



The Heart in Neurofibromatosis 1

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Neurofibromatosis type 1 (NF1) is one of the most common inherited disorders and affects one in 3000 people worldwide. It is a multisystem genetic disorder that is primarily associated with cutaneous, neurologic, and orthopedic manifestations. NF1 is caused by dominant loss-of-function mutations of the tumor suppressor *NF1* (Neurofibromin 1; MIM #613113), which is located at 17q11.2 and contains 60 translated exons over ~280 kb [1]. It is a completely penetrant Mendelian disease marked by an extremely variable clinical expressivity of the major features as well as the less frequent complications [2–4].

The major features of NF1 are multiple neurofibromas, café-au-lait macules, intertriginous freckling, Lisch nodules, tibial pseudarthrosis, and a tendency to develop benign and malignant tumors of the nervous system [5]. NF1 is diagnosed primarily on clinical grounds using the National Institutes of Health (NIH)

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diagnostic criteria [6]. The diagnosis of NF1 requires at least two out of the following seven NIH criteria:

- 6 or more café au lait macules (>0.5 cm in children or >1.5 cm in adults)
- 2 or more cutaneous/subcutaneous neurofibromas or one plexiform neurofibroma
- Intertriginous (axillary or groin) freckling
- Optic pathway glioma
- 2 or more Lisch nodules (iris hamartomas seen on slit lamp examination)
- Bony dysplasia (sphenoid wing dysplasia, bowing of long bone \pm pseudarthrosis)
- First degree relative with NF1

By using these criteria, the diagnosis of NF1 can be made based on physical examination and by evaluating the patient's family history. Genetic testing is not necessary if the diagnosis has already been clinically confirmed. These criteria usually appear in the following predictable order: café-au-lait macules (CALMs), axillary freckling, Lisch nodules, and neurofibromas. The characteristic osseous lesions usually develop within the first year of life, and the diagnosis of symptomatic optic glioma appears by 3 years of age [7]. However, these diagnostic criteria emerge in a variable manner, with some patients revealing criteria at a late onset and others never developing certain signs and symptoms. Definitive diagnosis may therefore be delayed by years. Indeed, although the NIH diagnostic criteria are useful for the diagnosis of NF1 in the majority of children at the age of 5 years, they are inadequate for reaching a diagnosis at an earlier age [7, 8]. Ninety-seven percent of NF1 patients meet the NIH criteria by the age of 8 years, and all do so by the age of 20 years [7]. On the other hand, only 50% of children younger than 2 years with sporadic NF1 fulfil a single NIH criterion, leading to delayed diagnosis [7].

Genetic testing is therefore useful for confirming the diagnosis in children who present with an unusual phenotype or those who do not meet the diagnostic criteria. Revising these diagnostic criteria is currently a hot topic in the NF1 community which believes that genetic testing should also be a diagnostic criteria; specially, that a positive genetic test shortens the period of diagnostic uncertainty which entails early appropriate surveillance. Furthermore, the clinical picture may be incomplete in some rare forms in adults, requiring genetic testing to confirm the NF1 diagnosis. Patients with limited and localized clinical features and no family history of NF1 may possibly have segmental NF1 [9].

Café-au-lait macules are large, generally oval, well-defined hyperpigmented macules which are usually present at birth and occur in >90% of patients (Figs. 7.1) [7]. CALMs may increase in number and size in early childhood [5]. They have no malignant potential. The majority of patients with NF1 (around 80%) will have more than 5 CALMs by 1 year of age. They become darker with sun exposure and fade with age or become obscured by numerous neurofibromas. Legius syndrome [10, 11], Noonan syndrome and constitutional mismatch repair-deficiency syndrome [12] are other syndromes which exhibit multiple CALMS [13]. Children with multiple CALMs and no other NF1 clinical feature and no family history of

Fig. 7.1 Café-au-lait macules are large, generally oval, well-defined hyperpigmented macules



NF1 might be referred to genetic testing to confirm a diagnosis of NF1. In a study of 71 patients younger than 20 years of age with six or more CALMs and no non-pigmentary criterion, 66.2% were confirmed to have NF1, 8.5% had Legius syndrome and 25.3% harbored no disease causing variant [14].

Neurofibromas (NF) are benign peripheral nerve sheath tumors and are the cardinal feature of NF1.

Cutaneous (or dermal) NFs are flesh-colored or purplish soft nodules that become pedunculated as they grow (Figs. 7.2 and 7.3). They usually start appearing during late adolescence and are found in the vast majority (>95%) of patients with NF1 [15]. They vary in number from a few lesions to thousands [15]. These tumors are benign and have no risk of malignant transformation, but sometimes lead to significant discomfort and cosmetic disfigurement. *Subcutaneous neurofibromas (or peripheral nodular NFs)* are firm discrete rubbery nodules bulging under the skin. They affect at least 20% of NF1 patients and usually develop during adolescence [16]. *Internal (nodular) NFs* are neurofibromas that cannot be felt by physical examination. They are associated with a high-risk phenotype necessitating closer monitoring and management [17]. *Plexiform neurofibromas* are congenital lesions found in 20 to 26% of individuals with NF1 [18]. They present as a subtle enlargement of soft tissue with a “wrinkled” texture or a patch of hyperpigmentation with or without hypertrichosis. A substantial increase in size occurs during the first

Fig. 7.2 Cutaneous neurofibromas are flesh-colored or purplish pedunculated soft nodules



decade of life and adolescence [19]. These tumors can invade surrounding structures, including muscle and bone which leads to substantial pain and bone destruction [20].

Malignant peripheral nerve sheath tumors (MPNSTs) are a subtype of sarcoma that arise in preexisting plexiform neurofibromas or subcutaneous NF (Fig. 7.4) [21]. The cumulative lifetime risk of developing MPNST in NF1 patients is 8–13% [21–23]. Seventy percent of MPNSTs are high-grade tumors that can metastasize widely and entail a poor prognosis [22]. Symptoms most suggestive of MPNST are a rapid increase in the size of an existing plexiform neurofibroma or alteration in its consistency from soft to hard, persistent or difficult to control pain, or new neurological deficit [22].

Intertriginous freckling is found in >80% of NF1 individuals [5, 15]. They may be found in any area where skinfolds are in apposition, including the axilla, intertriginous area, base of the neck, upper eyelid and under the breasts in women (Figs. 7.5) [24].

Optic pathway gliomas (OPG) are benign tumors seen in 15–20% [25–30] of children with NF1. They are slow growing tumors with a low potential of malignancy and they usually occur within the optic pathway including the optic nerve and optic chiasma [26, 31]. They are often indolent; however, due to their space

Fig. 7.3 Cutaneous neurofibromas are flesh-colored or purplish pedunculated soft nodules



occupying nature they can be locally invasive and become symptomatic [30, 32–35]. OPG can cause a rapid onset of proptosis leading to moderate-to-severe visual loss in the affected eye [25, 29, 36]. Precocious puberty can occur if the optic pathway tumor impinges on the optic chiasm [29, 37]. Therefore, all children diagnosed with NF1 should have a regular specific pediatric ophthalmological exam.

Lisch nodules are pigmented iris hamartomas that first appear around the age of 3 years and are found in 100% of patients by the age of 30 years [7, 38, 39]. They are asymptomatic 1–2 mm yellow-brown dome shaped papules of the iris; they are best visualized using careful slit-lamp examination of the non-dilated iris.

Congenital dysplasia of the long bones is a classic manifestation of NF1 and occurs in 7.2% of patients [40]. Bowing of long bones leads to a visible deformity and a fragile bone that is susceptible to fracture [41]. Repeated fractures that fail to heal can lead to pseudarthrosis (failure of primary union of the separate bone ends can create a false joint) [40, 42]. Bowing in an NF1 infant necessitates prompt radiographic examination and referral to an orthopedic surgeon.

Sphenoid wing dysplasia is a distinctive feature of NF1 found in a minority of patients (1–7%) [43].

The sphenoid bones comprise multiple ossification centers that fuse to become the important elements of the orbits. Sphenoid wing dysplasia is usually found early

Fig. 7.4 A malignant peripheral nerve sheath tumor (MPNST) over the shoulder



in life, is often unilateral and may progress over time. One complication of sphenoid wing dysplasia is a pulsating exophthalmos without visual loss; and an absent sphenoid wing can lead to herniation of the temporal lobe into the orbit [44]. The treatment of sphenoid wing dysplasia should be carried out by a multidisciplinary team including cranio-facial teams familiar with this complex surgery.

People with NF1 may also develop cardiac disease, however their prevalence, natural history, and pathogenesis have only recently received medical and scientific attention. This chapter focuses on the vast array of cardiac manifestations associated with NF1, including congenital and acquired hearing defects, as well as the role of Neurofibromin 1 in cardiac embryogenesis.

Congenital Heart Defects

The incidence and type of congenital heart defects in individuals with NF1 were long undefined and not well-characterized. The previously reported frequencies of congenital heart defects ranged from 0.4 to 8.6% in 8 large series of NF1 patients

Fig. 7.5 Skinfold freckling of the axilla



[45–52]. However, the diagnosis of both NF1 and congenital heart diseases was not clearly established in these patients and not clearly distinguished from Watson and NF1-Noonan syndromes.

It has been suggested that the seemingly conflicting data concerning the prevalence of cardiovascular malformations in NF1 is attributable to the fact that not all NF1 patients are systematically screened by means of echocardiography, especially that the cardiac defect is often mild and asymptomatic. Tedesco et al. were the first to evaluate the prevalence of cardiovascular abnormalities in patients with NF1 using echocardiography with color Doppler scan and found cardiac abnormalities in 13/48 young patients (27%) [53]. The same group later described the cardiac abnormalities in 13 out of a total of 69 young patients (18.8%) with NF1 [54]. This was followed by a study in the Turkish population whereby sixty-five NF1 patients were retrospectively studied, looking specifically at their standard electrocardiography and echocardiography. Congenital cardiac abnormalities were found in 11 patients (15.3%) [55]. However, all of these studies are limited by their small sample size.

Two large series have carefully looked at the cardiovascular malformation in patients with NF1. The first one reviewed the cardiovascular abnormalities, in

particular the cardiovascular malformations among 2,322 patients with well-established NF1 in the National Neurofibromatosis Foundation International Database (NNFFID) from 1991–98 [56]. A clear diagnosis of cardiovascular disease was found in 97/2,322 (4.2%) of patients with NF1. Cardiovascular malformations were found in 54/2,322 (2.3%) of these NF1 patients, of whom only two had Watson syndrome and only two had NF1-Noonan syndrome. When excluding patients with the latter syndromes, the overall frequency of cardiovascular malformations among NF1 patients was 2%. The second and more recent large series was a retrospective registry-based total population study conducted in Finland that evaluated the congenital anomalies in 465 children with NF1 [57]. NF1 children were found to have a significantly increased risk of congenital cardiovascular anomalies (adjusted OR 3.35), with congenital heart defects occurring in 1.8% of NF1 patients, a figure similar to that reported in the NNFFID study as well as in the studies by Carey et al. [49] and Schorry et al. [50] All of these data demonstrate a higher than expected frequency of congenital heart defects among patients with NF1, warranting early diagnosis in NF1 children to prevent potential long-term hemodynamic consequences.

In the NNFFID study, the predominant (43/54, 80%) cardiovascular malformations were classified as “flow defects,” whereby left or right heart obstruction or simple shunts are thought to be secondary to anomalous embryonic intracardiac hemodynamics [56]. Among these, the majority (25/43) had pulmonary stenosis, usually valvular. When compared to the patients in the Baltimore-Washington Infant Study (BWIS), pulmonary stenosis in NF1 patients constituted a much larger proportion of all cardiovascular malformations than expected. Indeed, the proportion of all cardiovascular malformations that were pulmonary stenosis was 4.3 times greater among NF1 patients. Similarly, individual case reports and smaller series also frequently reported pulmonary stenosis. Fortunately, pulmonary stenosis in patients with NF1 seems to be generally mild and not a source of serious morbidity. It is interesting to note that pulmonary stenosis is also a well-recognized feature of three clinical subtypes of NF1: NF1-Noonan syndrome, Watson syndrome, and individuals with large deletions of the NF1 gene [56, 58]. Pulmonary stenosis should therefore be considered in any NF1 patient with a systolic murmur.

Aortic coarctation was observed in 5/54 of patients in the NNFFID study and was 3.2 times greater among the NF1 patients than in the BWIS population. However, in 3 out of these 5 patients, the coarctation was a fusiform narrowing of the descending thoracic aorta, which is different from the typical juxtaductal shelf usually described [56]; suggesting that aortic coarctation is more of a NF1 vasculopathy rather than a true cardiovascular malformation.

It remains uncertain whether hypertrophic cardiomyopathy (HCM) constitutes a rare manifestation of NF1, or whether their simultaneous occurrence is simply related to chance. None of the reported patients in the NNFFID study had HCM which may have been due to the small sample size and insufficient statistical power

to identify this particular association. Lin et al. reviewed the different case reports in the literature: out of the 18 reported patients in the literature at that time, probably 14 had primary HCM (idiopathic hypertrophic subaortic stenosis and left ventricular). None of these patients had molecular analysis for either the NF1 or any of the common HCM mutations [59]. One patient had extrinsic compression by a neurofibroma which resulted in biventricular hypertrophy [60]. In 2 patients, HCM and NF1 were most likely segregating as independent autosomal dominant traits [61]. Histological examination of the myocardium was available in two patients (post-mortem examination [62] or biopsy [63]) revealing non-specific changes and fiber disproportion with interstitial fibrosis, respectively. Tedesco et al.'s echocardiography study detected two patients with septal to posterior left ventricular free wall ratio greater than 1.5, suggesting hypertrophic cardiomyopathy [53]. Another case report presented the case of an 18-year-old boy with NF1 with hypertrophic cardiomyopathy diagnosed using echocardiography showing the systolic anteward movement of the anterior leaflet of the mitral valve [64]. Further large series are needed to elucidate whether HCM is truly a rare manifestation of the NF1 mutation or simply a chance incident.

Interestingly, Class I conotruncal defects and Fallot's tetralogy were rare in the NNFID study, and there were no atrioventricular canal, anomalous pulmonary venous return, complex single ventricle and laterality defects detected [56]. This is in striking contrast to the Nf1 knockout mouse homozygous mutant embryos discussed below [65].

Table 7.1 describes the different congenital cardiac malformations detected in large series.

Table 7.1 Specific congenital cardiac malformations detected in large series found in literature

Authors	Lin et al. [56]	Tedesco et al. [53]	İncecik et al. [55]	Leppävirta et al. [57]
Year	2000	2002	2015	2018
Number of NF1 patients included	2322	48	65	465
Conotruncal, outflow	2			
Tetralogy of Fallot	2			
Pulmonary stenosis	25	1	1	2
Pulmonary valve stenosis	21		1	2
Pulmonary artery stenosis	1	1		
Aortic stenosis	2			1
Aortic valve regurgitation		2	1	1
Mitral valve regurgitation		2	5	
Mitral valve prolapse	1	1		
Tricuspid valve regurgitation			1	

(continued)

Table 7.1 (continued)

Authors	Lin et al. [56]	Tedesco e al. [53]	İncecik et al. [55]	Leppävirta et al. [57]
Secundum atrial septal defect	4	2	2	1
Ventricular septal defect	6		1	1
Patent ductus arteriosus	1			2
Coarctation thoracic aorta	5	1		
Atrial septal aneurysm		2		
Hypertrophic cardiomyopathy		2		

Genotype–Phenotype Correlation

The majority of *NF1* cases result from truncating mutations or whole gene deletions, causing an expected deterioration or absence of the RNA generated from the mutated allele. This leads to the total amount of protein being translated from a single allele only, creating a dosage effect [66].

For patients with intragenic *NF1* mutations which represent over 90% of all *NF1* cases, no straightforward genotype–phenotype correlations have been identified except for few exceptions [67].

NF1 and its Related Disorders (NF1-Noonan Syndrome (NFNS) and Watson Syndrome (WS))

Watson syndrome (WS) and NF1–Noonan syndrome (NFNS) were first described clinically and were later shown to be caused by *NF1* mutations [68–70]. Over 20 years ago, Watson had described three families with an autosomal dominant inheritance of pulmonary stenosis, multiple café-au-lait macules and lower intelligence [71]. Further studies showed that the Watson phenotype is distinct from that of *NF1*, as adult patients rarely had neurofibromas, if any [72].

Patients with NFNS, have overlapping features of both *NF1* and Noonan syndrome. Although at first thought to be a distinct condition, subsequent clinical [51] and molecular studies have proved that the majority of NFNS patients have *NF1* gene mutations only, mostly non-truncating mutations consisting of in-frame deletions as compared to typical *NF1* [73, 74].

A recent study investigated whether pulmonary stenosis is related to specific types of *NF1* gene mutations in *NF1*, NFNS and WS. The study examined the frequency of different *NF1* mutation types in a cohort of published and unpublished cases with *NF1*/NFNS/WS and pulmonary stenosis [66].

Compared with *NF1* in general, NFNS patients had statistically significant higher rates of pulmonary stenosis ($9/35 = 26\%$ vs $25/2322 = 1.1\%$). Further stratification of NFNS group according to the mutation type showed that the higher pulmonary stenosis rate appears to be affected specifically by non-truncating mutations. Eight out of twelve (66.7%) NFNS cases with non-truncating

mutations had pulmonary stenosis compared with only 1.1% pulmonary stenosis frequency in NF1 in general. In contrast, only 6.2% (1/16) of NFNS patients with a truncating mutation had pulmonary stenosis (not significantly different from NF1 patients). Looking specifically at individuals with NF1 and pulmonary stenosis, 8/11 (73%) of them had non-truncating mutations, which is largely higher than expected in the NF1 population (19% reported in NF1 cohorts). As for WS, out of the three available published cases with intragenic mutations, two of them had non-truncating mutations. Therefore, non-truncating mutations seem to play a specific role in pulmonary stenosis in NF1 and its related disorders. The authors postulated that unlike in truncating mutations whereby there is decay or absence of the RNA generated from the mutated allele with resulting dosage effect, non-truncating mutations do not result in loss of the NF1 protein. Instead it might result in an abnormally functioning protein with special cardiac effect. This suggests that the pulmonary stenosis is caused by an additional effect of the mutated protein instead of the common loss-of function effect. This effect may be similar to the cardiac effect of Noonan syndrome and other RASopathies and may characterize a particular role of these mutations in the Ras–MAPK pathway.

NF1 Microdeletion Syndrome (MIM 613675)

In 5–10% of patients, NF1 results from microdeletions that encompass the entire *NF1* gene and a variable number of flanking genes [75]. Patients carry a heterozygous deletion of 17q11.2 region usually spanning about 1–1.4 Mb [58, 76]. Typically, they present with a more severe phenotype compared with the one observed in NF1 with intragenic mutation [77].

Numerous studies have described cardiac defects in patients with NF1 microdeletions [52, 58, 77–85].

However, the overall frequency of cardiac defects in patients with NF1 microdeletions is still uncertain. Venturin et al. showed a prevalence of cardiovascular malformations of 18% in the subgroup of patients with NF1 microdeletion [58]. In another study, 8/28 (29%) NF1 microdeletion patients had cardiovascular anomalies [77]. The first thorough investigation of the frequency of heart defects in patients with NF1 microdeletions showed major cardiac abnormalities in 6 of 16 NF1 microdeletion patients whereas none of 16 patients with intragenic NF1 mutations in that study had heart defects [85].

The specific type and frequency of the heart defects described in patients with NF1 microdeletions are quite varied and are shown in Table 7.2.

It is worth mentioning that mitral valve prolapse and aortic dilatation are both associated with connective tissue abnormalities, now generally recognized as a part of NF1 microdeletion syndrome and have been reported in 12% in one series [83].

The higher frequency of cardiovascular malformations in NF1-microdeletions is most likely due to the haploinsufficiency of genes lying in the deletion interval, possibly involved in heart morphogenesis. One such gene includes *ADAP2* which encodes a protein belonging to the centaurins protein family. Expression analysis studies showed *ADAP2* murine ortholog expression in heart during

fundamental phases of cardiac morphogenesis [87]. The findings of this study suggested a correlation between ADAP2 haploinsufficiency and the presence of valve defects in NF1-microdeleted patients.

Specific Mutations

A specific mutation consisting of a 3-bp inframe deletion (c.2970–2972 delAAT) in exon 17 of the *NF1* gene has recently been found to be associated with a much milder NF1 phenotype with a lack of dermal neurofibromas, and increased rate of pulmonary stenosis [68].

Another genotype-phenotype correlation with cardiac involvement in NF1 are the missense mutations affecting arginine at position 1809. Patients present with pulmonary stenosis, Noonan-like features and developmental delay, but no external plexiform neurofibromas [88, 89]. Affected amino acids reside outside the GAP-related domain (GRD) domain.

A third and more recent genotype-phenotype correlation involves the constitutional missense mutation affecting one of five neighboring NF1 codons—Leu844, Cys845, Ala846, Leu847, and Gly848—located in the cysteine-serine-rich domain (CSRD).

Cardiovascular abnormalities observed in a studied group included hypertension, pulmonic stenosis, mitral valve stenosis, atrial septal defect, ventricular septal defect, Moyamoya disease, pericarditis carcinomatosa, mitral valve insufficiency, mild pulmonary insufficiency, and hypertrophic cardiomyopathy [90].

Acquired Heart Diseases

In addition to congenital heart defects, there is evidence that individuals with NF1 have an increased risk of developing acquired heart diseases. Diastolic function was evaluated in young patients with NF who were free of structural cardiovascular abnormalities using standard Doppler and Pulsed Doppler tissue imaging (DTI), which is a new technique that allows analysis of myocardial velocities and time intervals throughout the cardiac cycle [53]. The main finding of the study are that certain standard and DTI indexes are abnormal in patients with NF as compared to controls, indicating that patients with NF may have abnormal diastolic function. These findings were found in both mild and severe disease, suggesting that the suspected diastolic functional abnormalities may be independent of the clinical severity of NF1. The authors suggested that this may be attributed to cardiac myofibrillar dysplasia or, they may be secondary to abnormal vascular compliance [91].

The same group later evaluated the cardiac function of NF1 patients looking specifically at hypertension. The study revealed the presence of early cardiac morphologic and functional changes in young NF1 patients with hypertension. NF1 hypertensive patients had thicker myocardial walls, increased left ventricular mass index and increased atrial dimension compared with NF1 normotensive patients and healthy subjects [92]. Using standard Doppler, diastolic function was shown to be

significantly more impaired in NF1 hypertensives than normotensive NF1 patients and controls. However, using DTI technique (which detects changes induced by hypertension as well as those independent of blood pressure.), myocardial velocities in NF1 patients were found to be significantly higher than controls regardless of blood pressure. Hence the myocardial abnormalities are an early phenotype in patients with NF1 that presents independently of blood pressure and that can be identified using DTI. The authors here postulated that the high DTI values in NF1 normotensives could be explained by the abnormal sympathetic nerve supply to the heart based on the neural crest origin of NF. Furthermore, the presence of a significant decline in myocardial systolic velocity in NF1 hypertensives, when compared with normotensives, could be used as a measure for the long-term follow-up of those patients, since hypertension has been shown to decrease life expectancy in NF1 patients [93].

To sum up, patients with NF1 require a cardiologic assessment at regular intervals. Serial echocardiograms with standard and DTI measurements could provide important information about cardiac damage in NF1 patients diagnosed with hypertension.

Death from Cardiac Disease

Although heart diseases were found to be slightly lower than expected on death certificates of individuals with NF1 [94], sporadic cases of sudden death in adults

Table 7.3 Literature review of myocardial infarctions with the associated coronary artery anomalies in patients with NF1

Author	Age	Gender	Cardiac event	Pathology
Ruggieri et al. [101]	16	M	Presumed myocardial infarction	Aneurysm of the LAD
Kandarpa et al. [102]	30	M	Myocardial infarction	Coronary artery aneurysm
Daly et al. [96]	39	M	Myocardial infarction	Coronary artery aneurysm
Evrengul et al. [103]	17	F	Myocardial infarction	Coronary artery aneurysm
Fuchi et al. [99]	23	M	Myocardial infarction	Organic stenosis and spasm of LAD
Halper et al. [104]	38	M	Presumed myocardial infarction	Diffuse circumferential thickening of intima of coronary arteries; focal intimal smooth muscle nodules within intima of LAD
Kanter et al. [95]	2	M	Presumed myocardial infarction	Circumferential proliferation of the tunica intima LMCA and LAD
	7	M	Myocardial infarction	Nodular and circumferential proliferations of the tunica intima and tunica media of LMCA and LAD

LAD left anterior descending artery, LMCA left main coronary artery

with NF1 have been reported secondary to coronary artery involvement, vasospasm and myocardial infarction [95–99].

Cardiac diseases related to vascular lesions in patients with NF1 result mainly from an intrinsic pathology of the vessel walls. The NF1 vasculopathy most commonly affects the renal arteries; however, it has also been described in cardiac diseases. It consists of hyperplasia and luminal narrowing, with the proliferating cell type being smooth myocytes. Lesions have been categorized as pure intimal, advanced intimal, intimal-aneurysmal, and nodular [100]. Coronary imaging should therefore be considered in children and adults with NF1 who present with symptoms of angina such as chest pain and syncope.

Table 7.3 describes the different coronary artery anomalies described in NF1 patients in the literature in the form of case reports.

Molecular Basis of Cardiac Malformation and Dysfunction in NF1

The *Nf1* gene codes for neurofibromin, a large protein of 2800 aa. It includes a Ras-GTPase-activating domain (GAP) capable of accelerating the hydrolysis of GTP-bound Ras, thus down-regulating the activity of *Ras* proto-oncogenes which are important regulators of cell proliferation, growth, and differentiation [65, 105]. Hence, mutations that inactivate *Nf1* result in elevated levels of RAS signaling and increased cell proliferation. Further cellular functions for neurofibromin have also been recognized, including modulation of protein kinase A and cyclic adenosine monophosphate pathways [106–108]. The full-length of neurofibromin has been shown to bind to the scaffolding domain of Caveolin-1 [109], whereas the C-terminal region of Neurofibromin 1 has been shown to interact with a major class of heparan sulfate proteoglycans [110].

Neurofibromin is a ubiquitous protein [111] and plays a pivotal role in embryogenesis. The role of haploinsufficiency for neurofibromin in heart development and function has been the subject of many studies. Our understanding of the role of *Nf1* in embryogenesis stems from animal studies, essentially targeted mouse mutants and more recently zebrafish studies [65, 112–116]. All of these studies have shown that complex developmental mechanisms including neural crest migration are involved in the cardiac development in NF1.

Surprisingly enough, the earliest studies have shown that *Nf1* heterozygous mice do not exhibit any in utero cardiovascular malformations. However, *Nf1* knockout mouse homozygous mutant embryos have been found to die during midgestation due to outflow tract septation defects (double outlet right ventricle/membranous ventricular septal defect) and concurrent hyperproliferative enlarged endocardial cushions (the precursors of cardiac valves), that could impede forward blood flow [65, 112].

Zebrafish embryos displayed similar cardiovascular abnormalities to those seen in mouse models following transient knockdown of the orthologous *Nf1* genes (abnormal cardiac valves, presence of pericardial effusions, thinned myocardium) [116].

These cardiovascular malformations were previously thought to be due to abnormal migration of ectomesenchymal cells derived from the cranial neural crest [117]. However, more recent studies demonstrated using conditional gene inactivation that ablating the neurofibromin function specifically in neural crest did not cause cardiac defects. On the other hand, endothelial-specific inactivation of *Nf1* recapitulated the cardiovascular phenotype observed in *Nf1* knockout embryos: enlarged endocardial cushions, ventricular septal defects, double outlet right ventricle, thinned myocardium and pericardial effusions [113, 114]. These myocardial defects were not reproduced by tissue-specific inactivation of *Nf1* in myocardial cells indicating that signaling between the endocardium and myocardium is vital for proper myocardial development. Furthermore, loss of *Nf1* in the embryonic endocardium of these transgenic mice was then shown to be directly associated with an increase in activated GTP-bound Ras with the resulting stimulation of MAPK signaling leading to the abnormal enlargement of the outflow tract septum. Reconstitution of the neurofibromin GRD in endothelial cells is sufficient to rescue cardiovascular development and midgestational lethality in *Nf1* null embryos; however these mice succumb in the early postnatal period due to overgrowth of neural crest-derived tissues, suggesting that other domains outside the GRD are important for neural crest growth and homeostasis [114].

A more recent study also identified ectopic cardiac blood island formation as a new phenotype that arises in *Nf1*-deficient murine embryos and that this phenotype is a direct result of dysregulation of the Ras signaling pathway [118].

In a further study, neurofibromin was also shown to function as an important Ras-GAP in the myocardium of adult mice. Tissue-specific inactivation of *Nf1* in myocardial cells during mid-gestation led to no distinct phenotype at birth or in young adults, consistent with the aforementioned observations [65, 119]. However, as these cardiac *Nf1* deficient mice aged, they developed progressive cardiac hypertrophy, dilatation, fibrosis, and failure with secondary premature mortality. Reconstitution of the Ras-GAP activity in *Nf1* deficient myocardial cells led to the rescue of Ras activity and its downstream effectors, along with partial improvement of in the cardiac dysfunction and hypertrophy [119].

The role of Ras activation in cardiac hypertrophy is better highlighted when evaluating patients with Noonan, Costello, and Cardio-facial-cutaneous (CFC) syndromes. Each of these syndromes can be caused by activating mutations in components of the Ras-MAPK pathway and they are all strongly associated with cardiovascular defects including hypertrophic cardiomyopathy [120–122].

Parallels have been drawn with Noonan syndrome specifically as the spectrum of heart defects in mouse models of both NF1 and Noonan syndrome is markedly similar, including the enlarged endocardial cushions and double outlet right ventricle [123, 124]. These similarities imply that the in utero cardiovascular manifestations of *Nf1* null mice appear to result from endothelially driven hyperproliferation of the endocardial cushions associated with ERK activation.

Conclusion

Although once thought as a rare manifestation of NF1, cardiac malformations are now known to have a higher than expected frequency among patients with NF1 and occur in 2% of these patients. This warrants early diagnosis in NF1 children to prevent potential long-term hemodynamic consequences. Pulmonary stenosis was found to be the most common cardiac defect and related to non-truncating mutations in the *Nf1* gene. NF1 patients also present with cardiac function anomalies such as abnormal diastolic function which were found to be independent of the clinical severity of NF1. Furthermore, NF1 patients exhibit early cardiac functional changes independent of blood pressure which can be identified using DTI. Therefore, NF1 patients require cardiology assessment at regular intervals. Serial echocardiograms with standard and DTI measurements could provide important information about cardiac damage in NF1 patients, especially those diagnosed with hypertension. Animal studies have helped tremendously in understanding the role of *Nf1* in embryogenesis showing that complex developmental mechanisms are involved in cardiac development. Further experiments are needed to better understand disease pathogenesis of cardiovascular malformations in order to develop potential therapeutic strategies for patients with NF1.

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