Increased Plasma Amylase in the Family of a Patient with 3-Hydroxy-3-methylglutaryl-coenzyme A Lyase Deficiency

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A patient with 3-hydroxy-3-methylglutaryl-CoA lyase (HMG-CoA lyase, EC 4.1.3.4) deficiency presented consistently above-normal values of plasma amylase (EC 3.2.1.1). Activities measured were in the lower normal range in family members not proven heterozygotes and in the upper normal range in the proven heterozygotes. Heterozygosity was proven by intermediate HMG-CoA lyase activities determined in cultured fibroblasts and in lymphocytes in the parents and the paternal grandmother. Because all of the family members had diseases of the pancreas, colon, and liver, we question whether the heterozygote state contributes to the impaired function of these organs. Our findings of significantly increased amylase activities in the heterozygotes and the patient, in comparison with the other family members, support this hypothesis.

Additional Keyphrases: heritable disorders · enzyme activity

3-Hydroxy-3-methylglutaryl-CoA lyase (HMG-CoA lyase; EC 4.1.3.4) deficiency is an inherited disease of leucine catabolism with various symptoms. The cardinal clinical features include severe hypoglycemia, metabolic acidosis, hepatomegaly, lethargy, and coma. The absence of ketosis is characteristic (1, 2). Various newly developed methods of enzymatic diagnosis (3–5) have made it easier to investigate this disorder in large families (2).

We have previously reported on a family of a girl with HMG-CoA lyase deficiency who exhibited an attack of lethargy and who had liver enlargement, hypoglycemia, acidosis, and an initial increase of transaminases, but who developed normally (6, 7).

Because heterozygosity in general may influence the usual course of common diseases (8), because of a report by Wilson et al. (9) on a child with HMG-CoA lyase deficiency who presented an acute pancreatitis, and because the maternal grandfather of our patient had died from carcinoma of the pancreas, we examined pancreatic enzymes in the patient and her family.

Materials and Methods

We investigated the patient, her brother, her parents, her grandparents, and some of the siblings of her mother and father—15 persons in all (Figure 1).

Amylase was determined by two different laboratories several times in plasma from the patient and from all family members in whom enzyme studies for HMG-CoA lyase were performed. In Salzburg (Zentrallaboratorium der Landeskrankeanstalten), maltotetraose was used as substrate at 25 °C; in Berne, total and pancreatic amylase were determined by using 5-ethylidene-maltotetraoside p-nitrophenyl phosphate (Boehringer Mannheim, Mannheim, F.R.G.) as substrate and monoclonal antibodies at 30 °C (10). Lipase was measured according to Neumann et al. (11).

Organic acids in urine were converted to the trimethylsilyl derivatives and identified by gas chromatography/mass spectrometry (12).

The activity of HMG-CoA lyase in fibroblasts and lymphocytes isolated from whole blood was determined by the method of Gibson et al. (3).

Results

Total amylase in the patient’s plasma was determined nine times between ages 2.5 and 3.6 years and was always above normal. Pancreatic amylase, determined at the end of the period, was in the upper normal range, as were the values for total and pancreatic amylase in the heterozygotes in her family. Plasma amylase of nonaffected family members, although overlapping somewhat with the others, was on the average markedly lower than the amylase activities of the heterozygotes and the patient (Figure 2).

Despite the increased activity of plasma amylase, the patient did not present clinical or metabolic signs of pancreatic disease. Impairment of the liver (including hepatomegaly and above-normal values for the transaminases), acidosis, and hypoglycemia—typical symptoms of the disease before treatment (6)—were not detectable.

Of particular interest, however, the father, the mother, the maternal grandfather, and the paternal grandmother all had gastrointestinal diseases involving the pancreas (Table 1). The patient, the father, and the older brother presented a normal sonography of the pancreas.

We found no difference in the amounts of pancreatic lipase (determined in the patient, her mother, father,
and brother) measured in the homozygotes and the heterozygotes and in the noncarrier brother.

In an earlier study we showed that the activity of HMG-CoA lyase in cultured fibroblasts of the patient was reduced to 1.5% of normal values (6). In this study the HMG-CoA lyase activity in fibroblasts of the mother, the paternal grandmother, and the paternal grandfather was 62%, 54%, and 111%, respectively, of the normal mean value (Table 2). The activity of HMG-CoA lyase in lymphocytes isolated from whole blood supported the fibroblast data and confirmed that father, mother, and paternal grandmother were heterozygotes (Table 3).

Analysis of organic acids in urine, in particular 3-hydroxy-3-methylglutaric acid and 3-hydroxy-3-methylglutaconic acid, showed no differences between heterozygotes and noncarrier family members.

**Discussion**

Examination of fibroblasts and blood lymphocytes for HMG-CoA lyase activity identified the patient's father, mother, and paternal grandmother as heterozygous for HMG-CoA lyase deficiency. Because the maternal grandmother showed normal enzyme activity, the deceased grandfather most probably was a heterozygote. The urinary pattern of organic acids, however, did not allow this differentiation.

A particularly interesting observation in this family is the plasma amylase values. They were in the lower normal range in family members not proven to be heterozygotes, but showed a tendency to higher values in the heterozygotes and were above normal in the patient. Although the reported symptoms of the heterozygotes having diseases of the colon, liver, and pancreas differ, the question arises as to whether the heterozygous state contributes to an impaired function of these organs, as suggested by Vogel (8). The cause of the increase in plasma amylase in our patient and the three living heterozygotes is not clear, because none of them has clinical signs of pancreatic disease, and the activity of pancreatic isomylase, although in the upper normal range, was not above normal. Therefore, we must consider nonpancreatic sources of amylase.

Increased activities of total plasma amylase with normal values of pancreatic isomylase have been ob-
Table 3. Activity of HMG-CoA Lyase in Lymphocytes isolated from Whole Blood

<table>
<thead>
<tr>
<th>Subject</th>
<th>HMG-CoA lyase act., nmol min^-1 mg^-1 protein</th>
<th>Relative activity, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 4)</td>
<td>1.86 ± 0.65 (range 0.91–2.34)</td>
<td>100</td>
</tr>
<tr>
<td>Patient</td>
<td>0.24</td>
<td>13</td>
</tr>
<tr>
<td>Father</td>
<td>0.72</td>
<td>39</td>
</tr>
<tr>
<td>Mother</td>
<td>0.52</td>
<td>28</td>
</tr>
<tr>
<td>Brother</td>
<td>1.48</td>
<td>80</td>
</tr>
<tr>
<td>Paternal grandfather</td>
<td>1.10</td>
<td>59</td>
</tr>
<tr>
<td>Paternal grandmother</td>
<td>0.34</td>
<td>18</td>
</tr>
<tr>
<td>Maternal grandmother</td>
<td>1.46</td>
<td>78</td>
</tr>
<tr>
<td>II/6</td>
<td>2.80</td>
<td>151</td>
</tr>
<tr>
<td>II/3</td>
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<td>65</td>
</tr>
<tr>
<td>II/9</td>
<td>1.84</td>
<td>99</td>
</tr>
<tr>
<td>II/2</td>
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</tr>
<tr>
<td>II/3</td>
<td>1.12</td>
<td>60</td>
</tr>
<tr>
<td>II/4</td>
<td>2.10</td>
<td>113</td>
</tr>
<tr>
<td>II/5</td>
<td>1.39</td>
<td>75</td>
</tr>
</tbody>
</table>

All values are means of duplicate determinations on a single lymphocyte pellet. Propionyl-CoA carboxylase was assayed as a control for cellular viability, and results for all family members and the patient were within the range of four control subjects. The numbered individuals correspond to the identification in Fig. 1.

References

served in several gastrointestinal and hepatobiliary diseases (13). Similarly, in view of the various gastrointestinal symptoms of our patients, the increased plasma amylase activities probably are from nonpancreatic sources. On the other hand, pancreatitis has been observed in a child with HMG-CoA lyase deficiency (9) and in six children having an underlying disorder of branched-chain amino acid metabolism (14). Determination of total and pancreatic isomylase as well as lipase in patients with organic acidurias may therefore help to exclude pancreatic involvement in the presence of gastroenterological symptoms.