International Research Journal of Biological Sciences _____E-ISSN 2278-3202
Vol. **5(8),** 38-44, August **(2016)** *Int. Res. J. Biological Sci.*

NF1 Mutations and Clinical Manifestations in Neurofibromatosis Type 1 Patients in Tamil Nadu, South India

Eltahir Abdelrazig Mohamed Ali
Department of Human Genetics and Molecular Biology, Bharathiar University, Coimbatore-641046, India
eltahirgenome@yahoo.com

Available online at: www.isca.in, www.isca.me
Received 22nd April 2016, revised 11th June 2016, accepted 16th July 2016

Abstract

Neurofibromatosis type 1 (NF1) is a genetic disorder affecting approximately 1 in 3000 individuals. It is caused by heterozygous inactivation of the NF1 a tumor suppressor gene or deletions of the entire NF1 gene. This study exemplifies the clinical heterogeneity of 13 Indian patients with NF1 and highlights the variations in sites and types of mutations in NF1 gene in this population. The DNA was extracted from the blood samples and the entire coding region of NF1 gene was amplified and sequenced by the Sanger method. In the present study, the frequencies of neurofibromas and plexiform neurofibromas were 92.3% and 30.7% respectively. 84.6% had a short stature and 38.5% had a macrocephaly. Café-au-lait spots were in all the cases while scoliosis was in only 2 patients. 53.8% had a frequent headache and 23% had facial dysmorphism. All NF1 mutations identified in 11 patients in our study population have been reported in previous studies and no novel mutation has been detected. NF1 mutations were detected in exons 4, 13, 21, 29, and 46. The frequencies of point mutations were 36.4% nonsense, 36.4% missense and 27.2% frameshift. Variable expression of the same NF1 mutation made identification of genotype-phenotype correlations a daunting task.

Keywords: NF1 gene, Neurofibromas, Café-au-lait spots, Plexiform Neurofibromas, Axillary freckling.

Introduction

Neurofibromatosis type 1 (NF1) (also known as von Recklinghausen disease) is a multisystem, autosomal dominant disorder that affects approximately 1 in 3000 individuals worldwide[1]. Approximately 50% of NF1 cases are familial[2]. Neurofibromatosis type 1 is characterized by neurofibromas and plexiform neurofibromas, pigmented skin lesions called café-au-lait spots, axillary or inguinal freckling, Lisch nodules of the iris, pseudoarthrosis of the tibia, optic pathway gliomas, and many other minor features.

NF1 is caused by heterozygous loss-of-function mutations or deletions of the entire *NF1* gene. The *NF1* gene is a tumor suppressor gene and it encodes the neurofibromin, a GTPase-activating protein (GAP) for the p21 Ras family which acts as a negative regulator in Ras pathway by accelerating the conversion of active Ras-GTP into inactive Ras-GDP[3]. The genomic locus of *NF1* gene is 17q11.2, spanning approximately 350 kb of genomic DNA and is composed of 60 exons[4] . The *NF1* gene has a mutation rate higher than that of most of genes in human genome. More than 500 different types of mutation have been identified in *NF1* gene and most are unique to a particular family. Most small *NF1* deletions or point mutations are of paternal origin while large *NF1* mutations are thought to be of maternal origin. Loss of heterozygosity at the *NF1* locus has been shown by some but not all neoplasms associated with NF1 and this provide further evidence for the role of *NF1* as a tumor suppressor gene.

It has been and still difficult to identify genotype-phenotype correlations in NF1 because of variations in its expression in different patients and even in patients within the same family. Only two genotype-phenotype correlations have been identified for *NF1* gene, the first one is a large deletion encompassing the entire *NF1* locus (17q11.2) and neighboring genes. These recurrent deletions are commonly associated with severe symptoms comparing to the intragenic *NF1* mutations[5]. The second genotype-phenotype correlation is that the absence of cutaneous neurofibromas is associated with presence of a 3-bp inframe deletion (c.2970_2972 delAAT) in exon 17 of the *NF1* gene[6]. The objectives of the present study were to identify the *NF1* mutational spectrum in neurofibromatosis type 1 patients in Tamil Nadu, South India, to determine the clinical manifestations of NF1 and their frequencies as well as to identify genotype-phenotype correlations in the same group of patients.

Materials and Methods

Recruitment of NF1 Patients: The approval of the Institutional Ethics Committee of the Bharathiar University, Coimbatore, India, was obtained to conduct the study. The recruitment of subjects with NF1 from government hospitals in Coimbatore and Erode districts, Tamil Nadu State, South India, was performed by using the National Institutes of Health (NIH) diagnostic criteria for NF1, and written informed consent was obtained from the adult participants and from the children's parents or legal guardians. The medical history of all the

subjects and their families was obtained and the available medical records of the patients were reviewed to confirm the diagnosis of NF1.

Clinical Examination and Blood Sample Collection: The information regarding the clinical characteristics has been obtained from the available medical records of the subjects and physical examination has carried out to all the subjects to detect the NF1-related clinical features. From each subject participating in this study, 5 ml of blood has been drawn by venipuncture from an antecubital vein and the blood were collected in EDTA coated collection tubes for molecular genetic analysis, and stored at 4 °C.

Molecular Genetic Analysis: The whole genomic DNA was extracted by the salting out method following Miller et al.[7] method with slight modifications. The Primer 5 software was used for designing the forward primer 5'-gatgtaaaatgtcttacaag-3' and the reverse primer 5'-ctgccacctgtttgcgcact-3' required to amplify the entire *NF1* gene from the extracted genomic DNA. The Sanger sequencing method was used to sequence the amplified *NF1* gene by using Biomek FX Beckman fluid moving workstation and a nanoDrop 8000 spectrophotometer. Then the reaction plate was transferred to the ABI 3730 XL capillary sequencer a fully automated system from Applied Biosystems and the samples were electrophoresed.

Results and Discussion

Clinical Manifestations: A total of 13 NF1 patients have been evaluated in this study and out of the 13 patients, 8 were males and 5 were females with a male to female ratio of 1.6:1. Ten of the patients were from three unrelated families and the other 3 patients were also unrelated. There was a variation in the subjects' ages ranging from 9 months to 85 years with a mean age of 43.06 years. Positive family history in first-degree relatives was found in 10 patients (76.9%). All the patients have been clinically assessed, by taking the family history, physical examination, and the other clinical data have been obtained from the available medical reports of the patients. The National Institutes of Health (NIH) diagnostic criteria for NF1 have been used to confirm the diagnosis of the disorder and to evaluate the clinical features of the patients. The clinical manifestations of the study population are shown in Figures 1, 2, and 3 and their frequencies are summarized in Table-1. Most of the observations reported in this study were in conformity with the prevailing data. Café-au-lait spots of different sizes were observed in all the subjects (100%). This feature is usually the first sign of NF1- occurring in 99% of individuals with NF1[8] - that can be seen in young children, and it is the most important feature leading to diagnosis of NF1 Figure-3. One or two café-au-lait spots can be detected in about 20% of the general population but their number is significantly higher in individuals with NF1. 84.6% of the study population had skin-fold freckling, and this frequency is slightly below the frequency of freckling in the entire NF1 population (90%) [9].

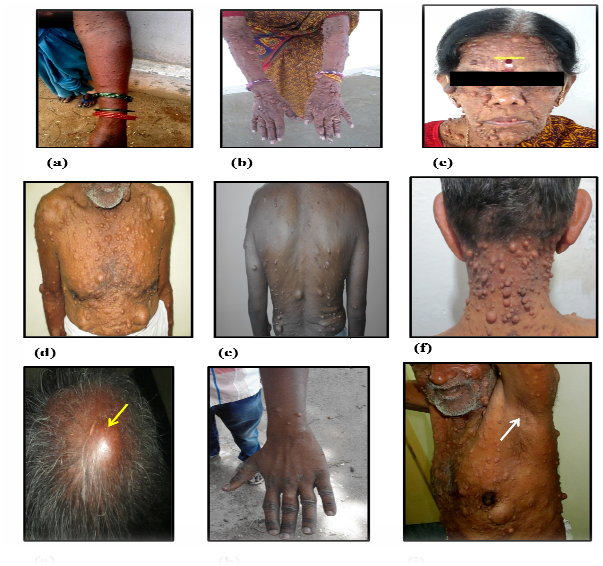

Figure-1
Cutaneous and subcutaneous neurofibromas in different parts of the body in some NF1 patients this study. The arrow in (g) shows a subcutaneous neurofibroma and the arrow in (i) shows axillary freckling

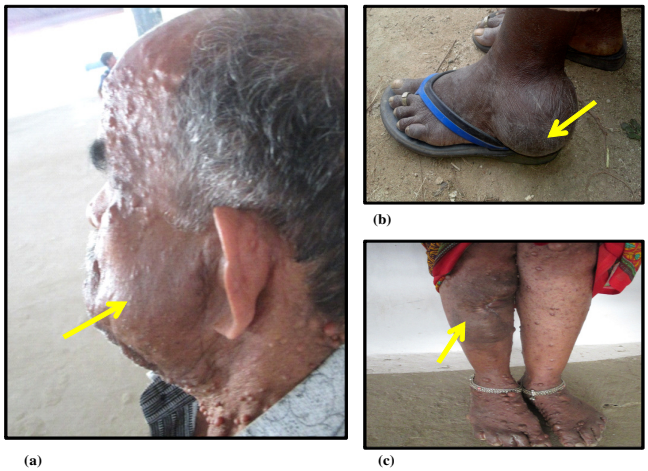

Figure-2
Plexiform neurofibromas in three NF1 patients in this study

Neurofibromas were observed in all the cases except the subject *C5,* and 61.5% of the patients had too numerous to count cutaneous neurofibromas and many subcutaneous neurofibromas and all of them were > 40 years [Figure-1]. Those with a very few number of neurofibromas were of ages < 30 which was consistent with the known fact that these dermal neoplasms are age-related. They usually occur after puberty and their size and number may increase with the increase in the age. Plexiform neurofibromas (PNFs) have been observed in 4 patients (30.7%) and it was far below the frequency of PNFs in the NF1 population (50%).

Table-1
Frequencies of the clinical features of NF1 patients in this Study

Features	C 1	C 2	C 3	C 4	C 5	C 6	C 7	C 8	C 9	C 10	C 11	C 12	C 13	No.	%
Age (years)	58	45	40	18	9 month	64	62	77	48	18	14	85	30		
Sex	M	F	F	M	M	M	F	M	M	M	F	F	M	8M/ 5F	61.5% / 38.5%
Family history	+	+	+	+	+	+	+	-	-	+	+	-	+	10	76.9%
Age of onset of neurofibromas	?	?	34	?	-	?	11	16	?	17	12	?	?		
Short stature	+	+	+	+	+	+	+	+	+	-	+	+	-	11	84.6%
Macrocephaly	+	-	-	-	-	+	+	+	-	-	-	+	-	5	38.5%
Café-au-lait spots	+	+	+	+	+	+	+	+	+	+	+	+	+	13	100%
Cutaneous neurofibromas	+	+	+	+	-	+	+	+	+	+	+	+	+	12	92.3%
No. of neuro fibromas (TNTC)	+	+	+	-	-	+	+	+	+	-	-	+	-	8	61.5%
Plexiform neurofibromas	-	-	+	+	-	+	+	-	-	-	-	-	-	4	30.7%
Axilary / inguinal freckling	+	+	+	-	-	+	+	+	+	+	+	+	+	11	84.6%
Lisch nodules	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE		
Optic gliomas	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE	NE		
Scoliosis	-	-	-	+	-	-	-	-	-	-	-	+	-	2	15.4%
Bone dysplasia	-	-	-	+	-	+	-	-	-	-	-	-	-	2	15.4%
Hypertension	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0%
Cardiac defect	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0%
Headache	+	+	+	-	-	+	+	+	-	-	-	+	-	7	53.8%
Facial dysmorphism	-	-	+		-	+	-	-	-	+	-	-	-	3	23%
Intellectual disability	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0%
Learning disabilities	NE	NE	NE	+	NE	NE	NE	NE	NE	+	+	NE	NE	3	23%
MPNSTs	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0%
Vision problem	-	-	-	-	-	+	-	+	+	-	+	+	+	6	46.2%
Hearing problem	-	-	-	-	-	+	+	-	-	-	-	+	-	3	23%
Seizures	-	-	-	-	-	-	-	-	-	-	-	-	-	0	0%
Thoracic deformity	-	-	-	+	-	-	-	-	-	-	-	-	-	1	7.6%
Large hands/feet		-	-	-	-	+	-	-	-	-	-	+	-	2	15.4%

C: case; MPNSTs: malignant peripheral nerve sheath tumors; NE: not evaluated; TNTC: too numerous to count; (?): unknown; (+): present;(-):absent.

The 4 patients were from two unrelated families, the case *C3* and her nephew *C4*, both had a PNF in the left foot but *C4* was severely affected with a PNF extending from his left thigh up to his foot. The patient *C6* had a facial PNF on the left side displacing the left side of mandible, and his sister *C7* had a PNF in the anterior part of her right leg which was surgically removed when she was 18 years. (Figure-2).

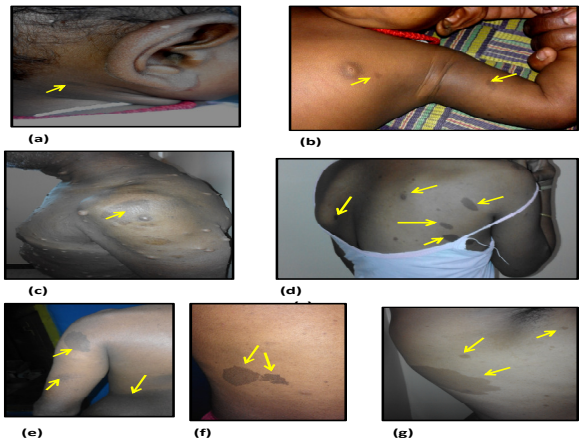

Figure-3
Café-au-lait spots of different sizes on the skin of some of NF1 patients in this Study

Some of the NF1 features such as short stature, macrocephaly and recurrent headache were present in a significant number of patients in this study. 84.6% of the patients had a short stature less than a 3rd percentile. In one family the father *C9* and his daughter *C11* had a short stature but his son *C10* had an average male stature (5 ft 6.9 in). In another family all individuals with NF1 had a short stature *C1, C2*, and *C3* including two sons of the subject *C1* (*C4* and *C5*). In a third family, both the subject *C6* and his sister *C7* had a short stature. In this study the frequency of short stature in NF1 patients is quite higher than what has been reported in the literature (30%) [9]. Macrocephaly has been observed in 38.5% of the patients and this falls within the range of the frequency of macrocephaly in patients with NF1 reported in previous studies[9], and it is important to mention that all the patients in this study who had a macrocephaly had also a short stature. 53.8% of the study population had a frequent headache. Both bone dysplasia and scoliosis were present in 15.4% of the patients, and thoracic deformity has been observed in only one case. Three unrelated patients (*C3, C6,* and *C10*) had facial dysmorphism. The facial dysmorphic features observed were prominent forehead, large and low-set ears, broad nasal bridge, deep set eyes, long inverted triangular face shape and epicanthic folds. Large hands and feet were in 15.4% of the cases.

Due to the lack of detailed clinical information in the available medical records of the NF1 patients in this study Due to the lack of detailed clinical information in the available medical records of the NF1 patients in this study and to the lack of clinical diagnosis of optic nerve gliomas and iris Lisch nodules, the presence or absence of these two NF1 manifestations in all the patients could not be ruled out. 46.2% of the patients had vision problems. Hearing problems have been observed in 23% of the study population. Although, vasculopathy, congenital heart defects, and hypertension are considered to be the most common cardiovascular manifestations of NF1, and cardiovascular disease has been reported to be a frequent cause of premature death in NF1 patients, no one of the study population showed any cardiovascular defect. Cases with seizures have been reported in a considerable number of individuals with NF1, but all the NF1 patients in the present study had no seizures.

Malignant peripheral nerve sheath tumors (MPNSTs) are the most frequent malignant neoplasms associated with NF1, occurring in approximately 8%-13% of NF1 patients[10], and up to 50% of MPNSTs present in NF1 patients. But in this study MPNSTs were not present in any NF1 patient. It has been described in published reports that learning disabilities were common in NF1 patients particularly in the children with NF1[11], and in the present study 23% of the cases had learning disabilities and they were all <20 years. Developmental delay was observed in only one patient (*C4*) and he was severely affected with a large PNF.

Molecular Analysis: DNA was extracted from the blood samples obtained from the NF1 patients and was amplified by polymerase chain reaction (PCR). A mutational screening of *NF1* at genomic DNA level was carried out for all except two patients (*C5* and *C12*) participated in this study using the chain termination method of DNA sequencing. All *NF1* mutations identified in the study population have been reported in previous studies [12-15] , and no novel mutation has been detected. In our study, *NF1* mutations were detected in exons 4, 13, 21, 29, and 46. The frequencies of point mutations were 36.4% missense, 36.4% nonsense, and 27.2% frame shift. A nonsense mutation c.5242C > T was detected in exon 29 in all the subjects in Family I (*C1, C2, C3,* and *C4*) except the proband *C5* who fulfilled the diagnostic criteria of NF1 but a blood sample was not collected from him for *NF1* mutational screening. Based on the fact that the affected members of a family usually have the same *NF1* mutation, this proband (*C5*) may also carry the same point mutation. In Family II, both the siblings had the same mutation c.2033_2034insC in exon 13. The father *C9* with numerous cutaneous and subcutaneous neurofibromas in Family III harbored a sporadic mutation c.601T >A in exon 4 and it was detected also in his son *C10* and daughter *C11* who were both mildly affected. A frame shift mutation c.7996_7997delAG in exon 46 was found in one patient *C8* while a missense mutation c.3548T >G in exon 21 was detected in the proband *C13* who had a father diagnosed with NF1. In this study no patient had *NF1* microdeletion syndrome. The *NF1* mutational analysis is summarized in Table-2.

Table-2
***NF1* mutational analysis in 11 NF1 cases**

Patient	Exon	Mutation	Type	Nucleotide change
C1	E29	c.5242C >T	NS	S
C2	E29	c.5242C >T	NS	S
C3	E29	c.5242C > T	NS	S
C4	E29	c.5242C > T	NS	S
C6	E13	c.2033_2034insC	FS	I
C7	E13	c.2033_2034insC	FS	I
C8	E46	c.7996_7997delAG	FS	D
C9	E4	c.601T >A	MS	S
C10	E4	c.601T > A	MS	S
C11	E4	c.601T >A	MS	S
C13	E21	c.3548T >G	MS	S

Clinical Profile of the Study Population: Neurofibromatosis type 1 (NF1) is a multisystem genetic disorder with an autosomal dominant inheritance, 100% penetrance, and variable expression. Point mutations in *NF1* gene or microdeletions of entire *NF1* gene are the causes of NF1. The hallmark signs of NF1 include café-au-lait spots (CALs), dermal neurofibromas, plexiform neurofibromas (PNFs), Lisch nodules of the iris, optic nerve gliomas, scoliosis, and bony dysplasia. The primary objectives of this study were to provide an exhaustive description of the phenotypic manifestations of NF1 in 13 South Indian patients with NF1 along with an analysis of *NF1* mutations in the study population. All the patients enrolled in the present study fulfilled the National Institutes of Health Consensus Criteria for the diagnosis of NF1. Approximately 50% of individuals with NF1 have de novo mutations[2], but in this study 76.9% of the cases had familial mutation and this may be due to the small group of NF1 patients participated and most of them were related. All the clinical features of the study population were similar to those reported in the literature, but some features showed higher frequencies than what has been revealed in previous studies.

In the present study, the frequencies of cutaneous neurofibromas and plexiform neurofibromas were 92.3% and 30.7% respectively. Numerous cutaneous and subcutaneous neurofibromas were in 61.5% of the cases and all of them were above 40 years, and mild cutaneous manifestation was in those who were below 30 years, reflecting the fact that these dermal lesions are age-related and they may increase in number and size over time[10]. PNFs have been described in published reports to be in approximately half of all NF1 patients, and to have the potential to transform into a MPNST in up to 10% of NF1 patients in their lifetime[9,10]. And this study showed a significantly lower frequency of PNFs than what has been reported in the literature and the absence of MPNSTs in all study population. Consistent with the literature, all NF1 patients in this report had café-au-lait spots (CALs) of different sizes and numbers, and two unrelated young patients (*C4* and *C11*) had multiple CALs of large sizes. The proband *C4* was severely affected while *C11* had mild clinical manifestations, but the number of CALs was not related to the severity of the condition. 84.6% of the patients had freckling in the axillary or inguinal region which is slightly lower than what has been reported in the general population of NF1 (90%) [8]. More than half of the subjects had a recurrent headache.

In this study, the lack of clinical information regarding the optic pathway gliomas and Lisch nodules made it impossible to identify the frequencies of these features, although it has been reported that approximately 15% - 20% of NF1 patients develop optic pathway gliomas and they can be detected by magnetic resonance imaging (MRI) at early ages but most are asymptomatic throughout life, and more than 90% of patients with NF1 aged > 15 years have Lisch nodules[16]. Some of the minor clinical characteristics of NF1 such as macrocephaly, short stature, and facial dysmorphism have been observed in a considerable number of NF1 patients in this study. Macrocephaly was in 38.5% of the cases and it was consistent with the frequency in published reports[9]. Short stature less than a 3rd percentile was observed in 84.6% of the patients and this frequency is significantly higher than the frequency of this feature in the general NF1population (30%). Facial dysmorphic features such as broad nasal bridge, prominent forehead, large and low-set ears, deep set eyes, long inverted triangular face shape and epicanthic folds were in 23% of the subjects. 15.4% of the patients had large hands and feet.

Scoliosis is thought to be the most common orthopedic feature in NF1 patients and it occurs in approximately 10% of patients[9]. In this study, 15.4% of the cases had scoliosis. The pathogenesis of this feature in NF1 is unknown but some investigators suggested its relationship to subsequent dysplastic bony elements and osteopenia[17]. Bone dysplasia is another common orthopedic manifestation of NF1 with a prevalence of 14% in NF1 population and usually manifesting within the first year of life[8]. The tibia is the most commonly affected bone bowing in an anterolateral direction. Congenital tibial pseudoarthrosis and overgrowth of a limb are other findings of NF1. Loss of heterozygosity of the *NF1* gene has been detected in osseous tissue from two NF1 patients with tibial pseudoarthrosis suggesting the association of this mutation with the pathogenesis of tibial pseudoarthrosis[18]. Only two cases in our report had bone dysplasia.

No one in the present study had cardiovascular manifestation of NF1, although a significant number of NF1 patients may develop congenital heart defects, vasculopathy and hypertension. Cardiovascular disease is a frequent cause of premature death in NF1patients. Hypertension is common among individuals with NF1, and it is more frequent in women with NF1 during pregnancy. The prevalence of hypertension in

NF1 increases with age[9], and renal artery stenosis from NF1 vasculopathy is thought to be the cause of hypertension. In a review of 2,550 NF1 patients, 2% of the cases had congenital heart defect (CHD), and the frequency of CHD in NF1 population was reported to be ranging from 0.4 to 6.4% [19]. Seizures have been observed in a significant number of NF1 patients in published reports, but all the 13 NF1 cases had no seizures.

An extensive review on the cognitive profile of NF1 revealed that academic deficiencies, particularly in reading and mathematics, a high frequency of attention deficit hyperactivity disorder (ADHD), and slightly lower intelligence quotients (IQs) are common in NF1, particularly in children with NF1. Approximately 30% - 65% of NF1 patients have learning disabilities (LDs) and the variation in the frequency is thought to be due to different definitions of LD used by different investigators[11]. The average IQ in NF1 patients is nearly 90, though approximately 5% are in the intellectual disability range [18], but all the subjects in the present study had no intellectual disability. We observed learning disabilities in 23% of the patients and developmental delay in only one case. The gastrointestinal manifestations of NF1 range from 5% - 25% [20], and only 5% are symptomatic. Symptoms may include bleeding, abdominal pain and symptoms associated with the presence of a tumor mass. Vision problems were significantly high in the present investigation (46.2%) while hearing problem was in 23% of the patients. Pectus carinatum, a thoracic deformity, was in only one subject.

***NF1* Mutations in the Study Population:** *NF1* gene has a large size (> 350 kb). It shows one of the highest mutation rates identified in the human genome. The large size of *NF1* gene, and the existence of pseudogenes, as well as the lack of clustered mutations make the detection of mutations in this gene a difficult task. In the present study, we analyzed *NF1* mutations in 11 patients with NF1 and identified five different mutations in exons 4, 13, 21, 29, and 46 and these mutations were reported in the literature [12-15].

The frequencies of missense, nonsense, and frame shift mutations were 36.4%, 36.4%, and 27.2% respectively. A nonsense mutation c.5242C >T was identified in exon 29 in all the affected members of Family I (*C1, C2, C3,* and *C4*) except the subject *C5*. Both the siblings in Family II had the same familial frame shift mutation c.2033_2034insC in exon 13. In Family III, the father *C9* and his son *C10* and daughter *C11* had the mutation c.601T > A in exon 4. The frame shift mutation c.7996_7997delAG in exon 46 and the missense mutation c.3548T > G in exon 21 have been detected in the probands *C8* and *C13* respectively.

Like most of the Mendelian disorders, the phenotypic expression of NF1 varies in NF1 families and even in the members of the same family who are affected with NF1. The existence of the same mutation in all family members with NF1 and the variation in the severity of their conditions, suggests that there is no clear-cut genotype-phenotype correlations, except two correlations reported in the literature. The association of *NF1* microdeletion with higher severity of NF1 clinical characteristics, and higher numbers of cutaneous neurofibromas at earlier ages as well as an elevated risk of developing MPNSTs than NF1 patients with intragenic mutations[5]. The second clear genotype-phenotype correlation is the association of the mutation c2970_2972delAAT in exon 17 with the absence of cutaneous neurofibromas[6].

Conclusion

In this study, there were no significant differences between males and females with NF1 in the frequencies of most of the clinical manifestations of NF1 except in the frequencies of headache, short stature, bone dysplasia and hearing problem. The frequency of short stature in males and females were 75% and 100% respectively. 80% of females had a frequent headache while only 37.5% of males had the same problem. Bone dysplasia was observed in 15.4% of males but was not in females. Hearing problem was more in females (40%) than in males (12.5%).

The most interesting findings of the present study were that there was a higher frequency of short stature (84.6%) than that in the general NF1 population; no one of the patients developed a MPNST, although most of them had benign subcutaneous neurofibromas and approximately one third had PNFs. The frequency of learning disabilities was significantly high in NF1 patients aged <20 years, and intellectual disability was not observed in any patient. Considerable proportion of females with NF1 (40%) experienced recurrent spontaneous abortions, and most occurred during the fifth month of gestation.

Intragenic *NF1* mutations have been found in 11 patients, and all these mutations have been reported previously by other investigators[12-15]. The mutations c.601T > A, c.2033_2034insC, c.3548T > G, c.5242C >T, and c.7996_7997delAG have been identified in exons 4, 13, 21, 29, and 46 respectively. No mutational hot spots have been identified. Both missense and nonsense mutations had the same frequency (36.4%) while the frequency of frame shift mutations was 27.2%. Each mutation was unique to a particular family, although there was a significant variation in the clinical manifestations of NF1 among affected individuals in the same family. This similarity in genetic defect and variability in expression led to inability to identify any genotype- phenotype correlation in the study population. This result agrees with the published reports.

The present study has revealed very interesting findings which will facilitate molecular diagnosis and genetic counseling in NF1 patients. Many of these findings were consistent with the literature. However, the study population was too small to permit a definitive conclusion. To best of my knowledge there is no any report on the genetic basis of NF1 in the Indian

population till the date, and most of the published reports focused on the clinical profile of NF1.Therefore, it is important to conduct a more detailed assessment on the genotypic and phenotypic aspects of NF1 in a large group of patients in India.

Acknowledgement

I would like to thank the patients and their families for participating in this study. I am grateful to Dr. V. Balachandar for his support during the course of this study and to Mr.A. Devaraj, Ms.D. ILakkiapavai and Mrs.Reem Rabih for their assistance during collection of samples.

References

1. McCormick F. (1995). #Ras signaling and NF1.# *Current opinion in genetics & development*, 5(1), 51-55.

2. Ferner R. E., Huson S. M., Thomas N., Moss C., Willshaw H., Evans D. G. and Kirby A. (2007). #Guidelines for the diagnosis and management of individuals with neurofibromatosis 1.# *Journal of medical genetics*, 44(2), 81-88.

3. Weiss B., Bollag G. and Shannon K. (1999). #Hyperactive Ras as a therapeutic target in neurofibromatosis type 1. #*American journal of medical genetics*, 89(1), 14-22.

4. Listernick R., Ferner R. E., Liu G. T. and Gutmann D. H. (2007). #Optic pathway gliomas in neurofibromatosis-1: Controversies and recommendations.# *Annals of neurology*, 61(3), 189-198.

5. Kluwe L., Siebert R., Gesk S., Friedrich R. E., Tinschert S., Kehrer-Sawatzki H. and Mautner V. F. (2004). #Screening 500 unselected neurofibromatosis 1 patients for deletions of the NF1 gene.# *Human mutation*, 23(2), 111-116.

6. Upadhyaya M., Huson S. M., Davies M., Thomas N., Chuzhanova N., Giovannini S. and Consoli C. (2007). #An absence of cutaneous neurofibromas associated with a 3-bp inframe deletion in exon 17 of the NF1 gene (c. 2970-2972 delAAT): evidence of a clinically significant NF1 genotype-phenotype correlation.# *The American Journal of Human Genetics*, 80(1), 140-151.

7. Miller S. A., Dykes D. D. and Polesky H. F. R. N. (1988). #A simple salting out procedure for extracting DNA from human nucleated cells.# *Nucleic acids research*, 16(3), 1215.

8. De Bella K., Szudek J. and Friedman J. M. (2000). #Use of the national institutes of health criteria for diagnosis of neurofibromatosis 1 in children.# *Pediatrics*, 105(3), 608-614.

9. Friedman J. M. (Ed.). (1999). #Neurofibromatosis: phenotype, natural history, and pathogenesis.# Johns Hopkins University Press.

10. Evans D. G. R., Baser M. E., McGaughran J., Sharif S., Howard E. and Moran A. (2002). #Malignant peripheral nerve sheath tumours in neurofibromatosis 1.# *Journal of medical genetics*, 39(5), 311-314.

11. Hyman S. L., Shores E. A. and North K. N. (2006). #Learning disabilities in children with neurofibromatosis type 1: subtypes, cognitive profile, and attention-deficit–hyperactivity disorder.# *Developmental Medicine & Child Neurology*, 48(12), 973-977.

12. Ars E., Kruyer H., Morell M., Pros E., Serra E., Ravella A. and Lazaro C. (2003). #Recurrent mutations in the NF1 gene are common among neurofibromatosis type 1 patients.# *Journal of medical genetics*, 40(6), e82-e82.

13. Ko J. M., Sohn Y. B., Jeong S. Y., Kim H. J. and Messiaen L. M. (2013). #Mutation spectrum of NF1 and clinical characteristics in 78 Korean patients with neurofibromatosis type 1.# *Pediatric neurology*, 48(6), 447-453.

14. Zeng K., Zhang Q. G., Liang L. P. and Liang Y. H. (2014). #Three Novel Missense Mutations of NF1 in Neurofibromatosis Type 1 Patient.# *Journal of Clinical & Experimental Dermatology Research*, 2014.

15. Rodríguez A. D., Moreno G. M., Santo-Domingo Y. M., Martín A. H., Roca J. E. S., Rojas M. R. F. and Argente J. (2015). #Características fenotípicas y genéticas en la neurofibromatosis tipo 1 en edad pediátrica.# *Anales de Pediatría*, 83(3), 173-182, Elsevier Doyma.

16. Huson S. U. S. A. N., Jones D. Y. L. A. N. and Beck L. (1987). #Ophthalmic manifestations of neurofibromatosis.# *British Journal of Ophthalmology*, 71(3), 235-238.

17. Schindeler A. and Little D. G. (2008). #Recent insights into bone development, homeostasis, and repair in type 1 neurofibromatosis (NF1).# *Bone*, 42(4), 616-622.

18. Stevenson D. A., Zhou H., Ashrafi S., Messiaen L. M., Carey J. C., D'Astous J. L. and Viskochil D. H. (2006). #Double inactivation of NF1 in tibial pseudarthrosis.# *The American Journal of Human Genetics*, 79(1), 143-148.

19. Lin A. E., Birch P. H., Korf B. R., Tenconi R., Niimura M., Poyhonen M. and Bonioli E. (2000). #Cardiovascular malformations and other cardiovascular abnormalities in neurofibromatosis 1.# *American journal of medical genetics*, *95*(2), 108-117.

20. Heuschkel R., Kim S., Korf B., Schneider G. and Bousvaros A. (2001). #Abdominal migraine in children with neurofibromatosis type 1: a case series and review of gastrointestinal involvement in NF1.# *Journal of pediatric gastroenterology and nutrition*, 33(2), 149-154.