On the association between Chiari malformation type 1, bone mineral density and bone related genes

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Keywords: Chiari malformation type 1; Bone mineral density

1. Introduction

Chiari malformation type 1 (C1M; OMIM 118420) is characterized by downward herniation of the cerebellar tonsils through the foramen magnum by at least 5 mm without involving the brain stem (Barkovich et al., 1986). This herniation results in compression of the neural tissue at the craniovertebral junction leading to neurological dysfunction, which may or may not be accompanied by syringomyelia or hydrocephalus (Millhorat et al., 1999; Urbizu et al., 2013; Speer et al., 2003). C1M presents in many cases as asymptomatic, and in many others with pain or headache within the occipital or upper cervical region, which can also be accompanied by ocular or otoneurological disturbances, lower cranial nerve signs, cerebellar ataxia or spasticity (McVige and Leonardo, 2014; Piper et al., 2019; Speer et al., 2003). The diagnosis is made through magnetic resonance imaging (MRI) or ultrasonography for the prenatal diagnosis (Efric and Eide, 2020; Holly and Batzdorf, 2019). Although it has been described that some cases of C1M may be the consequence of trauma, many cases can be congenital (Speer et al., 2003). It has been proposed that classical C1M is generated via an insufficient development of the paraxial mesoderm involving occipital bone sclerotomes, resulting in a smaller and shallow posterior fossa (PF), too small to accommodate the normal size of the cerebellum (Nishikawa et al., 1997; Marin-Padilla and Marin-Padilla, 1981). C1M is likely to have a genetic basis, based on familial aggregation, monozygotic twin studies and its overlap with known genetic conditions (Markunas et al., 2014; Mavinkurve et al., 2005; Szewka et al., 2006; Speer et al., 2003).

Abbreviations: C1M, Chiari Malformation type 1; HBM, High Bone Mass; BMD, Bone Mineral Density.

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Abbott et al., 2018; Speer et al., 2000). The main recent strategy to find the genetic basis of the disease and the inheritance pattern has involved the use of next generation sequencing (NGS) techniques such as whole exome sequencing (WES). These works have found several genes, including chromodomain genes (Sadler et al., 2021) and chromatin remodeling genes (Provenzano et al., 2021).

The fact that the cranial bone constriction is suspected to be the most common biologic mechanism leading to C1M suggests that variants in genes related to bone metabolism may be contributing to the malformation through an incorrect development of the cranial bone. This hypothesis is reinforced by the findings of several recent works which have found association of C1M with rare variants in genes involved in collagen metabolism (Uribu et al., 2021), in Wnt pathway genes (Merello et al., 2017), or in cranial bone sutures (Provenzano et al., 2021).

In this work, we have further investigated the relationship between C1M and bone metabolism, searching for rare variants in bone related genes through an in-house bone gene panel and, for the first time, exploring the association between bone mineral density and C1M, in a small series of C1M patients.

2. Material and methods

2.1. Study cohort, brain MRI and bone mineral density measurements

C1M cases, previously diagnosed at the Neurosurgery Service of the Hospital del Mar, were invited to participate in the study. Twelve patients agreed to participate, and in three cases, additional family members were also recruited (n = 6), four of whom were also affected with C1M. Diagnosis of C1M was based on brain-MRI (Achieva 3.0 T, Philips, Amsterdam, Netherlands) demonstration of downward herniation >5 mm of the cerebellar tonsils on a mid-sagittal T1-weighted image in the presence of signs or symptoms indicating neural compression at the cranio-vertebral junction, syringo-hydromyelia, cerebellar dysfunction or intracranial hypertension. All C1M cases and family members underwent brain MRI. The cohort characterization includes femoral neck and lumbar spine bone mineral density measurements obtained through dual-energy X-ray absorptiometry scans (DXA; QDR 4500 SL; Hologic, Waltham, MA, USA) and collection of blood samples (Table 1). All DXA measurements were performed prior to any treatment that could increase bone mass. For individuals CH7a and CH10 no DXA scan was available, for CH4 no T-score was available and for CH7 no Z-score was available (Table 1).

2.2. Genetic analyses

The genomic DNA of 12 unrelated C1M and their 6 relatives was isolated from peripheral blood leukocytes using Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA), according to the manufacturer’s instructions. The 12 unrelated C1M cases underwent targeted sequencing of the exonic and flanking intronic regions of 127 genes related with BMD in GWAS studies or associated with monogenic bone diseases included in an in-house bone related gene panel (IRN:1000011543, KAPA HyperChoice MAX 0.5 Mb T4, 12 rxn; Supplementary Table 1). Sequencing was performed at CNAG (Barcelona, Spain). Shortly, DNA was enzymatically fragmented and libraries were constructed, pooled and hybridized to the probesets. Captured fragments were sequenced in an Illumina NovaSeq 6000 sequencer. The reads were then aligned to the hg38 reference genome with BWA-mem, duplicate-marked, recalibrated and sorted before calling variants with GATK’s haplotype caller (V4) following GATK standard parameters. After quality-filtering following GATK recommended hard filters (htps://gatk.broadinstitute.org/hc/en-us/articles/360035890471-Hard-filtering-germline-short-variants), variants were annotated with Varat (Desvignes et al., 2018), and prioritized under the hypothesis of an autosomal dominant segregation.

Filtering included the exclusion of: variants located outside of the coding region (intergenic, 5’ and 3’ UTR, upstream, downstream, ncRNA or unknown variants); intronic variants not predicted to affect the splice site; synonymous variants; variants with a minor allele frequency  0.2; missense variants with less than two damaging or potentially damaging prediction scores out or four tools (CADD (V1.3), http://cadd.gs.washington.edu/; PROVEAN, http://provean.jcvi.org/; Polyphen-2, http://genetics.bwh.harvard.edu; SIFT, https://sift.bii.a-star.edu.sg/); or, for indel variants, those with a neutral SIFT indel score (https://sift.bii.a-star.edu.sg/). For the remaining variants, cosegregation in available family members (for CH2, CH7 and CH10) was examined by Sanger sequencing at the CCITUB genomics service (Genómica, Parc Cientific, Barcelona, Spain) using BigDye™ Terminator v3.1 Cycle Sequencing Kit, followed by detection on automated capillary sequencer models 3730 Genetic Analyzer and 3730xl Genetic Analyzer.

Table 1

Description of the Spanish C1M case series.

<table>
<thead>
<tr>
<th>ID</th>
<th>Sex</th>
<th>Age</th>
<th>Family relationships</th>
<th>Disease status</th>
<th>BMD (T-score)</th>
<th>BMD (Z-Score)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>LS</td>
<td>FN</td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>LS</td>
<td>FN</td>
</tr>
<tr>
<td>CH1</td>
<td>F</td>
<td>59</td>
<td></td>
<td>A</td>
<td>*</td>
<td>−2.6</td>
</tr>
<tr>
<td>CH2</td>
<td>F</td>
<td>65</td>
<td></td>
<td>A</td>
<td>−3.2</td>
<td>−1.5</td>
</tr>
<tr>
<td>CH2a</td>
<td>F</td>
<td>35</td>
<td>daughter of CH2</td>
<td>A</td>
<td>−0.5</td>
<td>−1.1</td>
</tr>
<tr>
<td>CH2b</td>
<td>F</td>
<td>68</td>
<td>sister of CH2</td>
<td>A</td>
<td>−2.8</td>
<td>−1.6</td>
</tr>
<tr>
<td>CH2c</td>
<td>F</td>
<td>71</td>
<td>sister of CH2</td>
<td>N</td>
<td>−1.4</td>
<td>−1.6</td>
</tr>
<tr>
<td>CH2d</td>
<td>F</td>
<td>72</td>
<td>sister of CH2</td>
<td>A</td>
<td>−4.1</td>
<td>−3.8</td>
</tr>
<tr>
<td>CH3</td>
<td>F</td>
<td>46</td>
<td></td>
<td>A</td>
<td>−0.3</td>
<td>−1.1</td>
</tr>
<tr>
<td>CH4</td>
<td>M</td>
<td>24</td>
<td></td>
<td>A</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CH5</td>
<td>F</td>
<td>45</td>
<td></td>
<td>A</td>
<td>+1.4</td>
<td>+1.5</td>
</tr>
<tr>
<td>CH6</td>
<td>M</td>
<td>46</td>
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<td>A</td>
<td>−0.6</td>
<td>−0.6</td>
</tr>
<tr>
<td>CH7</td>
<td>F</td>
<td>61</td>
<td></td>
<td>A</td>
<td>−2.5</td>
<td>−3.2</td>
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<tr>
<td>CH7a</td>
<td>F</td>
<td>63</td>
<td>sister of CH7</td>
<td>A</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CH8</td>
<td>M</td>
<td>47</td>
<td></td>
<td>A</td>
<td>−2</td>
<td>−1.9</td>
</tr>
<tr>
<td>CH9</td>
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<td>45</td>
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<td>A</td>
<td>+0.9</td>
<td>+0.1</td>
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<tr>
<td>CH10</td>
<td>M</td>
<td>74</td>
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<td>CH10a</td>
<td>F</td>
<td>NA</td>
<td>sister of CH10</td>
<td>N</td>
<td>−1.1</td>
<td>−0.5</td>
</tr>
<tr>
<td>CH11</td>
<td>F</td>
<td>36</td>
<td></td>
<td>NA</td>
<td>+0.8</td>
<td>−0.1</td>
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<tr>
<td>CH12</td>
<td>F</td>
<td>83</td>
<td></td>
<td>A</td>
<td>−2.4</td>
<td>−1.1</td>
</tr>
</tbody>
</table>


* Dysmorphic lumbar.
3. Results

3.1. Relationship between C1M and BMD

To study the relationship between C1M and BMD we have determined the BMD of a small group of C1M patients (Table 1). The average LS and FN BMD Z-score for the cases with available data was 0.16 and −0.058, respectively. Seven unrelated C1M cases had normal BMD according to the WHO diagnostic criteria based on the T-scores and Z-scores. Instead, following the same criteria, three unrelated C1M cases (CH1, CH2, CH7) and one of their relatives (CH2d) are classified as osteoporotic. On the other hand, a patient is considered to have high bone mass (HBM) phenotype when the sum of the LS and FM BMD Z-score values is higher than 4 (Little et al., 2002). According to this definition, only one patient (CH5) had values close to this threshold (sum Z-scores = 3.8), while the mean sum Z-score value for all 9 cases was +0.5 (Table 1).

3.2. Relationship between bone-related genes and C1M

To elucidate the relationship between variants in bone related genes and C1M, we sequenced the coding regions and intron boundaries of 127 genes through an in-house panel in 12 unrelated patients with C1M. After filtering (see Material and Methods) we identified 24 variants in 24 different genes in the 12 unrelated C1M patients (Supplementary Table 2). Seven of these 24 genes contain variants in more than one C1M patient (Table 2) and only one variant was present in two unrelated patients (p.P582H in DAAM2; Table 2).

Next, in three cases (CH2, CH7, CH10) with available DNA from family members, we tested the variants' cosegregation with the C1M phenotype (n = 6; Fig. 1 and “Coseg” column in Table 2 and Supplementary Table 2).

Two variants were consistent with cosegregation with the C1M phenotype in the families, p.R222C in WNT16 and p.L282I in DMP1, two were compatible with cosegregation only if assuming incomplete penetrance (i.e. they were present in both affected and healthy individuals; p.L347F in CRTAP and p.R371H in BMP1), and four did not cosegregate with the phenotype (i.e. they were absent in other affected family members; p.D478A in DMP1, p.I436T in FKB10, p.P582H in DAAM2 and p.R159L in SPP1; Table 2 and Supplementary Table 2). Unfortunately, the variants present in the remaining C1M cases could not be tested for cosegregation for lack of available relatives' samples.

After excluding variants that do not cosegregate with C1M, 26 variants in 23 different genes were present in at least one of the 12 unrelated C1M patients (Supplementary Table 2) and four genes presented variants in more than one C1M patient. These are WNT16, CRTAP, MYO7A and NOTCH2 (highlighted in bold in Table 2 and Fig. 2).

4. Discussion

Here, we present a first study on the possible relationship among C1M, variants in bone related genes and BMD, through the sequencing of a bone in-house panel and BMD determination in 12 unrelated patients with C1M.

We did not find a clear positive association between BMD and C1M. Even so, we have found 3 unrelated C1M patients with osteoporosis while one patient (CH5) presented a sum Z-score of 3.8, very close to the accepted threshold for HBM. Knowing that C1M can occur as an isolated malformation or as a part of a broader genetic entity (Spee et al., 2003), and many Mendelian diseases have been described to coexist with C1M, we may speculate that these extreme values of BMD (Osteoporotic or HBM) can be somehow related to C1M phenotype. The CH5 patient is of special interest because given the reported prevalence of the HBM phenotype of 0.2–1% (Sarrin et al., 2014; Gregson et al., 2012, 2013), under the hypothesis of no association it would have been highly unlikely to find a HBM case in this small cohort. Nevertheless, this could also be a spurious finding, as it is only one individual. In order to elucidate the relationship between the C1M and bone mass, it would be crucial to measure the LS and FN BMD of the C1M patients from previous publications (Boyles et al., 2006; Markunas et al., 2013; Urbizu et al., 2013; Markunas et al., 2014; Rosenblum et al., 2019; Gonçalves et al., 2019; Merello et al., 2017; Urbizu et al., 2021; Provenzano et al., 2021).

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Table 2: Genes with variants in more than one Chiari patient.

<table>
<thead>
<tr>
<th>Gene</th>
<th>BMD GWAS Disease</th>
<th>Variant</th>
<th>OMIM Disease</th>
<th>C1M patient</th>
<th>Coseg.</th>
<th>CADD</th>
<th>PP</th>
<th>PV</th>
<th>SIFT</th>
<th>MAF gnomAD</th>
<th>rs number</th>
</tr>
</thead>
<tbody>
<tr>
<td>WNT16</td>
<td>Y</td>
<td>p.R222C</td>
<td>CH10</td>
<td>Y</td>
<td>27.8</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td></td>
<td>0.0001995</td>
<td>rs773948299</td>
</tr>
<tr>
<td>CRTAP</td>
<td>N</td>
<td>p.C243G</td>
<td>CH12</td>
<td>NA</td>
<td>23.1</td>
<td>D</td>
<td>N</td>
<td>T</td>
<td></td>
<td>0.002061</td>
<td>rs11644834</td>
</tr>
<tr>
<td>DMP1</td>
<td>Y</td>
<td>p.L282I</td>
<td>CH10</td>
<td>Y</td>
<td>22.2</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td></td>
<td>0.0003853</td>
<td>rs148156611</td>
</tr>
<tr>
<td>DAAM2</td>
<td>Y</td>
<td>p.P582H</td>
<td>CH7</td>
<td>N</td>
<td>22.9</td>
<td>P</td>
<td>N</td>
<td>T</td>
<td>0.001564</td>
<td>rs15067991</td>
<td></td>
</tr>
<tr>
<td>FKB10</td>
<td>Y</td>
<td>p.R556C</td>
<td>CH11</td>
<td>NA</td>
<td>22.2</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td></td>
<td>0.0002014</td>
<td>rs759817118</td>
</tr>
<tr>
<td>MYO7A</td>
<td>N</td>
<td>p.M2008fs</td>
<td>CH6</td>
<td>NA</td>
<td>29.1</td>
<td>D</td>
<td>D</td>
<td>D</td>
<td>0.0001296</td>
<td>rs201928014</td>
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<tr>
<td>NOTCH2</td>
<td>N</td>
<td>p.R391L</td>
<td>CH4</td>
<td>NA</td>
<td>22.1</td>
<td>P</td>
<td>N</td>
<td>T</td>
<td>0.003978</td>
<td>rs37396789</td>
<td></td>
</tr>
</tbody>
</table>

In bold genes not discarded for cosegregation. BMD GWAS: Genes associated with BMD in GWAS; Y: Yes; N: No; OMIM DISEASE: Gene associated with human diseases according to OMIM; N: No; OI: Osteogenesis imperfecta; ARHR1: Hypophosphatemic rickets, autosomal recessive; 1; NPHS24: Nephrotic syndrome, type 24; BRKS1: Bruck syndrome 1; DFN: Deafness; USH1: Usher syndrome, type 1B; ALGS2: Alagille syndrome 2; HJCYS: Hajdu-Cheney syndrome; Coseg: Cosegregation in the available families (CH2, CH7, CH10); Y: Yes; Variant present in other C1M cases in the family and/or absent in healthy family members; P: Incomplete penetrance: variant found in a healthy family member; N: No; Variant absent in other C1M cases in the family; CADD: http://cadd.gs.washington.edu; PP: Polyphen-2 http://genetics.bwh.harvard.edu/pph2/; D: Probably damaging; P: Possibly damaging; B: Benign; NA: Not available; PV: PROVEAN http://provean.jcvi.org/; D: Deleterious; T: Tolerated; NA: Not available; +: Tested with SIFT indels https://sift.bi.a-star.edu.sg/www/SIFT_indels2.html; MAF: minor allele frequency from gnomAD V2.1.1.
Sadler et al., 2021), thus increasing the size of the cohort of patients with C1M and BMD.

Regarding the relationship between variants in bone-related genes and C1M, we have found 4 such genes each with variants in two unrelated C1M patients. These are WNT16, CRTAP, MYO7A and NOTCH2 (Table 2 and Fig. 2). In WNT16, two missense variants, p.S260P and p.R222C, have been found in patients CH4 and CH10, respectively, the latter absent in CH10’s healthy sister (CH10a), thus co-segregating with the variant (p.R1036Q) in CH3 and a missense variant (p.R654C) in CH11 (Table 2 and Fig. 2).

Taking all this together, it is tempting to speculate that rare variants in WNT16, LRP5 and DAAM2, alone or in combination with other variants, could be responsible for C1M in these individual cases.

Another gene present in two C1M cases is CRTAP (variants p.C243G and p.L347F, in patients CH12 and CH2, respectively). Nevertheless, the p.L347F variant is present in all available CH2 family members (Fig. 1), including the healthy sister (CH2c), which would require the assumption of incomplete penetrance. CRTAP, a member of the multifunctional complex P3H1-CRTAP-CypB, is involved in posttranslational modifications of fibrillar collagen. CRTAP loss-of-function variants are associated with osteogenesis imperfecta type VII in humans, with a phenotype that includes craniofacial bone defects (Morello et al., 2006; Baldridge et al., 2010; Xu et al., 2020; Barbirato et al., 2015; Valli et al., 2012). Interestingly, the p.C243G variant is modifying the first cysteine of the third CXXXC domain of the CRTAP protein, an important domain that could have disulfide isomerase activity (Fig. 2; Ishikawa and Bächinger, 2013). The involvement of this gene in osteogenesis imperfecta could partially explain the low BMD present in all the studied individuals with CRTAP variants. In family CH2, where all family members carry the p.L347F variant, two of them show a clear osteoporosis and the remaining three show osteopenia (Table 1) while patient the CH12, carrying variant p.C243G, shows an osteopenia phenotype (Table 1). Considering all this together, it is tempting to speculate that the variants in CRTAP may be contributing to a decrease in BMD and possibly to C1M, too.

In MYO7A, we have found a frameshift variant (p.M2008fs) in CH6 and a missense variant (p.R654C) in CH11 (Table 2 and Fig. 2). MYO7A encodes an unconventional myosin which is an actin-based motor molecule with ATPase activity, important in intracellular movement (reviewed in Williams and Lopes, 2011). Variants in the MYO7A gene have been associated with different forms of inherited deaf-blindness or deafness (Weil et al., 1995; Gibson et al., 1995; Hildebrand et al., 2010;
In conclusion, while we did not find a clear association of BMD with the C1M, we have found variants in more than one C1M patient in four interesting genes -WNT16, CRTAP, MYO7A and NOTCH2- involved in craniofacial development or previously associated with C1M.
Altogther, we consider the relationship of C1M with bone biology a very interesting issue, and we provide a few pieces of evidence supporting it. We encourage the C1M research community to pursue this effort.

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Availability of data and material

All the data are available upon request.

Ethics approval and consent to participate

All procedures performed were in accordance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Both the Bioethics Committee of Universitat de Barcelona and the Clinical Research Ethics Committee of Parc de Salud MAR have emitted a favourable bioethical statement regarding the present research. Written informed consents were obtained from the participants in both instances.

CRediT authorship contribution statement

NMG, DG, SB were involved in the study conception and design. LM and DMLG recruited the Chiari 1 malformation cohort and made bone mineral density measurements. NMG, MC, JDP and RR were involved in the design of the bone gene panel and the sequencing of the patients and relatives. The first draft of the manuscript was written by NMG, DG, SB, MC, JDP and RR and all authors commented on previous versions of the manuscript and all authors read and approved the final version.

Declaration of competing interest

Núria Martínez-Gil, Leonardo Mellibovsky, Demian Manzano-Lopez Gonzalez, Juan David Patiño, Monica Cozar, Raquel Rabionet; Daniel Grinberg and Susanna Balcells, authors of the above-mentioned manuscript, jointly declare they hold no conflicts of interest regarding the research and results presented in it.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jbonr.2022.101181.

References


