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Variants in Chromatin Remodeling Genes Are Involved in Patients With Chiari Malformation Type 1

Ferruccio Romano[1] | Maria Cerminara[2] | Patrizia De Marco[2] | Michele Iacomino[2] | Marco Di Duca[2] | Domenico Tortora[3,4] | Marco Pavanello[5] | Gianluca Piatelli[5] | Marcello Scala[2] | Federico Zara[2,3] | Aldamaria Puliti[2,3] | Valeria Capra[1]

[1]Genomics and Clinical Genetics Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy | [2]Medical Genetics Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy | [3]Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health, University of Genoa, Genoa, Italy | [4]Neuroradiology Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy | [5]Neurosurgery Unit, IRCCS Istituto Giannina Gaslini, Genoa, Italy

Correspondence: Ferruccio Romano (ferruccioromano@gaslini.org)

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ABSTRACT

Objectives: Chiari malformation type 1 (CMI) is defined by the herniation of cerebellar tonsils of 5 mm or more, with possible neurological consequences, including compression of the neural tissue and/or anomalies in cerebral spinal fluid circulation. The etiology of CMI is not fully elucidated, with both genetic and environmental factors being involved. Several genes and pathways involved in bone development are pointed out like genes of the WNT, FGF, and BMP signaling pathways. More recently, the crucial role played by chromatin remodeling genes in the pathogenesis of CMI has increasingly emerged.

Methods: In this paper, we discuss a familial case of CMI and a single patient, harboring variants in chromatin remodeling genes, identified by whole exome sequencing.

Results: The first is a family with three affected members and one sibling with a cerebellar tonsil herniation of < 5 mm. The three CMI patients harbor a heterozygous missense variant in the *SETD2* gene, whose truncating variants are responsible for Luscan–Lumish syndrome. A second variant in *HP1BP3*, a gene not previously associated with human pathology, with evidence of skeletal anomalies in mice models, was found in the three patients and also in the girl with a herniation of < 5 mm. The second case is a proband with a de novo variant in *KMT2A*, associated with Wiedemann–Steiner syndrome, in which anomalies of the craniocervical junction are described.

Discussion: We highlight the importance of chromatin remodeling genes in both isolated and syndromic CMI and suggest the potential role of *HP1BP3* as a possible modifier gene in CMI pathogenesis, even if this association needs to be further clarified.

1 | Introduction

Chiari malformation type I (CMI) is a brain disorder characterized by the observation, on cranial mid-sagittal magnetic resonance imaging (MRI), of a cerebellar tonsil herniation of at least 5 mm below the foramen magnum (Barkovich et al. 1986). This results in a direct compression of the neural tissue at the craniovertebral junction and, often, cerebrospinal fluid

Ferruccio Romano and Maria Cerminara have equally contributed.

disturbances, which can cause other related conditions such as syringomyelia (SM; ORPHA # 3280) or secondary hydrocephalus and neurological symptoms (Mueller and Oro 2004). CMI affects individuals of every ethnicity. The clinical presentation of CMI is usually delayed till the third or fifth decade (Milhorat et al. 2010). With the advance in imaging technology, CMI has been detected at increasing rates in younger population (Greenlee et al. 2002). In adults, a higher incidence is generally observed for females, while in pediatric cases, this incidence is more evenly distributed among sexes (Milhorat et al. 2010; Tubbs et al. 2011).

Evidence in favor of a genetic contribution comes from at least three sources: twin studies, familial aggregation, and cosegregation of CMI with known genetic conditions (Speer et al. 2003). The majority of CMI cases are believed to be sporadic. However, familial cases with autosomal-dominant inheritance with decreased penetrance or autosomal recessive have been reported. CMI has been associated with 90 Mendelian genetic disorders and, for some of them, associated genes have been identified and hypothesized to have pleiotropic effects (Afifi et al. 1988; Cohen Jr. and Kreiborg 1992; Dooley et al. 1993; Caldemeyer et al. 1995; Dietz et al. 1991; Iglesias-Osma et al. 1997). Despite evidence for a genetic component, genetic studies for CMI have been limited. In fact, family studies have been hindered due to the rare disease prevalence and the small proportion of familial cases.

Although multiple mechanisms have been proposed, including cranial constriction, cranial settling, spinal cord tethering, intracranial hypertension, and intraspinal hypotension (Milhorat et al. 2010), CMI is thought to be caused by an underdeveloped occipital bone. Marin-Padilla and collaborators first postulated that a paraxial mesodermal insufficiency is the cause of CMI (Marin-Padilla and Marin-Padilla 1981). An underdeveloped occipital bone, possibly due to the underdevelopment of the occipital somite originating from the paraxial mesoderm, induces overcrowding of the hindbrain in the posterior cranial fossa (PCF). Little is known about the molecular mechanisms, which regulate how the whole cranium is shaped. Mouse models currently available for genetic research include hundreds of unique inbred strains and genetically engineered mutants. Several signaling pathways are implicated in proper skull ossification and growth in mice and humans, including the fibroblast growth factor (FGF), hedgehog (HH), Wnt, and BMP signaling (Lin and Hankenson 2011).

Recent genetic studies in humans have begun to decipher CMI pathogenesis. Exome sequencing (ES) of two multiplex Italian CMI families identified candidate genes, implicating impaired Wingless-related integration site (WNT) signaling in PCF bone development (Merello et al. 2017). Rare variants of collagen genes, such as *COL7A1*, *COL5A2*, *COL6A*, *or COL1A2*, encoding for proteins of the extracellular matrix of the bone, have also been associated with CMI (Mekbib et al. 2023). Recently, the ES analysis of two large cohorts of patients identified another interesting class of candidate genes, the chromatin remodeling genes, influencing gene expression (Aref-Eshghi et al. 2020; Provenzano et al. 2021). Given the significant genetic and clinical heterogeneity of CMI, further studies are warranted to increase our understanding of this disorder. Our study, even if carried out only on two families, provides support to the role played by chromatin remodeling genes in isolated CMI pathogenesis, both in inherited and sporadic cases.

2 | Materials and Methods

2.1 | Editorial Policies and Ethical Considerations

The present study was reviewed and approved by the Ethics Committee of the Italian Regione Liguria (P.R. code IGG-VACA). The authors have acquired the subject's family's written informed consent for publication of the details of their medical case.

2.2 | Clinical Assessment

CMI patients and families were recruited at the Neurosurgery Clinic of the Giannina Gaslini Children's Hospital, Genoa, Italy. Diagnosis of CMI was based on MRI demonstration of a downward herniation of >5 mm of the cerebellar tonsils on a mid-sagittal T1-weighted image. Complete multidisciplinary (neurological, clinical, and clinical genetic) evaluations were performed.

2.3 | Exome Sequencing

Genomic DNA was isolated from peripheral blood samples. The ES analysis was performed for all the members of Family A and Family B. The ES was performed using the xGen Exome Research Panel v1.0—IDT KIT, consisting of 429,826 probes (approximately 19,396 genes), and carried out by the Italian Institute of Technology (IIT) in Genoa, Italy. The FASTQ files were aligned to the GRCh37/hg19 human reference genome using the Burrows–Wheeler Aligner, package version 6.1. Variant calling was performed with Genome Analysis Tool Kit (GATK4), and variants were annotated with Annovar (database updated May 27, 2019) (Wang, Li, and Hakonarson 2010). We prioritized variants based on specific criteria. For Family A, according to an autosomal-dominant inheritance mechanism, only heterozygous variants shared by all affected members were considered; for Family B, de novo dominant and recessive variants were selected. We also prioritized variants with a frequency of less than 0.1% in ExAC/GnomAD v2.1.1/1000g2015; predicted as deleterious or damaging by PolyPhen and SIFT; and with a CADD prediction value of at least 20. Variants classified as pathogenic, likely pathogenic, or variants of uncertain significance (VUS), as defined by the Franklin classification tool (https://franklin.genoox.com, Franklin by Genoox) based on the American College of Medical Genetics guidelines (Richards et al. 2015), were then analyzed. Further manual evaluation of variants was performed using data reported in public databases, such as ClinVar, OMIM, Mouse Genome Database (MGD), and PubMed. Finally, variants in genes known to play a role in biological processes likely altered in CMI, according to the literature (Merello et al. 2017; Mekbib et al. 2023; Provenzano et al. 2021; Capra et al. 2009; Urbizu et al. 2021; Sadler et al. 2021), and enclosed in Gene Ontology (GO) biological process categories, such as bone morphogenesis (GO:0060349) and skeletal system morphogenesis (GO:0048705), were considered.

3 | Results

3.1 | Case Presentations

3.1.1 | Case A

This is a case of familial CMI. A 10-year-old male child (II-3) came to our attention. The child complained of frequent episodes of cephalalgia and paresthesia of hands and feet. Neurological evaluation was otherwise not significant. Brain MRI led to the diagnosis of asymmetric CMI: herniation of the left cerebellar tonsil of 9 mm and the right tonsil of 4.5 mm. The child underwent decompression surgery, with partial resolution of symptoms. Episodes of milder paresthesias and headaches were still reported and are now under investigation.

Pedigree collection highlighted the recurrence of CMI in the family (Figure 1). The elder brother (now aged 16 years old, II-1) was reported with language delay, attention deficit, oppositional behavior, and learning difficulties. After recurrent lower back and lower limb pain, brain and spinal MRIs were performed, highlighting CMI (herniation of 12 mm) and scoliosis. After surgical decompression, symptoms persisted. Later on, the boy also showed episodes of a sudden loss of consciousness, hemiplegia or limb stiffness, and eye revulsion. The postcritic period was described with agitation and aggressiveness. Only mild and no

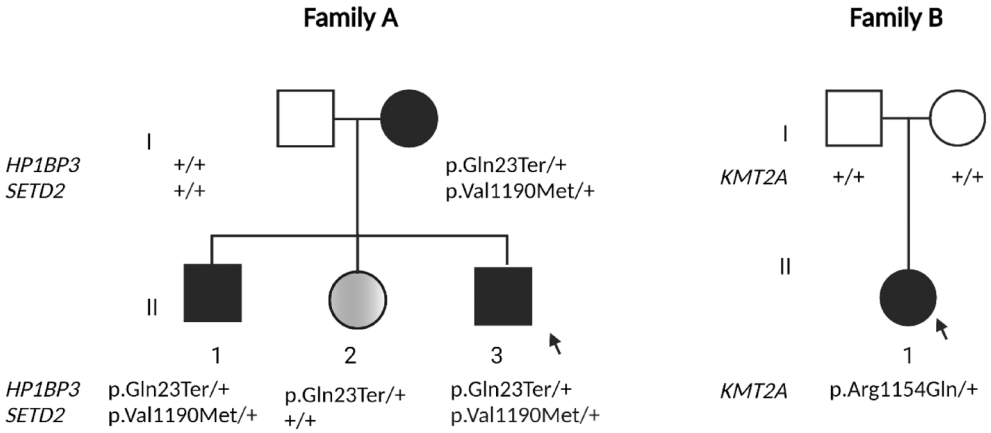

FIGURE 1 | Pedigree of Family A and Family B. Implicated genes and their variants, indicated as amino acid changes, are shown. Arrows indicate the probands. Gray symbol indicates a mild phenotype (tonsillar herniation < 5 mm; II-2 Family A). +: Wild-type genotype.

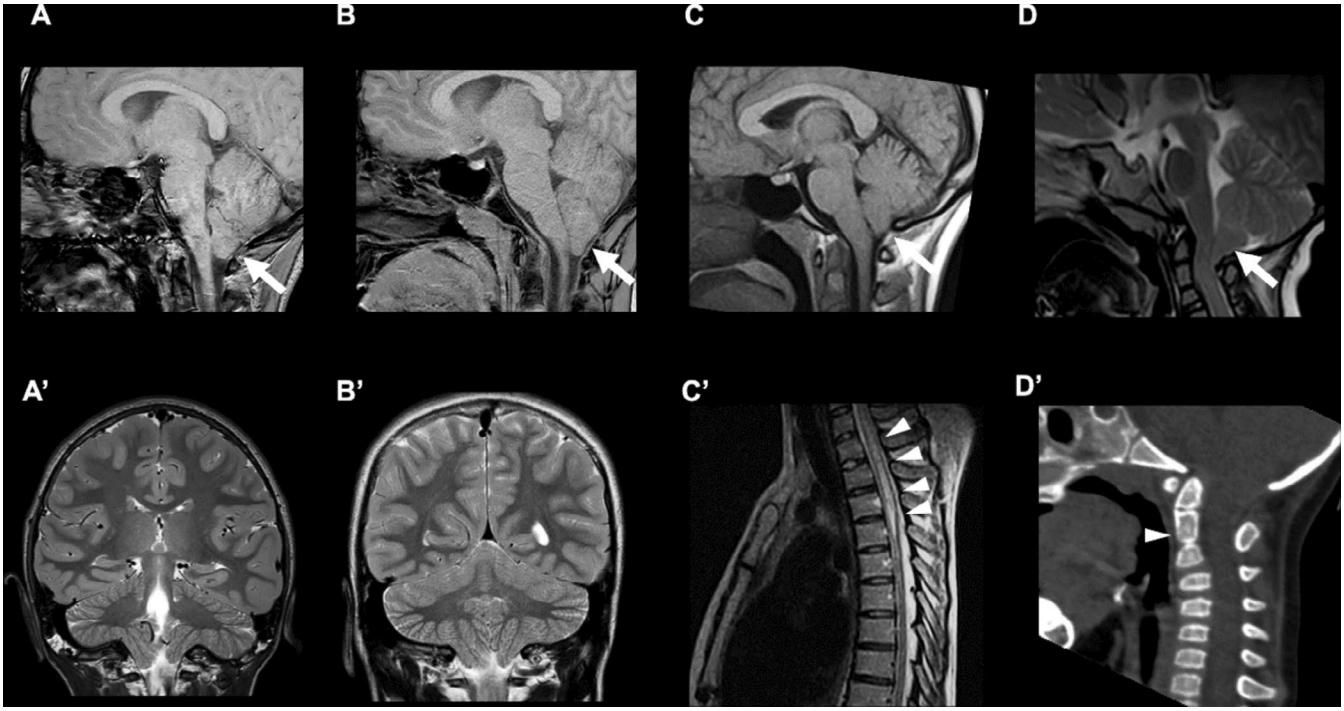

FIGURE 2 | MR images of Family A and B members. Sagittal T1 weighted and coronal T2 weighted MR images of: 16-year-old patient (II-2) showing tonsillar ectopia with pointed appearance (white arrow) (A, A′); of the younger brother (II-1) showing tonsillar ectopia with pointed appearance (white arrow) (B, B′); of the mother (I-1) showing tonsillar ectopia (white arrow) and syringomyelia at the cervical–thoracic junction (arrowheads) (C, C′). Sagittal T2-weighted MR image of the Family B proband showing tonsillar ectopia through the foramen magnum (white arrow) (D); sagittal reconstruction of CT scan showing fusion of C2 and C3 vertebrae (arrowhead) leading to craniocervical instability (D′).

specific anomalies were reported by EEGs. Antiepileptic drugs were used with no success. The boy is now under neuropsychiatric investigation. Differential diagnosis is an issue between epilepsy and suspect conversion disorder.

A sister (II-1), referred in apparent good health, underwent a brain MRI for familial CMI: herniation of cerebellar tonsils was detected but was less than 5 mm.

The mother (I-1) had undergone decompression surgery at the age of 24, after a diagnostic brain MRI that revealed bilateral tonsil herniation of 5 mm on the left and 6.5 mm on the right, associated with syringomyelia within the spinal cord at the cervical–thoracic junction. Brain MRI of affected individuals is shown (Figure 2).

3.1.2 | Case B

In this case, the patient is a girl, the sole offspring of two healthy, unrelated parents (Figure 1). Familial anamnesis highlighted a paternal aunt with pregnancy interruption for fetal Dandy–Walker malformation (normal male karyotype). The girl was born at term after an uneventful pregnancy. Growth parameters at birth were reported within the normal range. APGAR scored 8 and 9. The perinatal period was referred clinically unremarkable except for durable constipation.

Head and trunk control were achieved at 3 and 6 months of age, respectively. The girl was noticed with difficulties in postural changes and began to walk at 18 months, with clumsiness and a wide-based gait. Language was delayed and slowly progressing with the main difficulties in making sentences. The girl presented with mild psychomotor delay in the absence of behavioral manifestations. Intermittent exotropia of the left eye was also reported.

Persistent torticollis was referred after a falling episode. After neurological evaluations, the patient underwent a brain and spine MRI, which detected CMI. Head CT also highlighted craniocervical junction instability with a fusion of C2 and C3 vertebrae. Posterior decompression surgery and occipitocervical fixation were performed.

Genetic evaluation at the age of 3 years and 6 months highlighted normal growth parameters: height 94 cm (18th pc), weight 13.5 kg (18th pc), and HC 48.5 cm (34th pc). Dysmorphic signs were noticed, including flat philtrum, bulbous nose, thickened palmar subcutaneous tissue, hyperthricosis of the back and upper limbs, sandal gap, and bilateral ankle laxity. The inability to jump and wide-based gait were persistent. Brain MRI of the patient and her mother is presented (Figure 2).

3.2 | Variant Interpretation

3.2.1 | Genetic Analysis of Family A

Considering only variants having an impact on the protein function (frameshift, nonsense, splicing, and missense variants), the ES analysis revealed that affected members shared 82 variants, and among them, the proband and his affected brother were carriers of a missense variant in the *SETD2* (SET domain containing 2) gene (NM_014159.7: c.3568G>A p.Val1190Met), inherited from the mother and absent in the sister, who presents with a borderline phenotype (tonsil herniation below 5 mm), and in the unaffected father. The variant was present at a very low frequency in the dataset of the general population, and it was classified as a variant of uncertain clinical significance (VUS) by ClinVar in a patient with the Luscan–Lumish syndrome (LSS, #616831). This syndrome, mostly caused by truncating variants of *SETD2*, is characterized by postnatal overgrowth, macrocephaly, obesity, intellectual disability, and behavioral issues. Other neurological findings are CMI and syringomyelia. *SETD2* variants, all of the missense type, were identified in 5 patients with isolated and syndromic CMI by Provenzano (Provenzano et al. 2021). *SETD2* encodes for a histone methyltransferase that is specific for lysine-36 of histone H3 (Sun et al. 2005), and methylation of this residue is associated with active chromatin. In addition to histones, it also mediates methylation of other proteins, such as tubulins like TUBA1B (Park et al. 2016), which is required for normal mitosis and cytokinesis and may be a specific tag in cytoskeletal remodeling.

When we included the proband's sister as a mildly affected member in the autosomal-dominant model, we could identify a heterozygous stop variant in the *HP1BP3* gene (NM_001372052.1: c.67C>T: p.Gln23Ter) transmitted by the mother to all three children. The pathogenicity prediction models, SIFT and PolyPhen, reported this variant as "deleterious" and "damaging," respectively. In addition, the variant showed a high CADD (combined annotation-dependent depletion) score (Phred-score = 36). This variant has not yet been reported in the available variant databases. According to the American College of Medical Genetics and Genomics (ACMG) guidelines, it is classified as VUS (PM2; PVS1) (Table 1). *HP1BP3* encodes a heterochromatin protein 1 binding protein 3, which is involved in chromatin remodeling (Hayashihara et al. 2010), osteoclast and osteoblast formation, and the IGF1 pathway (Garfinkel et al. 2015). *HP1BP3* plays a key role in normal growth and bone development by regulating transcription of the endocrine components of IGF-1, a major endocrine pathway that regulates both body growth and bone acquisition. Consistent with the gene's specific function in bone, mice lacking *Hp1bp3* expression (*Hp1bp3*$^{-/-}$) show dramatic impairment in bone development and structure (Garfinkel et al. 2015).

3.2.2 | Genetic Analysis of Patient B

In Family B, the proband showed a de novo missense variant of *KMT2A (MLL)* gene (NM_001197104.2: c.3461G>A p.Arg1154Gln) classified as pathogenic, according to the American College of Medical Genetics and Genomics (ACMG; PM1, PP2, PM2, PM5, PP3, PP5). *KMT2A* encodes for a histone methyltransferase that is specific for lysine 4 of histone H3 (H3K4) and mediates chromatin modifications associated with epigenetic transcriptional activation. The p.(Arg1154Gln) variant is located in the zinc finger domain (PF02008) of the encoded protein that binds to nonmethyl-CpG dinucleotides (Figure 3). This variant showed a high CADD score (Phred-score = 33.00) and was predicted as intolerant by MetaDome (0.21 score), a

TABLE 1 | Overview of the genes and variants found in the patients.

	Family A		Family B
Gene	***SETD2***	***HP1BP3***	***KMT2A***
Genomic position (GRCh38)	chr3:47121068	chr1:20780374	chr11:118478093
Variant	NM_014159.7: c.3568G>A: p.Val1190Met	NM_001372052.1: c.67C>T: p.Gln23Ter	NM_001197104.2: c.3461G>A: p.Arg1154Gln
Exon number	Exon 3 of 21	Exon 2 of 13	Exon 5 of 36
Inheritance	Affected mother	Affected mother	De novo
Present in ClinVar	Yes (ID1432340)	No	No
GnomAD frequency	AF: 0.000022	AF: 0.0 (GnomAD v2.1.1); AF: 0.0 (GnomAD v4.0.0)	NA
pLI/ZScore	1/0.45 (GnomAD v2.1.1); 1/1.72 (GnomAD v.4.1.0)	0.99/2.21 (GnomAD v2.1.1); 1/1.74 (GnomAD v4.1.0)	1/6.23 (GnomAD v2.1.1); 1/8.77 (GnomAD v4.1.0)
SIFT/PolyPhen/CADD phred	Deleterious/benign/11	Deleterious/damaging/36	Deleterious/NA/33
Franklin prediction (ACMG criteria)	Uncertain significance VUS (PM2, BP4)	Uncertain significance VUS (PM2; PVS1)	Pathogenic P (PM1, PP2, PM2, PM5, PP3, PP5)
Metadome	Not applicable	Not applicable	Intolerant (0.21)
MGI database	Setd2^{tm1Zhch} (MGI: 4437215) Mice phenotype: embryonic lethality, pharyngeal arch hypoplasia, abnormal head, development, growth retardation, open neural tube, abnormal blood vessels	Hp1bp3$^{tm1a(EUCOMM)Wtsi}$ (MGI: 4432659) Hp1bp3$^{-/-}$ mice phenotype: growth/size/body	Kmt2a$^{tm1(AFF1)Ksy}$ (MGI: 96995) Mice phenotype: behavior, craniofacial, growth/size/body, hearing/vestibular/ear, hematopoietic, immune, integument, limbs/digits/tail, mortality/aging, neoplasm
OMIM disease	Luscan–Lumish syndrome (616831; AD)	No	Wiedemann–Steiner syndrome (605130; AD)

Note: GnomAD v2.1.1. PM2—(moderate) absent from controls (or at extremely low frequency if recessive) in Genome Aggregation Database; PVS1—(very strong) null variant (nonsense, frameshift, canonical ±1 or 2 splice sites, initiation codon, single or multiexon deletion) in a gene, where LOF is a known mechanism of disease; PM1—(moderate) located in a mutational hot spot and/or critical and well-established functional domain (e.g., active site of an enzyme) without benign variation; PP2—(supporting); missense variant in a gene that has a low rate of benign missense variation and in which missense variants are a common mechanism of disease; PP3—(supporting) multiple lines of computational evidence support a deleterious effect on the gene or gene product (conservation, evolutionary, splicing impact, etc.); BP4—(benign supporting) for a missense or a splice region variant, computational prediction tools unanimously support a benign effect on the gene.
Abbreviation: AF, allele frequency.

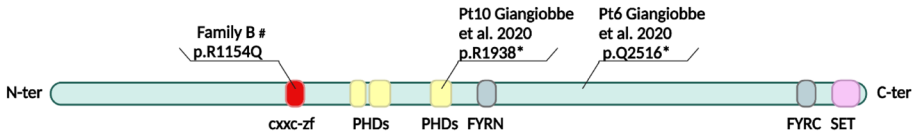

FIGURE 3 | Graphic representation of the KMT2A protein structure. The figure shows the variant present in Family B and the two variants already described in patients with Chiari malformation I (Pt6 and Pt10 reported in Giangiobbe et al. (2020)). #This variant is also reported in ClinVar in a patient with the Wiedemann–Steiner syndrome (RCV002248724.2). CXXC-ZF, CXXC-motif zinc fingers; PHD, plant homeodomain zinc fingers; FYRN/FYRC, PheTyr-rich N/C-terminal domain; SET, Su(var)3–9/enhancer-of-zeste/trithorax domain (catalytic methylation domain). *It indicates a STOP variant.

software that integrates publicly available data of human genetic variation. The same variant was reported in ClinVar as pathogenic in a patient with Wiedemann–Steiner syndrome (RCV002248724.2). Wiedemann–Steiner Syndrome (WDSTS, OMIM # 605130) is an autosomal-dominant congenital malformation syndrome mainly characterized by hypertrichosis cubiti, dysmorphic facial features, intellectual disability, behavioral difficulties, and possible bone malformations. Loss-of-function mutations, also missense, of *KMT2A* have been identified in WDSTS patients with craniovertebral junction malformations and CMI (Giangiobbe et al. 2020). Thus, given the phenotype of the proband, a diagnosis of WDSTS was established.

4 | Discussion

Both syndromic and isolated CMI have strong genetic bases, although the CMI etiology is multifactorial. Recent evidence indicates that genes associated with CMI can be theoretically divided into two main groups: (a) chromatin remodeling genes and (b) genes involved in the development of the skull and craniovertebral junction. These include genes involved in the sutures of the cranial bones and in microcephaly, in the closure of the neural tube, and in RASopathy (Provenzano et al. 2021). The boundaries between these two gene groups are only theoretical and traced for schematic purposes. Lines are extremely blurred, as the same gene can be involved both in chromatin remodeling and bone/skull/craniovertebral development.

In this study, we discuss two clinical cases with CMI, in which chromatin remodeling genes appear to have a key role. Chromatin remodeling is crucial in modulating gene expression. Histone post-translational modifications (PTMs) by specific enzymes, for example, histone acetyltransferases, deacetylases, methyltransferases, and kinases, modulate chromatin dynamism. The application of ES to cohorts of people with intellectual disabilities, autism spectrum disorders, and other neurological anomalies has shown that a growing number of constitutional disorders is associated with germline variants of genes involved in chromatin regulation (Provenzano et al. 2021).

Here, we report an autosomal-dominant CMI family, in which a missense *SETD2* variant is segregated with the disease. Even if this variant is classified as VUS, it is rare and with mild deleterious predictions: the p.Val1190Met variant in SETD2 is classified as a VUS according to the ACMG. In Family A, this variant is shared by all affected members and by the sister with a tonsillar herniation of <5 mm. The fact that it is very uncommon in the GnomAD database seems to account for a possibly deleterious role (PM2); however, the bioinformatic predictions are variable, with only a few of them stating this variant as deleterious and others assessing uncertain or benign effects (BP4). The same *SETD2* variant was described in a patient with the Luscan–Lumish syndrome (LLS, #616831), an intellectual disability disorder with overgrowth, macrocephaly, and CMI. However, affected members of Family A presented only with isolated CMI and they do not have signs of overgrowth or obesity. The elder brother (II-3) only shows some of the clinical features defining the Luscan–Lumish syndrome (OMIM #616831): the principal complaint is represented by CMI, and also mild learning difficulties and behavioral issues, including sporadic aggressiveness and suspect conversion disorder, are reported. Other typical characteristics of LSS are lacking like dysmorphisms, macrocephaly, obesity, and hyperlaxity of hands. We cannot exclude that this boy is affected by a very mild form of the syndrome, displaying only some of its typical clinical marks. Truncating (frameshift and nonsense) loss-of-function variants, mainly de novo, account for the majority of cases with LSS. Five *SETD2* missense variants have been reported in patients with isolated nonsyndromic forms of CMI, suggesting that missense variants of *SETD2*, which do not destabilize the protein function, may play a critical role in the development of hindbrain malformation. The p.Val1190Met change identified in Family A affects a moderately conserved Valine located in the N-terminal region and outside the SETD domain that mediates the methylation of histone H3-lysine 36.

Members of Family A were also carriers of a novel heterozygous stop variant (p.Gln23Ter) in the *HP1BP3* gene, which has not been associated with human disease, however. This variant, also segregating in the sister who presented a milder malformation, is considered deleterious by pathogenicity prediction missense variant tools and has never been reported. *HP1BP3* encodes for a histone H1-related protein having a role in chromatin structure and transcriptional regulation. Mice with loss-of-function mutations of the gene ($Hp1bp3^{-/-}$ mice) exhibit a phenotype characterized by severe anomalies in bone development and structure (Garfinkel et al. 2015). *HP1BP3* plays a key role in normal growth and bone development by regulating the transcription of the endocrine components of IGF-1, a major endocrine pathway that regulates both body growth and bone acquisition (Garfinkel et al. 2015). Its role is demonstrated in osteoblast and osteoclast functions (Hayashihara et al. 2010). These findings support the role of *HP1BP3* as a modifier gene for CMI occurrence, given that *SETD2* and *HP1BP3*, as two methyltransferases of histones, are involved in the same biological processes, such as chromatin modeling and transcriptional elongation. Direct interactions

between these two genes are absent in BioGRID, STRING, or IntAct databases. However, they share similar functions on chromatin remodeling and gene expression regulation; hence, we cannot exclude a possible combined contribution to the CMI phenotype via different networks/pathways. The way they lead to CMI still remains poorly understood.

In total, 127 variants, including missense, splicing, framesfift, nonsense variants, are shared among affected members of Family A. Considering the highest CADD scores, we identified a missense variant with a CADD of 20 in the *RFWD3* gene, c.1352G>A (p.Arg451Gln). Biallelic variants of *RFWD3* cause Fanconi anemia (FA), complementation group W; some patients with the FA group W have Chiari malformation. However, based on current knowledge, no cases of monoallelic *RFWD3* variants are reported in CMI patients, thus seemingly excluding its potential role in the patient's clinical picture. When we hypothesized an autosomal-recessive inheritance for Family A, no interesting variants were found.

A pathogenic, de novo, missense variant of *KMT2A* has been identified in Family B: the c.3461G>A: p.(Arg1154Gln) variant found in a little girl, affected by CMI, psychomotor and language delay, dysmorphisms, hypertrichosis cubiti and of the back, and constipation. No other genetic variant consistent with the clinical phenotype was identifiable in Family B. Differently from the case of Family A, the *KMT2A* variant displays a clear pathogenic role and seems to explain the whole clinical phenotype of the proband. The clinical picture of Patient B is consistent with a diagnosis of Wiedemann–Steiner syndrome, even if no consensus clinical diagnostic criteria are currently available for this syndrome. To date, this is the third reported case of a patient harboring a disease-related variant in *KMT2A*, also affected by CMI (Giangiobbe et al. 2020). In the work by Giangiobbe and colleagues, two Wiedemann–Steiner syndrome patients with two different stop variants (p.Arg1938* and p.Gln2516*) were reported with CMI. No apparent correlation can be inferred between the variants identified in our patient and the two reported by Giangiobbe. Moreover, the p.(Arg1154Gln) variant described in our patient was already reported in ClinVar as pathogenic in a patient with Wiedemann–Steiner syndrome, growth deficiency, and mental retardation with facial dysmorphism (RCV002248724.2), with no mention of other clinical details provided in the database (e.g., craniocervical junction disorders). *KMT2A* encodes a histone-lysine N-methyltransferase that participates in specific complexes mediating the methylation of lysine 4 of histone H3 (H3K4me) and the acetylation of lysine 16 of histone H4 (H4K16ac), tags for epigenetic transcriptional activation (Giangiobbe et al. 2020; Dou et al. 2005). Known targets include the *HOX* genes, a family of transcription factors essential for normal embryonic development: they regulate segment specification along the body axis, thus also controlling the positional identity of prevertebral bodies at the craniovertebral junction (Giangiobbe et al. 2020; Pang and Thompson 2011). Furthermore, it is known that *KMT2A* interacts with other proteins having a role in histone-mediated chromatin remodeling, such as CREBBP. Variants in the *CREBBP* gene are associated with Rubinstein–Taybi syndrome 1 (OMIM # 180849), in which CMI is a frequent finding (Giangiobbe et al. 2020; Lee et al. 2015). This also supports the role of chromatin remodeling genes in syndromic and isolated CMI.

The role of genetics and epigenetics in the pathophysiology of CMI requires further studies in order to be elucidated. In a recent study by Provenzano and colleagues, on the genetics of CMI, two main classes of genes in association with CMI were highlighted: those directly associated with the development of skull and craniovertebral junction and those involved in chromatin remodeling.

Variants in chromatin remodeling genes can be identified in a wide spectrum of clinical issues, including neurodevelopmental disorders, intellectual disability, and congenital malformations. In many cases, these genes are the same whose somatic variants, sometimes even the same variant, drive cancer (Provenzano et al. 2021). The specific impact of chromatin remodeling genes on the formation of the craniovertebral junction remains elusive. Episignatures of patients with syndromes possibly related to CMI show varying degrees of abnormal methylation (Aref-Eshghi et al. 2020; Provenzano et al. 2021): for example, there is evidence of an intermediate episignature alteration detectable in Wiedemann–Steiner syndrome. This may suggest that the proper development of the craniovertebral junction requires the interplay of multiple genes, each of them finely activated or silenced with a specific space–time pattern during embryogenesis (Provenzano et al. 2021).

In a recent case series, it has been highlighted that CMI, both syndromic and isolated, depends on variants in chromatin remodeling genes in 25 patients out of 45: among isolated cases, mainly inherited missense or inframe variants were revealed, not seemingly destabilizing the whole protein function, whereas few cases were due to de novo, protein-truncating variants. The authors suggested that haploinsufficiency could be the predominant mechanism (Provenzano et al. 2021). As regards the inheritance model, both familial and de novo CMI cases are reported, sharing similar pathogenic mechanisms. Knowledge is still elusive about possible shared comorbidities between familial and de novo CMI cases; however, we cannot exclude them. Further studies are needed to confirm these findings.

The missense variants identified in our study are in genes (*KMT2A*, *STED2*), whose protein-truncating variants are involved in severe neurodevelopmental syndromes. We can speculate that the paucity of syndromic signs possibly detectable in our patients is due to hypomorphic variants, whereas protein-truncating variants in the same genes account for more severe clinical manifestations. This appears to be in line with the current literature's opinion (Aref-Eshghi et al. 2020; Provenzano et al. 2021).

To conclude, we report useful genetic data in patients with CMI, harboring missense variants in chromatin remodeling genes, *SETD2* and *KMT2A*. We speculate *HP1BP3* as a possible modifier gene to be considered for CMI etiology, given the evidence supporting the role of *HP1BP3* in bone development. Functional studies will be needed to foster comprehension about this possible association. Future studies with a major numerousness of patients, along with functional approaches, are required to gain better insight into the genetics of CMI and the role of chromatin-regulating genes. This will also be useful for better clinical management of syndromic patients in whom CMI may be underrated.

Author Contributions

F.R. and M.C. drafted the article, revised the literature, and contributed to clinical and genetic data analysis. P.D.M., M.I., and M.D.D. performed genetic analysis and collected genetic data. D.T. reviewed brain MRI scans and analyzed the neuroimaging spectrum. M.P. and G.P. collected clinical data. F.Z. and M.S. critically revised the article. V.C. and A.P. conceived the study, supervised the work, and critically revised the article.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are openly available in the Decipher database (https://www.deciphergenomics.org/) with the following accession numbers: 531752 (Family A) and 531754 (Family B).

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Supporting Information

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