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BRIEF REPORT

Heterozygous variants in *CTR9*, which encodes a major component of the PAF1 complex, are associated with a neurodevelopmental disorder

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ABSTRACT

Purpose: CTR9 is a subunit of the PAF1 complex (PAF1C) that plays a crucial role in transcription regulation by binding CTR9 to RNA polymerase II. It is involved in transcription-coupled histone modification through promoting H3K4 and H3K36 methylation. We describe the clinical and molecular studies in 13 probands, harboring likely pathogenic *CTR9* missense variants, collected through GeneMatcher.

Methods: Exome sequencing was performed in all individuals. CTR9 variants were assessed through 3-dimensional modeling of the activated human transcription complex Pol II-DSIF-PAF-SPT6 and the PAF1/CTR9 complex. H3K4/H3K36 methylation analysis, mitophagy assessment based on tetramethylrhodamine ethyl ester perchlorate immunofluorescence, and RNA-sequencing in skin fibroblasts from 4 patients was performed.

Results: Common clinical findings were variable degrees of intellectual disability, hypotonia, joint hyperlaxity, speech delay, coordination problems, tremor, and autism spectrum disorder. Mild dysmorphism and cardiac anomalies were less frequent. For 11 *CTR9* variants, de novo occurrence was shown. Three-dimensional modeling predicted a likely disruptive effect of the variants on local CTR9 structure and protein interaction. Additional studies in fibroblasts did not unveil the downstream functional consequences of the identified variants.

Conclusion: We describe a neurodevelopmental disorder caused by (mainly) de novo variants in *CTR9*, likely affecting PAF1C function.

Introduction

The PAF1 complex (PAF1C) is a highly conserved transcriptional regulator that consists of PAF1, CTR9, LEO1, CDC73, RTF1, and WDR61. CTR9 plays a crucial role in the transcriptional function of PAF1C by binding to the RNA polymerase II. PAF1C is also involved in transcription-coupled histone modification, including H2B monoubiquitination as well as H3K4 and H3K36 methylation,[1,2] and recruits the ATP-dependent chromatin remodeling enzyme CHD1.[3] In yeast, Paf1C, especially through Paf1 and Ctr9, has been shown to be involved in the induction of mitophagy, ie, a gatekeeper of cellular metabolism that controls degradation of superfluous or damaged mitochondria.[4] In zebrafish, deletion of *ctr9* leads to abnormal heart and neural crest development through dysregulation of Notch signaling.[5,6] Studies of mutant *Ctr9* in Drosophila show a key role in nervous system development.[7] In addition, in rat brain, *Ctr9* was shown to interact with dopamine transporters in dopaminergic neurons,

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independent of Paf1C, suggesting a dual nuclear and cytoplasmic function.[8]

Although PAF1C appears crucial for development, developmental disorders have not been associated with genetic variants in most of its components. Only *LEO1* variants were recently linked to neurodevelopmental disease, including autism spectrum disorder (ASD), developmental delay, and neurobehavioral problems.[9]

Because heterozygous *CTR9* variants have been reported in 4 unrelated families with Wilms tumor, *CTR9* was previously identified as a Wilms tumor predisposition gene.[10] In 3 of these families the pathogenic variants affected the consensus splice site, resulting in an in-frame exon 9 deletion, whereas in the fourth family a truncating variant in exon 2 was identified. Further studies in affected tissue derived from these respective patients showed absence of the wild-type *CTR9* allele, indicative of a second hit in the tumor.[10]

Three independent de novo *CTR9* missense variants c.1405G>A, p(Glu469Lys),[11] c.2488C>T, p.(Arg830Trp),[12] and c.2515C>T, p(Arg839Trp)[13] were previously identified as singletons in 3 large intellectual disability (ID),[11] ASD,[12] and developmental disorder[13] cohorts respectively, however, without phenotypic details.

In this article, we report 13 patients, with (predominantly de novo) heterozygous *CTR9* missense variants, suffering from a neurodevelopmental disorder characterized by varying degrees of ID, neurodevelopmental delay, hypotonia, fatigability, behavioral abnormalities including ASD, anxiety and aggressive behavior, cardiac anomalies, and mild facial dysmorphism.

Materials and Methods

Patients and samples

Patient collection was established using the web-based platform GeneMatcher.[14] The study was approved by the Institutional Ethics Review Boards and informed consents were obtained from the legal guardians of all participating subjects. A separate informed consent for publication of identifiable photographs was provided.

Exome sequencing

Exome sequencing (ES), data annotation, and variant filtering were performed at different centers. Details on ES methodology for each case are described in Supplemental Data 1. Variants were classified on the basis of American College of Medical Genetics and Genomics/Association for Molecular Pathology guidelines for variant classification.[15]

Structural modeling of *CTR9* variants

Available structures of human CTR9 were used; ie, 2 electron microscopy structures of the activated human transcription complexes Pol II-DSIF-PAF-SPT6 (Protein DataBase [PDB]: 6TED [residues 3-892][16] and 6GMH [residues 3-892][17]), both with 3.1 Å resolution. To evaluate the effect of N-terminal CTR9 variants, the cryogenic electron microscopy (cryo-EM) structure of the human PAF1/CTR9 complex (2.53 Å resolution), containing residues 3 to 244 of CTR9 and residues 57 to 116 of PAF1 (PDB: 5ZYQ[18]) was used. Variant impact on protein stability was studied using FoldX 5.0.[19] Side chain replacements (wild type to variant) were performed on the 5ZYQ cryo-EM structure and were repeated 5 times for each variant. Differences in energy between the folded and unfolded state, referred to as protein stability, were calculated for the wild-type and mutant protein and averaged over 5 mutant structures. Visualization and manipulation of the structures was performed using the UCSF Chimera software.[20]

Functional analysis of *CTR9* variants

Methodologies used for fibroblast culture, H3K4 and H3K36 methylation analysis, mitochondrial membrane potential analysis, RNA extraction, and RNA-sequencing (RNA-seq) are described in Supplemental Data 2.

Results

Identification of *CTR9* variants

Trio-based ES in case 1 and both unaffected parents revealed the presence of heterozygous de novo p.(Glu15Lys) variant in *CTR9* (OMIM 609366; RefSeq accession number NM_014633.5; hg19) in the patient. This variant is absent in Genome Aggregation Database (gnomAD) v2.1.1, affects an amino acid residue that is highly conserved up to *Drosophila melanogaster* (Supplemental Figure 1), and is predicted to be damaging by multiple in silico prediction programs (Supplemental Table 1). Using GeneMatcher, we identified 12 additional individuals with *CTR9* missense variants and neurodevelopmental phenotypes. In 10 of these individuals, the variant was *de novo*. In case 3, the variant was inherited from the affected father and in case 10, only absence in maternal DNA was shown. Except for 2 variants (p.[Asn455Ser] [1/251322] and p.[Arg878Gln] [1/249646] in case 9 and 12 respectively), all variants were absent in gnomAD v2.1.1. Moreover, all affect highly conserved residues and in silico predictions largely suggest deleterious effects (Supplemental Figure 1 and Supplemental Table 1). Interestingly, the p.(Glu376Lys) variant, affecting a CpG dinucleotide, was found in 3 de novo cases, suggesting a mutational hotspot. Variant positions are depicted in Figure 1A and Supplemental Figure 2.

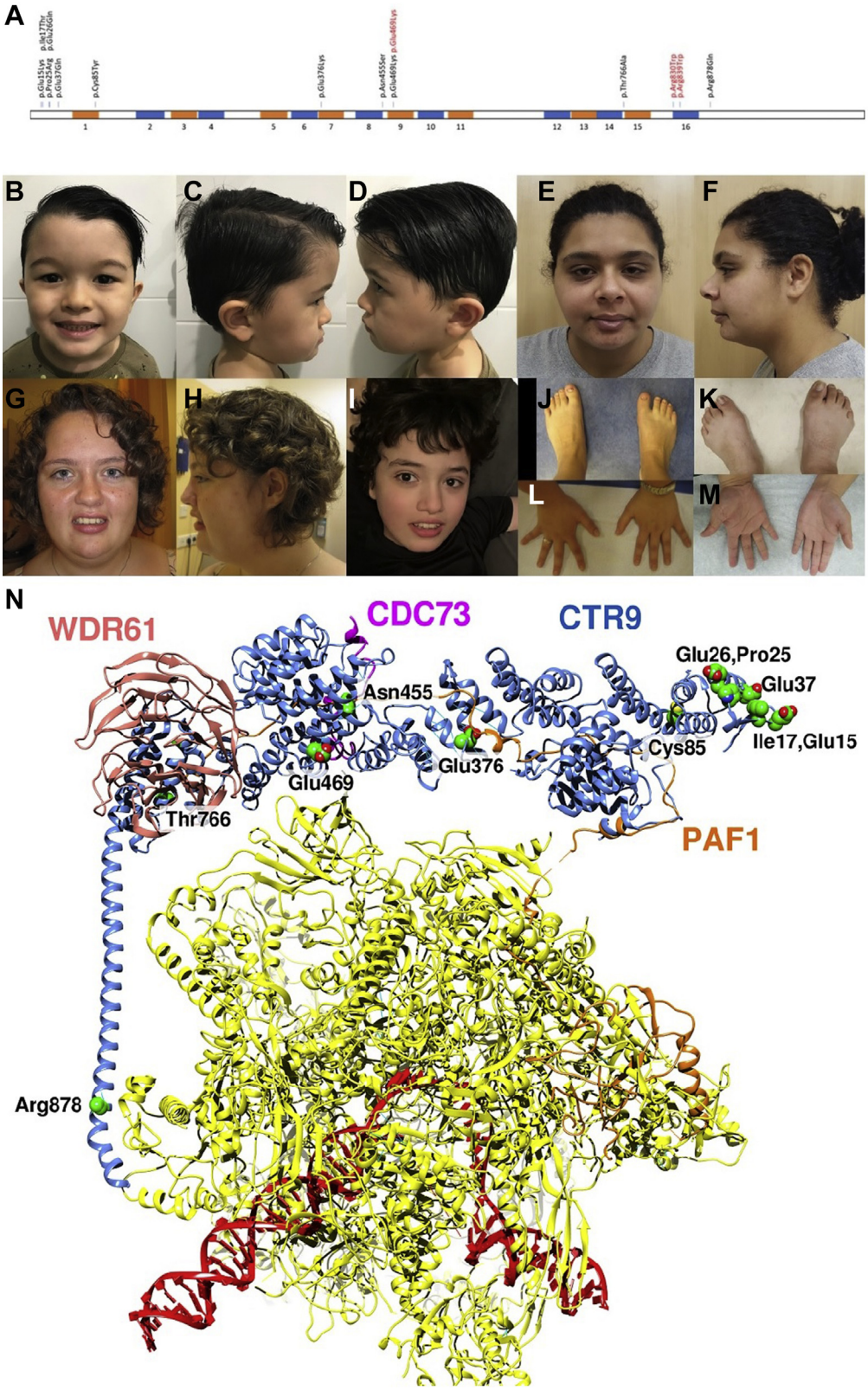

Figure 1 CTR9 variant localization, clinical pictures, and modeling of CTR9 structure and mutated residues. A. Localization of CTR9 variants at protein level. Representation of the CTR9 protein, including the localization of the tetratricopeptide repeats in orange/ blue, numbered 1-16. Previously reported missense variants are indicated in red;[13-15] variants reported in this paper are presented in black (Reference Sequence [RefSeq] accession number NM_014633). B-M. Mild dysmorphism in individuals harboring *CTR9* variants. (B, C, D) Facial features of case 1 showing a high forehead, arched eyebrows, and a left-sided ear pit with slightly low-set ears. (E, F) Facial features of case 10, showing arched eyebrows, a full nasal tip, retrognathia, and full lips. (G, H) Facial features of case 4, comprising a high forehead

Clinical description of individuals harboring *CTR9* variants

We describe 13 cases with heterozygous *CTR9* missense variants presenting with a variable neurodevelopmental disorder. Clinical features are summarized in Table 1 and individual cases are described in Supplemental Data 3.

Cognition

All cases showed impaired cognitive abilities to variable extent. ID was present in all but 3 cases (4, 10, and 12), ranging from mild to severe. Although case 4 and case 10 did not meet the strict criteria for ID (total intelligence quotient > 70), both showed significant impairment in other neurodevelopmental domains. The least affected case (case 12) had an intelligence quotient of 93, but experiences learning difficulties, whereas both parents are highly educated.

Neurodevelopment

Motor development was complicated by hypotonia in 9 cases, and in 6 cases, joint hyperlaxity was obvious. Other recurrent findings included speech delay ($n = 9$), coordination problems or tremor ($n = 6$), and muscular weakness ($n = 3$).

Brain magnetic resonance imaging, performed in 6 cases, showed nonspecific abnormalities in 2, comprising delayed myelination, short/thin corpus callosum, and ventricular dilatation.

Behavior

ASD was present in 4 cases, of which, 2 showed developmental regression and severe behavioral problems with aggression during late childhood. Psychotic episodes, aggression, automutilation, attention deficit hyperactivity disorder, stereotypies, anxiety, and mood disorders were also reported in a subset of cases.

Visceral findings

In total, 4 patients showed cardiac abnormalities comprising infantile thoracic aortic aneurysm, ventricular septal defect, mild pulmonary valve stenosis, and supravalvular aortic stenosis presenting in childhood with normal findings on an early echocardiogram.

Dysmorphic features

Variable nonspecific, subtle dysmorphic features were noted (Figure 1B-M); some recurrent features included hypertelorism, micro/retrognathia, and a broad, flat nasal bridge. A total of 9 cases showed feet abnormalities comprising club feet, varus position of the forefoot, flat feet, sandal gap, and clinodactyly, although some of these foot features are also common in the general population.

Other

Five cases had failure to thrive and/or feeding problems, including infantile feeding difficulties and selective feeding. Fatigability and unexplained pain were recurrently seen.

In silico structural modeling of *CTR9* variants

Positions of the affected residues on the cryo-EM structure of CTR9 are depicted in Figure 1N. A detailed analysis of the variants is presented in Supplemental Data and Supplemental Figures 3, 4, and 5, but in general, the variants described in this article can be divided into 3 categories on the basis of their probable consequences for the structure of the PAF1C complex. A first group of variants most probably destabilizes the local CTR9 protein structure. Variants p.(Ile17Thr), p.(Pro25Arg), p.(Glu26Gln), p.(Glu37Gln), and p.(Cys85Tyr) most likely destabilize the N-terminal part of CTR9, possibly also influencing the interactions of CTR9 with PAF1 protein (Supplemental Figure 3, Supplemental Table 2). A second group of variants is predicted to perturb the PAF1 interactions in the tetratricopeptide repeats regions. The p.(Glu469Lys), p.(Glu376Lys), p.(Asn455Ser), and p.(Thr766Ala) variants most probably influence the interactions of CTR9 with PAF1 protein either directly or indirectly, by inducing changes in the local conformation of the tetratricopeptide repeats region domain of CTR9 (Supplemental Figure 4). Finally, a third class of variants influences the interactions with other PAF1C complex subunits. The variant p.(Glu15Lys) does not influence the structural stability of CTR9 (Supplemental Table 2). Therefore we assume that its phenotype is likely caused by an effect on the yet unknown interactions on the N-terminal part of CTR9. The substitution of Arg878 to Gln weakens the interactions of CTR9 "trestle" with Pol II subunit E (Supplemental Figure 5).

Functional analysis of CTR9 variants

H3K4/H3K36 methylation analysis, mitochondrial quality assessment, and RNA-seq in fibroblasts did not provide conclusive evidence for downstream functional

with broad eyebrows, attached ear lobules, full alae nasi, full lips, a high palate, and a prominent chin. (I) Facial features of case 5, showing flared eyebrows and a full lower lip. (J, K, L, M) Hands and feet of cases 4 (J, L) and 10 (K, M). Case 10 shows mild hallux valgus and broad feet with short toes. N. Structure of CTR9 in RNA PAF II complex and the positions of mutated residues. Cryogenic electron microscopy (cryo-EM) structures of the activated RNA Pol-II complex (6TED.pdb, 5ZYQ.pdb) with positions of CTR9 mutated residues analyzed in this study. Crystallographic structure of N-terminal part of CTR9 (5ZYQ.pdb) was overlapped with the cryo-EM structure of the complex. CTR9 protein is presented in cornflower blue, WDR61 protein in salmon, PAF1 protein in orange, CDC73 in magenta, RNA in red, and rest of the complex in yellow. Wild-type residues of CTR9, analyzed in this study, are presented in green color in sphere representation. The heteroatoms of analyzed residues are colored as follows: oxygen in red, nitrogen in blue, and sulfur in yellow.

Table 1 Clinical features of patients harboring *CTR9* variants

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10	Case 11	Case 12	Case 13
CTR9 variant (NM_014633.5; hg19)	c.43G>A, p.(Glu15Lys)	c.109G>C, p.(Glu37Gln)	c.50T>C, p.(Ile17Thr)	c.1405G>A, p.(Glu469Lys)	c.254G>A, p.(Cys85Tyr)	c.76G>C, p.(Glu26Gln)	c.1126G>A, p.(Glu376Lys)	c.1126G>A, (p.Glu376Lys)	c.1364A>G, p.(Asn455Ser)	c.2296A>G, p.(Thr766Ala)	c.74C>G p.(Pro25Arg)	c.2633G>A p.(Arg878Gln)	c.1126G>A, p.(Glu376Lys)
Inheritance	De novo	De novo	Paternal inheritance	De novo	De novo	De novo	De novo	De novo	De novo	Unknown (not maternal)	De novo	De novo	De novo
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M
Growth parameters													
Birth weight, g	3830	3460	3650	3575 (+1 SD)	3850 (+0.1 SD)	ND	950 (28 WG)	ND	2750	2270 (32 WG)	3100	Average	2350 (34 WG)
Age at assessment	6 y 10 m	9.5 y	18 m	18 y	12 y	20 y	3 y 3 m	15 y	31 m	22 y	17 m	7 y	6 y
Height, cm (SD)	109 (–2.1 SD)	138 (+0.49 SD)	78 (–1.18 SD)	160 (–1.7 SD)	151 (+0.2 SD)	150.3 (–1.99 SD)	98 (+0.51 SD))	ND	93.7 (+0.39 SD)	153.8 (–1.46 SD)	77.5 (–0.49 SD)	120.1 (–1.6 SD)	121.7 (+1 SD)
Weight, kg (SD)	17.5 (–2 SD)	34.7 (+ 0.5 SD)	9.9 (–1.55 SD)	79.2 (+3 SD)	31 (–2 SD)	68.3 (+0.67 SD)	13.2 (–0.75 SD)	ND	13.7 (+0.08 SD)	80.5 (+1.41 SD)	9.7 (–1.04 SD)	24.9 (–0.1 SD)	29 (+3 SD)
HC, cm (SD)	ND	54.5 (+1.87 SD)	50 (+1.5 SD)	56.8 (+1 SD)	ND	ND	52 (+2 SD)	ND	47.1 (–1.39 SD)	53.8 (-0.47 SD)	50 (+ 2.83 SD)	53.8 (+1.4 SD)	52.5 (+0.5 SD)
Hypertelorism	–	+	+	–	+	–	+	ND	ND	–	–	+	–
Broad and flat nasal bridge	–	–	+	–	+	ND	–	ND	+	–	–	+	–
Joint hyperlaxity	+	+++	–	+	–	ND	+	ND	ND	+	+	–	–
Skeletal findings	Overpronation of the forefoot, slight pectus excavatum, flat feet	Varus of the forefoot, flat feet	–	Flat feet	Flat feet	ND	Flat feet	ND	ND	Hallux valgus, brachydactyly, flat feet	Pronated feet, second toe overlapping first and third toes, clinodactyly of the fifth toes, flat feet	Mild clinodactyly of the fifth finger, sandal gap both feet, flat feet	Rigidity of the lower limbs, tapered fingers, clinodactyly of the fifth digits
Other dysmorphic features	Slightly velvety skin	Rounded forehead, flat, long philtrum, wide mouth, low-set ears, retrognathia	ND	–	Velvety skin	ND	ND	ND	Epicanthal folds, bulbous nose with hypoplastic nasal tip, retrognathia	Tapered fingers	Deep set eyes, prominent forehead, upslanted palpebral fissures, broad nasal tip	Epicanthal folds, sparse lateral eyebrows, long and flat philtrum, thin upper lip, retrognathia	Synophrys, small mouth, almond eyes, inverted nipples
Delayed early milestones	+	+	+	–	–	–	+	+	+	+	+	–	+
Hypotonia	+	+	+	–	–	–	+	+	+	+	+	–	+
Motor delay	+	+	+	±	–	–	+	ND	+	+	+	–	+
Speech delay	+	+	–	–	+	+	+	–	+	+	+	–	+
Regression	–	–	–	–	+	+	–	–	±	ND	ND	–	ND
Intellectual disability	Learning difficulties	+, mild	ND yet	Disharmonic IQ (VIQ 103, PIQ 75)	+ (severe)	+	+, mild to moderate	+, mild	+	+, FSIQ 77	ND	–, IQ 93, learning difficulties	+, IQ between 58-70
ASD	–	–	–	+	+	+	ND	ND	ND	ND	ND	–	+
Behavioral abnormalities	–	–	Tantrums, agitation, ADHD	Psychotic episodes	Aggressive behavior, automutilation, ADHD		–	ADHD	Stereotypies with hand flapping	Mood disorder, OCD, PTSD, reactive attachment disorder, ADHD	ND	Hand flapping when happy, social-emotional delay	Social-emotional delay, anxiety, ADHD
Other neurological findings	Balance problems	Balance problems with gait difficulties	ND	Balance/ coordination problems, muscle weakness	–	ND	ND	Uncoordination, numbness in feet, muscle weakness	Balance difficulties	History of dystonia, tremors, muscle weakness	ND	–	–
Brain MRI	ND	Normal	Short CC, delayed myelination	ND	Normal	Normal	Delayed myelination, thin CC, ventricular dilatation	ND	No MRI	Normal	No MRI, normal head ultrasound	No MRI	No MRI

(continued)

Table 1 Continued

	Case 1	Case 2	Case 3	Case 4	Case 5	Case 6	Case 7	Case 8	Case 9	Case 10	Case 11	Case 12	Case 13
Cardiovascular findings	TAA, BAV, brain vessel tortuosity	–	Subaortic valve stenosis	Normal cardiac evaluation	–	VSD	ND	ND	Normal ultrasound at 1 week	History of mild PVS	Normal fetal ultrasound	Normal cardiac evaluation	–
Ocular findings	–	Astigmatism, hypermetropia	–	Intermittent esotropia, anisometropia	Abnormal ocular movements	–	ND	ND	Esotropia, bilateral, astigmatism	Glasses	ND	–	–
Unexplained pain	–	Unexplained pain in legs	ND	Unexplained pain (back and legs)	Unexplained pain episodes	ND	ND	ND	ND	ND	ND	–	Unexplained pain in knee
Easy fatigability	ND	+	ND	+	ND	ND	+	+	+	ND	ND	+	ND
Additional findings	FTT, eczema, constipation		ND	Constipation	FTT since age 10 y, phases of anorexia/ hyperphagia	Neonatal hepatomegaly and hemolytic anemia	FTT in the first 2 years of life, poor muscle mass	Constipation	Neonatal feeding difficulties	IBS type issues, headaches, dizziness, hot/ cold intolerance	FTT in the first few weeks, constipation	–	Infantile feeding difficulties

ADHD, attention deficit hyperactivity disorder; *ASD*, autism spectrum disorder; *BAV*, bicuspid aortic valve; *CC*, corpus callosum; *F*, female; *FSIQ*, Full-Scale Intelligence Quotient; *FTT*, failure to thrive; *HC*, Head Circumference; *IBS*, irritable bowel syndrome; *IQ*, intelligence quotient; *OCD*, obsessive compulsive disorder; *M*, male; *MRI*, magnetic resonance imaging; *ND*, not documented; *PIQ*, Performance Intelligence Quotient; *PTSD*, post-traumatic stress disorder; *PVS*, pulmonary valve stenosis; *TAA*, thoracic aortic aneurysm; *VIQ*, Verbal Intelligence Quotient; *VSD*, ventricle septum defect; *WG*, weeks of gestation.

consequences of the likely disease-causing variants (Supplemental Data 4 and Supplemental Figures 6-8).

Discussion

We report heterozygous missense variants in *CTR9*, a main component of the PAF1C, in 13 unrelated cases presenting with a neurodevelopmental disorder. De novo occurrence of the variants was documented in 11 cases; in 1 case the variant was inherited from a mildly affected father and in 1 case only the mother was available for testing. The missense variants are spread over the *CTR9* gene. A hotspot variant (p.Glu376Lys) was recurrent in 3 cases (case 7, 8, and 13). The only consistent finding between these 3 cases seems to be developmental delay and ID. Overall, the neurodevelopmental disorder in our cases includes variable ID (ranging from borderline intelligence to mild/moderate) and delayed motor and speech development. In several cases, motor development is hampered by hypotonia and joint hypermobility. Behavioral problems are prominent in the phenotypic spectrum, with reports of ASD, sometimes associated with regression, aggressive and self-injurious behavior, attention deficit hyperactivity disorder, anxiety, and mood disorders.

Interestingly, only missense variants were identified, suggesting a dominant-negative effect of the variants in our cohort. The constraint *z*-score for missense variants in CTR9 in the gnomAD v2.1.1 is 4.3, suggesting intolerance to missense variation. The hypothesized dominant-negative effect is supported by the prior identification of loss-of-function variants in patients with Wilms tumor in the absence of neurodevelopmental problems.[10] Homozygous knockout mice from Wellcome Trust Sanger Institute consortium ($Ctr9^{tm1b(EUCOMM)Wtsi}/Ctr9^{tm1b(EUCOMM)Wtsi}$) showed embryonic lethality (http://www.informatics.jax.org/marker/phenotypes/MGI:109345).

In yeast, Paf1c is a known regulator of H2B monoubiquitylation, mediated by the Rad6/Bre1 complex, and of H3K4 and H3K36 di and trimethylation, mediated by Set1 and Set2 respectively.[1] Pathogenic variants in the human orthologues of these genes/complexes under PAF1C regulation including *UBE2A* (*Rad6*), *SETD1A*/*SETD1B* (*Set1*), and *SETD2* (*Set2*) were recently associated with neurodevelopmental disorders.[21-23] Associated phenotypes show an important overlap with our *CTR9* cases, including ID, developmental delay, ASD, and other psychiatric and behavioral manifestations, including aggressive outbursts (Supplemental Table 3). We were unable to show differential H3K4 or H3K36 methylation in *CTR9* fibroblasts compared with controls. However, because of the phenotypic resemblance, we hypothesize a brain-specific effect of *CTR9* variants on H3K4 and H3K36 methylation, explaining the absence of differential methylation of H3K4 and H3K36 in fibroblasts. The same may be true for mitophagy dysregulation, as was shown for *UBE2A* pathogenic variants but could not be shown in *CTR9* patient fibroblasts. Future

studies are needed to investigate this hypothesis of brain-specific differential H3K4 and H3K36 methylation and/or mitophagy dysregulation in *CTR9*-variants.

In 4 cases (case 1, 3, 6, and 10), variable cardiac abnormalities were identified, comprising thoracic aortic aneurysm, subaortic valve stenosis, ventricular septal defect, and pulmonary valve stenosis. PAF1C was previously shown to play a role in cardiogenesis in zebrafish by regulating the developmental potential of the lateral plate mesoderm, cardiomyocyte numbers, and heart tube elongation.[6] Zebrafish *ctr9*^{LA961} mutants showed severe defects in morphogenesis of the primitive heart tube.[6] Interestingly, PAF1C was shown to regulate NOTCH signaling in zebrafish.[5] Although, owing to its role during cardiogenesis,[24] impaired NOTCH signaling might contribute to the cardiac phenotype in our cases, this hypothesis could not be supported by our RNA-seq analysis. Altogether, a cardiac evaluation seems warranted in patients harboring *CTR9* pathogenic variants.

In vivo studies, eg, in zebrafish, could be useful to further study the pathogenicity of the identified *CTR9* variants, which however are beyond the scope of this study.

We conclude that heterozygous, mainly de novo missense variants in *CTR9* cause a novel neurodevelopmental disorder characterized by variable ID and neurodevelopmental delay that can be associated with hypotonia; fatigability; behavioral abnormalities, including ASD, anxiety, aggressive behavior; cardiac anomalies; and mild dysmorphism. Future studies to elucidate the underlying pathogenic mechanism are warranted.

Data Availability

The authors declare that the data supporting the findings of this study are available within the article and its supplementary material. Raw sequencing data are available from the corresponding authors on request if in line with the provided consent of the families.

Variants have been submitted to ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/):

- c.43G>A, p.(Glu15Lys) Accession ID: SCV001738054
- c.109G>C, p.(Glu37Gln) Accession ID: SCV0017 38055
- c.50T>C, p.(Ile17Thr) Accession ID: SCV001738056
- c.1405G>A, p.(Glu469Lys) Accession ID: SCV001738057
- c.254G>A, p.(Cys85Tyr) Accession ID: SCV001738058
- c.76G>C, p.(Glu26Gln) Accession ID: SCV0017 38059
- c.1126G>A, p.(Glu376Lys) Accession ID: SCV0017 38060
- c.1364A>G, p.(Asn455Ser) Accession ID: SCV001738061
- c.2296A>G, p.(Thr766Ala) Accession ID: SCV00 1738062
- c.74C>G p.(Pro25Arg) Accession ID: SCV001738063
- c.2633G>A p.(Arg878Gln) Accession ID: SCV0017 38064

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Ethics Declaration

Data were obtained in a diagnostic setting at individual centers after Institutional Review Board (IRB) approval. The main IRB in this study is the IRB of the University Hospital Antwerp,

Belgium. Informed consent to publish individuals' clinical information and photographs was obtained from the parents of the individuals reported in this article.

Conflict of Interest

Amber Begtrup is an employee of GeneDx, Inc. Stylianos E. Antonarakis and Emmanuelle Ranza are cofounders of MediGenome. All other authors declare no conflicts of interest.

Additional Information

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References

1. Crisucci EM, Arndt KM. The roles of the Paf1 complex and associated histone modifications in regulating gene expression. *Genet Res Int*. 2011;2011:707641. http://doi.org/10.4061/2011/707641.
2. Wood A, Schneider J, Dover J, Johnston M, Shilatifard A. The Paf1 complex is essential for histone monoubiquitination by the Rad6-Bre1 complex, which signals for histone methylation by COMPASS and Dot1p. *J Biol Chem*. 2003;278(37):34739–34742. http://doi.org/10.1074/jbc.C300269200.
3. Tomson BN, Arndt KM. The many roles of the conserved eukaryotic Paf1 complex in regulating transcription, histone modifications, and disease states. *Biochim Biophys Acta*. 2013;1829(1):116–126. http://doi.org/10.1016/j.bbagrm.2012.08.011.
4. Zheng L, Shu WJ, Li YM, et al. The Paf1 complex transcriptionally regulates the mitochondrial-anchored protein Atg32 leading to activation of mitophagy. *Autophagy*. 2020;16(8):1366–1379. http://doi.org/10.1080/15548627.2019.1668228.

5. Akanuma T, Koshida S, Kawamura A, Kishimoto Y, Takada S. Paf1 complex homologues are required for Notch-regulated transcription during somite segmentation. *EMBO Rep*. 2007;8(9):858–863. http://doi.org/10.1038/sj.embor.7401045.
6. Langenbacher AD, Nguyen CT, Cavanaugh AM, Huang J, Lu F, Chen JN. The PAF1 complex differentially regulates cardiomyocyte specification. *Dev Biol*. 2011;353(1):19–28. http://doi.org/10.1016/j.ydbio.2011.02.011.
7. Bahrampour S, Thor S. Ctr9, a key component of the Paf1 complex, affects proliferation and terminal differentiation in the developing Drosophila nervous system. *G3 (Bethesda)*. 2016;6(10):3229–3239. http://doi.org/10.1534/g3.116.034231.
8. De Gois S, Slama P, Pietrancosta N, et al. Ctr9, a protein in the transcription complex Paf1, regulates dopamine transporter activity at the plasma membrane. *J Biol Chem*. 2015;290(29):17848–17862. http://doi.org/10.1074/jbc.M115.646315.
9. Wang T, Hoekzema K, Vecchio D, et al. Large-scale targeted sequencing identifies risk genes for neurodevelopmental disorders. *Nat Commun*. 2020;11(1):4932. Published correction appears in *Nat Commun*. 2020;11(1):5398. https://doi.org/10.1038/s41467-020-18723-y.
10. Martins AG, Pinto AT, Domingues R, Cavaco BM. Identification of a novel CTR9 germline mutation in a family with Wilms tumor. *Eur J Med Genet*. 2018;61(5):294–299. http://doi.org/10.1016/j.ejmg.2017.12.010.
11. Lelieveld SH, Reijnders MR, Pfundt R, et al. Meta-analysis of 2,104 trios provides support for 10 new genes for intellectual disability. *Nat Neurosci*. 2016;19(9):1194–1196. http://doi.org/10.1038/nn.4352.
12. De Rubeis S, He X, Goldberg AP, et al. Synaptic, transcriptional and chromatin genes disrupted in autism. *Nature*. 2014;515(7526):209–215. http://doi.org/10.1038/nature13772.
13. Deciphering Developmental Disorders Study. Prevalence and architecture of de novo mutations in developmental disorders. *Nature*. 2017;542(7642):433–438. http://doi.org/10.1038/nature21062.
14. 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.
15. Richards S, Aziz N, Bale S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med*. 2015;17(5):405–424. http://doi.org/10.1038/gim.2015.30.
16. Vos SM, Farnung L, Linden A, Urlaub H, Cramer P. Structure of complete Pol II-DSIF-PAF-SPT6 transcription complex reveals RTF1 allosteric activation. *Nat Struct Mol Biol*. 2020;27(7):668–677. http://doi.org/10.1038/s41594-020-0437-1.
17. Vos SM, Farnung L, Boehning M, et al. Structure of activated transcription complex Pol II-DSIF-PAF-SPT6. *Nature*. 2018;560(7720):607–612. http://doi.org/10.1038/s41586-018-0440-4.
18. Xie Y, Zheng M, Chu X, et al. Paf1 and Ctr9 subcomplex formation is essential for Paf1 complex assembly and functional regulation. *Nat Commun*. 2018;9(1):3795. http://doi.org/10.1038/s41467-018-06237-7.
19. Delgado J, Radusky LG, Cianferoni D, Serrano L. FoldX 5.0: working with RNA, small molecules and a new graphical interface. *Bioinformatics*. 2019;35(20):4168–4169. http://doi.org/10.1093/bioinformatics/btz184.
20. Pettersen EF, Goddard TD, Huang CC, et al. UCSF Chimera—a visualization system for exploratory research and analysis. *J Comput Chem*. 2004;25(13):1605–1612. http://doi.org/10.1002/jcc.20084.
21. Hiraide T, Nakashima M, Yamoto K, et al. De novo variants in SETD1B are associated with intellectual disability, epilepsy and autism. *Hum Genet*. 2018;137(1):95–104. http://doi.org/10.1007/s00439-017-1863-y.
22. Luscan A, Laurendeau I, Malan V, et al. Mutations in SETD2 cause a novel overgrowth condition. *J Med Genet*. 2014;51(8):512–517. http://doi.org/10.1136/jmedgenet-2014-102402.
23. Singh T, Kurki MI, Curtis D, et al. Rare loss-of-function variants in SETD1A are associated with schizophrenia and developmental disorders. *Nat Neurosci*. 2016;19(4):571–577. http://doi.org/10.1038/nn.4267.
24. MacGrogan D, Münch J, de la Pompa JL. Notch and interacting signalling pathways in cardiac development, disease, and regeneration. *Nat Rev Cardiol*. 2018;15(11):685–704. http://doi.org/10.1038/s41569-018-0100-2.