Original Article

Cardiac characterization of 16 patients with large \textit{NF1} gene deletions


The aim of this study was to characterize cardiac features of patients with neurofibromatosis 1 (NF1) and large deletions of the \textit{NF1} gene region. The study participants were 16 patients with large \textit{NF1} deletions and 16 age- and sex-matched NF1 patients without such deletions. All the patients were comprehensively characterized clinically and by echocardiography. Six of 16 \textit{NF1} deletion patients but none of 16 non-deletion NF1 patients have major cardiac abnormalities (\(p = 0.041\)). Congenital heart defects (CHDs) include mitral insufficiency in two patients and ventricular septal defect, aortic stenosis, and aortic insufficiency in one patient each. Three deletion patients have hypertrophic cardiomyopathy. Two patients have intracardiac tumors. NF1 patients without large deletions have increased left ventricular (LV) diastolic posterior wall thickness (\(p < 0.001\)) and increased intraventricular diastolic septal thickness (\(p = 0.001\)) compared with a healthy reference population without NF1, suggestive of eccentric LV hypertrophy. CHDs and other cardiovascular anomalies are more frequent among patients with large \textit{NF1} deletion and may cause serious clinical complications. Eccentric LV hypertrophy may occur in NF1 patients without whole gene deletions, but the clinical significance of this finding is uncertain. All patients with clinical suspicion for NF1 should be referred to a cardiologist for evaluation and surveillance.

Conflict of interest

The authors declare no conflict of interest.

Neurofibromatosis 1 (NF1) is an autosomal dominant tumor suppressor gene disorder that occurs with an incidence of 1 in 3000. NF1 is characterized by benign peripheral nerve sheath tumors and pigmentation abnormalities, but learning difficulties, skeletal alterations and cardiovascular disease may also occur in affected individuals (1).

Approximately 5\% of all NF1 patients have large deletions of the entire NF1 locus and adjacent region. There are currently three identified types of recurrent NF1 deletions that differ in span of the deleted segment, namely type 1 (1.4 Mb), type 2 (1.2 Mb), and type 3 (1.0 Mb) microdeletions. Type 1 microdeletions encompass 14 genes including NF1. These ‘large’ or ‘whole gene’ deletions (also called ‘NF1 microdeletions’) are associated with more severe manifestations than other kinds of NF1 mutations (2–6). Such manifestations are, for example, cardiac abnormalities, higher tumor
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A past medical history concerning cardiac problems was obtained. We defined the patient to have cardiac disease if a CHD was diagnosed in the prenatal and postnatal period by ultrasound examination or if another cardiac problem that required hospitalization and/or medical treatment was diagnosed after symptoms became apparent later in life. CHDs were defined according to the most recent American Heart Association criteria (18–20). Patients were also screened clinically and echocardiographically for other cardiac findings (defined as all cardiac abnormalities other than CHDs) that had been reported in NF1 patients previously (10, 11). All patients underwent clinical examination (auscultation, estimation of the quality of peripheral pulses, inspection for signs of heart dysfunction, or cyanosis). Examiners were not blinded with respect to the presence/absence of NF1 whole gene deletions.

All patients underwent two-dimensional echocardiography and Doppler scanning using a Vivid 7 device (General Electric, Munich, Germany). Examinations were performed by a pediatric cardiologist (TSM) specialized in transthoracic echocardiography who was blinded to the clinical findings and past medical history of the subjects. A 3–5 MHz transducer was used for two-dimensional guided M-mode and Doppler scan imaging. Left ventricular (LV) analysis included measurement of interventricular septum thickness (IVSd), LV posterior wall thickness (LVPWd), LV internal diameter diastolic septal thickness (LVIDd), and LV internal diameter systolic thickness (LVId) by two-dimensional guided M-mode in parasternal short-axis view. Each variable was indexed for body surface area (BSA) (calculated using the Dubois method) by dividing each variable with BSA (21). Age and BSA adjusted z-scores were calculated in patients 16 years and older using published normal reference values (21). As published by Van de Veire et al. (21) and briefly described here, the normal reference values were obtained in a cohort of presumed healthy Belgian individuals referred to the Department of Cardiology at Ghent University in 2004 for a cardiovascular checkup. Individuals were prospectively screened by echocardiography and values of individuals with a normal trans-thoracic echocardiogram, without structural heart disease and with a negative cardiovascular history were gathered and used for comparison. Valvular regurgitation was measured by color Doppler scan. The degree of valvular regurgitation was visually classified as none (0), physiological (I°), mild (II°), moderate (III°), or severe (IV°) (22). Physiological (I°) mitral, tricuspid or pulmonary regurgitation was not considered to be an abnormality (23).

Methods

Sixteen patients with constitutional large deletions of NF1 and 16 NF1 patients without such deletions agreed to participate and were investigated in this study. All fulfilled the standard clinical diagnostic criteria for NF1 (16). Fifteen of the 16 patients with large deletions of NF1 were previously described in a clinical study (5). These 15 patients have constitutional non-mosaic type-1 NF1 gene deletions as determined by screening of microsatellite markers, multiplex ligation dependent probe amplification and fluorescence in situ hybridization, described previously (5). The sixteenth subject with a type-2 deletion, Patient 1a, has previously been described as Patient 2429 by Roehl et al. (17). Breakpoint analysis indicated that the deletion is mediated by non-allelic homologous recombination between intron 8 of the SUZ12 gene and the SUZ12P pseudogene, which flank opposite ends of the NF1 gene. Neither fluorescence in situ hybridization analysis of blood samples nor microsatellite marker analysis of buccal DNA yielded evidence for somatic mosaicism in this patient (17). Each of the 16 control NF1 patients without a large deletion was consecutively ascertained in our Neurofibromatosis Clinic and matched to a different large deletion patient by sex and age within 2 years. Large deletion was excluded in these 16 control NF1 patients based on heterozygosity of one or more intragenic NF1 microsatellite markers. The study was approved by the Ethics Committee of the Hamburg Medical Chamber, and all patients gave informed consent to participate.

Statistics

Frequencies of cardiac abnormalities in patients with and without large deletions were compared using Mc Nemar’s test. Statistics were performed on unadjusted echocardiographic variables, indexed echocardiographic variables, and normalized echocardiographic burden, malignancy, facial dysmorphic features, hyper-flexibility of joints, generalized muscle hypotonia, scoliosis, and mental retardation (5, 6).

The NF1 gene product, neurofibromin, plays a role in cardiac development (7, 8) and haploinsufficiency of the SUZ12 and CENTA2 genes, which lie in the region involved in these NF1 microdeletions, may also influence the development of the heart (9).

The prevalence of congenital heart defects (CHDs) in people with NF1 has not been clearly defined, but estimates range from 2% (based on medical histories reported to an international database) to 27% (based on echocardiographic study of clinical series) (10, 11). However, the type of NF1 mutation, whether intragenic or a large NF1 gene deletion, was not determined in any of these studies. Clinical investigations revealed a high frequency (18–21%) of cardiac anomalies (5, 9, 12) although the patients were not routinely evaluated by a cardiologist or with echocardiography. The pathologies observed in these patients included pulmonic stenosis, other valvular abnormalities, septal defects and cardiomyopathy (5, 13–15). In this study, we systematically and comprehensively characterized cardiac status by history, clinical examination, and echocardiography in 16 patients with large NF1 deletions and 16 age- and sex-matched control NF1 patients without such deletions. All echocardiographic findings in the NF1 patients were compared to normal population standards.
variables because of the age-restriction in the published normal reference population. The paired $t$-test was used to analyze unadjusted and BSA-indexed LVIDd, LVPWd, IVSd, LVIDs, and fractional shortening (FS) in NF1 large deletion cases and NF1 controls without deletion. It is also used to eliminate the effect of sex and reduce the effect of age on the variables examined. The unpaired $t$-test was used to analyze LVIDd, LVPWd, IVSd, LVIDs, and FS z-scores, which were normalized for BSA and age. The one-sample $t$-test was used to compare LVIDd, LVPWd, IVSd, LVIDs and FS z-scores from NF1 large deletion cases or NF1 controls without deletion to the general population. All tests were two-tailed and $p$ values <0.05 were considered to be statistically significant.

Results

Sixteen patients (nine females and seven males) with germine whole gene NF1 deletions and 16 age- and sex-matched NF1 patients, whose mutation is not a large deletion of the NF1 locus were included. Six (38%) of 16 NF1 deletion patients but none of 16 non-deletion NF1 patients had major cardiac abnormalities. Major cardiac abnormalities were significantly more frequent among patients with non-mosaic large deletions than in NF1 patients with other constitutional NF1 mutations (0/16; $p=0.041$; Table 1). CHDs included ventricular septal defect (VSD) and aortic stenosis in one patient each. Mitral insufficiency was found in two patients and aortic insufficiency in one patient. Three patients had hypertrophic cardiomyopathy (HCM) and two had intracardiac tumors. All cardiac findings are listed in Table 1.

The VSD in Patient 13a was detected at birth but was always asymptomatic and resolved by the age of 2.5 years. Patient 14a was diagnosed with right ventricular HCM at birth. She had low oxygen saturations (50%–60%) and required oxygen supplementation and hospitalization in the neonatal intensive care unit for 3 weeks. Her HCM resolved by the age of 5 years. Two patients each had three cardiac lesions (aortic insufficiency I–II, mitral insufficiency I–II, and an intracardiac tumor in deletion Patient 5a and mild mitral insufficiency, mild aortic stenosis, and LV HCM in deletion Patient 8a). The cardiac abnormalities persisted in both the patients to their most recent evaluations at 19 and 25 years, respectively. Patient 8a developed secondary concentric LV HCM and atrial dilatation. Both individuals had multiple episodes of syncope at rest and during exercise that developed during adolescence. In Patient 8a, the syncopal episodes are thought to be related to his relative outflow obstruction. In Patient 5a, a cause has not been identified yet. However, in both patients vasovagal syncope, orthostatic hypotension, and primary arrhythmias were ruled out. They did not receive any pharmacologic treatment.

Weight and height of paired cases and controls did not differ significantly between the large deletion and non-deletion NF1 patients. Echocardiographic measurements for the NF1 large deletion patients and non-deletion NF1 patients in comparison to a healthy reference population are listed in Table 2. In comparison to the reference population without NF1, NF1 patients with large deletions, who are 16 years or older do not have significantly different IVSd, LVIDd, LVPWd, LVIDs, or FS. Interestingly, in comparison to the reference population without NF1, NF1 patients without large deletions, who are 16 years or older have increased LVPWd ($p<0.001$) and increased IVSd ($p=0.001$) suggestive of eccentric LV hypertrophy.

Echocardiographic measurements for the NF1 large deletion patients in comparison to non-deletion NF1 patients are listed in Table 3. NF1 patients with large deletions have a decreased LVPWd ($p=0.001$) and IVSd ($p=0.01$) when compared with non-deletion NF1 patients of the same sex and a similar age. FS, BSA-indexed LVIDd, and BSA-indexed LVPWs systolic posterior wall thickness did not significantly differ between the two groups.

Discussion

This is the first comprehensive echocardiographic characterization of genetically confirmed patients with non-mosaic large NF1 deletions. Recent descriptions of the clinical phenotype in patients with large NF1 deletions support the frequent occurrence of CHDs and other cardiac abnormalities among these patients (5, 9, 13), but these studies did not include a systematic comparison to NF1 patients without large deletions. We have performed such a comparison and showed that CHDs and other cardiac abnormalities are more frequent among patients with non-mosaic large deletions of NF1 than among other NF1 patients, whereas specifically increased LVPWd and IVSd suggestive of LV hypertrophy seems to be more frequent among patients without such deletions. Given this study is retrospective, it may be that some of the transient cardiac findings in the microdeletion group might have been underreported in the matched non-microdeletion group. However, clinically significant CHDs would have been probably detected and documented.

Our study shows that cardiac abnormalities in NF1 deletion patients may cause serious complications requiring treatment and hospitalization. Three of six large deletion patients with cardiac abnormalities had symptomatic lesions. The cardiac findings in one of these patients resolved during childhood. The other two symptomatic patients are still having cardiac abnormalities and develop syncope at rest and during exercise. NF1 patients with large NF1 gene deletions should be screened for CHDs at birth. Lesions that may require medical attention and could lead to symptoms like those in the studied patients are right ventricular (RV) HCM in the perinatal period, aortic stenosis with secondary LV HCM later in life, and mitral and aortic valve insufficiency with intracardiac tumor. Echocardiographic and/or clinical follow-up of these patients may be useful to monitor the cardiac status.

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Table 1. Cardiac findings in 16 patients with whole gene NF1 deletions. Patients with 1.4 or 1.2 Mb (Patient 1a) deletions of NF1

<table>
<thead>
<tr>
<th>ID</th>
<th>Sex</th>
<th>Age</th>
<th>CHD</th>
<th>Valve insufficiency</th>
<th>Cardiomyopathy</th>
<th>Cardiac tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>M</td>
<td>18</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>Pulmonary subvalvular tumor, 17 × 17 mm</td>
</tr>
<tr>
<td>5a</td>
<td>F</td>
<td>19</td>
<td>–</td>
<td>Aortic valve insufficiency I–II°, mitral valve insufficiency I–II°</td>
<td>–</td>
<td>LV intracardial tumor, 11 × 14 mm</td>
</tr>
<tr>
<td>7a</td>
<td>F</td>
<td>15</td>
<td>–</td>
<td>–</td>
<td>Restrictive hypertrophic cardiomyopathy</td>
<td></td>
</tr>
<tr>
<td>8a</td>
<td>M</td>
<td>25</td>
<td>Aortic stenosis II°</td>
<td>Mitral valve insufficiency II°</td>
<td>Dilated left atrium, secondary concentric LV hypertrophic cardiomyopathy</td>
<td>–</td>
</tr>
<tr>
<td>13a</td>
<td>F</td>
<td>14</td>
<td>Ventricular septal defect</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>14a</td>
<td>F</td>
<td>6</td>
<td>–</td>
<td>–</td>
<td>RV hypertrophic cardiomyopathy</td>
<td>–</td>
</tr>
</tbody>
</table>

CHD, congenital heart defects; F, female; LV, left ventricular; M, male; RV, right ventricular.

Table 2. z-Scores of standard echocardiographic parameters in NF1 patients with and without whole gene deletions compared to a healthy reference population without NF1

<table>
<thead>
<tr>
<th></th>
<th>Mean (SD)</th>
<th>Confidence intervals</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>NF1 deletion cases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVSD z-score</td>
<td>0.2 (1.5)</td>
<td>−0.77–1.22</td>
<td>0.624</td>
</tr>
<tr>
<td>LVIdD z-score</td>
<td>0.4 (1.5)</td>
<td>−0.52–1.28</td>
<td>0.373</td>
</tr>
<tr>
<td>LVPWd z-score</td>
<td>0.2 (1.4)</td>
<td>−0.71–1.05</td>
<td>0.679</td>
</tr>
<tr>
<td>LVIDs z-score</td>
<td>0.3 (1.6)</td>
<td>−0.91–1.60</td>
<td>0.546</td>
</tr>
<tr>
<td>FS z-score</td>
<td>0.4 (0.6)</td>
<td>−0.01–0.72</td>
<td>0.058</td>
</tr>
<tr>
<td>NF1 non-deletion cases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IVSD z-score</td>
<td>1.0 (0.8)</td>
<td>0.54–1.52</td>
<td>0.001</td>
</tr>
<tr>
<td>LVIdD z-score</td>
<td>0.1 (0.9)</td>
<td>−0.44–0.59</td>
<td>0.743</td>
</tr>
<tr>
<td>LVPWd z-score</td>
<td>2.2 (1.2)</td>
<td>1.49–2.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LVIDs z-score</td>
<td>−0.3 (0.8)</td>
<td>−0.80–0.16</td>
<td>0.178</td>
</tr>
<tr>
<td>FS z-score</td>
<td>0.5 (0.9)</td>
<td>−0.03–1.02</td>
<td>0.064</td>
</tr>
</tbody>
</table>

FS, fractional shortening; ind, body surface area-indexed values; IVSD, interventricular diastolic septum thickness; LVIdD, left ventricular diastolic internal dimension; LVIDs, left ventricular systolic internal dimension; LVPWd, left ventricular diastolic posterior wall thickness; n, number of patients; NF1, neurofibromatosis 1; SD, standard deviation; z-score, z-score were calculated from reference values of the normal population. Bold numbers indicate p < 0.05 considered to be statistically significant.

and look for secondary cardiac complications, which may develop later in life.

In our study, the distribution of CHDs was slightly different than those previously reported in the literature. Pulmonary valve stenosis is the most frequently reported cardiac finding in patients with large NF1 deletions; it has been reported in four genetically confirmed cases (4, 14, 15). However, none of the 16 large deletion patients in this study had pulmonic stenosis, which might be due to the number of patients recruited in the study. Single cases with VSD and HCM, both of which were observed in our study, were previously found in this patient population (13). Larger systematic studies of NF1 patients with large deletions will be necessary to characterize the frequency and spectrum of cardiovascular disease more accurately.

Intracardiac neurofibromas have been reported in four genetically not ascertained NF1 cases (24–26). We had two patients with intracardiac tumors. Both were not detected at birth but in the course of surveillance and suggest that the tumors might have grown with time. However, in our cases, they are not hemodynamically relevant and on recent serial echocardiographic examinations, the size of the tumors were stable. Intracardiac tumors can be a cardiac and/or oncologic problem. Management of these tumors is limited by the sparse knowledge about their natural history, including histologic origin and growth dynamics, retrieved from rare case reports in the literature. Hence, these tumors should be approached by both specialties in regards to hemodynamic significance and tumor progression, respectively.

NF1 patients are heterozygous for NF1, with one mutant and one normal copy of the gene, and they are thought to be haploinsufficient for neurofibromin. While neurofibromin is known to be involved in the
cardiomyopathy. Unfortunately, as we could normalize only patients 16 years or older accurately, only Patient 8a was included in the analysis between patients and the normal reference population. It is of note however that the raw unadjusted values and indexed values included all patients and the non-deletion NF1 patients still had increased LVPWd and IVSd. These findings have not been reported in the literature before and future studies are needed to further investigate this observation and its clinical significance. Common causes of eccentric LV hypertrophy in the general population are volume strain due to athletic training or long-standing aortic valve regurgitation. However, these factors are unlikely to contribute to our findings in non-deletion NF1 patients. Vasculopathy, which is known to be associated with NF1 (28), could be considered as a possible cause. Recent studies provide evidence for vascular inflammation and neointima formation in both NF1 patients and murine Nf1+/- models (28). Inflammation and subsequent rigidity in vascular structure could increase pressure strain and contribute to cardiac changes. Hypertension is known to be relatively frequent in NF1 patients (29), but we did not measure blood pressure routinely in these patients.

Although this study is the largest one of its kind reported to date, the numbers of deletion and non-deletion NF1 patients evaluated are still small. Our results show that cardiac abnormalities seem to be more frequent among patients with constitutional large deletions of NF1 and may cause serious clinical complications. Eccentric LV hypertrophy appears to be more frequent in NF1 patients without whole gene deletions, but the clinical significance of this finding is uncertain. As molecular detection of NF1 large rearrangement is not often performed at birth, patients with clinical suspicion for NF1 (regardless of deletion or non-deletion) should undergo an electrocardiography (EKG), blood pressure measurement, and a trans-thoracic echocardiography at the time of diagnosis, and may require referral to a cardiologist. Further studies are necessary to see whether patients with cardiac screening at birth may benefit from early intervention.

**Acknowledgements**

This work was funded by the ‘Deutsche Krebshilfe’ grants (106982 and 108793). Special thanks to Dr Windt for patient recruitment and Dr Van de Veire for providing echocardiography reference values for the normal population.

**References**


