HHS Public Access

Author manuscript

Genet Med. Author manuscript; available in PMC 2020 November 18.

Published in final edited form as:

Genet Med. 2020 February ; 22(2): 427–431. doi:10.1038/s41436-019-0639-2.

The pleiotropy associated with de novo variants in *CHD4, CNOT3,* and *SETD5* extends to moyamoya angiopathy

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SUPPLEMENTARY INFORMATION

The online version of this article (https://doi.org/10.1038/s41436-019-0639-2) contains supplementary material, which is available to authorized users.

DISCLOSURE

The authors declare no conflicts of interest.

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Abstract

Purpose: Moyamoya angiopathy (MMA) is a cerebrovascular disease characterized by occlusion of large arteries, which leads to strokes starting in childhood. Twelve altered genes predispose to MMA but the majority of cases of European descent do not have an identified genetic trigger.

Methods: Exome sequencing from 39 trios were analyzed.

Results: We identified four de novo variants in three genes not previously associated with MMA: *CHD4*, *CNOT3*, and *SETD5*. Identification of additional rare variants in these genes in 158 unrelated MMA probands provided further support that rare pathogenic variants in *CHD4* and *CNOT3* predispose to MMA. Previous studies identified de novo variants in these genes in children with developmental disorders (DD), intellectual disability, and congenital heart disease.

Conclusion: These genes encode proteins involved in chromatin remodeling, and taken together with previously reported genes leading to MMA-like cerebrovascular occlusive disease (*YY1AP1*, *SMARCAL1*), implicate disrupted chromatin remodeling as a molecular pathway predisposing to early onset, large artery occlusive cerebrovascular disease. Furthermore, these data expand the spectrum of phenotypic pleiotropy due to alterations of *CHD4*, *CNOT3*, and *SETD5* beyond DD to later onset disease in the cerebrovascular arteries and emphasize the need to assess clinical complications into adulthood for genes associated with DD.

Keywords

moyamoya angiopathy; exome sequencing; developmental disorders; chromatin remodeling

INTRODUCTION

Moyamoya angiopathy (MMA) is a rare cerebrovascular disease characterized by progressive bilateral stenosis of the distal internal carotid arteries with the development of a compensatory collateral vessel network. MMA leads to strokes starting in childhood through the fourth decade of life resulting in a range of neurologic deficits. Occlusive lesions in the large arteries of MMA patients contain neointimal cells that positively stain for smooth muscle cell (SMC) markers, but lack lipid and inflammatory cell deposition typical of atherosclerosis,[1] suggesting that MMA is triggered by pathways distinct from those responsible for atherosclerosis. The risk factors associated with MMA also differ from those for atherosclerosis and include traumatic injury, hypertension, irradiation of the brain, chemotherapy, and hyperthyroidism.[2] A genetic predisposition for MMA is supported by the fact that 7–12% MMA patients have similarly affected family members[2] and an increased incidence of MMA in Asian countries is due to a founder variant in *RNF213*.[3] Lastly, a number of genetic syndromes are associated with an increased risk for MMA, including neurofibromatosis type I (OMIM 162200, due to *NF1* pathogenic variants),[4] Alagille syndrome (OMIM 118450, *JAG1*),[5] X-linked moyamoya syndrome (OMIM 300845, *BRCC3* and *MTCP/MTCP1NB* deletions),[6] MMA with achalasia and hypertension (OMIM

615750, *GUCY1A3*),[7] Schimke immuno-osseous dysplasia (OMIM 242900, *SMARCAL1*), and microcephalic osteodysplastic primordial dwarfism type II (OMIM 210720, *PCNT*).[8] Bilateral stenosis of the distal internal carotid arteries also occurs with smooth muscle dysfunction syndrome (OMIM 613834, *ACTA2* pathogenic variants)[9] and Grange syndrome (OMIM 602531, biallelic *YY1AP1* loss-of-function variants).[10] These genetic findings highlight potential molecular pathways leading to MMA, including activation of proproliferative pathways (*NF1*) and loss of nitric oxide signaling (*GUCY1A3*), and implicate involvement of SMCs (*ACTA2*).[7]

MATERIALS AND METHODS

This study was approved by the Institutional Review Board at the University of Texas Health Science Center at Houston. Unrelated sporadic and familial cases of MMA were recruited from the United States, France, and Germany, and informed consent was obtained from all study participants. Diagnosis of MMA was based on magnetic resonance imaging, computed tomography, or cerebral angiography (Supplemental Materials and Methods). Clinical history and demographics were abstracted from medical records, public databases, or obtained by patient report. The cohort includes 197 unrelated probands (172 bilateral, 24 unilateral, and 1 unknown) and 125 relatives (21 affected and 104 clinically healthy) including a total of 39 trios. Individuals diagnosed with established Mendelian diseases predisposing to MMA were excluded from the study. Patients with *RNF213* variants were not excluded since *RNF213* is considered to be a susceptibility gene for MMA rather than responsible for Mendelian MMA. Exome sequencing assay was performed by the Center for Mendelian Genomics (University of Washington, USA) or Integragen core (Evry, France) on Illumina platforms (Supplemental Materials and Methods). To limit the detection of false de novo variants, we employed stringent quality criteria by restricting our analysis to variants with a high-quality score (Q Phred score ≥30) and a read depth ≥20× in all members of a same trio. We limited our analysis to the following types of variants: nonsense, nonsynonymous, canonical splice site, and indels in coding regions. Variants with a minor allelic frequency exceeding 0.01 in public databases (dbSNP 144, 1000 Genomes Phase 3, Exome Sequencing Project [ESP6500-SIV2], ExAC [0.3], gnomAD) were excluded. In silico prediction tools MutationTaster, SIFT, and PolyPhen-2 were used in the variant curation process; missense variants predicted benign by more than one of these tools were excluded from further analysis. Variants with Combined Annotation Dependent Depletion pathogenicity (CADD) scores ≤20 in non–de novo trios cases were excluded. Remaining variants were validated using Sanger sequencing (Supplemental Materials and Methods).

RESULTS

To further identify genes and cellular pathways involved in the molecular pathogenesis of MMA, exome sequencing was pursued for 39 affected probands with MMA of unknown genetic etiology (average age of disease onset 15 years, range: 0.5 months–54 years) and their unaffected parents. After rigorous filtering, 39 genes harboring rare de novo variants were identified in 26 MMA trios (2 trios had 3 de novo variants, 12 trios had 2 de novo variants, 12 trios had 1 de novo variant). Thirteen trios did not have de novo variants that

met these criteria (Table S1). Three of these variants were in *RNF213*, and one was a variant of unknown significance in *NF1* (Table S1).

In four MMA patients, we identified de novo variants in three genes, *CHD4*, *CNOT3*, and *SETD5*, previously found to harbor pathogenic de novo variants in children with developmental disorders (DD) (Table 1, Fig. 1). The affected probands had clinical features that overlapped with these previously reported cases (Table S3). *CHD4* de novo variants were reported in children with congenital heart disease, developmental delay, and Sifrim–Hitz–Weiss syndrome (OMIM 617159).[11,12] We identified a de novo CHD4 variant, p.Ala1178Val (absent in exome databases; CADD score 23.9) in a patient diagnosed with bilateral MMA at 10 years of age with a history of developmental delay, congenital heart defect, abnormal gait, and mildly dysmorphic facies (Tables S3 and S4). Importantly, the fact that MMA was previously reported in a child with intellectual disability due to a de novo *CHD4* variant, p.Arg1127Gln, further supports an association of CHD4 variants with MMA (Tables S3 and S4).[11] *CNOT3* de novo variants have also been identified in children with DD and autism spectrum.[12] Two MMA probands in our cohort harbor de novo variants in *CNOT3*, p.Gln215* and p. Leu522Ile (absent in exome databases; CADD scores 39 and 17.9, respectively). The de novo variant, p.Gln215*, was identified in a woman diagnosed with bilateral MMA at 20 years of age; she had a history of intellectual disability, autism spectrum disorder, and mildly dysmorphic facies (Figure S1B and C, Tables S3 and S4). De novo *SETD5* variants, primarily loss-of-function (LoF) variants, have been found in children with intellectual disability, developmental delay, skeletal abnormalities, and variable dysmorphic features (OMIM 615761).[12,13] A de novo *SETD5* haploinsufficiency variant, p.Glu661Lysfs*5 (Fig. 1), was identified in a patient diagnosed with bilateral MMA at 10 years of age with a history of developmental delay, polydactyly, and mild dysmorphic facial features (Tables S3 and S4). Collectively, the phenotypic overlap between our MMA patients harboring de novo variants and those previously reported with neurodevelop-mental disorders indicates that the pleiotropy associated with de novo variants in *CHD4*, *CNOT3*, and *SETD5* should include MMA.

To further validate an association between rare variants in *CHD4*, *CNOT3*, and *SETD5* and MMA, exome data from 158 unrelated MMA cases and 43 relatives (18 with MMA, and 25 unaffected) were filtered and validated as previously described. Rare *CHD4* variants were confirmed in six additional MMA cases, including five missense variants and one splice donor site variant (Table 1). RNA analysis of leukocytes confirmed that the splice site variant is associated with aberrant splicing of exon 11, leading to a frameshift and nonsense-mediated decay. *CHD4* is intolerant to both missense (*Z*-score = 7.05) and LoF variants (probability of being LoF variant intolerant score [pLI] = 1) in the general population. Of the seven rare *CHD4* variants in the MMA cases, four were absent in gnomAD and three were reported with very low frequencies (minor allele frequency [MAF] < $1.62e^{-5}$) (Table 1). The majority of *CHD4* variants associated with DD cluster in the central region of the protein involved in the helicase function of *CHD4*. Interestingly, three of the variants present in MMA patients (p.Ile1741Val, p. Tyr1758Cys, and p.Pro1880Ser) are located in the C-terminal part of the protein, which is known to interact with pericentrin, encoded by the *PCNT* gene.[14] Homozygous LoF variants in *PCTN* predispose to MMA (Fig. 1).[8] Of the seven individuals with rare *CHD4* cases in this study, all had bilateral MMA (Figure S1A;

Tables S2, S3, and S4). Intellectual disability was observed in one of the additional cases (Table S4).

A *CNOT3* missense variant, p.Gly304Ser, with a MAF of $4.76e^{-4}$ (CADD score 27.2) was identified in two unrelated MMA patients (Table 1). *CNOT3* is another gene intolerant to both missense (*Z*-score = 3.89) and LoF variation (pLI = 1). The two patients who harbor the p.Gly304Ser variant, presented with unilateral MMA at 5 and 22 years of age, but these cases lacked developmental delay and other clinical findings previously associated with de novo CNOT3 variants.

Two missense variants, p.Asp339Tyr and p.Arg767Cys, were identified in *SETD5* in unrelated MMA cases. The variant, p.Asp339Tyr, is absent in gnomAD, and p.Arg767Cys is present at an extremely low frequency (Table 1). *SETD5* is intolerant to LoF variation (pLI = 1) but not missense variation (*Z*-score = −0.04). All three cases with *SETD5* rare variants had bilateral MMA (Tables S2, S3, and S4). In addition to MMA, the patient harboring p.Asp339Tyr had stenosis of other arteries and hypertension (Table S4).

DISCUSSION

These data indicate that rare variants in *CHD4* and *CNOT3* predispose to MMA in the presence and absence of DD; additional data are required to validate an association between *SETD5* rare variants and MMA. *CHD4* and *CNOT3*, along with a previously published gene, *YY1AP1*,[10] are all associated with MMA-like occlusive vascular disease, developmental delay, skeletal changes, and dysmorphic facies. All of these genes encode proteins involved in chromatin remodeling. *CHD4* encodes a helicase that is the main component of the nucleosome remodeling and histones deacetylase (NuRD) repressor complex, which pairs adenosine triphosphate (ATP)-dependent chromatin remodeling with histone deacetylase activity. The NuRD complex is involved in the regulation of gene expression during differentiation of pluripotent stem cells.[15] *CNOT3* encodes the subunit 3 of the CCR4-NOT transcription complex, which executes poly(A)-tail shortening of messenger RNA (mRNA) in the cytoplasm and its conditional deletion results in the formation of autophagic vacuoles and cardiomyocyte death, leading to lethal heart failure accompanied by long QT intervals.[16] CNOT3 also controls the acetylation level of histones H3 and H4.[17] Inhibition of histone acetylases was shown to reverse the heart defects observed in heterozygous knockout mice suggesting a mechanistic link to epigenetic remodeling.[18] Therefore, it remains unclear whether the effect on transcription is direct or indirect through mRNA deadenylation. We previously reported LoF variants in *YY1AP1* in individuals with Grange syndrome, which is characterized by MMA-like occlusion in the carotid arteries, along with occlusive lesions in the renal, celiac, and coronary arteries.[10] *YY1AP1*, encoding YY1-associated protein 1, is an enhancer of Yin Yang 1 (*YY1*) transcriptional activity; both are components of the INO80 chromatin remodeling complex. Interestingly, *SETD5* encodes SET domain-containing protein 5 and family of proteins with lysine methyltransferase, but SETD5 has not been shown to have methyltransferase activity. SETD5 interacts closely with the histone deacetylase-containing NCoR complex, and loss of Setd5 increases histone acetylation at transcription start sites.[19]

The association of MMA or MMA-like with rare variants in *CHD4*, *CNOT3*, *SETD5*, and *YY1AP1* indicates that disrupted chromatin remodeling is a predisposing factor for non-atherosclerotic occlusive cerebrovascular disease. The differentiation of progenitor cells into functional cells in organs requires chromatin remodeling for tissue-specific gene expression patterns. Deficiency of *Chd4* in mice is known to disrupt neuronal differentiation; neural progenitor cells in *Chd4*-deficient mice precociously exit the cell cycle, fail to differentiate, and die.[15] In contrast, an interaction between retinal ganglion cells and endothelial cells critical for development of the mouse retina vasculature is dependent on Setd5. Setd5 in retinal ganglion cells represses *Sema3a*, thus allowing expression of miR-126–5p, which regulates angiogenesis by protecting adjacent endothelial cells from apoptosis.[20] Deficiency of Setd5 in these ganglion cells decreases production of miR-126–5p and leads to an underdeveloped retinal vasculature. Thus, disruption of *CHD4*, *CNOT3*, *SETD5*, and *YY1AP1* potentially predisposes to MMA through altered differentiation of the cells in the arterial wall or altered expression of regulatory factors in the adjacent neuronal cells.

These data expand the phenotypic pleiotropy resulting from *CHD4*, *CNOT3*, and *SETD5* de novo variants to include childhood to young adult–onset occlusive cerebrovascular disease. Additional studies to assess the penetrance of MMA are required before we can recommend imaging for all the patients carrying de novo variants in *CHD4*, *CNOT3*, and *SETD5* (Table S4). It is also important to note that individuals with pathogenic variants may present with MMA in adulthood without history of developmental defects. Furthermore, these results suggest that pathogenic variation in other genes that disrupt chromatin remodeling and lead to development defects may be associated with other complications not evident until later in life. Finally, identifying the genetic triggers responsible for MMA large artery occlusive lesions raises the possibility that “atherosclerotic” lesions in ischemic stroke patients in general may result from aberrant cellular chromatin remodeling.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGEMENTS

We are grateful to the patients and their family members for participating in this study. This study was funded by the Henrietta B. and Frederick H. Bugher Foundation (D.M.M.), the Texas Heart Institute Fibromuscular Dysplasia Project (D.M.M.), INSERM (E.T.-L.), the American Heart Association (18POST34020031; A. P.) and the Fondation pour le Recherche Médicale (S.G.). Exome sequencing was funded by the National Human Genome Research Institute and the National Heart, Lung, and Blood Institute grant HG006493 to D. A.N., M.J.B., and Suzanne Leal. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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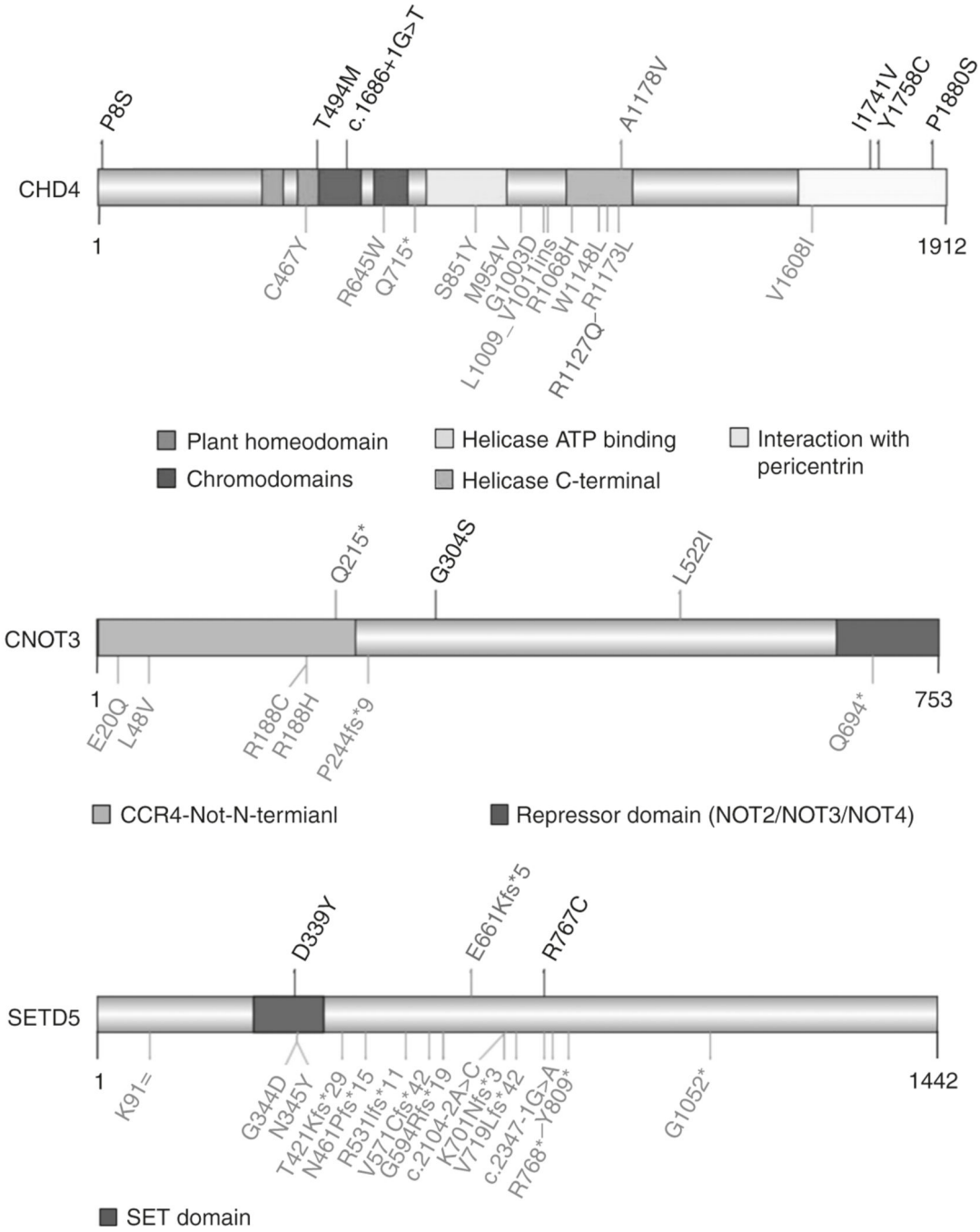

Fig. 1. Schematic representation of domains of the proteins encoded by *CHD4, CNOT3,* and *SETD5* and the location of the rare variants identified in individuals with either moyamoya angiopathy (MMA) or developmental disorders.

Above the protein schematics are the location of rare de novo variants (red) and variants (black) identified in singleton cases in the MMA cohort. Below the protein schematics are variants identified in children with intellectual disability, congenital heart defects, or developmental disorders (blue).[11,12] The red variant in the CHD4 schematic was identified in three unrelated patients, including the patient described with intellectual disability and

moyamoya disease[11] (CHD4: NP_001264.2, CNOT3: NP_055331.1, SETD5: NP_001073986.1). *ATP* adenosine triphosphate.

Table 1

Rare variants in *CHD4*, *CNOT3*, and *SETD5* identified in MMA patients

Gene	Variant cDNA	Variant protein	CADD	ExAC	gnomAD	Segregation	Family
CHD4	**c.3533C>T**	**p.Ala1178Val**	**23.9**	**0**	**0**	**De novo**	**MM182**
CHD4	c.22C>T	p.Pro8Ser	23.1	0	0	Proband[a]	MM117
CHD4	c.1481C>T	p.Thr494Met	24.9	0	$1.22e^{-5}$	Inherited from unaffected mother	M102
CHD4	c.1686+1G>T	-	24.7	0	0	Proband[a]	M136
CHD4	c.5221A>G	p.Ile1741Val	24.5	0	$7.25e^{-6}$	Proband[a]	M108
CHD4	c.5273A>G	p.Tyr1758Cys	26.5	$1.60e^{-3}$	$1.62e^{-5}$	Proband[a]	M123
CHD4	c.5638C>T	p.Pro1880Ser	24.8	0	0	Absent in mother[b]	M155
CNOT3	**c.643C>T**	**p.Gln215***	**39**	**0**	**0**	**De novo**	**MM165**
CNOT3	**c.1564C>A**	**p.Leu522Ile**	**17.9**	**0**	**0**	**De novo**	**MM150**
CNOT3	c.910G>A	p.Gly304Ser	27.2	$1.174e^{-3}$	$4.76e^{-4}$	Inherited from unaffected father	MM078
CNOT3	c.910G>A	p.Gly304Ser	27.2	$1.174e^{-3}$	$4.76e^{-4}$	Absent in father[b]	MM112
SETD5	**c.1981delG**	**p.Glu661Lysfs*5**	**-**	**0**	**0**	**De novo**	**MM166**
SETD5	c.1015G>T	p.Asp339Tyr	27.8	0	0	Proband[a]	M257
SETD5	c.2299C>T	p.Arg767Cys	33	$6.415e^{-5}$	$7.67e^{-5}$	Proband[a]	M011

CHD4: NM_001273.3, *CNOT3*: NM_014516.1, *SETD5*: NM_001080517.1.

cDNA complementary DNA, *MMA* moyamoya angiopathy.

[a]Only the proband was available for analyses.

[b]Only one parent was available for analyses.

The bold value used to highlight the de novo variants.