2024, Volume 4, Issue 2, Page No: 20-29
ISSN: 3108-4826

Society of Medical Education & Research

Journal of Medical Sciences and Interdisciplinary Research

Rare Co-occurrence of Two Mutational Variants in NF1: Molecular Testing Reveals Diagnostic Surprises

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Abstract

Neurofibromatosis type 1 (NF1; MIM #162200), commonly called von Recklinghausen disease, is a frequently encountered genetic condition transmitted via autosomal dominant inheritance. It is characterized by a spectrum of neurocutaneous symptoms, such as café-au-lait patches, freckling in skin folds, various types of neurofibroma (including dermal and plexiform), as well as neurological and skeletal complications. The condition affects approximately 1 in every 3,000 to 4,000 people. Separately, cone-rod dystrophies constitute a broad category of retinal disorders that involve degeneration of both cone and rod photoreceptors and are often driven by mutations in over 100 identified genes. One such gene, CRX, is associated with autosomal dominant forms of the disease. This paper highlights an unusual clinical scenario: a patient initially diagnosed with NF1 during infancy later presented with visual impairments—specifically, myopia and astigmatism—by the age of 15 years. Genetic screening revealed two distinct variants: a pathogenic splice-site mutation in the NF1 gene (c.3871-2A) and a potentially pathogenic alteration in the CRX gene (c.119G>A). This rare combination of variants, identified through molecular diagnostics, challenges the assumption that clinical assessment alone is sufficient in NF1 cases and highlights the role of genomic analysis in refining diagnosis and management strategies.

Keywords: NF1, Cone-rod retinal degeneration, Genetic testing, Dual gene variants, Clinical-genomic correlation

Introduction

Neurofibromatosis type I (NF1) belongs to a group of neurocutaneous disorders, which also includes conditions such as von Hippel-Lindau disease, Sturge-Weber syndrome, and tuberous sclerosis complex [1, 2]. NF1 arises due to pathogenic alterations in the NF1 gene, located on chromosome 17q11.2. These mutations result in hyperactivation of the RAS/MAPK signaling cascade, ultimately contributing to cutaneous and skeletal abnormalities [3]. Clinically, NF1 is primarily recognized by the presence of café-au-lait macules and neurofibromas, which differ significantly in their number, size, and anatomical distribution [4, 5]. Alongside Gardner and Cowden syndromes, NF1 is among the most frequently observed neoplastic hamartoma syndromes [6]. The condition shows nearly complete penetrance, but its phenotypic expression is

Access this article online https://smerpub.com/

Received: 01 June 2024; **Accepted:** 11 September 2024

How to cite this article: Kowalski TW, Reis LB, Andreis TF, Ashton-Prolla P, Rosset C. Rare Co-occurrence of Two Mutational Variants in NF1: Molecular Testing Reveals Diagnostic Surprises. J Med Sci Interdiscip Res. 2024;4(2):20-9. https://doi.org/10.51847/H2qQlZTYO7

highly variable, even among members of the same family.

Diagnosis is typically based on clinical findings, as outlined by the National Institutes of Health (NIH) in 1988, which remain the foundation for identifying affected individuals [7]. Because the manifestations of NF1 emerge progressively and are strongly influenced by age, prolonged observation is often necessary before a definitive diagnosis can be made. Approximately 97% of affected individuals meet the NIH clinical criteria by the age of 8 [8, 9]. In recent years, genetic testing has gained prominence in confirming NF1 and has been incorporated into the revised diagnostic guidelines issued in 2021 [10, 11].

Cone-rod dystrophy (CORD), by contrast, represents a heterogeneous set of inherited retinal disorders with overlapping clinical features, complicating both diagnosis and treatment strategies [12]. These dystrophies typically begin with reduced central vision and impaired color perception, eventually progressing to peripheral vision loss. Although symptoms often begin in childhood—commonly during school age—as blurred vision that does not improve with corrective lenses, many individuals are not formally diagnosed until adulthood [13]. As the disease advances, night vision becomes compromised due to rod cell degeneration. Additional symptoms may include light sensitivity and color discrimination issues. Over 90 genes and multiple inheritance modes—autosomal dominant, autosomal recessive, and X-linked—have been linked to CORD. Among the genes associated with autosomal dominant cone-rod dystrophy (adCORD), CRX (OMIM #602225) is one of the most studied. This gene encodes a photoreceptor-specific transcription factor critical for the development and function of cone and rod cells. Variants in CRX are known to cause a range of disorders, including cone-rod dystrophy, macular dystrophy, and Leber's congenital amaurosis [14].

This article presents a rare and incidental co-occurrence of two genetic variants—one in NF1 and the other in CRX—within a single patient who was clinically diagnosed with NF1 during infancy. Despite NF1 typically being confirmed through clinical criteria, molecular testing, in this case, revealed an additional likely pathogenic variant in the CRX gene. This unexpected result underscores the value of integrating molecular diagnostics into routine clinical assessment, as it can reveal additional or coexisting conditions that may otherwise go undetected. Accurate molecular diagnosis is vital for effective genetic counseling, early intervention, and informed family risk assessment.

Materials and Methods

Case description

This report focuses on a 19-year-old female patient who was referred to the Bihor Regional Center for Medical Genetics for genetic counseling following a recommendation from her primary care physician during infancy.

Laboratory analyses

The initial biochemical workup included assessments of carbohydrate and lipid metabolism (cholesterol, triglycerides, and general lipid profile), protein metabolism (total protein levels and serum protein electrophoresis), and mineral levels (phosphorus, calcium, and magnesium). Hormonal testing and alpha-fetoprotein levels were also evaluated.

Imaging studies

Radiological investigations consisted of a cerebral magnetic resonance imaging (MRI) scan and optical coherence tomography (OCT) of the eyes.

Molecular genetic testing

Informed consent was obtained from the patient's mother before participation. Genomic DNA was extracted and sequenced using the Illumina MiSeq system, applying the TruSight One Sequencing Panel for targeted next-generation sequencing. Library preparation, DNA fragmentation, and sequence capture were conducted at the Regional Genomic Centre in Timisoara, Romania. Bioinformatic processing involved multiple tools: sequence alignment via BWA (v0.7.9a-isis-1.0.1), variant calling through SAMtools (v0.1.18), and GATK (v1.6-23-gf0210b3), and variant annotation using Isis software (v2.5.2.3).

Data interpretation utilized public genomic resources, including the UCSC Genome Browser, OMIM, and DGV. The variant filtration strategy included all known pathogenic variants recorded in HGMD® and ClinVar (class 1), alongside rare variants with a minor allele frequency (MAF) below 1% based on ExAC data. Only variants linked to the clinical features observed in the patient were retained. Variant classification followed the American College of Medical Genetics and Genomics (ACMG) 2015 guidelines [1].

Results and Discussion

Patient background

The subject is the second child born to a young, non-consanguineous couple. A detailed family history revealed that the paternal grandfather, father, and paternal uncle presented with multiple café-au-lait macules and dermal neurofibromas.

From birth, the patient exhibited six café-au-lait macules on the chest and abdomen, measuring between 7 × 4 cm and 2 × 1.5 cm, which were strongly indicative of NF1. Over time, the number of pigmented lesions increased, and additional features such as axillary freckling and dorsal neurofibromas emerged. At age 15 years, she was evaluated by ophthalmology due to myopia and astigmatism. Lisch nodules were later identified. At 16, she experienced a generalized tonic-clonic seizure lasting around 30 seconds.

Clinical assessment at age 17 years

At the time of her most recent evaluation, the patient measured 166 cm in height and weighed 66 kg—within normal ranges. Physical examination revealed more than 10 café-au-lait spots distributed across the anterior and posterior trunk, neck, abdomen, and limbs. Additional findings included multiple thoracic neurofibromas and freckling in the axillary regions **(Figures 1–4)**.

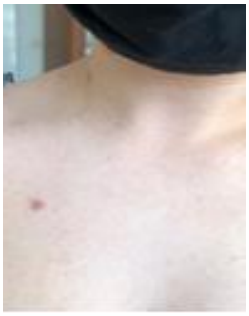


Figure 1. Cutaneous neurofibroma

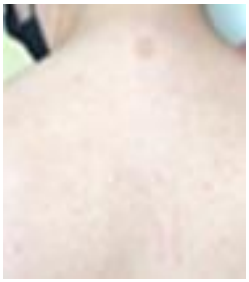


Figure 2. Posterior thoracal café au lait spots

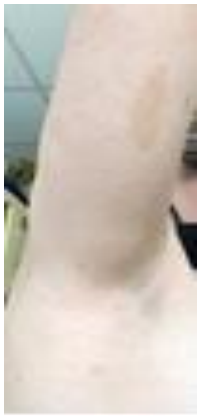


Figure 3. Axillary freckles

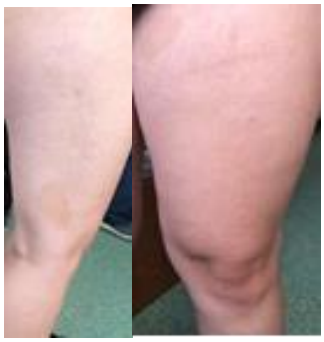


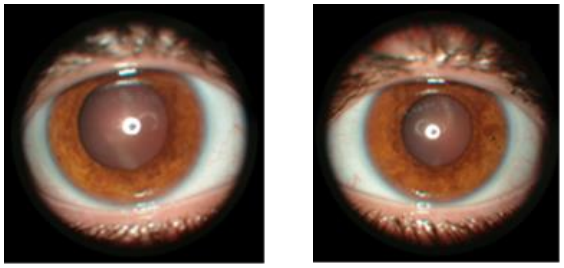
Figure 4. Café au lait spots on the lower limbs

Laboratory investigations

Biochemical, hematological, and endocrine evaluations—including assessments of thyroid hormone levels—yielded results within normal reference ranges. Additionally, serum alpha-fetoprotein, a key tumor marker, was found to be within normal limits, suggesting no evidence of malignancy.

Interdisciplinary consultations and imaging studies

An ophthalmologic assessment confirmed the presence of both myopia and astigmatism. Corrected visual acuity was measured at 0.8 in the right eye and 1.0 in the left. Anterior segment examination identified Lisch nodules in both eyes **(Figure 5)**. Fundoscopic evaluation showed well-defined optic disc margins and a normal macular reflex. Optical coherence tomography (OCT), conducted using the Nidek DuoScan 3300, demonstrated a preserved and structurally normal macular profile. Visual field testing indicated no abnormalities in either eye.



a) b)

Figure 5. Lisch nodules: a) right eye, and b) left eye.

Neuroimaging findings

Brain MRI revealed multiple well-circumscribed lesions, exhibiting hyperintensity on T2-weighted and FLAIR sequences and isointensity on T1-weighted sequences. These were located in the anterior right mesencephalon (measuring approximately 5–7 mm), bilaterally in the thalamic regions (approximately 9 mm on the right and 7 mm on the left), and in the right capsulo-lenticular area (measuring around 5 mm), with the latter demonstrating characteristics suggestive of glioma. No perilesional edema was observed. The corpus callosum appeared slightly enlarged, yet without signal abnormalities. Electroencephalography (EEG) did not reveal any pathological graphoelements.

Molecular genetic testing

Next-generation sequencing via panel analysis identified two distinct genetic variants. The first, a heterozygous splice site variant (c.3871-2A > T) in the NF1 gene, demonstrated 89X sequence coverage. This loss-of-function variant is absent from large-scale population datasets (e.g., ExAC and GnomAD), indicating its rarity. Predictive bioinformatic tools—BayesDel_addAF, DANN, EIGEN, FATHMM-MKL, MutationTaster, and scSNV-Splicing—all support its pathogenicity, with no tools indicating a benign effect. Based on these findings, and following ACMG guidelines, this variant is classified as pathogenic.

The second variant, detected in the CRX gene, is a heterozygous missense change (c.119G > A) with a read depth of 224X. Like the NF1 variant, this change is absent from ExAC and GnomAD, supporting its potential clinical significance. Predictive algorithms—including BayesDel_addAF, DANN, DEOGEN2, EIGEN, FATHMM-MKL, LIST-S2, M-CAP, MVP, MutationAssessor, MutationTaster, PrimateAI, and SIFT—uniformly suggest a pathogenic impact, with no benign predictions reported. According to ACMG criteria, this mutation is interpreted as *likely pathogenic* and warrants phenotypic correlation.

Gene profiles

NF1 gene

The *NF1* gene resides on chromosome 17q11.2, spanning approximately 350 kb and encompassing 55 constitutive and 5 alternatively spliced exons [15]. It encodes neurofibromin, a multi-domain protein integral to the negative regulation of the RAS/MAPK pathway. Loss-of-function mutations disrupt this control, leading to excessive cellular proliferation and tumorigenesis [16]. Over 3,600 pathogenic variants have been documented in the Human Gene Mutation Database (HGMD), including microdeletions, CNVs, and splice-altering mutations [17, 18]. While pathogenic variants account for over 0.5% of the general population, private or rare mutations constitute more than 46% [19, 20]. Approximately 90% of these mutations are intragenic; large deletions affecting the entire gene are rare [21].

CRX gene

Located on chromosome 19q13.33, the *CRX* gene belongs to the homeobox gene family and plays a crucial role in the development and maintenance of photoreceptor cells in the retina [22]. It encodes a 299-amino-acid transcription factor involved in the maturation of rods and cones. The gene spans ~25 kb and includes four exons [23]. Pathogenic mutations are typically missense or in-frame deletions, with over 45 mutations currently associated with retinal diseases such as cone-rod dystrophy (CORD), Leber congenital amaurosis, and retinitis pigmentosa [12, 24–27]. CRX mutations are responsible for 5–10% of autosomal dominant cases of CORD [15, 25].

Clinical considerations in neurofibromatosis

Neurofibromatosis represents a spectrum of neurocutaneous syndromes with multi-organ involvement. It includes NF1 (90% of cases), NF2-related schwannomatosis (3%), and rare forms such as SMARCB1- and LZTR1-related schwannomatosis (< 1%) [28]. As part of the broader RASopathy group, NF1 shares molecular pathways with conditions like Noonan, Legius, Costello, and cardiofaciocutaneous syndromes [10, 29]. NF1 is inherited in an autosomal dominant manner, though approximately half of cases are attributed to de novo mutations. The disorder exhibits complete penetrance but with variable expressivity, and it is progressive—typically associated with a 15% reduction

in life expectancy. Our patient fulfilled NIH diagnostic criteria, displaying numerous café au lait macules, axillary freckling, cutaneous neurofibromas, and MRI-confirmed gliomas. Molecular testing confirmed the presence of a pathogenic *NF1* variant, supporting the clinical diagnosis.

Cutaneous features in NF1

Café au lait macules and freckling

Café au lait macules (CALMs) often represent the earliest visible marker of NF1, typically present at birth. These pigmented skin lesions increase in number and size in early childhood, enlarging proportionally with growth. Another hallmark of the disease is the appearance of axillary and inguinal freckling, which tends to emerge later, usually between the ages of 3 and 5 years. These freckles are small, pigmented lesions clustered in skin folds and are present in approximately 70% of individuals with NF1.

Neurofibromas

Neurofibromas are benign peripheral nerve sheath tumors that manifest as soft dermal papules or subcutaneous nodules, varying in size and pigmentation [30]. Two principal forms exist: cutaneous and plexiform neurofibromas. Cutaneous neurofibromas typically emerge during puberty and may continue to proliferate in size and number until early adulthood. In contrast, plexiform neurofibromas—pathognomonic for NF1—are congenital and occur in about 30% of affected individuals. These lesions carry a higher risk of transforming into malignant peripheral nerve sheath tumors (MPNSTs) [31].

In our patient, multiple cafés au lait macules were noted across the neck, thoracic and abdominal regions, buttocks, and lower limbs. Axillary freckling was first observed at age 11 years, coinciding with the initial appearance of cutaneous neurofibromas.

Ophthalmologic manifestations in NF1

Ophthalmic involvement in NF1 can complicate diagnosis and treatment due to variable presentations. The most frequently associated central nervous system tumor is optic pathway glioma (OPG), typically a low-grade neoplasm identified in around 15% of children with NF1 [32]. Recent advances in ocular imaging modalities have expanded the spectrum of recognized NF1-associated eye findings, including choroidal abnormalities (CAs), hyperpigmented patches, and retinal vascular changes. Diagnostic criteria have evolved, now permitting the presence of two or more cutaneous neurofibromas to substitute for Lisch nodules. Choroidal abnormalities occur more commonly in adults (80–90%) but are also seen in children, albeit at a lower prevalence (60–78.6%). These abnormalities are considered more sensitive markers than Lisch nodules in pediatric cases [16, 33, 34].

In our case, the patient exhibited ocular refractive errors—namely, myopia and astigmatism—as well as bilateral Lisch nodules on ophthalmologic examination.

Neurological involvement in NF1

Brain gliomas are frequently encountered in NF1 patients and are often asymptomatic, with incidental detection during routine imaging [1]. These tumors are seen in 2–5% of cases, with onset possible at any age, although the mean age at diagnosis is around 13 years. NF1 patients in early adulthood exhibit a 10- to 50-fold elevated risk of developing aggressive brain tumors [35].

The patient presented here had multiple gliomas on brain MRI at age 15 years and experienced a generalized tonic-clonic seizure (grand mal) at age 16 years. Although the electroencephalogram did not demonstrate abnormal activity, the seizure may have been linked to a lesion involving the corpus callosum. The observed hypertrophy of this structure may reflect microscopic changes not yet discernible on imaging.

Genotype-phenotype relationship in NF1

To date, there is no definitive genotype-phenotype correlation in NF1. However, increasing numbers of studies seek to clarify this relationship by analyzing mutational profiles. In a retrospective cohort of 38 NF1 patients, Well *et al*. [36] reported that large deletions encompassing the entire *NF1* gene correlated with more severe clinical features, including accelerated tumor growth and greater tumor burden. As a result, the authors recommended close clinical surveillance and consideration of MEK inhibitor therapy in selected cases [36].

Similarly, Peduto *et al*. supported the association between gross deletions and severe phenotypes, emphasizing that not all pathogenic variants have uniform clinical effects [3, 21]. Although the splice site mutation identified in our patient has not been reported in population databases, its novelty currently prevents a precise genotype-phenotype association from being established. Nonetheless, its classification as pathogenic warrants clinical correlation and long-term monitoring.

Treatment of neurofibromatosis type 1 (NF1)

Surgical management

Surgical excision remains the cornerstone of treatment for neurofibromas in NF1, although it is associated with a notably high recurrence rate following resection [28]. Despite this limitation, surgery remains the only potentially curative option. The decision to operate depends on several factors, including the neurofibroma's size, anatomical location, growth dynamics, radiological features, and the patient's overall health status [37].

Pharmacologic therapy

Targeted drug therapies have emerged as promising options, particularly in managing inoperable plexiform neurofibromas in pediatric populations. In 2020, the FDA approved selumetinib, a selective MEK1/2 inhibitor, marking a significant advancement in the treatment of children over two years old with symptomatic, inoperable plexiform tumors [38]. Another candidate, rapamycin, an mTOR pathway inhibitor, has shown potential due to its role in modulating AKT signaling, offering a secondary therapeutic pathway for plexiform neurofibromas.

Cone-rod dystrophies (CORD)

Genetic inheritance

Ongoing research in cone-rod dystrophies primarily focuses on identifying additional causative genes, understanding their molecular roles, and refining clinical trial endpoints. Currently, over 30 genes associated with CORD are cataloged in the RetNet database (http://www.sph.uth.tmc.edu/retnet/) [14]. These are monogenic disorders transmitted via autosomal dominant (adCORD), autosomal recessive (arCORD), or X-linked (xlCORD) patterns. For adCORD, mutations have been reported in at least 10 genes, including CRX [23]. For arCORD, mutations have been linked to 13 genes, and additional loci have been mapped on chromosomes 1q12-q24, 10q26, 18q21.1-q21.3, and NF1. The X-linked variant is attributed to mutations in exon ORF15 of the RPGR gene located at Xp21.1 [39].

Clinical characteristics

CORD is characterized by retinal pigment abnormalities, primarily affecting the macular region. Unlike retinitis pigmentosa (or rod-cone dystrophy, RCD), where rod cells are primarily affected first, CORD involves early degeneration of cone photoreceptors. This leads to progressive central vision loss, reduced color discrimination, and decreased central visual field sensitivity. As the disease advances, patients may experience peripheral vision loss and nyctalopia. According to Hamel [13], CORD typically manifests in childhood but is often diagnosed in adulthood. Fujinami-Yokokawa *et al*. [40], in a study of 730 Japanese families with inherited retinal disorders, identified 18 individuals from 13 families with CRX-associated retinal degeneration. The average age of onset was 45 years (ranging from 15 to 77 years), with one case beginning at 15 years and another showing late onset at 45 years [40]. In the present case, the patient harbors a likely pathogenic CRX variant that currently lacks a direct clinical correlation. She has been followed for myopia and astigmatism since age 15 years, without any signs suggestive of cone-rod dystrophy. However, given the possibility of delayed symptom onset, ongoing ophthalmologic surveillance remains essential.

Diagnosis of cone-rod dystrophy

Diagnosis is based on a comprehensive ophthalmological workup, including fundus photography, optical coherence tomography (OCT), and full-field electroretinography (ffERG). ffERG allows for classification into three categories: those with normal responses (macular dystrophy, MD), those with cone dysfunction (CD), and those with both cone and rod involvement (CORD) [41]. In our patient's case, ffERG findings were within normal limits, placing her in the MD category.

Management and prognosis

At present, no approved treatments can halt disease progression or restore lost vision in CORD. However, identifying pathogenic variants—such as those in CRX—is essential for accurate diagnosis, genetic counseling, prognosis, and participation in clinical trials [42]. Current management strategies focus on slowing disease progression, addressing complications, and providing support for eventual vision loss [13]. The patient continues to receive corrective treatment for myopia and astigmatism, with no evidence of CORD thus far. Nevertheless, due to the variant's pathogenic potential, close ophthalmologic follow-up is warranted.

Genotype-phenotype correlation in CORD

Establishing genotype-phenotype correlations in CORD remains challenging. Mutations in the same gene can result in a wide spectrum of clinical manifestations, while different genes can produce overlapping phenotypes. Additionally, patients initially diagnosed with MD may later progress to CORD or even show features mimicking

retinitis pigmentosa (RP) [40, 43, 44]. The rare missense variant (c.119G > A) identified in our patient was previously reported by Carss *et al*. as being associated with inherited retinal disorders [45]. Interestingly, Oh *et al*. [46] reported the same variant in two unrelated patients with pigmented paravenous retinochoroidal atrophy, but without NF1. Although this variant is considered deleterious and has a very low population frequency, its precise phenotypic outcome is still unclear [46]. In our case, the patient has only mild refractive errors, whereas Oh *et al*.'s cases involved more severe retinal changes, making it difficult to draw a direct genotype-phenotype correlation [46].

Association between NF1 and cone-rod dystrophy

Reports linking NF1 with cone-rod dystrophy (CORD) are exceedingly rare in the literature. The earliest documented case was published by Kylstra *et al*. [47], who first suggested an association between the two conditions. Zobor *et al*. [48] described another case involving NF1 with cone-rod dystrophy in the context of an autosomal recessive disorder, accompanied by a CNNM4 gene mutation and amelogenesis imperfecta. The case presented here is novel in that it documents, for the first time, the coexistence of a pathogenic NF1 mutation and a likely pathogenic variant in the CRX gene. Although the patient currently exhibits no clinical signs of CORD, the possibility of a delayed onset remains, highlighting the intricacies of genetic interplay. This finding reinforces the importance of continuous, multidisciplinary follow-up and thorough genomic investigation in complex clinical presentations.

Conclusion

The diagnosis and ongoing management of neurofibromatosis require a multidisciplinary approach that incorporates both clinical evaluation and genetic investigation. While clinical features remain crucial for diagnosis, unusual findings should prompt broader diagnostic considerations. Molecular analysis, as shown in this case, can uncover unexpected genetic combinations—in this instance, rare concurrent mutations in NF1 and CRX. Despite the patient's current lack of cone-rod dystrophy symptoms, vigilant ophthalmological monitoring remains essential for the early detection of potential future manifestations. This case exemplifies the significance of personalized medicine, where treatment and surveillance strategies are tailored to the patient's unique genotypic profile. The identification of coexisting pathogenic variants in different disease-associated genes underlines the importance of comprehensive genetic screening in individuals with atypical or overlapping phenotypes.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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