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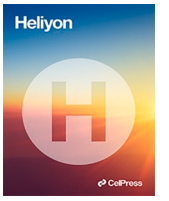

Neurofibromatosis type 1: Clinical characteristics and mutation spectrum in a North Indian cohort

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ABSTRACT

Background: Neurofibromatosis type 1 (NF1) is a unique, highly penetrant neuro-cutaneous disorder with a wide range of manifestations. Though the clinical diagnosis of NF1 is straight forward, there can be other disorders which mimic NF1, especially its cutaneous features. Here we describe the clinical and mutation spectrum of a series of individuals whose primary diagnosis was NF1 or NF1 related disorders.

Methods: We have screened 29 unrelated individuals who fulfilled the clinical criteria of NF1. Whole exome sequencing (WES) was done in all individuals except one with suspected microdeletion syndrome with NF1 in whom Cytogenetic microarray (CMA) was done.

Results: Out of 29 suspected patients, 25 had germline pathogenic/likely pathogenic variants involving *NF1* gene. Five novel and 20 known variants in coding and non-coding regions were identified, among them 7 variants were deletions (28%), 7 nonsense (28%), 3 splice-site (12%), 4 missense (16%), 2 duplications (8%) and 2 (8%) were contiguous deletions. In those where NF1 variants were not detected, 3 had neurofibromatosis type 2 (NF2) and 1 rare autosomal recessive form of Elher Danlos syndrome.

Conclusion: We hereby present the wide range of manifestations in different age groups and the mutation spectrum ranging from small scale variants to contiguous gene deletion syndromes involving *NF1* gene. We highlight the usefulness of molecular testing and its importance in tumor surveillance and genetic counseling in this disorder.

1. Introduction

Neurofibromatosis (NF1) is a well identifiable, autosomal dominant, neuro-cutaneous disorder; with a prevalence of 1 in 2500–3000 [1]. There are more than 100 genetic conditions and multiple congenital anomaly syndromes that has the characteristic skin manifestation of NF1, café au lait macules (CALMs) or other individual features of neurofibromatosis 1 (NF1). Though rarely

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confused with NF1, there can be a diagnostic dilemma in certain conditions.

Neurofibromin is a large gene with 282 kb size and 58 exons. This gene acts as a tumour suppressor gene [2]; hence pathogenic variant in this gene leads to tumour predisposition. More than 3000 different disease-causing variants have been described [3]. Many disease-causing variants are novel, and about half are de novo. NF1 is characterized by cutaneous manifestations in the form of multiple café au lait macules, intertriginous freckling, multiple cutaneous neurofibromas, and various other disorders like optic nerve and other central nervous system gliomas, malignant peripheral nerve sheath tumors, scoliosis, tibial dysplasia, vasculopathy, and various neurological, gastrointestinal, endocrine, or pulmonary disease [4]. Recently (2021) revised diagnostic criteria have been defined for neurofibromatosis type 1 diagnosis which has a high sensitivity and specificity [5]. Though pathogenic variant may not be necessary to establish a diagnosis of NF1, it may help in anticipating clinical severity. Management is largely supportive care, US FDA has recently approved selumetinib (KOSELUGO, AstraZeneca) for paediatric patients, 2–18 years of age, with neurofibromatosis type 1 (NF1) who have symptomatic, inoperable plexiform neurofibromas (PN) [6]. Here we report some rare findings in *NF1* mutation proven cases and compare these with previously reported literature. We report novel variants and some disorders where NF1 was considered as the diagnosis as per clinical criteria.

2. Material and methods

2.1. Patients

The study included 29 consecutive unrelated clinically suspected NF1 patients referred over a period of 2 years (January 2021 to December 2022) to the Medical Genetics units of two North-Indian tertiary care centres. All patients and families who had provided consent and compliance for at least 3 visits (pre-test counseling, sampling and post-test counseling) were included in the study. Detailed medical history including three generation-pedigree were obtained. All patients, parents and siblings where available were subjected to complete clinical examination. Four adult patients (19 years and above) with suspected NF1 had undergone ophthalmological assessment including slit lamp examination. Peripheral blood samples of all 29 participants, as well as clinical data and photographs of patients, were obtained after written informed consent from all participants and from parents or legal guardians of children under the age of 18. Neurofibromatosis 1 (NF1) was suspected and later on proven in individuals as per the revised diagnostic criteria [5].

2.2. Ethical approval and funding

The study was a part of a larger study approved by the Institutional ethics committee (IEC No: NK/6910/Study/483) of one Institution and the other Institute was the collaborator. The project was partially funded under the above study. Some tests and investigations were carried out as part of clinical testing.

2.3. Genetic testing

Genomic DNA was extracted from peripheral blood leukocytes from affected individuals using Qiagen DNA extraction kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. DNA extracted from blood was used to perform targeted gene capture using a custom capture kit. The libraries were sequenced to mean depth of >80–100X on Illumina sequencing platform. The sequences obtained were aligned to human reference genome (GRCh38) using BWA aligner [7]. The germline variants identified in the sample were deeply annotated using VariMAT pipeline. Gene annotation of the variants was performed using VEP program against the Ensemble release 99 human gene model [8]. In addition to SNVs and small Indels, copy number variants (CNVs) are detected from targeted sequence data using the Exome Depth method. This algorithm detects CNVs based on comparison of the read-depths in the sample of interest with the matched aggregate reference dataset. Clinically relevant pathogenic/likely pathogenic variants in both coding and non-coding regions were annotated using published variants in literature and a set of diseases databases: ClinVar, OMIM, HGMD, LOVD, DECIPHER (population CNV) and SwissVar [9–14]. Common variants were filtered based on allele frequency in 1000Genome Phase 3, gnomAD (v3.1 & 2.1.1), dbSNP (GCF_000001405.38. Non-synonymous variants effect was calculated using multiple algorithms such as PolyPhen-2, SIFT, MutationTaster2 and LRT. Clinically significant variants were used for interpretation and reporting. Variants identified were classified as per latest ACMG guidelines [15].

Cytogenetic microarray (CMA) was performed in one child using Cytoscan 750K array in view of dysmorphism and suspicion of microdeletion after clinical genetic evaluation. CNV scoring was done as per latest technical standards for interpretation and reporting of CNV by ACMG and ClinGen [16].

Clinical, demographic data (gender, age at presentation, family history), genetic data results, type of variants, interpretation of variant pathogenicity and scoring as per ACMG criteria, mutation site in NF1 protein, of enrolled patients were reviewed and described.

3. Results

3.1. Clinical manifestations

Demographic profile and overview of clinical findings that could be seen in our cohort is shown in Table 1 and Fig. 1. Total 64% (n

= 16) presented in the prepubertal (before 8 years) age. Out of those who presented before 8 years of age, revised diagnostic criteria (more than two clinical criteria before genetic testing) were met by only 4 patients. Lowest age was 10 months and among patients who met revised diagnostic criteria, before testing was 2 years (P13). Overall, 16 (64%) were male, 4 (16%) had positive clinical family history and 21 (84%) were sporadic. Mean age in sporadic group was 8.90 years ± 3.682 and those with a positive family history was 18.5 years ± 13.583. Hence sporadic cases came to clinical attention and diagnosed earlier as compared to familial cases.

However, it was not significantly evident from clinical features that sporadic cases were severe than familial cases. In fact, familial cases had intellectual disability (ID) with macrocephaly (P16), cutaneous neurofibromas (P7), brainstem glioma (P25), cutaneous markers (P19) as presenting features that are present early in life as well.

Clinical features in patients with confirmed NF1 were highly variable at presentation (Figs. 1, 2a–d and 3a–c). Twenty percent had mild features in form of only cutaneous lesion such as CALMs or cutaneous neurofibromas or freckling and had primarily cosmetic concern at time of presentation. Forty percent were evaluated as part of dysmorphism and intellectual disability with or without cutaneous features. They did not have any other complaint at time of first presentation to clinic. Ten patients (40%) presented with major systemic problems and on evaluation were found to have clinical features suggestive of NF1.

We have categorised *NF1* mutation positive patients (N = 25) into different categories according to the type of pathogenic/likely pathogenic variant we found after genetic testing and described their clinical features (Supplementary Table 2).

NFNS phenotype overlap with NF1: It was interesting to note that 8 patients (32%) had facial features of Noonan syndrome (P10, P13, P17, P20, P21, P22, P26, P29) Fig. 3(a–c).

A child (P22) with NFNS features had unidentified bright objects on neuroimaging (MRI brain) which has been found to be associated in NF1 in various studies and this has been proposed as point for diagnostic criteria for NF1 [17,18].

One of patient with NFNS phenotype (P29) presented at 6 years of age with left sided hemiparesis. At 3 years of age there was history of stroke with left sided hemiparesis. He was operated thrice in a period of 6 months after the episode for intracranial bleeding. CT Angio had showed intracranial narrowing of vasculature and formation of collaterals. There was significant narrowing of abdominal aorta and some narrowing of bilateral renal arteries. He had hypertension and seizures, left ventricular hypertrophy, right MCA chronic infarct, stenosis of anterior circulation arteries with chronic ischaemic changes and old infarcts in the cerebral hemispheres bilaterally. He had history of mild developmental delay with DQ of around 70 in all domains. There were facial features of Noonan syndrome with droopy eye lids, epicanthic folds, retrognathia, low set posteriorly rotated ears with fleshy ear lobules, pectus excavatum, multiple café-au-lait macules and freckles all over the body. Height was at - 1.7 SD. Screening for hot spot mutation in exon 3 and 13 of *PTPN11* gene was normal. Later Exome Sequencing revealed a nonsense variant in *NF1* gene. Moya Moya disease has previously been described to occur in NF1 patients [19].

3.2. Molecular testing results

Molecular details of variants found in cohort are listed in Supplementary Table 2. Twenty-five different *NF1* pathogenic/likely pathogenic variants were identified in 25 families. The variants were distributed throughout the exonic and intronic region and no hotspot region was identified. Different variants identified are represented pictorially in Fig. 4 and tabulated in Supplementary Table 2. Among 25 variants, 4 were missense (16%), 7 nonsense (28%), 2 insertion (8%), 7 deletion (28%), 2 contiguous gene deletions (8%), 3 splice site variants (12%) (Fig. 5). Majority of variants were truncating variants. Five variants were novel and 20 were known and previously reported in databases. Novel variants were: P7: c.7744C>T (p.Gln2582Ter), P21: c.430dup (p.Ser144fsTer12), P15: c.5059_5060del (p.Arg1687GlyfsTer7), P19: c.6290delT (p.Arg2098fsTer31), P17: c.4773-2A>G. Novel variants were submitted to ClinVar and accession ID are mentioned in Table 2.

All the variants were classified as per ACMG guidelines [15]. Recently developed ACMG scoring system [20] was used to give the

Table 1
Demographic profile and clinical features of *NF1* positive patients.

Clinical feature	N (%)	
Total NF1 positive patients	25	
Age <8 yrs.	16 (64%)	Diagnostic criteria met before genetic testing = 4 (25%)
Lowest age at which diagnostic criteria met clinically (two or more clinical features) before genetic testing was 2 years		Diagnostic criteria not met before genetic testing = 12 (75%)
>8 years	9 (36%)	Diagnostic criteria met before genetic testing = 6 (66.67%)
		Diagnostic criteria not met before genetic testing = 3 (33.33%)
Family history	4 (16%)	
Gender (M)	16 (64%)	
Female (F)	(9) (36%)	
Congenital Heart Defect (CHD)	4 (16%)	
Café au lait	25 (100%)	
Freckling	6 (24%)	
Cutaneous neurofibroma	4 (16%)	
Lisch nodules	2 of 4 adults (19 years and above) tested (50%)	

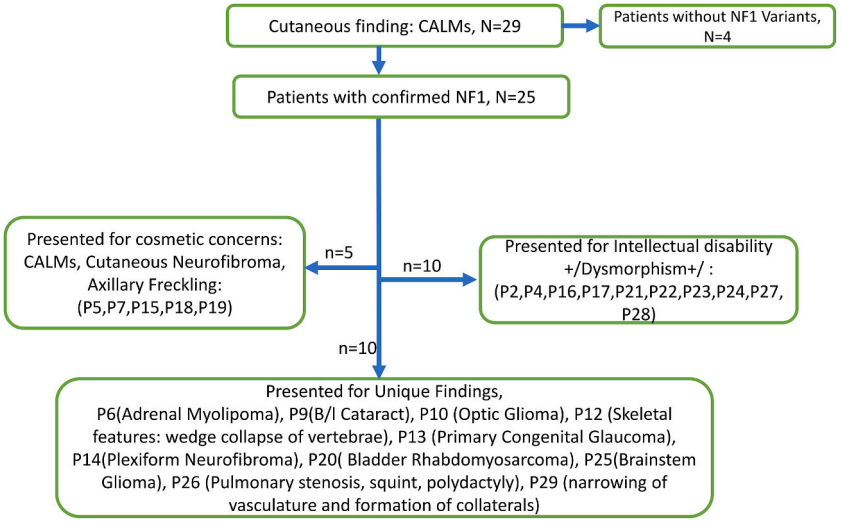

Fig. 1. Presenting complaints of patients positive for *NF1* variants at time of initiation of evaluation.

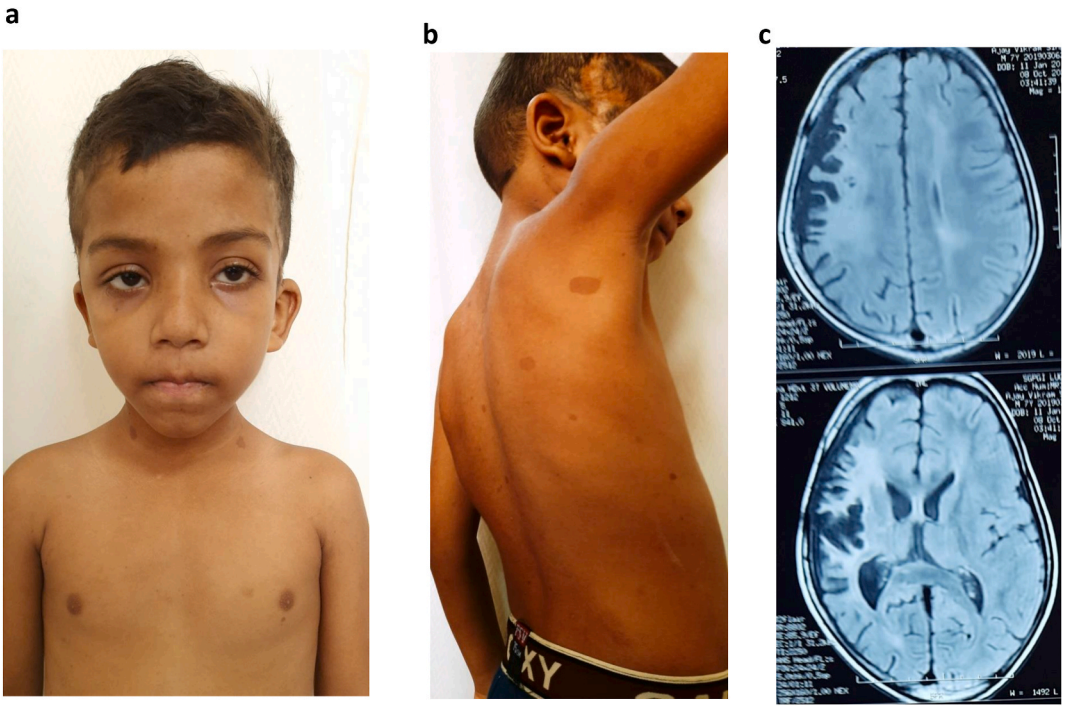

Fig. 2. Skin and intracranial manifestations in NF1. a) Café-au-lat macules in the back in a child with large deletion of exons 1 to 36 in *NF1* b) Bilateral optic glioma and focal areas of signal intensity in the cerebellar white matter in the above child c) Café-au-lat macules in another child with nonsense variant c.5305C > T (p.Arg1769Ter) d) Neurofibromas and freckling in the back of an adult female with nonsense variantc.7744C > T (p.Gln2582Ter).

points to each variant and is given in Table 2. All of the novel variants were causing an unambiguous pathogenic effect on the NF1 protein (missense, nonsense, and nucleotide changes at splice site). CNV were classified using CNV scoring system as per latest technical standards for interpretation and reporting CNV by ACMG and ClinGen [16].

3.3. Detailed phenotyping

Detailed clinical features and variants identified for all patients are summarized in Supplementary Tables 1 and 2. CALMs were

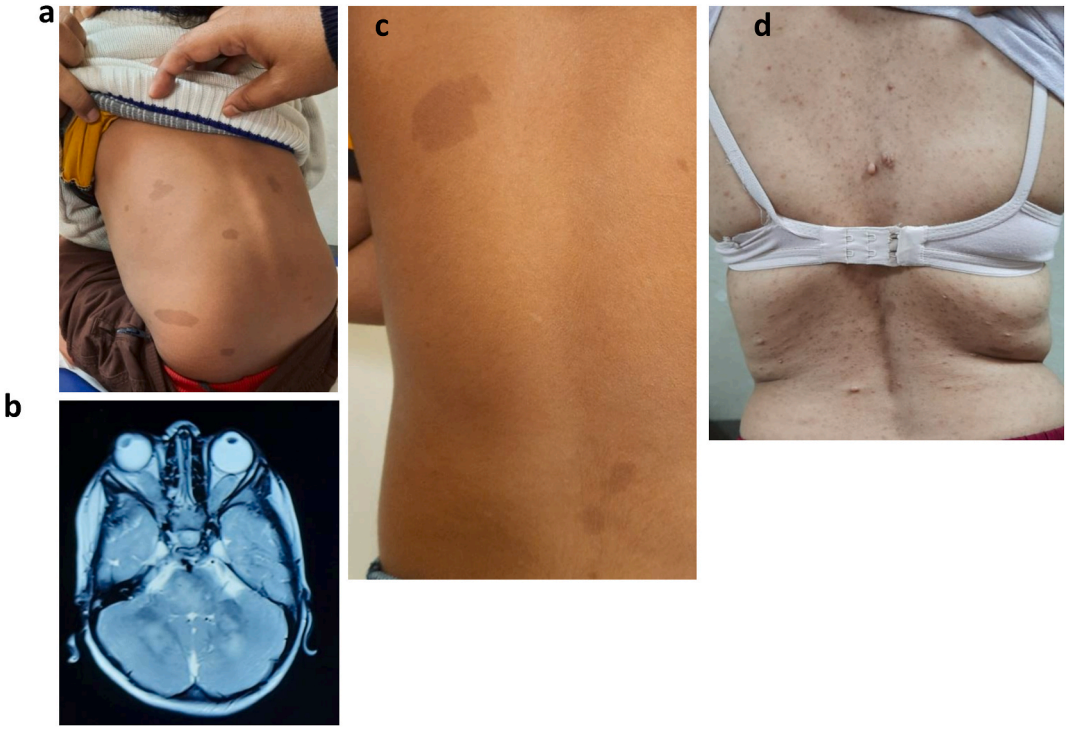

Fig. 3. Eight-year-old child with NF1 Noonan phenotype. a) Facial features of Noonan syndrome with droopy eyelids. B) Multiple café-au-lat macules c) MRI brain (T1) showing old infarct with volume loss mainly on the right side leading to left hemiparesis.

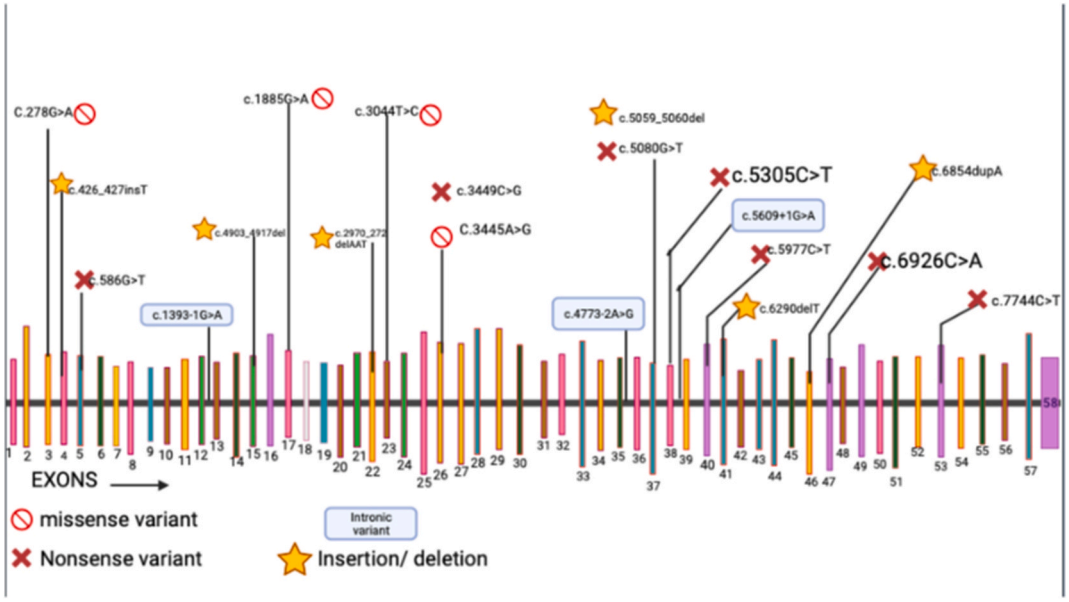

Fig. 4. Pictorial representation of variants found in study cohort.

found in all type of sequence variations, as it was present in all patients. An attempt to correlate ACMG score with phenotype was done. Patients with predominantly cosmetic problems and those with additional dysmorphism had scores in all ranges from 9 to 17. Hence, there was no clearcut correlation of score obtained by variants with phenotype of patients. However, it was interesting to note that patients who had additional unique findings had scores in higher range $\geq$10 points or they had larger deletions.

Another significant feature was tumors or severe phenotype was associated with patients having large deletions. Example: P10 had optic glioma, P6 had adrenal myolipoma, P20 had bladder rhabdomyosarcoma, P14 had plexiform neurofibroma. Overall

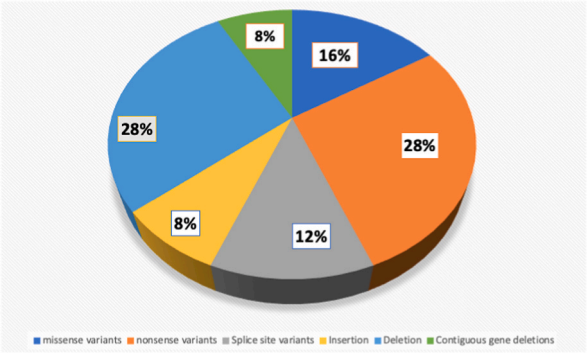

Fig. 5. Frequency of different type of variants found in cohort.

Table 2
Novel variants in detail.

Patient ID	Exon Transcript (NM_001042492.3)	cDNA	Amino acid change	ACMG classification Scoring	ACMG Criteria	Classification	Novel/known
P7	Exon 53	c.7744C > T	p.Gln2582Ter	9points = 9P-0B	PVS1, PM2	LP	Novel SCV004015026
P17	Intron 35	c.4773-2 A > G	–	9points = 9P-0B	PVS1, PM2	LP	Novel SCV004015019
P21	Exon 4	c.430dup	p. Ser144PhefsTer12	9points = 9P-0B	PVS1, PM2	LP	Novel SCV004015025
P15	Exon 37	c.5059_5060del	p. Arg1687GlyfsTer7	9points = 9P-0B	PVS1, PM2	LP	Novel SCV004015021
P19	Exon 41	c.6290delT	p.Arg2098fsTer31	9points = 9P-0B	PVS1, PM2	LP	Novel SCV004015020

malignancies were found to be associated with deletions only (P6, P10, P14, P20) except in P25 who had a duplication.

3.4. Patients without NF1 pathogenic variants

Out of 29 patients who were clinically suspected to have NF1, 4 patients were found to have alternate diagnosis or no variant identified (Table 3). Three patients were having neurofibromatosis type 2 (NF2), the term recently recommended being NF2-related schwannomatosis. In one patient, no variant was identified in NF1 or NF2 gene (P1); he however, had subsequent neuroimaging findings suggestive of NF2. Two patients were proven by molecular testing and assigned the diagnosis of NF2 (P3, P11) and one had autosomal recessive form of EDS (Ehlers-Danlos syndrome, classic-like, 1; # 606408) (P8), arising from a homozygous likely pathogenic variant *TNXB*: c.-8-1G>A. She had a neurofibroma in the back, café-au-lait spots all over the body and axillary freckling and met the diagnostic criteria of NF1, she also had joint laxity and easy bruisability.

3.5. Other important observations

Out of 9 patients who presented after 8 years of age, none had a missense variant. Those who had severe phenotype requiring surgical intervention in any form, had nonsense variant (P29), deletions (P6, P10, P20, P14) or splice site variants (P12, P13). Skeletal features were seen with splice site variant (P12).

4. Discussion

NF1 is an autosomal dominant disorder with variable presentation. Revised diagnostic criteria were put forth in 2021 [5], various studies have suggested that it has reduced the time to diagnose NF1 [22]. Around 97% are postulated to meet diagnostic criteria by 8 years of age [21]. Only 66.67% of patients greater than 8 years of age met the diagnostic criteria in our cohort. Twenty percent patients had only mild phenotype, consisting of cosmetic concerns in form of CALMs or cutaneous neurofibromas of freckling. Remaining 80% had additional features such as intellectual disability and dysmorphism (40%) and malignancies (40%). Those patients who had severe phenotype more commonly had deletions followed by splice site and missense variants.

Missense variants reported previously had severe phenotype. In literature, variant p.Cys93Tyr has been reported to be associated with pheochromocytoma with metastasis, scoliosis and neurofibromas [23–25]. Our patient (P18) had seizure and cutaneous features. He is young and might develop more severe features later on. Variant p.Gly629Arg has been reported to have alopecia areata, vitiligo,

Table 3
Cases where mutations involving NF1 were not detected.

Patient ID	Gender	Family history	Age (year)	Clinical Features	Mutation
P1	Male	No	13	Proximal flexion deformity in second, third and fourth fingers of the right hand and decreased extension of fifth finger. Café au lait spot, cutaneous neurofibromas, gaze evoked nystagmus. MRI brain and spine showed multiple tumors/schwannomas in cervical region and acoustic neuroma. Probably had NF2.	No sequence variations in NGS based tests, MLPA for *NF1* was normal
P3	Male		35	Café-au-lait spots, subcutaneous nodules at right temple and right arm and left iliac crest region, hearing loss (left > right), Proptosis involving left eye, flexion deformity at interphalangeal joints, difficulty in walking and frequent falls. MRI brain showed bilateral vestibular schwannomas and left trigeminal (ophthalmic division) schwannomas	Heterozygous NF2: c.949_950delinsC (p. Glu317GlnfsTer5)
P11	Male	Yes (Father and elder brother)	14	Multiple swellings and pigmentation all over the body with café-au-lait macules.	Heterozygous *NF2*: c.169C > T (p. Arg57Ter)
P8	Female	Yes (elder sibling)	20	Single neurofibroma in the back, café-au-lait spots all over the body and axillary freckling. Easy bruisability and joint laxity was present, there was a similarly affected elder sibling	Homozygous *TNXB*: c.-8-1G > A

pseudoarthrosis of tibia [26,27]. Our patient with same variant P22 had global developmental delay, ptosis, coarse facies suspected to be NFNS spectrum. p.Leu1015Pro has been found to have peripheral nerve sheath tumor in 13–58-year age group [28]. Patient (P16) reported here had only macrocephaly with development delays. Phenotype of another missense variant p.Met1149Val (P26) corroborated with previously reported cases with NFNS spectrum [29]. An explanation for the variation in phenotype in our cohort with that described in literature could be age at molecular diagnosis. P18, P22, P16 had age at presentation 5, 2 and 3 years respectively and phenotype described in literature has been for older patients. Out of 7 nonsense variants described here, p. Gln2582Ter was novel variant and had neurofibromas in back, skin lesions. Another variant p.Glu196Ter was found to be associated with unique features of narrowing of vasculature and formation of collaterals, left ventricular hypertrophy, narrowing of abdominal aorta and renal arteries which has never been reported previously with this variant. P2- p.Ser1150Ter, P5- p.Gln1993Ter, P24- p. Ser2309Ter have been previously reported to have cutaneous features predominantly [30,31]. Out cohort additionally was noticed to have intellectual disability and macrocephaly associated to these variants.

Two out of four small deletions detected in out cohort, pArg1687Glyfs*7 (c.5059_5060del) (P15), p.Arg2098fs (c.6290delT) (P19) among deletions were novel. Among previously reported deletions: p.Tyr1635_Tyr1639del (c.4903_4917del) (P27), p.Met992del (c.2970_2972delAAT) (P9) former had similar phenotype as previously reported in literature [32]. Our patient had cutaneous findings along with cortical cataract and vision problems, which has not been mentioned in previous studies [33,34].

Two patients had insertions, one of them was novel p.Ser144fs*12 (c.430dup), patient (P21) was suspected to have NFNS clinical phenotype i.e. short stature, pectus excavatum, excessive palmar creases. Second (P25)- p.Tyr2285Ter (c.6854dupA) has been previously reported in more than 10 individuals [28,33]. This sequence change creates a premature translational stop signal (p.Tyr2264*) in the *NF1* gene leading to an absent or disrupted protein product [30,35].

Pasmant et al. have demonstrated association of learning disabilities with microdeletions and contiguous gene deletions in NF1 patients. We also noticed the same in the 4 patients who had contiguous gene deletion syndromes involving the whole *NF1* gene in our cohort. They had a mixture of intellectual disability, dysmorphism, genital and cardiac structural defects in different permutations and combinations. P14 had largest deletion of 1.93 Mb, encompassing 33 genes. Previously reported cases with deletion within this region had ambiguous genitalia, cardiac defect, anorectal anomalies. Index patient had only plexiform neurofibroma. It was interesting to note 3 out of four larger deletions (P6, P20, P14) had tumors: adrenal tumor, bladder rhabdomyosarcoma, plexiform neurofibroma respectively. It has been previously postulated in literature that increased risk of tumors may be due to inactivation of a second tumor suppressor gene located within 1.4 Mb microdeletion found in most NF1 micro deleted patients [36]. Napolitano et al. showed a slight significant inverse correlation between age at diagnosis and delayed acquisition of psychomotor skills with residual multi-domain cognitive impairment [37].

In our study, most of the variations were spread throughout different domains of gene while Koczkowska et al. have shown genotype-phenotype correlation at the NF1 codons 844–848 [38]. Among those where we didn't find pathogenic/likely pathogenic variant involving *NF1* gene, 3 had pathogenic/likely pathogenic variants in other genes and so were assigned alternate diagnosis; 2 of NF2 and 1 of EDS. The recent term suggested for NF2 is NF2-related schwannomatosis [39]. There are other putative genes *SMARCB1* and *LZTR1*, both located centromeric to NF2 on chromosome 22 responsible for familial schwannomatosis. The child (P1) where we could not find pathogenic variants in *NF1, NF2* or other schwannomatosis genes by exome sequencing and no pathogenic deletions/duplications in *NF1* gene, probably had NF2-related schwannomatosis. We assume that child might be having a de-novo variant in *NF2* gene with mosaicism, a postzygotic event found in more than 60% of such individuals [40].

It is interesting to note that in one of our patients (P8), we had an initial diagnosis of both NF1 and Ehler Danlos syndrome (EDS). She fulfilled clinical criteria of NF1. After molecular testing and reviewing her clinical features and family history we assigned her the diagnosis of Ehlers-Danlos syndrome, classic-like, 1 [41], which is inherited in autosomal recessive fashion. The classic form of EDS and most other forms are autosomal dominant in inheritance. There is a possibility that she has a dual diagnosis with a cryptic NF1

variant; however, we would like to highlight that there are various disorders including EDS, which mimic NF1 in view of the cutaneous features.

5. Conclusion

NF1 is a multidomain molecule which regulates several intracellular processes, including the RAS-MAP kinase pathway/RAS-ERK-CREB pathway. The function of *NF1* gene as a tumor suppressor gene and its dysregulation leading to various tumors throughout the human body is well known. We hereby present the wide range of manifestations at different age groups and the pathogenic/likely pathogenic variant spectrum ranging from small scale variants to contiguous gene deletion syndromes involving the *NF1* gene. We have also tried to correlate the disease severity with variant types, thereby highlighting the usefulness of molecular testing and genetic counseling in suspected NF1 and NF1 related disorders. Further studies are required in larger cohort with in depth functional analysis of the molecular defects to fully understand the mechanism of tumor formation and associated symptoms, thereby assisting development of therapeutic targets in this disorder.

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Data availability statement

The authors have given all the data set in form of tables in the manuscript. Data associated with this study has been deposited into a publicly available repository ClinVar with Accession numbers SCV004015026, SCV004015019, SCV004015025, SCV004015021, SCV004015020.

CRediT authorship contribution statement

Priyanka Srivastava: Writing – review & editing, Supervision, Project administration, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Shifali Gupta:** Writing – original draft, Methodology, Formal analysis, Data curation. **Chitra Bamba:** Visualization, Methodology, Investigation, Formal analysis, Data curation. **Roshan Daniel:** Software, Formal analysis, Data curation. **Parminder Kaur:** Resources, Investigation, Formal analysis. **Anupriya Kaur:** Visualization, Resources, Project administration, Formal analysis, Data curation. **Inusha Panigrahi:** Writing – review & editing, Resources, Investigation, Data curation. **Kausik Mandal:** Writing – original draft, Supervision, Project administration, Investigation, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2023.e23685.

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