Haploinsufficiency of the Notch Ligand DLL1 Causes Variable Neurodevelopmental Disorders

Björn Fischer-Zirnsak,[1,23] Lara Segebrecht,[1,2,23] Max Schubach,[1,2] Perrine Charles,[3] Emily Alderman,[4] Kathleen Brown,[5] Maxime Cadieux-Dion,[6,7] Tracy Cartwright,[8] Yanmin Chen,[9] Carrie Costin,[10] Sarah Fehr,[11] Keely M. Fitzgerald,[12,13] Emily Fleming,[14] Kimberly Foss,[15] Thoa Ha,[16] Gabriele Hildebrand,[1] Denise Horn,[1] Shuxi Liu,[9] Elysa J. Marco,[17] Marie McDonald,[18] Kirsty McWalter,[9] Simone Race,[19] Eric T. Rush,[14] Yue Si,[9] Carol Saunders,[6,7,13] Anne Slavotinek,[16] Sylvia Stockler-Ipsiroglu,[19] Aida Telegrafi,[9] Isabelle Thiffault,[6,7,13] Erin Torti,[9] Anne Chun-hui Tsai,[5,22] Xin Wang,[9] Muhammad Zafar,[18] Boris Keren,[3] Uwe Kornak,[1] Cornelius F. Boerkoel,[4] Ghayda Mirzaa,[20,21] and Nadja Ehmke[1,*]

Notch signaling is an established developmental pathway for brain morphogenesis. Given that Delta-like 1 (DLL1) is a ligand for the Notch receptor and that a few individuals with developmental delay, intellectual disability, and brain malformations have microdeletions encompassing *DLL1*, we hypothesized that insufficiency of *DLL1* causes a human neurodevelopmental disorder. We performed exome sequencing in individuals with neurodevelopmental disorders. The cohort was identified using known Matchmaker Exchange nodes such as GeneMatcher. This method identified 15 individuals from 12 unrelated families with heterozygous pathogenic *DLL1* variants (nonsense, missense, splice site, and one whole gene deletion). The most common features in our cohort were intellectual disability, autism spectrum disorder, seizures, variable brain malformations, muscular hypotonia, and scoliosis. We did not identify an obvious genotype-phenotype correlation. Analysis of one splice site variant showed an in-frame insertion of 12 bp. In conclusion, heterozygous *DLL1* pathogenic variants cause a variable neurodevelopmental phenotype and multi-systemic features. The clinical and molecular data support haploinsufficiency as a mechanism for the pathogenesis of this *DLL1*-related disorder and affirm the importance of DLL1 in human brain development.

The evolutionarily conserved Notch signaling pathway operates in many different developmental, homeostatic, and disease processes. Cell-cell contact-mediated Notch receptor-ligand interactions release the Notch intracellular domain (NICD), which enters the nucleus and stimulates transcription of target genes.[1] All mammalian Notch ligands are transmembrane proteins and interact through the extracellular Delta-Serrate-LAG2 (DSL) and amino-terminal (NT) domains with the epidermal growth factor (EGF) repeats 11-12 of the extracellular domain of Notch. Modification of the EGF repeats by β3-*N*-acetylglucosaminyltransferases, encoded by the three mammalian Fringe orthologs Lunatic Fringe (*Lfng*), Manic Fringe (*Mfng*), and Radical Fringe (*Rfng*), enhances or inhibits Notch-ligand activation.[2] Notch signaling is highly sensitive to relative levels of ligands and receptors through feedback loops as well as the mechanism of *cis* inhibition and *trans* activation. Various other regulatory mechanisms like expression profiles of ligands and receptors, post-translational events, and integration with other signaling pathways account for the diversity of Notch signaling outcomes.[1,3]

Several *in vivo* and *in vitro* studies have shown a role for the Notch ligand Delta-like 1 (DLL1) in development of the central nervous system (CNS) as well as in that of somites and lymphocytes.[4–14] *DLL1* (MIM: 606582) is highly expressed in neuronal precursor cells during embryogenesis.[15] It regulates neuronal differentiation through oscillation and lateral inhibition such that differentiating neurons inhibit neighboring cells from undergoing neuronal differentiation by expressing Notch signals in an oscillatory manner.[3,16,17] Consequently, Notch signaling forms cellular fields and influences brain morphogenesis. Supporting its role in neuronal development, loss of Dll1 in mice increases

[1]Charité – Universitätsmedizin Berlin, Berlin 13353, Germany; [2]Berlin Institute of Health (BIH), Berlin 10117, Germany; [3]Department of Genetics, Assistance Publique - Hôpitaux de Paris, Hôpital Pitié-Salpêtrière, Paris 75013, France; [4]Department of Medical Genetics, University of British Columbia, Vancouver, BC V6H 3N1, Canada; [5]Department of Pediatrics, The Children's Hospital, University of Colorado School of Medicine, Aurora, CO 80045, USA; [6]Center for Pediatric Genomic Medicine, Children's Mercy Hospital, Kansas City, MO 64108, USA; [7]Department of Pathology and Laboratory Medicine, Children's Mercy Hospitals, Kansas City, MO 64108, USA; [8]Neuroscape Center, Departments of Neurology, Pediatrics, Physiology, Radiology, and Psychiatry, University of California, San Francisco, CA 94158, USA; [9]GeneDx, Gaithersburg, MD 20877, USA; [10]Akron Children's Hospital, Akron, OH 44302, USA; [11]Praxis für Humangenetik Tübingen, Tübingen 72076, Germany; [12]Division of Child Neurology, Department of Pediatrics, Children's Mercy Hospital & Clinics, Kansas City, MO 64108, USA; [13]University of Missouri–Kansas City School of Medicine, Kansas City, MO 64108, USA; [14]Division of Clinical Genetics, Children's Mercy Hospital & Clinics, Kansas City, MO 64108, USA; [15]Division of Genetic Medicine, Seattle Children's Hospital, Seattle, WA 98105, USA; [16]Division of Genetics, Department Pediatrics, University of California, San Francisco, CA 94143-2711, USA; [17]Cortica Healthcare, San Rafael, CA 94903, USA; [18]Department of Pediatrics, Division of Medical Genetics, Duke University Medical Center, Durham, NC 27710, USA; [19]Division of Biochemical Genetics, Department of Pediatrics, University of British Columbia, BC Children's Hospital, Vancouver, BC V6H 3N1, Canada; [20]Center for Integrative Brain Research, Seattle Children's Research Institute, Seattle, WA 98101, USA; [21]Department of Pediatrics, University of Washington, Seattle, WA 98195, USA; [22]Section of Genetics, Department of Pediatrics, University of Oklahoma Health Sciences Center, Oklahoma City, OK 73104, USA
[23]These authors contributed equally to this work
*Correspondence: nadja.ehmke@charite.de
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Table 1. Clinical Features of Individuals with Heterozygous *DLL1* Pathogenic Variants

Individual	1 (F1/II-1)	2 (F2/II-4)	3 (F2/II-1)	4 (F2/II-2)	5 (F2/I-2)	6 (F3/II-1)	7 (F4/II-1)	8 (F5/II-1)	9 (F6/II-1)	10 (F7/II-1)	11 (F8/II-1)	12 (F9/II-1)	13 (F10/II-1)	14 (F11/II-1)	15 (F12/II-1)	Summary of Clinical Features
Gender	f	f	m	m	f	m	m	f	m	m	f	f	m	f	m	7f/8 m
Age at last visit	3 y	2 y	4 y	5 y	u	8 y	16 y	2 y	2 y	9 y	7 y	35 y	33+2 w	8 y	3 y	birth-35 y
DD/ID (HP:0012758 / HP:0001249)	+	–	–	+	+	+	+	+	+	+	+	+	NA	+	+	12/14
ASD (HP:0000729)	–	–	+	u	u	–	+	–	+	+	+	–	NA	–	+	6/14
ADHD (HP:0007018)	–	u	u	u	u	+	+	–	–	+	–	–	NA	–	–	3/14
Stereotypic behavior (HP:0000733)	–	ND	ND	ND	ND	–	–	+	(+)	–	(+)	–	NA	–	+	4/14
Seizures (HP:0001250)	–	u	u	u	u	+	+	–	–	–	+	+	NA	+	+	6/14
Muscular hypotonia (HP:0001252)	+	ND	ND	ND	ND	+	–	–	(+)	+	+	–	NA	–	+	6/14
Ataxia (HP:0001251)	+	ND	ND	ND	ND	+	–	–	–	–	–	+	NA	–	+	4/14
Abnormal brain MRI (HP:0012443)	+	+	+	ND	ND	+	+	+	+	ND	+	+	+	+	–	11/15
Ventriculomegaly (HP:0002119)	+	–	+	ND	ND	+	–	mild	mild	ND	+	mild	+	–	–	8/15
Hydrocephalus (HP:0000238)	–	+	+	ND	ND	+	–	–	–	ND	–	–	–	–	–	3/15
Abnormal corpus callosum (HP:0001273)	–	–	–	ND	ND	+	+	+	+	ND	+	–	–	+	–	6/15
Cortical dysplasia (HP:0002539)	–	–	–	ND	ND	–	(+)	+	–	ND	–	–	–	(+)	–	3/15
Migration defect (HP:0002269)	–	–	–	ND	ND	–	–	–	–	ND	–	PVNH	–	–	–	1/15
Other brain abnormality	–	–	+	ND	ND	+	–	–	+	ND	–	–	–	+	–	4/15

(*Continued on next page*)

Table 1. ***Continued***

Individual	1 (F1/II-1)	2 (F2/II-4)	3 (F2/II-1)	4 (F2/II-2)	5 (F2/I-2)	6 (F3/II-1)	7 (F4/II-1)	8 (F5/II-1)	9 (F6/II-1)	10 (F7/II-1)	11 (F8/II-1)	12 (F9/II-1)	13 (F10/II-1)	14 (F11/II-1)	15 (F12/II-1)	Summary of Clinical Features
Abnormal prenatal brain imaging	+	+	u	u	u	+	–	+	–	–	+	–	+	–	–	6/15
Abnormal vertebrae (HP:0003468)	ND	ND	ND	ND	ND	sc	sc, ky	sc, ky, sd	ND	ND	sc	ND	–	ND	ND	4/15
Microcephaly (HP:0000252)	–	ND	ND	ND	ND	–	–	–	–	+	–	–	–	ND	+	2/15
Macrocephaly (HP:0000256)	–	ND	ND	ND	ND	+	–	–	–	–	–	–	–	ND	–	1/15
Facial dysmorphism (HP:0000271)	(+)	+	ND	ND	ND	–	+	+	+	+	+	–	+	–	–	8/15
Other (details in Table S1)	+	+	+	+	–	+	+	+	+	+	+	+	–	–	+	12/15
Type of sequence variant	nonsense	nonsense	nonsense	nonsense	nonsense	nonsense	nonsense	nonsense	nonsense	nonsense	nonsense	splice site	splice site	missense	deletion	
Inheritance	*de novo*	affected mother	affected mother	affected mother	u	affected father	u	u	*de novo*	*de novo*	not maternal	*de novo*	*de novo*	*de novo*	*de novo*	

Abbreviations: F, family; f, female; m, male; y, years; w, weeks of gestation; DD, developmental delay; ID, intellectual disability; ASD, autism spectrum disorder; ADHD, attention deficit hyperactivity disorder; NA, not applicable; ND, not determined; u, unknown; PVNH, periventricular nodular heterotopia; (+), subtle/borderline; +, present; –, absent; sc, scoliosis; ky, kyphosis; SD, segmentation defect.

Table 2. Molecular Data of Individuals with Heterozygous *DLL1* Pathogenic Variants (GenBank: NM_005618.3; ENST00000366756.3)

Individual	Sequence Variant (c.DNA)	Amino Acid Change	*DLL1* Deletion	Inheritance
1	c.1492G>T	p.Glu498*	–	*de novo*
2	c.231C>A	p.Cys77*	–	affected mother
3	c.231C>A	p.Cys77*	–	affected mother
4	c.231C>A	p.Cys77*	–	affected mother
5	c.231C>A	p.Cys77*	–	unknown
6	c.1525C>T	p.Arg509*	–	affected father
7	c.2013_2014del	p.Glu673Glyfs*15	–	unknown
8	c.50_51del	p.Cys17Serfs*108	–	unknown
9	c.2013_2014del	p.Glu673Glyfs*15	–	*de novo*
10	c.1401_1405dup	p.Cys469Serfs*70	–	*de novo*
11	c.543_570dup	p.Phe191Thrfs*50	–	not maternal
12	c.54+1G>A	p.Gln18_Val19insIleGlyGlyGln	–	*de novo*
13	c.54_54+1insTAGTCG	p.Val19*	–	*de novo*
14	c.536G>T	p.Cys179Phe	–	*de novo*
15	–	–	arr[GRCh37]6q27 (170591663-170713885)x1; 122 kb deletion of *DLL1* and *FAM120B*	*de novo*

neuronal differentiation and causes CNS hyperplasia and an increased number of neurons,[5,8,18] and Delta-Notch signaling disrupting mutations in the *DLL1* zebrafish ortholog *DeltaA* (*dlA*) cause a wide range of pattering defects in the hindbrain and overproduction of neurons due to the lack of lateral inhibition.[12]

In the past, overlapping microdeletions of the chromosomal region 6q27 with a smallest region of overlap encompassing the genes *DLL1, THBS2* (MIM: 188061), *PHF10* (MIM: 613069), *ERMARD* (MIM: 615532), and others have been identified in individuals with developmental delay (DD), intellectual disability (ID), and brain malformations. These genes have been discussed as candidates for brain malformations,[19] but statistical analysis of large population sequencing data indicates that only *DLL1* is very likely intolerant to loss of function (LoF).[20] Combined with the functions of DLL1 and its homologs and the prior report of a *de novo* nonsense variant in an individual with autism spectrum disorder (ASD), this led us to hypothesize that insufficiency of DLL1 causes a human neurodevelopmental disorder.[19,21–23] We report 15 individuals from 12 unrelated families with DD, ID, ASD, seizures, brain malformations, and other multi-system features with heterozygous pathogenic variants in *DLL1* (Figure 1A). These findings establish *DLL1* as disease-associated gene and delineate the *DLL1*-related phenotypes.

Individuals with *DLL1* pathogenic variants were identified in several genetic centers across the world by exome sequencing (see Supplemental Subjects and Methods). The cohort was identified through GeneMatcher.[24] Referring physicians were asked to provide details on the medical history of the individuals. The referring clinicians obtained informed consent for genetic testing, publication of clinical and genetic data, re-evaluation of MR images, functional analysis of blood cells, and publication of facial photographs (if applicable). The ethics board of the involved institutions approved the study (Charité Berlin: protocol EA2/177/18; Clinical Research Ethics Board at the University of British Columbia [Vancouver, BC, Canada]: protocol H15-00092).

The clinical and molecular data of the 15 individuals with *DLL1* pathogenic variants (GenBank: NM_005618.3) are summarized in Tables 1 and 2. Detailed clinical descriptions of the individuals are provided in the Supplemental Note and Table S1. The most common features in our cohort were DD/ID (12/14), ASD (6/14), other behavioral abnormalities, seizures (6/14), and brain MRI abnormalities (11/15). We reviewed all but three brain MR images of the individuals; Figure 2 shows representative brain MR images. Brain abnormalities were non-specific and included hydrocephalus, ventriculomegaly, anomalies of the corpus callosum, cortical dysplasia, a small cerebellum/pons, and periventricular nodular heterotopias (PVNH) identified in single individuals. Two individuals had microcephaly, and one individual had macrocephaly due to severe hydrocephalus. Three individuals had kyphosis and/or scoliosis and spine radiographs of one individual with lumbar scoliosis (individual 8, F5/II-1 in Figure 1A) showed a segmentation defect of the lumbar spine (L2 half vertebra) and mild asymmetry of the S1 vertebra (Figures 1B). Clinical facial photographs of individuals 1, 6, 7, 9, and 11 (F1/II-1, F3/II-1, F4/II-1, F6/II-1, and F8/II-1 in Figure 1A, respectively) were available for comparison. Some individuals had mild facial

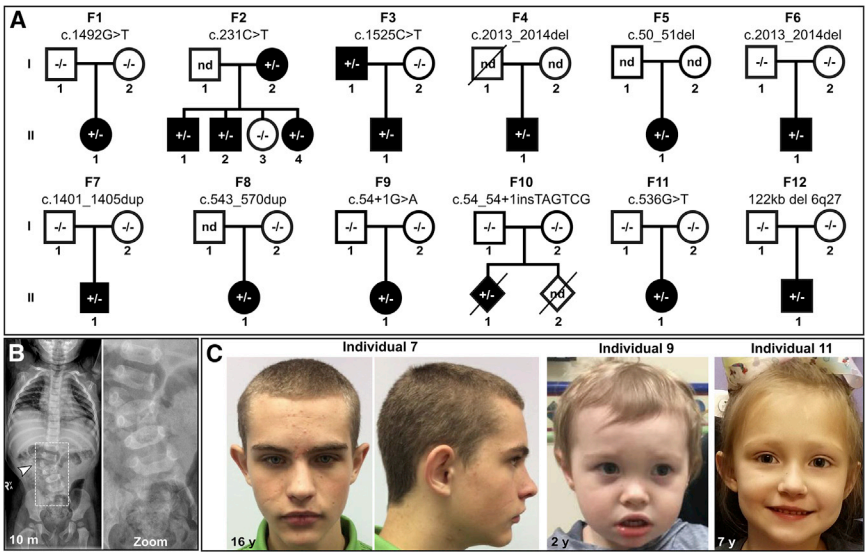

Figure 1. Pedigrees of the Affected Individuals and Phenotypic Presentation

(A) Twelve unrelated families with *DLL1* pathogenic variants. The *DLL1* variants occurred *de novo* in families (F) 1, 6, 7, 9, 10, 11, and 12. The *DLL1* variant co-segregated with the phenotype in F2 and F3. Inheritance is unknown due to lack of parenteral DNA samples for F4, F5, and F8.

(B) Radiographs of individual (I) 8 (F5/II-1 in A) at the age of 10 months demonstrate lumbar scoliosis (34°) due to a segmentation defect of the lumbar spine; the vertebral malformations included an incomplete fusion of the vertebral arch in L1, a right L2 hemivertebra, an abnormally shaped L3, and an asymmetric S1.

(C) Facial photographs of I7 (F4/II-1), I9 (F6/II-1), and I11 (F8/II-1) showing upslanted palpebral fissures and mild retrognathia (I7), a prominent forehead, upslanted palpebral fissures, epicanthal folds, broad and flat nasal bridge, full cheeks, everted upper lip, and full lips (I9) as well as upslanted palpebral fissures (I11).

dysmorphism, but we could not identify a distinctive facial gestalt associated with *DLL1* pathogenic variants. The facial dysmorphism found in more than one individual was upslanted palpebral fissures (Figure 1C).

We identified five different nonsense variants, two splice site variants affecting the canonical splice donor site of intron 2, one missense variant, and one 122 kb microdeletion encompassing *DLL1* and *FAM120B* (Figure 3A). Additional pathogenic variants and rare variants of unknown significance that were identified in some individuals are listed in Table S2. The same nonsense variant c.2013_2014del was present in the unrelated individuals 7 and 9. The variants were absent from public databases including gnomAD[20] (v.2.1). The variants were *de novo* in seven individuals; four individuals inherited the variants from an affected parent and inheritance is unknown in five individuals due to incomplete parental testing. All variants of unknown inheritance were nonsense variants and therefore classified as disease causing. All predicted premature stop codons are located before the penultimate exon and probably cause nonsense-mediated decay (Figure 3A). The only missense variant we found affects Cys179, the third amino acid in the DSL domain of DLL1 (individual 14, F11/II-1 in Figure 1A). This cysteine is invariably conserved in all Notch ligands (Figure 3B). No other amino acid changes affecting Cys179 or other highly conserved cysteines of the DSL domain of DLL1 and other Notch ligands are present in gnomAD or ClinVar, suggesting reduced tolerance to variation in this important domain (Figure 3B).[20,25–27] No LoF variants in *DLL1* are reported in control cohorts, indicating intolerance to LoF (gnomAD pLI score = 1.0). In individual 15 (F12/II-1 in Figure 1A) with the microdeletion of *DLL1*, the only other gene located within the deletion was *FAM120B* (MIM: 612266), a gene not known to be associated with neurodevelopmental issues and is expected to be tolerant to LoF (gnomAD pLI score = 0), suggesting that the deletion of *DLL1* causes the phenotype in this individual. Our preliminary genotype-phenotype analysis of this cohort did not identify significant correlations partly due to the small size of this cohort (Table 1).

For functional analysis of the splice site variant c.54+1G>A, we investigated mRNA extracted from LCLs of individual 12 (F9/II-1 in Figure 1A; see Supplemental Methods). Sanger sequencing of the exon-intron border of exon 2/intron 2 showed a retention of 12 intronic base pairs (bp), resulting in an in-frame insertion of four amino acids (p.Gln18_Val19insIleGlyGlyGln, Figure 3C). This insertion is located immediately downstream of the signal peptide of DLL1 (amino acids 1–17).[25] Analysis of the mutant sequence with Signal Peptide 4.0 predicted a shift of the cleavage site and a mature protein containing four additional amino acids at the N terminus.[28] Individual 13 (F10/II-1 in Figure 1A) was a fetus and material for RNA analysis was not available. Human Splicing Finder[29] predicted that the insertion c.54_54+1insTAGTCG leads to an alternative donor splice site after the insertion, resulting in a premature stop codon (p.Val19*, Figure 3D).

This study identified *de novo* and dominantly inherited heterozygous *DLL1* pathogenic variants (nonsense, missense, splice site, and gene deletion) as a cause of DD, ID, ASD, seizures, variable brain malformations, and scoliosis. The sequence variants identified and previous findings support haploinsufficiency as the mechanism for disease pathogenesis. The phenotype in our cohort shows great overlap with the phenotype of individuals with 6q27 deletions.[19] Multiple studies have delineated the roles of Notch signaling in neuronal development including neuronal migration.[3,30] The presence of neurodevelopmental features (ID/ASD and others) in our cohort further supports the neurodevelopmental role of DLL1, a critical node in this pathway. Brain malformations identified in our cohort included ventriculomegaly, hydrocephalus, abnormalities

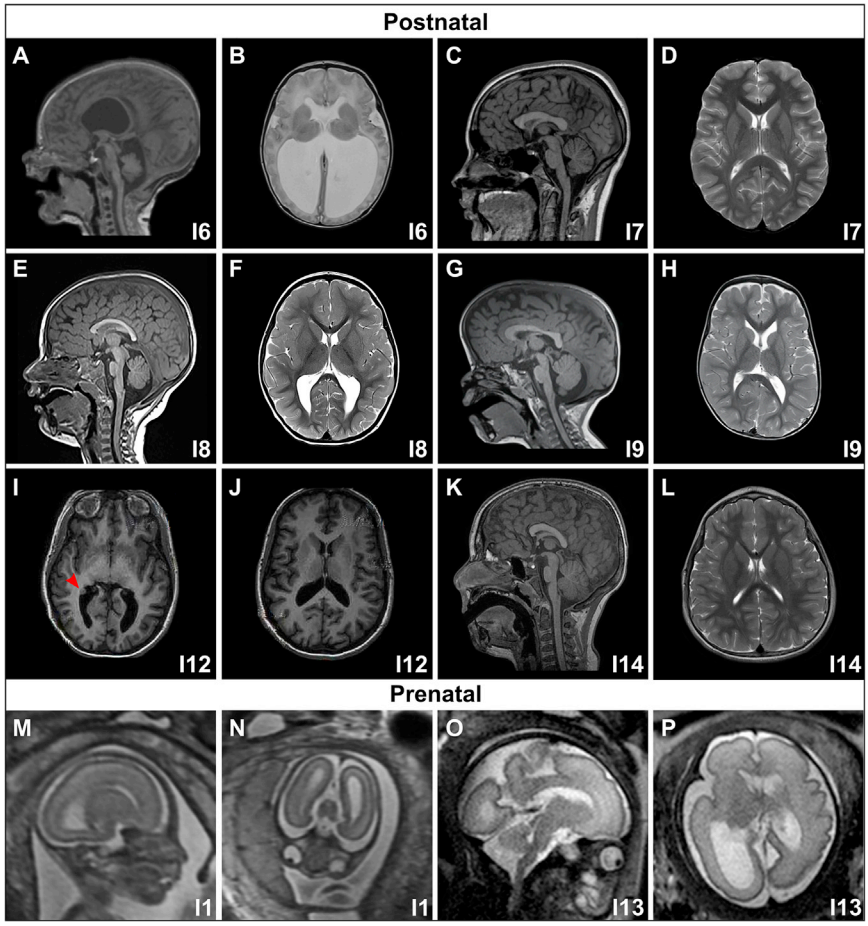

Figure 2. Representative Brain MR Images of the *DLL1* Cohort
(A and B) T1-midsagittal (A) and T2-axial (B) images of I6 showing severe hydrocephalus with a thin/stretched corpus callosum, thin brainstem, small cerebellum, and compression of the overlying cortex.
(C and D) T1-midsagittal (C) and T2-axial (D) images of I7 showing a mildly short and thick corpus callosum, and subtle areas of cortical dysplasia.
(E and F) T1-midsagittal (E) and T2-axial (F) images of I8 showing a mildly dysplastic corpus callosum, mild cortical dysplasia, and mild ventriculomegaly.
(G and H) T1-midsagittal (G) and T2-axial (H) images of I9 showing relatively large brain size with a prominent forehead, mildly dysplastic corpus callosum, and mildly dilated ventricles.
(I and J) T1-axial images of I12 showing PVNH (red arrowhead, I) with mild ventriculomegaly.
(K and L) T1-midsagittal (K) and T2-axial (L) images of I14 showing a mildly short and dysplastic corpus callosum, mildly small pons, and subtle cortical dysplasia.
(M and N) Prenatal T2 sagittal (M) and axial (N) images of I1 showing ventriculomegaly, with no good mid-sagittal views.
(O and P) Prenatal T2 sagittal (O) and axial (P) images of I13 showing asymmetric ventriculomegaly.

of the corpus callosum, mild cortical dysplasia, and a small cerebellum/pons. Notably, one individual had PVNH, which was also reported in patients with 6q27 deletions[21] and that we hypothesize is due to aberrant neuronal migration caused by *DLL1* haploinsufficiency.

It has also been proposed that variants in *DLL1* are associated with holoprosencephaly in humans. This hypothesis was based on the detection of 6q deletions encompassing *DLL1* and most particularly on the detection of an in-frame deletion and a missense variant in probands with holoprosencephaly.[21,31] However, both sequence variants (c.1802_1804del [p.Asp601_Ile602insVal] and c.2117C>T [p.Ser706Leu]) were inherited from a healthy parent. In current databases (gnomAD), c.1802_1804del has an allele frequency of 0.003 and is present in 13 individuals in a homozygous state, and c.2117C>T is present in four individuals in a heterozygous state. Only one of the deletions detected in individuals with holoprosencephaly is restricted to 6q27 (patient 4 in Dupé et al.[21]); the others are larger deletions that contain additional genes. Therefore, haploinsufficiency of other genes located in the deleted region could be causative for holoprosencephaly in these individuals. Further, on careful examination of brain MR imaging in our cohort, we did not detect holoprosencephaly. Taken together, further evidence is needed to confirm an association of *DLL1* haploinsufficiency with holoprosencephaly.

Among the individuals described herein, one had a missense variant (individual 14). The amino acid change p.Cys179Phe affects the third amino acid of the highly conserved DSL domain. Cys179 is predicted to participate in a disulfide bond with Cys188.[25] Disulfide bonds are important for protein folding and stability and are lost if a cysteine is replaced by any other amino acid.[32] Therefore, it is possible that the variant p.Cys179Phe affects correct folding of the DSL domain of DLL1 and interferes with physiological Notch signaling. Amino acid changes at the same position in the DSL domain of JAG1 (GenBank: NM_000214.2; p.Cys187Ser and p.Cys187Tyr) were found in two individuals with Alagille syndrome.[33,34] Combined with the phenotype of individual 14, this information suggests that the *de novo* variant p.Cys179Phe is disease causing and causes LoF.

In contrast to the large number of studies on *in vivo* and *in vitro* knockout (KO) models, there are few reports on *DLL1* haploinsufficiency. Rubio-Aliaga et al. found *Dll1* haploinsufficient mice to be smaller with altered fat tissue and lean mass ratio, higher energy uptake and metabolized energy, probably due to hyperactivity. The authors suspected a neurological phenotype as the cause of the hyperactivity, but this has not been analyzed in detail.[35] These results suggest that this mouse model might reflect the human phenotype at least partially and be suitable for

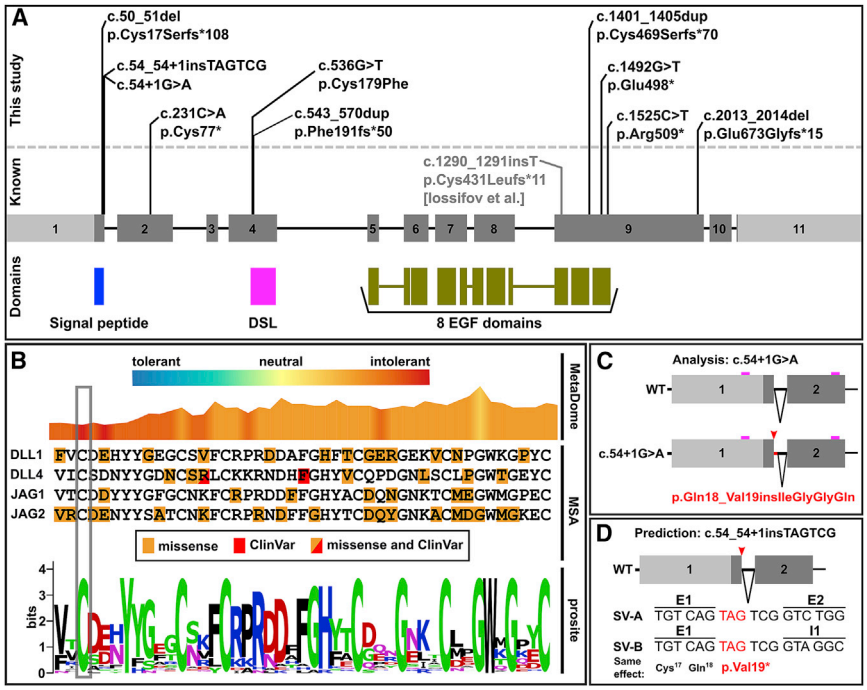

Figure 3. Spectrum of *DLL1* Pathogenic Variants and Functional Interpretations

(A) Schematic of the *DLL1* gene with *DLL1* variants annotated. A previously described LoF variant is indicated in gray.[23] The DLL1 protein domains are listed below.

(B) Sequence and variant characteristics of the DSL domain. The missense variant GenBank: NM_005618.3; p.Cys179Phe of I14 is highlighted in gray. Top: visualization of the MetaDome score.[26] Center: multi sequence alignment (MSA) of the DSL domain of genes annotated with DSL domain by Prosite (DLL1, DLL4, JAG1, JAG4). Positions with missense variants in gnomAD version 2.1 as well as pathogenic ClinVar variants are labeled (no frameshift and nonsense variants are present in gnomAD). Bottom: Conservation of the DSL domain (Prosite entry PS51051). Cysteine is conserved among DSL domains. The MetaDome score indicates the deleterious nature of any amino acid changes at this position.

(C) Scheme of the consequence of the splice site variant identified in I12. Alternative splicing of the allele carrying c.54+1G>A leads to the retention of 12 intronic base pairs (bp), resulting in an in-frame insertion of four amino acids (p.Gln18_Val19insIleGlyGlyGln).

(D) Predicted consequence of the insertion c.54_54+1insTAGTCG identified in I13. Human Splicing Finder[29] predicted that the insertion c.54_54+1insTAGTCG leads to an alternative donor splice site after the insertion, resulting in two different predicted transcript forms. Both lead to a premature stop codon. Boxes present exons, lines present introns; light gray boxes: untranslated regions; dark gray boxes: coding sequence; small boxes in magenta in (C): location of the sequencing primers; red arrows: location of the splice variants; black triangles: intronic sequence removed by splicing; SV-A: splice variant A; SV-B: splice variant B. E1 and E2 represent sequence from exon 1 and 2 respectively, I1 represents intron 1.

future investigations of the pathomechanism. Haploinsufficient mice also had metabolic and immunological abnormalities, and *DLL1* is important for lymphocyte development.[7,35] The individuals in this cohort did not have obvious abnormalities of their metabolism or immune system; only one individual (individual 8) had recurrent infections, which can be due to various factors. However, since the metabolic and immunological function of this cohort was not specifically investigated, a minor effect of the *DLL1* pathogenic variants on the metabolism and the immune system cannot be excluded at present.

Oscillatory expression of *DLL1* is essential for somite segmentation *in vivo*. A complete KO of *Dll1* in mice leads to defects of somite compartmentalization and epithelialization, inhibition of oscillatory *Dll1* expression in mice causes severe segmentation defects, and zebrafish *DeltaD* (*dlD*) mutants show disrupted somitogenesis.[5,16,36,37] KO of *Lfng* in mice, which enhances Notch activation from Dll1, has a similar effect,[38,39] and biallelic mutations in *LFNG* (MIM: 602576) cause spondylocostal dysostosis 3 (MIM: 609813) in humans.[40] In contrast, reduced *Dll1* expression in mice is associated with scoliosis and mild vertebral defects.[35,41,42] Four individuals in our cohort had scoliosis, and one had a segmentation defect of the spine. These results suggest that *DLL1* dosage at critical times during development is needed for correct somite segmentation.

To date, three other Notch ligands have been associated with human monogenic diseases: monoallelic pathogenic variants in *JAG1* (MIM: 601920) causing Alagille syndrome 1 (MIM: 118450)[43,44] and tetralogy of Fallot (MIM: 187500),[45] monoallelic pathogenic variants in *DLL4* (MIM: 605185) causing Adams-Oliver syndrome 6 (MIM: 616589),[46] and biallelic pathogenic variants in *DLL3* (MIM: 602768) causing spondylocostal dysostosis 1 (MIM: 602768).[47] Our data show that *DLL1* pathogenic variants are predominantly associated with a neurodevelopmental phenotype.

In summary, our study confirms heterozygous *DLL1* pathogenic variants as the cause of a variable neurodevelopmental phenotype and other multi-system features. This is consistent with the known role of DLL1 in the CNS and contributes to the understanding of Notch signaling in human development. Future studies are required to delineate fully the *DLL1*-associated phenotype, and functional *in vivo* and *in vitro* studies are needed to uncover the pathomechanism. Of particular interest are the effect of *DLL1* haploinsufficiency on lateral inhibition and oscillatory expression, and why the phenotype is predominantly neurological although DLL1 is essential for somite formation.

Supplemental Data

Supplemental Data can be found online at https://doi.org/10.1016/j.ajhg.2019.07.002.

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Declaration of Interests

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Web Resources

Bcftools, https://www.htslib.org/doc/bcftools.html
dbSNP, https://www.ncbi.nlm.nih.gov/snp/
GenBank, https://www.ncbi.nlm.nih.gov/genbank/
MGI, http://www.informatics.jax.org/
Mutalyzer, https://mutalyzer.nl/
OMIM, https://www.omim.org/
PubMed, https://www.ncbi.nlm.nih.gov/pubmed/
UCSC Genome Browser, https://genome.ucsc.edu/
Zebrafish Information Network, https://zfin.org

References

1. Bray, S.J. (2016). Notch signalling in context. Nat. Rev. Mol. Cell Biol. *17*, 722–735.
2. Kakuda, S., and Haltiwanger, R.S. (2017). Deciphering the Fringe-Mediated Notch Code: Identification of Activating and Inhibiting Sites Allowing Discrimination between Ligands. Dev. Cell *40*, 193–201.
3. Louvi, A., and Artavanis-Tsakonas, S. (2006). Notch signalling in vertebrate neural development. Nat. Rev. Neurosci. *7*, 93–102.
4. Bettenhausen, B., Hrabě de Angelis, M., Simon, D., Guénet, J.L., and Gossler, A. (1995). Transient and restricted expression during mouse embryogenesis of Dll1, a murine gene closely related to Drosophila Delta. Development *121*, 2407–2418.
5. Hrabě de Angelis, M., McIntyre, J., 2nd, and Gossler, A. (1997). Maintenance of somite borders in mice requires the Delta homologue DII1. Nature *386*, 717–721.
6. Campos, L.S., Duarte, A.J., Branco, T., and Henrique, D. (2001). mDll1 and mDll3 expression in the developing mouse brain: role in the establishment of the early cortex. J. Neurosci. Res. *64*, 590–598.
7. Hozumi, K., Negishi, N., Suzuki, D., Abe, N., Sotomaru, Y., Tamaoki, N., Mailhos, C., Ish-Horowicz, D., Habu, S., and Owen, M.J. (2004). Delta-like 1 is necessary for the generation of marginal zone B cells but not T cells in vivo. Nat. Immunol. *5*, 638–644.
8. Marklund, U., Hansson, E.M., Sundström, E., de Angelis, M.H., Przemeck, G.K., Lendahl, U., Muhr, J., and Ericson, J. (2010). Domain-specific control of neurogenesis achieved through patterned regulation of Notch ligand expression. Development *137*, 437–445.
9. Kawaguchi, D., Yoshimatsu, T., Hozumi, K., and Gotoh, Y. (2008). Selection of differentiating cells by different levels of delta-like 1 among neural precursor cells in the developing mouse telencephalon. Development *135*, 3849–3858.
10. Hiraoka, Y., Komine, O., Nagaoka, M., Bai, N., Hozumi, K., and Tanaka, K. (2013). Delta-like 1 regulates Bergmann glial monolayer formation during cerebellar development. Mol. Brain *6*, 25.
11. Dunwoodie, S.L., Henrique, D., Harrison, S.M., and Beddington, R.S. (1997). Mouse Dll3: a novel divergent Delta gene which may complement the function of other Delta homologues during early pattern formation in the mouse embryo. Development *124*, 3065–3076.
12. Riley, B.B., Chiang, M.Y., Storch, E.M., Heck, R., Buckles, G.R., and Lekven, A.C. (2004). Rhombomere boundaries are Wnt signaling centers that regulate metameric patterning in the zebrafish hindbrain. Dev. Dyn. *231*, 278–291.
13. Okigawa, S., Mizoguchi, T., Okano, M., Tanaka, H., Isoda, M., Jiang, Y.J., Suster, M., Higashijima, S., Kawakami, K., and Itoh, M. (2014). Different combinations of Notch ligands and receptors regulate V2 interneuron progenitor proliferation and V2a/V2b cell fate determination. Dev. Biol. *391*, 196–206.
14. Mahler, J., Filippi, A., and Driever, W. (2010). DeltaA/DeltaD regulate multiple and temporally distinct phases of notch signaling during dopaminergic neurogenesis in zebrafish. J. Neurosci. *30*, 16621–16635.
15. Cao, J., Spielmann, M., Qiu, X., Huang, X., Ibrahim, D.M., Hill, A.J., Zhang, F., Mundlos, S., Christiansen, L., Steemers, F.J., et al. (2019). The single-cell transcriptional landscape of mammalian organogenesis. Nature *566*, 496–502.
16. Shimojo, H., Ohtsuka, T., and Kageyama, R. (2011). Dynamic expression of notch signaling genes in neural stem/progenitor cells. Front. Neurosci. *5*, 78.
17. Artavanis-Tsakonas, S., Rand, M.D., and Lake, R.J. (1999). Notch signaling: cell fate control and signal integration in development. Science *284*, 770–776.
18. Yun, K., Fischman, S., Johnson, J., Hrabe de Angelis, M., Weinmaster, G., and Rubenstein, J.L. (2002). Modulation of the notch signaling by Mash1 and Dlx1/2 regulates sequential specification and differentiation of progenitor cell types in the subcortical telencephalon. Development *129*, 5029–5040.
19. Peddibhotla, S., Nagamani, S.C., Erez, A., Hunter, J.V., Holder, J.L., Jr., Carlin, M.E., Bader, P.I., Perras, H.M., Allanson, J.E., Newman, L., et al. (2015). Delineation of candidate genes responsible for structural brain abnormalities in patients with terminal deletions of chromosome 6q27. Eur. J. Hum. Genet. *23*, 54–60.

20. Karczewski, K.J., Francioli, L.C., Tiao, G., Cummings, B.B., Alföldi, J., Wang, Q., Collins, R.L., Laricchia, K.M., Ganna, A., Birnbaum, D.P., et al. (2019). Variation across 141,456 human exomes and genomes reveals the spectrum of loss-of-function intolerance across human protein-coding genes. bioRxiv. https://doi.org/10.1101/531210.
21. Dupé, V., Rochard, L., Mercier, S., Le Pétillon, Y., Gicquel, I., Bendavid, C., Bourrouillou, G., Kini, U., Thauvin-Robinet, C., Bohan, T.P., et al. (2011). NOTCH, a new signaling pathway implicated in holoprosencephaly. Hum. Mol. Genet. *20*, 1122–1131.
22. Li, Y., Choy, K.W., Xie, H.N., Chen, M., He, W.Y., Gong, Y.F., Liu, H.Y., Song, Y.Q., Xian, Y.X., Sun, X.F., and Chen, X.J. (2015). Congenital hydrocephalus and hemivertebrae associated with de novo partial monosomy 6q (6q25.3→qter). Balkan J. Med. Genet. *18*, 77–84.
23. Iossifov, I., Ronemus, M., Levy, D., Wang, Z., Hakker, I., Rosenbaum, J., Yamrom, B., Lee, Y.H., Narzisi, G., Leotta, A., et al. (2012). De novo gene disruptions in children on the autistic spectrum. Neuron *74*, 285–299.
24. Sobreira, N., Schiettecatte, F., Valle, D., and Hamosh, A. (2015). GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum. Mutat. *36*, 928–930.
25. UniProt Consortium (2018). UniProt: the universal protein knowledgebase. Nucleic Acids Res. *46*, 2699.
26. Wiel, L., Baakman, C., Gilissen, D., Veltman, J.A., Vriend, G., and Gilissen, C. (2019). MetaDome: Pathogenicity analysis of genetic variants through aggregation of homologous human protein domains. Hum. Mutat. Published online May 22, 2019. https://doi.org/10.1002/humu.23798.
27. Landrum, M.J., Lee, J.M., Benson, M., Brown, G.R., Chao, C., Chitipiralla, S., Gu, B., Hart, J., Hoffman, D., Jang, W., et al. (2018). ClinVar: improving access to variant interpretations and supporting evidence. Nucleic Acids Res. *46* (D1), D1062–D1067.
28. Petersen, T.N., Brunak, S., von Heijne, G., and Nielsen, H. (2011). SignalP 4.0: discriminating signal peptides from transmembrane regions. Nat. Methods *8*, 785–786.
29. Desmet, F.O., Hamroun, D., Lalande, M., Collod-Béroud, G., Claustres, M., and Béroud, C. (2009). Human Splicing Finder: an online bioinformatics tool to predict splicing signals. Nucleic Acids Res. *37*, e67.
30. Conti, V., Carabalona, A., Pallesi-Pocachard, E., Parrini, E., Leventer, R.J., Buhler, E., McGillivray, G., Michel, F.J., Striano, P., Mei, D., et al. (2013). Periventricular heterotopia in 6q terminal deletion syndrome: role of the C6orf70 gene. Brain *136*, 3378–3394.
31. Dubourg, C., Carré, W., Hamdi-Rozé, H., Mouden, C., Roume, J., Abdelmajid, B., Amram, D., Baumann, C., Chassaing, N., Coubes, C., et al. (2016). Mutational Spectrum in Holoprosencephaly Shows That FGF is a New Major Signaling Pathway. Hum. Mutat. *37*, 1329–1339.
32. Betts, M.J., and Russell, R.B. (2003). Amino Acid Properties and Consequences of Substitutions. Bioinformatics for Geneticists (John Wiley & Sons, Ltd.), pp. 289–316.
33. Jurkiewicz, D., Popowska, E., Gläser, C., Hansmann, I., and Krajewska-Walasek, M. (2005). Twelve novel JAG1 gene mutations in Polish Alagille syndrome patients. Hum. Mutat. *25*, 321.
34. Crosnier, C., Driancourt, C., Raynaud, N., Dhorne-Pollet, S., Pollet, N., Bernard, O., Hadchouel, M., and Meunier-Rotival, M. (1999). Mutations in JAGGED1 gene are predominantly sporadic in Alagille syndrome. Gastroenterology *116*, 1141–1148.
35. Rubio-Aliaga, I., Przemeck, G.K., Fuchs, H., Gailus-Durner, V., Adler, T., Hans, W., Horsch, M., Rathkolb, B., Rozman, J., Schrewe, A., et al. (2009). Dll1 haploinsufficiency in adult mice leads to a complex phenotype affecting metabolic and immunological processes. PLoS ONE *4*, e6054.
36. van Eeden, F.J., Granato, M., Schach, U., Brand, M., Furutani-Seiki, M., Haffter, P., Hammerschmidt, M., Heisenberg, C.P., Jiang, Y.J., Kane, D.A., et al. (1996). Mutations affecting somite formation and patterning in the zebrafish, Danio rerio. Development *123*, 153–164.
37. Kawamura, A., Koshida, S., Hijikata, H., Ohbayashi, A., Kondoh, H., and Takada, S. (2005). Groucho-associated transcriptional repressor ripply1 is required for proper transition from the presomitic mesoderm to somites. Dev. Cell *9*, 735–744.
38. Zhang, N., and Gridley, T. (1998). Defects in somite formation in lunatic fringe-deficient mice. Nature *394*, 374–377.
39. Evrard, Y.A., Lun, Y., Aulehla, A., Gan, L., and Johnson, R.L. (1998). lunatic fringe is an essential mediator of somite segmentation and patterning. Nature *394*, 377–381.
40. Sparrow, D.B., Chapman, G., Wouters, M.A., Whittock, N.V., Ellard, S., Fatkin, D., Turnpenny, P.D., Kusumi, K., Sillence, D., and Dunwoodie, S.L. (2006). Mutation of the LUNATIC FRINGE gene in humans causes spondylocostal dysostosis with a severe vertebral phenotype. Am. J. Hum. Genet. *78*, 28–37.
41. Cordes, R., Schuster-Gossler, K., Serth, K., and Gossler, A. (2004). Specification of vertebral identity is coupled to Notch signalling and the segmentation clock. Development *131*, 1221–1233.
42. Sparrow, D.B., Chapman, G., Smith, A.J., Mattar, M.Z., Major, J.A., O'Reilly, V.C., Saga, Y., Zackai, E.H., Dormans, J.P., Alman, B.A., et al. (2012). A mechanism for gene-environment interaction in the etiology of congenital scoliosis. Cell *149*, 295–306.
43. Oda, T., Elkahloun, A.G., Pike, B.L., Okajima, K., Krantz, I.D., Genin, A., Piccoli, D.A., Meltzer, P.S., Spinner, N.B., Collins, F.S., and Chandrasekharappa, S.C. (1997). Mutations in the human Jagged1 gene are responsible for Alagille syndrome. Nat. Genet. *16*, 235–242.
44. Li, L., Krantz, I.D., Deng, Y., Genin, A., Banta, A.B., Collins, C.C., Qi, M., Trask, B.J., Kuo, W.L., Cochran, J., et al. (1997). Alagille syndrome is caused by mutations in human Jagged1, which encodes a ligand for Notch1. Nat. Genet. *16*, 243–251.
45. Eldadah, Z.A., Hamosh, A., Biery, N.J., Montgomery, R.A., Duke, M., Elkins, R., and Dietz, H.C. (2001). Familial Tetralogy of Fallot caused by mutation in the jagged1 gene. Hum. Mol. Genet. *10*, 163–169.
46. Meester, J.A., Southgate, L., Stittrich, A.B., Venselaar, H., Beekmans, S.J., den Hollander, N., Bijlsma, E.K., Helderman-van den Enden, A., Verheij, J.B., Glusman, G., et al. (2015). Heterozygous Loss-of-Function Mutations in DLL4 Cause Adams-Oliver Syndrome. Am. J. Hum. Genet. *97*, 475–482.
47. Bulman, M.P., Kusumi, K., Frayling, T.M., McKeown, C., Garrett, C., Lander, E.S., Krumlauf, R., Hattersley, A.T., Ellard, S., and Turnpenny, P.D. (2000). Mutations in the human delta homologue, DLL3, cause axial skeletal defects in spondylocostal dysostosis. Nat. Genet. *24*, 438–441.