Introduction

Methylmalonic acidemia (MMA) belongs to the bigger group of organic acidaemias (OAs) and is an autosomal recessive disorder of propionate catabolism. The overall incidence is estimated around 1:50 000 in Europe. Isolated methylmalonic acidemia can be caused by a spectrum of defects: a reduced or absent function of methylmalonyl-CoA mutase due to a mutation in the gene itself (MMUT), a deficiency of its active cofactor 5-deoxyadenosylcobalamin (CblA, CblB, CblD) or of methylmalonyl-CoA epimerase (MCE, encoded by MCEE).

Clinical presentation varies based on the severity of the enzyme defect and can present as early as the neonatal period with acute deterioration of their general clinical condition or in late-onset cases of MMA may present at any age with an unspecific and heterogeneous clinical picture.

MMA patients develop a range of long-term complications. We were interested to systematically review their bone health. There are several known risk factors for low BMD in this population: chronic acidosis, protein restricted diet, amino acid mixture administration, renal impairment, and reduced mobility.

We conducted the first longitudinal study of bone health in a cohort of 20 MMA paediatric patients by means of biochemical parameters, clinical data and DXA scan reports from 2015 to 2021.

Materials and methods

Biochemical parameters, clinical data and DXA scan reports for each patient were obtained from Electronic Patient Records system available in the centre. A bespoke questionnaire was sent to MMA families to investigate the mobility status of their affected child and the fracture history.

All statistical analysis was performed using Python 3.7.9. We ran univariate and multivariate regressions to establish links between bone health and potential risk factors. Beta sensitivities and their t-statistics were computed from these regressions. Two-sided p-values below 10% were considered statistically significant. Adjusted R square to control for the loss of degree of freedom in multivariate regressions was calculated. Regression analysis was conducted both on raw data and on ranked values.
Results

Data from 2015 to 2021 were collected for 20 patients affected by Methylmalonic aciduria:

- Age range was 5 to 18 years, median age 11.8 years.
- Gender distribution was 11/20 males (55%), 9/20 females (45%).
- The distribution in regards of disease subtype was heterogenous: 35% MMAA, 30% MMAB, 25% mutase, 5% CblD deficiency and 5% (1/20) unknown).
- 12/20 (60%) presented in the neonatal period, 35% had an infantile onset and 1/20 was diagnosed due to an affected sibling and therefore was asymptomatic at time of diagnosis.

We collected data of a total of 54 observations. 5/20 patients had 4 observations, 7/20 had 3 observations, 5/20 had 2 observations and 3/20 had only one observation (Figure 1).

Bone health related bloods were overall in the normal range (Table 1). Ionized calcium median value was 1.22 mmol/L (nv. 1.15-1.30 mmol/L) PTH median value 4.6 pmol/L (nv. 1.6-6.9 pmol/L), vitamin median value 68 nmol/L (50-120 nmol/L), ALP median level 269 U/L (nv. 145-320 U/L). Plasma MMA median value 80.35 umol/L, SD 943. GFR median value 69 mo/min/1.73.

Assessment of mobility. 15/20 subjects could walk independently and had no reduced mobility. 4/20 patients were severely impaired, and wheelchair bound and 1/20 could walk with support.

Bone fractures. Only 1 patient had experienced more than 3 long bone fractures by the age of 19y. This patient also had a BMD Z-score < -2 and is the only one in this study that fulfilled the criteria for osteoporosis in children. Of note, none of our patients experienced vertebral fractures.

DXA results. Bone health was evaluated with BMD z-score on DXA scan. We decided to use the BMD Z-score, rather than the z-score adjusted for the stature, since this was not available for all the patients. We reckon that this could bring to an underestimation of BMD in our cohort.

The median BMD Z-score was -1.85 in our MMA patients.

Based on the gender, male had BMD Z-score mean -1.88 SD and a median - 1.9 SD; female had a BMD Z-score mean -1.64 SD and a median - 1.6 SD.

![Figure 1. Distribution of observations per MMA subtype](image)

| Table 1. Biochemical parameters |
|-----------------------------|----------------|--------|
| **Mean** | **Median** | **SD** |
| Ionized calcium (1.15-1.30 mmol/L) | 1.22 | 1.22 | 0.06 |
| PTH (1.6 - 6.9 pmol/L) | 7.85 | 4.6 | 7.5 |
| Vitamin D (50-120 nmol/L) | 79.59 | 68 | 43.81 |
| ALP (145-320 U/L) | 257.56 | 269 | 70.37 |
| Plasma MMA (0.00-0.28 umol/L) | 637.4 | 80.35 | 943 |
| GFR (>90 ml/min) | 60 | 69 | 32 |
BMD Z-score. Based on the BMD Z-score values, patients were divided into three groups:

(I) 10/20 (50%) had a BMD Z-score < -2 SD (mean BMD z-score -3.52 SD); (II) 7/20 (35%) had a BMD Z-score between -2 and -1 SD (mean BMD z-score -1.43 SD); (III) 3/20 (15%) had a BMD Z-score > -1 (mean BMD z-score 0.46 SD).

Correlation Bone Mineral density and plasma MMA. There was a difference in plasma MMA levels across the three subgroups (Figure 2). In order to understand whether there was a correlation between MMA and BMD we ran a univariate regression of BMD on MMA which proved to be statistically significant: beta -0.0006, t-stat -3.45, p value 0.05% (Figure 3). Nearly 20% of BMD variation was explained by MMA plasma level (R squared 0.19).

Due to the high dispersion of the sample, we controlled the variation ranking the MMA levels.

We ran a univariate regression of BMD on ranked MMA: beta -0.04, t-stat -3.79, p value 0.01% (Figure 4). R squared 0.22 (Figure 4).

Correlation Bone Mineral density and GFR. We also evaluated the correlation between renal function and bone health. We therefore ran a univariate regression of BMD on GFR: beta 0.018, t-stat 3.55, p value 0.03% (Figure 5). Even in this case, almost 20% of BMD variation was explained by GFR (R squared 0.21).
Multivariate regression of BMD on GFR and MMA ranked

In order to establish which of the two variables was prominent risk factors in influencing bone health, we ran a multivariate regression of BMD on GFR and MMA ranked (see Table 2). To bring the two variables on the same variation it was decided to use the ranked values of them.

<table>
<thead>
<tr>
<th></th>
<th>Beta</th>
<th>T-stat</th>
<th>P-value %</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR rank</td>
<td>0.009</td>
<td>0.53</td>
<td>59.19</td>
</tr>
<tr>
<td>Plasma MMA rank</td>
<td>-0.04</td>
<td>-2.58</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Table 2. Multivariate regression of BMD on ranked GFR and MMA

In our cohort MMA is the prominent risk factor in explaining the BMD variation.

GFR loses its explanatory power when considered in conjunction with MMA. In fact, MMA impacts 4 times more than GFR in absolute terms.

The adjusted R squared was 0.22, not far from the 20% variation of BMD explained by MMA itself.

Even when using non-ranked values, the p values for MMA remains <10%, whereas the GFR correlation with BMD is not statistically significant anymore.

Summary

- We demonstrated an overall reduced bone mineral density in our cohort of MMA patients.
- High MMA plasma levels should be considered a risk factor per se for impaired bone health, regardless of GFR.
- Though this is a recognized complication for the disease, this is the first longitudinal cohort study reported in the paediatric population.

Conclusion

On systematic review by a longitudinal study of bone health in pediatric patients affected by Methylmalonic acidemia, we have shown that these patients are at increased risk of low bone mineral density and osteoporosis, potentially affecting their quality of life. Along with the well-known risk factors for reduced bone density in this population, we showed a new risk factor, specific for this disease, such as high level of methyl malonic acid in plasma. This finding requires further studies about its pathophysiology, its possible employment in the bone health follow up and curative approaches, but definitely states its relevance in identifying which MMA patients are at higher risk for long term osteopenia and osteoporosis.

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