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Chiari malformation type I: what information from the genetics?

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Abstract

Purpose Chiari malformation type I (CMI), a rare disorder of the craniocerebral junction with an estimated incidence of 1 in 1280, is characterized by the downward herniation of the cerebellar tonsils of at least 5 mm through the foramen magnum, resulting in significant neurologic morbidity. Classical CMI is thought to be caused by an underdeveloped occipital bone, resulting in a posterior cranial fossa which is too small to accommodate the normal-sized cerebellum. In this review, we dissect the lines of evidence supporting a genetic contribution for this disorder.

Methods We present the results of two types of approaches: animal models and human studies encompassing different study designs such as whole genome linkage analysis, case-control association studies, and expression studies. The update of the literature also includes the most recent findings emerged by whole exome sequencing strategy.

Results Despite evidence for a genetic component, no major genes have been identified and the genetics of CMI is still very much unknown. One major challenge is the variability of clinical presentation within CMI patient population that reflects an underlying genetic heterogeneity.

Conclusions The identification of the genes that contribute to the etiology of CMI will provide an important step to the understanding of the underlying pathology. The finding of a predisposing gene may lead to the development of simple and accurate diagnostic tests for better prognosis, counseling, and clinical management of patients and their relatives.

Keywords Chiari type I malformation (CMI) · Syringomyelia (SM) · Hindbrain · Tonsillar ectopia · Posterior cranial fossa (PCF) · Autosomal dominant/recessive inheritance · Whole exome sequencing (WES)

Introduction

Chiari malformation type I (CMI; ORPHA268882) is an heterogeneous condition characterized by the caudal descent of the cerebellar tonsils of at least 5 mm below the foramen magnum [1]. CMI causes neurological dysfunction by direct compression of the neural tissue at the craniovertebral junction and, often, obstruction of the cerebrospinal fluid (CSF) outflow. The block of CSF flow is thought to be responsible for the progressive formation of a cystic cavity within the spinal cord, called syringomyelia that is present in approximately 65–75% of patients with CMI (SM; ORPHA3280) or secondary hydrocephalus [2].

Not all cases of CMI detected on imaging are symptomatic and tonsillar descent is often regarded as an incidental neuroradiological finding. The severity does not correlate with the degree of herniation, with some asymptomatic cases presenting with prominent herniation [3, 4].

CMI affects individuals of every race and ethnicity. The onset of symptomatology is delayed till the third or fifth decade [5, 6]. With the advent of magnetic resonance imaging (MRI), the number of pediatric patients diagnosed with CMI is increasing [7, 8]. In adults, females are more frequently affected. Gender effects may be due to hormonal status or genetic variants affecting disease susceptibility and progression. In pediatric cases, there are no differences of CMI incidence among sexes and this could indicate a different etiology for pediatric and adult cases [5, 9, 10].

The true CMI prevalence is unknown. From a retrospective investigation of almost 22,000 brain MRI, the CMI prevalence

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was estimated as 1/1280 (175 out of 22,591) [4]. Thus, CMI can be considered as a rare disease. However, patients undergoing MRI are not representative of the general population and the condition may be much more common, as seen by studies reviewing large numbers of MRI performed for unrelated conditions in asymptomatic patients [11, 12].

Is CMI genetic?

CMI may have a genetic origin only in some cases. Evidence in favor of a genetic contribution comes from twin studies, familial aggregation, and co-segregation of CMI with known genetic conditions [12]. If monozygotic, identical twins are more concordant for a condition than dizygotic (non-identical) twins; a genetic contribution to the trait can be inferred. The majority of the twin studies for CMI found a higher concordance between monozygotic with respect to dizygotic twins. Some studies found that twins were concordant only with respect to the diagnosis of CMI, although they were discordant for other factors, including the presence of SM, age of onset, extent of tonsillar herniation, and symptoms severity [13–15].

Familial aggregation or clustering refers to the identification of families where more individuals are affected. While 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 [5]. Speer et al. [16] identified 31 families in which 2 or more individuals were affected with or without SM. Imaging analysis of family members revealed that 21% of asymptomatic first-degree relatives also had CMI/SM. Milhorat and colleagues [5] reported that among 364 symptomatic patients, 12% had at least one affected relative. In a large retrospective review of 500 CMI pediatric patients undergoing decompressive surgery, only 3% of patients had a positive family history for CMI [11].

Co-inheritance of CMI with more than twenty syndromes has been reported including achondroplasia, Klippel-Feil syndrome, primary basilar impression, and Goldenhar syndrome [17–25]. The majority of these disorders affect bony development supporting the hypothesis that CMI is mesodermal in origin and most of the causative genes, having pleiotropic effects, have been identified. Milhorat et al. [26] prospectively studying 2813 patients with CMI reported that 5% of the patients had both a hereditary disorder of connective tissue and CMI [26]. Case reports of CMI occurring with dermatological, hormonal, and other neurological and various chromosomal disorders have also been noted [27–29]. Syndromic cases account for fewer than 1% of total CMI cases [12]. For these cases, genetic counseling with respect to the recurrence of the underlying syndrome is warranted, although the risk for transmission of CMI to relatives remains unclear.

CMI is a complex trait whose inheritance cannot be explained by the Mendelian transmission of a single genetic variant. Most likely, CMI has a polygenic architecture based on the small effects of multiple genetic variants that all together impact the disease risk. Furthermore, environmental factors may interact with genes modulating the phenotype and influencing symptoms, disease severity, and progression.

What causes CMI?

Although multiple mechanisms have been proposed including cranial constriction, cranial settling, spinal cord tethering, intracranial hypertension, and intra-spinal hypotension [9], CMI is thought to be caused by an a underdeveloped occipital bone due to an abnormal development of the occipital somites originating from the paraxial mesoderm [30]. This insufficiency induces overcrowding in the posterior cranial fossa (PCF), which is the most posterior part of the skull base housing the brainstem and cerebellum. Overcrowding of the hindbrain and resulting displacement of CSF likely contributes to the array of neurological symptoms seen in CMI.

The occipital bone is one of the first bones of the skull to develop and consists of four parts surrounding the foramen magnum: the basioccipital, supraoccipital, and two lateral parts (exoccipital). Morphometric measurements in CMI patients demonstrated that supraoccipital, exoccipital, and basioccipital segments can be underdeveloped and hypoplastic in some patients, albeit to varying degrees [31].

The occipital bone is formed from the cephalic mesoderm segmented into somites and it has both membranous and cartilaginous origin with ossification occurring as early as week 9 of fetal gestation [32]. These events are dependent on complex interactions between genes and molecules with pathologies resulting from disruption of this delicate process.

Little is known about the molecular mechanisms which regulate how the whole cranium is shaped. Mouse models and studies of the genetic basis of human hereditary skeletal diseases have implicated several signaling pathways in proper skull ossification and growth, including the fibroblast growth factor (FGF), Hedgehog (HH), Wnt, and BMP signaling [33]. The role of FGF signaling in ossification is well known, as mutations in the FGF signaling pathway cause a number of skeletal disorders, including craniosynostosis, the premature fusion of one or more cranial sutures [34–37]. The phenotypic consequences of defective signaling can develop at different stages during differentiation and maturation of chondrocytes, osteoblasts, and osteoclasts. Studies of human skeletal disorders have identified transcription factors that are crucial for patterning and formation of cartilage. The transcription factor sex-determining region Y (SRY)-box 9 (Sox9) is a master transcriptional factor that regulates early differentiation of mesenchymal cells into the chondrocyte lineage. Impaired

Sox9 function causes severe chondrodysplasia in humans and mice [38]. Osteoblast maturation is regulated by another transcription factor, Runx2. *Runx2*-null mice have no osteoblasts and consequently bone tissue. Mutations of the *Runx2* gene in humans cause cleidocranial dysplasia [39]. Since only a small subset of causative mutations for congenital skull defects has been identified, large gaps in our knowledge remain about the genetic basis underlying development and postnatal of the cranial bones.

Animal models of CMI

Animal models offer opportunities for increased understanding of human diseases. Underdevelopment of basichondrium and cerebellar herniation in hamsters can be induced by the administration of a single dose of *vitamin A* on the eighth gestation day. This experiment enabled Marin-Padilla and collaborators to demonstrate that impairing of posterior fossa development could result in CMI occurrence [30]. Moreover, vitamin A–induced model of CMI implicated retinol metabolism in the pathogenesis of CMI.

Occipital bone hypoplasia and secondary SM similar to human CMI is a common condition in brachycephalic toy breed dogs [40, 41]. The dog model is the only known naturally occurring animal model for CMI and represents a powerful tool for the identification of defective genes involved in the human disorder. In fact, canine CMI is a less heterogeneous condition than the human one and it is known to have a strong heritable component. The number and identity of genes predisposing to canine CMI have been determined by a quantitative trait locus (QTL) approach study in a cohort of 74 dogs of the Griffon Bruxellois (*BG*) breed. The study identified 2 regions on *Canis familiaris* autosomes (CFA), CFA2 and CFA14, that were strongly associated to height of the cranial fossa. The QTL region on CFA2 contained the *Sall-1* gene, encoding for a transcription factor, whose human orthologue is mutated in the Townes-Brocks syndrome associated to CMI [42]. A second whole genome association study in 65 Cavalier King Charles (*CKCS*) dogs identified two loci on CFA22 and CFA26 significantly associated with a reduced volume and altered orientation of the caudal cranial fossa. Candidate genes within these regions were *protocadherin 17* (*PCDH17*) and *ZW10-interactor* (*ZWINT*) on CFA22 and CFA26, respectively. *PCDH17* encodes for a member of the cadherin superfamily of cell adhesion proteins, specifically expressed in the brain and the spinal cord [43].

Very recently, Solis-Moruno et al. [44] reported *Nico* as the first chimpanzee diagnosed with a natural form of CMI. His genome was sequenced and a private missense mutation of *LRP5* was identified that could explain its phenotype. *LRP5* protein is implicated in Wnt signaling pathway, a highly conserved process that controls bone density and bone metabolism.

Genetic studies in humans

Despite evidence for a genetic component, genetic studies for CMI have been limited due mainly to the rare disease prevalence and the small proportion of familial cases. Candidate gene approach, whole genome linkage analysis, case-control association studies, expression studies on dura mater tissue samples, and eQTL approach were carried out. One major challenge is the variability of clinical presentation within the CMI patient population. Inter- and intra-familial differences in the severity of symptoms, response to surgery, presence of associated conditions, age of onset, and the extent of tonsillar herniation had substantial implications during the design of a genetic study. When significant associations were found in human genetic studies, stratified analysis among phenotypic homogenous patient groups was performed (Table 1).

A genome-wide linkage study conducted in 23 families with 71 affected individuals showed association to chromosomes 9 and 15 [46]. Interestingly, the region on chromosome 15q21.1-q22.3 harbored a biologically plausible gene, *fibrillin 1*, which is mutated in patients with Shprintzen-Goldberg syndrome, a rare syndrome with craniosynostosis and brain anomalies including CMI [23]. The only other whole genome screen conducted to date was published by Markunas et al. Using 66 non-syndromic CMI multiplex families, they performed a stratified analysis based on clinical criteria in order to reduce phenotypic heterogeneity. This resulted in increased evidence for linkage to regions on chromosomes 8 and 12 that harbored the growth differentiation factors, *GDF6* and *GDF3*, previously implicated in Klippel-Feil syndrome which is diagnosed in roughly 3–5% of CMI patients [45]. A case-control association study looking specifically at common variants (SNPs) across 58 candidate genes involved in paraxial mesoderm development found associations with 3 genes (*CDX1*, *FLT1*, *ALDH1A2*), only when analyses were restricted to a subset of patients with a small PCF [47].

Dura mater samples obtained during decompressive surgery from CMI patients have been used to perform expression studies. In fact, dura mater, the outermost meningeal layer, is considered a source of factors modulating development and growth of cranial bones. Markunas et al. [48] used cranial base morphometrics and whole gene expression analysis on both blood and dura mater samples to identify disease genes for CMI. By clustering analysis, PCF morphology was found heritable and influenced by genetic factors taking part in biological pathways, including the ribosome, spliceosome, proteasome, RNA degradation, and oxidative phosphorylation. However, there was little concordance observed between the blood and dura mater expression profiles.

Table 1 Genes involved in human CMI and strategies for the gene identification

Gene	Function	Signaling pathway	Strategy	Reference
GDF6 (growth differentiation factor 6)	Bone formation	TGF-beta	Whole linkage	[45]
GDF3 (growth differentiation factor 3)	Embryonic development	TGF-beta	Whole linkage	[45]
FBN1 (fibrillin 1)	Constituent of the extracellular matrix	TGF-beta, BMP	Whole linkage	[46]
CDX1 (caudal type homeobox 1)	Transcriptional regulator	Wnt	Case-control association	[47]
FLT1 (fms-related tyrosine kinase 1)	Receptor of vascular growth factors	VEGF	Case-control association	[47]
ALDH1A2 (aldehyde dehydrogenase 1 family member A2)	Aldehyde dehydrogenase	Retinoic acid	Case-control association	[47]
CREBBP (CREB-binding protein)	Transcriptional activator	TGF-beta	Whole linkage	[48]
EP300 (E1A binding protein P300)	Transcriptional activator	TGF-beta	Whole linkage	[48]
ATF4 (activating transcription factor 4)	Transcriptional activator	–	Whole linkage	[48]
IPO8 (importin 8)	Nuclear protein import	–	eQTL	[49]
XYLT1 (xylosyltransferase 1)	Biosynthesis of glycosaminoglycan chains		eQTL	[49]
PRKAR1A (protein kinase cAMP-dependent type I regulatory subunit alpha)	Phosphorylation of different target proteins	–	eQTL	[49]
DKK1 (Dickkopf Wnt signaling pathway inhibitor 1)	Inhibitor of Wnt signaling	Wnt	WES	[50]
LRP4 (low-density lipoprotein receptor-related protein 4)	Inhibitor of Wnt signaling	Wnt	WES	[50]
BMP1 (bone morphogenetic protein 1)	Bone formation	BMP	WES	[50]

eQTL, expression quantitative loci studies; *WES*, whole exome sequencing

Expression quantitative loci trait (eQTL) analysis aims to discover genetic variants that explain variations in gene expression levels. eQTL analysis of whole blood and dura mater tissues from 43 individuals with CMI identified 239 eQTLs, with 79% of these shared by both tissues. This approach implicated genes involved in bone development (*IPO8*, *XYLT1*, and *PRKAR1A*) and in ribosomal pathways related to marrow and bone dysfunction as potential candidates in the development of CMI [49].

Whole exome sequencing in CMI patients

Rare variants (RVs) (either structural variants, small insertion/deletions, or SNVs) of moderate to large effect are expected to play a role in the pathogenesis of CMI. Next-generation sequencing methods represent a powerful approach for the investigation of RVs. We screened two CMI families, with at least two affected individuals, to identify RVs by means of whole exome sequencing (WES) [50]. WES enriches ~1% of genomic DNA that encodes proteins. Because of issues associated with data volume and cost effectiveness, WES is the more popular sequencing platform for identifying disease-causing mutations [51]. In the WES platform, the coding regions of the entire genome are captured from sheared genomic DNA fragments, using labeled hybridization probes that bind to the protein-coding sequences [52]. The raw sequencing data from WES can be processed using well-established pipelines. A fundamental limitation of WES is that it is unable to assess non-coding regions; however, many disease-causing genes have been identified using this technology. Moreover, NGS-based procedures require a smaller number of families than linkage analysis.

Preliminary volumetric analysis from preoperative MRI images of pediatric patients of the two families used for WES confirmed a smaller PCF volume with respect to the age-related control group. The index cases of the first family were two dizygotic twins, both affected by CMI and scaphocephaly. MRI scans of the twins' parents showed a reduced PCF volume in the father, although there were no signs of caudal displacement of the cerebellar tonsils. In the second family, both the mother and the son were affected by CMI. The son also presented with facial dysmorphisms, clinodactyly, and psychomotor delay. Prioritization of the WES variants identified 3 variants of biologically plausible genes in the two families. All the candidate variants were missense mutations. The *DKK1* c. 121 G>A (p.A41T), LRP4 c.2552C>G (p.T851R), and *BMP1* c.941G>A (p.R314H) segregated in all affected individuals and were absent in the controls. *DKK1* is involved, through its inhibition of the WNT signaling pathway, in the head induction, skeletal development, and limb patterning [53]. *DKK1* encodes for a secreted protein that is a high affinity ligand for LRP6. *DKK1* mutant mice die at the birth, exhibiting absence of cephalic neural-crest-derived head structures and forelimb

malformations [54]. Although the DKK1 p.A41T substitution falls in a functionally uncharacterized portion of the protein, it lies in a conserved amino acid motif, NAIKN (amino acids 40–44) that is present in multiple DKK proteins and is conserved among species. *LRP4* belongs to the low density lipoprotein receptor gene family, whose members are involved as signal transducers or modulators of several fundamental pathways, including BMP, TGF-beta, PDGF, and canonical Wnt signaling. LRP4 reduces Wnt signaling during bone development. Lrp4 binds the two secreted Wnt modulators, Dkk1 and sclerostin, and is expressed by murine bone, specifically by osteoblasts [55]. Mice carrying *Lrp4* mutations have impaired bone growth and increased bone turnover and fusion of digits at the hind and fore limbs (polysyndactyly) [56]. The mutation p.T851R affected the LDL receptor class B repeat 7 that is part of the third β-propeller domain; it was never reported and it is a patient-specific mutation. Mutational analysis by Sanger sequencing of a cohort of 65 sporadic CMI cases and 100 controls could identify a missense mutation in exon two of *DKK*1, the c.359G>T (p.R120L) in two sporadic CMI patients. The p. R120L was a de novo variant because it was not present in the parents and it is a patient-specific mutation because it was not found in controls. *DKK1* p.R120L could explain only a minority (3%; 2/65) of the sporadic CMI cases. Our findings confirmed the role of WNT signaling in the correct development of the cranial mesenchyme originating the PCF. Our pilot study could serve as an entry point for future studies of exome sequencing in a larger cohort of familial cases.

Conclusions

CMI and its frequently associated conditions are more common disorders than previously recognized. However, the genetics of CMI is still very much unknown. The connection of rare genetic variations to a complex phenotype like CMI remains challenging. Genetic contributions are likely to be multigenic, involving genes that influence cranial base and craniocervical morphology, size of the cerebellum, CSF production, and absorption. Environmental factors and genetic modifiers may also be responsible for the final phenotype. Whole exome sequencing on large patient cohorts has the potential to successfully enhance our understanding of CMI pathogenesis. Characterizing the genetic contribution to CMI could yield very valuable insights into the etiology of the disorder. Such work would provide genetic counselors with a clearer understanding of the risk of CMI in relatives of affected patients.

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Compliance with ethical standards

Conflict of interest The authors report no conflicts of interest.

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