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Clinical and Genotype-Phenotype Investigation of the Neurofibromatosis Type 1 Paediatric Population in Hungary, Diagnostic and Follow-up Protocols

PhD Thesis

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List of Abbreviations

ACMG/AMP – American College of Medical Genetics and Genomics / Association for Molecular Pathology

ADHD – Attention Deficit Hyperactivity Disorder

AKT (PKB): Ak strain transforming (*Protein Kinase B*)

ASD – Autism Spectrum Disorder

BRAF – v-Raf murine sarcoma viral oncogene homolog B (RAF kinase B isoform)

CALMs – Café-au-Lait Macules

CHL – Conductive Hearing Loss

CNF – Cutaneous Neurofibroma

CNS – Central Nervous System

CNV – Copy Number Variant

CSF1R (CSF1-R) – Colony-Stimulating Factor 1 Receptor

c-KIT (KIT) – proto-oncogen, receptor tyrosine kinase;

ERK1/2 – Extracellular Signal-Regulated Kinase ½

FDA – Food and Drug Administration

GDP – guanosine diphosphate

GTP – guanosine triphosphate; FASI – Focal Areas of Signal Intensity

ID – Intellectual Disability

MAPK – Mitogen-Activated Protein Kinase

MEK1/2 (MAP2K1/2) – MAPK/ERK kinase MEK – Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase

MLPA – Multiplex ligation-dependent probe amplification

MPNST – Malignant Peripheral Nerve Sheath Tumour

MRI – Magnetic resonance imaging

mTOR – mammalian Target Of Rapamycin

NF1 – Neurofibromatosis Type 1

NF2 – Neurofibromatosis Type 2

NGS – Next-Generation Sequencing

NIH – National Institutes of Health

OPG – Optic Pathway Glioma

PAK1 – p21-Activated Kinase 1

PDGFR (PDGF-R): Platelet-Derived Growth Factor Receptor

PI3K – Phosphoinositide 3-Kinase

PNF – Plexiform neurofibroma

RAF – Rapidly Accelerated Fibrosarcoma

RAC (RAC1): Ras-related C3 botulinum toxin substrate

RAS – Rat Sarcoma

RTK – Receptor Tyrosine Kinase; PET – Positron Emission Tomography

SHL – Sensorineural Hearing Loss

SPRED1 – Sprouty-related, EVH1 domain-containing protein 1

UBO – Unidentified Bright Object

VEGFR (VEGF-R) – Vascular Endothelial Growth Factor Receptor

WES – Whole exome sequencing

1. Introduction

Neurofibromatosis type 1 (NF1) (MIM #162200) is an autosomal dominant neurocutaneous disorder with significant clinical variability and complications. The incidence of NF1 is estimated at 1 in 3000 to 4000 individuals globally, making it one of the most common inherited genetic disorders associated with café-au-lait macules (CALMs) (Lammert et al., 2005; Duong et al., 2011; Veres et al., 2023; Tamura et al., 2021; Wilson et al., 2021). NF1 is primarily caused by mutations in the neurofibromin gene *(NF1*), located on chromosome 17q11.2 (NF1, NM_000267). This tumour suppressor gene plays a critical role in regulating cell growth, and its mutation can lead to uncontrolled cellular proliferation and tumour development (Tamura, et al., 2021). Recent studies also suggest that immune dysregulation may contribute to NF1 pathogenesis, as neurofibromin participates in RAS–MAPK–mediated immune modulation; however, such alterations appear to be mainly local, tumour microenvironment–related phenomena rather than systemic autoimmunity or immunodeficiency (Torres et al., 2016; Karmakar et al., 2017; Wei et al., 2020; White et al., 2024).

Due to the large size of the *NF1* gene, which encompasses 350 kb of the genome and contains 61 coding exons, it is susceptible to a wide range of mutations, leading to de novo mutations in up to 50% of cases (Pálla et al., 2023; Bettegowda et al., 2021). The remaining 50% are inherited in an autosomal dominant manner from an affected parent (Cassiman et al., 2013; Wilson et al., 2020; Saleh et al., 2023). 85–90% of the mutations are point mutations, 5–10% are microdeletions, and 2% are exon deletions or duplications (Büki et al. 2023; Tamura et al., 2021; Veres at el., 2023). The disease typically progresses over time since childhood and shows nearly complete penetrance with age (Bettegowda et al., 2021; Pálla et al., 2023; Alkindy et al., 2012). Interestingly, affected individuals, even those with the same germline *NF1* gene mutation or even within members of the same family, manifest with a wide range of clinical manifestations (Komlósi et. al. 2011, Tang et al., 2023, Miller et al., 2019). The disorder affects both males and females equally, with manifestations appearing in early childhood. According to the literature, 50% of patients demonstrating characteristic clinical signs by the age of 1 year and 97% by the age of 8 years (Veres et al., 2023; Wilson et al., 2021). A significant concern associated with NF1 is the decreased life expectancy of patients, which can be reduced by 8-15 years

compared to the general population. This reduction is primarily attributed to vascular diseases and malignancies, including female breast cancer under 50 years and malignant peripheral nerve sheath tumours (MPNSTs) (Legius et al., 2021; Tamura et al., 2021; Duong et al., 2011; Miller et al., 2019).

In 1987, the National Institutes of Health (NIH) established diagnostic criteria for NF1, which were revised in 2021 to enhance diagnostic accuracy. These criteria include:

- Six or more café-au-lait macules (CALMs, ≥5 mm in prepubertal individuals);
- Axillary or inguinal freckling (Crowe sign);
- Two or more neurofibromas or one plexiform neurofibroma (PN);
- Optic pathway glioma;
- Lisch nodules (iris hamartomas) or choroidal abnormalities;
- A distinctive osseous lesion (e.g., sphenoid dysplasia, anterolateral tibial bowing, or pseudarthrosis of a long bone);
- A heterozygous pathogenic NF1 variant confirmed by genetic testing (Legius et al., 2021).

Diagnosis can be established if the patient meets at least two of these criteria, while a child with a parent diagnosed with NF1 requires only one criterion (Legius et al., 2021). In cases where only pigmentary features are present, at least one of the two (café-au-lait macules or freckling) must be bilateral. Under such circumstances, Legius syndrome should also be considered in the differential diagnosis, and mosaic NF1 may likewise fulfil diagnostic criteria (Legius et al., 2021).

Table 1. Major clinical features of Neurofibromatosis type 1 with typical onset age, prevalence, and key clinical notes.

Clinical Feature	Typical Onset Age	Approximate Prevalence	Clinical Notes / Comments
Café-au-lait macules (CALMs)	Infancy (often present at birth)	>99% by age 1	Earliest diagnostic sign; number and size increase in early childhood, then stabilize.
Axillary/inguinal freckling (Crowe sign)	3–5 years	>85% by age 7	Pathognomonic for NF1; often appears after CALMs and before neurofibromas.
Cutaneous neurofibromas	Adolescence → adulthood	~90% in adults	Benign peripheral nerve sheath tumours; increase in number with age.
Plexiform neurofibromas	Congenital or early childhood	20–50%	May cause disfigurement or compressive symptoms; risk of malignant transformation (MPNST).
Lisch nodules (iris hamartomas)	3-7 years	50-90%	Pigmented iris nodules, characteristic but asymptomatic; detected by slit-lamp exam.
Optic pathway glioma (OPG)	1–7 years	5–25%	May cause vision loss or proptosis; requires regular ophthalmologic follow-up.
Skeletal abnormalities (e.g., scoliosis, tibial dysplasia, sphenoid wing dysplasia)	Childhood	30–50%	Scoliosis most common; tibial pseudarthrosis in ~5%.
Cognitive/behavioural disorders (ADHD, LD, ASD features)	Childhood – early school age	30–81%	Common cause of morbidity; requires neuropsychological evaluation.
MRI T2 hyperintensities (FASI)	2–10 years	60–90% in children	Non-neoplastic brain changes; usually regress by adulthood.
Malignant peripheral nerve sheath tumour (MPNST)	Adolescence → adulthood	5–10% (lifetime risk)	Arises from plexiform neurofibromas; requires vigilance and imaging if symptomatic.

1.1. Clinical characteristics

1.1.1. Skin manifestations

1.1.1.1. Café-au-lait macules

Café-au-lait macules (CALMs) are often the earliest clinical signs of NF1 and can be observed as early as infancy. By one year of age, 99% of individuals with NF1 exhibit six or more CALMs, which may increase in number during early childhood and subsequently stabilize or even fade (Table 1) (Nasi et al., 2023; Ly et al., 2019; Wang et al., Ben-Shachar et al., 2017). CALMs are typically oval-shaped macules with well-defined borders resembling the "coast of California" (Figure 1A). They are slightly darker than the surrounding skin, and vary in size from 1 to 3 cm (Ozarslan et al., 2021; Albaghdadi et al., 2022; Ly et al., 2019). They usually occur all over the body but are not found on the palms or soles of the feet (Lalor et al., 2020; Miller et al., 2019, Veres et al., 2025; Ozarslan et al., 2021; Albaghdadi et al., 2022). However, atypical CALMs may present with irregular shapes resembling the "coast of Maine," or display a marked pigmentation contrast with surrounding skin. In instances where atypical CALMs are observed without the presence of classic CALMs, differential diagnoses for other conditions should be

considered (Miller et al., 2019, Veres et al., 2025b). Neither the size nor the number of café-au-lait macules is directly correlated with the severity of the disease (Solares et al., 2022).

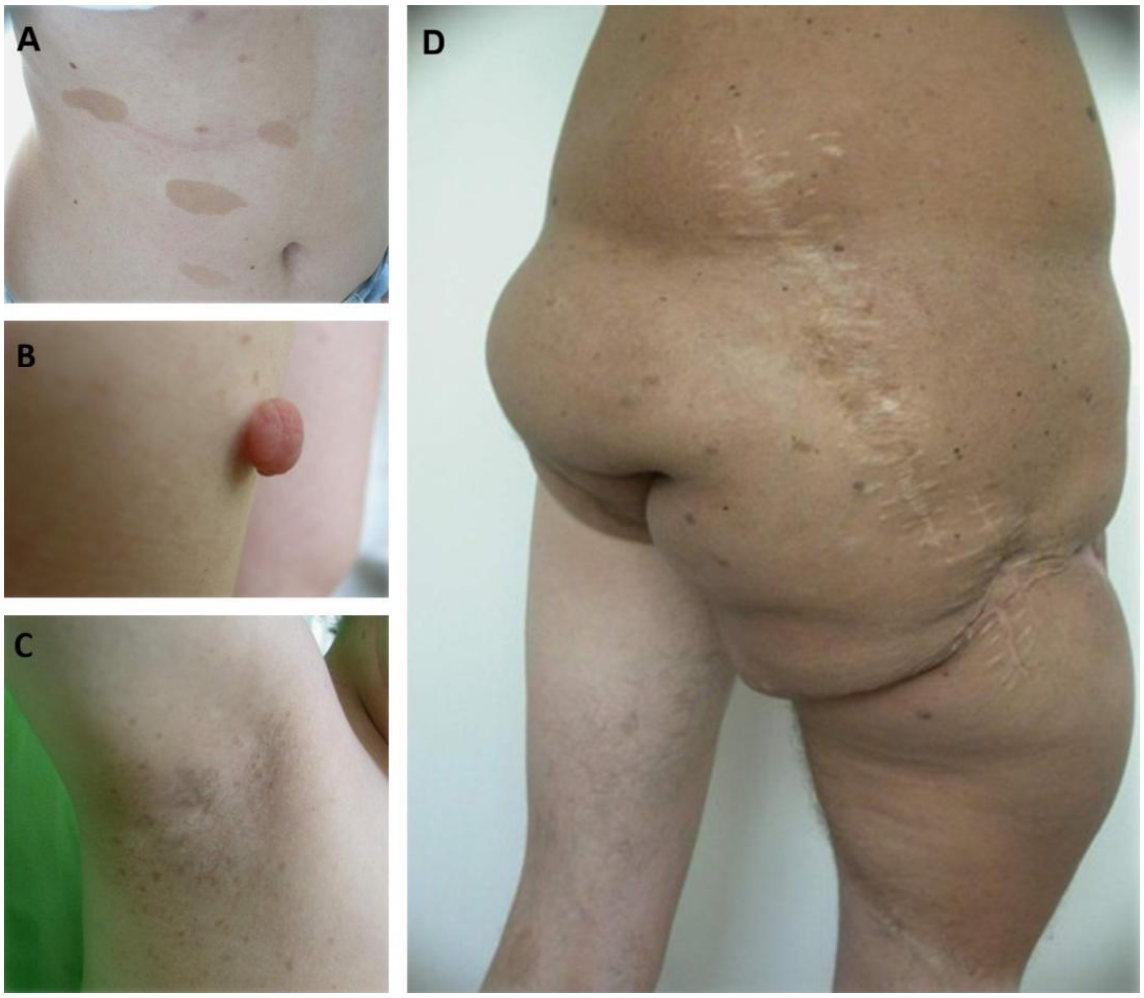

Figure 1. Characteristic cutaneous manifestations of NF1: (A) café-au-lait macules, (B) cutaneous neurofibroma, (C) axillary freckling (Crowe sign), and (D) extensive plexiform neurofibroma causing marked disfigurement.

1.1.1.2. Freckling

Axillary and inguinal freckling (Crowe sign), characterized by small (1–4 mm), clustered pigmented macules, is a pathognomonic feature of NF1 (Figure 1C) (Wang et al., 2021; Ly et al., 2019; Ozarslan et al., 2021; Albaghdadi et al., 2022; Anderson, et al., 2020). Typically appearing between three and five years of age, often after CALMs and before neurofibromas, it's present in over 85% of NF1 patients by age seven (Table 1) (Wang et al., 2021; Ly et al., 2019; Miller et al., 2019; Lammert et al., 2005). Axillary or inguinal

freckling is one of the seven cardinal diagnostic features of NF1 (Ozarslan et al., 2021; Albaghdadi et al., 2022).

1.1.1.3. Neurofibromas

Neurofibromas are benign tumours characterized by a diverse composition of cell types, including Schwann cells, perineural cells, fibroblasts, mast cells, macrophages, neuronal axonal processes, and extracellular matrix components such as collagen (Solares, 2022). These tumours typically become more prominent during adolescence and continue to develop into adulthood, with the prevalence of over 90% of adult patients with NF1 (Table 1) (Wang et al., 2021; Veres et al., 2011; Jouhilahti et al., 2011; Ortonne et al., 2018; Ozarslan et al., 2021; Ly et al., 2019; Miller et al., 2019). Neurofibromas can arise on the skin, nerves, and other tissues. They often present as cutaneous neurofibromas (CNFs), which are soft, non-tender, purplish or skin-coloured lesions, and may appear in large quantities, sometimes numbering in the hundreds or thousands (Figure 1B) (Miller et al., 2019; Ly et al., 2019; Chen et al., 2019; Jouhilahti et al., 2011; Ortonne et al., 2018). The morphological presentation of CNFs varies, encompassing nascent/latent, flat, sessile, globular, and pedunculated lesions. These lesions are typically soft in consistency, nontender on palpation, and range in size from 0.5 to 30 mm (Ortonne et al., 2018; Ly et al., 2019). Despite their benign nature, CNFs may lead to pruritus, pain, and cosmetic concerns (Solares et al., 2022).

1.1.1.4. Plexiform neurofibromas

Plexiform neurofibromas (PNFs), affecting 20% to 50% of patients with NF1 (Veres et al., 2023; Ly et al., 2019; Gross et al., 2018; Gross et al., 2020; Armstrong et al., 2023), are histologically benign tumours of the peripheral nerve sheath, with the potential to transform into malignant peripheral nerve sheath tumours (MPNSTs) in approximately 8% to 13% of NF1 patients (Table 1) (Kehrer-Sawatzki et al., 2017). PNFs are typically congenital and grow slowly, except during periods of early childhood and pregnancy (Gross et al., 2018; Veres et al., 2023a; Ly et al., 2018). These tumours can lead to significant disfigurement, visible deformities, pain, and local compression of adjacent structures, resulting in functional impairment of nerves, vasculature, and airways (Figure 1D) (Veres et al., 2023a; Gross et al., 2018; Wang et al., 2021; Chen et al., 2019). Recent evidence indicates that individuals with non-mosaic large NF1 deletions exhibit a

markedly higher tumour burden, correlating with an increased propensity for malignant transformation into MPNSTs (Kehrer-Sawatzki et al., 2017, Wang et al., 2021). Magnetic resonance imaging (MRI) plays a critical role in the early detection and monitoring of asymptomatic internal PNFs, thus facilitating timely intervention and management (Pálla et al., 2022; Veres et al., 2023a). If a tumour is persistently painful, associated with neurological dysfunction, or exhibits rapid growth, malignant transformation must be excluded (Miller et al., 2019).

Further skin symptoms like naevus anaemicus, pseudoatrophic macules, glomus tumour, and juvenile xanthogranulomas are also more frequently seen in NF1 patients (Legius et al., 2021; Nasi et al., 2023; Wilson et al., 2021; Miller et al., 2019)

1.1.2. Ocular manifestations

Ocular manifestations are also significant in NF1, four of the established diagnostic cardinal criteria are assessed through ophthalmological screening: Lisch nodules, optic pathway glioma, a distinctive osseous lesion (sphenoid dysplasia), and the (orbital) plexiform neurofibroma (Cassiman et al., 2013).

1.1.2.1. Lisch nodules

Lisch nodules are a hallmark feature of NF1 and represent its most frequent ocular manifestation (Wang et al., 2021; Veres et al., 2023a). Lisch nodules are benign, pigmented, gelatinous lesions located on the iris, typically identified during slit-lamp examination. They can vary in colour, ranging from creamy white in darker irides to brown in lighter shades like blue and green (Cassiman et al., 2013). They tend to cluster in the inferior hemifield of the iris, likely due to the sunlight-shielding effect of the upper eyelid, and light-coloured irides typically exhibit more nodules than dark ones, possibly due to the photo-protective benefits of pigmentation (Cassiman et al., 2013). Lisch nodules usually begin to develop around the age of 2.5 years and are generally asymptomatic. Around fifty percent of NF1 toddlers harbour nodules, and by the age of 30, this number increases to over 90 % (Table 1) (Rauen et al., 2013; Cassiman et al., 2013; Maharaj et al., 2014; Wiliam et al., 2007). They are rarely seen in patients without NF1. The presence of Lisch nodules alongside café-au-lait spots serves as a diagnostic criterion for NF1, distinguishing it from other syndromes characterized by multiple CALMs (Cassiman et al., 2013, Wang et al., 2021; Veres et al., 2023b). In addition to their

diagnostic relevance, Lisch nodules have also been linked to specific mutation types, with previous genotype–phenotype correlation studies showing a significantly higher prevalence in individuals with frameshift mutations (22.6% vs. 9.1%), as reported by Stella et al. (2018), in agreement with earlier findings (Sabbagh et al., 2013; Castle et al., 2003).

1.1.2.2. Optic pathway gliomas

Optic pathway gliomas (OPGs) represent the most common glioma associated with NF1, primarily diagnosed in children under 7 years of age (mean age at OPG diagnosis is 4.5 years) (Table 1) (Carvalho et al., 2023; Cassiman et al., 2013; Tang et al., 2023, Dunning-Davies et al., 2016; Campen et al., 2019; Wang et al., 2021; Ly et al., 2019). Notably, approximately one-third of children diagnosed with optic pathway gliomas have underlying NF1. Among children with NF1, the reported prevalence of OPGs ranges from 5% to 25% (Cassiman et al., 2013; Tang et al., 2023). OPGs are typically classified as benign low-grade pilocytic astrocytomas. NF1-associated OPGs can arise within any segment of the visual pathway, predominantly arise on the prechiasmatic optic nerve, with or without chiasmic involvement, and rarely invade the optic radiations. In contrast, sporadic OPGs are more frequently found at the chiasm and often extend beyond the optic pathway. Additionally, bilateral optic nerve gliomas are more commonly observed in NF1 patients (Cassiman et al., 2013). Although most patients remain asymptomatic, symptoms develop in approximately 20–30% of cases, depending on the tumour's location. Among those who become symptomatic, roughly one-third may require intervention (Ly et al., 2019; Campen et al., 2019). Clinical manifestations may include decreased visual acuity, visual field defects, unilateral proptosis, strabismus, relative afferent pupillary defect, and optic disc oedema or atrophy (Carvalho et al., 2023; Cassiman et al., 2013; Tang et al., 2023). In rare cases, posterior involvement may cause obstructive hydrocephalus, leading to symptoms such as headache, nausea, and vomiting (Campen et al., 2018). OPGs affecting the optic chiasm can even lead to bi-temporal vision loss or endocrinologic abnormalities, such as precocious puberty due to hypothalamic involvement (Tang et al., 2023; Cassiman et al., 2013). Data indicate that female patients with OPGs are more prone to disease progression and may be more likely to require treatment; however, identical surveillance protocols are recommended for both sexes (Miller et al., 2019). The diagnosis is confirmed by magnetic resonance imaging (MRI) of the brain. Compared to

sporadic cases, OPGs in NF1 patients generally exhibit a less aggressive nature and demonstrate differing responses to (radio)therapy (Cassiman et al., 2013).

1.1.2.3. Orbital plexiform neurofibromas

Orbital plexiform neurofibromas are characterized by eyelid swelling and mechanical ptosis. Although histologically benign, these hamartomas can lead to significant visual impairment and ocular motility issues due to their expansive growth (Cassiman et al., 2013). Interestingly, congenital glaucoma is often diagnosed in the eye on the affected side, although the underlying reasons for this association remains unclear (Cassiman et al., 2013).

Orbital neurofibromas often present with skull deformities, particularly dysplasia or absence of the greater wing of the sphenoid, which can lead to direct communication between the orbit and the middle cranial fossa, potentially causing pulsating exophthalmos or enophthalmos due to intracranial movement of orbital content. The natural history of these lesions is not well understood, but they typically exhibit growth during childhood, with surgical intervention tailored to the patient's residual visual function and the extent of bony and soft tissue involvement, guided by limited case studies (Cassiman et al., 2013).

1.1.3. Skeletal manifestations

Skeletal abnormalities associated with NF1 affect approximately 50% of individuals, presenting in early childhood and including short stature, scoliosis, chest-wall deformities, osteopenia, osteoporosis, localized bone dysplasia (sphenoid wing, long bone), and pseudoarthrosis (a false joint from long bone fracture non-union), which can impact mobility and quality of life significantly (Table 1) (Lammert et al., 2005; Solares et al., 2022; De Lucia et al., 2011; Elefteriou et al., 2009; Ly et al., 2019; Wang et al., 2021). Macrocephaly is commonly observed in patients with NF1 in the absence of congenital hydrocephalus; however, acquired hydrocephalus may develop as a result of aqueductal stenosis (Miller et al., 2019). Postnatal growth delays may be seen in one-third of children, along with a slightly reduced pubertal growth spurt, although growth velocity typically remains normal. Scoliosis occurs in 21% to 49% of patients and often progresses rapidly, while long-bone dysplasia can lead to tibial bowing and pseudarthrosis (Anderson et al., 2020; Nastase et al., 2021; De Lucia et al., 2011). Anterolateral tibial bowing and

tibial dysplasia are present in about 5% of NF1 patients (Nastase et al., 2021). Additionally, both children and adults with NF1 demonstrate decreased bone mineral content compared to age-matched controls, with osteoporosis developing earlier, especially in postmenopausal women (De Lucia et al., 2011). Nevertheless, fracture risk is only slightly elevated, often related to inadequate vitamin D levels (Miller et al., 2019).

1.1.4. Neurological complications

Neurological and cognitive complications are among the most common clinical features in individuals with NF1, encompassing a wide spectrum of structural, functional, and developmental manifestations. Patients are more prone to headaches (particularly migraines) and seizures (often secondary to structural lesions) than the general population (Miller et al., 2019). Other notable neurological involvements include brain gliomas, intracranial malformations, aneurysms, and moyamoya syndrome (Sánchez et al., 2022; Scala et al., 2021; Kang et al., 2022). Mild motor developmental delays, speech and language difficulties are also commonly reported; while these may improve with therapy, social communication challenges and heightened anxiety often persist (Sánchez et al., 2022; Miller et al., 2019). Moreover, cognitive impairments are especially prevalent in children with NF1, with up to 81% affected (Sánchez et al., 2022; Miller et al., 2019). Learning disabilities affect up to 75% of cases, particularly in mathematics and reading (Ly et al., 2019; Wang et al., 2021). Attention-deficit/hyperactivity disorder (ADHD) is diagnosed in approximately 40% of children according to some sources (Sánchez et al., 2022; Isenberg et al., 2013; Domon-Archambault et al., 2018), whereas others report rates as high as 50% (Table 1) (Miller et al., 2019). Intellectual disability (ID) (IQ <70) occurs in 4–8% of individuals, a slight increase compared to the general population (De Lucia et al., 2011; Elefteriou et al., 2009). These findings emphasize the need for early screening, supportive interventions, and long-term educational and psychological support.

1.1.4.1. Characteristic MRI Findings

Magnetic resonance imaging (MRI) plays a central role in the evaluation of neurological involvement such as optic nerve gliomas and brain tumours in NF1 (Sánchez et al., 2022; Kang et al., 2022). A characteristic finding is the presence of T2-weighted hyperintense lesions—historically termed unidentified bright objects (UBOs), now more commonly referred to as focal areas of signal intensity (FASIs) (Sánchez et al., 2022; Zhang et al., 2015; Kang et al., 2022). These lesions, typically located in the basal ganglia, brainstem,

and cerebellum, emerge between ages 2 and 10 and usually regress by early adulthood (Table 1) (Miller et al., 2019; Anders et al., 2022; Pillay-Smiley et al., 2023). Although their clinical significance remains uncertain, they are frequently observed in NF1 (Sánchez et al., 2022; Zhang et al., 2015; Kang et al, 2022). If a T2 hyperintensity demonstrates contrast enhancement or mass effect, a low-grade glioma must be considered. Additional MRI-detectable abnormalities in NF1 include optic pathway gliomas and intracranial vascular malformations such as aneurysms or moyamoya syndrome. MRI thus remains the imaging modality of choice for identifying and monitoring brain involvement in NF1 (Sánchez et al., 2022; Kang et al., 2022).

1.1.5. Cardiological and vascular involvement

Individuals with NF1 are associated with a range of congenital cardiac anomalies, vascular stenoses or aneurysms, and cerebrovascular lesions. The most common issues include renal artery stenosis, leading to elevated hypertension in paediatric patients, essential hypertension, and congenital heart defects, particularly pulmonic stenosis. Cerebrovascular abnormalities occur in 2.5% to 6% of children with NF1, commonly manifesting as stenotic lesions in cerebral arteries, which may progress to increased stroke risk, particularly in those with Moyamoya syndrome (Miller et al., 2019).

1.1.6. Tumours associated with NF1

NF1 is associated with a decreased life expectancy, typically reduced by 8-15 years compared to the general population, primarily due to cardiovascular complications and malignancies. The incidence of cancer in patients with NF1 is five to ten times higher than in the general population (Solares et al., 2022; Perrino et al., 2024; Kratz et al., 2015). The most frequent neoplasms in children include malignant peripheral nerve sheath tumours, low- to high-grade gliomas, pheochromocytomas, and leukaemia (Miller et al., 2019; Solares et al., 2022).

1.1.6.1. Malignant peripheral nerve sheath tumours

Malignant peripheral nerve sheath tumours (MPNSTs) are aggressive soft tissue sarcomas that typically arise from malignant transformation of existing plexiform or rarely nodular neurofibromas in 8% to 13% of NF1 patients (Table 1) (Miller et al., 2019, Solares et al., 2022; Perrino et al., 2024). These tumours, commonly affect the limbs and trunk.

MPNSTs are locally aggressive neoplasms with a high potential for metastasis and are a leading cause of mortality in NF1 (Miller et al., 2019; Solares et al., 2022).

1.1.6.2. Central nervous system tumours

Central nervous system (CNS) tumours, primarily low-grade pilocytic astrocytomas (most commonly optic pathway gliomas), are among the most frequent neoplasms and significant causes of mortality in NF1 (Solares et al., 2022). While optic pathway gliomas are the most prevalent, low-grade gliomas may also occur in other locations (~5%), such as the basal ganglia, cerebellum, or brainstem. Rarely, high-grade gliomas can develop at younger ages (1–2%). These tumours may be asymptomatic or present with increased intracranial pressure.

1.1.6.3. Breast cancer

Women with NF1 exhibit a significantly increased risk of developing breast cancer at an earlier age than the general population, with those under 50 years experiencing a fivefold increased risk, more advanced disease at diagnosis, and higher mortality rates (Solares et al., 2022). In contrast, the risk of breast cancer in men with NF1 appears to be low (Solares et al., 2022).

1.1.6.4. Pheochromocytoma

Pheochromocytomas are catecholamine-secreting tumours originating from the chromaffin cells of the adrenal medulla, which, although rare in the general population, are more common in individuals with NF1 (Solares et al., 2022; Perrino et al., 2024).

1.1.6.5. Gastrointestinal stromal tumours

Gastrointestinal stromal tumours (GISTs) are uncommon mesenchymal neoplasms that occur more frequently in patients with NF1 than in the general population, primarily affecting the small intestine, particularly the jejunum and ileum (Solares et al., 2022).

1.1.6.6. Other tumours

Patients with NF1 are at increased risk of developing characteristic malignant neoplasms during paediatric ages, such as embryonal rhabdomyosarcoma and juvenile myelomonocytic leukaemia, with a poorer prognosis compared to the general population. Other notable tumours include undifferentiated pleomorphic sarcoma, melanoma, and ovarian cancer (Solares et al., 2022; Miller et al., 2019).

1.1.7. Other features

According to the multisystem involvement - although their presence is not critical for diagnosis - several other clinical symptoms can be observed, such as craniofacial features (macrocephaly and hypertelorism), short stature, hormonal imbalances (e.g., hyperthyroidism or endocrine tumours), and hypertension (Scala et al., 2021; Wang et al. 2021; Sánchez et al., 2022). Even a phenotypic overlap with characteristic features of Noonan syndrome, a distinctive NF1 clinical variant known as neurofibromatosis-Noonan syndrome (NFNS) exists (Scala et al., 2021).

2. Aims and Objectives

This doctoral study aims to provide a comprehensive clinical and genetic characterization of paediatric patients diagnosed with NF1, who were examined as inpatient and/or outpatient at the Heim Pál National Paediatric Institute, Budapest, Hungary, between January 1, 2010 and January 31, 2025. The analysis is based on a retrospective review of medical records, including clinical, radiological and molecular genetic data. The overarching aim is to enhance diagnostic accuracy and develop evidence-based surveillance strategies for NF1 patients, and to establish genotype–phenotype correlations with a focus on mutation-specific risk profiles and age-dependent clinical expression patterns.

The specific objectives of the study are as follows:

1. To characterize the clinical spectrum of the Hungarian paediatric NF1 population in comparison with international cohorts, with particular emphasis on the age of onset and overall prevalence of the major diagnostic features.
2. To investigate genotype–phenotype associations across different NF1 variant types (frameshift, nonsense, splice-site, missense, and copy number variants), with the aim of stratifying the spectrum of phenotypic severity.
3. To develop a practical diagnostic and follow-up protocol tailored to paediatric NF1, integrating observed clinical features, genetic findings, and current international recommendations.

Based on these objectives, the following hypotheses were formulated:

1. We hypothesize that the clinical spectrum of the Hungarian paediatric NF1 population is largely comparable to that of international cohorts, particularly regarding the prevalence and age-related appearance of the diagnostic criteria.
2. We hypothesize that, within the Hungarian NF1 cohort, after large deletions (CNVs), splice-site variants are associated with the most pronounced and severe clinical phenotypes compared with other variant types.
3. We hypothesize that, based on the clinical and genetic characteristics of the Hungarian paediatric NF1 population, a diagnostic and follow-up protocol can be

developed that is better tailored to the national patient cohort than approaches relying solely on international recommendations.

3. Materials and Methods

3.1. Study Design and Patient Cohort

This retrospective study was conducted at the Heim Pál National Paediatric Institute (Budapest, Hungary) and was approved by the institutional ethics committee (project no. KUT—45/2024). The analysis included patients evaluated between January 1, 2010, and January 31, 2025, based on outpatient or inpatient clinical records.

Two complementary subcohorts were established during the course of the study. The first group, primarily comprising hospitalized patients, included 204 individuals evaluated up to October 30, 2024. The second cohort extended the dataset until January 31, 2025, and also incorporated outpatients, resulting in a total of 262 individuals assessed for NF1 or multiple café-au-lait macules (CALMs).

Following the application of inclusion and exclusion criteria—such as the presence of fewer than six CALMs without other NF1 features, or an alternative diagnosis (e.g., Peutz–Jeghers syndrome, tuberous sclerosis)—231 patients met at least one NIH diagnostic criterion for NF1, of whom 175 fulfilled at least two criteria and were included in the final clinical analysis. These 175 patients (83 males, 92 females; mean age 15.2 years, range 1–35 years) represented the core cohort for detailed clinical characterization.

3.2. Clinical Data Collection

The analysis encompassed clinical, genetic, and radiological evaluations, based on a comprehensive review of medical records. Dermatological examination was conducted in all patients. In addition, neurological, ophthalmologic, orthopaedic, and audiological evaluations were routinely performed. Depending on clinical severity, patient age, and specific symptoms, the following investigations were also undertaken: cerebral and spinal MRI, chest and abdominal MRI, abdominal ultrasonography, skeletal X-ray survey, echocardiography, and psychological assessment. Due to the retrospective nature of the analysis, not all examinations were conducted in every patient.

The age distribution of the clinically diagnosed NF1 cohort was as follows: 0–6 years (n=29), 7–12 years (n=43), 13–18 years (n=39), and over 18 years (n=64). Although some individuals had reached adulthood by the time of data collection, all were originally managed within the paediatric care system, and data were collected retrospectively.

3.3. Genetic Testing and Variant Classification

Genetic testing was performed in a subset of patients with a clinical diagnosis of NF1. Due to limited national access to molecular diagnostics, genetic analysis was conducted in 88 of the 175 patients who fulfilled at least two NIH diagnostic criteria. Of the 88 tested individuals, 70 (79.5%) received a positive molecular diagnosis, 12 yielded negative results, and 6 had inconclusive findings, including two extended panel tests still in progress.

Genetic analysis for the study cohort was conducted using two distinct sequencing techniques. For approximately half of the patient samples, Next-Generation Sequencing (NGS) was employed. This high-throughput approach allowed for the comprehensive examination of multiple gene regions simultaneously, providing a broad overview of potential genetic variants. The remaining patient samples underwent sequencing via the Sanger method, a gold standard for accuracy in validating specific gene mutations. This technique was utilized to confirm variants identified by NGS and to ensure the fidelity of sequencing results in targeted gene regions. By integrating both NGS and Sanger sequencing methods, we ensured robust and reliable detection of genetic variants, enhancing the overall validity and precision of our findings. Testing was performed at the Department of Medical Genetics, University of Pécs, following written informed consent.

Detected variants were classified into five molecular categories: nonsense, frameshift, missense, splice-site, and copy number variants (CNVs), and were interpreted according to ACMG/AMP (American College of Medical Genetics and Genomics / Association for Molecular Pathology) guidelines.

Genotype–phenotype correlation analyses were conducted exclusively within a well-defined subcohort of 204 patients evaluated between January 1, 2010, and October 30, 2024. Of these, 148 individuals met at least two NIH diagnostic criteria, and 70 underwent genetic testing. In this subgroup, 58 index cases (83%) were confirmed to carry a pathogenic or likely pathogenic NF1 variant. Of these, 13 were excluded due to lack of consent or unavailable data. The remaining 45 individuals with confirmed and available pathogenic variants formed the basis of the genotype–phenotype correlation study.

Of note, among the 12 patients with negative genetic results in the subcohort, clinical suspicion of NF1 remained high. For these individuals, further testing—such as whole-

gene sequencing, RNA analysis, or extended gene panels—is planned or underway to clarify the molecular background. However, in pediatric cases, the possibility of alternative NF1-like or overlapping conditions must also be carefully considered, as diagnostic accuracy is often limited at an early age; therefore, differential diagnostic evaluation in this direction is also warranted (Komlósi et al. 2011, Pinti E. et al, 2021).

Among the 45 genetically confirmed cases, single nucleotide variants (SNVs) were identified in 40 individuals (88.9%), and CNVs in 5 (11.1%). In 17 patients (37.7%), small insertions, deletions, or indels resulting frameshifts were identified, in 11 patients (24.4%) nonsense mutations, in 8 patients (17.8%) splice-site mutations, and in 4 patients (8.9%) missense variants. This genetically characterized subgroup served as the basis for all genotype–phenotype correlation analyses presented in this study.

3.3.1. Additional molecular analyses in a patient with extensive plexiform neurofibroma

In one genetically confirmed NF1 case presenting with an exceptionally severe and segmentally distributed plexiform neurofibroma, extended molecular investigations were performed on DNA extracted from both peripheral blood leukocytes and tumour tissue.

3.3.1.1. Next-Generation Sequencing-based multi-gene panel sequencing

Targeted sequencing of the *NF1, NF2, RAF1, KIT, SPRED1, SMARCB1,* and *PTPN11* genes was carried out using a custom-designed QIAseq DNA panel (Qiagen, Hilden, Germany). Library preparation followed the manufacturer's instructions, and sequencing was performed on an Illumina MiSeq platform (Illumina, San Diego, CA, USA) with paired-end 150 bp reads.

3.3.1.2. Multiplex ligation-dependent probe amplification

Multiplex ligation-dependent probe amplification (MLPA) assays (SALSA MLPA probemixes P081-D1 and P082-C2; MRC-Holland, Amsterdam, The Netherlands) were used to detect large deletions or duplications in the *NF1* gene. Probes covered all exons and selected upstream, downstream, and intronic regions, including the *OMG* gene located within intron 36 of *NF1*. Analyses were performed on 100–200 ng DNA from both peripheral blood and plexiform neurofibroma tissue, with three control genomic DNA samples. Capillary electrophoresis was conducted on an ABI 3130 Genetic

Analyzer (Life Technologies, Carlsbad, CA, USA), and Coffalyser software (MRC-Holland) was used for data interpretation. A signal ratio deviation exceeding 30% was considered indicative of copy number variation.

3.3.1.3. Whole exome sequencing

Whole exome sequencing (WES) was performed on DNA from unaffected skin, peripheral blood, and a second plexiform neurofibroma sample. Libraries were prepared using the Illumina DNA Preparation with Enrichment Kit and sequenced on an Illumina NovaSeq 6000 platform with paired-end 100 bp reads. The mean coverage depth across target regions was 100.4×–123.5×. Reads were aligned to the GRCh37/hg19 reference genome using Burrows–Wheeler Aligner, and processed with GATK algorithms (Sentieon Inc., San Jose, CA, USA) for duplicate removal, local realignment, base quality recalibration, and variant calling. Variant analysis focused on the *NF1* gene, with coverage statistics calculated from aligned reads.

3.4. Statistical Analysis

Due to the limited number of genetically characterized cases, formal statistical hypothesis testing was not performed. Instead, descriptive analyses were applied to evaluate the distribution of clinical features across different age groups, sexes, and NF1 variant types. Genotype–phenotype correlations were explored by comparing the relative frequency of specific clinical manifestations within each molecular variant category. Results are presented as proportions and percentages.

4. Results

4.1. The clinical spectrum of the Hungarian paediatric NF1 population

We retrospectively reviewed data from a cohort of 262 individuals diagnosed with multiplex CALMs or NF1 at the Heim Pál National Paediatric Institute, between January 1, 2010, and January 31, 2025. During the analysis, 31 patients were excluded for having fewer than six CALMs without any additional clinical signs of NF1, as well as those diagnosed with alternative conditions such as Peutz-Jeghers syndrome or tuberous sclerosis, and those with insufficient material for analysis. The remaining 231 individuals met at least one diagnostic criterion for NF1 according to the National Institutes of Health (NIH) criteria, of which 175 patients fulfilled at least two criteria for diagnosis. Among the 175 patients with positive clinical diagnoses, 88 subjects were analysed by genetic test to prove the diagnosis. Of these 88 patients, 70 received positive genetic test results, while 12 had negative results. Two of the negative results were sent for an extended panel examination, which is still in progress, and an additional four genetic test results had not yet been returned by the time of this analysis.

A total of 175 patients with a confirmed diagnosis of Neurofibromatosis type 1 (NF1) were included in this study. The cohort comprised 83 males (47.4%) and 92 females (52.6%), with a mean age of 15.2 years (range 1-35 years). The age distribution was as follows: 0-6 years (n=29), 7-12 years (n=43), 13-18 years (n=39), and >18 years (n=64) at the time of our study; however, because this is a retrospective analysis of a paediatric hospital database, most of the data were collected while the patients were still in their paediatric years.

Table 2. Prevalence of Clinical Features in NF1 Patients in the following age groups: 0-6 years, 7-12 years, 13-18 years, and over 18 years.

	All NF1 patients (N=175)	0–6 years (N=29)	7–12 years (N=43)	13–18 years (N=39)	Over 18 years (N=64)
Male:female	83:92 (47.4%):(52.6%)	16:13 (55.2%):(44.8%)	27:16 (62.8%):(37.2%)	18:21 (46.2%):(53.8%)	22:42 (34.4%):(65.6%)
≥6 CALMs	175 (100%)	29 (100%)	43 (100%)	39 (100%)	64 (100%)
Skinfold freckling	50 (28.6%)	5 (17.2%)	13 (30.2%)	9 (23%)	23 (35.9%)
Neurofibromas	62 (35.4%)	1 (3.4%)	16 (37.2%)	15 (38.5%)	30 (46.9%)
Plexiform neurofibromas	31 (17.7%)	0 (%)	9 (20.9%)	9 (23%)	13 (20.3%)
Lisch nodules	63 (36%)	5 (17.2%)	12 (27.9%)	19 (48.7%)	27 (42.2%)
Optic pathway gliomas	20 (11.4%)	1 (3.4%)	7 (16.3%)	5 (12.8%)	7 (10.9%)
Skeletal manifestations	67 (38.2%)	8 (27.6%)	21 (48.8%)	17 (43.6%)	21 (32.8%)
Scoliosis	35 (20%)	0 (0%)	7 (16.3%)	13 (33.3%)	15 (23.4%)
Neurological complications (seizures, migrain)	13 (7.4%)	0 (0%)	3 (6.9%)	1 (2.6%)	9 (14%)
Cognitive/learning disabilities, behavioural disturbances	44 (25.1%)	3 (10.3%)	12 (27.9%)	13 (33.3%)	18 (28.1%)
Fine motor disturbances, motor developmental delays, coordination disorders	20 (11.4%)	1 (3.4%)	9 (20.9%)	6 (15.4%)	4 (6.3%)
NF1 specific brain MRI finding (FASI)	98 (56%)	9 (31%)	31 (72%)	28 (71.8%)	30 (46.9%)
Intracranial LOW GRADE glioma (optic glioma not included)	14 (8%)	2 (6.9%)	4 (9.3%)	4 (10.3%)	4 (6.3%)
Conductive hear impairment	9 (5.1%)	3 (10.3%)	3 (6.9%)	2 (5.1%)	1 (1.6%)
Endocrine involvement	3 (1.7%)	0 (0%)	1 (2.3%)	2 (5.1%)	0 (0%)
Cardiological manifestation	11 (6.3%)	1 (3.4%)	3 (6.9%)	4 (10.3%)	3 (4.7%)
Macrocephalia	7 (4%)	2 (6.9%)	3 (6.9%)	1 (2.6%)	1 (1.6%)
Hypertonia	3 (1.7%)	1 (3.4%)	0 (0%)	1 (2.6%)	1 (1.6%)
Arnold Chiari malformation	1 (0.6%)	0 (0%)	0 (0%)	1 (2.6%)	0 (0%)
Pheochromocytoma	1 (0.6%)	0 (0%)	0 (0%)	0 (0%)	1 (1.6%)
MPNST, death	1 (0.6%)	0 (0%)	0 (0%)	1 (2.6%)	0 (0%)
Adnex tumour	1 (0.6%)	0 (0%)	0 (0%)	0 (0%)	1 (1.6%)
First degree relative	25 (14.3%)	5 (17.2%)	9 (20.9%)	6 (15.4%)	5 (7.8%)

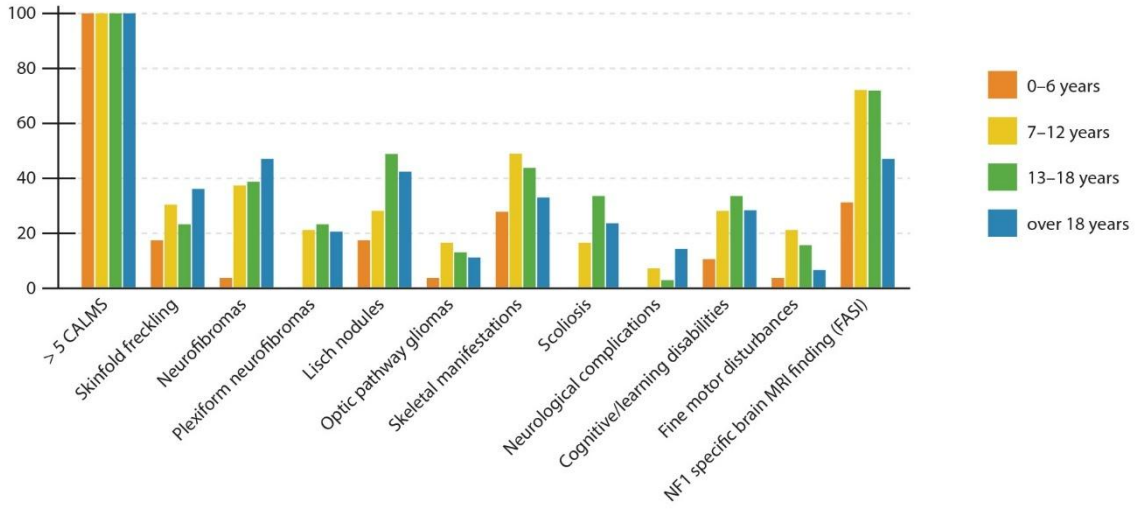

Figure 2. Percentage distribution of the frequency of the most common NF1-related clinical features in our cohort in the following age groups: 0-6 years, 7-12 years, 13-18 years, and over 18 years.

4.1.1. Cutaneous Involvements

CALMs were detected in all patients who fulfilled at least two diagnostic criteria for NF1. Freckling was observed in 28.6% of our patients (50 out of 175), with the lowest prevalence of 17.2% (5 out of 29) in the 0–6-year age group, while it was most prevalent in the adult population at 35.9% (23 out of 64). Neurofibromas (35.4% overall, 62/175) began to appear more frequently in the 7–12-year age group (37.2%, 16/43), with their prevalence steadily increasing to a maximum of 46.9% (30/64) in the adult population. Plexiform neurofibromas (Figure 3D) were absent in the youngest age group, while their prevalence was approximately 20% in the other age groups (Figure 2, Table 2).

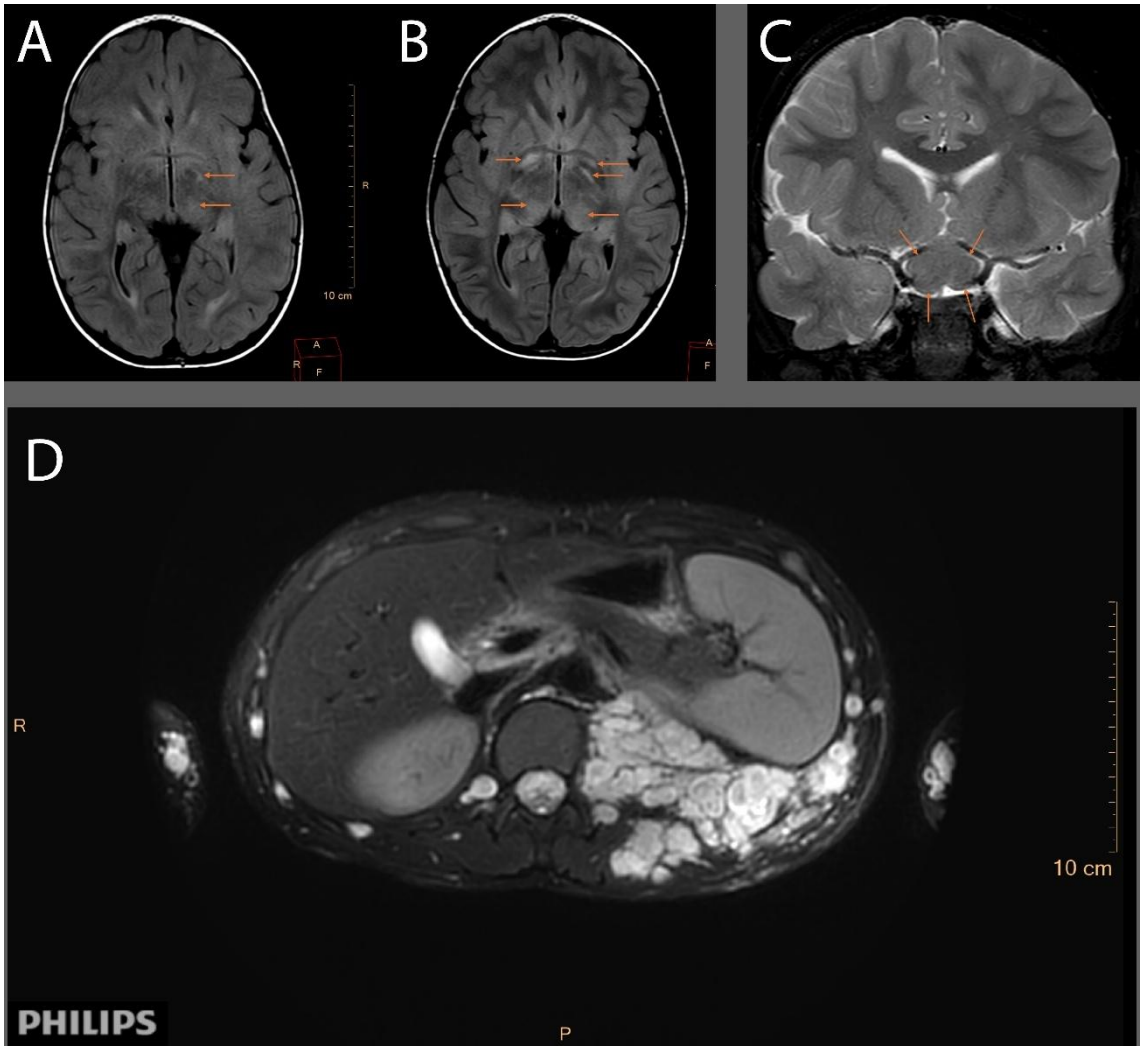

Figure 3. A-B. Axial T2 FLAIR MR images at the level of basal ganglia taken at the age of 2 years (A) and at the age of 5 years (B) showing multiple bilateral foci of high signal areas in the lentiform nuclei and thalami (arrows). The number and intensity of the lesions have progressed in the follow-up period.

C. 5 years old boy. Coronal T2 weighted MR image showing an isointense mass enlarging the optic chiasm: optic pathway glioma (arrows).

D. 14 years old boy. Axial T2 weighted MR image at the level of lower chest wall. Bulky T2 hyperintense mass in the left dorsal chest wall following the course of intercostal nerves represents a typical plexiform neurofibroma (arrows).

4.1.2. Ocular Manifestations

This study identified Lisch nodules in 36% of patients overall (63/175), with an age-dependent prevalence ranging from 17.2% (5/29) in the 0–6-year age group to 42.2% (27/64) in those older than 18 years. Optic pathway gliomas (Figure 3C) were observed in 11.4% of patients (20/175), exhibiting the highest prevalence (16.3%, 7/43) within the 7–12-year age group (Figure 2, Table 2).

4.1.3. Skeletal Manifestations

The prevalence of skeletal malformations varied across age groups: 27.6% (8/29) in the 0–6-year group, 48.8% (21/43) in the 7–12-year group, and 43.6% (17/39) (in the 13–18-year group. Scoliosis, representing 20% (35/175) of the total cohort, demonstrated the highest prevalence (33.3%, 13/39) in the 13–18-year age group (Figure 2, Table 2).

4.1.4. Neurological Complications

Neurological complications, such as seizures and migraine, were relatively infrequent (7.4%, 13/175), while cognitive/learning disabilities and behavioural disturbances were more prevalent (25.1%, 44/175), with the highest rate (33.3%, 13/39) observed in the 13–18-year age group. Fine motor and coordination difficulties affected 11.4% of patients (20/175). Characteristic NF1-associated brain MRI findings (FASI) were identified in 56% of patients (98/175) (Figure 3A-B), increasing to approximately 72% (31/43) in the 7–18-year age range. Low-grade gliomas (excluding optic pathway gliomas) were detected in 8% of patients (14/175) (Figure 2, Table 2).

4.1.5. Other findings

In contrast to NF2, where sensorineural hearing loss is primarily associated with acoustic neuroma, the examined patient group demonstrated conductive hearing loss in 9 subjects (5.1%). Among these, 3 were in the under-6 age group (10.3%), 3 were in the 7-12 age range (6.9%), 2 were aged 13-18 (5.1%), and 1 was over 18 years old (1.6%). Additionally, there was one case of sensorineural hearing loss observed (1/175, 0.57%) (Figure 2, Table 2).

Endocrine involvement, cardiological manifestations, macrocephaly, hypertonia, and Arnold-Chiari malformation were observed in a small number of cases, and a single case of pheochromocytoma was also detected. One patient developed a malignant peripheral

nerve sheath tumour (MPNST), ultimately leading to death at the age of 18 years old (Figure 2, Table 2).

A positive family history of NF1 was reported in 14.3% of patients (25/175).

4.2. Genotype-phenotype associations across different NF1 variant types (frameshift, nonsense, splice-site, missense, and copy number variants)

In accordance with our second hypothesis, we conducted a genotype–phenotype correlation analysis. In this part of the study, we retrospectively reviewed data from a cohort of 204 individuals meeting at least 1 diagnostic National Institutes of Health (NIH) criterion for NF1. Among these, 148 subjects fulfilled ≥2 NIH criteria. Genetic examination was undertaken in 70 individuals, of whom 58 index cases were confirmed to have an *NF1* pathogenic variant. Out of the 58 positive tests, 13 were not available for inclusion in this study. In 12 patients, no pathogenic NF1 variant was detected during the initial genetic test. An extended genetic test is planned for these individuals in the future (Figure 4).

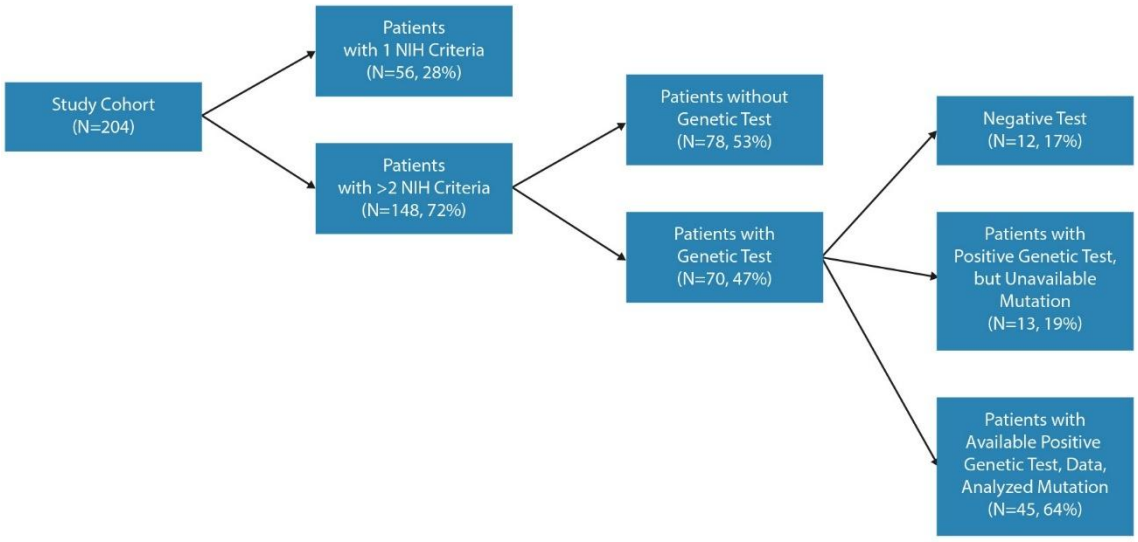

Figure 4. Number of patients involved in our study.

The age of enrolled subjects ranged from 1 to 33 years (mean 16 years) with 135 patients (66.2%) being in the paediatric age group (≤18 years). Age distribution showed 78 patients (38.2%) under 12 years of age (49 males, 29 females), 57 patients (27.9%)

between 12 and 18 years of age (28 males, 29 females), and 69 patients (33.8%) over 18 years of age (30 males, 39 females) (Figure 5). Among the 204 patients, 107 (52.5%) were male, and 97 (47.5%) were female. Positive genetic testing was present in a subset of 58 patients. In this group, there were 32 males (55.2%) and 26 females (44.8%). Among these 58 patients 45 subjects had pathogenic variants available for us to include in our research, of whom 21 were male (46.7%) and 24 were female (53.3%) (Figure 5).

Figure 5. Age and Gender Distribution in NF1 Patients of our Cohort: (**a**) Gender Distribution of our Cohort, (**b**) Gender Distribution of Patients with Positive Genetic Test, (**c**) Gender Distribution of Patients with Available Positive Mutation analysed in the study, (**d**) Gender Distribution of Patients under 12 years of age, (**e**) Gender Distribution of Patients between 12 and 18 years, (**f**) Gender Distribution of Patients over 18 years of age.

Among the 45 patients with detected pathogenic mutation, causative single nucleotide variants (SNVs) were detected in 40/45 (88,89%), copy number variations (CNVs) in

5/45 (11,1%) cases. We identified 17 (37.7%) frameshifting small insertions, deletions or indels, 11 (24.4%) nonsense mutations, 8 (17.8%) splice site mutations, and 4 (8.9%) missense variants (Figure 6).

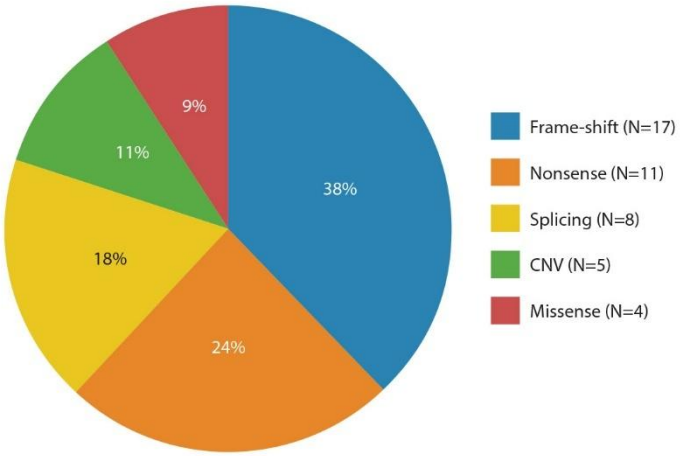

Figure 6. Relative proportion of the different types of variants among the *NF1*-mutations identified.

The analysis revealed some significant associations between specific NF1 mutations and various clinical manifestations.

Table 3. Prevalence of Clinical Features in NF1 Patients in the following age groups: under 12 years, from 12 to 18 years, and over 18 years.

	All positive genetic test results (N=58)	Under 12 years (N=28)	12-18 years (N=20)	Over 18 years (N=10)
Subjects with positive genetic test	58 (100%)	28 (100%)	20 (100%)	10 (100%)
Male:female	32:26 (55.2%:44.8%)	18:10 (64.3%:35.7%)	10:10 (50%:50%)	4:6 (40%:60%)
≥6 CALMs	58 (100%)	28 (100%)	20 (100%)	10 (100%)
Skinfold freckling	15 (25.9%)	7 (25%)	3 (15%)	5 (50%)
Neurofibromas	16 (27.6%)	7 (25%)	8 (40%)	1 (10%)
Plexiform neurofibromas	11 (19%)	5 (17.9%)	5 (25%)	1 (10%)
Malignant peripheral nerve sheath tumor	1 (1.7%)	0 (0%)	0 (0%)	1 (10%)
Death	1 (1.7%)	0 (0%)	0 (0%)	1 (10%)
Lisch nodules	19 (32.8%)	6 (21.4%)	7 (35%)	6 (60%)
Optic glioma	9 (15.5%)	2 (7.1%)	5 (25%)	2 (20%)
Skeletal abnormalities	16 (27.6%)	4 (14.3%)	9 (45%)	3 (30%)
Pseudarthrosis	4 (6.9%)	1 (3.6%)	2 (10%)	1 (10%)
NF1 specific brain MR findings	34 (58.6%)	17 (60.7%)	12 (60%)	5 (50%)
Seizures	3 (5.2%)	0 (0%)	1 (5%)	2 (20%)
Cognitive/learning disabilities, behavioural disturbances	13 (22.4%)	4 (14.3%)	5 (25%)	4 (40%)
Fine motor disturbances, motor developmental delay	11 (19%)	5 (17.9%)	5 (25%)	1 (10%)
Macrocephaly, craniofacial dysmorphism	5 (8.6%)	4 (14.3%)	0 (0%)	1 (10%)
Endocrinology	1 (1.7%)	0 (0%)	0 (0%)	1 (10%)
Cardiologic involvement	4 (6.9%)	2 (7.1%)	2 (10%)	0 (0%)
First degree relative	6 (10.3%)	3 (10.7%)	3 (15%)	0 (0%)

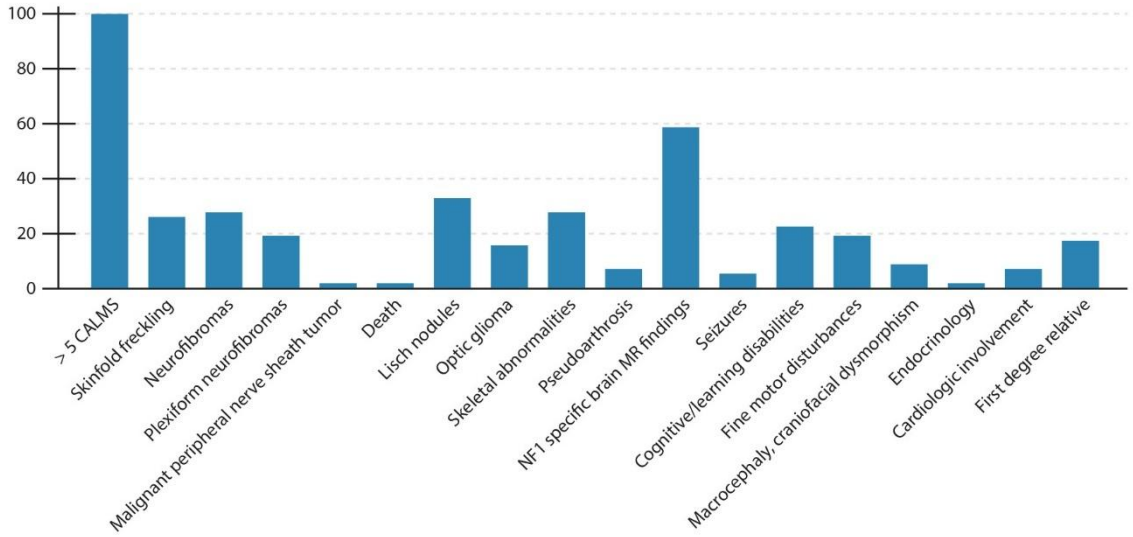

Figure 7. Percentage distribution of the frequency of the most common NF1-related clinical features in the cohort of patients with positive test results.

Table 4. Prevalence of Clinical Features in Neurofibromatosis Type 1 Patients Classified by Frameshift, Nonsense, Splice-Site, CNV, and Missense Mutations (Percentages calculated within each variant subgroup).

	Mutation-positive individuals (N=58)	Available, analyzed mutations (N=45)	Frame-shift (N=17)	Nonsense (N=11)	Splicing (N=8)	CNV (N=5)	Missense (N=4)
Male:female	32:26 (55.2%:44.8%)	21:24 (46.7%:53.3%)	8:7 (53.3%:6.7%)	4:7 (36.4%:63.6%)	4:4 (50%:50%)	1:4 (20%:80%)	3:1 (75%:25%)
≥6 CALMs	58 (100%)	45 (100%)	17 (100%)	11 (100%)	8 (100%)	5 (100%)	4 (100%)
Skinfold freckling	15 (25.9%)	12 (26.7%)	3 (17.6%)	3 (27.3%)	2 (25%)	4 (80%)	0 (0%)
Neurofibromas	16 (27.6%)	15 (33.3%)	4 (23.5%)	4 (36.4%)	3 (37.5%)	4 (80%)	0 (0%)
Plexiform neurofibromas	11 (19%)	8 (17.8%)	4 (23.5%)	0 (0%)	3 (37.5%)	1 (20%)	0 (0%)
Malignant peripheral nerve sheath tumor	1 (1.7%)	1 (2.2%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)
Death	1 (1.7%)	1 (2.2%)	0 (0%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)
Lisch nodules	19 (32.8%)	16 (35.6%)	6 (35.3%)	4 (36.4%)	4 (50%)	2 (40%)	0 (0%)
Optic glioma	9 (15.5%)	6 (13.3%)	3 (17.6%)	1 (9.1%)	1 (12.5%)	1 (20%)	0 (0%)
Skeletal abnormalities	16 (27.6%)	14 (31.1%)	5 (29.4%)	3 (27.3%)	4 (50%)	3 (60%)	0 (0%)
Pseudarthrosis	4 (6.9%)	3 (6.7%)	1 (5.9%)	0 (0%)	2 (25%)	0 (0%)	0 (0%)
NF1 specific brain MR findings	34 (58.6%)	25 (55.6%)	6 (35.3%)	7 (63.6%)	5 (62.5%)	5 (100%)	2 (50%)
Seizures	3 (5.2%)	2 (4.4%)	0 (0%)	2 (18.2%)	0 (0%)	0 (0%)	0 (0%)
Cognitive/learning disabilities, behavioural disturbances	13 (22.4%)	10 (22.2%)	4 (23.5%)	3 (27.3%)	1 (12.5%)	1 (20%)	1 (25%)
Fine motor disturbances, motor developmental delay	11 (19%)	9 (20%)	2 (11.8%)	2 (18.2%)	1 (12.5%)	3 (60%)	1 (25%)
Macrocephaly, craniofacial dysmorphism	5 (8.6%)	3 (6.7%)	2 (11.8%)	0 (0%)	0 (0%)	1 (20%)	0 (0%)
Endocrinology	1 (1.7%)	1 (2.2%)	1 (5.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Cardiologic involvement	4 (6.9%)	3 (6.7%)	2 (11.8%)	1 (9.1%)	0 (0%)	0 (0%)	0 (0%)
First degree relative	10 (17.2%)	5 (11.1%)	3 (17.6%)	1 (9.1%)	1 (12.5%)	0 (0%)	0 (0%)

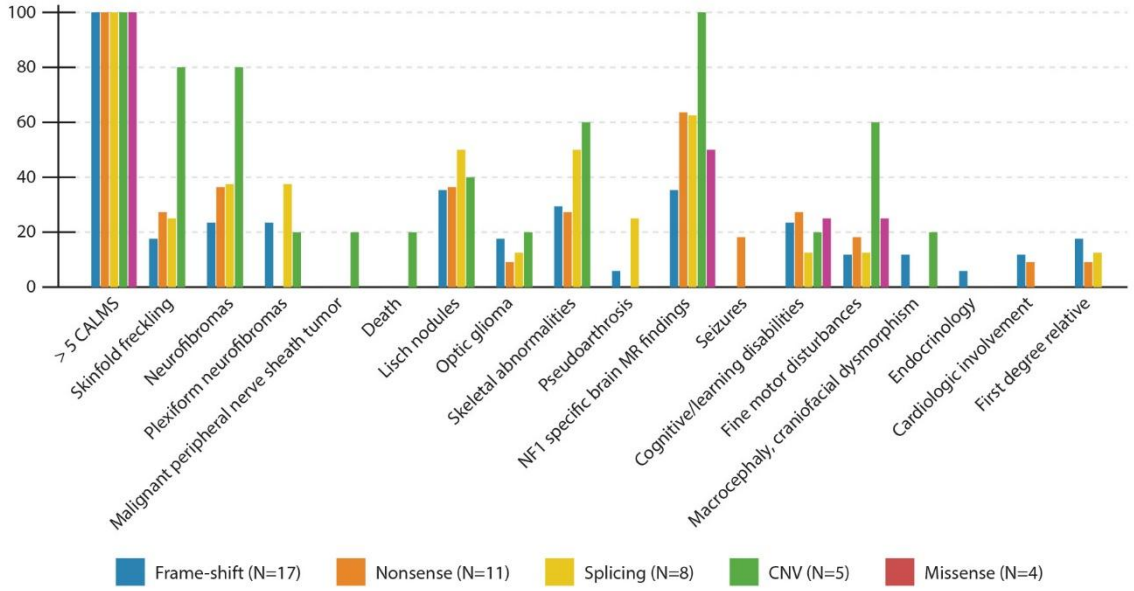

Figure 8. Percentage distribution of the frequency of the most common NF1-related clinical features in the different variant types of our patients. Frameshift (N = 17) (dark blue colour), Nonsense (N = 11) (brown colour), Splice-Site (N = 8) (light blue colour), CNV (N = 5) (green colour), Missense (N = 4) (red colour).

4.2.1. Skin Manifestations

4.2.1.1. CALMS and Freckling

Multiple CALMs were observed in all subjects in our cohort (100%), while skinfold freckling was present in 26% (15/58) and 26.7% (12/45) of the patients. Freckling was identified in 25% (7/28) of children under 12 years of age, 15% (3/20) of adolescents aged 12-18 years, and 50% (5/10) of adults over 18 years of age (Table 3). Examining the mutation types, the highest prevalence of freckling was among patients with CNVs (80%, 4/5), while subjects with other mutation types did not reveal significant correlation (frameshift 3/17, 17.6%, nonsense 3/11, 27.3%, splice site 2/8, 25%). Patients with missense variants negatively correlated with freckling, although just 4 patients belong to this group (Table 4, Figure 8).

4.2.1.2. Neurofibromas and Other Tumours

Neurofibromas were observed in 16/58 (27.6%) of patients, of which 7 was already detected in the youngest generation (25%), 8 in patients at the age of 12-18 years (40%) and 1 in the adult group (10%) (Table 3). Neurofibromas were observed in subjects with

CNV (4/5, 80%), splicing (3/8, 37.5%), nonsense (4/11, 36.4%) and less common in patients with frameshift mutation (4/17, 23.5%). No neurofibroma was detected in subjects with missense variants (0/4, 0%) (Table 4, Figure 8).

Plexiform neurofibromas were less common (19%), with a notable absence in nonsense (0/11) and missense (0/4) mutation carriers. Plexiform neurofibroma was detected in patients with frame shift mutation (4/11, 23.5%), splice site mutation (3/8, 37.5%) and CNV (1/5, 20%) (Table 4, Figure 8). Plexiform neurofibromas were observed in 17.9% (5/28) of patients under 12 years of age, 25% (5/20) of patients aged 12-18 years, and 10% (1/10) of patients over 18 years of age (Table 3).

One patient with microdeletion syndrome developed a malignant peripheral nerve sheath tumour (MPNST), ultimately leading to death. Internal neurofibromas were also seen in this cohort, predominantly in patients with CNV (Table 4, Figure 8).

4.2.2. Ocular Manifestations

Lisch nodules were detected in all age groups with a growing incidence: 6/28, 21.4% in younger than 12 years, 7/20, 35% in patients between 12 and 18 years and 6/10, 60% in the adult population (Table 3). Lisch nodules were found in 32.8% (19/58) and 35.6% (16/45) of patients with positive genetic test, with splice site mutations demonstrating the highest frequency (50%, 4/8) and missense mutation the lowest (0%) (Table 4, Figure 8).

Among patients with a confirmed pathogenic NF1 variant, optic gliomas were reported in 15.5% (9/58) overall, and in 13.3% (6/45) of those included in the clinical correlation analysis. The frequency of optic pathway gliomas was 7.1% (2/28) in the under-12 age group, 25% (5/20) in the 12-18 age group, and 20% (2/10) in the over-18 age group Table 3). Among the mutation types, the highest frequency was in patients with CNV (20%, 1/5) without significancy (Table 4, Figure 8).

4.2.3. Skeletal Manifestations

Skeletal abnormalities were found in 27.6% (16/58) and 31.1% (14/45) of patients with positive genetic test and were associated with whole gene deletions (3/5, 60%), splicing (4/8, 50%), frameshift (5/17, 29.41%) and nonsense variants (3/11, 27.27%) (Table 4, Figure 8). Scoliosis (13/45, 28.9%) and pectus excavatum (6.6%, 3/45) were notable

skeletal manifestations. The age distribution of the research sample was as follows: 14.3% (4/28) of children were under 12 years of age, 45% (9/20) of adolescents aged 12–18 years, and 30% (3/10) of adults were over 18 years of age (Table 3).

Arthralgia or arthritis was not more common in any of the mutation types (whole gene deletions (1/5, 20%), nonsense (2/11, 18.18%), splicing (1/8, 12.5%), and frameshift variants (1/17, 5.8%). Pseudarthrosis was present in 6.9% (4/58) and 6.7% (3/45) of patients, and they were exclusively in splice-site (2/8, 25%) and frameshift variant (1/17, 5.9%) carriers. Valgus deformity was present in one patient with a frameshift mutation (1/17, 5.9%). Patients with the missense variant did not have any skeletal or joint involvement (0/4, 0%) (Table 4, Figure 8).

4.2.4. Neurological and Brain MRI Findings

The presence of specific structural brain lesions found by brain magnetic resonance imaging (MRI) was observed in 58.6% (34/58) of genetically confirmed cases and in 55.6% (25/45) of those with detailed clinical analysis. The prevalence of positive cranial MRI was highest in patients with CNV (5/5, 100%), followed by nonsense (7/11, 63.6%), splicing (5/8, 62.5%), missense (2/4, 50%), and frameshift variants (6/17, 35.3%) (Table 4, Figure 8). Notably, intracranial abnormalities were detected across all age groups, with similar frequencies: 60.7% (17/28) in children under 12 years, 60% (12/20) in adolescents aged 12–18 years, and 50% (5/10) in adults over 18 years (Table 3).

Epilepsy was observed in a small number of patients, affecting 5.2% (3/58) of the genetically confirmed cases and 4.4% (2/45) of those included in the clinical evaluation. In the latter group, both individuals with seizures harboured nonsense variants (2/11, 18.2%) (Table 4, Figure 8). Regarding age distribution, one affected patient was an adolescent (12–18 years; 1/20, 5%), while two were adults over 18 years of age (2/10, 20%) (Table 3).

4.2.5. Learning Disability and Other Cognitive Disorders

Cognitive or learning disabilities and behavioural disturbances were identified in 22.4% (13/58) of genetically confirmed patients and in 22.2% (10/45) of those included in the clinical evaluation. Fine motor disturbances and motor developmental delays were observed in 19% (11/58) and 20% (9/45) of patients, respectively. In the genetically

confirmed group, no significant association was found between cognitive/behavioural symptoms and mutation type (frameshift: 4/17, 23.5%; nonsense: 3/11, 27.3%; splice-site: 1/8, 12.5%; CNV: 1/5, 20%; missense: 1/4, 25%). In contrast, fine motor disturbances and motor delay were more frequently associated with CNVs (3/5, 60%) compared to other variant types (frameshift: 1/17, 11.8%; nonsense: 2/11, 18.2%; splice-site: 1/8, 12.5%; missense: 1/4, 25%) (Table 4, Figure 8).

4.2.6. Other Features

Craniofacial dysmorphism and macrocephaly were observed in 8.6% (5/58) and 6.7% (3/45) of patients, respectively, with a higher frequency among those carrying CNVs (20%, 1/5).

Endocrinological abnormalities were detected in only one patient, who carried a frameshift variant (5.9%, 1/17).

Cardiac involvement was found in 6.9% (4/58) and 6.7% (3/45) of patients. Among these, two had frameshift variants (11.8%) and one had a nonsense variant (9.1%)

First-degree relatives with neurofibromatosis type 1 (NF1) were identified in 10 out of 58 patients (17.2%) with positive genetic test and in 5 out of 45 patients (11.1%) with known mutations. The incidence of positive family history was similar among frameshift (3/17, 17.6%), nonsense (1/11, 9.1%), and splicing (1/8, 12.5%) mutations; however, patients with CNV and missense mutations did not have any first-degree relatives with NF1 (Table 4, Figure 8).

4.2.7. Investigation of the genetic background of an extensive plexiform neurofibroma

Among the genetically confirmed NF1 cases, one patient presented with an exceptionally severe and segmentally distributed plexiform neurofibroma, representing a rare and clinically remarkable phenotype within our cohort. The 12-year-old girl exhibited an extremely extensive plexiform neurofibroma with segmental distribution over the entire right arm, extending to the right shoulder, right chest wall and mediastinum, superimposed on classic cutaneous lesions of NF1 as café-au-lait macules and multiple small cutaneous neurofibromas (Figure 9A-C). The large plexiform neurofibroma was associated with significant functional impairment and cosmetic disfigurement. Phenotypically, additional NF1-related manifestations included Lisch nodules, scoliosis

with pronounced pectus excavatum, and mild left ventricular dysfunction without the need for treatment. No cognitive or behavioural abnormalities were observed. MRI imaging revealed tumour extension from the lung apex to the upper mediastinum along the right side of the thoracic spine, with further lesions tracking the right brachial plexus down to the wrist, infiltrating both muscle and subcutaneous tissues (maximum width: 3.5 cm) (Figure 10A-C). Cranial MRI showed no significant intracranial abnormalities.

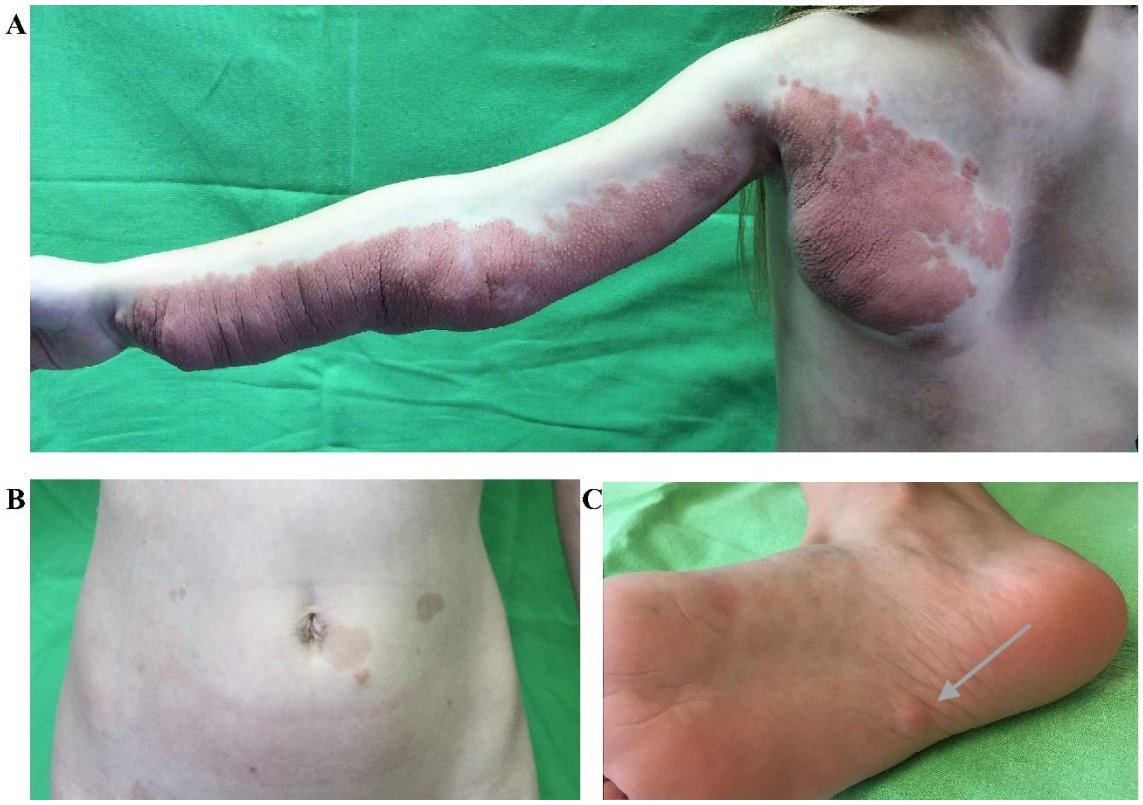

Figure 9. **A**. Well-demarcated, brownish-livid soft tumour mass with several mobile, tender nodules on the medial side of the right arm from the palm to the axillary region with a segmental distribution, extending to the right side of the chest and pronounced pectus excavatum as a representation of plexiform neurofibroma.

B. Multiple, homogeneously hyperpigmented macules on the chest and trunk consistent with CALMs.

C. Small, soft, skin-coloured cutaneous neurofibroma (arrow) on the sole.

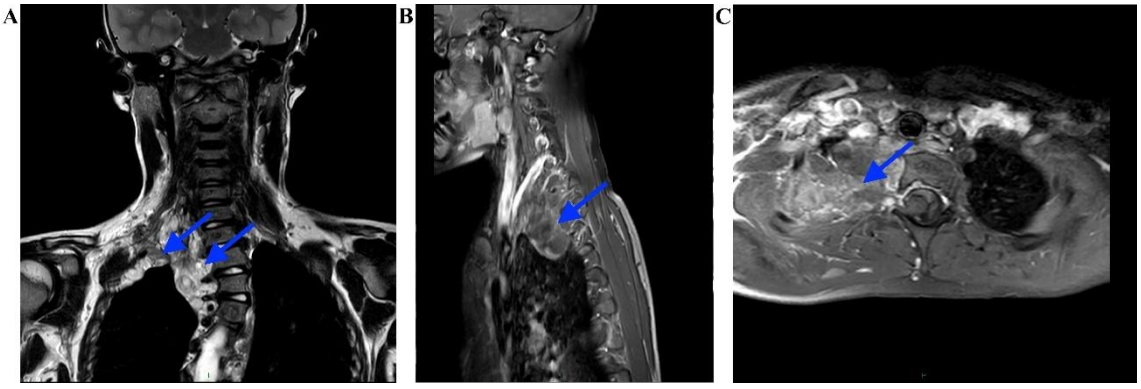

Figure 10. MRI scan of the chest shows a large heterogenous T2 hyperintense tumour (blue arrows) consisting of multiple nodules on the right side of the thoracic spine, extending from the lung apex to the upper mediastinum. Consequential left convex scoliosis is also seen. **A**. T2 weighted, coroneal view. **B**. contrast-enhanced T1, sagittal view. **C**. and contrast-enhanced T1, axial view.

Molecular genetic analysis was performed following pre-test clinical genetic counselling and written informed consent. DNA was extracted from both the plexiform neurofibroma tissue (forearm lesion) and peripheral blood lymphocytes. Targeted next-generation sequencing (NGS) of *NF1, NF2, RAF1, KIT, SPRED1, SMARCB1* and *PTPN11* identified a pathogenic *NF1* splice-site variant (c.2326-2A>G; HGMD Accession Number: CS030307; [NM_001042492.3]) in both tumour and blood DNA, confirming its germline origin (Figure 11). No pathogenic variants were detected in the other analysed genes. The presence of the *NF1* variant was confirmed by Sanger sequencing.

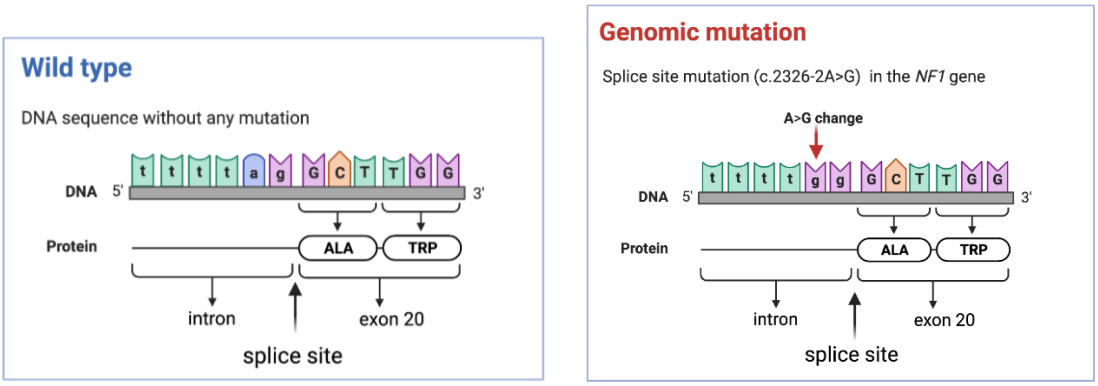

Figure 11. Schematic illustration of the genomic mutation in the NF1 gene: splice-site

mutation (c.2326-2A>G) in the intron before exon 20, compared to wild type. The figure was created with BioRender.com

To investigate a possible second-hit mechanism, multiplex ligation-dependent probe amplification was performed to detect loss of heterozygosity (LOH) due to deletion of one *NF1* allele. No copy number variation was identified in either tumour or blood DNA; however, the sensitivity of MLPA does not allow detection of low-level mosaicism (<20%).

Given the absence of detectable copy number changes, whole-exome sequencing was subsequently undertaken on DNA from unaffected skin, peripheral blood, and a second tumour sample. Bioinformatic analysis filtered for the *NF1* gene revealed a novel frameshift variant, c.4933dupC (p.Leu1645Profs*7) [NM_001042492.3], present exclusively in the plexiform neurofibroma at a variant allele frequency (VAF) of 16% (Figure 12). According to ACMG criteria (PVS1, PM2), this mutation was classified as likely pathogenic. Retrospective re-analysis of the NGS panel data with a modified bioinformatic pipeline also identified this variant in the tumour DNA, supporting its somatic origin.

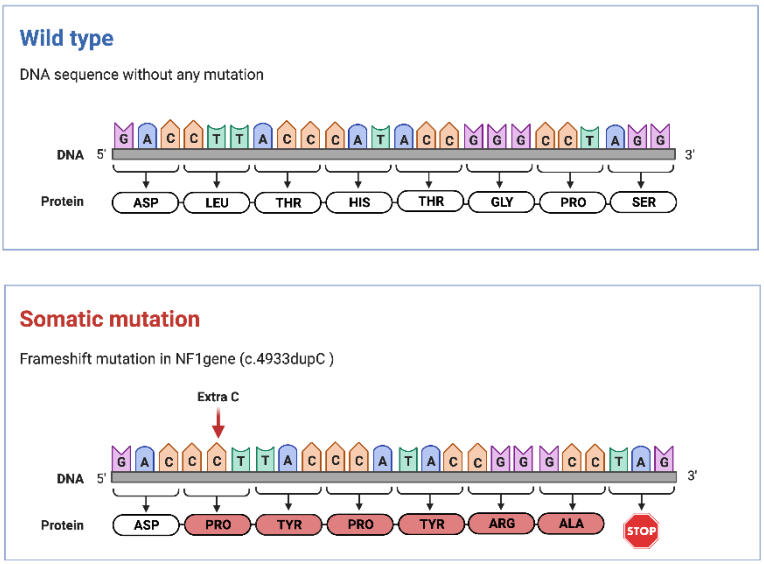

Figure 12. Schematic illustration of the somatic mutation in the *NF1* gene: a novel frameshift variant (c.4933dupC/p.Leu1645Profs*7/) exclusively in plexiform

neurofibroma with a 16% variant allele frequency compared to wild type. The figure was created with BioRender.com

Together, these findings demonstrate a germline *NF1* splice-site variant in combination with a somatic second-hit frameshift mutation, consistent with the "two-hit" hypothesis and providing a molecular explanation for the patient's exceptionally severe, segmentally distributed plexiform neurofibroma phenotype.

4.3. Development of a Practical Diagnostic and Follow-up Protocol for Paediatric NF1

Given the heterogeneity of NF1 in childhood, establishing a structured, age-adapted approach to diagnosis and follow-up is essential for optimising patient outcomes. Based on the clinical spectrum observed in our Hungarian paediatric cohort and in accordance with current international recommendations, we developed a practical protocol aimed at facilitating early diagnosis, identifying high-risk subgroups, and guiding systematic surveillance. The protocol is designed for integration into everyday paediatric and specialist practice, ensuring timely recognition and management of NF1-related complications from infancy through adolescence. It is anticipated that comprehensive dissemination and consistent implementation of the proposed diagnostic protocol across primary and specialist care levels will significantly improve the identification and follow-up of paediatric NF1 cases in the coming years.

Table 5. Comparison of the frequency of key clinical features in the Hungarian NF1 cohort and international literature data, including café-au-lait macules (Lammert et al, 2005, Nasi et al, 2023), freckling (Miller et al, 2019, Ly et al, 2019), neurofibromas (Miller et al, 2019, Ly et al, 2019, Ozarslan et al, 2021), plexiform neurofibromas (Miller et al, 2019, Moodley et al, 2024, Veres et al, 2023), Lisch nodules (Cassimann et al, 2013, Ly et al, 2019, Maharaj et al, 2014, Rauen et al, 2013), optic pathway gliomas (Cassiman et al, 2013, Carvalho et al, 2023, Tang et al, 2023), skeletal abnormalities (Lammert et al, 2005, Miller et al, 2019, Ly et al, 2019, Solares et al, 2022), scoliosis (Miller et al, 2019, Tabata et al, 2020, Solares et al, 2022), cognitive/behavioural disorders (Miller et al, 2019, Sánchez et al, 2022), seizures and migraine (Miller et al, 2019, Sánchez et al, 2022), T2 hyperintensities (FASI) (Miller et al, 2019, Pillay-Smiley et al, 2023, Anders et al, 2022), low-grade gliomas (Miller et al, 2019, Solares et al, 2022), and hearing loss (Solares et al, 2022, Yun et al, 2021, Idowu et al, 2023).

	Hungarian Cohort (n=175)	International Data (range or mean)
≥6 CALMs	100%	~99% (by age 1)
Skinfold freckling	28.6%	>85% (by age 7)
Neurofibromas	35.4%	90% (adults)
Plexiform neurofibromas	17.7%	20–50%
Lisch nodules	36%	50–90%
Optic pathway gliomas (OPG)	11.4%	5–25%
Skeletal manifestations	38.2%	30–50%
Scoliosis	20%	20–40%
Cognitive/behavioural disturbances	25.1%	30–81% (ADHD/ASD/ID)
Seizures and migraine	7.4%	4–13%
FASI (MRI T2 hyperintensities)	56%	60–90% (children)
Low-grade gliomas (non-OPG)	8%	2–14%
Hearing loss (conductive and sensorineural)	5.7%	4-19%

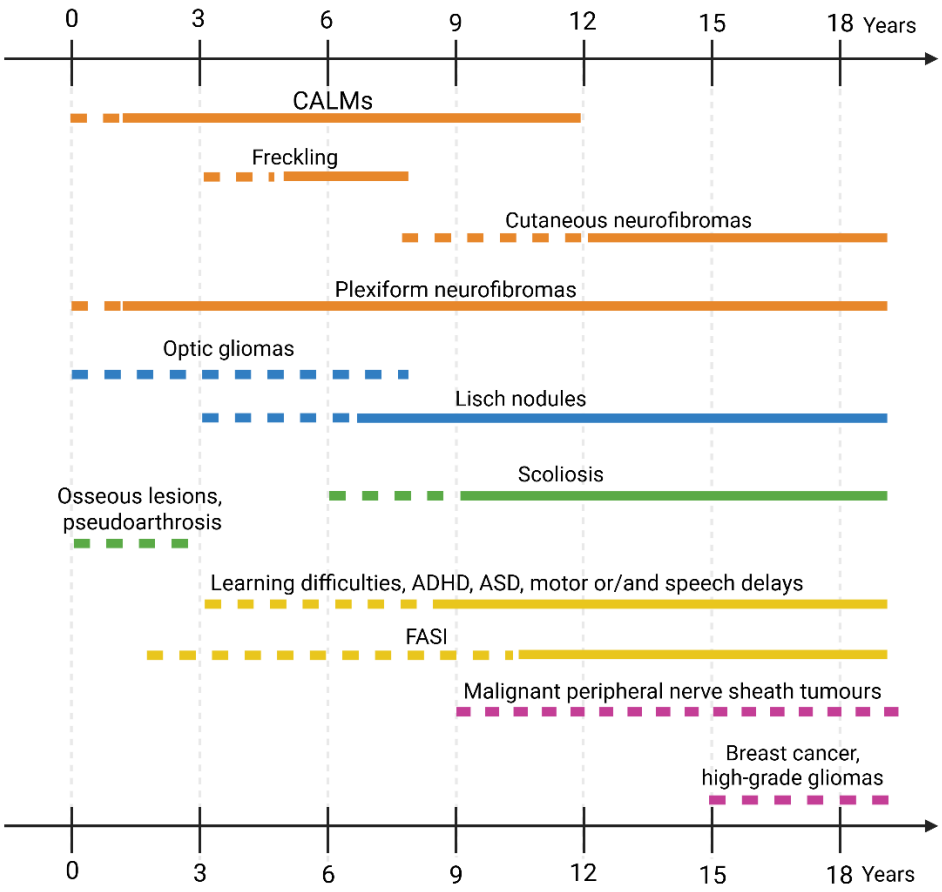

Figure 13. Timeline of Clinical Manifestations in Neurofibromatosis Type 1 by Age.

The figure illustrates the typical timing of first appearance (dashed lines) and subsequent progression or increasing prevalence (solid lines) of key NF1-related manifestations from birth to 18 years. Colour coding denotes clinical features: pigmentary and cutaneous (orange), ocular (blue), skeletal (green), neurodevelopmental and brain MRI findings (yellow), and malignancies (purple).

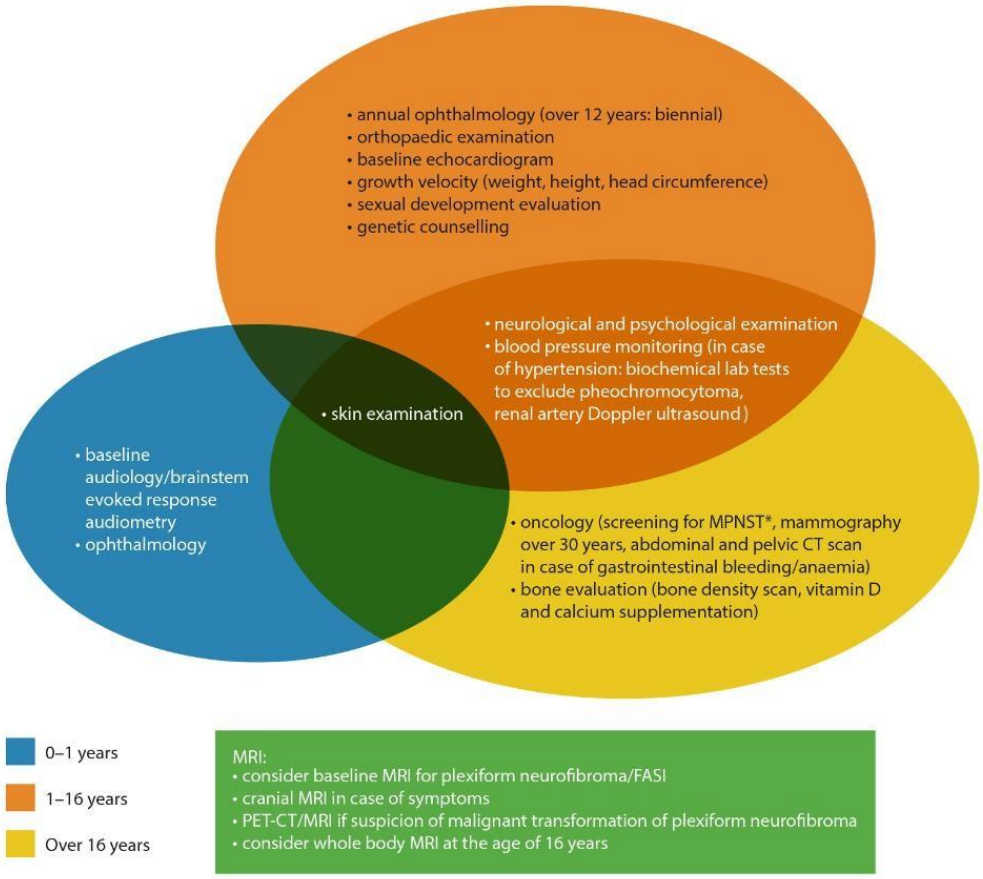

Figure 14. Age-specific recommendations for initial evaluation and clinical follow-up in individuals with neurofibromatosis type 1. The overlapping ellipses represent clinical assessments recommended at different age groups: infancy (0–1 year), childhood and adolescence (1–16 years), late adolescent and adulthood (over 16 years). Skin examination is a universal requirement across all ages. MRI indications are summarized separately, highlighting key points such as baseline imaging, symptom-driven evaluation, and whole-body MRI at age 16.

* MPNST: Malignant Peripheral Nerve Sheath Tumour

Table 6. Age-specific multidisciplinary follow-up recommendations for individuals with NF1, corresponding to Figure 14.

Examination / Specialty	0–1 year	1–16 years	>16 years
Dermatology / Physical examination	Annual	Annual	Annual
Ophthalmology	Annual	Annual until age 12, then every 2 years	Every 3–5 years or as indicated
Neurology	At baseline and as indicated	Annual (clinical exam ± MRI if symptomatic)	Every 1–2 years
Otolaryngology (ENT)	At baseline and as indicated	Annual hearing and airway screening if symptomatic or with plexiform NF of head/neck, ask about hearing problems at every visit	As indicated
Psychology / Neurocognitive assessment	Developmental screening	At school entry and during transitions	As indicated
Orthopaedics	Annual or as indicated	Annual	Bone densitometry every 3-5 years
Cardiology	At baseline	At baseline and as indicated	As indicated
Blood pressure		At every visit	At every visit
Endocrinology / Growth and puberty	Growth monitoring (weight, height, head circumference)	Annual growth, puberty, menstrual cycle monitoring	As indicated
Oncology	—	Clinical vigilance for rapid growth of neurofibromas; MRI if suspicious	Annual review in adult clinic; imaging if pain or enlargement
MRI (brain / spine)	Only if symptomatic	If symptomatic or progressive lesion	If new neurological signs

4.3.1. Cutaneous involvements

In our cohort, all patients presented with at least six **CALM**s, reinforcing their established role as one of the earliest diagnostic signs of NF1 (Figure 14, Table 6). By one year, 99% of NF1 patients exhibit six or more CALMs, with both the number and size increasing in early childhood before stabilizing (Nasi et al., 2023) (Figure 13, Table 5). CALMs are benign melanotic macules that do not necessitate intervention. However, in cases of significant cosmetic or psychological burden, various treatment modalities, including topical depigmenting agents, intense pulsed light (IPL), radiofrequency therapy (RF), and laser therapies (QS Nd:YAG, Alexandrite, Ruby, Pulsed Dye Laser), may be considered (Miller et al., 2019; Veres et al., 2025b). Nevertheless, these approaches are associated with high recurrence rates and potential adverse effects such as hypo- or hyperpigmentation and scarring (Veres et al., 2025b).

According to the literature, **axillary and/or inguinal freckling** typically appears between the ages of 3 and 5 years, with over 85% of NF1 patients developing these features by the age of 7 (Miller et al., 2019; Nasi et al., 2023; Ly et al., 2019) (Figure 13). In our cohort, the onset of freckling was slightly later than reported, however, a marked increase was

observed during the early school years (Table 2). This pattern most likely reflects improved clinical recognition with age rather than a true biological delay.

The third diagnostic criteria, the **neurofibromas** are known to develop progressively, typically emerging during adolescence and continuing to increase in number throughout adulthood, with a reported prevalence exceeding 90% in adult NF1 patients (Figure 13, Table 5) (Miller et al., 2019; Ly et al., 2019; Ozarslan et al., 2021). In our cohort, neurofibromas began to appear in the 7–12-year age group, with their prevalence increasing with age, reaching its highest proportion in adulthood (Table 2). Although cutaneous neurofibromas (CNFs) are benign, their visibility, associated pruritus, and pain can profoundly impair quality of life, leading to physical discomfort, emotional distress, and social self-consciousness, thereby warranting therapeutic attention. If needed, surgical excision remains the primary therapeutic approach, although laser ablation (CO_2, Alexandrite, Nd:YAG lasers) and electrodessication have been utilized despite limited long-term efficacy data (Miller et al., 2019; Saleh et al., 2023). Investigational therapies, including systemic agents (everolimus, hydroxychloroquine, chloroquine), intralesional injections (1% deoxycholic acid, 1% polidocanol), and mast cell-targeting treatments (tranilast, ketotifen), are under evaluation (Saleh et al 2023). Additional emerging strategies, such as photodynamic therapy, vitamin D supplementation, and NFX-179 Topical Gel, hold promise for future management (Saleh et al., 2023; Sarin et al., 2024; Quirk et al., 2021).

Compared with the typically small-sized cutaneous neurofibromas, **plexiform neurofibromas** are associated with substantially greater morbidity. In our cohort, their prevalence was approximately 20%, aligning with the reported range of 20–50% in the literature, although their absence in the youngest age group likely reflects limitations in early clinical recognition (Table 5) (Miller et al., 2019; Moodly et al., 2024; Veres et al., 2023). PNFs typically present at birth or during early infancy and may cause substantial visible deformity, pain, and functional impairment due to compression of adjacent structures (Moodly et al., 2024; Veres et al., 2025a; Veres et al., 2023a). Early detection—most reliably achieved through imaging, particularly MRI—is critical for timely intervention (Veres et al., 2023a; Pálla et al., 2023). Clinical features that should raise suspicion for malignant transformation include rapid or disproportionate tumour growth, new or worsening pain (especially nocturnal pain), neurological deficits (motor weakness,

paraesthesia), changes in tumour consistency (new focal firmness), and increased metabolic activity on FDG-PET imaging (Miller et al., 2019).

The management of PNFs remains challenging, with surgical excision being the primary approach; however, complete removal is often not feasible due to tumour infiltration. Recently, medical therapies—particularly for inoperable cases—have gained importance. In 2020, the FDA approved the MEK inhibitor selumetinib for treating symptomatic, progressive, inoperable PNFs in children over 2 years of age, demonstrating efficacy in reducing tumour volume and alleviating symptoms (Gross et al., 2020; Armstrong et al., 2023). Selumetinib and other MEK inhibitors (binimetinib, mirdametinib, trametinib) target the MAPK signalling pathway, whose overactivation in *NF1* results from loss of neurofibromin, a multifunctional GTPase-activating protein (GAP) that accelerates the hydrolysis of RAS-bound GTP and thereby suppresses downstream signalling (Pinna et al., 2015; Legius et al., 2021) (Figure 15). Beyond MEK inhibition, additional targeted approaches are under investigation, including tyrosine kinase inhibitors—some of which also target VEGFR - (cabozantinib, imatinib, dasatinib, sunitinib, nilotinib) and mTOR inhibitors (sirolimus, everolimus) (Staedtke et al., 2024; Armstrong et al., 2023; Tamura et al., 2021; Wilson et al., 2021). While these therapies hold promise, reported clinical outcomes to date remain modest (Carvalho et al., 2023; Moodley et al., 2024).

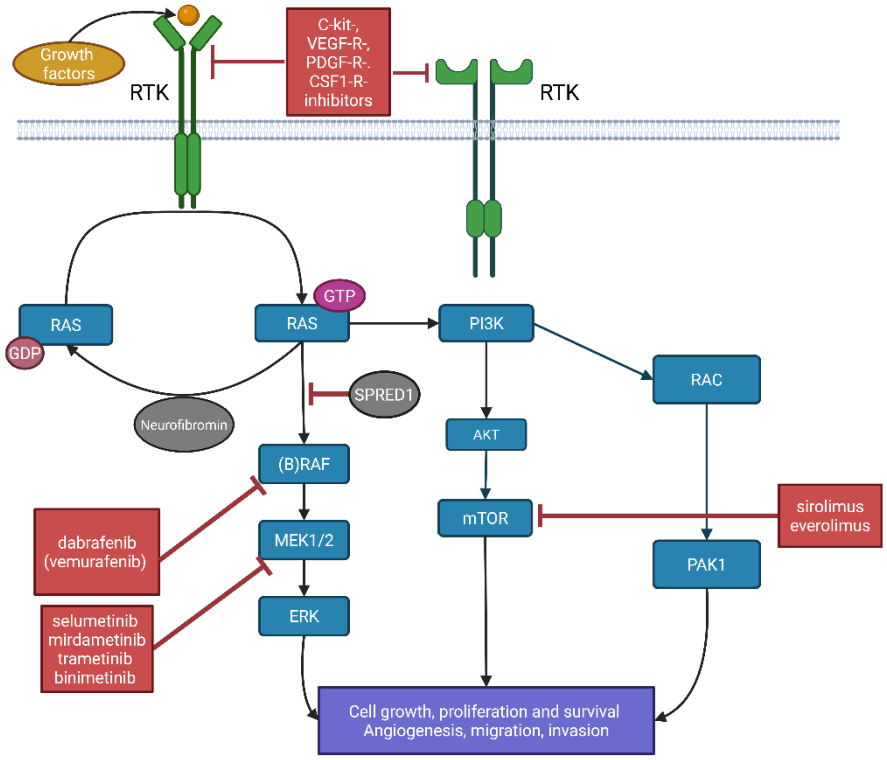

Figure 15. NF1 Pathomechanism and Potential Targeted Treatment Options. Created in https://BioRender.com

AKT (PKB): Ak strain transforming (*Protein Kinase B*); **BRAF**: *v*-Raf murine sarcoma viral oncogene homolog B (RAF kinase B isoform); **CSF1R (CSF1-R)**: Colony-Stimulating Factor 1 Receptor; **c-KIT (KIT)**: proto-oncogen, receptor tyrosine kinase; **ERK1/2**, Extracellular Signal-Regulated Kinase 1/2; **GDP**: guanosine diphosphate; **GTP:** guanosine triphosphate; **MAPK**: Mitogen-Activated Protein Kinase; **MEK1/2 (MAP2K1/2):** MAPK/ERK kinase; **mTOR**: mammalian Target Of Rapamycin; **NF1**: Neurofibromin 1; **PAK1**: p21-Activated Kinase 1; **PI3K**: Phosphoinositide 3-Kinase; **RAF**: Rapidly Accelerated Fibrosarcoma; **RAC (RAC1):** Ras-related C3 botulinum toxin substrate; **RAS:** Rat Sarcoma; **RTK:** Receptor Tyrosine Kinase; **SPRED1**: Sprouty-related, EVH1 domain-containing protein 1; **VEGFR (VEGF-R)**: Vascular Endothelial Growth Factor Receptor; **PDGFR (PDGF-R)**: Platelet-Derived Growth Factor Receptor.

Beyond these hallmark manifestations, other NF1-associated cutaneous abnormalities—such as naevus anaemicus, pseudoatrophic macules, glomus tumours, and juvenile

xanthogranulomas—were also identified with increased frequency in our cohort, in line with previous observations (Miller et al., 2019; Anderson, et al., 2020). Given the progressive nature of NF1-related skin lesions, annual dermatological assessments are essential to enable early identification of new or evolving manifestations and to guide timely, clinically indicated interventions (Solares et al., 2022). Accordingly, in line with our proposed protocol, **the presence of more than six CALMs should prompt initiation of annual dermatological follow-up from infancy onward** (Figure 14, Table 6).

4.3.2. Ocular manifestations

In our cohort, the prevalence of Lisch nodules (36%, 63/175) was comparable to some published series (Scala et al., 2021) but lower than others (Ly et al., 2019; Maharaj et al., 2014), most likely due to underreporting and underdetection, particularly in patients examined by non-specialist ophthalmologists (Table 5). Nonetheless, their increasing frequency with age mirrors the literature, where they are present in ~50% of NF1 toddlers and over 90% of adults (Figure 13, Table 5) (Cassiman et al., 2013; Maharaj et al., 2014; Rauen et al., 2013). The detection rate of 17.2% in the youngest group highlights the importance of early ophthalmologic screening (Figure 14, Table 6). Given their rarity in individuals without NF1, Lisch nodules remain a key diagnostic criterion, aiding in differentiating NF1 from other CALM-associated syndromes (Cassiman et al., 2013).

Optic pathway gliomas represent the most frequent gliomas in NF1, typically diagnosed before the age of seven (mean onset 4.5 years) (Figure 13) (Cassiman et al., 2013; Carvalho et al., 2023; Tang et al., 2023; Dunning-Davies et al., 2016). They occur in 5–25% of NF1 patients and account for roughly one-third of all OPG cases (Cassiman et al., 2013; Tang et al., 2023). The 11.4% prevalence in our series is consistent with these ranges, with a relatively stable age distribution, supporting the view that OPGs develop predominantly in early childhood (Table 5) (Ly et al., 2019., Wang et al., 2021).

In line with current international guidelines, **annual ophthalmologic examinations** are recommended until the age of eight; however, as optic pathway gliomas were diagnosed at a slightly later age in our Hungarian cohort, we suggest extending **annual screening until 12 years of age, followed by biennial visits** thereafter in asymptomatic patients. (Figure 14, Table 6) (Tang et al., 2023; Miller et al., 2019; de Blank et al., 2017; Kotch et al., 2024). Assessment should include visual acuity, colour vision, visual fields, ocular

motility, and alignment, alongside inspection of the eyelids, orbits, pupils, irises, and fundi. In symptomatic cases, MRI is indicated, and follow-up should occur every three months during the first year, with intervals extended upon disease stability (Tang et al., 2023; Miller et al., 2019, de Blank et al., 2017; Kotch et al., 2024). Routine baseline MRI for asymptomatic OPG detection is not recommended (Tang et al., 2023; Solares et al., 2022), although some experts suggest biannual ophthalmologic examinations before age six due to the difficulty of symptom recognition in younger children (Miller et al., 2019; Caen et al., 2015).

Regular monitoring of growth, weight, pubertal progression, and endocrine function is essential in NF1 patients to allow early recognition or exclusion of hypothalamic and chiasmal tumours, with serologic evaluation performed as indicated (Figure 14, Table 6) (Kotch et al., 2024).

Management of OPGs generally relies on careful observation for stable lesions. When intervention is warranted, vincristine and carboplatin remain the standard first-line regimen, with vinblastine or vinorelbine as alternatives. Novel targeted approaches—such as mTOR inhibition (everolimus), anti-angiogenic therapy (bevacizumab), and MEK inhibition (selumetinib, binimetinib, trametinib)—are emerging as promising options. Combination protocols (e.g., trametinib with everolimus, hydroxychloroquine, or dabrafenib; vinblastine with selumetinib) are under active clinical investigation (Amato et al., 2023; Kotsch et al., 2024). Surgery is seldom indicated given limited benefit, and radiotherapy is generally avoided due to risks of cerebrovascular events, neurocognitive decline, and secondary malignancies (Saleh et al., 2023; Tang et al., 2023; Miller et al., 2019).

4.3.3. Skeletal manifestations

Skeletal abnormalities affect approximately 30–50% of NF1 patients in the literature, most commonly scoliosis, while tibial pseudarthrosis is reported in about 2–5% of cases (Miller et al., 2019; Tabata et al., 2020; Solares et al., 2022; Ly et al., 2019). In our cohort, skeletal manifestations were documented in 38.2% of patients, with scoliosis at the lower prevalence limit (20%) and pseudarthrosis underreported (2.3%) (Table 2, 5). The lower frequency in the adult subgroup may reflect both under documentation and reduced access to orthopaedic follow-up outside specialised NF1 care.

In accordance with current international recommendations, and as outlined in our proposed protocol (Figure 14, Table 6), we recommend annual orthopaedic evaluations for all paediatric NF1 patients, with targeted imaging where clinically indicated. Given the risk of progressive scoliosis and fractures with poor healing, scoliosis screening should be part of every routine visit, and tibial radiography is advised before walking age in children at risk for long-bone dysplasia. Bone health monitoring—including serum vitamin D and parathyroid hormone (PTH) levels—should be performed every 3–5 years, complemented by densitometry when indicated (Miller et al., 2019; Solares et al., 2022).

Scoliosis management remains challenging; while bracing is standard, severe or progressive curves often necessitate surgical correction. Macrocephaly is common in NF1, typically without congenital hydrocephalus, although acquired hydrocephalus can develop due to aqueduct stenosis (Miller et al., 2019).

4.3.4. Neurological complications

Neurological manifestations are common in NF1, yet in our cohort the prevalence of seizures and migraines (7.4%) was lower than typically reported, whereas cognitive and behavioural disturbances (25.1%, rising to 33.3% in adolescents) were higher than in the general population but below some studies reporting 30–81% for ADHD, ASD, and moderate-to-severe cognitive impairment (Table 5) (Miller et al., 2019; Sánchez et al., 2022; Isenberg et al., 2013). This discrepancy may partly reflect underdiagnosis, influenced by social stigma surrounding neurodevelopmental disorders in Hungary and restricted access to psychiatric records. Fine motor and coordination difficulties (11.4%) further underscore the association between NF1 and motor developmental challenges.

These findings reinforce the importance of regular neurological assessments, particularly in childhood and adolescence, when cognitive and behavioural difficulties are most apparent. In line with current international guidelines and our proposed protocol, **we recommend targeted annual neurological evaluation during childhood and adolescence, including detailed neurodevelopmental assessment, and immediate brain MRI in cases of severe headaches, new-onset seizures, or focal neurological signs** (Figure 14, Table 6) (Miller et al., 2019; Sánchez et al., 2022).

Focal areas of signal intensities generally appear between ages 2 and 10. In our cohort, their prevalence reached 72% among children aged 7–18 years, closely matching published data (Figure 12, Table 5) (Miller et al., 2019; Pillay-Smiley et al., 2023; Anders et al., 2022), and their natural regression by the second decade was also observed. When contrast enhancement or mass effect is present, low-grade gliomas—particularly in the brainstem—should be considered (Miller et al., 2019; Pillay-Smiley et al., 2023; Anders et al., 2022). The prevalence of low-grade gliomas (excluding optic pathway gliomas) in our cohort was 8%, consistent with reported NF1-related CNS tumour rates. These gliomas, typically located in the basal ganglia, cerebellum, or brainstem, are generally indolent, with clinical symptoms such as headaches, hydrocephalus, or cranial nerve deficits occurring in only one-third or fewer of cases. Most can be managed conservatively, with only a small proportion requiring treatment (Miller et al., 2019).

High-grade gliomas are rare in NF1 (1–2%) and may develop at younger ages. When intervention is necessary, conventional oncologic treatments remain standard, although recent studies suggest that MEK inhibitors (selumetinib, trametinib, binimetinib) may offer additional therapeutic benefit (Solares et al., 2016).

4.3.5. Other findings

The relatively low prevalence of certain manifestations in our cohort—such as endocrine abnormalities and pheochromocytoma—aligns with their known rarity in NF1. Nevertheless, given the potential severity of these complications, vigilant monitoring is warranted.

In line with our proposed protocol, all children with NF1 should undergo **annual comprehensive physical examinations**, including systematic assessment of weight, height, growth velocity, sexual development, and head circumference. Close tracking of growth patterns is particularly important, as short stature, growth acceleration, or early puberty may warrant **prompt endocrinology referral** and brain MRI (Figure 14, Table 6) (Miller et al., 2019).

4.3.6. Cardiological and vascular involvement

NF1 is associated with a broad spectrum of cardiovascular anomalies, including congenital heart defects (most commonly pulmonic stenosis), vascular stenoses, aneurysms, and cerebrovascular abnormalities such as Moyamoya syndrome, which

significantly increases stroke risk (Miller et al., 2019). Hypertension is a frequent finding, often secondary to renal artery stenosis, though essential hypertension is also observed. In line with our proposed protocol, **blood pressure measurement is recommended at least annually in all age groups**, preferably on both arms. **Cardiology evaluation should be performed at the time of first NF1 diagnosis**, whenever symptoms or physical examination findings suggest cardiac involvement, and in cases of known cardiac pathology, at a frequency determined by the cardiologist. Newborns should be screened for congenital heart defects, with echocardiography performed when indicated. In cases of persistent hypertension, targeted vascular imaging—such as Doppler ultrasonography or magnetic resonance angiography—should be performed to assess for renovascular abnormalities; in patients with refractory hypertension, evaluation for pheochromocytoma is also warranted. For confirmed Moyamoya syndrome, revascularization surgery and prophylactic aspirin may be warranted to reduce stroke risk (Figure 14, Table 6) (Miller et al., 2019).

4.3.7. Hearing loss

Hearing loss affects approximately 4–19% of individuals with NF1, with conductive hearing loss being more frequent than sensorineural forms, often resulting from plexiform neurofibromas in the head and neck region—findings consistent with our cohort (Table 5) (Yun et al., 2021; Idowu et al., 2023). In contrast, NF2 is predominantly associated with sensorineural hearing loss due to bilateral vestibular schwannomas. An **initial audiological evaluation** is advisable in any patient presenting with multiple café-au-lait macules to help differentiate NF1 from NF2 (Figure 14, Table 6). While routine audiological screening is not generally recommended after NF1 diagnosis, audiometric testing may be considered up to the age of 5–7 years, and targeted assessments should be performed if symptoms arise. **At each follow-up, clinicians should actively enquire about hearing changes or related symptoms** (Solares et al., 2022; Yun et al., 2024).

4.3.8. Tumours associated with NF1

NF1 is associated with a decreased life expectancy, which can be reduced by 8–15 years compared to the general population (Duong et al., 2011; Tamura et al., 2021; Miller et al., 2019), although our structured diagnostic and follow-up protocol may help improve long-term outcomes. The reduction in life expectancy among individuals with NF1 is primarily attributed to vascular diseases and malignancies, with a five- to ten-fold higher cancer

incidence (Solares et al., 2022; Perrino et al., 2024). Common tumours in NF1 patients include malignant peripheral nerve sheath tumours, gliomas, pheochromocytomas, and leukaemia (Miller et al., 2019; Solares et al., 2022). Women with NF1 have a significantly higher risk of breast cancer, diagnosed earlier and with more advanced disease, while men show a low risk (Solares et al, 2022). Breast cancer screening should begin at age 30, with MRI considered for women aged 30–50 (Solares et al., 2022) (Figure 14, Table 6). Gastrointestinal stromal tumours are also more frequent in NF1, typically in the small intestine, with screening recommended when clinical suspicion arises (Solares et al., 2022). NF1 patients are also at risk for other malignancies, such as rhabdomyosarcoma, juvenile myelomonocytic leukaemia, melanoma, and ovarian cancer, but routine screening for these is not supported (Miller et al., 2019; Solares et al., 2022).

4.3.8.1. Malignant peripheral nerve sheath tumours

Malignant peripheral nerve sheath tumours are aggressive sarcomas with a high metastatic potential, contributing substantially to morbidity and mortality in NF1 (Miller et al., 2019; Solares et al., 2022). There is currently no universally accepted screening protocol, but high-risk patients may benefit from periodic whole-body diffusion-weighted MRI. **Any rapid growth of a neurofibroma, new or worsening pain, changes in consistency, or the onset of neurological symptoms should prompt immediate evaluation with diffusion-weighted MRI or PET-CT, followed by histological confirmation** if indicated (Figure 14, Table 6) (Miller et al., 2019; Solares et al., 2022). Treatment requires wide surgical excision, often in combination with radiotherapy and chemotherapy. Several targeted strategies—including mTOR, MEK, and tyrosine kinase inhibitors (e.g., selumetinib with sirolimus, everolimus with bevacizumab)—are under investigation, but long-term outcomes remain poor, with a 5-year survival rate of less than 20% (Miller et al., 2019; Solares et al., 2022; Wei et al., 2023).

4.3.9. Genetic testing

Genetic testing for NF1 plays a pivotal role in diagnostic confirmation, family screening, and genetic counselling. While it is generally not required in children who already fulfil the NIH clinical criteria, it is particularly valuable in suspected cases with incomplete phenotypic expression, such as those presenting only with CALMs before the onset of other features (Tamura et al., 2021; Miller et al., 2019). Current methods achieve a sensitivity of approximately 95%, offering high diagnostic reliability, although a negative

result does not definitively exclude NF1 (Miller et al., 2019). In atypical presentations—such as isolated plexiform neurofibromas—germline testing may yield negative results, with pathogenic variants detectable only in affected tissue, reflecting mosaicism. Although genotype–phenotype correlations offer potential for risk stratification, their clinical applicability remains limited due to the pronounced inter- and intrafamilial variability, underscoring the need for further research into mutation-specific clinical patterns (Komlósi et. al., Veres et al., 2025a; Pacot et al., 2021; Büki et al., 2021). In line with our proposed protocol, we recommend **genetic testing in all patients with diagnostic uncertainty, atypical features, or when early molecular confirmation could influence surveillance strategies**, thereby facilitating timely risk stratification, targeted counselling, and integration into a coordinated, multidisciplinary NF1 care pathway.

5. Discussion

This PhD dissertation is based on a comprehensive 15-year dataset (2010–2024) collected at the Department of Dermatology, Heim Pál National Paediatric Institute, encompassing one of the largest systematically evaluated Hungarian paediatric NF1 cohorts to date. The research focused on three main objectives: (1) to characterise the clinical presentation and age-related evolution of NF1-associated manifestations in childhood, (2) to explore genotype–phenotype correlations in a genetically confirmed subgroup, and (3) to develop a practical, age-adapted diagnostic and follow-up protocol for paediatric NF1, integrating our findings with current international recommendations.

From the 261 individuals referred with suspected NF1, 231 met at least one NIH diagnostic criterion, and 175 fulfilled at least two, establishing the diagnosis on clinical grounds. This full cohort formed the basis for the detailed analysis of age-dependent cutaneous and systemic features, which in turn informed the development of the proposed diagnostic and follow-up protocol.

A genetically tested **subgroup of 70 patients** underwent molecular analysis for NF1 variants, yielding an 82.8% detection rate, in line with international reports (Veres et al., 2025; van Minkelen et al., 2014; Stella et al., 2018; Zhang et al., 2015). From these, 45 patients with confirmed pathogenic or likely pathogenic variants were included in genotype–phenotype correlation analyses. The mutational spectrum was dominated by single nucleotide variants (n = 40, 88.9%), with copy number variations detected in 5 patients (11.1%). Variants were identified by Next Generation Sequencing and/or Sanger sequencing, ensuring comprehensive detection and confirmatory accuracy.

Given the paediatric profile of our institution, the mean age in the clinically diagnosed NF1 cohort (n = 204) was 15.2 years (range 1–35), with 154 patients (66.7%) classified within the paediatric age group (≤18 years). In the genetically tested subgroup, the mean age was slightly higher, at 16 years (range 1–33), with 135 patients (66.7%) belonging to the paediatric age group (≤18 years).

Sex distribution was nearly balanced in both cohorts: in the overall clinically diagnosed group, males represented 47.4% and females 52.6%, whereas in the genetically tested subgroup a slight male predominance was observed (55.2% male, 44.8% female). These proportions align with most published NF1 epidemiological data (Veres et al., 2025; Lalor et al., 2020; Scala et al., 2021; Bianchessi et al., 2015), although other series have reported

a mild female predominance (De Luca et al., 2007; Sánchez et al., 2022), suggesting that the true sex ratio may vary according to geographic region and ascertainment methodology.

The age-stratified analysis of our cohort highlights the dynamic and progressive nature of NF1 manifestations throughout childhood and adolescence. As expected, café-au-lait macules were universally present in patients fulfilling at least two NIH diagnostic criteria, while axillary or inguinal freckling appeared later and with lower prevalence, reaching its peak in adulthood (35.9%), which may in part reflect under recognition or incomplete documentation in earlier clinical records.

Neurofibromas, rare in early childhood (3.4%), showed a marked increase from the 7–12-year age group, plateauing at approximately 47% in adulthood. However, the observed prevalence in individuals over 18 years was lower than reported in previous studies, possibly due to the limited number of adult patients in our primarily paediatric cohort (Ortonne et al., 2018; Wang et al., 2021; Ly et al., 2019). It is noteworthy that nearly all of these now-adult patients were children during the data-collection phase of our 15-year study period, introducing a potential bias.

The prevalence of plexiform neurofibromas (~20%) was consistent with published ranges (20–50%), and their absence in the youngest age group likely reflects underdiagnosis (Solares, 2022; Wang et al., 2021). In light of the progressive nature of these lesions, annual physical examinations including comprehensive dermatological assessment remain essential to ensure timely recognition and management of NF1-related cutaneous complications (Solares, et al., 2022; Wang et al., 2021; Veres et al., 2025; Jouhilahti et al., 2011; Ortonne et al., 2018; Ly et al., 2019).

Ocular manifestations showed distinct age-related patterns. The prevalence of Lisch nodules increased with age, from 17.2% in the youngest group to 42.2% in adults, consistent with their gradual accumulation over time (Maharaj et al., 2014; Ly et al., 2019). Nevertheless, the overall rate in our cohort (36%) remained below that reported in several other studies (Pacot et al., 2021; Maharaj et al., 2014; Ly et al., 2019), a difference that might be explained by variations in study design, patient selection criteria, or under detection in routine ophthalmological screening. In contrast to previous findings (Stella et al., 2018; Sabbagh et al., 2013; Castle et al., 2003), the highest frequency of Lisch nodules in our series was not seen in patients with frameshift variants but in those with

splice-site variants (50%, 4/8). This discrepancy may be partly attributable to the relatively small size of our genotyped subgroup, which could have limited the detection of certain trends. However, it may also reflect under recognition of Lisch nodules in other mutation categories or, potentially, a genuinely higher phenotypic severity associated with splice-site variants in our Hungarian cohort.

Optic pathway gliomas were identified in 11.4% of patients, consistent with literature data (Campen et al., 2019; Wang et al., 2021; Ly et al., 2019; Giugliano et al., 2019; Tabata et al., 2020). The highest prevalence was observed in the 7–12-year age group (16.3%), followed by adolescents aged 13–18 years (12.8%), with markedly lower rates in the youngest (3.4%) and adult (10.9%) cohorts. This age distribution deviates from the widely reported pattern that most OPGs are diagnosed before the age of seven (Campen et al., 2019; Wang et al., 2021; Ly et al., 2019), which in our setting may be attributable to delayed diagnosis, under detection, incomplete documentation, or variability in imaging practices over the 15-year study period. In accordance with current international guidelines, annual ophthalmological examinations are recommended up to 8 years of age; however, based on the age distribution of optic pathway gliomas observed in our Hungarian cohort, we propose extending annual screening until 12 years of age, followed by biennial follow-up thereafter. Targeted neuroimaging is indicated in the presence of visual symptoms or an atypical disease course to enable timely detection and intervention. (Ly, et al., 2019).

Skeletal abnormalities were identified in 38.2% of our patients, which is consistent with the 30–50% prevalence range reported in the literature (Ly et al., 2019; Wang et al., 2021; Tabata et al., 2020; Delucia et al., 2011; Elefteriou et al., 2009). Within our cohort, scoliosis was the most frequent skeletal manifestation, affecting 20% of patients, which aligns with the reported prevalence range of 20-40% (Ly et al., 2019; Wang et al., 2021; Delucia et al., 2011; Elefteriou et al., 2009; North et al., 1993). Pseudarthrosis, although rare (6.7%), closely matched the ~5% rate described in the literature (Ly et al., 2019; Wang et al., 2021; Delucia et al., 2011; Elefteriou et al., 2009; North et al., 1993). Notably, most skeletal abnormalities were documented in adolescents aged 13–18 years (43.6%), whereas the incidence was lower in early childhood (27.6%), despite this being the age at which certain abnormalities, such as pseudarthrosis, are typically detected. Interestingly,

in our genotyped subgroup, pseudarthrosis clustered among patients with splice-site variants, raising the possibility of a mutation-specific risk profile. These findings reinforce the need for systematic orthopaedic assessment at diagnosis, with annual clinical evaluation during growth, and targeted imaging when indicated, to facilitate timely recognition and management of NF1-related skeletal complications.

Neurological involvement in NF1 encompasses a wide spectrum, ranging from radiological findings without clinical impact to disabling seizures, tumours, and neurodevelopmental disorders. In our cohort, the prevalence of seizures and migraines (7.4%) was lower than reported in many international series, whereas cognitive and behavioural disturbances were more frequent, affecting 25.1% of patients and rising to 33.3% in adolescents (Miller et al., 2019; Sánchez et al., 2022). Although these rates are elevated compared to the general population, they remain below the 30–81% prevalence described elsewhere, which may in part reflect underdiagnosis due to societal stigma surrounding neurodevelopmental disorders in Hungary and limited access to psychiatric records (Miller et al., 2019; Sánchez et al., 2022). Fine motor and coordination difficulties, observed in over 10% of patients, further support the recognised association between NF1 and motor developmental impairment.

Focal areas of signal intensities were common, detected in 72% of children aged 7–18 years, consistent with their early onset and stability throughout childhood, followed by regression in early adulthood (Miller et al., 2019; Pillay-Smiley et al., 2023; Anders et al., 2022). Low-grade gliomas outside the optic pathway were present in 8% of cases, most often in the basal ganglia, cerebellum, or brainstem, and were generally indolent (Miller et al., 2019; Solares et al., 2022). High-grade gliomas were rare, in line with published data. These observations emphasise the need for structured neurological follow-up, including annual neurodevelopmental assessment during childhood and adolescence, and prompt neuroimaging in the presence of severe headaches, new-onset seizures, or focal neurological deficits, as reflected in our proposed protocol.

Beyond the more common cutaneous, ocular, skeletal, and neurological manifestations, our cohort also demonstrated less frequent yet clinically significant systemic complications, including conductive hearing loss, cardiovascular anomalies, endocrine disturbances, and macrocephaly. Although individually uncommon, these findings

underscore the multisystemic nature of NF1 and the necessity of coordinated, multidisciplinary management. In our proposed protocol, this is addressed through structured growth monitoring (including weight, height, growth velocity, pubertal status, and head circumference), regular blood pressure measurement with further vascular assessment when indicated (e.g., Doppler ultrasound, MRA), exclusion of pheochromocytoma in refractory hypertension, targeted endocrinological and cardiological evaluations, and selective audiological screening in early childhood (Solares et al,. 2022; Yun et al., 2021; Idowu et al., 2023). Malignancies other than gliomas were rare in our series; however, given the five- to ten-fold increased lifetime cancer risk in NF1, their recognised contribution to morbidity and mortality necessitates continued vigilance (Solare et al., 2022; Perrino et al., 2024). Early breast cancer screening and gastrointestinal evaluation when clinically indicated remain important preventive strategies. Other malignancies, including rhabdomyosarcoma, juvenile myelomonocytic leukaemia, melanoma, and ovarian cancer, also occur with increased frequency, although routine screening for these is not currently recommended (Miller et al., 2019; Solare et al., 2022). The single malignant peripheral nerve sheath tumour identified in an adolescent patient in our cohort illustrates both the rarity and severity of malignant transformation in NF1 (Miller et al., 2019; Solare et al., 2022; Perrino et al., 2024). Our recommendations therefore highlight the need for heightened clinical awareness of warning signs suggestive of tumour progression and for prompt diagnostic imaging and histopathological confirmation when malignant change is suspected.

In parallel with optimising clinical surveillance, the role of molecular genetic testing in NF1 has expanded considerably in recent years, serving not only as a confirmatory tool in diagnostically challenging cases, but also as a potential means of prognostic stratification and personalised follow-up planning. According to current guidelines and our proposed protocol, genetic testing is indicated when: (1) the clinical presentation is inconclusive and the NIH criteria are not yet fully met, particularly in young children with isolated pigmentary features; (2) a definitive diagnosis is required for prenatal or preimplantation genetic counselling; (3) differentiation from other RASopathies or NF1-like syndromes (e.g., Legius syndrome,

Noonan syndrome) is necessary; or (4) atypical manifestations raise suspicion for mosaic NF1 or segmental involvement (Miller et al., 2019; Tamura et al., 2021).

Furthermore, the availability of molecular techniques has broadened the scope of prenatal diagnostics in NF1, which, while technically feasible and increasingly performed, continues to pose ethical and prognostic challenges due to the lack of reliable genotype–phenotype correlations. In addition to chorionic villus sampling, amniocentesis, and non-invasive cfDNA testing, preimplantation genetic diagnosis (PGD) also represents a viable option for families with known pathogenic NF1 variants undergoing assisted reproduction. Recent large-scale data indicate that, in families with a known parental NF1 mutation, the pathogenic variant is detected in approximately 40–60% of tested fetuses (Pacot et al., 2024; van Minkelen et al., 2014).

Beyond its diagnostic applications, molecular testing forms the basis for genotype–phenotype correlation studies, which aim to identify mutation-specific risks and enable more accurate prognostic assessment. Although establishing robust correlations remains challenging due to the large size of the *NF1* gene, the wide mutational spectrum, and the marked inter- and intrafamilial variability, selected associations have been consistently reported — for example, large deletions with more severe phenotypes and early-onset tumour burden, or certain missense variants with predominantly pigmentary manifestations (Komlósi et. al. 2011, Pasmant et al., 2012). Expanding this knowledge base is essential, as emerging targeted therapies and potential gene-based interventions will increasingly rely on patient stratification by genetic profile. This consideration formed the basis for our genotype–phenotype analysis in the genetically confirmed subgroup of our cohort.

The distribution of pathogenic variant types was as follows: frameshift (n = 17, 37.8%), nonsense (n = 11, 24.4%), splice-site (n = 8, 17.8%), missense (n = 4, 8.9%), and copy number variations (n = 5, 11.1%) (Veres et al., 2025).

Frameshift variants, representing the largest group (37.8%) like in most of the studies showed a notable association with several features (Stella et al., 2018; Bianchessi et al., 2015; Zhang et al., 2015; Alkindy et al., 2012). The presence of intracranial findings in patients with frameshift mutations was observed in 35.3% (6/17) of the sample, which is lower than the average prevalence of brain abnormalities found in other mutation types (55.5%) and reported in the literature (Kang et al., 2022; Sánchez et al., 2022; Zhang et

al., 2015; Solmaz et al., 2021). Lisch nodules were identified in 6 out of 17 patients (35.3%); however, their occurrence did not demonstrate a stronger association with frameshift variants compared to other mutation types reported in former studies (Stella et al., 2018; Sabbagh et al., 2013; Castle et al., 2003). The presence of scoliosis (29.4%, 5/17) in this group also warrants further investigation given the known association between skeletal abnormalities and NF1 (Scala et al., 2021). Although the results obtained in this subgroup statistically were not significant, it is worth noting that pseudarthrosis (5.9%, 1/17) was observed in one patient. Motor developmental delays and fine motor disturbances were observed in 11.8% (2/17) of patients with frameshift variants, while cognitive and learning disabilities were detected in 23.5% (4/17) of patients. These findings are significantly lower than those reported in the literature, similar to our observations in other mutation groups (Sánchez et al., 2022; Isenberg et al., 2013). Although cardiovascular malformations are more commonly associated with microdeletion syndrome (Wang et al., 2021; Kehrer-Sawatzki et al., 2020), in our cohort, the detection rate of cardiologic involvement (mild mitral valve insufficiency) was the highest in subjects with frameshift mutations at 11.7% (2/17).

The **stop-gain mutation** was the second most common (24.4%) pathogenic mutation type in our cohort, which was consistent with several studies (Stella et al., 2018), although some other studies reported nonsense mutation to be the most common NF1 pathogenic variant (Scala et al., 2021; Calì et al., 2016, Polgár et al., 2011). The nonsense mutation group showed a relatively lower frequency of severe manifestations. Intracranial findings were common (63.6%, 7/11), confirming the findings in the literature (Sánchez et al., 2022; Scala et al., 2021; Solmaz et al., 2021), but other severe features, such as plexiform neurofibromas, were notably absent. The rates of motor developmental delay/fine motor disturbances (18.2%, 2/11), cognitive/learning disabilities (27.3%, 3/11), and scoliosis (27.3%, 3/11) were similar to those observed in other mutation categories.

The **splice-site mutation** group (17.8%) demonstrated a notable presence of pseudarthrosis (25%, 2/8), suggesting a potential association between this mutation type and this specific skeletal abnormality; however, further investigation in larger cohorts is necessary to establish its clinical significance. Additionally, skeletal abnormalities were observed in 50% (4/8) of patients, alongside a notable presence of intracranial findings in 62.5% (5/8) of patients, aligning with known manifestations of NF1 (Alkindy et al., 2012;

Ly et al., 2019; Wang et al., 2021; Solmaz et al., 2021). Lisch nodules were also reported in 50% (4/8) of patients with splice-site variants, although previous studies have indicated a slightly lower incidence (Scala et al., 2021; Alkindy et al., 2012). The prevalence of plexiform neurofibromas was the highest in this group (3/8, 37.5%) compared to other mutation types, but the presence of cognitive or learning disabilities was limited to only one case (12.5%), which is remarkably lower than reported in former studies (Alkindy et al., 2012). While we detected notably high percentages of various clinical features within this cohort, the limited sample size necessitates caution in drawing firm conclusions. The greater severity of phenotypes associated with splicing variants compared to frameshift or other loss-of-function variants may be explained by disrupted pre-mRNA processing, leading to aberrant transcripts that evade nonsense-mediated decay (NMD) and exert dominant-negative effects. Factors such as the genetic diversity within our Hungarian cohort, variations in modifier genes or regulatory elements, environmental and epigenetic influences, variant distribution, and methodological differences may also contribute to the observed severity (Pasmant et al., 2012, Szudek et al., 2002). Evidence from studies of monozygotic twins also indicates that, while pigmentary and cognitive features tend to show high concordance, tumour-related manifestations can vary markedly even among individuals with identical NF1 mutations, emphasizing the contribution of modifier genes, somatic second hits, and epigenetic mechanisms (Rieley et al., 2011; Sites et al., 2017).

Notably, similar findings of heightened severity linked to splice-site variants have been reported in Duchenne muscular dystrophy, highlighting the broader significance of this mechanism in genetic disorders (Yang et al., 2019). Larger-scale studies are essential to confirm these associations and explore their clinical implications.

In our study, patients with **missense variants** were present with a milder clinical phenotype, which was consistent with the literature, although the small number of patients (n = 4) with missense mutation limits definitive conclusions (Pinna et al., 2015; Koczkowska et al., 2020; Trevisson et al., 2019; Rojnueangnit et al., 2015; Zhu et al., 2024). While half of patients (50%, 2/4) exhibited NF1-specific intracranial findings, no other striking clinical features like neurofibroma/plexiform neurofibroma (0%, 0/4), skeletal involvement (0%, 0/4), pseudarthrosis (0%, 0/4), optic glioma (0%, 0/4), or Lisch nodule (0%, 0/4) were observed, except the presence of one case of cognitive/learning

disabilities (25%, 1/4) and one case of motor developmental delay (25%, 1/4). Larger cohorts will be required to establish further meaningful genotype–phenotype correlations.

NF1 microdeletion syndrome, which involves the deletion of large segments of the *NF1* gene and its flanking region, is associated with more severe phenotypes, as evidenced by our findings in a large number of neurofibromas (Büki et al., 2023; Büki et al., 2021; Pacot et al., 2021; De Luca et al., 2007; Well et al., 2021; Zhu et al., 2024). Four out of five patients (80%) with CNV had numerous neurofibromas all over the body, including internal ones for instance in the liver and ovarium. In one patient, paravertebral neurofibromas caused aqueduct stenosis leading to hydrocephalus, while another developed a malignant peripheral nerve sheath tumour, resulting in severe neurological symptoms, paresis, and eventually death at the age of 18. Contrary to the literature, plexiform neurofibromas were found in only one (20%) of the patients in our cohort, although whole-body MRI was not routinely performed on all of our patients (Kehrer-Sawatzki et al., 2020; Pacot et al.,2021; De Luca et al., 2007; Corsello et al., 2018; Well et al., 2021; Zhu et al., 2024). In our study, NF1-specific intracranial findings were detected in all patients (100%), although only one (20%) out of five children with CNV investigated by head MRI had an optic pathway glioma. This finding is consistent with the literature, indicating that there is no increased risk of optic glioma in children with *NF1* microdeletions compared to the general NF1 population (Kehrer-Sawatzki et al., 2020; Pacot et al., 2021). As reported in the literature, motor developmental delays and fine motor disturbances were observed in three out of five patients (60%) (Kehrer-Sawatzki et al., 2020; Lorenzo et al., 2010; Pardej et al., 2021). Skeletal abnormalities were detected in three (60%) out of five patients, of whom two (40%) had scoliosis and one (20%) had pectus excavatum. This aligns with previous findings, which reported scoliosis in 43% and pectus excavatum in 30% of patients with microdeletions (Mautner et al., 2010; Mensink et al., 2006). Valgus deformity was also identified in one (20%) of our patients. Café-au-lait macules were present in all CNV cases, and skinfold freckling also had a high appearance (80%) in this group. Additional clinical manifestations, like dysmorphic facial features, including macrocephaly and Noonan-like facial features, were observed in just one patient (20%) with CNV, which is significantly below the data as reported formerly (Kehrer-Sawatzki et al., 2020; Büki et al., 2021; Büki et al., 2017; Sánchez et al., 2022). In contrast to the literature, cognitive disability was noted in only

one patient (20%), which may be attributed to the limited size of our cohort (Pacot et al., 2021)."

Beyond cohort-level trends, an illustrative case in our series underscores the diagnostic and pathogenetic insights that can emerge from integrating sequencing-based approaches into the evaluation of NF1. In this patient, a known pathogenic splice-site variant (c.2326-2A>G) was detectable in blood, unaffected skin, and a massive segmentally distributed plexiform neurofibroma, while a second, likely pathogenic frameshift variant (c.4933dupC, p.Leu1645Profs*7) was identified exclusively in tumour tissue at low variant allele frequency (16%), consistent with mosaic distribution due to admixture of non-neoplastic cells and the heterozygous state of the mutation in Schwann cells. This constellation is consistent with superimposed mosaicism — the coexistence of generalised NF1 with segmentally aggravated disease due to an early postzygotic mutational event — and provides direct support for the two-hit model of tumorigenesis (Knudson et al., 1971; Happle et al., 1993; Happle et al., 2016; De Raedt et al., 2003; Upadhyaya et al., 2008). In this patient, the coexistence of a germline splice-site variant and a second somatic frameshift mutation within the tumour is consistent with biallelic NF1 inactivation rather than classic loss of heterozygosity, representing a molecular correlate of superimposed mosaicism. In NF1, one allele typically carries a germline pathogenic variant, and tumour formation requires a somatic “second hit” leading to complete loss of function. Loss of heterozygosity (LOH) has been reported in 20–70% of PNFs, with the highest rates in MPNSTs (>90%) (Upadhyaya et al., 2008; Pemov et al., 2020), supporting the hypothesis that early NF1 inactivation in undifferentiated precursor cells may account for the diversity of tumour types and anatomical locations (Serra et al., 1997). Importantly, such mosaic lesions are considered at higher risk for malignant transformation, underscoring the need for early molecular characterisation and risk-adapted surveillance (Ferner et al., 2007; Pemov et al., 2020).

In the course of final dataset verification with our collaborating geneticists, minor corrections were applied to the classification of two variants — including the reclassification of one missense to nonsense and the clarification that one presumed CNV represented an intragenic deletion/inversion — to ensure maximal accuracy without altering the overall trends or conclusions.

Further investigation is required to determine whether the trends observed in our cohort extend to other mutation categories and to assess their applicability in broader NF1 populations. The heterogeneity in the prevalence of clinical features across different variant types underscores the complexity of the NF1 genotype–phenotype relationship and the multifactorial nature of its clinical expression. It should also be noted that certain clinically important complications, such as malignancies, could not be adequately assessed in the present analysis due to their low prevalence, highlighting the need for larger datasets to explore these associations in depth.

This study has several limitations that warrant consideration. First, the relatively small size of the genotyped subgroup reflects both the historically limited availability of NF1 genetic testing in Hungary and the modest size of the national population, which may have reduced the statistical power to detect subtle genotype–phenotype associations. Second, the retrospective design, spanning a 15-year period, means that patient ages were calculated at the time of manuscript preparation and may not correspond to the ages at which specific clinical assessments were performed, potentially underestimating the prevalence of features that emerged later. Third, the lack of complete, standardised multidisciplinary evaluations — including neurological, ophthalmological, orthopaedic, cardiological, and audiological examinations, as well as uniform access to cranial and whole-body MRI — may have led to underreporting of certain manifestations. Finally, while rigorous data verification was undertaken, a limited number of minor data corrections were necessary following supplementary genetic review, reflecting the inherent challenges of long-term retrospective data integration.

Despite these constraints, this work represents the largest systematically evaluated paediatric NF1 cohort reported from Hungary and provides novel insights into potential genotype–phenotype correlations, including variant-specific trends that may inform future risk stratification. Beyond the genetic aspects, our findings support the first hypothesis that the clinical manifestations of NF1 follow recognisable age-dependent patterns, underlining the necessity of longitudinal surveillance throughout childhood and adolescence. The second hypothesis, addressing genotype–phenotype relationships, revealed certain mutation-specific tendencies, while also highlighting the inherent variability that necessitates larger, prospectively collected cohorts to establish more robust associations. Finally, the third hypothesis is reflected in the development of an age-

adapted diagnostic and follow-up protocol, integrating both international recommendations and cohort-specific observations. Collectively, these results not only contribute to the understanding of NF1 in the Hungarian population but also emphasise the importance of harmonising clinical, genetic, and protocol-driven approaches to improve patient outcomes and to lay the foundation for future genotype-informed personalised care in NF1.

6. Conclusions

This thesis investigated neurofibromatosis type 1 in the Hungarian paediatric population from three perspectives: clinical spectrum, genotype–phenotype associations, and an age-adapted diagnostic and follow-up protocol.

Our findings confirmed age-dependent trajectories of NF1 manifestations, underscoring the need for structured, age-specific surveillance. The genetic analysis suggested variant-related tendencies: splice-site and CNV mutations were linked to more severe phenotypes, missense variants to milder ones, and frameshift/nonsense variants showed intermediate presentations. Although limited by sample size, these results highlight the importance of genetic stratification and the need for larger, multicentre studies. The proposed protocol integrates these insights to enhance multidisciplinary care and early detection of complications.

Overall, the results support all three hypotheses and provide the first systematically evaluated dataset on paediatric NF1 in Hungary, forming a foundation for future precision-medicine approaches.

7. Summary

This doctoral work addressed three hypotheses: (1) that the clinical spectrum of the Hungarian paediatric NF1 population is largely comparable to that of international cohorts; (2) that genotype–phenotype associations reveal mutation-specific severity patterns; and (3) that these findings enable the development of an age-adapted follow-up protocol.

Hypothesis 1 was partly supported: the Hungarian paediatric NF1 cohort showed a generally similar clinical profile to international data, with age-related patterns following comparable trends. Café-au-lait macules were almost universal, while freckling, cutaneous neurofibromas, Lisch nodules, and cognitive and behavioural disturbances appeared later and somewhat less frequently. In contrast, the occurrence of plexiform neurofibromas, optic pathway gliomas and skeletal manifestations closely matched international findings. Minor discrepancies may partly reflect the retrospective design and potential underdetection.

Hypothesis 2 was also supported: splice-site variants were associated with disproportionately severe phenotypes (skeletal, intracranial abnormalities, plexiform neurofibromas, Lisch nodules, pseudarthrosis), while microdeletions conferred the most severe multisystemic involvement, and missense variants milder profiles. These tendencies point to aberrant splicing and modifier genes as possible mechanisms but require larger studies.

Hypothesis 3 was realised in a structured, multidisciplinary, age-adapted follow-up protocol integrating clinical trajectories, genetic categories, and international recommendations, aiming to improve diagnostic accuracy, early complication detection, and personalised care.

In conclusion, this thesis provides the first integrated dataset on paediatric NF1 in Hungary, reveals variant-associated severity patterns, and proposes a clinically applicable protocol, laying the groundwork for prospective multicentre research and precision medicine.

Table 7. NF1 gene variants identified in 45 patients. Variants are shown with cDNA and protein nomenclature according to both NM_000267.3 and the MANE transcript NM_001042492.3, together with mutation type and novelty. Current literature recommends the use of the MANE transcript for standardized reporting.

Patient ID	NM_000267.3 transzkript	NM_001042492.3 MANE transzkript	Novelty	Variant classification
Patient 1.	c.6792C>A heterozygote p.(Tyr2264*)	c.6855C>A heterozygote p.(Tyr2285*)	known	nonsense
Patient 2.	NF1 17q11.2 microdeletion syndrome			CNV
Patient 3.	NF1 17q11.2 microdeletion syndrome			CNV
Patient 4.	c.5190delC heterozygote p.(Val1732Phefs*12)	c.5253del heterozygote p.(Val1753Phefs*12)	known	frameshift
Patient 5.	c.2326-2A>G heterozygote	c.2326-2A>G heterozygote	known	splice-site
Patient 6.	c.4267A>G heterozygote p.Lys1423Glu	c.4330A>G heterozygote p.Lys1444Glu	known	missense
Patient 7.	c.5224C>T heterozygote p.(Gln1742*)	c.5287C>T heterozygote p.(Gln1763*)	known	nonsense
Patient 8.	c.480-1G>T heterozygote	c.480-1G>T heterozygote	novel	splice-site
Pateint 9.	c.2560C>T heterozygote p.(Gln854*)	c.2560C>T heterozygote p.(Gln854*)	known	nonsene
Patient 10.	c.5262_5265delATCA heterozygote p.(Val1756Phefs*2)	c.5325_5328del p.(Val1777Phefs*2)	known	frameshift
Patient 11.	c.4269+1G>A heterozygote	c.4332+1G>A heterozygote	novel	splice-site
Patient 12.	NF1 17q11.2 microdeletion syndrome			CNV
Patient 13.	c.1140del heterozygote p.(Val381Phefs*6)	c.1140del heterozygote p.(Val381Phefs*6)	known	frameshift
Patient 14.	c.889-1G>T heterozygote	c.889-1G>T heterozygote	known	splice-site
Patient 15.	c.3301_3302del heterozygote p.(Gln1101Valfs*4)	c.3301_3302del heterozygote p.(Gln1101Valfs*4)	known	frameshift
Patient 16.	c.1318C>T heterozygote p.(Arg440*)	c.1318C>T heterozygote p.(Arg440*)	known	nonsene
Patient 17.	c.1667_1670delATAG heterozygote p.(Asp556Alafs*11)	c.1667_1670delATAG heterozygote p.(Asp556Alafs*11)	known	frameshift
Patient 18.	c.2761delG heterozygote p.(Val921Trpfs*3)	c.2761delG heterozygote p.(Val921Trpfs*3)	novel	frameshift
Patient 19.	c.5710G>T heterozygote p.(Glu1904*)	c.5773G>T heterozygote p.(Glu1925*)	novel	nonsene
Patient 20.	c.6434delG heterozygote p.(Gly2145Alafs*34)	c.6497delG heterozygote p.(Gly2166Alafs*34)	novel	frameshift
Patient 21.	c.4984_4990delinsGGAG heterozygote p.(Asn1662_Trp1664delinsGlyGly)	c.5047_5053delinsGGAG heterozygote p.(Asn1683_Trp1685delinsGlyGly	novel	in-frame indel
Patient 22.	c.1845+1G>C heterozygote	c.1845+1G>C heterozygote	novel	splice-site
Patient 23.	c.3721C>T heterozygote p.(Arg1241*)	c.3721C>T heterozygote p.(Arg1241*)	known	nonsene
Patient 24.	c.1541_1542delAG heterozygote p.(Gln514Argfs*43)	c.1541_1542delAG heterozygote p.(Gln514Argfs*43)	known	frameshift
Patient 25.	c.653T>A heterozygote p.(Val212Asp)	c.635T>A heterozygote p.(Val212Asp)	novel	missense
Patient 26.	c.6641+1G>C heterozygote	c.6704+1G>C	known	splice-site
Patient 27.	c.4537C>T heterozygote p.(Arg1513*)	c.4600C>T heterozygote p.(Arg1534*)	known	nonsene
Patient 28.	c.4375delC heterozygote p.(Asp1460Ilefs*2)	c.4438delC heterozygote p.(Asp1481Ilefs*2)	known	frameshift
Patient 29.	NF1 17q11.2 microdeletion syndrome			CNV
Patient 30.	c.499_502delTGTT heterozygote p.(Cys167Glnfs*10)	c.499_502delTGTT heterozygote p.(Cys167Glnfs*10)	known	frameshift
Patient 31.	c.2991-1G>A heterozygote	c.2991-1G>A heterozygote	known	splice-site
Patient 32.	c.1238C>G heterozygote p.(Ser413*)	c.1238C>G heterozygote p.(Ser413*)	known	nonsene
Patient 33.	c.3703C>T heterozygote p.(Gln1235*)	c.3703C>T heterozygote p.(Gln1235*)	known	nonsene
Patient 34.	c.2557C>T heterozygote p.(Gln853*)	c.2557C>T heterozygote p.(Gln853*)	known	nonsene
Patient 35.	c.6841C>T heterozyote p.(Gln2281*)	c.6904C>T heterozygote p.(Gln2302*)	known	nonsene
Patient 36.	c.1466A>G heterozygote p.(Tyr489Cys)	c.1574A>G heterozygote p.(Tyr525Cys)	known	missense
Patient 37.	c.4752_4761del heterozygote p.(Ile1584Metfs*16)	c.4825_4834del heterozygote p.(Ile1605Met fs*16)	novel	frameshift
Patient 38.	c.5527_5528del heterozygote p.(Ser1843Valfs*2)	c.5590_5591del heterozygote p.(Ser1864Phefs*2)	novel	frameshift
Patient 39.	c.3457_3460del heterozygote p.(Leu1153Metfs*4)	c.3457_3460del heterozygote p.(Leu1153Metfs*4)	known	frameshift
Patient 40.	c.2271_2272del heterozygote p.(Arg758Serfs*9)	c.2271_2272del heterozygote p.(Arg758Serfs*9)	known	frameshift
Patient 41.	c.1765C>T heterozygote p.(Gln589*)	c.1765C>T heterozygote p.(Gln589*)	known	nonsene
Patient 42.	c.1845+1G>T heterozygote	c.1845+1G>T heterozygote	known	splice-site
Patient 43.	c.2033dupC heterozygote p.(Ile679Aspfs*21)	c.2033dupC heterozygote p.(Ile679Aspfs*21)	known	frameshift
Patient 44.	c.952_953delGA heterozygote p.(Glu318Lysfs*11)	c.952_953delGA heterozygote p.(Glu318Lysfs*11)	known	frameshift
Patient 45.	c.246_247delTC heterozygote p.(Gln83Valfs*23)	c.246_247delTC heterozygote p.(Gln83Valfs*23)	known	frameshift

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9. Bibliography of the candidate's publications

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11. ETHICAL APPROVAL

This study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the Heim Pál National Paediatric Institute, Budapest, Hungary (project no. KUT—45/2024).