Genetics in Medicine (2024) 26, 101126

ELSEVIER

Genetics in Medicine
An Official Journal of the ACMG

www.journals.elsevier.com/genetics-in-medicine

ARTICLE

DISP1 deficiency: Monoallelic and biallelic variants cause a spectrum of midline craniofacial malformations

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ARTICLE INFO

Article history:
Received 7 June 2023
Received in revised form
21 March 2024
Accepted 21 March 2024
Available online 24 March 2024

Keywords:
Congenital nasal piriform aperture stenosis
Holoprosencephaly
Midline defects
SHH
Solitary median maxillary central incisor

ABSTRACT

Purpose: *DISP1* encodes a transmembrane protein that regulates the secretion of the morphogen, *Sonic hedgehog,* a deficiency of which is a major cause of holoprosencephaly (HPE). This disorder covers a spectrum of brain and midline craniofacial malformations. The objective of the present study was to better delineate the clinical phenotypes associated with division transporter dispatched-1 (*DISP1*) variants.

Methods: This study was based on the identification of at least 1 pathogenic variant of the *DISP1* gene in individuals for whom detailed clinical data were available.

Results: A total of 23 *DISP1* variants were identified in heterozygous, compound heterozygous or homozygous states in 25 individuals with midline craniofacial defects. Most cases were minor forms of HPE, with craniofacial features such as orofacial cleft, solitary median maxillary central incisor, and congenital nasal pyriform aperture stenosis. These individuals had either monoallelic loss-of-function variants or biallelic missense variants in *DISP1*. In individuals with severe HPE, the *DISP1* variants were commonly found associated with a variant in another HPE-linked gene (ie, oligogenic inheritance).

The Article Publishing Charge (APC) for this article was paid by University Rennes.

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doi: https://doi.org/10.1016/j.gim.2024.101126

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Conclusion: The genetic findings we have acquired demonstrate a significant involvement of *DISP1* variants in the phenotypic spectrum of midline defects. This underlines its importance as a crucial element in the efficient secretion of *Sonic hedgehog*. We also demonstrated that the very rare solitary median maxillary central incisor and congenital nasal pyriform aperture stenosis combination is part of the DISP1-related phenotype. The present study highlights the clinical risks to be flagged up during genetic counseling after the discovery of a pathogenic *DISP1* variant.

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Introduction

Holoprosencephaly (HPE, OMIM236100) is a congenital brain malformation that results from aberrant forebrain cleavage and is characterized by incomplete or absent separation of the 2 cerebral hemispheres.[1] The disease encompasses a continuum of brain malformations associated with midline craniofacial malformations ranging from extremely severe forms of alobar HPE with severe facial abnormalities (eg, cyclopia and proboscis) to milder forms of lobar HPE with milder craniofacial malformations (eg, orofacial cleft).[2,3] The study of familial forms of HPE has allowed for the identification of microforms, which are formed with no cerebral malformations at the level of the division of the cerebral hemispheres but presenting variable degrees of anomalies along the midline of the face. These microforms are characterized by craniofacial defects: orofacial cleft (ie, cleft lip and/or palate), congenital nasal pyriform aperture stenosis (CNPAS), a solitary median maxillary central incisor (SMMCI), pituitary malformation, and hypotelorism. Although not a midline malformation, microcephaly is also 1 of the clinical features within the HPE spectrum'.[2,3] These features are typically observed in mildly affected family members carrying a sonic hedgehog (*SHH* - HGNC: 10848) variant.[2,3]

The great variety of clinical manifestations makes the molecular diagnosis particularly challenging. Because most reported pathogenic variants display incomplete penetrance and variable expressivity, genetic modifiers are now emerging as key modulators of the disease phenotype. These genetic modifiers might correspond to hypomorphic missense variants, which are often discounted as benign because they are also present in unaffected or slightly affected family members.[4,5] Most of the known pathogenic variants affect genes belonging to the SHH pathway, which has a key role in the pathogenesis of HPE.[6,7]

One of the essential components in the SHH pathway is the resistance-nodulation-division transporter dispatched-1 (DISP1, MIM607502), which deploys SHH molecules from producing cells and is absolutely required for the long-range signaling that drives the SHH gradient.[8-12] DISP1 is similar in structure to SHH receptor (patched-1 [PTCH1]) and has 12 transmembrane helices (TMs), 2 extracellular domains, a 170-residue N-terminal intracellular domain and a 300-residue C-terminal intracellular domain.[13] At the plasma membrane, SHH is first bound by DISP1 and then released into the extracellular space by the chaperone signal peptide-CUB-EGF domain-containing protein 2 (SCUBE2-MIM611747). Several recent studies have shed light on the mechanism by which DISP1 mobilizes SHH from the producing cells.[13-15] The rate of SHH export is dependent on a Na^+ gradient, which, in turn, is modulated by conserved polar residues in the TMs.[14] In line with the transporter's known biological function, *Disp1*-deficient mice exhibit defective SHH signaling and severe HPE.[12,16] The Shh protein is locked in its site of synthesis (ie, the notochord and prechordal plate), which results in the absence of brain ventralization. This suggests that haploinsufficiency or loss of function (LoF) is the disease mechanism in HPE. Accordingly, some pathogenic variants in *DISP1* (HGNC: 19711) have been identified in 4 different families.[17,18] The varying inheritance patterns and phenotypic diversity in those families indicate the need for additional genetic studies of *DISP1* role in the pathogenesis of HPE.

In this report, we present findings from the largest cohort of HPE individuals with germline *DISP1* variants segregating with anterior midline defects ($n = 25$). We further characterize the clinical spectrum of the *DISP1*-related HPE phenotype, providing insights into the possible underlying disease mechanism, and describing various modes of inheritance.

Materials and Methods

Ethics statement and participants

Thanks to a multicenter multinational matchmaking collaboration based on GeneMatcher[19] and additional calls through the French Rare developmental diseases network (AnDDI-RARE) and international networks (ERN-ITHACA: https://ern-ithaca.eu), we were able to collect biological and clinical data from 20 families.

Inclusion in the study was based on the identification of at least 1 putative pathogenic *DISP1* variant (Table 1). For 11 of the 20 families, the variant was identified by the Molecular Genetics and Genomics Laboratory at Rennes University Medical Center (Rennes, France). Referring clinicians were sent a questionnaire to collect relevant information on their patient's personal and family medical

Table 1 In silico prediction and population allele frequencies of rare *HPE-gene* variants identified in this cohort

Genomic Variant (GRCh37)	cDNA Variant	Protein Variant	Individual	Allele Frequency (gnomAD Database)[a]	Prediction Scores				
					Grantham Distance[b]	SIFT[c]	PhyloP[d]	CADD[e]	VEST4[f]
DISP1 variants (NM_032890.3 ENST00000284476.7; NP_116279.2)									
NC_000001.10:g.223116414del	c.249del	p.(Pro84Leufs*8)	F1-A	0	NA	NA	NA	NA	NA
NC_000001.10:g.223116596C>G	c.431C>G	p.(Ser144Cys)	F9-A; F9-B	0	112	D (0.01)	5.752	24.8	0.473
NC_000001.10:g.223163993C>T	c.566C>T	p.(Pro189Leu)	F16-A; F16-B	1.20E-04	98	D (0)	7.905	28.4	0.984
NC_000001.10:g.223165373G>A	c.810G>A	p.(Trp270*)	F2-A	0	NA	NA	NA	38	0.264
NC_000001.10:g.223165370_223165373dup	c.807_810dup	p.(Ser271Metfs*4)	F3-A	0	NA	NA	NA	NA	NA
NC_000001.10:g.223175826A>G	c.1087A>G	p.(Asn363Asp)	F15-A	0	23	D (0)	8.735	25.9	0.79
NC_000001.10:g.223176096A>C	c.1357A>C	p.(Met453Leu)	F14-A	0	15	T (0.44)	7.001	20.2	0.516
NC_000001.10:g.223176164G>A	c.1425G>A	p.(Trp475*)	F4-A	0	NA	NA	NA	38	0.443
NC_000001.10:g.223176396G>A	c.1657G>A	p.(Glu553Lys)	F15-A	1.20E-05	56	T (0.47)	6.656	23.3	0.918
NC_000001.10:g.223176941C>A	c.2202C>A	p.(Tyr734*)	F5-A	2.39E-05	NA	NA	NA	36	0.477
NC_000001.10:g.223176975T>C	c.2236T>C	p.(Ser746Pro)	F11-A	0	74	D (0.02)	8.017	27.3	0.812
NC_000001.10:g.223177297T>C	c.2558T>C	p.(Ile853Thr)	F13-A; F13-B	1.42E-05	89	D (0)	7.969	26.1	0.898
NC_000001.10:g.223177438G>A	c.2699G>A	p.(Ser900Asn)	F18-A	1.59E-05	46	D (0.04)	8.07	22.7	0.243
NC_000001.10:g.223177637G>A	c.2898G>A	p.(Trp966*)	F6-A	3.99E-06	NA	NA	NA	43	0.45
NC_000001.10:g.223177867C>T	c.3128C>T	p.(Ser1043Leu)	F13-A; F13-B	1.59E-05	145	D (0)	7.858	27.4	0.903
NC_000001.10:g.223177878G>A	c.3139G>A	p.(Gly1047Ser)	F12-A	3.98E-06	56	D (0)	9.94	27.7	0.97
NC_000001.10:g.223177941C>T	c.3202C>T	p.(Arg1068*)	F7-A	7.96E-06	NA	NA	NA	41	0.463
NC_000001.10:g.223177972G>A	c.3233G>A	p.(Arg1078His)	F14-A; F19-A	3.61E-04	29	T (0.06)	9.94	28.5	0.846
NC_000001.10:g.223177984C>T	c.3245C>T	p.(Ala1082Val)	F8-A	2.12E-05	64	D (0.02)	7.858	25.9	0.915
NC_000001.10:g.223177996C>G	c.3257C>G	p.(Ala1086Gly)	F20-A	0	60	T (0.23)	7.858	23.3	0.717
NC_000001.10:g.223178026T>C	c.3287T>C	p.(Met1096Thr)	F17-A; F17-B; F17-C	5.91E-3	81	D (0)	7.969	23.6	0.925
NC_000001.10:g.223178697C>T	c.3958C>T	p.(Gln1320*)	F10-A	0	NA	NA	NA	37	0.244
NC_000001.10:g.223179147del	c.4408del	p.(Glu1470Argfs*18)	F12-A	0	NA	NA	NA	NA	NA
SHH variants (NM_000193.4 ENST00000297261.7; NP_000184.1)									
NC_000007.13:g.155595943G>T	c.1040C>A	p.(Pro347Gln)	F17-A; F17-B; F17-C	0	76	D (0)	9.170	27.4	0.92
FGFR1 variants (NM_023110.3 ENST00000447712.7; NP_075598.2)									
NC_000008.10:g.38272332T>A	c.1942A>T	p.(Ile648Phe)	F8-A	0	21	D (0)	1.076	26.7	0.785
PTCH1 variants (NM_000264.5 ENST00000331920.11; NP_000255.2)									
NC_000009.11:g.98240366T>A	c.1318A>T	p.(Ile440Phe)	F13-A	0	21	D (0.03)	4.847	24	0.9
SUFU variants (NM_016169.4 ENST00000369902.8; NP_057253.2)									
NC_000010.10:g.104359301C>T	c.1022C>T	p.(Pro341Leu)	F6-A	1.99E-05	98	T (0.12)	3.625	25.3	0.462

cDNA, complementary DNA; *D*, deleterious; *NA*, not applicable; *T*, tolerated; *VEST4*, Variant Effect Scoring Tool 4.

[a]Version v2.1.1, accessed in September 2021.

[b]Score: 0-215.

[c]v6.0.2; Score: 0-1; Deleterious if <0.05.

[d]100way_vertebrate v4.3; Score: −20.0-10.0.

[e]GRCh37-v1.6; Deleterious if >20.

[f]Score: 0-1; Deleterious if >0.5.

histories. Most of the patients originated from France ($N = 11$), whereas others came from The Netherlands, the United States, Germany, Turkey, Saudi Arabia, and India. Four families described previously were also included in the present study.[17,18]

The patients and/or their parents or legal representatives had given their written, informed consent for genetic analysis, the sharing of anonymized clinical and molecular information for research purposes, and the publication of photographs. The research protocol fulfilled the requirements of the local institutional ethics committee (Rennes Hospital, France). The study complied with the principles set out in the Declaration of Helsinki.

Clinical data on individuals with *DISP1* variants

A description of the patient's facial appearance was obtained in all cases, and photographs for publication were available for 6 patients. Additional information on the patients' phenotypes and pedigrees is provided as Supplemental Information (Supplemental Figure 2). We divided the cohort into 2 phenotypic classes: mild HPE (microforms and lobar forms; described in Table 2[17,18] and Supplemental Data) and severe HPE (alobar and semi-lobar forms; described in Table 3[18] and Supplemental Data).

Exome sequencing

Routine singleton, trio, or quartet exome sequencing was applied by all genetic laboratories, using their preferred diagnostic protocols and platforms. Sequence reads were aligned with the GRCh37 human reference genome assembly. *DISP1* variants were described according to the RefSeq NM_032890.3 reference sequence. Chromosomal microarray analysis was performed on the patients (Supplemental Data).

Genetic analyses and pathogenicity assessment of *DISP1* variants

Candidate variants were selected by focusing on protein-altering and splice-site variants. Patient-associated *DISP1* germline single-nucleotide variants were analyzed in a semiautomated bioinformatics pipeline (genome version hg19). The Genome Aggregation Database (gnomAD v2.1, the control data set) was used to assess variant allele frequency. The candidate variants were defined as rare (minor allele frequency <0.01) or protein-truncating/missense variants predicted to be pathogenic by the majority of the in silico tools used by our pipeline. Enrichment of likely pathogenic *DISP1* rare variants in cases versus controls (gnomAD v2.1) was assessed in a Wilcoxon rank sum test. A structural image was generated using the iCn3D software suite (web server, v.3.22.1) and the AlphaFoldDB model Q96F81; iCn3D was also used to deduce the impact of missense variants of interest on DISP1's predicted 3-dimensional structure. Full details of the bioinformatics methods are given in the Supplemental Data.

Results

Description of the cohort of individuals with midline anomalies and *DISP1* variants

As a result of an international collaboration, we recruited 25 individuals (from 20 unrelated families) with 1 or more *DISP1* variants and an HPE phenotype. A total of 23 different *DISP1* variants were identified (Table 1). Fourteen (61%) missense substitutions, 6 (26%) truncating nonsense variants, and 3 (13%) frameshift variants were found heterozygotes (12 families), compound heterozygotes (4 families) or homozygotes (4 families). All variants were inherited from asymptomatic parents, except for a c.249del p.(Pro84Leufs*8) variant inherited from a father with mild intellectual disability. Five of these variants had been described previously: c.2202C>A p.(Tyr734*) and c.1425G>A p.(Trp475*) by Roessler et al[17] and c.1087A>G p.(Asn363Asp), c.1657G>A p.(Glu553Lys), and c.3287T>C p.(Met1096Thr) by Mouden et al.[18] Schematic views of the *DISP1* gene and the variants' distribution are shown in Figure 1A and B.[14] There were 11 private variants (6 truncating and 5 missense variants), ie, not present in any of the 141,456 healthy individuals documented in gnomAD.[20] The remaining variants were ultrarare to rare, with gnomAD minor allele frequencies ranging from 5.91E−03 to 3.98E−06. None of the variants was reported in a homozygous state in gnomAD (Table 1). These results suggest that the identified *DISP1* variants are extremely rare in healthy individuals and may therefore be involved in the disease mechanism. No significant evidence of any other gene alteration was found in 15 of the 20 families. In the remaining 5 families, an additional alteration was identified in *SHH*, *PTCH1* (HGNC: 9585), *SIX3* (HGNC: 10889), *SUFU* (HGNC: 16466), or *FGFR1* (HGNC: 3688) (Tables 1-3).[17,18] These genes have all been previously linked to HPE.[4]

In silico characterization of HPE-associated *DISP1* variants

The main isoform of DISP1 comprises 1524 amino acids encoded by 10 exons, the last of which corresponds to nearly 80% of the gene's coding regions (Figure 1A[14]). No other isoforms with a significant biological role have been described for DISP1. The 23 variants presented here were classed as likely pathogenic by most of the in silico tools used in our platform (Table 1). Most of the variants were substitutions (14 missense variants and 6 nonsense variants) and were predicted to be among the 1% most deleterious

Table 2 Clinical information about lobar or microform holoprosencephaly affected patients with *DISP1* variant(s)

Patient ID	Age	Gender	HPE	Orofacial cleft	SMMCI	CNPAS	Microcephaly	Other Brain Anomalies	*DISP1* Inheritance	*DISP1* Variant(s) V1;V2	Oligogenism Additional SNV/CNV (HPE GENES)	Publication
F1-A	10 years	male	microform	+	-	-	-	-	Monoallelic	p.(Pro84Leufs*8)	-	This study
F2-A	22 months	male	microform	-	+	+	**−2 SD**	-	Monoallelic	p.(Trp270*)	-	This study
F3-A	12 months	female	microform	-	-	-	**−3 SD**	-	Monoallelic	p.(Ser271Metfs*4)	-	This study
F4-A	N/A	female	microform	+	-	-	N/A	-	Monoallelic	p.(Trp475*)	-	Roessler et al[17]
F5-A	N/A	female	microform	+	+	-	N/A	-	Monoallelic	p.(Tyr734*)	-	Roessler et al[17]
F6-A	fetus MTP 20+6 GW	female	microform	+	-	-	-	Olivary bodies pachygyria	Monoallelic	p.(Trp966*)	*SUFU* p.(Pro341Leu)	This study
F7-A	2 months	female	lobar	+	-	-	**−3 SD**	-	Monoallelic	p.(Arg1068*)	-	This study
F8-A	5 years	male	microform	-	+	+	-	Incomplete corpus callosum	Monoallelic	p.(Ala1082Val)	*FGFR1* p.(Ile648Phe)	This study
F9-A	7 years	male	microform	-	-	-	**−3 SD**	Cerebellar atrophy	Biallelic, Consanguineous	p.(Ser144Cys) p.(Ser144Cys)	-	This study
F9-B	4 years	female	microform	-	-	-	**−3 SD**	-	Biallelic, Consanguineous	p.(Ser144Cys) p.(Ser144Cys)	-	This study
F10-A	22 years	male	microform	+	-	-	**−3,5 SD**	-	Biallelic, consanguineous parents	p.(Gln1320*) p.(Gln1320*)	-	This study
F11-A	10 months	male	microform	-	+	+	-	-	Biallelic	p.(Ser746Pro) p.(Ser746Pro)	-	This study
F12-A	7 years	male	microform	+	-	-	-	Chorio-retinal coloboma	Biallelic	p.(Gly1047Ser) p.(Glu1470argfs*18)	-	This study
F13-B	11 years	male	microform	-	+	+	-	-	Biallelic	p.(Ile853Thr); p.(Ser1043Leu)	-	This study
F14-A	17 years	female	microform	+	-	-	-	Empty sella turcica	Biallelic	p.(Met453Leu); p.(arg1078His)	-	This study
F15-A	8 years 8 months	female	lobar	+	-	-	-	-	Biallelic	p.(Asn363Asp); p.(Glu553Lys)	-	Mouden et al[18]

CNPAS, congenital nasal piriform stenosis; *GW*, gestation weeks; *HPE*, holoprosencephaly; *MTP*, medical termination of pregnancy; *N/A*, not available *SMMCI*, single median maxillary central incisor.

Table 3 Clinical information about alobar or semi-lobar holoprosencephaly affected fetuses (medical termination of pregnancy) with *DISP1* variant(s)

Patient ID Article	Number of Affected Patient(s) in Family	Gender	HPE	Face	Consanguinity	*DISP1* Inheritance	*DISP1* Variant(s) v1;v2	Oligogenism Additionnal SNV/CNV (HPE Genes)	Publication
F16-A	2	male	alobar	Cyclopia, proboscis	+	Biallelic Consanguineous	p.(Pro189Leu) p.(Pro189Leu)	-	This study
F16-B	2	male	alobar	Cyclopia, proboscis	+	Biallelic Consanguineous	p.(Pro189Leu) p.(Pro189Leu)	-	This study
F13-A	2	male	alobar	Median cleft/premaxillary agenesis	-	Biallelic	p.(Ile853Thr) p.(Ser1043Leu)	*PTCH1* p.(Ile440Phe)	This study
F17-A	3	male	semi-lobar	Proboscis	-	Monoallelic	p.(Met1096Thr)	*SHH* p.(Pro347Gln)	Mouden et al[18]
F17-B	3	female	alobar	Median cleft/premaxillary agenesis	-	Monoallelic	p.(Met1096Thr)	*SHH* p.(Pro347Gln)	Mouden et al[18]
F17-C	3	female	alobar	N/A	-	Monoallelic	p.(Met1096Thr)	*SHH* p.(Pro347Gln)	Mouden et al[18]
F18-A	1	female	alobar	Single nostril nose, median cleft lip without cleft palate	-	Monoallelic	p.(Ser900Asn)	4,5 Mb *de novo* microdeletion 2p21p16.3 (including *SIX3*)	This study
F19-A	1	female	alobar	hypotelorism, flat and narrow nose and nonvisible nostril openings	-	Monoallelic	p.(Arg1078His)	-	This study
F20-A	1	female	semi-lobar	Median cleft/premaxillary agenesis	-	Monoallelic	p.(Ala1086Gly)	-	This study

HPE, Holoprosencephaly; *N/A*, not available.

substitutions in the human genome (Combined Annotation Dependent Depletion score >20, mean score = 29.65, Table 1).[21] The substitutions presented significantly higher Combined Annotation Dependent Depletion and Variant Effect Scoring Tool 4 scores than those found in healthy individuals documented in gnomAD ($P < 1.617E{-}06$ and $P < .00414$, respectively, in Wilcoxon's sum-rank test; Figure 1C[14]). Notably, the majority of missense single-nucleotide variants were in highly conserved regions in terms of both the nucleotide sequence (PhyloP100way_vertebrate > 7.56) (11/14) and the amino acid sequence (sorting intolerant from tolerant - SIFT < 0.05) (10/14) levels.[22] None of the in silico prediction tools used in the laboratory (MaxEntScan, SPiP, spliceAI, and AbSplice) predicted a potential effect on splicing for any of the *DISP1* missense variants reported here (Supplemental Table 1). One of the 6 nonsense variants, (p.Trp270*), is in *DISP1* exon 5 and is predicted to result in null protein expression and thus *DISP1* haploinsufficiency. In contrast, the premature stop codons in the last exon ($n = 5$) are predicted to escape from nonsense-mediated decay and thus to lead to a truncated protein.[23] However, 4 of these stop codons (p.(Trp475*), p.(Tyr734*), p.(Trp966*), p.(Arg1068*), and p.(Gln1320*)) would be expected to eliminate the TM11 domain of DISP1 and therefore would probably be functionally null alleles or severe LoF variants.

Given the complex etiology of the *DISP1*-related phenotype and the known function of DISP1 in SHH release, these variant impact prediction analyses support that *DISP1* LoF variants are involved in the pathogenesis of HPE.

Impact of *DISP1* variants on SHH release

We next investigated the possible impact of *DISP1* variants on SHH release by mapping the variants to the protein's known functional domains, active sites, and critical residues (Figure 1B and D).[14]

The 3 frameshift variants (p.(Pro84Leufs*8), p.(Ser271Metfs*4), and p.(Glu1470Argfs*18)) were private and were predicted to alter *DISP1* transcription. Given their exonic position, p.(Pro84Leufs*8) and p.(Ser271Metfs*4) are likely to lead to LoF. P.(Glu1470Argfs*18) is located near the protein's C-terminal and may engender a truncated protein. However, the truncation would encompass 20 amino acid (including 2 serine residues) that are highly conserved in all vertebrate species (PhyloP) and are involved in the basolateral localization of DISP1 (essential for its long-range activity).[7]

We identified a cluster of 6 missense variants in the TM10-TM11 domains around the Na+ binding sites (Figure 1B).[14] Four of these variants are located in the DISP1 active sites that are critical for the Na+ gradient (Figure 1D).[14] The variants p.(Gly1047Ser) and p.(Ser1043Leu) map close to the polar residue Asp1051 in ion site 1 (Figure 1E).[14] p.(Gly1047Ser) modifies an important supporting amino acid in ion site 1, whereas p.(Ser1043Leu) might result in a major conformational change near the ion site (Grantham distance: 145; substitution of a non-charged polar residue by a hydrophobic residue) (Table 1). The p.(Ala1086Gly) and p.(Ala1082Val) variants are predicted to result in conformational changes in the critical motif (ion sites 2 and 3, Asp572/573, TM4; Figure 1D and E).[14] The p.(Arg1078His) variant is predicted to alter certain specific bonds and thus disrupt the folding and conformation of ion site 1 (Figure 1D and E,[14] Supplemental Figure 1). These variants might disrupt the protein's function because the Na^+ flux is needed to power the extraction of SHH from the plasma membrane.[14] The variant p.(Asn363Asp) is in 1 of the 5 highly conserved N-linked glycosylation sites in the extracellular domains.[14] The 7 remaining missense variants lead to diverse outcomes, including structural changes (p.(Pro189Leu) and p.(Ser746Pro)), the removal of a hydrophobic residue (p.(Ile853Thr)), a change in the charge on a residue (p.(Glu553Lys)), a change from a charged residue to an uncharged residue (p.(Ser900Asn)), and the addition or removal of a sulfur-containing residue (p.(Ser144Cys) and p.(Met453Leu), respectively). Taken as a whole, these analyses of variant-to-protein structures converge toward a pathogenic impact on the intrinsic activity of DISP1, affecting aspects such as sub-cellular localization, Na+ or ligand binding/release, or overall structural integrity, which would impair the functioning of the SHH pathway.

DISP1 variants are associated with craniofacial morphological features of mild HPE

Most of the individuals in the cohort had mild HPE: 16 (64%) of the 25 individuals (including a fetus) had a microform ($n = 14$) or lobar HPE ($n = 2$). The craniofacial anomalies were midline malformations, such as orofacial cleft (9 out of 16), an SMMCI (5 out of 16) and CNPAS (4 out of 16) (Table 2[17,18]); all these clinical features, alongside microcephaly (present in 8 out of 14), are typical of the mild end of the HPE spectrum.[2]

In line with previous case reports,[2] brain MRI did not provide evidence for any cerebral hemisphere malformations in the 14 individuals with microforms. However, 3 of 12 had microcephaly but no delay in psychomotor development. Furthermore, F8-A had a short incomplete corpus callosum, F9-A had a cerebellar atrophy, and F14-A had an empty sella turcica and panhypopituitarism (diagnosed before the age of 1) treated with growth hormone, thyroid hormone, and hydrocortisone. One patient (F6-A) had olivary body pachygyria in the medulla oblongata, with a normal midline in an autopsy. F12-A had additional features consisting of bilateral chorioretinal coloboma, right microphthalmia, and hypospadias. For the 2 individuals with lobar HPE, the diagnosis was confirmed by MRI after the

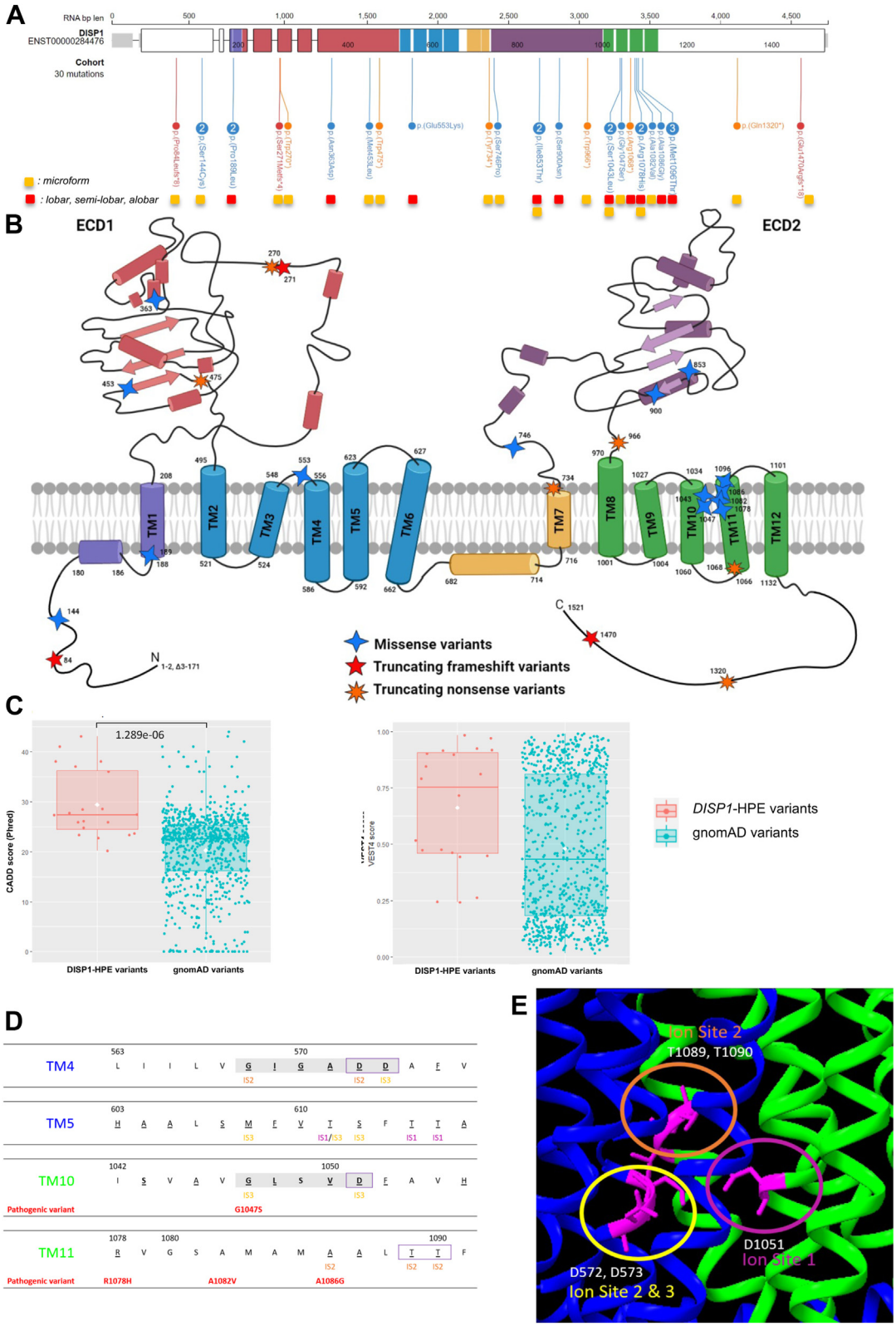


Figure 1 Genetic aspects of affected individuals with *DISP1* variants. A. A 1-dimensional, schematic representation of the *DISP1* transcript, with the locations of the patients' variants. Missense, nonsense, and frameshift variants are colored in blue, orange, and red, respectively. Extracellular domains (ECD)1 (red) and ECD2 (pink) are the extracellular domains. The preTM1 and TM1 domains are shown in purple, the TM2 to TM6 domains in blue, the preTM7 and TM7 domains in yellow, and the TM8 to TM12 domains in green. Orange square under a variant indicates that it has been identified in an individual with lobar or microform; red square indicates that it has been identified in an individual with alobar or semi-lobar HPE. B. A 2-dimensional topological diagram of human DISP1, showing secondary

observation of mild intellectual disability, cleft palate, and dysmorphia in 1 case[18] and a broad right cleft lip and cleft palate at birth and microcephaly in the other.

DISP1 is a major genetic component of SMMCI- and CNPAS-associated features

When considering the 14 individuals with microforms of HPE, 4 had the very rare combination of CNPAS and SMMCI: F2-A carried a heterozygous truncating variant, F8-A carried a heterozygous missense variant, F10-A carried a homozygous missense variant and an additional *FGFR1* variant, and F12-B carried compound heterozygous *DISP1* variants.

The SMMCI develops and erupts exactly on the midline of the maxillary arch in both the primary and permanent dentitions, and the estimated incidence is 1 per 50,000 live births.[24] For all 4 individuals (Table 2[17,18]), this incisor was the only centrally positioned tooth (Figure 2). CNPAS is a very rare cause of neonatal respiratory distress. After diagnosis in the context of neonatal respiratory distress (because infants are obligate nasal breathers), CNPAS usually requires surgical treatment early in life. On an axial craniofacial CT scan, the diagnosis is confirmed by a pyriform aperture width <11 mm (measured at the inferior meatus) in a term neonate.[25] In our study, the 4 unrelated individuals had a horizontal narrowing of the side walls, which resulted in a smaller aperture (Figure 2) and thus CNPAS at the most anterior aspect of the bony nasal airway.[26]

When CNPAS was present in our cohort, it was always combined with an SMMCI ($n = 4$ out of 4). This very rare association has been described previously in individuals with *SHH* variants; hence, our observations strongly suggest that DISP1 dysfunction leads to a patent impairment in SHH signaling.

Overall, the 14 individuals with mild HPE had specific craniofacial features, with 2 main forms: (1) an orofacial cleft (64%) or (2) the rare combination of SMMCI and CNPAS features (30%).

Minor HPE phenotypes are associated with either monoallelic *DISP1* haploinsufficiency or biallelic missense *DISP1* variants

Of the 16 individuals with microform or lobar HPE, 8 had a monoallelic *DISP1* variant and 8 had biallelic variants. All these variants were inherited from parents with no typical HPE craniofacial features (ie, no clefts, SMMCI, CNPAS, or hypotelorism).

In the 8 families with a monoallelic variant, 7 individuals carried variants leading to premature protein truncation (2 frameshift variants and 5 nonsense variants). The frameshift variant p.(Pro84Leufs*8) in F1-A was inherited from the father, who had a learning disability (Figure 2 and Supplemental Figure 2). The remaining individual (F8-A) had a monoallelic missense variant p.(Ala1082Val) that was predicted to have a significant impact on SHH release (Figure 1E).[14] Notably, pangenomic analysis revealed the inheritance of a missense variant in the HPE-linked gene *FGFR1* from the other (asymptomatic) parent (ie, oligogenic inheritance).

Of the 7 families with recessive inheritance and minor HPE, 2 were consanguineous (F9 and F10) with asymptomatic parents (cousins). A brother (F9-A) and a sister (F9-B) had the biallelic missense variant p.(Ser144Cys) and 1 individual (F10-A) had the biallelic nonsense variant p.(Gln1320*) that was predicted to produce an almost complete protein. Another individual (F10-A) had a biallelic missense variant p.(Ser746Pro) inherited from nonconsanguineous parents. The other 4 individuals with biallelic variants in *DISP1* were compound heterozygotes and carried 8 different variants; 7 of the 8 were missense variants (Table 1), and the last one was a frameshift variant (p.(Glu1470Argfs*18) in F11-A) at the end of the protein and was predicted to lead to only partial LoF.

In general, when a monoallelic *DISP1* variant was found in individuals with mild HPE, it was either a nonsense or frameshift variant that typically led to haploinsufficiency. In contrast, the biallelic variants were mainly missense variants that disrupted the structure of the protein and probably only led to partial LoF of DISP1.

The *DISP1*-related severe HPE phenotype involves additional genetic factors

Nine of the 25 individuals with 1 or more *DISP1* variants were fetuses with antenatal signs of failure of the prosencephalon to divide. Seven of the 9 had the most severe form of HPE (alobar HPE), characterized by a single ventricle and no separation of the cerebral hemispheres. The 2 remaining cases corresponded to semilobar HPE, with partial separation of the cerebral hemispheres (Table 3[18] and

structure elements. C. In silico pathogenicity prediction scores (Combined Annotation Dependent Depletion and Variant Effect Scoring Tool 4) for the DISP1 variants identified in our cohort and those present in gnomAD. The scores were compared in a Wilcoxon rank sum test. D. A 1-dimensional representation of the set of coordinating ligands for each of the 3 Na+ ions. Three polar residues interact directly with the Na+ ion at D1051 (ion site 1), D572 (ion site 2), and D573 (ion site 3). The 3-dimensional conformation of the 3 residues is supported by neighboring, conserved residues in other TM domains. IS1/IS2/IS3: ion site; magenta boxes: polar amino acids in Figure 1D; a gray background: a critical motif; red: a pathogenic variant in our cohort; underlined AAs: highly conserved AAs. E. A 3-dimensional representation of critical ion sites in DISP1: the set of polar residues (shown in magenta) within helices TMD4 (D572, D573/ion sites 2 and 3, yellow circle), TMD10 (D1051/ion site 1, purple circle), and TMD11 (T1089, T1090/ion site 2, orange circle). A salt bridge between TMD4 and TMD10 alternates with a hydrogen bond between TMD4 and TMD11. The successful operation of this pocket is required for DISP1's function.[14]

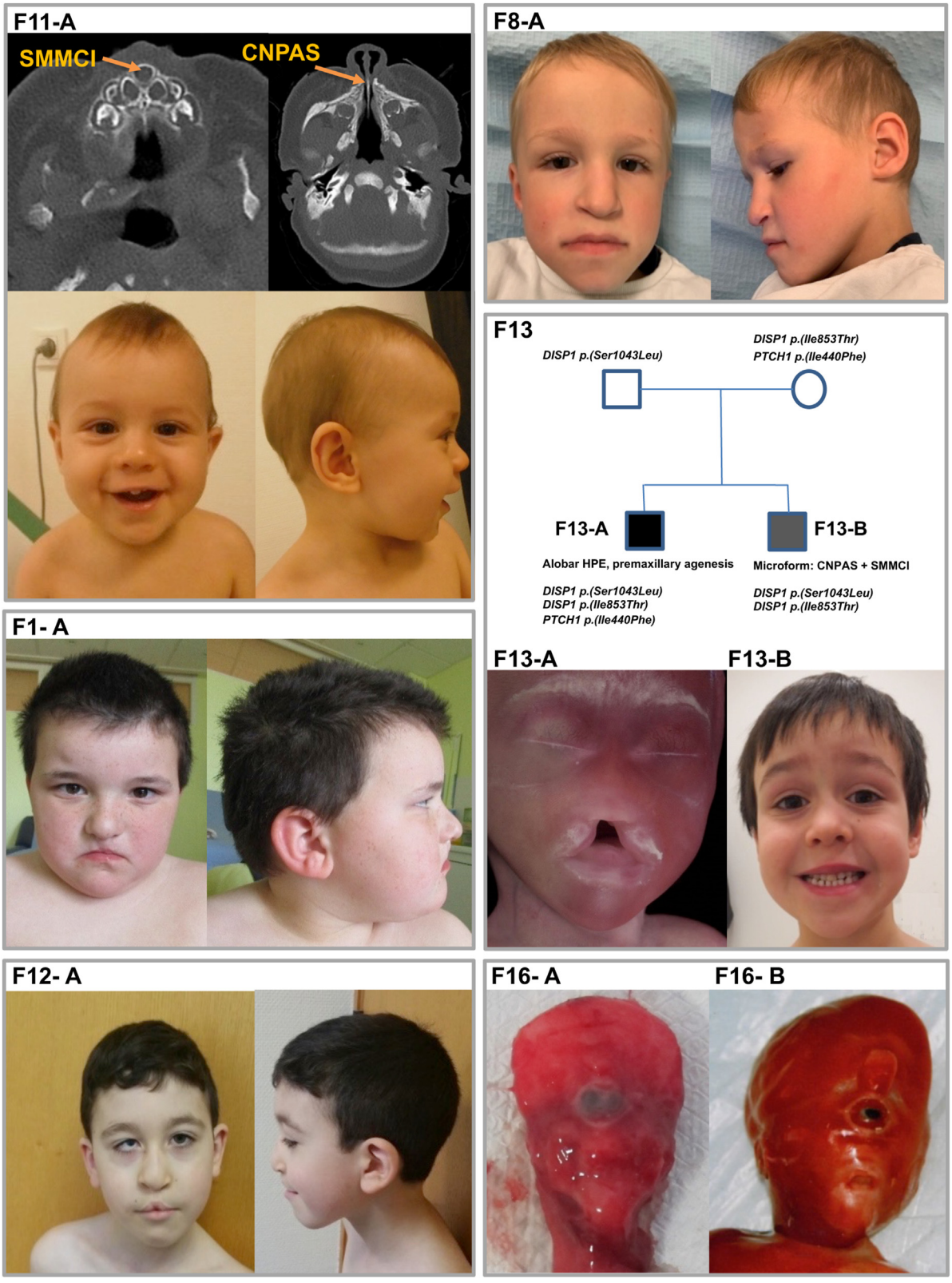


Figure 2 Photographs of 8 patients described in this study and a CT scan slice of patient #F11-A highlighting single median maxillary central incisor and congenital nasal pyriform aperture stenosis (SMMCI and CNPAS). F1-A, and F12-A are HPE patients with orofacial cleft. F8-A, F11-A, and F13-B are microform HPE patients with SMMCI and CNPAS. Family 13 and 16 have 2 affected siblings. For further details, see the Supplemental Data.

Supplemental Data). These brain malformations were associated with severe craniofacial malformations, including cyclopia ($n = 2$), proboscis ($n = 3$), an arhinia ($n = 1$), or severe median cleft/premaxillary agenesis (a specific and rare type of orofacial cleft; $n = 4$). Overall, these findings show that impairments in DISP1 can also contribute to the severe HPE phenotype.

It has been previously proposed that HPE can arise from a combination of multiple inherited variants.[4] Here, 5 out of 9 patients with severe HPE had oligogenic inheritance involving *DISP1* and variants in other known HPE-linked genes from the SHH pathway (eg, *SIX3*, *SHH*, and *PTCH1*). The family comprising 3 siblings with alobar or semi-lobar HPE and DISP1/SHH oligogenic inheritance (F17-A, F17-B, and F17-C) was described previously by Mouden et al.[18] In this family, the relatives exhibiting only the SHH variant present solely microcephaly with hypotelorism.

Oligogenic inheritance with a microdeletion including *SIX3* (a 4.5 Mb de novo 2p21p16.3 heterozygous microdeletion) and the *DISP1* variant p.(Ser900Asn) was found in F18-A (alobar HPE). *SIX3* alterations typically result in severe HPE in the majority of instances (74%).[2] Nevertheless, a previous report has indicated incomplete penetrance for family cases of HPE linked to complete deletions of *SIX3* gene.[27] Therefore, determining the extent to which *SIX3* or *DISP1* contributes to the manifestation of the severe phenotype observed in the F18-A family is a challenge.

In family F13, quartet exome sequencing revealed biallelic *DISP1* variants—p.(Ile853Thr) and p.(Ser1043Leu)—in 2 siblings (F13-A and F13-B), each inherited from a different parent. F13-A (with alobar HPE) was the older brother of F13-B (microform, SMMCI, and CNPAS) and carried an additional variant in the HPE-linked gene *PTCH1* (p.(Ile440Phe)) inherited from his mother and that was absent in F13-B (Figure 2). Overall, these results suggest that in individuals with a severe HPE phenotype, the *DISP1* variant would be associated with a deleterious variant in a second HPE-linked gene.

However, 2 siblings with severe HPE (F-16A and B) from a consanguineous family had a biallelic *DISP1* missense variant p.(Pro189Leu). In this multiplex family with 3 affected fetuses from 2 branches (Figure 2), the consanguineous parents of the third fetus were both found to be heterozygous, whereas their fetus could not be tested. Further exome analyses did not reveal other variants in known HPE-linked genes. Two other fetuses (F-19A and F-20A) with severe HPE were found to carry a monoallelic missense variant in *DISP1* p.(Arg1078His) and p.(Ala1086Gly) but no other gene variants identified by exome sequencing. These findings imply that severe phenotypes could potentially be linked to variants only in *DISP1*.

Discussion

Here, we described the largest cohort to date of individuals with an HPE phenotype and *DISP1* variants. Our results highlighted several new genetic characteristics of the HPE spectrum.

Monoallelic, hypomorphic *SHH* variants are the most commonly detected and best-understood pathogenic changes observed in patients with anterior midline defects. This observation is consistent with SHH's essential function in forebrain and craniofacial patterning; it is well known that the disruption of SHH's spatiotemporal gradient accounts for the very broad clinical spectrum in HPE.[28-30] Because DISP1 is responsible for the establishment of this SHH gradient,[8] haploinsufficiency or impairment of its function disrupts the SHH gradient in animal models.[31,32] It is therefore not surprising to find hypomorphic *DISP1* variants in individuals with an HPE phenotype. However, very few of these variants have been described previously.[17,18]

Of the 23 *DISP1* variants described here, 9 (39%) were frameshift or nonsense variants that led to LoF or a truncated protein. The missense variants ($n = 14$, 61%) were predicted to have a deleterious impact on DISP1 and thus were likely to impair the SHH gradient. Seven of the 14 missense variants were located in TM10 or TM11, and the affected residues were predicted to influence the Na^+ gradient required for SHH export.[14] Furthermore, variants found in these regions of the protein are more often associated to severe HPE (Figure 1A).[14]

Previously, Roessler et al[17] attempted to assess the functional impact of *DISP1* variants by evaluating DISP1 ability to transport SHH through the utilization of a Renilla-tagged Shh reporter within *Drosophila* cells. The researchers observed abnormally low *DISP1* activity for nonsense variants but not for missense variants.[17] This finding emphasizes the challenge of using an in vitro system to evaluate the slight decrease in efficiency that modifying a single amino acid can have on the function of a protein. Based on current data, the American College of Medical Genetics labels 8 out of the 23 DISP1 variants described here as pathogenic and likely pathogenic (Supplemental Table 2). Nevertheless, despite the absence of functional data, our findings, relying on phenotypic overlap and in silico analysis, suggest that the remaining 14 variants could reasonably be classified as likely pathogenic.

Similar to *SHH* variants, *DISP1* variants are associated with a broad spectrum of severity in the brain and craniofacial manifestations of HPE. Given the critical role of the SHH gradient in early brain development,[6] our results suggest that *DISP1* variants can lead to mild HPE (microform and lobar forms) or severe HPE. The reduction in DISP1 activity would therefore lead to variable but significant impairments of SHH release.

Remarkably, *DISP1* variants were biallelic in 40% of the families studied. Biallelic inheritance had never been described in families with *SHH* variants, which led to the assumption whereby the latter are lethal for embryonic development in humans.[7,33]

Furthermore, most of the individuals with mild HPE carried monoallelic LoF variants (frameshift or nonsense variants) or biallelic missense variants of the *DISP1* gene. This observed mixture of monoallelic and biallelic *DISP1*-variants is intriguing, particularly since the great majority of individuals with biallelic *DISP1*-variants display a mild HPE phenotype. We do not have a satisfactory explanation for this, although it is reasonable to postulate that a monoallelic LoF variant exerts a greater impact on the SHH gradient compared with a monoallelic missense variant. We suspect that only the addition of 2 missense variants resulting in a slight decrease in the activity of the protein would lead to a clinically obvious phenotype. Our results also suggest that the combination of a LoF variant with a second variant in the *DISP1 trans* allele would create a major disturbance in the SHH gradient and would lead to early miscarriage. Alternatively, for SHH,[4,5] there could be involvement of tissue-specific modifier genes that have not been described yet.

Monoallelic *SHH* variants have been described in affected individuals with a severe HPE phenotype (50%) or with microform HPE (50%),[2,3] whereas we mainly observed *DISP1* variants in individuals with mild HPE (64%). Our observations lead us to believe that the severe HPE is caused by the combination of a *DISP1* variant with an additional alteration in an HPE-linked gene. Half of the individuals with severe HPE in this study were found to carry an additional variant or copy-number variation involving an HPE-linked gene (eg, *SHH*, *PTCH1*, and *SIX3*). This observation suggests that some *DISP1* variants are only pathogenic in oligogenic combinations because it has been established that HPE has oligogenic inheritance.[4] However, our exome analysis did not reveal any alterations in other genes in 3 of the 6 families with severe HPE and *DISP1* missense variants. Either these variants alone are damaging enough to affect the SHH gradient, leading to a severe phenotype, or there might be a genomic alteration within a non-coding region. A genome analysis of these families might provide some answers. It should be noted that all these variants, which are extremely rare in the population, are inherited and therefore present in at least 1 healthy parent. We can therefore expect these variants to have occurred de novo in some of the ancestors. Our results encourage us to raise awareness among clinicians of the importance of extending molecular diagnosis to grandparents to improve information on segregation.

Microform HPEs are significantly underdiagnosed because the affected individuals do not have the characteristic brain malformation. Most of these microforms are detected only when a severe case of HPE occurs in the same family.[3,34] In particular, orofacial clefts are relatively frequent and often non-syndromic. Cleft lip and cleft palate are nevertheless within the spectrum of SHH deficiency,[35] as demonstrated in several mouse models.[36] Our results indicate that these clefts are caused by low SHH activity; when cleft lip or cleft palate are combined with either microcephaly, coloboma/microphthalmia, or endocrine anomalies (hypopituitarism), the physician should specifically consider an SHH-related disorder. Similarly, CNPAS was thought to be an isolated phenomenon before it was identified in association with SMMCI and then a defect of SHH signaling.[37,38] Our results indicate that CNPAS formation can be a consequence of an SHH signaling deficiency. The combination of SMMCI with a suggestive facial phenotype (ie, including hypotelorism) is well known to clinical geneticists and pediatricians[34,39,40] and is 1 of the criteria for performing an HPE-linked gene panel/exome analysis. We recommend that the combination of CNPAS with other features suggestive of HPE should prompt the medical team to focus on the SHH pathway genes, including DISP1. Moreover, conducting a systematic examination of brain imaging in parents carrying a *DISP1* variant would be pertinent to identify potential subclinical brain structural differences. This exploration could likely expand the phenotypic spectrum associated with *DISP1*-related diseases.

Conclusion

Our work provided new insights into the natural history and clinical heterogeneity of the HPE spectrum, including the inheritance pattern, the nature of the variants, the presence of additional genetic factors, and the prevalence of clinical features.

This significantly improves our knowledge of *DISP1*-related phenotypes. It is noteworthy that *DISP1* is a major gene in the pathogenesis of the rare CNPAS-SMMCI combination.

Data Availability

The *DISP1* variants and associated phenotypes have been submitted to the ClinVar database at https://www.ncbi.nlm.nih.gov/clinvar/. Further data supporting the study's findings are available from the corresponding authors upon request (valerie.dupe@univ-rennes.fr).

Acknowledgments

The authors are grateful to the members of the "AnDDirares" study network for rare developmental disorders, the GeneMatcher team, the clinicians involved in the patient care, the biologists and bioinformatics specialists involved in the molecular diagnoses, and the families having participated in the study. The authors acknowledge the "Centre de Ressources Biologiques (CRB) Santé of Rennes" (CHU

Rennes BB-0033-00056) for managing patient samples. Several authors of this publication are members of the European Reference Network for Developmental Anomalies and Intellectual Disability (ERN-ITHACA) and FHU Genomeds (https://www.gem-excell.fr/recherche-et-innovation/fhu-genomeds/).

Funding

This work was funded by the Agence Nationale de la Recherche (ANR-22-CE17-0064) and CHU de Rennes (35RC20_8894 EXOMEDIANE).

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Conceptualization: A.L., C.D., V.D., S.O.; Data Curation: A.L., E.P., L.A., M.F., P.R., C.D., W.C.; Formal Analysis: A.L., P.R., A.K., V.D.; Funding Acquisition: A.L., P.R., V.D., S.O.; Investigation: A.L., P.R., W.C., V.P., H.G., C.D., E.P., L.A., C.Q.; Methodology: A.L., P.R., A.K., M.D.T., V.D.; Project Administration: V.D., S.O.; Resources: V.P., H.G., M.F., E.P., L.A., C.Q., S.W., J.B., M.B., J.M.v.H., K.G., M.A.-S., E.M., S.D.M., H.K., U.A., A.E.Q.C., S.X., M.J., N.A., W.H., C.V., L.F., E.V.-D., C.P., H.S., F.L., L.M., R.A.J., S.J.P., A.D., A.S.S., B.K., B.R., C.D.; Software: P.R., A.K., W.C., M.D.T.; Supervision: V.D., S.O.; Visualization: A.L., P.R., V.P., V.D.; Writing-original draft: A.L., P.R., A.K., V.D.; Writing-review and editing: A.L., P.R., B.R., M.F., M.D.T., C.D., S.O., V.D.

Ethics Declaration

The patients and/or their parents or legal representatives gave their written, informed consent for genetic analysis, the sharing of anonymized clinical and molecular information for research purposes, and the publication of photographs. All institutions involved in human participant research received local IRB approval. Human samples were obtained from the processing of biological samples through the Centre de Ressources Biologiques (CRB) Santé of Rennes BB-0033-00056. The research protocol was conducted under French legal guidelines and fulfilled the requirements of the local institutional ethics committee.

The study complies with the principles set out in the Declaration of Helsinki.

Conflict of Interest

All authors declare no conflicts of interest.

Additional Information

The online version of this article (https://doi.org/10.1016/j.gim.2024.101126) contains supplemental material, which is available to authorized users.

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References

1. Orioli IM, Castilla EE. Epidemiology of holoprosencephaly: prevalence and risk factors. *Am J Med Genet C Semin Med Genet*. 2010;154C(1):13-21. http://doi.org/10.1002/ajmg.c.30233
2. Mercier S, Dubourg C, Garcelon N, et al. New findings for phenotype-genotype correlations in a large European series of holoprosencephaly cases. *J Med Genet*. 2011;48(11):752-760. http://doi.org/10.1136/jmedgenet-2011-100339
3. Solomon BD, Bear KA, Wyllie A, et al. Genotypic and phenotypic analysis of 396 individuals with mutations in Sonic Hedgehog. *J Med Genet*. 2012;49(7):473-479. http://doi.org/10.1136/jmedgenet-2012-101008
4. Kim A, Savary C, Dubourg C, et al. Integrated clinical and omics approach to rare diseases: novel genes and oligogenic inheritance in holoprosencephaly. *Brain*. 2019;142(1):35-49. http://doi.org/10.1093/brain/awy290
5. Hong M, Krauss RS. Modeling the complex etiology of holoprosencephaly in mice. *Am J Med Genet C Semin Med Genet*. 2018;178(2):140-150. http://doi.org/10.1002/ajmg.c.31611
6. Lupo G, Harris WA, Lewis KE. Mechanisms of ventral patterning in the vertebrate nervous system. *Nat Rev Neurosci*. 2006;7(2):103-114. http://doi.org/10.1038/nrn1843
7. Dubourg C, Kim A, Watrin E, et al. Recent advances in understanding inheritance of holoprosencephaly. *Am J Med Genet C Semin Med Genet*. 2018;178(2):258-269. http://doi.org/10.1002/ajmg.c.31619
8. Etheridge LA, Crawford TQ, Zhang S, Roelink H. Evidence for a role of vertebrate Disp1 in long-range Shh signaling. *Development*. 2010;137(1):133-140. http://doi.org/10.1242/dev.043547
9. Burke R, Nellen D, Bellotto M, et al. Dispatched, a novel sterol-sensing domain protein dedicated to the release of cholesterol-modified hedgehog from signaling cells. *Cell*. 1999;99(7):803-815. http://doi.org/10.1016/s0092-8674(00)81677-3
10. Tukachinsky H, Kuzmickas RP, Jao CY, Liu J, Salic A. Dispatched and scube mediate the efficient secretion of the cholesterol-modified hedgehog ligand. *Cell Rep*. 2012;2(2):308-320. http://doi.org/10.1016/j.celrep.2012.07.010
11. Hall ET, Cleverdon ER, Ogden SK. Dispatching sonic hedgehog: molecular mechanisms controlling deployment. *Trends Cell Biol*. 2019;29(5):385-395. http://doi.org/10.1016/j.tcb.2019.02.005
12. Kawakami T, Kawcak T, Li YJ, Zhang W, Hu Y, Chuang PT. Mouse dispatched mutants fail to distribute hedgehog proteins and are defective in hedgehog signaling. *Development*. 2002;129(24):5753-5765. http://doi.org/10.1242/dev.00178
13. Chen H, Liu Y, Li X. Structure of human Dispatched-1 provides insights into Hedgehog ligand biogenesis. *Life Sci Alliance*. 2020;3(8):e202000776. http://doi.org/10.26508/lsa.202000776
14. Wang Q, Asarnow DE, Ding K, et al. Dispatched uses Na^+ flux to power release of lipid-modified Hedgehog. *Nature*. 2021;599(7884):320-324. http://doi.org/10.1038/s41586-021-03996-0
15. Li W, Wang L, Wierbowski BM, et al. Structural insights into proteolytic activation of the human Dispatched1 transporter for Hedgehog morphogen release. *Nat Commun*. 2021;12(1):6966. http://doi.org/10.1038/s41467-021-27257-w
16. Ma Y, Erkner A, Gong R, et al. Hedgehog-mediated patterning of the mammalian embryo requires transporter-like function of dispatched. *Cell*. 2002;111(1):63-75. http://doi.org/10.1016/s0092-8674(02)00977-7
17. Roessler E, Ma Y, Ouspenskaia MV, et al. Truncating loss-of-function mutations of DISP1 contribute to holoprosencephaly-like microform features in humans. *Hum Genet*. 2009;125(4):393-400. http://doi.org/10.1007/s00439-009-0628-7
18. Mouden C, Dubourg C, Carré W, et al. Complex mode of inheritance in holoprosencephaly revealed by whole exome sequencing. *Clin Genet*. 2016;89(6):659-668. http://doi.org/10.1111/cge.12722
19. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. *Hum Mutat*. 2015;36(10):928-930. http://doi.org/10.1002/humu.22844
20. Karczewski KJ, Francioli LC, Tiao G, et al. The mutational constraint spectrum quantified from variation in 141,456 humans. *Nature*. 2020;581(7809):434-443. http://doi.org/10.1038/s41586-020-2308-7
21. Rentzsch P, Schubach M, Shendure J, Kircher M. CADD-Splice-improving genome-wide variant effect prediction using deep learning-derived splice scores. *Genome Med*. 2021;13(1):31. http://doi.org/10.1186/s13073-021-00835-9
22. Pollard KS, Hubisz MJ, Rosenbloom KR, Siepel A. Detection of nonneutral substitution rates on mammalian phylogenies. *Genome Res*. 2010;20(1):110-121. http://doi.org/10.1101/gr.097857.109
23. Zhao Y, Lin J, Xu B, Hu S, Zhang X, Wu L. MicroRNA-mediated repression of nonsense mRNAs. *Elife*. 2014;3:e03032. http://doi.org/10.7554/eLife.03032
24. Hall RK. Solitary median maxillary central incisor (SMMCI) syndrome. *Orphanet J Rare Dis*. 2006;1:12. http://doi.org/10.1186/1750-1172-1-12
25. Belden CJ, Mancuso AA, Schmalfuss IM. CT features of congenital nasal piriform aperture stenosis: initial experience. *Radiology*. 1999;213(2):495-501. http://doi.org/10.1148/radiology.213.2.r99oc38495
26. Moreddu E, Le Treut-Gay C, Triglia JM, Nicollas R. Congenital nasal pyriform aperture stenosis: elaboration of a management algorithm from 25 years of experience. *Int J Pediatr Otorhinolaryngol*. 2016;83:7-11. http://doi.org/10.1016/j.ijporl.2016.01.011
27. Stokes B, Berger SI, Hall BA, et al. SIX3 deletions and incomplete penetrance in families affected by holoprosencephaly. *Congenit Anom (Kyoto)*. 2018;58(1):29-32. http://doi.org/10.1111/cga.12234
28. Mercier S, David V, Ratié L, Gicquel I, Odent S, Dupé V. NODAL and SHH dose-dependent double inhibition promotes an HPE-like phenotype in chick embryos. *Dis Model Mech*. 2013;6(2):537-543. http://doi.org/10.1242/dmm.010132
29. Marcucio RS, Young NM, Hu D, Hallgrimsson B. Mechanisms that underlie co-variation of the brain and face. *Genesis*. 2011;49(4):177-189. http://doi.org/10.1002/dvg.20710
30. Petryk A, Graf D, Marcucio R. Holoprosencephaly: signaling interactions between the brain and the face, the environment and the genes, and the phenotypic variability in animal models and humans. *Wiley Interdiscip Rev Dev Biol*. 2015;4(1):17-32. http://doi.org/10.1002/wdev.161
31. Tian H, Jeong J, Harfe BD, Tabin CJ, McMahon AP. Mouse Disp1 is required in sonic hedgehog-expressing cells for paracrine activity of the cholesterol-modified ligand. *Development*. 2005;132(1):133-142. http://doi.org/10.1242/dev.01563

32. Nakano Y, Kim HR, Kawakami A, Roy S, Schier AF, Ingham PW. Inactivation of dispatched 1 by the chameleon mutation disrupts Hedgehog signalling in the zebrafish embryo. *Dev Biol*. 2004;269(2):381-392. http://doi.org/10.1016/j.ydbio.2004.01.022
33. Lo HF, Hong M, Krauss RS. Concepts in multifactorial etiology of developmental disorders: gene-gene and gene-environment interactions in holoprosencephaly. *Front Cell Dev Biol*. 2021;9:795194. http://doi.org/10.3389/fcell.2021.795194
34. Odent S, Atti-Bitach T, Blayau M, et al. Expression of the Sonic hedgehog (SHH) gene during early human development and phenotypic expression of new mutations causing holoprosencephaly. *Hum Mol Genet*. 1999;8(9):1683-1689. http://doi.org/10.1093/hmg/8.9.1683
35. Setó-Salvia N, Stanier P. Genetics of cleft lip and/or cleft palate: association with other common anomalies. *Eur J Med Genet*. 2014;57(8):381-393. http://doi.org/10.1016/j.ejmg.2014.04.003
36. Lipinski RJ, Holloway HT, O'Leary-Moore SK, et al. Characterization of subtle brain abnormalities in a mouse model of Hedgehog pathway antagonist-induced cleft lip and palate. *PLoS One*. 2014;9(7):e102603. http://doi.org/10.1371/journal.pone.0102603
37. Arlis H, Ward RF. Congenital nasal pyriform aperture stenosis. Isolated abnormality vs developmental field defect. *Arch Otolaryngol Head Neck Surg*. 1992;118(9):989-991. http://doi.org/10.1001/archotol.1992.01880090105027
38. Tavin E, Stecker E, Marion R. Nasal pyriform aperture stenosis and the holoprosencephaly spectrum. *Int J Pediatr Otorhinolaryngol*. 1994;28(2-3):199-204. http://doi.org/10.1016/0165-5876(94)90012-4
39. Nanni L, Ming JE, Du Y, et al. SHH mutation is associated with solitary median maxillary central incisor: a study of 13 patients and review of the literature. *Am J Med Genet*. 2001;102(1):1-10. http://doi.org/10.1002/1096-8628(20010722)102:1<1::aid-ajmg1336>3.0.co;2-u
40. Garavelli L, Zanacca C, Caselli G, et al. Solitary median maxillary central incisor syndrome: clinical case with a novel mutation of sonic hedgehog. *Am J Med Genet A*. 2004;127A(1):93-95. http://doi.org/10.1002/ajmg.a.20685