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COMPLEX MULTIDISCIPLINARY FOLLOW-UP OF CHILDREN WITH NEUROFIBROMATOSIS TYPE 1

KOMPLEXNÝ MULTIDISCIPLINÁRNY PRÍSTUP K DEŤOM S NEUROFIBROMATÓZOU TYPU 1

Original research article

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Abstract Neurofibromatosis type 1 (NF1) is one of the most common autosomal dominant disorders with mainly mild cutaneous manifestations. Some patients with NF1, however, develop severe complications such as progressive optic pathway glioma, plexiform neurofibroma or malignant peripheral nerve sheath tumour. Due to potentially progressive and asymptomatic course of the disease, patients with NF1 require a regular multidisciplinary follow-up in coordination with various specialties and early intervention. In this article, we summarise our long-term experience with multidisciplinary follow-up of NF1 patients in the Centre for Neurofibromatosis Type 1 patients at the Children's University Hospital in Bratislava.

Slovak abstract Neurofibromatóza typ 1 (NF1) je jedným z najčastejších autozómovo dominantných ochorení, prevažne s miernymi kožnými prejavmi. U skupiny pacientov s NF1 však pozorujeme závažné komplikácie, ako je progresívny glióm zrakového nervu, plexiformný neurofibróm, alebo malígne nádory obalov periférnych nervov. Vzhľadom na potenciálne progresívny a asymptomatický priebeh ochorenia, vyžadujú pacienti s NF1 pravidelné multidisciplinárne sledovanie v spolupráci s rôznymi špecialistami. V tomto článku sme zhrnuli naše dlhoročné skúsenosti z multidisciplinárneho sledovania pacientov s NF1 v centre pre neurofibromatózu typ 1 v detskej fakultnej nemocnici s poliklinikou v Bratislave.

Keywords *Neurofibromatosis, genetic disorder, variable clinical manifestation, diagnostic criteria, genetic counselling, malignancy, follow-up, broad cooperation with specialists*

Kľúčové slová *neurofibromatóza typu 1, variabilné klinické prejavy, diagnostické kritériá, genetické poradenstvo, tumor, klinické sledovanie, multidisciplinárny prístup*

INTRODUCTION

Neurofibromatosis type 1 (NF1), previously known as von Recklinghausen's disease, has an incidence of 1–5 in 10 000 inhabitants (http://www.orpha.net/). It mostly manifests with cutaneous signs such as multiple café-au-lait macules, axillar and inguinal freckling and various types of neurofibroma. Other signs affect the ocular region (Lisch nodules of the iris, optic pathway gliomas (OPGs)) or skeletal system (pseudoarthrosis, or bowing of tibia and pathologic scoliosis). The most serious complication is the malignant transformation of neurofibromas and a spontaneous outbreak of malignancy due to the decrease of tumour suppressor effect of neurofibromin.

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GENETICS AND CLINICAL MANIFESTATIONS OF NF1

NF1 gene is one of the largest genes in human genome composed of 57 constitutive and at least three alternatively spliced exons, and it spans over about 280 kb of genomic DNA. The protein product of *NF1* gene neurofibromin is expressed especially in ectodermal tissues (skin, nerves and bones) and it functions as a "tumour suppressor protein" via activation of Ras-GTPase (Martin et al. 1990, Yunoue et al. 2003). Impaired expression of neurofibromin thus leads to a persistent increased activity of Ras oncogene and subsequent increased cell proliferation (DeClue et al. 1992).

NF1 is inherited in autosomal dominant manner, and the incidence of new mutations is up to 50%, which signifies that *NF1* gene has a high mutation speed (Yunoue et al. 2003). Although one mutation in the NF1 gene is the only factor required to initiate the NF1 syndrome, it has been shown that the pathoetiology of the multiple manifestations of this disease in different organ systems seems increasingly complex. The bi-allelic inactivation of the NF1 gene through a "second hit" seems to be of crucial importance to the development of certain manifestations, such as neurofibromas, café-au-lait macules and glomus tumours described below. In each case, the second hit involves only one cell type, which is subsequently clonally expanded in a discrete lesion (Joubilahti et al. 2011). In summary, neurofibromatosis can have a variable clinical manifestation even within the family carrying the same mutation. Therefore, in order to diagnose the patient in childhood, it is necessary to recognise the main clinical features.

Café-au-lait spots are the most common sign of the disease and contribute to the early diagnosis. These skin hyperpigmentations with sharp edges have a uniform character in an individual. They can vary in number and size (from few millimetres to few centimetres in diameter) on every body area apart from the capilicium, eyebrows, palms and soles (Landau et al. 1999, Friedman 2002). The number of hyperpigmentations does not correlate with the severity of the disease (Korf et al. 2002). As seen in Figure 1, they can be present at birth, but usually appear by the age of six (DeBella et al. 2000). **Freckling** is typically localised in the groin and axillary area and occurs at the age of three to five years (Gutmann et al. 1997). Café-au-lait spots and freckling do not cause any discomfort, and neurofibromatosis can escape the diagnosis in this stage.

Lisch nodules are avascular hamartomas of the iris, which are patognomic for NF1. These lesions measure 0.5–2 mm (Figure 2) and they are present in about 80% of patients with NF1 (Otsuka et al. 2001). They have no effect on the visual acuity and do not require any treatment. **Neurofibromas** are benign tumours of the nerve tissue; they usually begin to appear in early puberty. Most of them are clinically silent; however, some of them can cause persistent pruritus, deformation of limbs or face, and functional disorders. The symptomatology depends on the level of invasion into surrounding tissues. The most severe type of neurofibromas is **plexiform neurofibroma**. This is a congenital lesion and in most cases remains clinically silent. The growth of congenital plexiform neurofibromas is unpredictable; in some cases, it may suddenly start behaving aggressively. Given the generally higher risk of transformation to **malignant peripheral nerve sheath tumour (MPNST)**, early diagnosis and follow-up of plexiform neurofibromas are essential (Evans et al. 2002).

MPNST is the most frequent and most serious malignant complication in patients with NF1. The lifetime risk of developing MPNST in an NF1 patient is 10%, which means it is 1000–10 000-fold higher in an individual with neurofibromatosis compared with general population (McGaughran et al. 1999). Tumours can occur on any body part, but mostly on proximal parts of upper and lower extremities and the trunk, and behave aggressively and invasively (Friedrich et al. 2007).

OPG is the most frequent tumour of the central nervous system and occurs in approximately 15–20% of patients with NF1 (Listernick et al. 1989, Lund and Skovby 1991, Czyzyk et al. 2003). It is a benign, non-metastatic neoplasia of astrocytes, which may be asymptomatic but can sometimes lead to progressive axial protrusion of the eye bulbus and visual impairment. Optic nerve glioma is mostly localised in the area of optic chiasma and prechiasma although it can appear in the whole course of optic nerve (Figure 4). Other benign brain tumours derived from astrocytes are MRI hyperintensive lesions in the brain – hamartomas, also known as **neurofibromatosis bright objects (NBOs)**, or unidentified bright objects. They appear in approximately 60–70% of children with NF1 (Brown et al. 1987). Despite their high frequency among NF1 patients, attempts to include this sign in the diagnostic criteria failed (Lopes et al. 2008). NBOs are located mainly in the basal ganglia, brain stem and cerebellum and often disappear in adulthood (Gill et al. 2006).

NBOs in general were suspected to cause **cognitive impairment** such as hyperactivity, ADHD, lower intellect and spaciomotor functions impairment (Feldmann et al. 2003). However, only lesions in thalamus were proved to have this effect (Hyman SL. 2007). Deficit in various cognitive functions is present in approximately 30–65% of patients with NF1 (North et al. 1997).

Typical skeletal dysplasias – **antero-posterior bowing and pseudoarthrosis of tibia** – are included in the diagnostic criteria. They typically occur at birth or during the first year of life (Figure 3).

The diagnosis of neurofibromatosis can be made based on the presence of at least two of the seven clinical criteria listed in table 1 (National Institutes of Health Consensus Development Conference Statement: Neurofibromatosis 1988, Gutmann et al. 1997). The manifestation of NF1 signs and complications of NF 1 typically occur at a certain age, although they might appear at any time during the course of life. Knowing the mean age of peek incidence of NF1 signs does not allow categorising patients but can help to select adequate diagnostic methods in the follow-up. Various signs of NF1 and the peek incidence are listed in table 2. If available, the *NF1* gene mutation identification can provide an important tool for definitive confirmation of clinical diagnosis of NF1.

THERAPEUTIC OPTIONS IN NF1

Currently, there is no causal therapy of NF1 or a therapy that would slow down the progression of the disease. Cafe-au-lait spots, freckling and Lisch nodules do not require treatment. Surgical removal of neurofibromas is recommended if they cause discomfort, pain, or oppression of surrounding tissues and/or vital organs. Otherwise, it is not routinely performed due to the risk of regrowth.

Management of OPGs remains challenging because their clinical course is difficult to predict. They are mostly asymptomatic, and only about 5% of patients have complications (Blazo et al. 2004, Singhal et al. 2002, Sharif et al. 2006). In most patients, OPGs have a clinically silent period of stagnation, and therefore initial management strategy of gliomas is observation. There have also been reports about spontaneous regression of optic glioma in adulthood (Brzowski 1992, Parsa et al. 2001). In case of rapid growth, enhancement (using the gadolinium contrast in the MRI) or onset of clinical symptoms, chemotherapeutic drugs – vincristine and carboplatin – are the treatment of choice (Mahoney et al. 2000, Hsu et al. 2008). Surgical extirpation is an option for large gliomas causing protrusion.

Treatment of the most severe complication of neurofibromatosis – malignant peripheral nerve sheath tumor - MPNST – is another major challenge. A total surgical resection combined with radiotherapy is the only effective method of treatment because these tumours are mostly resistant to chemotherapy. Intervention should be prompt due to increased tendency to tumour metastasis (Vraa et al. 1998, Yang 1998).

NF1 manifests in various forms of severity, and it is not possible to predict the course of the disease in an individual. The strategy for management of patients with NF1 worldwide is "watchful waiting" requiring coordinated cooperation of several specialties. The supervising specialist (paediatrician, neurologist, oncologist or clinical geneticist) should be experienced in recognising potentially threatening conditions in these patients and starting early treatment. Centralised observation of the clinical course of the disease with such significant heterogeneity is an important step towards more effective care for children with neurofibromatosis.

MULTIDISCIPLINARY FOLLOW-UP OF CHILDREN WITH NF1 IN SLOVAKIA

Since 1990, a systematic monitoring of patients with NF1 has been taking place in the second paediatric clinic in cooperation with other departments of Children's University Hospital. In 2008, there were 70 patients regularly examined. In 2013, the number increased up to 220 patients followed-up regularly. Clinical examination consisted of regular paediatric examination in 6- or 12-month intervals. The frequency of examinations

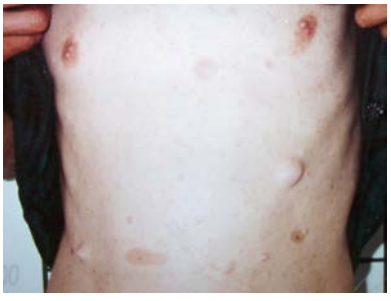

Figure 1. Café-au-lait macules and subcutaneous neurofibromas

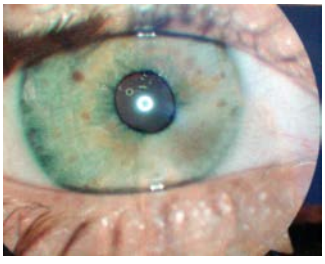

Figure 2. Lisch nodules of iris

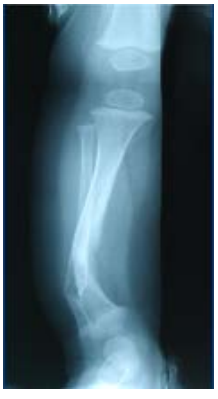

Figure 3. Pseudoarthrosis of tibia

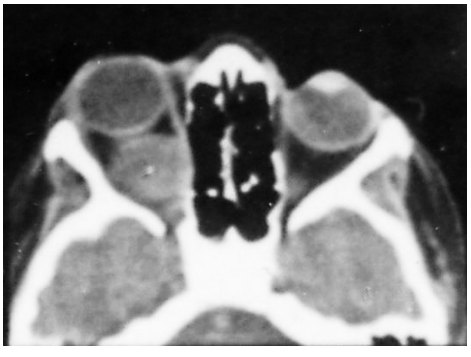

Figure 4. Optic pathway glioma (CT imaging)

Table 1. Diagnostic criteria of neurofibromatosis (NIH 1988, Gutmann et al. 1997)

1.	Six and more café-au-lait spots larger than 5 mm in pre-pubertal age or larger than 15 mm in post-pubertal age
2.	Freckling in the axilla or groin
3.	Two or more Lisch nodules (iris hamartomas)
4.	Optic pathway glioma
5.	Two or more neurofibromas of any type or one or more plexiform neurofibroma
6.	Distinctive skeletal lesion (dysplasia of the sphenoid bone or dysplasia or thinning of long bone cortex)
7.	A first-degree relative with NF1

was chosen individually according to the clinical condition and symptoms of the patient with regard to the age-specific occurrence of some signs (see table 2). The first interview reveals the presence or absence of family history of NF1. The clinical examination that follows is aimed at the manifestations of the disease and the basic physical parameters (weight, height, blood pressure). Laboratory tests include blood count, leukocyte differential and biochemical parameters (blood glucose, creatinine, uric acid, urea, liver transaminases (AST, ALT), serum amylase (AMS), total cholesterol, LDL, HDL, sodium, potassium and chloride levels). Endocrinological examination includes measuring of thyroid hormones (TSH, fT4) and pituitary hormones in case of short stature or precocious puberty (FSH, LH) and if hypertension occurs, we measure cortisol and catecholamines.

Ultrasound examination of abdominal organs is performed with a particular focus on the pathological masses in the abdomen (neurofibromas, tumours), liver, spleen (hepatoma), kidney (Wilms' tumour), adrenal gland (pheochromocytoma) and stenosis often presenting with hypertension. X-ray of lower extremities is carried out only in patients with lower leg deformity or limb length asymmetry (pseudoarthrosis of the tibia, anterior-posterior bending of the tibia, cystic lesions in long bones). MRI reveals tumour processes in the brain tissue and optic pathways (optic nerve gliomas, tumour processes in the orbit, NBOs in the brain, hydrocephalus). MRI is also used as a modality if a plexiform neurofibroma grows near the spinal canal.

Specialist examinations: Neurological examination focuses on the investigation of focal symptomatology and muscle tone. Ophthalmologic examination discloses presence or absence of Lisch nodules of the iris and tests the visual acuity, colour vision and visual fields.

Examination of hearing using tympanometry or Audiotory Steady State Response (ASSR) is used in the differential diagnosis of neurofibromatosis type 2. Dermatological examination helps to assess café-au-lait spots and the extent of neurofibromas.

The assessment of posture, limb length and symmetry (antero-posterior bowing of tibia, tibial pseudoarthrosis) is made during the orthopaedic examination.

Psychological or psychiatric examination is needed especially in management of various speech disorders, mental retardation, emotional lability and hyperactivity.

Genetic diagnostics is an important part of the complex care of NF1 patients worldwide. It is indicated in patients meeting diagnostic criteria of NF1 (NIH 1988, Gutmann et al. 1997). Patients who can benefit from excluding NF1 (patients with OPG or brain glioma) are an exception to this rule. There are several modalities of molecular analyses used. We use molecular analysis based on cDNA sequencing in combination with MLPA, which proved to be efficient in the diagnostics of majority mutations, including mutations affecting splicing (Messiean et al. 2012, Nemethova et al. 2013).

The inclusion of genetic diagnostics proved to be an effective diagnostic tool from various points of view. In case of a positive result, we included the patient in the study focused on finding genotype–phenotype correlations. In case of a negative result, the follow-up of the patient was adjusted for further differential diagnostics.

RESULTS

Recently, we published the results of a long-term follow-up and molecular testing of 124 Slovak *NF1* patients from 102 families (Nemethova et al. 2013). After re-evaluation of the NIH diagnostic criteria (NIH 1988, Gutmann et al. 1997), we showed that 108 individuals from 86 families exhibited typical NF1 features and remained in the study group. In these patients, we summarised clinical and molecular data. All 108 patients had café-au-lait spots, 85% had axillary and/or inguinal freckling, 61% had neurofibromas, 36% had Lisch nodules of the iris, 31% had OPG and 5% had a typical skeletal disorder; in 51% of patients, first-degree relative was also affected. The incidence of signs is shown in Figure 5. In 86 cases that fulfilled NIH diagnostic criteria, 78 different *NF1* mutations were identified by cDNA sequencing and/or MLPA analysis, out of which 39 were novel (Nemethova et al. 2013).

In this group of patients, we observed a higher incidence of OPGs (31%), compared with previously published incidence of 15% (Listernick et al. 1989, Lund and Skovby 1991, Czyzyk et al. 2003) and lower incidence of Lisch nodules. It is still unclear what the reasons underlying behind these significant differences are. There might be regional specificities, which are yet to be investigated.

After the assessment of the clinical picture and mutational status of all 124 patients, we attempted to search for the correlation between them. In general, it is difficult to find

*Table 2. Signs of neurofibromatosis type 1 (*NF1*), frequency and typical onset (Ferner et al. 2007)*

Signs of NF1	Frequency (%)	Age of onset
Café-au-lait macules	>99	0–2 years
Freckling in intertriginous areas	67	3–5 years
Cutaneous neurofibromas	>95 of adults	>7 years, mostly after puberty
Nodular neurofibromas	48	Rarely before 10 years, in puberty
Plexiform neurofibromas		
All lesions	30	0–18 years
Lesions of head and neck	1.2	0–1 year
Lesions of the truncus and limbs	5.8	0–5 years
Lisch nodules of iris	90–95	>3 years
Macrocephaly	45	Congenital
Short stature	31,5	Congenital
Intellectual disability		
Severe	0.8	0–5 years
Medium	2.4	0–5 years
Mild/learning disability	29.8	0–5 years
Epilepsy		
Unknown cause	4.4	Lifetime risk
Secondary in NF1	2.2	Lifetime risk
Hypsarrhytmia	1.5	0–5 years
Tumours of central nervous system (CNS)		
Optic pathway glioma	1.5*	0–20
Other tumours of CNS	1.5	Lifetime risk
Spinal neurofibromas	1.5	Lifetime risk
Aqueductal stenosis – hydrocephalus	1.5	Lifetime risk <30 years
Malignancy		
Malignant peripheral nerve sheath tumour	1.5**	Lifetime risk
Rhabdomyosarcoma	1.5	Lifetime risk
Gastrointestinal stromal tumours	2.2	Lifetime risk
Pheochromocytoma	0.7	>10 years
Duodenal carcionoid	1.5	>10 years
Juvenile myelomonocyte leukaemia	<1	0–18 years
Carcinoma of the breast in women	Relative risk 3× higher for women <30 years	
Skeletal lesions		
Scoliosis requiring surgery	4.4	0–18 years
Mild scoliosis	5.2	0–18 years
Long bone dysplasia	3.7	0–2 years
Infiltration of the spine by neurofibromas	10	Lifetime risk
Sphenoidal dysplasia	<1	Congenital
Renal artery stenosis	1.5	Lifetime risk
Juvenile xanthogranuloma	0.7	0–1 year
Congenital glaucoma	0.7	0–1 year
Lateral meningocele	<1	Lifetime risk
Cerebrovascular disease	<1	Childhood
Glomus in periungual area	<1	Adulthood
* Optic nerve glioma occurs in 15% of children with MRI of the brain (Czyzyk et al. 2003)		
** Lifetime risk is about 8–13% (Evans et al. 2002)		

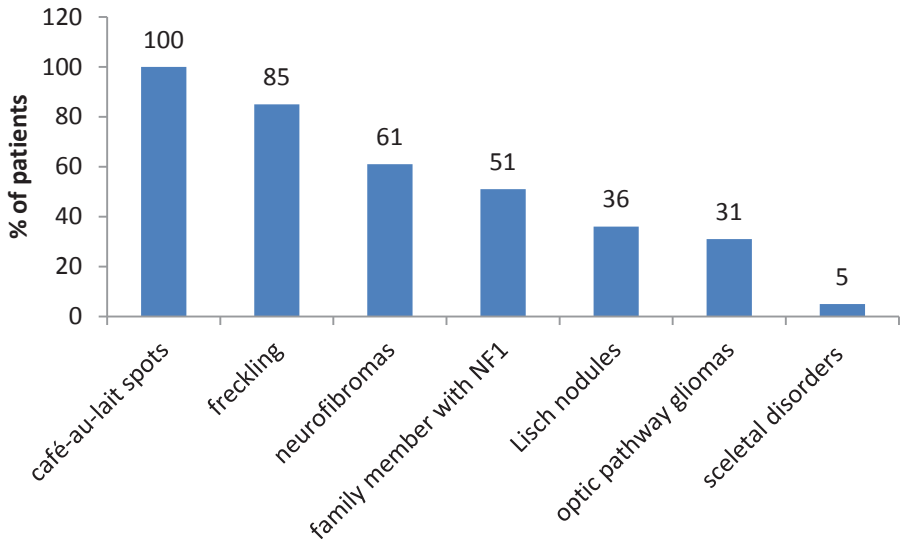

*Figure 5. Signs of neurofibromatosis type 1 (*NF1*) in the group of all tested 108 patients with* NF1 *(figure adjusted according to Nemethova et al. 2013)*

this kind of correlations in NF1 due to the rapidly increasing number of mutations as well as the varying clinical picture even within one family. Only few genotype–phenotype correlations in NF1 have been published, and it is known that there are also several other complex molecular mechanisms in question regarding the variable phenotype of the diseases. One of them was a suspected correlation between the mutation in the first tertile of the gene and the incidence of OPGs (Sharif et al. 2011). Our study that is coordinated together with the Departments of radiology and ophthalmology confirmed the clustering of mutations in the 5'tertile of the *NF1* gene in patients with an OPG and also revealed higher incidence of NF1 disease features (freckling and neurofibromas) in this group (Bolcekova et al. 2013). These data represent new knowledge and contribute to the already published genotype–phenotype correlations (Kayes et al. 1994, De Raedt et al. 2003, Kluwe et al. 2003, Upadhyaya et al. 2007).

In conclusion, we confirm that long-term monitoring of children with neurofibromatosis in Slovakia is important. The idea of the Centre for Children with NF1 at the Children's Faculty Hospital as well as the idea of Centre of Expertise for Rare Disease was a reaction to the need of a systematic follow-up by specialists experienced in detecting early signs of potentially harmful progression of NF1. Another positive aspect of a centralised health care is also its accessibility to the patients and their parents who were previously dependent on local health specialists who rarely examine patients with NF1. Currently, we are not able to treat neurofibromatosis as such; however, some complications (OPG, plexiform neurofibromas) can be treated or limited if they are recognised early.

There is still much that is unknown about NF1, but collecting sufficient data about the course of the disease can lead to the improvement in the diagnostics and more effective care for these patients.

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