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Clinical and Molecular Analyses in 8 New Craniofrontonasal Syndrome Families: Revisiting the Mild End of the Phenotypic Spectrum in Females

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What is already known on this topic?

- *Craniofrontonasal syndrome is a rare X-linked-dominant disorder caused by **EFNB1** pathogenic variants. Affected females display coronal craniosynostosis, facial asymmetry, woolly hair, and skeletal anomalies in addition to frontonasal dysplasia findings, whereas males paradoxically display a less severe phenotype with mild hypertelorism.*

What does this study adds on this topic?

- *This study adds to the genetic and mutational landscape of CFNS by providing the largest Turkish cohort to date. Our deep phenotyping data allowed for the documentation of previously unreported or rarely observed features in affected males and females. The identification of 2 mildly affected female patients highlights the importance of recognizing this rare phenomenon for early diagnosis and accurate genetic counseling.*

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ABSTRACT

Objective: Craniofrontonasal syndrome (CFNS) is a rare X-linked disorder caused by *EFNB1* pathogenic variants. It is characterized by coronal synostosis, facial asymmetry, hypertelorism, bifid nasal tip, woolly hair, longitudinal nail ridging, and skeletal anomalies in heterozygous females. Hemizygous males paradoxically display a less severe phenotype. This study aims to further define the clinical and mutational spectrum of CFNS in 8 new families.

Materials and Methods: Nine female and 2 male patients were included. After detailed phenotypic characterization, Sanger sequencing of *EFNB1* was performed, followed by deletion–duplication analysis of mutation-negative patients.

Results: Universal findings in affected females included wide nasal bridge, hypertelorism, and nasal tip abnormalities. Clinical features were consistent with literature data in the majority, except for 2 females with a mild phenotype resembling hemizygous males. Rare or previously undescribed features included enlarged cisterna magna, nasolacrimal duct obstruction, mesoaxial polydactyly, small echogenic kidney, and hypoplastic labia minora in females, and metopic groove, columellar skin pit, and absent midline mustache hair in males. Genetic analyses identified 6 *EFNB1* variants, including a novel variant, heterozygous in females and hemizygous in males. One female patient with a classical CFNS phenotype had no identifiable *EFNB1* variant.

Conclusion: This study of the largest CFNS cohort from Türkiye expands the phenotypic spectrum in affected males and females and contributes to the genetic landscape of *EFNB1* variants. Two females of the cohort with a phenotype at the mildest end of the CFNS spectrum emphasize the importance of recognizing atypical CFNS presentations for timely diagnosis and accurate genetic counseling.

Keywords: Craniofrontonasal syndrome, EFNB1, clinical paradox, mild female phenotype

INTRODUCTION

Frontonasal dysplasia (FND) represents a heterogeneous group of disorders with common clinical characteristics including cranium bifidum, hypertelorism, wide nasal bridge, and bifid nasal tip.[1] These phenotypic features arise from disruptions in the development of the facial primordia during embryogenesis.[2] Among the FND spectrum with known genetic etiopathogenesis, the most well-defined condition is craniofrontonasal syndrome

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(CFNS, MIM# 304110), an X-linked dominant (XLD) disorder with an estimated prevalence of less than 1 in 100 000 live births.[3] Craniofrontonasal syndrome is associated with loss-of-function variants in the *EFNB1* gene (Ephrin-B1, MIM* 300035), located on Xq13.1.[4,5] Ephrin-B1 ligands interact with Eph-B receptor tyrosine kinases on adjacent cells, mediating cell contact-dependent bidirectional signaling.[4] This interaction plays a crucial role in cell adhesion and migration, required for the normal development of craniofacial structures.

Affected females with CFNS typically display FND features of hypertelorism and a bifid or broad nasal tip, along with additional manifestations such as coronal craniosynostosis, orbital dystopia and craniofacial asymmetry, thoracic skeletal anomalies, partial or complete syndactyly, longitudinal ridging of the nails, and dry, woolly hair.[6] Less frequent findings include cleft lip and/or palate, axillary pterygium, scoliosis, asymmetric lower limb shortening, diaphragmatic hernia, and underdevelopment of the corpus callosum. Despite the XLD inheritance pattern of CFNS, hemizygous males are paradoxically less severely affected than females.[3,7] While isolated hypertelorism is often the only clinical feature in males, cleft lip and/or palate, dental malocclusion, ridging of the nails, and diaphragmatic hernia have occasionally been described. Heterozygous females or mosaic males have 2 distinct cell populations, expressing either wild-type or mutant EFNB1.[8] Disrupted communication between these 2 cell groups interferes with proper cell alignment and tissue patterning, resulting in the characteristic craniosynostosis and facial asymmetry of CFNS. In contrast, hemizygous males with uniformly deficient cells exhibit normal alignment due to the promiscuity of the Eph/ephrin system.[9] This phenomenon is named "cellular interference" and explains the counterintuitive sex-based difference in disease severity.

The *EFNB1* gene comprises 5 exons, with exons 2 and 3 encoding the extracellular ephrin domain-mediating Eph receptor binding. The Human Gene Mutation Database (HGMD) lists 152 disease-associated *EFNB1* variants, predominantly occurring within exons 2 and 3 encoding the ephrin domain: 47% are single nucleotide variants, and the remaining constitute a wide spectrum comprising small insertions and deletions (indels), splicing variants, large copy number variations, and 5' untranslated region (UTR) variants.[10,11] About 20% of CFNS patients remain without a molecular diagnosis, suggesting potential involvement of other genetic or regulatory factors.[3,12,13] A recent report has described the first CFNS case caused by a large inversion, separating *EFNB1* from its potential enhancer and altering the gene's regulatory landscape.[14] No genotype–phenotype correlations have yet been established, underscoring the complexity of CFNS pathogenesis.[11]

The objective of this study is to expand our understanding of CFNS through comprehensive clinical and molecular characterization in a cohort of 9 females and 2 males from 8 CFNS families, including a mother–daughter pair and 2 father–daughter pairs. Notably, we report on an unusual mild CFNS phenotype in 2 *EFNB1* female heterozygotes and discuss possible mechanisms underlying this rare observation.

MATERIALS AND METHODS

Patients

Eight unrelated probands with CFNS or mild FND findings and a normal female karyotype were retrospectively recruited through the Medical Genetics Department at İstanbul University İstanbul Medical Faculty, between 2006 and 2012. The clinical inclusion criterion was the presence of marked hypertelorism along with at least one of the following features: coronal craniosynostosis, facial asymmetry, wide nasal bridge, and nasal tip anomalies. Parents of the probands were clinically examined when available and included in the study when FND features were present. Demographic information, including gender and age, was recorded for each patient. Family history and detailed pedigree analysis were also documented. A comprehensive personal history comprising growth, developmental milestones, and previous disease history was collected. Clinical data were assessed retrospectively, encompassing anthropometric measurements, biochemical and radiological findings, and surgical and medical treatments. The standard deviation scores (SDSs) for anthropometric measurements were calculated using national reference data.[15] All probands underwent echocardiographic evaluation and renal ultrasound. Extended family members were evaluated only by clinical history and facial photographs. The study received approval from the Institutional Review Board of İstanbul University (Date: 30.06.2009, No.: 2029). Written informed consent for genetic testing and publication of clinical photographs was obtained from all subjects or their legal guardians in accordance with the Helsinki Declaration.

Genetic Analyses

Genomic DNA was extracted from peripheral blood lymphocytes of the probands and their available parents using a commercial kit (MagNa Pure, Roche). Coding exons and flanking regions of *EFNB1* [NM_004429.5] were sequenced using the Sanger method (ABI3500). Multiplex ligation-dependent probe amplification (MLPA) was performed for patients with no sequence variants, using the P080 Craniofacial kit (MRC Holland, Amsterdam, the Netherlands). Initial classification of the variants was based on the predicted impact on the protein, novelty, segregation, and conservation. The pathogenicity of a splice variant was evaluated using *in silico* splice prediction tools.[16] Familial segregation studies were conducted via targeted Sanger sequencing when parental DNA was available. The variants were recently reclassified using the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) 2015 criteria, according to the Association for Clinical Genomic Science (ACGS) Best Practice Guidelines for Variant Classification in Rare Disease 2024.[17,18]

RESULTS

Cohort Characteristics and Affected Family Members

The age at last clinical assessment of the probands ranged between 2.5 months and 15 years, with an average of 65.5 months. The parents of Patient (P) 8 were not available for clinical evaluation. P2 and P1 represent an affected mother–daughter pair, while P5 and P10 are the affected fathers of P4 and P9, respectively (Figure 1). The mother of P5, and the

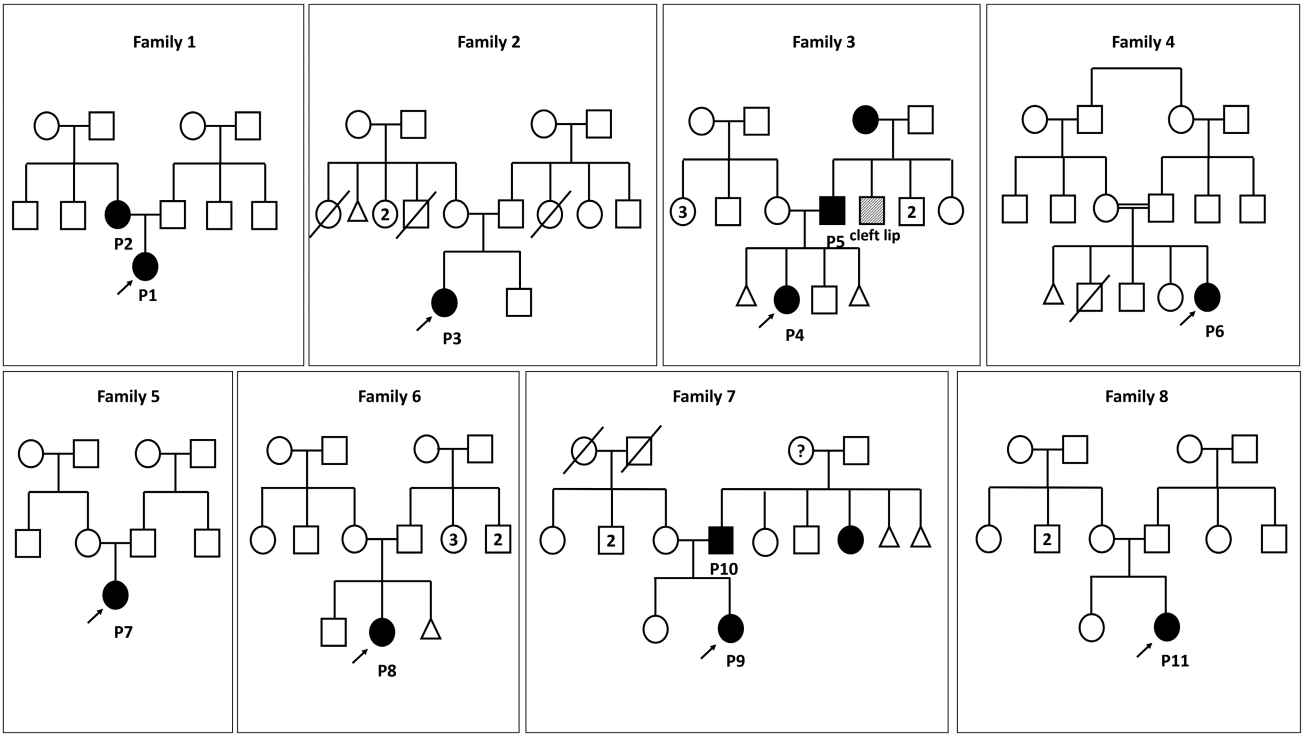


Figure 1. Pedigrees showing the probands and clinically affected individuals within the families. Squares represent males, circles represent females, filled symbols denote clinically affected individuals, triangles indicate spontaneous abortions, and arrows mark the probands. Patient numbers are provided below the respective symbols. Numbers within symbols indicate the count of healthy individuals of each gender. The question mark in the paternal grandmother of P9 denotes she was considered affected based on clinical history.

mother and sister of P10 were found to display a classic female CFNS phenotype with severe hypertelorism, facial asymmetry, and orbital dystopia identified through facial photographs, but they were not available for clinical examination or molecular testing. Clinical features are summarized in Table 1 and elaborated in the Supplementary material.

Molecular Findings

We identified 6 germline *EFNB1* [NM_004429.5] variants in 7 families: c.325C>T, p.(Arg109Cys) in Patients 1 and 2; c.496C>T, p.(Gln166*) in P3; c.196C>T, p.(Arg66*) in Patients 4, 5, and 7; c.406+4A>G, p.(?) in Patient 6; c.406+1G>A, p.(?) in Patients 9 and 10; and c.217_231delinsCCGAGCAGAAG, p.(Tyr73Profs*84) in P11 (Table 2). We failed to detect disease-causing variants in P8. Our molecular results confirmed maternal inheritance in Family 1, paternal inheritance in Families 3 and 7, and indicated *de novo* occurrence in the remaining 4 families (Figure 1).

Clinical Features of Affected Females

Antenatal and Natal Findings

The antenatal history of 1 patient was complicated with oligohydramnios, and another by maternal hypertension treated with methyldopa. The average gestational age at birth was 39.3 weeks: 6 patients were born at term, and 1 at 36 weeks of gestation. One patient was born large for gestational age, and the rest had birth weights appropriate for gestational age. The average birth weight was 3079 g (range: 2.2-4.25 kg), with a mean SD value of −0.26 ± 0.92. Length and occipitofrontal circumference (OFC) measurements at birth were within normal limits for the 2 patients whose data were available. Two patients required short-term neonatal intensive care unit (NICU) care for transient respiratory issues.

Growth

Growth data were unavailable for Patients 2 and 8. In the remaining patients, at the last evaluation at an average of 49.1 months, mean ± SD values for weight, length/height, and OFC according to national growth charts were 0.07 ± 0.66 (range −1.3 to 1.0), 0.03 ± 0.53 (range −1.9 to 1.2), and −1.77 ± 0.90 (range −4.2 to −0.3), respectively.[15] One patient was overweight, another was underweight, and the rest had body mass index (BMI) values within the healthy range. Length and height values were within the normal range for all patients. No patient had an OFC measurement above the mean; only 2 displayed microcephaly.

Craniofacial Features

Frontonasal dysplasia features of wide nasal bridge, hypertelorism, and nasal tip anomalies (bifid or broad nasal tip and columellar indentation) were observed in all affected females. Other consistent features were facial asymmetry (n = 7/9), coronal craniosynostosis (n = 6/9), frontal bossing (n = 6/9), brachycephaly (n = 6/9), high palate (n = 6/9), orbital dystopia (n = 5/9), strabismus (n = 5/7), and ptosis (n = 5/9). Additional findings included cranium bifidum (n = 4/9), widow's peak (n = 4/9), upslanted palpebral fissures (n = 4/9), downslanted palpebral fissures (n = 3/9), low anterior hairline (n = 3/9), low-set ears (n = 3/9), dry, woolly hair (n = 3/9), and epicanthal folds (n = 2/9) (Figure 2). Capillary hemangioma of the facial midline, medially absent eyelashes on the left eyelid, maxillary

Table 1. Detailed Clinical Features Observed in the 2 Male and 9 Female Patients of this Cohort

Index Patient	**Patient 1**	**Patient 2**	**Patient 3**	**Patient 4**	**Patient 5**	**Patient 6**	**Patient 7**	**Patient 8**	**Patient 9**	**Patient 10**	**Patient 11**
Gender	Female	Female	Female	Female	Male	Female	Female	Female	Female	Male	Female
Clinical diagnosis	Classical CFNS	Mild CFNS	Classical CFNS	Classical CFNS	Male CFNS	Mild CFNS	Classical CFNS	Classical CFNS	Classical CFNS	Male CFNS	Classical CFNS
EFNB1 variant	c.325C>T		c.496C>T	c.196C>T		c.406+4A>G	c.196C>T	–	c.406+1G>A		c.217_231delins CCGAGCAGAAG
	p.(Arg109Cys)		p.(Gln166*)	p.(Arg66*)		p.(Arg66*)	p.(?)		p.(Tyr73Profs*84)	Affected family member	Mother (P2)
Daughter (P1)	–	Father (P5)	Daughter (P4)	–	–	–	Father (P10)	Daughter (P9)	–	Birth parameters	GW at birth
40		40	38		36	42		38		41	Weight, g (SDS)
3050 (−0.7)		3070 (−0.7)	2600 (−1.3)		2200 (−1.0)	3780 (1.0)		2600 (−1.3)		4250 (2.2)	Birth length, cm (SDS)
					45 (−1.0)					54 (2.3)	OFC, cm (SDS)
					31.5 (−1.0)					36.5 (1.6)	Growth parameters
Age at last evaluation	2.5 mos	32 yrs	4 yrs 7 mos	7 yrs 1 mo	41 yrs	5 yrs	6 days	15 yrs	10 yrs	38 yrs	21 mos
Weight, kg (SDS)	5.7 (0.4)		16.3 (−0.5)	19 (−1.3)		21 (0.9)	3.91 (1.0)		27 (−0.9)		13 (0.9)
BMI (SDS)	15.8 (−0.1)		14.8 (−0.5)	12.6 (−2.4)		18.3 (1.6)	15.6 (1.4)		15.7 (−0.6)		16.1 (−0.6)
Length/height, cm (SDS)	60 (0.7)		105 (−0.3)	123 (0.3)		107 (−0.5)	50 (−0.1)		131 (−1.1)		90 (1.2)
OFC, cm (SDS)	39 (−0.3)		46.5 (−2.7)	49.6 (−1.3)		48 (−1.8)	34 (−0.8)		46.5 (−4.2)		46 (−1.3)
Craniofacial findings											
Coronal craniosynostosis	+	–	+	+	–	–	+	+	+, operated	–	+, operated
Cranium bifidum	+	–	–	–	–	–	+	–	+	–	+
Frontal bossing	+	–	+	+	–	–	–	+	+	–	+
Dry, woolly hair	–	–	–	–	–	–	–	+	+	–	+
Low anterior hairline	–	–	–	–	–	–	+	–	+	–	+
Widow's peak	–	+	+	–	–	–	–	–	+	+	+
Facial asymmetry	+	–	+	+	–	–	+	+	+	–	+
Orbital dystopia	–	–	+	+	–	–	–	+	+	–	+
Strabismus	–	–	+	+	–	–	–	+	+	–	+
Hypertelorism	+	+	+	+	+	+	+	+	+	+	+
Ptosis	–	–	+	+	–	–	–	+	+	–	+
Palpebral fissure slant	N	N	Up	Up	N	Up	N	L up, R down	Down	Down	Down
Epicanthal folds	–	–	–	–	–	–	–	–	+	–	+
Wide nasal bridge	+	+	+	+	+	+	+	+	+	–	+
Broad or bifid nasal tip	+	ci	+	+	–	ci	+	+	+	ci	+
Maxillary hypoplasia	–	–	–	+	–	–	–	–	–	–	–
High palate	+	+	+	+	–	–	–	+	+	+	–
Low-set ears	–	–	–	+	–	–	+	–	+	–	–
Brachycephaly	–	–	+	+	–	–	+	+	+	–	+

(*Continued*)

Table 1. Detailed Clinical Features Observed in the 2 Male and 9 Female Patients of this Cohort (Continued)

Index Patient	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11
Skeletal deformities											
Short neck	–	–	+	+	–	–	–	+	+	–	+
Sloping or narrow shoulders	–	–	+	+	–	–	–	+	+	–	+
Sprengel deformity	–	–	+	–	–	–	–	+	–	–	–
Limited shoulder abduction	na	–	+	–	–	–	na	+	–	–	–
Limb anomalies											
Broad thumb or hallux	–	–	+	–	–	–	+	+	–	–	+
Polydactyly	–	–	uh, mesoaxial	–	–	–	–	–	uf, postaxial	–	–
Cutaneous syndactyly	–	–	+	–	–	–	–	–	–	–	–
Longitudinal nail grooves	–	+	–	–	–	–	+	+	+	–	+
Neurological features and development											
Cranial MRI				N		N			CCH		pCCA, ECM
Motor delays	na		–	Borderline		–	na		–		–
Sitting (mos)	na		7	9		7	na		8		7
Walking (mos)	na		15	18		16	na		14		12
Speech delays	na		–	Mild		–	na		Moderate		–
First words (mos)	na		13	24		10	na		27		14
First 2-word sentences	na		22	31		18	na		48		
Intellectual disability	na	–	–	–	–	–	na	–	Mild	–	na
Other											
Low-set nipples	–	–	–	–	–	–	–	–	+	+	+
Unilateral breast hypoplasia	–	–	+	–	–	–	–	+	–	–	–
NL duct obstruction	–	–	+	–	–	–	–	–	–	–	+
Other			Anterior ectopic anus	Maxillary hypoplasia, generalized joint hyper-extensibility, prominent finger pads, hypoplastic labia minora	Metopic groove, absence of mustache hair on the midline	Midline facial hemangioma		Medially absent eyelashes of L eyelid, midline notching of upper lip, thoracic kyphosis	Hyperextensibility of the PIP joints, anomalous positioning of toes, echogenic small R kidney	Columellar skin pit, long smooth philtrum, thin upper lip	Accessory upper labial frenula, pectus deformity, scoliosis

BMI, body mass index; ci, columellar indentation; CCH, corpus callosum hypoplasia; CFNS, craniofrontonasal syndrome; ECM, enlarged cisterna magna; down, downslanting; EFNB1, Ephrin B1; GW, gestational week; L, left; mos, months; N, normal; na, not applicable; NL, nasolacrimal; OFC, occipitofrontal circumference; pCCA, partial agenesis of the corpus callosum; PIP, proximal interphalangeal; SDS, standard deviations; blank, not available; +, present; –, absent; R, right; uf, unilateral foot; uh, unilateral hand; up, upslanting; yrs, years.

Table 2. Detailed Description and Classification of EFNB1 Variants Identified in the Cohort

Family	Patient	Nucleotide Change	Mutation Status	Segregation	Genomic Position (hg38)	Location	Mutation Type	Protein Change	ACMG/AMP Evidence	Previously Reported in	Classification
F1	P1	c.325C>T	het	Familial	chrX-68838813	exon 2	Missense	p.(Arg109Cys)	PS4_mod, PM2, PP2, PP3	PMIDs: 18627045, 27884935	LP
	P2		het								
F2	P3	c.496C>T	het	*de novo*	chrX-68839753	exon 3	Nonsense	p.(Gln166*)	PVS1, PS4_mod, PM2, PM6	PMID: 23335590, 16685650	P
F3	P4	c.196C>T	het	familial	chrX-68838684	exon 2	Nonsense	p.(Arg66*)	PVS1, PS2, PS4_mod, PM2	PMID: 26586496, 34602953, 16685650, 15959873	P
	P5		hem								
F4	P6	c.406+4A>G	het	*de novo*	chrX-68838898	intron 2	Splicing	p.(?)	PM6_sup, PM2, PP3	ClinVar Accession: VCV002500875.1	VUS
F5	P7	c.196C>T	het	*de novo*	chrX-68838684	exon 2	Nonsense	p.(Arg66*)	PVS1, PS2, PS4_mod, PM2	PMID: 26586496, 34602953, 16685650, 15959873	P
F7	P9	c.406+1G>A	het	familial	chrX-68838895	intron 2	Splicing	p.(?)	PVS1, PS4_sup, PM2	PMIDs: 15166289	P
	P10		hem								
F8	P11	c.217_231delins CCGAGCAGAAG	het	*de novo*	chrX-68838705_68838719	exon 2	Frameshift	p.(Tyr73Profs*84)	PVS1, PM2, PM6	no	P

F, family; hem, hemizygous; het, heterozygous; LP, likely pathogenic; P, patient; P, pathogenic.

hypoplasia, midline notching of upper lip, and accessory upper labial frenula were each observed once.

Skeletal and Limb Anomalies

Polysyndactyly was noted in 2 patients: mesoaxial synpolydactyly of the right hand in one and postaxial polydactyly of the left foot in the other (Figure 2). Additional features included short neck (n = 5/9), sloping or narrow shoulders (n = 5/9), longitudinal nail grooves (n = 5/9), broad thumbs or halluces (n = 4/9), unilateral Sprengel deformity (n = 2/9), and limited shoulder abduction (n = 2/7). Pectus deformity, thoracic kyphosis, scoliosis, generalized joint hyperextensibility, hyperextensibility of the distal interphalangeal joints, prominent finger pads, and anomalous positioning of toes were each observed once.

Developmental Features and Brain Imaging

Developmental milestones were recorded in 5 patients aged 21 months-10 years. Seven patients were old enough for the evaluation of intellectual performance, aged 55 months to 32 years. One patient had a borderline delay in the acquisition of early motor milestones, and 2 had a history of language delay. P9 demonstrated mild intellectual disability (ID). Brain MRI was performed on 4 patients, identifying corpus callosum hypoplasia in P9 with ID, and partial corpus callosum agenesis with enlarged cisterna magna in P11 with normal development.

Additional Systemic Findings

Nasolacrimal duct obstruction, unilateral breast hypoplasia, and low-set nipples were each observed in 2 patients (n = 2/9). A small, echogenic right kidney with decreased concentration function, hypoplastic labia minora, and anterior ectopic anus were each observed in one patient.

DISCUSSION

Here, we describe 9 female and 2 male patients from 8 new CFNS families. One female patient was negative for *EFNB1* variants, and the molecular diagnosis was confirmed in the remaining patients, comprising 1 novel and 5 previously reported *EFNB1* variants.

Molecular Characteristics and EFNB1 Variant Classification

All variants we identified in the present cohort were clustered in the genomic region encompassing exons 2-3, which encode the Eph binding domain, in line with the literature. Four of these were previously reported in CFNS patients, consisting of 2 stop-gain variants, a missense variant, and a canonical splice-site variant.[3,4,13] All 4 are classified as pathogenic or likely pathogenic according to the ACMG/AMP 2015 criteria (Table 2).

We report a novel out-of-frame indel variant c.217_231delins CCGAGCAGAAG in P11 with classical CFNS, which is classified as pathogenic due to one very strong and 2 moderate pieces of evidence: PVS1 (null variant in a gene where loss of function is the disease mechanism), PM2 (not reported in population databases), and PM6 (*de novo* in a patient with phenotype consistency, no family history, and parentage is assumed).

The c.406+4A>G variant was identified in P6, who has findings consistent with mild CFNS. It was submitted to ClinVar (VCV002500875.1) as a variant of uncertain significance (VUS) in an affected individual reported to have callosal agenesis



Figure 2. Clinical images of affected individuals. (A) P1 (at 2.5 months of age), and her mildly affected mother (P2) are shown. Note the facial dysmorphic features of P1 including mild facial asymmetry, frontal bossing, hypertelorism, wide and depressed nasal bridge, and broad nasal tip. The mother displays hypertelorism, a wide nasal bridge, and columellar indentation. (B,C) The images of P3 at 4 years and 7 months of age demonstrate marked facial asymmetry with downward displacement of the right orbit, a widow's peak, frontal bossing, hypertelorism, bilateral ptosis (more pronounced on the right), upslanted palpebral fissures, a wide and depressed nasal bridge, a bifid nasal tip, anteverted nares, and a wide columella. (D) P4 exhibits marked facial asymmetry with downward positioning of the right orbit, frontal bossing, severe hypertelorism, exotropia, maxillary hypoplasia, a wide nasal bridge, a broad and bifid nasal tip, underdeveloped nares, a thin upper lip vermilion, and low-set, prominent ears. (E) P4's mildly affected father (P5) displays a metopic groove, a wide nasal bridge, mild hypertelorism, and an absence of mustache growth along the midline. (F) P6 at infancy displays hypertelorism and a capillary hemangioma on the glabella. At 5 years of age (G), she exhibits long palpebral fissures, hypertelorism, a wide nasal bridge, and columellar indentation. (H) P8 at 15 years of age exhibits facial asymmetry with downward displacement of the right orbit, woolly hair, frontal bossing, underdeveloped supraorbital ridges, medial flaring of the eyebrows, and absence of eyelashes on the medial part of the left eyelid, marked hypertelorism, upslanted left palpebral fissure, downslanted right palpebral fissure, wide and depressed nasal bridge, broad and bifid nasal tip, thick lip vermilions, and midline notching of the upper lip. (I) P11 at 21 months of age presents facial asymmetry, woolly hair, widow's peak, tall and prominent forehead, hypertelorism, bilateral ptosis, left epicanthus, wide and depressed nasal bridge, and bifid nasal tip. (J) Longitudinal grooving of the fingernails of P2. (K) Broad thumb, mesoaxial polydactyly with 6 fingers, and total cutaneous syndactyly affecting the third, fourth, and fifth fingers of P3.

and developmental delay, but we refrained from using the PS4 evidence because the reported phenotype only partially overlaps with CFNS.[19] Parental studies indicated de novo occurrence for P6, who had a consistent but not highly specific CFNS phenotype, and her parentage was not molecularly confirmed (PM6_sup). As a result, the c.406+4A>G variant still classifies as a VUS, even after the application of PM2 and PP3 (multiple lines of pathogenic in silico evidence).

P8 had no *EFNB1* variants but was included in the cohort since she had a classical CFNS phenotype with very specific facial and systemic features. A normal constitutional karyotype ruled out potential large rearrangements causing regulatory *EFNB1* disturbances as a cause of CFNS in P8.

Clinical Characteristics of Affected Females

Previous cohorts have described the constant features of the classic CFNS phenotype as hypertelorism and longitudinal nail ridging/splitting.[6,20] We observed nail abnormalities in only 5 of 9 female patients, diverging from the literature data. Remarkable hypertelorism was present in all, in agreement with the previous reports. Other universal findings were a wide nasal bridge and a spectrum of nasal tip abnormalities ranging from a wide/bifid nasal tip to mild columellar indentation. Coronal craniosynostosis was present in only 5 affected females, 2 of whom had undergone surgical treatment for craniosynostosis at 6 and 8 months of age, respectively. While most of the characteristic craniofacial features of CFNS were observed only in a subset of the cohort (Table 1), the majority of female probands could be diagnosed using the gestalt diagnostic approach. Patients 6 and 2 represent exceptions, as they presented with an unusually mild phenotype for female CFNS.

A comprehensive review of 115 females and 18 males with CFNS showed that 98% of clinically diagnosed females and 17/18 males had normal intelligence.[9] Another study suggested developmental delays in at least 12% of 59 patients, without specification of gender distribution and degree of delay.[3] In our cohort, mean ages for key developmental milestones in affected females were: unsupported sitting at 7.6 months, walking at 15 months, speaking first words at 17.6 months, and building 2-word sentences at 29.8 months. P4 had normal intellectual performance at 7 years of age, despite having a history of borderline motor and mild language delays. Only 1 patient demonstrated ID.

Two affected females had corpus callosum anomalies, a finding reported with 7%-12% prevalence in previous CFNS cohorts.[4,9] The presence of ID was not correlated with corpus callosum abnormalities. Enlarged cisterna magna in P11 was previously unreported as part of the CFNS spectrum.

Various anogenital malformations and urinary tract abnormalities have been reported among individuals with CFNS, including imperforate anus, hypospadias, septate vagina, congenital uterine anomalies, vesicoureteral reflux, and duplex, fused, or pelvic kidneys.[21-23] To the best of our knowledge, anterior ectopic anus, hypoplastic labia minora, and small echogenic kidney reported in 1 patient each in our cohort have not been previously reported. Skeletal features in our cohort generally align

with existing literature.[6] Notably, P3 displayed mesoaxial polydactyly of the right hand, which was reported only once as part of the CFNS spectrum.[24]

Characteristics of 2 Female EFNB1 Heterozygotes with Mild Presentations

P2 had a mild facial phenotype without craniosynostosis or notable asymmetry, almost comparable to a hemizygous male. A clinical diagnosis of CFNS was made due to the presence of longitudinal nail ridging and because her daughter (P1) displayed a more pronounced CFNS phenotype with craniosynostosis. P6 also lacked a classical CFNS phenotype, and her preliminary diagnosis at initial clinical evaluation was mild FND due to the presence of hypertelorism, a wide nasal bridge, and columellar indentation. *EFNB1* sequencing was only performed to pursue exclusion diagnosis.

Mild clinical features in heterozygous females with *EFNB1* variants are quite rare, with only 2 descriptions in the literature. The first is a female with minimal clinical manifestations and heterozygous p.(Pro54Leu), who had constitutional levels of mutation in blood but lower levels (~20%) in hair roots.[3] The initial assumption that this mild CFNS phenotype was due to low mosaicism levels was discarded by evidence from females and males displaying classical CFNS despite similar levels of mosaicism. Another hypothesis that skewed X chromosome inactivation (XCI) may account for a milder phenotype was contrasted by evidence from another mildly affected female with almost no CNFS features and heterozygous p.(Glu210*), displaying almost random XCI in blood.[21] Her monozygotic twin sister with skewed XCI had classical CFNS. The weak correlation between mosaicism levels and XCI status reported in these 2 patients from the literature may indicate variability of these factors across unsampled tissues or the presence of unknown modifying factors.

We initially speculated that leaky splicing from the *EFNB1* c.406+4A>G variant might explain the mild phenotype in P6, as co-expression of wild-type and misspliced transcripts could reduce cellular interference. However, similar mild phenotypes in females with missense or nonsense variants in *EFNB1* suggest this is unlikely. Unfortunately, we could not assess XCI status in our 2 mildly affected patients.

Characteristics of 2 Male Patients

While the female CFNS phenotype has been well delineated, clinical findings in hemizygous males have not been thoroughly documented. Two male patients of this cohort, as naturally expected, were diagnosed after their daughters due to the presence of mild hypertelorism and a wide nasal bridge. Additional findings included widow's peak, metopic groove, columellar indentation, columellar skin pit, absence of moustache hair on the midline, and low-set nipples, further expanding the clinical spectrum associated with hemizygous *EFNB1* variants.

Craniosynostosis was assessed based on craniofacial shape abnormalities and the presence of a coronal groove, and 3D-computed tomography was not performed in the majority of cases. Our molecular methodology did not allow for the assessment of mosaicism or XCI status, and the effect of the consensus splicing variant on RNA could not be evaluated through functional studies. Lastly, as exome sequencing did not exclude combined phenotypes in patients with uncommon features, further studies are needed to determine whether these features should be considered part of the CFNS phenotype.

CONCLUSION

To the best of our knowledge, our study represents the largest CFNS cohort from Türkiye with 9 affected females and 2 males. It contributes to the genetic landscape of CFNS with a novel *EFNB1* variant, expands the female CFNS phenotype with findings such as enlarged cisterna magna, nasolacrimal duct obstruction, small echogenic kidney, hypoplastic labia minora, and anterior ectopic anus, while reinforcing mesoaxial polydactyly as a CFNS feature. Two male patients in our cohort displayed previously unreported features, including metopic groove, columellar skin pit, absence of midline mustache hair growth, and low-set nipples. Notably, 2 mildly affected female patients of this cohort represent further examples of the rarely reported, mild CFNS phenotype in females, emphasizing the importance of recognizing atypical presentations for timely diagnosis and optimal management. Lastly, phenotypic differences observed in a mother–daughter pair provide additional evidence of intrafamilial variability in CFNS.

Availability of Data and Materials: The data that support the findings of this study are available on request from the corresponding author.

Ethics Committee Approval: This study was approved by the institutional review board of İstanbul University (approval number: 2029; date: June 30, 2009).

Informed Consent: Written informed consent was obtained from the subjects or their legal guardians who agreed to take part in the study.

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Author Contributions: Concept – U.A., H.K.; Design – U.A., H.K.; Supervision – Z.O.U., H.K.; Resources – U.A., H.K.; Materials – U.A., Y.A., F.P., H.K.; Data Collection and/or Processing – U.A., Y.A., F.P., Z.O.U., H.K.; Analysis and/or Interpretation – U.A., Z.O.U., H.K.; Literature Search – U.A.; Writing – U.A.; Critical Review – H.K.

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REFERENCES

1. Wu E, Vargevik K, Slavotinek AM. Subtypes of frontonasal dysplasia are useful in determining clinical prognosis. Am J Med Genet A. 2007;143A(24):3069-3078. [CrossRef]

2. Farlie PG, Baker NL, Yap P, Tan TY. Frontonasal dysplasia: towards an understanding of molecular and developmental aetiology. Mol Syndromol. 2016;7(6):312-321. [CrossRef]
3. Twigg SR, Matsumoto K, Kidd AM, et al. The origin of EFNB1 mutations in craniofrontonasal syndrome: frequent somatic mosaicism and explanation of the paucity of carrier males. Am J Hum Genet. 2006;78(6):999-1010. [CrossRef]
4. Twigg SR, Kan R, Babbs C, et al. Mutations of ephrin-B1 (EFNB1), a marker of tissue boundary formation, cause craniofrontonasal syndrome. Proc Natl Acad Sci U S A. 2004;101(23):8652-8657. [CrossRef]
5. Wieland I, Jakubiczka S, Muschke P, et al. Mutations of the ephrin-B1 gene cause craniofrontonasal syndrome. Am J Hum Genet. 2004;74(6):1209-1215. [CrossRef]
6. van den Elzen ME, Twigg SR, Goos JA, et al. Phenotypes of craniofrontonasal syndrome in patients with a pathogenic mutation in EFNB1. Eur J Hum Genet. 2014;22(8):995-1001. [CrossRef]
7. Feldman GJ, Ward DE, Lajeunie-Renier E, et al. A novel phenotypic pattern in X-linked inheritance: craniofrontonasal syndrome maps to Xp22. Hum Mol Genet. 1997;6(11):1937-1941. [CrossRef]
8. Twigg SR, Babbs C, van den Elzen ME, et al. Cellular interference in craniofrontonasal syndrome: males mosaic for mutations in the X-linked EFNB1 gene are more severely affected than true hemizygotes. Hum Mol Genet. 2013;22(8):1654-1662. [CrossRef]
9. Wieacker P, Wieland I. Clinical and genetic aspects of craniofrontonasal syndrome: towards resolving a genetic paradox. Mol Genet Metab. 2005;86(1-2):110-116. [CrossRef]
10. Stenson PD, Mort M, Ball EV, et al. The Human Gene Mutation Database (HGMD((R))): optimizing its use in a clinical diagnostic or research setting. Hum Genet. 2020;139(10):1197-1207. [CrossRef]
11. Wieland I, Makarov R, Reardon W, et al. Dissecting the molecular mechanisms in craniofrontonasal syndrome: differential mRNA expression of mutant EFNB1 and the cellular mosaic. Eur J Hum Genet. 2008;16(2):184-191. [CrossRef]
12. Wieland I, Reardon W, Jakubiczka S, et al. Twenty-six novel EFNB1 mutations in familial and sporadic craniofrontonasal syndrome (CFNS). Hum Mutat. 2005;26(2):113-118. [CrossRef]
13. Wallis D, Lacbawan F, Jain M, et al. Additional EFNB1 mutations in craniofrontonasal syndrome. Am J Med Genet A. 2008; 146A(15):2008-2012. [CrossRef]
14. Kakadia PM, Fritz B, Bohlander SK. Craniofrontonasal syndrome in a patient with an inv(X)(p22.2q13.1), separating EFNB1 from its enhancer. Eur J Hum Genet. Published online December 7, 2024. 2024. [CrossRef]
15. Neyzi O, Bundak R, Gökçay G, et al. Reference values for weight, height, head circumference, and body mass index in Turkish children. J Clin Res Pediatr Endocrinol. 2015;7(4):280-293. [CrossRef]
16. de Sainte Agathe JM, Filser M, Isidor B, et al. SpliceAI-visual: a free online tool to improve SpliceAI splicing variant interpretation. Hum Genomics. 2023;17(1):7. [CrossRef]
17. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015;17(5):405-424. [CrossRef]
18. Durkie M, Cassidy E-J, Berry I, et al. ACGS best practice guidelines for variant classification in rare disease. 2024. Available at: https://www.acgs.uk.com/media/12533/uk-practice-guidelines-for-variant-classification-v12-2024.pdf. Accessed December 18, 2024.
19. National Center for Biotechnology Information. ClinVar; [VCV002500875.1]. Available at: https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV002500875.1. Accessed December 23, 2024.
20. Farkas LG, Munro IR. Anthropometric Facial Proportions in Medicine. Springfield, IL: Charles C Thomas; 1987.
21. Bukowska-Olech E, Gawliński P, Jakubiuk-Tomaszuk A, et al. Clinical and molecular characterization of craniofrontonasal syndrome: new symptoms and novel pathogenic variants in the EFNB1 gene. Orphanet J Rare Dis. 2021;16(1):286. [CrossRef]
22. Kawamoto HK, Heller JB, Heller MM, et al. Craniofrontonasal dysplasia: a surgical treatment algorithm. Plast Reconstr Surg. 2007;120(7):1943-1956. [CrossRef]
23. Acosta-Fernández E, Zenteno JC, Chacón-Camacho OF, et al. Extracranial midline defects in a patient with craniofrontonasal syndrome with a novel EFNB1 mutation. Am J Med Genet A. 2020;182(5):1223-1229. [CrossRef]
24. Gürsoy S, Hazan F, Öztürk T, Çolak R, Çalkavur Ş. Evaluation of sporadic and familial cases with craniofrontonasal syndrome: a wide clinical spectrum and identification of a novel EFNB1 gene mutation. Mol Syndromol. 2021;12(5):269-278. [CrossRef]

SUPPLEMENTAL FILE WITH AN EXPANDED DESCRIPTION OF CLINICAL REPORTS

FAMILY 1

Patient 1

Patient 1 was a 2.5-month-old female, the only child of apparently healthy, unrelated parents (G1P1). Her mother (Patient 2) was found to be mildly affected with subtle CFNS features. Following an uncomplicated pregnancy, Patient 1 was born via spontaneous vaginal delivery (SVD) at the 40th gestational week (GW) with a birth weight (BW) of 3050 g (−0.74 SDS). At 2 months of age, she had a weight of 5700 g (0.40 SDS), a length of 60 cm (0.73 SDS), and OFC of 39 cm (−0.29 SDS). On palpation, she had a large bony defect extending from above the brow to a wide anterior fontanel measuring 5 cm × 5 cm, consistent with cranium bifidum. She displayed bilateral coronal craniosynostosis, mild facial asymmetry, frontal bossing, hypertelorism, wide and depressed nasal bridge, a broad nasal tip, and a high palate. Neuromotor development was age-appropriate, with head control being achieved at 2 months of age. Cranial and abdominal ultrasounds showed normal results.

Patient 2

Patient 2 is the mother of Patient 1. History and anthropometric measurements are not available. She had dysmorphic craniofacial features comprising widow's peak, hypertelorism, a wide nasal bridge, columellar indentation, and a high palate. She had longitudinal grooving of the fingernails, with otherwise normal systemic examination findings. Mental performance was normal.

FAMILY 2

Patient 3

Patient 3 was a 3.5-month-old female, firstborn to apparently healthy, unrelated parents (G2P2). Family history was non-contributory. Following an uncomplicated pregnancy, she was born via caesarean section at the 40th gestational week, with a weight of 3070 g (−0.68 SD). She experienced no neonatal adaptation problems, but polysyndactyly of the right hand was noted at the first examination. At 3.5 months of age, her weight was 5600 g (−0.60 SDS), length 61 cm (0.04 SDS), and OFC 37 cm (−2.75 SDS). The size of the anterior fontanel was 0.5 cm × 0.5 cm, and she had bilateral coronal craniosynostosis. Physical examination was remarkable for marked facial asymmetry with downward positioning of the right orbit, widow's peak, frontal bossing, hypertelorism, bilateral ptosis more prominent on the right, upslanted palpebral fissures, wide and depressed nasal bridge, bifid nasal tip, anteverted nares, wide columella, and a high palate. She had broad thumbs, right-sided mesoaxial polydactyly with 6 fingers with the fourth and extra digit having 2 phalanges and a well-developed nail structure, total cutaneous syndactyly involving the third, fourth, and fifth fingers, and secondary camptodactyly of the third finger. Additional findings included short neck, left-sided breast hypoplasia with inverted nipple, limited right shoulder abduction with Sprengel's deformity, bilateral plantar grooves of the feet, and an anterior ectopic anus. Transfontanel, abdominal, and renal ultrasonography revealed normal results. Ophthalmological examination with fundoscopy showed exotropia and left nasolacrimal duct obstruction. At 4 years and 7 months of age, weight was 16.3 kg (−0.50 SDS), height 105 cm (−0.32 SDS), BMI 14.8 (−0.46 SDS), and OFC 46.5 cm (−2.7 SDS). Developmental milestones were acquired on time, with unsupported sitting at 7 months, unsupported walking at 15 months, speaking single words at 13 months and first sentences at 22 months, and achievement of sphincter control at 2 years 7 months. Mental performance was age appropriate.

FAMILY 3

Patient 4

Patient 4 was a female aged 7 years and 1 month, the first child of second-degree cousins (G4P2A2). The apparently healthy mother had a history of 2 spontaneous abortions in the early second trimester. The patient's father (Patient 5) was noted to have hypertelorism and a wide nasal bridge, suggestive for the male CFNS phenotype. Her paternal uncle had a history of cleft lip. Facial photographs of her paternal grandmother demonstrated facial asymmetry with severe hypertelorism, but she was not available for clinical examination. After an uneventful pregnancy, she was born via SVD at the 38th gestational week, with a weight of 2600 g (−1.27 SD). No neonatal adaptation problems were reported. Language and developmental milestones were mildly delayed: she sat without support at 9 months, walked without support at 18 months, spoke first words at 24 months and 2-word sentences at 2 years and 7 months, and achieved sphincter control at 3.5 years of age. She had normal mental performance. At 7 years and 1 month of age, her weight was 19 kg (−1.28 SDS), height 123 cm (0.29 SDS), and OFC 49.6 cm (−1.27 SDS). She had bilateral asymmetric coronal craniosynostosis, facial asymmetry with downward positioning of the right orbit, frontal bossing, brachycephaly, severe hypertelorism, exotropia, maxillary hypoplasia, wide nasal bridge, broad and bifid nasal tip, underdeveloped nares, broad columella, thin upper lip vermilion, high palate, and low-set, prominent ears. Other notable findings were a short neck, hyperextensibility of the elbow, wrist, distal interphalangeal and proximal interphalangeal joints, persistent fetal finger pads, and hypoplastic labia minora. Cranial MRI showed normal brain structures.

Patient 5

Patient 5 is the father of Patient 4. History and anthropometric measurements are not available. He had dysmorphic craniofacial features comprising a metopic groove, wide nasal bridge, mild hypertelorism, and absence of moustache hair on the midline. Systemic examination was otherwise normal, and he had normal intelligence.

FAMILY 4

Patient 6

Patient 6 was a 5-year-old female, the fourth child of apparently healthy first-degree cousins (G5P4A1). The mother had a history of a spontaneous abortion at the 10th gestational week and a male child stillborn at 6 months of gestation. Following a pregnancy complicated with oligohydramnios, Patient 4 was born via caesarean section at the 36th gestational week with first and fifth minute APGAR scores of 9 and 10. At birth, her weight was 2200 g (–1.05 SDS), length 45 cm (–1 SDS), and OFC 31.5 cm (–1.01 SDS). A capillary hemangioma on the glabella was noted, which disappeared completely over the years. At 12 days of age, she was interned in the level II NICU for 5 days due to an apneic seizure. Work-up including electroencephalogram, cranial ultrasound, abdominal ultrasound, echocardiogram, and ophthalmological consultation revealed normal findings. She did not require the use of antiepileptic drugs, and the seizures did not recur. Developmental milestones were achieved on time: she sat unsupported at 7 months, walked unsupported at 16 months, spoke first words at 10 months, and built 2-word sentences at 18 months of age. At 5 years of age, her weight was 21 kg (0.93 SDS), height 107 cm (–0.46 SDS), and OFC 48 cm (–1.84 SDS). She demonstrated long palpebral fissures and mild FND findings comprising hypertelorism, a wide nasal bridge, and columellar indentation. Systemic examination was otherwise normal. Cranial MRI showed normal brain structures, and intellectual performance was age appropriate.

FAMILY 5

Patient 7

Patient 7 was a 6-day-old female, the only child of apparently healthy, unrelated parents (G1P1). Family history was not contributory. Following an uncomplicated pregnancy, she was born via SVD at the 42nd gestational week, with a weight of 3780 g (1.04 SDS). She experienced no neonatal adaptation problems. At 6 days of age, her weight was 3910 g (1.01 SDS), length 50 cm (–0.06 SDS), and OFC 34 cm (–0.75 SDS). On examination, she had cranium bifidum extending to an enlarged anterior fontanel measuring 6 cm × 5 cm. Dysmorphic craniofacial features included facial asymmetry, low anterior hairline, prominent forehead, hypertelorism, wide nasal bridge, bifid nasal tip, wide columella, deep philtrum, thin upper lip vermilion, and low-set ears. Great toes were broad, and the systemic examination was otherwise normal.

FAMILY 6

Patient 8

Patient 8 was a 15-year-old female, the second child of apparently healthy, unrelated parents (G3P2A1). She was born at home following an unfollowed pregnancy, and anthropometric measurements at birth or on later evaluation were unavailable. Intellectual performance was normal, and she was attending a regular school with good grades. She had menarche at 12 years of age, and secondary sexual characteristics were well developed. At 15 years old, physical examination revealed coronal craniosynostosis, brachycephaly, facial asymmetry with downward displacement of the right orbit, dry, woolly hair, frontal bossing, underdeveloped supraorbital ridges, medial flaring of eyebrows, absence of eyelashes on the medial part of the left eyelid, marked hypertelorism, upslanted left palpebral fissure, downslanted right palpebral fissure, wide and depressed nasal bridge, broad and bifid nasal tip, thick lip vermilions, midline notching of upper lip, and a high and narrow palate with dental crowding. Additional abnormal findings included a short neck, sloping shoulders with Sprengel's deformity and limited abduction, unilateral breast hypoplasia, thoracic kyphosis, broad left thumb, longitudinal grooves of fingernails, and left alternating exotropia.

FAMILY 7

Patient 9

Patient 9 was a 10-year-old female, the second child of unrelated parents (G2P2). The patient's father (Patient 10) was noted to have hypertelorism along with additional mild FND findings, suggestive for the male CFNS phenotype. Her paternal aunt and paternal grandmother were observed to have remarkable CFNS findings of facial asymmetry, orbital dystopia, and bifid nose, as indicated by facial photographs and history, respectively. Both were unavailable for clinical examination. Following an uncomplicated pregnancy, she was born via SVD at the 38th gestational week, with a birth weight of 2600 g (–1.27 SDS). In the postnatal period, cranium bifidum, coronal craniosynostosis, a high palate, and postaxial polydactyly on the left foot were detected. She had undergone surgery for craniosynostosis at 6 months of age. Preoperative cranial MRI revealed hypoplasia of the corpus callosum. An abdominal ultrasound showed a small right kidney with increased parenchymal echogenicity. 99mTc-MAG3 scintigraphy showed that the right kidney had normal excretion function with decreased, heterogeneous concentration function. Follow-up with routine biochemistry showed normal renal function. She acquired motor skills on time, such as unsupported sitting at 8 months and unsupported walking at 14 months. However, she spoke her first words at 27 months and first sentences at the age of 4 years and was attending an inclusive education program due to mild learning disabilities. At 10 years of age, her weight was 27 kg (–0.87 SDS), height 131 cm (–1.06 SDS), and OFC 46.5 cm (–4.17 SDS). She had facial asymmetry with downward displacement of the right orbit. Dysmorphic facial features included brachycephaly, thick, woolly hair, low anterior hairline, widow's peak, frontal bossing, underdeveloped supraorbital ridges, marked hypertelorism, downslanted palpebral fissures, bilateral epicanthus, strabismus, wide nasal bridge, broad nasal tip, anteverted nares, long and smooth philtrum, high palate, crowded teeth, and low-set, posteriorly rotated ears. Abnormal skeletal features comprised a short neck, pectus carinatum, low-set nipples, hyperextensibility

of the proximal interphalangeal joints, clinodactyly of the fourth and fifth fingers of the right hand, longitudinal grooving of fingernails, anomalous positioning of toes, and postaxial polydactyly of the left foot.

Patient 10

Patient 10 is the father of Patient 9. History and anthropometric measurements are not available. He had dysmorphic craniofacial features comprising widow's peak, mild hypertelorism, downslanted palpebral fissures, long and smooth philtrum, a skin pit on the columella, columellar indentation, thin upper lip vermilion, and a high, narrow palate. He displayed low-set nipples, with otherwise normal systemic examination findings. He had normal intellectual performance.

FAMILY 8

Patient 11

Patient 11 was a 21-month-old female, the second child of apparently healthy, unrelated parents (G2P2). After a pregnancy complicated with hypertension, which was well-managed with methyldopa, she was born via SVD at the 41st gestational week. Birth weight was 4250 g (2.22 SDS), birth length 54 cm (2.31 SDS), and OFC 36.5 cm (1.63 SDS). She experienced transient tachypnea of the newborn, which necessitated a 2-day stay in level II NICU. At 3 months, cranial MRI showed partial agenesis of the corpus callosum and an enlarged cisterna magna, and 3-dimensional cranial CT revealed bilateral coronal craniosynostosis with cranium bifidum. At 8 months of age, she underwent corrective surgery for craniosynostosis and surgical treatment due to lacrimal duct obstruction. She had high myopia and right exotropia. Developmental skills were acquired timely: she sat without support at 7 months, spoke single words at 12 months, and walked unsupported at 14 months. At 21 months, weight was 13 kg (0.86 SDS), height was 90 cm (1.15 SDS), and OFC was 46 cm (−1.31 SDS). She had facial asymmetry, woolly hair, low anterior hairline, widow's peak, tall and prominent forehead, hypertelorism, bilateral ptosis, left epicanthus, wide and depressed nasal bridge, bifid nasal tip, and accessory upper labial frenula. Additional features were a short neck with narrow and sloping shoulders, low-set nipples, mild dextroscoliosis at the lower thoracic level, and longitudinal ridges of thumbnails.