressor gene, as two distinct events, is necessary for the development of a malignancy.

In patients with NF1, without mosaicism, all cells already present a mutated allele of *NF1*, hence only another one somatic mutation is necessary to develop a tumour phenotype.

The loss of heterozygosity, frequently secondary to somatic rearrangements, genetic deletions and recombination, is associated with the allele that does not segregate with the disease (Upadhyaya *et al*., 2004).

1.2.3 Gene mutations

The *NF1* gene has a very elevate mutational rate and *de novo* mutations cause around the half of NF1 cases. So far over 1300 gene mutations have been identified: they are heterogeneous in dimension and type, ranging from massive deletion to single-base substitution.

Around 5-10% of NF1 patients present a pathogenic mutation constituted by a heterozygous deletion involving the *NF1* gene and a variable number of contiguous genes, called *microdeletion* (Kluwe *et al*., 2004).

1.2.4 Genotype and phenotype correlations

Many studies have investigated possible correlations between a single *NF1* gene mutation and its associated clinical picture, but so far only three genotype-phenotype correlations have been identified.

In subjects presenting microdeletions, NF1 clinical picture seems to be more severe.

The *microdeletion syndrome* is characterized by:

- craniofacial dismorphisms: in these patients they are significantly more frequent than in those without microdeletion (75% vs.15%) (Mautner *et al*., 2010);

- intellective deficit: IQ is lower than in other NF1 patients, with an higher incidence or intellective retardation (38% vs. 6-8%) (Mautner *et al*., 2010), probably secondarily to an alteration of brain development (Venturin *et al*., 2004);

- bones abnormalities: scoliosis, pectus escavatum and brachydactyly are more recurrent (Spiegel *et al*., 2005);

- congenital heart defects: pulmunary artery stenosis, interatrial and interventricular septal defects, valvular abnormalities, hypetrophic cardiomyopathy and patent ductus arteriosus have higher prevalence in these patients (Tinschert, 2008);

- cutaneous and plexiform neurofibromas: they develop precociously and in elevated number in these patients (Riva *et al*., 1996; Tonsgard *et al*., 1997); they also present an higher incidence of malignant peripheral nerve sheet tumours with a lifetime risk of 16-26% (vs. 8-13%)(De Raedt *et al*., 2003);

- acceleration in bone maturation: they usually present a 1-2 year older bone age (Tinschert, 2008).

Also in patients with microdeletion syndrome there is yet a phenotypic variability, which may be related to the different length of gene portion deleted and to the different roles played by contiguous genes involved.

The second identified genotype-phenotype correlation is between an *inframe deletion* of 3 bp in exon 17 of the *NF1* gene, and a milder clinical picture, presenting only with CALS and without neurofibromas or other signs development (Upadhyaya *et al*., 2007).

Recently p.Arg1809Cys substitution has been associated with a mild phenotype characterized by CALs and freckling, without neurofibromas, Lisch nodules and NF1-associated malignancies.

1.3 NF1 CLINICAL MANIFESTATIONS

1.3.1 Skin changes

Cafè au lait spot, CALs

CALs are round or ovoidal skin spot with an homogenous pigmentation and net borders. They are asymptomatic and their areas may vary from a few millimeter square to tens of centimeters square and their colour changes with the patient skin colour.

They are the most common clinical signs of NF1, affecting more than 95% of patients and their pathogenesis seem to be related to mutations in the second allele of the *NF1* gene in melanocytes (De Schepper *et al*., 2007).

There is not recognized correlation between the number of CALs and the disease severity (Emery & Rimoin, 2002), nor between CALs site and site where other manifestations are more likely to appear.

CALs may be present at birth or appear in the first months of life and then they grow in number and dimension until puberty. During adulthood they usually decolorate and become less recognizable.

Although they constitute a diagnostic criterium when they are present in a number larger than 5 and with a diameter superior to 0.5 cm before puberty and 1.5 cm after puberty, they are not a pathognomonic sign of NF1. One CAL may be present in around 2.5% of newborns and in 25% of young children, yet the development of CALs after the age of 6 years is less frequent.

On the other hand the presence of 6 or more CALs, even without the association of other signs, is highly suggestive of NF1 and an high percentage of children presenting 6 or more CALs will develop other NF1 signs in the following years, justifying the planning of an annual follow-up (Nunley *et al*., 2009).

CALs may also be present in subjects affected with McCune-Albright syndrome and LEOPARD syndrome, hence other signs have to be investigated carefully.

Freckling

Freckling are multiple small pigmented spots, with a 1 to 3 mm diameter, that look like smaller CALs. They appear at around 4 years of age (Friedman, 2002) in around 90% of NF1 patients, in the axillary or groin region. They may also

involve the neck, intermammillary and perioral areas and the trunk. They are considered "atypical" since they involve sun-unexposed skin and they appear darker and larger than in normal subjects; they are pathognomonic of NF1.

Juvenile xanthogranuloma
Juvenile xanthogranulomas are more frequently observed in children affected with NF1 than in normal subjects with a prevalence of 1:5 or 1:6. They are clinically different from those in subjects not affected with NF1; they appear in early childhood (within the age of 2), they are small yellow papulae, with a diameter of few millimetres, varying in number and involving primarily the head skin. They usually present a spontaneous regression.

A correlation between juvenile xanthogranulomas and juvenile myelomonocytic leukemia has been suggested (Zvulunuv *et al*., 1995), but these findings seem to be inconsistent (Burgdorf *et al.*, 2004).

1.3.2 Ocular manifestations

Lisch nodules
Lisch nodules are hyperpigmented maculas of iris surface, also known as hamartomas. They are asymptomatic, with no impact on visual function and no need for treatment. They have been first described at the beginning of XX century (Snell & Treacher Collins, 1903) and later associated to NF1 (Goldstein & Wexler, 1930; Lisch, 1937; Sakurai, 1935). They are easily detected with slit lamp examination as small, gelatinous, defined elements on iris surface. They are frequently pigmented and usually placed bilaterally in the lower hemifield due to the sun-protective effect of the upper eyelid (Ragge *et al*., 1993).

Lisch nodule prevalence increases gradually with age: they appear at around the age of 2 and they are recognizable in the 50% of paediatric subjects with NF1 and 90% in adults (Cassiman *et al*., 2013). They usually appear later than CALs but before than neurofibromas and they are a useful diagnostic criteria in paediatric patients with negative NF1 familiar history.

They are constituted, as neurofibromas, by pigmented cells, fibroblastic-like cells and mast cells (Richetta *et al*., 2004).

Optic pathway glioma

Optic pathway gliomas (OPGs) will be treated in section 1.4.

1.3.3 Tumours

Patients affected with NF1present a predisposition to tumours development, particularly concerning peripheral and central nervous system.

Neurofibromas

Neurofibromas are non-malignant tumours originating from peripheral nerves sheet; they are composed by Schwann cells, which are considered the main neoplastic cell in neurofibromas, but also fibroblasts, perineural cells and mast cells.

They are divided in cutaneous, sub-cutaneous, plexiform and spinal. Their number and localization is unpredictable.

Cutaneous neurofibromas are pigmented, soft skin growths that may be sessile or pedunculated. They appear during puberty and reach a prevalence of 99% in adulthood (Ferner *et al*., 2007); they grow in number and size during pregnancy.

They are usually more frequently localized in the trunk but they may appear in every site. They do not grow significantly in size (diameter varying usually from 2 mm to 3 cm) and do not undergo into malignant transformation, but they may represent a severe aesthaetical issue for patients.

Sub-cutaneous or nodular neurofibromas are less common. They are harder and though they are usually asymptomatic, in case of compression they may also manifest with paraesthesias and pain irradiating in nerve territory.

They may present malignant transformation.

Neurofibromas causing discomfort or aesthaetical problems may be surgically removed; they may nevertheless reform or cause hypertrophic scars.

Plexiform neurofibromas grow along nerves courses and they are usually congenital. They are present in around 30% of patients and may clinically manifest at birth, during childhood and adulthood although some of them, deeply localized, are asymptomatic.

Their development in unpredictable; they may undergo very fast growth, especially during puberty and stop growing also for a long time (Ferner *et al*., 2007).

Due to their development along nerve trunks or plexuses, they may cause severe neurological deficits and physical deformity.

Superficial plexiform neurofibromas present as sub-cutaneous, usually multi-nodular, poorly defined swellings, whose size may vary from a few centimeters of extension to the involvement of whole body districts. They are frequently associated with skin hyperpigmentation of the area and/or hypertrichosis and they may be misdiagnosed as congenital melanocytic nevus.

Deep, large plexiform neurofibromas may remain asymptomatic for a long time before presenting with neurological deficits or as space-occupying lesions.

Diagnosis is clinical, yet MRI is a necessary diagnostic investigation.

Disfiguring facial plexiform neurofibromas appear before the age of 3 years; surgery removal of the lesion is technically difficult due to the infiltration of nerve trunks and of near-by structures and to their high vascularization. These issues bring along a high risk of massive bleeding during surgery and of neurological deficit after surgery (Ferner *et al.*, 2007).

Schwann cells in plexiform neurofibromas present a biallelic loss of the *NF1* gene, while the other tumours cells as fibroblasts, mast cells and endothelial cells are heterozygous for *NF1* mutations.

Since mast cells may produce cytokines and growth factors, they might contribute to the creation of tumour microenvironment (Yang *et al.*, 2012), yet Schwann cells play a crucial role in the onset and the development of neurofibromas.

Around 10% of plexiform neurofibromas undergo malignant transformation in malignant peripheral nerve sheath tumour (MPNST) yet the biallelic mutation of the *NF1* gene is necessary for the transformation (Jouhilahti *et al.*, 2011) and the mutation timing is crucial for tumour development (Larizza *et al.*, 2009).

Malignant peripheral nerve sheath tumours: MPNST

Malignant peripheral nerve sheath tumours (MPNST) are the most frequently NF1-associated malignant tumours. NF1 patients present a risk of developing MPNS 100 times higher than general population (Walker *et al.*, 2006).

MPNST incidence *per* year is 0.16% in NF1 patients and around 1-2% of them develops MPSNT during lifetime (0.001% in general population) with a cumulative risk of 8-13% (Evans *et al.*, 2002).

Mean onset age, 20-35 years, is significantly lower than in subject non-affected by NF1 (62 years) and so is survival rate (21% vs 42% respectively).

Lower survival rate is partially due to diagnostic delay secondary to the earlier discovery of a rapidly growing swelling in non-NF1 subjects (Evans *et al*., 2002) and to the higher aggressive behavior with metastatic spreading of NF1-realted MPNST (39% vs. 16%) (Ducatman *et al.*, 1986).

In NF1 patients, MPNSTs usually, but not exclusively, develop from a plexiform neurofibroma (Evans *et al.*, 2002).

MPNST development should be suspected when a plexiform or sub-cutaneous neurofibroma becomes persistently and intensely painful, when it grows or changes texture rapidly and when neurological deficits appear (Ferner *et al.*, 2007).

Patients with familiar or personal history of cancer, *NF1* gene deletion, OPG, an high number of deep or sub-cutaneous neurofibroma or who previously underwent radiotherapy should be strictly monitored for MPNST.

PET scan with 18FDG is an highly specific and sensitive test that may help in differentiating MPNST from plexiform neurofibroma.

MPNST treatment is surgical, adjuvant radiotherapy is used when the tumour present a diameter larger than 5 cm, it is an high grade lesion or when surgical removal is incomplete. Chemotherapy is applied as neo-adjuvant treatment or when metastases are present.

In MPSNT, the cellular cycle is altered with *P53* mutations that are responsible of malignant progression of this tumour (Larizza *et al.*, 2009).

Gastrointestinal stromal tumours (GIST)

Gastrointestinal stromal tumors (GIST) affect 3.9 to 25% of NF1 patients, being the most common gastrointestinal tumours in NF1. Compared to sporadic form, GIST appear at younger age, they usually develop in duodenum and small intestine and they are multiple. They are frequently diagnosed incidentally, they usually show a non-aggressive behaviour. yet in some cases they may give metastasis (Miettinen and Lasota, 2011).

Pheochromocytoma

Pheochromocytoma affects up to 14.6% of NF1 patients (Zinnamosca *et al.*, 2011) and 20-30% of NF1 patients with high blood pressure. Mean onset age is around 40 years of age; they have a benign progression, they are usually localized in suprarenal glands and their treatment is surgical.

Brest cancer

Women affected with NF1 present a 5 times higher risk of breast cancer than non-NF1 women, in particular at age younger than 50 years (Sharif *et al.*, 2007). They also present an higher mortality rate (Evans *et al.*, 2011). An imaging screening has been hence suggested in high risk population (Madanikia *et al.*,2012).

Juvenile myelomonocytic leukaemia (JMML)

Juvenile myelomonocytic leukaemia (JMML) is a chronic myeloproliferative disorder affecting typically young children: more than 95% of cases are diagnosed before age 4. Patients affected with NF1 present a 200-300 times higher risk of developing JMML (Side *et al.*, 1998).

1.3.4 Neuroradiological signs

Brain areas with altered-signal called unidentified bright objects (UBOs) are revealed by MRI scan in at least 60% of NF1 patients. They are hyperintense in T2-weighted images, they do not present a compressive effect on surrounding structures and they do not show a post Gadolinium enhancement.
Frequently, they are localized in the globus pallidus, thalamus and cerebellum; more rarely, in subcortical white matter, cortex, hippocampus and amigdala (Hsieh,*et al.*, 2011).
UBOs are patognomonic signs in NF1and are asymptomatic. Histologically they seem to be caused by vacuolar change of myelin and intramyelinic edema associated to glial proliferation with hyperplastic or dysplastic features (Dipaolo *et al.*, 1995).
They appear during childhood, they do not present consistent changes in number,

sites and size during puberty, and then they disappear around 20 years of age (Kraut *et al.*, 2004). Their correlation with learning disabilities has been investigated but without consistent results (Ozonoff, 1999).

1.3.5 Bones alteration

Short stature and macrocephalia are common signs in children with NF1, affecting respectively 13% and 24% of subjects (considering abnormal features those beyond 2 standard deviation from the mean value adjusted for age) (Szudek *et al.*, 2000).

Scoliosis affects 10-26% of patients, involving more frequently cervical thoracic spine. It is divided in dystrophic and idiopathic.

Dystrophic scoliosis is usually associated to kyphosis, it has a precocious onset (before the age of 10) and it involves 4 to 6 segments and causes distortion of vertebrae and ribs. It is rapidly progressive and may request early surgical correction (Alwan *et al.*, 2005); it may be associated to an underlying plexiform neurofibroma and in most severe cases it brings along respiratory difficulties (Ferner *et al.*, 2007).

Neurological complication may occur, secondary to spinal cord compression (Williams *et al.,* 2009).

Idiopathic scoliosis onsets during puberty, it is not progressive, it involves usually 8 to 10 segments and it clinically similar to non NF1-assocaited scoliosis. It may progress to a dystrophic form; clinical and imaging follow-up is hence mandatory.

Sphenoid dysplasia involves more commonly the greater wing that results partially or totally absent; it is usually asymptomatic and it is diagnosed by clinical and radiological examination. It is frequently associated to a periorbiral plexiform neurofibroma and some patients may present a pulsating exophthalmos.

Long bones dysplasia is present in around 2% of NF1 patients and it involves mainly the tibial bone with deformity and thinning of cortical layer; omolateral fibulae may also be involved. Also femoral, radial, ulnar, homeral and clavicular bones may be affected.

It usually onsets during the first months of life and it is associated with pathological fractures with complicated and delayed healings that may need

surgical treatment and in most severe cases also amputation.

Patient with dysplasia without fractures may uses orthesis as a prophylactic measure until complete skeletal growth, when fractures are less probable (Alwan *et al.*, 2005).

Early tibial dysplasia may allow a precocious diagnosis of NF1 (Morcaldi *et al.*, 2013).

1.3.6 Epilepsy and other neurological manifestations

Epilepsy prevalence in NF1 subjects is 4.2 to 6%, two times higher than in the general population, with a 9.5% of prevalence of non-provoked seizures (Ostendorf *et al.*, 2013).

Crisis are usually focal and a 75% of epileptic patients present focal EEG abnormalities (Ostendorf *et al.*, 2013).

This increased epileptic risk may be due to neurofibromin expression in cerebral cortex during embryonic development that is involved in neurotransmission and synapses formation. Hence abnormal neurofibromin expression may cause the development of an altered neural network with a lowered epileptic threshold (Hsieh *et al.*, 2011).

Epilepsy in NF1 is usually well-controlled with pharmacological therapy, unlike epilepsy associated with other neurocutaneous diseases such as Tuberous sclerosis and Sturge-Weber syndrome (Kulkantrakorn and Geller, 1998).

Children with NF1 are more frequently affected by attention deficit hyperactivity disorder (ADHD), autism, behavioural and psycho-social problems.

ADHD, diagnosed according to DSM-IV criteria, is 3 times more frequent in NF1 children than in their relatives and general population (Williams *et al.*, 2009).

Mean Intelligence Quotient (*QI*) in NF1 subjects is frequently lower than general population (Ferner *et al.*, 2007, Schrimsher *et al.*, 2003, Levine *et al.*, 2006). A severe intellective deficit (IQ <70) is present in 4-8% of NF1 subjects vs. 3% in general population (Hyman *et al.*, 2005).

Learning disabilities of different degrees are present in 30-60% of NF1 children (Cutting and Levine, 2010; Hyman *et al.*, 2005; Ozonoff, 1999). Learning disabilities are diagnosed when the child cannot develop an academic potential independently from his social-economical and cultural background and in absence of neurological or other medical issues. IQ may be normal in these patients and learning disabilities may concern writing and reading difficulties, visuospatial problems, working memory impairment and attention deficits (Ferner *et al.*, 2007).

1.3.7 Cardiovascular abnormalities

Cardiovascular abnormalities are present in 2% of NF1 patients, though the incidence could be higher if an ultrasound screening was performed in all subjects (Tedesco *et al.*, 2002).

The most common *cardiac alteration* is pulmonary artery stenosis that represents 25% of all cardiac abnormalities in NF1.

Vasculopathies in NF1 included stenosis, aneurisms, dysplasias and arteriovenous malformation and they represent the second more frequent death cause in NF1. The most frequent vasculopathy is renal artery stenosis, which affects around 1% of NF1 patients. Other vessel abnormalities may involve cerebral arteries such as internal carotid, middle or anterior cerebral artery and may cause ectasia, stenosis, aneurisms and Moyamoya phenomenon and may bring along parenchyma ischaemic alterations and clinical neurological manifestations.

Arterial hypertension is a cause of morbidity and mortality and must be closely followed-up every year. It is frequently secondary to renal artery stenosis, particularly in children, but also to pheochromocytoma and coarctation (Williams *et al.*, 2009)

1.4 OPTIC PATHWAY GLIOMAS

1.4.1 Epidemiology and clinical manifestations

OPG affect up to 20% of children affected with NF1 (DeBella K, *et al*., 2000) and usually manifests during the first decade of life, though later onset is well documented (Listernick R, *et al.*, 2004).

They are grade I pilocytic astrocytoma, not different histologically from other gliomas or gliomas in subjects not affected by NF1.

OPG in NF1 present usually a more indolent course compared to sporadic ones (Guillamo JS *et al.*, 2003) but it may present a hazardous evolution with severe impairment of visual function and potentially life-threatening behaviour (Balcer *et al.*, 2001)

Around the 50% of OPG is symptomatic; it usually presents with impairment of visual acuity, papillary abnomarmalities, visual field reductions, atrophy or aedema of optic nerve, proptosis or strabismus. In more severe cases, especially when OPG involves the chiasmatic region, neurological symptoms may surface, such as neurological deficits, hydrocephalus, development delay, precocious puberty (Cassiman *et al,.* 2013), intracranial hypertension and also death.

Since visual function impairment seems to constitute the first sign of OPG onset, patients with NF1 should undergo ophthalmologic screening examination every 6-12 months, especially since young children are not reliable in referring visual acuity impairment.

Screening planning may vary with age and clinical picture and examination includes visual acuity and visual field assessment, colour vision test, ocular motility and fundus oculi evaluation and slit lamp examination (Listernick *et al*.,1997) according to the subject compliance (around one third of the patients can not undergo a reliable visual acuity assessment due to young age or cognitive disabilities).

Since the compliance-related reliability of the ocular examination, new test as optical coherence tomography (OCT) has been recently applied as screening tool. OCT allows the measurement of the optic nerve fibers layer thickness in the retina. It has been shown that the thickness of this layer is reduced in NF1 patients with OPG and a recent work (Parrozzani *et al*., 2013) has revealed that this

thinning of the layer precede the onset of ocular clinical manifestations. NF1 patients without OPG present no OCT alteration.

1.4.2 Neuroradiological classifications

OPG are classified according to the MRI involvement of the pre-chiasmatic tracts of optic nerves, monolaterally or bilaterally, or also the chiasmatic-ipothalamic region and the posterior optic pathway (Taylor T *et al*., 2008).

No consistent data seem to indicate a correlation between site and tumour progression and no other prognostic factors of OPG behaviour have been clearly identified; tumour evolution hence remains unpredictable so far (Segal *et al*., 2010; Astrup *et al,*. 2003; King *et al.*, 2003; Lama *et al*., 2007; Thiagalingam e*t al*., 2004)

1.4.4 Current treatments

Although most OPGs present an indolent behavior, treatment is indicated in presence of neuroradiological and/or clinical progression. Therapy options include chemotherapy and surgery. Chemotherapy is usually based on vincristine and carboplatin combinations, that results well-tolerated and presents a moderately low neurotoxicity.

Surgery is applied only in cases of obstructive hydrocephalus or severe proptosis.

Radiotherapy was once used but it is not more applied due to cerebro-vascular complications, collateral effects on intellective abilities and the significantly increased risk of a second tumor development in the involved area (Listernick *et al*., 2007).

1.5 FUNCTIONAL MRI

The functional magnetic resonance imaging (fMRI) is a non-invasive imaging tool which allows the measurement of blood oxygen level dependent (BOLD) signal in different brain areas during resting state or during the performance of specific cognitive tasks.

Although task-based approaches on fMRI have been more explored in a first time, resting-state fMRI has recently emerged as a powerful tool for functional brain analysis since it allows the examination of multiple functional circuits simultaneously, without the requirement of selecting a priori hypothesis.

fMRI in resting state

Imaging the brain during resting state, characterized by the absence of tasks to perform,reveals large-amplitude spontaneous low-frequency (<0.1 Hz) fluctuations in the fMRI signal that are temporally correlated across functionally related areas (Biswal et al., 1995; Fox et al., 2007; Margulies et al., 2007; Smith et al., 2009)Different brain areas connected in functional networks are hence identified due to temporal synchrony and inherent coherence of BOLD in the activation or resting state.

These neural networks with synchronous activity result altered in cases of cortical dysfunction (Assaf et al, 2010).

The images obtained by fMRI must undergo preprocessing including motion correction and spatial filtering (Biswal et al, 2010) and then, to identify the different major functional networks, an independent component analysis (ICA) may be applied using temporal concatenation to find independent patterns in multivariate data.

Among the better defined brain functional networks is the visual network (Smith et al. 2009), that involves medial, occipital pole, and lateral visual areas.

2. AIM OF THE STUDY

The aim of our study was to analyze the possible functional modifications of the visual network in patients affected by NF1 and OPG.

For this purpose, we investigated through fMRI the visual networks in NF1 patients with OPG, in NF1 patients without OPG and in healthy controls. In addition, NF1 patients were subdivided according to OPG size and localization and according to the impairment of visual function

3. PATIENTS AND METHODS

3.1 Cohort selection

We included in our study patients affected byNF1 diagnosed accordingly to the National Institute of Health criteria National Institutes of Health Consensus Development Conference, 1988).

Ethical committee approval was obtained before the beginning of the study; all our patients, or their parents or tutors in cases of minors, gave written informed consent.

All patients were regularly attending our NF1 Clinic in the Clinical Genetics Unit of the Department of Woman and Child Health of the University of Padova or the Paediatric Neuro-Oncology Unit of the University of Genova.

We selected patients affected with NF1 who already presented an indication to perform brain MRI, who did not required pharmacological sedation during the scan and who had not undergone any therapy for OPG.

All patients with OPG were submitted to brain MRI for follow-up purposes while patients without OPG underwent brain MRI for the onset of headache or the investigation of learning disabilities, facial plexiform neurofibroma or ocular abnormalities.

Fifty-seven patients were enrolled (mean age at brain MRI scan 13.31 ± 6.07 years, range 3-34; 31 females).

Patients were further subdivided between those with and without neuroradiological evidence of OPG.

Patients with OPG were subdivided accordingly to the anatomical Dodge classification (Dodge *et al.*, 1958): one group including patients with OPG involving only the optic nerves, one group with OPG involving the optic nerves and the chiasmatic region (including also the hypothalamus) and one group with OPG involving also the posterior optic pathways (including all post-geniculate structures).

Among the 57 NF1 patients, 35 harbored an OPG.

Of the 35 patients with OPG (mean age 12.9±5.6, 19 female and 16 male), the OPG involved:

- in 15 (42.8%) only the optic nerves (mean age 13.3±7.5, 6 female and 9 male);

- in 20 (57.2%) also the chiasmatic area (mean age 12.6±3.2, 13 female and 7 male)

Of the latter, 5 NF1 patients also reached the posterior optic pathways (lateral geniculate bodies and optic radiation).

Eleven (19.3%) NF1 patients OPG (mean age 13.9±5.9, 6 female) presented altered visual acuity while 46 (80.7%) NF1 patients (mean age 10.4±4.2, 25 female) had normal visual acuity.

Table IV *Cohort of NF1 patients*

Pt.	sex	age (yrs)	OPG site	visual acuity
1	male	15	chiasmatic	normal
2	female	13,3	no glioma	normal
3	male	9	chiasmatic	altered
4	female	6,45	optic nerves	normal
5	male	10,3	optic nerves	normal
6	female	7,8	optic nerves	altered
7	male	13,1	no glioma	normal
8	male	8,4	no glioma	normal
9	male	14,3	no glioma	normal
10	female	14,3	no glioma	normal
11	female	8,9	no glioma	normal
12	female	10,4	chiasmatic	altered
13	male	7,2	no glioma	normal
14	female	8	no glioma	normal
15	male	4	chiasmatic	normal
16	female	10,6	chiasmatic	normal
17	male	34,5	optic nerves	normal
18	male	12,6	chiasmatic	normal
19	female	16,4	no glioma	normal
20	male	15,6	optic nerves	normal
21	female	16,9	chiasmatic	normal

22	male	3,3	no glioma	normal
23	female	9,5	chiasmatic	altered
24	female	5,7	optic nerves	normal
25	female	14,3	chiasmatic	altered
26	female	15,5	chiasmatic	normal
27	female	9,8	chiasmatic	normal
28	female	11,8	optic nerves	normal
29	female	14,2	chiasmatic	normal
30	female	22,6	no glioma	normal
31	male	8,3	no glioma	normal
32	male	14,5	chiasmatic	normal
33	female	14,2	chiasmatic	normal
34	female	9	no glioma	normal
35	male	13,3	optic nerves	altered
36	female	10,6	chiasmatic	altered
37	male	15,5	chiasmatic	normal
38	male	31,3	no glioma	normal
39	male	15	optic nerves	normal
40	female	13,7	optic nerves	normal
41	male	6,6	chiasmatic	altered
42	male	8,8	optic nerves	altered
43	female	17,8	chiasmatic	normal
44	female	25,8	no glioma	normal
45	female	14,9	chiasmatic	normal
46	female	16,6	chiasmatic	normal
47	female	17,2	optic nerves	altered
48	male	2,8	optic nerves	altered
49	male	9,3	no glioma	normal
50	female	11,7	no glioma	normal
51	male	15,9	no glioma	normal
52	female	25,2	no glioma	normal
53	male	11,9	no glioma	normal
54	female	12,5	no glioma	normal
55	female	10,4	no glioma	normal
56	male	17,8	optic nerves	normal
57	male	18,3	optic nerves	normal

Brain MRI scans of 19 patients non-affected by NF1 and without any evidence of brain parenchymal abnormalities (11 females and 8 males; mean age at brain MRI scan 11.23±3.92) were used as control subjects.

Clinical data were collected for all patients with particular focus on visual acuity measurement at the last ophthalmological follow-up evaluation.

3.2 Resting state functional MRI

Patients and controls underwent brain MRI scans with a 1.5T MRI (Achieva; Philips Medical Systems,Best, the Netherlands) with a standard quadrature head coil. Different imaging sequences were acquired during the scan to achieve images useful both for the clinical management of the patients and the analysis of the optic pathway.

The MR imaging study protocol included:

- 3D T1-weighted imaging (TR/TE, 20/3.8 ms; flip angle, 20°;section thickness, 1 mm; acquired voxel size, 1x1 mm; reconstructed voxel size, 0.66x0.66 mm; acquisition matrix,212x210; reconstructed matrix, 320x320; acquisition time, approximately 7 minutes);

- Fluid-attenuated inversion-recovery (TR/TE/TI, 10,000/140/2800 ms; echo-train length, 53; flip-angle, 90°; section thickness,5 mm; acquisition voxel, 0.90x1.15 mm; reconstructed voxel, 0.9x0.9 mm; acquisition time, 3 minutes 20 seconds);

- Diffusion tensor images acquired with single-shot echo-planar diffusion-weighted imaging (TR/TE, 11,114/80 ms; acquisition matrix, 112x110; echo-train length, 59; reconstructed matrix, 128x128; acquisition voxel, 2x2 mm; reconstructed voxel, 1.75x1.75x2 mm; sensitivity encoding p reduction,2; section-thickness, 2 mm without gap; NEX, 2; acquisition time, 12 minutes 24 seconds). The axial sections covered the whole brain including the cerebellum. The diffusion-sensitizing gradients were applied along 32 non collinear gradient-encoding directions with maximum b=800 s/mm2. One additional image without diffusion gradients (b=0 s/mm2) was also acquired.

- Resting-state fMRI data with 250 continuous functional volumes(TR/TE,

2216/50 ms; flip angle, 90°; 21 axial sections; acquisition matrix, 96x96; reconstructed matrix, 128x128;acquisition voxel, 2.4x2.4 mm; reconstructed voxel, 1.8x1.8 mm; section thickness, 5.5 mm; gap between sections, 0.5mm; acquisition time; 8 minutes 27 seconds).

During the scan, subjects were requested to remain still, stay awake, and keep their eyes open.

3.3 fMRI data processing

Resting-state scans were preprocessed by using both Analysis of Functional Neuro- Images (version AFNI_2010_10_19_1028; http://afni.nimh.nih.gov/afni) and fMRI of the Brain Software Library (FSL, Version 4.1.9; http:/ www. fmrib. ox.ac.uk/fsl). Preprocessing was performed as described by Biswal (Biswalet al, 2010) and in Neuroimaging Informatics Tools and Resources Clearinghouse(www.nitrc.org/projects/fcon_1000).

The first 5 volumes of every scan were discarded to remove possible stabilization effects. Preprocessing consisted of motion correction by using Fourier interpolation (volume registration by using least-squares alignment of 3 translational and 3rotational parameters); spatial smoothing by using a 6-mm full width at half maximum Gaussian kernel; mean-based intensity normalization of all volumes; linear and quadratic detrending; and spatial normalization via estimation of a linear transformation from the individual functional space to Montreal NeurologicalInstitute-152 (MNI152) standard brain space according to each individual's high-resolution anatomic image.

A high-pass filter setting of 200 seconds (<0.005 Hz) was used to reduce very low-frequency artifacts such as scanner draft.

Five patients with NF1 and 4 control subjects displayed a single brief movement of head displacement >3mm or 3° during scanning. We decided to remove the interested volumes (about20–30 volumes in each patient) before undergoing preprocessing, to prevent issues in the identification of the networks.

We then decide to exclude these 9 subjects from the ICA, but not from the dual regression analyses [http://en.pudn.com/downloads226/sourcecode/math/ detail1062122_e].

Temporal-concatenation group ICA analysis was used to generate 25 group-level components of the dataset by Multivariate Exploratory Linear Optimized Decomposition into Independent Components (MELODIC, FSL) (Zuo et al., 2010) in the group of controls. We decided to consider only the control subjects to better identify the networks, since we speculated that the presence of parenchymal alterations due to OPG might hinder the correct network recognition.

Before statistical inference, we identified correctly the medial (Figure 1) and ventral (Figure 2) visual network, corresponding to medial, occipital

pole, and lateral visual areas, by visual inspection and by comparison with available maps in the literature (Oakes *et al*., 2007).

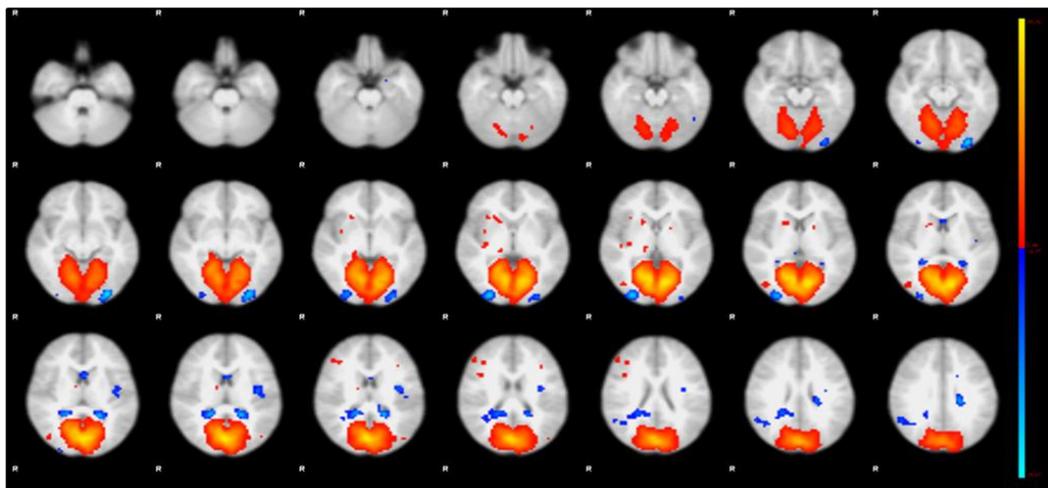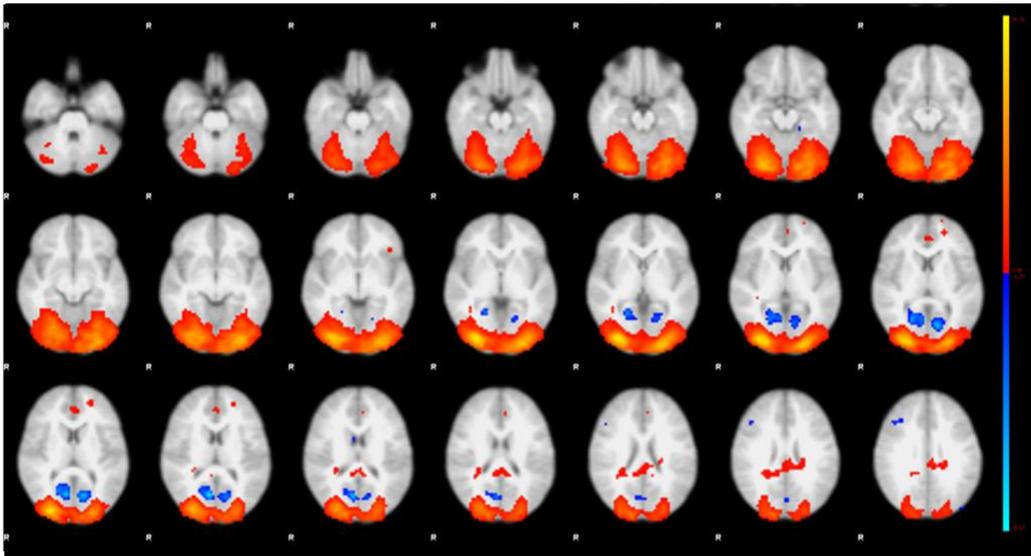**Figure 1** *fMRI image of medial visual network*

Figure 2 *fMRI image of ventral visual network*

The dual-regression approach was used to obtain a connectivity map for each of the 2 components and for each subject.

The standardized maps obtained by dual regression were used to perform group comparisons.

Nonparametric permutation testing (5000 permutations) was used for statistical analysis of spatial maps, by the TFCE method for multiple comparisons and thresholding at P<0.05.
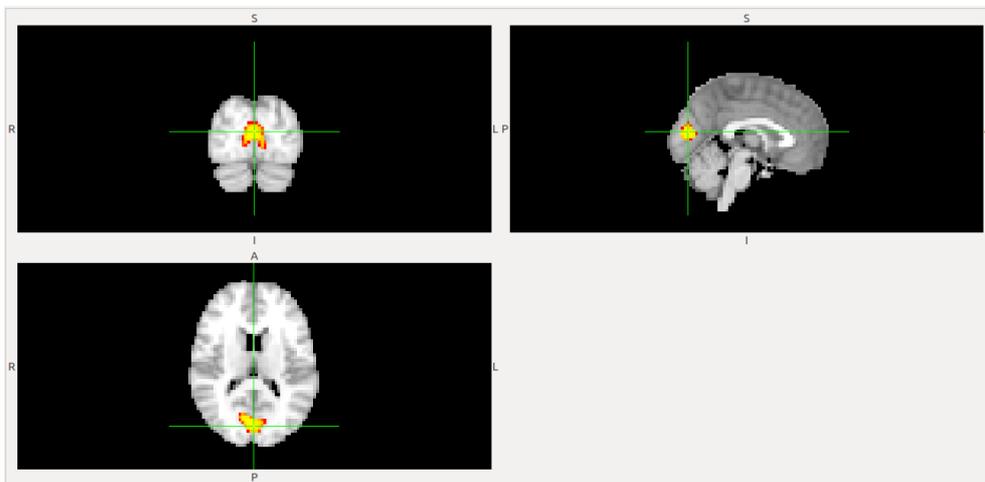
Group comparisons were performed between:

- NF1 patients vs. controls

- NF1 patients with OPG vs. controls

- NF1 patients without OPG vs. controls

- NF1 patients with OPG limited to the optic nerves vs. controls

- NF1 patients with OPG involving the optic nerves and the chiasmatic region vs. controls

- NF1 patients with OPG involving also posterior optic pathways vs. controls

- NF1 patients with normal visual acuity vs. controls

- NF1 patients with altered visual acuity vs. controls

4. RESULTS

4.1 OPG and visual network

Comparing the connectivity of medial and ventral visual networks among NF1 patient subgroups and control subjects, we found a reduced connectivity in NF1 patients with OPG limited to the optic nerves (one cluster, size 87 voxels, peak 27, 18, 26) in the medial visual network. The area of reduced connectivity involved the paramedian cuneus bilaterally in the occipital lobe (Figure 3).

Figure 3 *area of reduced connectivity in the medial visual network of NF1 patients with OPG limited to optic nerves*

No significant difference could be indentified in these patients when considering the ventral visual network.
No significant differences were found comparing NF1 patients vs. controls, NF1 patients with OPG vs. controls, NF1 patients without OPG vs. controls and NF1 patients with OPG involving the optic nerves and the chiasmatic region, including or not also the posterior optic pathways vs. controls.

4.2 Visual acuity and visual network

The connectivity of medial and ventral visual network in patients with NF1 with or without visual impairment presented no significant difference when compared with control subjects, all with no visual abnormalities.

5. DISCUSSION

OPG is the most common NF1-associated central nervous system tumour, affecting about 15% of patients. Although it usually shows a more indolent course in NF1 subjects compared to sporadic cases (Balcer *et al.*, 2001), it may present a hazardous evolution with severe impairment of visual function and potentially life-threatening behaviour.

Possible prognostic factors have been repeatedly investigated (Astrup, 2003; King *et al*., 2003; Thiagalingam *et al*., 2004; Lama *et al*., 2007; Taylor *et al.*, 2008; Segal *et al*., 2010) but none has been identify consistently so far.

In this study, we analyzed the possible impact of OPG on the visual network in patients with NF1; to our knowledge, visual network had never been investigated with fMRI in patients with NF1 before.

De Blank *et al* (2013) examined *via* diffusion tensor imaging the white matter tract integrity of the visual pathway in patients with NF1 and OPG. Their data seem to indicate a correlation between a decrease of the fractional anisotropy in optic radiations and abnormal visual acuity; this also seem to be predictive of visual acuity loss during the following year. No correlation was found between the integrity of the pre-chiasmatic optic pathways and the visual acuity.

In our cohort we detected a significant reduction of the network connectivity only in patients with OPG limited to the optic nerves. Moreover, there was no correlation between the impairment of visual acuity and connectivity of the visual networks.

Actually, more significant abnormalities were expected in patients affected with OPG involving largely the optic pathways, particularly the post-geniculate tracts.

Previous studies detected significant remodelling of the medial visual network in blind patients affected by Alstrom syndrome, suggesting that visual deafferentation can impact on the neural connectivity of the primary visual cortex (Manara *et a.l*, 2014). These findings were not replicated in NF1 patients with chiasmatic or retrochiasmatic OPG, probably due to the relatively mild visual impairment in our cohort, as the patients affected with more aggressive OPG with severe visual impairment and hence indication for treatment, were excluded due to previous chemotherapy. In contrast, NF1 patients with OPG showed visual network changes only when the optic nerves were primarily involved. Interestingly, the majority of optic nerve OPG (12/15 cases) presented monolateral involvement. Since optic nerves fibres cross over in the optic chiasma, each nerve is connected bilaterally to the primary visual cortex; hence a monolateral damage of optic nerves should imply less deafferentation of the visual cortex compared to bilateral OPG. Yet, considering the absence of connectivity alterations in patients with chiasmatic (bilateral) region involvement, the unbalance in the bilateral afference to the visual cortex may have caused more alteration then the deafferentation itself.

Similar alterations have in fact have been found in patients with asymmetric or unilateral optic neuritis (Lopes *et al.*, 2015), even if in that case an increased connectivity was found in patients visual cortex vs. control, possibly due to the different nature of nerve damage. A reduced connectivity was instead found in patients with asymmetric glaucoma (Dai *et al.* 2013) confirming that the monolateral eye involvement might impact more severely the primary visual network.

A follow-up study will help us clarify the possible predictive role of an altered visual network connectivity in the OPG prognosis.

REFERENCES

Alwan S, Tredwell SJ, Friedman JM. Is osseous dysplasia a primary feature of neurofibromatosis 1 (NF1)? *Clin Genet*. 2005;67:378-90.

Astrup J, Natural history and clinical management of optic pathway glioma. *Br J Neurosurg*. 2003;17:327-35.

Balcer LJ, Liu GT, Heller G, Bilaniuk L, Volpe NJ, Galetta SL, et al. Visual loss in children with neurofibromatosis type 1 and optic pathway gliomas: relation to tumor location by magnetic resonance imaging. *Am J Ophthalmol*. 2001;131:442-5.

Biswal B, Yetkin FZ, Haughton VM, Hyde JS. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med*. 1995; 34:537-41.

Biswal BB, Mennes M, Zuo XN, Gohel S, Kelly C, Smith SM, et al. Toward discovery science of human brain function. *Proc Natl Acad Sci U S A*. 2010;107: 4734-9.

Boyanapalli M, Lahoud OB, Messiaen L, Kim B, Anderle de Sylor MS, Duckett SJ, et al. Neurofibromin binds to caveolin-1 and regulates ras, FAK, and Akt. *Biochem Biophys Res Commun*. 2006;340:1200-8.

Burgdorf WH1, Zelger B. JXG, NF1, and JMML: alphabet soup or a clinical issue? *Pediatr Dermatol*. 2004;21:174-6.

Cassiman C, Legius E, Spileers W, Casteels I. Ophthalmological assessment of children with neurofibromatosis type 1. Eur J Pediatr. 2013;172:1327-33.

Cutting LE, Levine TM. Cognitive profile of children with neurofibromatosis and reading disabilities. *Child Neuropsychol*. 2010;16:417-32

Dai H, Morelli JN, Ai F, Yin D, Hu C, Xu D, et al. Resting-state functional MRI: functional connectivity analysis of the visual cortex in primary open-angle glaucoma patients. *Hum Brain Mapp*. 2013;34:2455-63.

De Blank PM, Berman JI, Liu GT, Roberts TP, Fisher MJ. Fractional anisotropy of the optic radiations is associated with visual acuity loss in optic pathway gliomas of neurofibromatosis type 1. *Neuro Oncol*. 2013;15:1088-95.

De Raedt T, Brems H, Wolkenstein P, Vidaud D, Pilotti S, Perrone F, et al. Elevated risk for MPNST in NF1 microdeletion patients. *Am J Hum Genet*. 2003; 72:1288-92.

De Schepper S, Maertens O, Callens T, Naeyaert JM, Lambert J, Messiaen L. Somatic mutation analysis in NF1 café au lait spots reveals two NF1 hits in the melanocytes. *J Invest Dermatol*. 2008;128:1050-3.

DeBella K, Szudek J, Friedman JM. Use of the national institutes of health criteria for diagnosis of neurofibromatosis 1 in children. *Pediatrics*. 2000;105:608-14.

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;195:721-4.

Ducatman BS, Scheithauer BW, Piepgras DG, Reiman HM, Ilstrup DM. Malignant peripheral nerve sheath tumors. A clinicopathologic study of 120 cases. *Cancer*. 1986;57:2006-21.

Evans D, Baser M, Mcgaughran J, Sharif S, Howard E, Moran A. Malignant peripheral nerve sheath tumours in neurofibromatosis 1. *Journal of medical genetics*. 2002; 39:311-314.

Evans DG, O'Hara C, Wilding A, Ingham SL, Howard E, Dawson J, et al. Mortality in neurofibromatosis 1: in North West England: an assessment of actuarial survival in a region of the UK since 1989. *Eur J Hum Genet*. 2011;

19:1187-91.

De Schepper S, Maertens O, Callens T, Naeyaert JM, Lambert J, Messiaen L. Somatic mutation analysis in NF1 café au lait spots reveals two NF1 hits in the melanocytes. *J Invest Dermatol*. 2008;128:1050-3.

Fahsold R, Hoffmeyer S, Mischung C, Gille C, Ehlers C, Kücükceylan N, et al. Minor lesion mutational spectrum of the entire NF1 gene does not explain its high mutability but points to a functional domain upstream of the GAP-related domain. *Am J Hum Genet.* 2000;66:790-818.

Ferner RE, Huson SM, Thomas N, Moss C, Willshaw H, Evans DG, et al. Guidelines for the diagnosis and management of individuals with neurofibromatosis 1. *J Med Genet*. 2007;44:81-8.

Ferner RE. The neurofibromatoses. *Pract Neurol*. 2010;10:82-93.

Fox MD, Raichle ME. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci*. 2007;8:700–711

Friedman, J. Neurofibromatosis 1: Clinical Manifestations and Diagnostic Criteria. *Journal of child neurology.* 2002;17:548-554.

Garicochea B, Giorgi R, Odone VF, Dorlhiac-Llacer PE, Bendit I. Mutational analysis of N-RAS and GAP-related domain of the neurofibromatosis type 1 gene in chronic myelogenous leukemia. *Leuk Res.* 1998;22:1003-7.

Guillamo JS, Créange A, Kalifa C, Grill J, Rodriguez D, Doz F, et al. Prognostic factors of CNS tumours in Neurofibromatosis 1 (NF1): a retrospective study of 104 patients. *Brain*. 2003;126:152-60.

Hattori S, Maekawa M, Nakamura S. Identification of neurofibromatosis type I gene product as an insoluble GTPase-activating protein toward ras p21. *Oncogene*. 1992 ;7:481-5.

Hsieh HY, Fung HC, Wang CJ, Chin SC, Wu T. Epileptic seizures in neurofibromatosis type 1 are related to intracranial tumors but not to neurofibromatosis bright objects. *Seizure*. 2011;20:606-11

Huson S. Neurofibromatosis: emerging phenotypes, mechanisms and management. *Clin Med*. 2008;8:611-7.

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

Izawa I, Tamaki N, Saya H. Phosphorylation of neurofibromatosis type 1 gene product (neurofibromin) by cAMP-dependent protein kinase. *FEBS Lett*. 1996;382: 53-9.

Jouhilahti EM, Peltonen S, Heape AM, Peltonen J. The pathoetiology of neurofibromatosis 1. *Am J Pathol.* 2011;178:1932-9

King A, Listernick R, Charrow J, Piersall L, Gutmann DH. Optic pathway gliomas in neurofibromatosis type 1: the effect of presenting symptoms on outcome. *Am J Med Genet A*. 2003;122:95-9.

Kluwe L, Siebert R, Gesk S, Friedrich RE, Tinschert S, Kehrer-Sawatzki H, et al. Screening 500 unselected neurofibromatosis 1 patients for deletions of the NF1 gene. Hum Mutat. 2004;23:111-6.

Knudson AG Jr. Mutation and cancer: statistical study of retinoblastoma. *Proc Natl Acad Sci U S A*. 1971;68:820-3.

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;129:113-9.

Kulkantrakorn and Geller. Seizures in neurofibromatosis 1. *Pediatric neurology*. 1998;19: 347-350.

Lama G, Esposito Salsano M, Grassia C, Calabrese E, Grassia MG, Bismuto R, et al. Neurofibromatosis type 1 and optic pathway glioma. A long-term follow-up. *Minerva Pediatr*. 2007;59:13-21.

Larizza L, Gervasini C, Natacci F, Riva P. Developmental abnormalities and cancer predisposition in neurofibromatosis type 1. *Curr Mol Med*. 2009;:634-53.

Levine TM, Materek A, Abel J, O'Donnell M, Cutting LE. Cognitive profile of neurofibromatosis type 1. *Semin Pediatr Neurol*. 2006;13:8-20.

Listernick R, Louis DN, Packer RJ, Gutmann DH. Optic pathway gliomas in children with neurofibromatosis 1: consensus statement from the NF1 Optic Pathway Glioma Task Force. *Ann Neurol*. 1997;41:143-9.

Listernick R, Ferner RE, Piersall L, Sharif S, Gutmann DH, Charrow J. Late-onset optic pathway tumors in children with neurofibromatosis 1. *Neurology*. 2004;63: 1944-6.

Listernick R, Ferner RE, Liu GT, Gutmann DH. Optic pathway gliomas in neurofibromatosis-1: controversies and recommendations. *Ann Neurol*. 2007;61: 189-98.

Lopes FC, Alves-Leon SV, Godoy JM, de Souza Batista Scherpenhuijzen S, Fezer L, Gasparetto EL. Optic Neuritis and the Visual Pathway: Evaluation of Neuromyelitis Optica Spectrum by Resting-State fMRI and Diffusion Tensor MRI. *J Neuroimaging*. 2015 Jan 15

Madanikia SA, Bergner A, Ye X, Blakeley JO. Increased risk of breast cancer in women with NF1. *Am J Med Genet A*. 2012;158:3056-60

Manara R, Salvalaggio A, Favaro A, Palumbo V, Citton V, Elefante A, et al. Brain changes in Kallmann syndrome. *AJNR Am J Neuroradiol*. 2014;35:1700-6.

Mangoura D, Sun Y, Li C, Singh D, Gutmann DH, Flores A, Ahmed M, et al. Phosphorylation of neurofibromin by PKC is a possible molecular switch in EGF receptor signaling in neural cells. *Oncogene*. 2006;25:735-45.

Margulies DS, Kelly AM, Uddin LQ, Biswal BB, Castellanos FX, Milham MP. Mapping the functional connectivity of anterior cingulate cortex. *Neuroimage*. 2007;37:579-88.

Masocco M, Kodra Y, Vichi M, Conti S, Kanieff M, Pace M, et al. Mortality associated with neurofibromatosis type 1: a study based on Italian death certificates (1995-2006). *Orphanet J Rare Dis*. 201; 6:11

Mautner VF, Kluwe L, Friedrich RE, Roehl AC, Bammert S, Högel J, et al. Clinical characterisation of 29 neurofibromatosis type-1 patients with molecularly ascertained 1.4 Mb type-1 NF1 deletions. *J Med Genet*. 2010;47:623-30.

Mezer A, Yovel Y, Pasternak O, Gorfine T, Assaf Y. Cluster analysis of resting-state fMRI time series. *Neuroimage*. 2009;45:1117-25.

Miettinen and Lasota. Histopathology of gastrointestinal stromal tumor. *Journal of Surgical Oncology*. 2011; 104: 65-873.

Morcaldi G, Clementi M, Lama G, Gabrielli O, Vannelli S, Virdis R, et al. Evaluation of tibial osteopathy occurrence in neurofibromatosis type 1 Italian patients. *Am J Med Genet A*. 2013;161:927-34.

National Institutes of Health Consensus Development Conference Statement: neurofibromatosis. Bethesda, Md., USA, July 13-15, 1987. *Neurofibromatosis*. 1988;1:172-8

Nunley KS, Gao F, Albers AC, Bayliss SJ, Gutmann DH. Predictive value of café au lait macules at initial consultation in the diagnosis of neurofibromatosis type 1. *Arch Dermatol*. 2009;145:883-7

Oakes TR, Fox AS, Johnstone T, et al. Integrating VBM into the general linear model with voxelwise anatomical covariates. *Neuroimage* 2007;34:500-08.

Ostendorf AP, Gutmann DH, Weisenberg JL. Epilepsy in individuals with neurofibromatosis type 1. *Epilepsia*. 2013;54:1810-4.

Ozonoff, S. Cognitive impairment in neurofibromatosis type 1. *American journal of medical genetics*. 1999; 89:45-52.

Parrozzani R, Clementi M, Kotsafti O, Miglionico G, Trevisson E, Orlando G, et al. Optical coherence tomography in the diagnosis of optic pathway gliomas. *Invest Ophthalmol Vis Sci*. 2013;54:8112-8.

Pasmant E, Masliah-Planchon J, Lévy P, Laurendeau I, Ortonne N, Parfait B, et al. Identification of genes potentially involved in the increased risk of malignancy in NF1-microdeleted patients. Mol Med. 2011;17:79-87.

Ragge NK, Falk RE, Cohen WE, Murphree AL. Images of Lisch nodules across the spectrum. *Eye (Lond)*. 1993;7:95-101.

Rasmussen SA, Yang Q, Friedman JM. Mortality in neurofibromatosis 1: an analysis using U.S. death certificates. *Am J Hum Genet*. 2001;68:1110-8.

Riccardi VM. Type 1 neurofibromatosis and the pediatric patient. Curr Probl Pediatr. 1992;22:66-106.

Richetta A, Giustini S, Recupero SM, Pezza M, Carlomagno V, Amoruso G, et al. Lisch nodules of the iris in neurofibromatosis type 1. *J Eur Acad Dermatol Venereol*. 2004;18:342-4.

Riva P, Castorina P, Manoukian S, Dalprà L, Doneda L, Marini G, et al. Characterization of a cytogenetic 17q11.2 deletion in an NF1 patient with a contiguous gene syndrome. *Hum Genet*. 1996;98:646-50.

Schrimsher GW, Billingsley RL, Slopis JM, Moore BD 3rd. Visual-spatial performance deficits in children with neurofibromatosis type-1. *Am J Med Genet A*. 2003;120:326-30.

Segal L, Darvish-Zargar M, Dilenge ME, Ortenberg J, Polomeno RC. Optic pathway gliomas in patients with neurofibromatosis type 1: follow-up of 44 patients. *J AAPOS*. 2010;14:155-8.

Sharif S, Moran A, Huson SM, Iddenden R, Shenton A, Howard E, et al. Women with neurofibromatosis 1 are at a moderately increased risk of developing breast cancer and should be considered for early screening. *J Med Genet.* 2007;44:481-4.

Shen MH, Harper PS, Upadhyaya M. Molecular genetics of neurofibromatosis type 1 (NF1). *J Med Genet.* 1996;33:2-17.

Side LE, Emanuel PD, Taylor B, Franklin J, Thompson P, Castleberry RP, et al. Mutations of the NF1 gene in children with juvenile myelomonocytic leukemia without clinical evidence of neurofibromatosis, type 1. *Blood*. 1998;92:267-72.

Smith SM, Fox PT, Miller KL, Glahn DC, Fox PM, Mackay CE, et al. Correspondence of the brain's functional architecture during activation and rest. *Proc Natl Acad Sci U S A*. 2009;106:13040-5.

Spiegel M, Oexle K, Horn D, Windt E, Buske A, Albrecht B, et al. Childhood overgrowth in patients with common NF1 microdeletions. *Eur J Hum Genet*. 2005;13:883-8.

Szudek J, Birch P, Friedman JM. Growth charts for young children with neurofibromatosis 1 (NF1). *Am J Med Genet*. 2000;92:224-8.

Tadini G, Milani D, Menni F, Pezzani L, Sabatini C, Esposito S. Is it time to change the neurofibromatosis 1 diagnostic criteria? *Eur J Intern Med*. 2014;25: 506-10.

Taylor T, Jaspan T, Milano G, Gregson R, Parker T, Ritzmann T, et al. Radiological classification of optic pathway gliomas: experience of a modified functional classification system. *Br J Radiol*. 2008;81:761-6.

Tedesco MA, Di Salvo G, Ratti G, Natale F, Calabrese E, Grassia C, et al. Arterial distensibility and ambulatory blood pressure monitoring in young patients with neurofibromatosis type 1. *Am J Hypertens*. 2001;14:559-66.

Thiagalingam S, Flaherty M, Billson F, North K. Neurofibromatosis type 1 and optic pathway gliomas: follow-up of 54 patients. *Ophthalmology*. 2004;111:568-77.

Tonsgard JH, Yelavarthi KK, Cushner S, Short MP, Lindgren V. Do NF1 gene deletions result in a characteristic phenotype? *Am J Med Genet*. 1997;73:80-6.

Trevisson E, Forzan M, Salviati L, Clementi M. Neurofibromatosis type 1 in two siblings due to maternal germline mosaicism. *Clin Genet*. 2014;85:386-9.

Upadhyaya M1, Shaw DJ, Harper PS. Molecular basis of neurofibromatosis type 1 (NF1): mutation analysis and polymorphisms in the NF1 gene. *Hum Mutat*. 1994;4:83-101.

Upadhyaya M, Han S, Consoli C, Majounie E, Horan M, Thomas NS, et al. Characterization of the somatic mutational spectrum of the neurofibromatosis type 1 (NF1) gene in neurofibromatosis patients with benign and malignant tumors. *Hum Mutat*. 2004;23:134-46.

Upadhyaya M, Huson SM, Davies M, Thomas N, Chuzhanova N, Giovannini S, et al. An absence of cutaneous neurofibromas associated with a 3-bp inframe

deletion in exon 17 of the NF1 gene (c.2970-2972 delAAT): evidence of a clinically significant NF1 genotype-phenotype correlation. *Am J Hum Genet.* 2007;80:140-51.

Venturin M, Guarnieri P, Natacci F, Stabile M, Tenconi R, Clementi M, et al. Mental retardation and cardiovascular malformations in NF1 microdeleted patients point to candidate genes in 17q11.2. *J Med Genet.* 2004;41:35-41.

Viskochil D, Buchberg AM, Xu G, Cawthon RM, Stevens J, Wolff RK, et al. Deletions and a translocation interrupt a cloned gene at the neurofibromatosis type 1 locus. *Cell.* 1990;62:187-92.

Viskochil D, White R, Cawthon R. The neurofibromatosis type 1 gene. *Annu Rev Neurosci.* 1993;16:183-205.

Walker M, Gabikian P. Benign and malignant pathology in neurofibromatosis type 1. *Neurology.* 2006;67:E13.

Wallace MR, Marchuk DA, Andersen LB, Letcher R, Odeh HM, Saulino AM, et al. Type 1 neurofibromatosis gene: identification of a large transcript disrupted in three NF1 patients. Science. 1990;249:181-6.

Williams VC, Lucas J, Babcock MA, Gutmann DH, Korf B, Maria BL. Neurofibromatosis type 1 revisited. *Paediatrics.* 2009;123:124-33.

Yang FC, Staser K, Clapp DW. The plexiform neurofibroma microenvironment. *Cancer Microenviron.* 2012;5:307-10.

Zinnamosca L, Petramala L, Cotesta D, Marinelli C, Schina M, Cianci R, et al. Neurofibromatosis type 1 (NF1) and pheochromocytoma: prevalence, clinical and cardiovascular aspects. *Arch Dermatol Res.* 2011;303:317-25.

Zuo XN, Kelly C, Adelstein JS, Klein DF, Castellanos FX, Milham MP. Reliable intrinsic connectivity networks: test-retest evaluation using ICA and dual

regression approach. *Neuroimage*. 2010;49:2163-77.

Zvulunov A, Barak Y, Metzker A. Juvenile xanthogranuloma, neurofibromatosis, and juvenile chronic myelogenous leukemia. World statistical analysis. *Arch Dermatol*. 1995;131:904-8.

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