Urinary Uroporphyrin and Coproporphyrin Monitoring for the Assessment of Future Cancer Risk in Porphyria

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erythrocyte porphobilinogen deaminase (PBG-D), uroporphyrin (UP),
coproporphyrin(CP)

ABSTRACT

Background: Porphyrias may trigger carcinogenesis and require precautions to minimize the cancer risk, however there has been no clinical benchmark to predict such complications.

Methods: The cancer death percentages were investigated in acute intermittent porphyria (AIP) and variegate porphyria (VP). Urinary uroporphyrin (UP), coproporphyrin (CP) and erythrocyte porphobilinogen deaminase (PBG-D) activity were analyzed in AIP and UP to assess the etiological impact of porphyria on carcinogenesis.

Results: VP showed a nearly 4-fold significant increase of cancer death risk when compared to Japanese national cancer death percentage in 2007 (OR = 4.33; 95% CI: 1.91 to 9.81; p < 0.01). The proportion of CP/UP >6.0 in VP (3/5, 60.0%) was significantly higher than that in AIP (0/6, 0%, Chi-square test, p=0.026). The proportion of PBG-D activity < 20.9 nmol UP/ml RBC/hr, below low end reference range in AIP (4/6, 66.7%) was significantly higher than that in VP (0/5, 0%, Chi-square test, p=0.022).

Conclusions: CP/UP > 6.0 can be a good prospective approach to assessment for future cancer risk in VP. Periodical examination of these indices will be helpful for the long-term convalescence management of porphyria.

INTRODUCTION

Porphyrias are a group of rare inherited diseases due to enzyme defect in the biosynthesis steps of heme production developing during life and produce too much porphyrin and insufficient heme. There are seven types of porphyria (acute intermittent porphyria; AIP, variegate porphyria; VP, eryth-
ropoietic protoporphyria; EPP, hereditary coproporphyria; HCP, aminolevulinate dehydratase deficiency porphyria; ADP, porphyria cutanea tarda; PCT, and congenital erythropoietic porphyria; CEP) and these can be distinguished by genetic cause, clinical symptoms, acute and non-acute type. Two major types of clinical manifestations of porphyric people are skin problems (skin sensitivity to sunlight, severe pain and damage after sunlight exposure), and life threatening acute attack (abdominal pain, stomach cramps and nausea) [1-8].

Although recent development of early detection method and medical treatment of porphyria enables limitation of disease progression, improvement of patient life expectancy [4-6], it unveils other complicated diseases. Since Lithner and Wetterberg first suggested the association between hepatocellular carcinoma and AIP in 1984 from Sweden [7], porphyrias are believed to be important trigger carcinogenesis and risk factors for various cancers, including hepatocellular carcinoma and liver disease. Thus, porphyric patients may require specific precautions to ensure optimum treatment of the malignancy and minimize the cancer risk [8-10].

The present study was designed to investigate the cancer death of individuals from 9 porphyria families (4 AIP families and 5 VP families) in Kyushu district, Japan. The odds ratio (OR) and corresponding 95% confidence interval (CI) of cancer death associated with porphyria vs Japanese national cancer death rate was evaluated. Urinary uroporphyrin (UP), coproporphyrin (CP) and erythrocyte porphobilinogen deaminase (PBG-D) (also known as hydroxymethylbilane synthase (HMBS)) activity of AIP and VP subjects was analyzed to assess the etiological impact of porphyria on carcinogenesis.

MATERIALS and METHODS

Subjects
Families with inherited AIP and VP in Kyushu district, Japan were studied by the questionnaires, interviews and clinical laboratory examinations.

Analysis of OR and CI of cancer death associated with porphyria
The ancestor records were reviewed by interviews and questionnaire with surviving members of 9 porphyria families (4 AIP families and 5 VP families) to examine their cause of death. The numbers of deceased ancestors and cancer death among them except the spouses from non-porphyria families were counted to obtain the cancer death percentage. The cancer death percentage was compared with the Japanese national cancer death percentage of 30.4 % in 2007 to examine OR and CI.

Analysis of urinary heme precursor concentration
Urine samples were collected from 11 subjects in the latent phase (6 subjects from one AIP family and 5 subjects from one VP family above described; 5 male, 6 female; age range, 5-73 years). The samples were maintained in dark test bottles and stored in a deep freezer until analysis. Then the samples were thawed by incubating at 37°C and dissolved by shaking (Termo Mini TM-100). After centrifugation, the supernatant was used for porphyrin analysis by HPLC (Intelligent HPLC System LC-800 series, JASCO, Japan) with appropriate pretreatment and analysis condition adjustment. UP (uroporphyrinogen ; URO, heptacarboxyrophephyrin; HEPTA, hexacarboxyphophyrin; HEMA and pentacarboxyphophyrin; PENTA) and CP (coproporphyrinogen-I and III and ; CP-I and III) were quantified as µg/g creatinine.

Analysis of erythrocyte PBG-D activity
Blood samples were collected from same 11 subjects above described and 2-ml venous blood samples were collected in sterile Venoject heparin vacutainers, diluted 1:35 with ice-cold distilled water and then left in a refrigerator for hemolysis. After centrifugation, 350 µl of supernatant was taken for PBG-D activity analysis. The PBG-D activity was assayed by converting porphobilinogen into uroporphyrin as described by Meyer et al. [11].

Ethics
The study was conducted according to the Helsinki Declaration of Human Experimentation, and it was approved by the society for porphyrin research.

Statistics
Statistical analysis was undertaken using unpaired t-test and chi-square test. A p value of < 0.05 was considered to be statistically significant. All data analyses were conducted using PASW (formerly SPSS) 17.0 statistical software package.

RESULTS
There observed 14 deceased subjects including 5 subjects of cancer death (2 rectum cancers, 2 gastric cancers and 1 gall bladder cancer) in AIP families and cancer death percentage of AIP family was 35.7%. The risk of cancer death in AIP family was not significantly different when compared to the Japanese national data (OR = 1.27; 95% CI: 0.42 to 3.83;
There observed 26 deceased subjects including 17 subjects of cancer death (7 gastric cancers, 4 liver cancers, 2 uterine cancers, 1 rectum cancer, 1 colon cancer, 1 bladder cancer and 1 gall bladder cancer) in VP families and cancer death percentage of VP family was 65.3%. There was a nearly 4-fold significant increase of cancer death risk in VP family when compared to the Japanese national data (OR = 4.33; 95% CI: 1.91 to 9.81; p < 0.01). The risk of cancer death in VP family was not significantly different from AIP family (OR = 3.40; 95% CI: 0.87 to 13.2; p = 0.078).

As shown in Table 1 and 2, both AIP and VP subjects showed extremely high urinary UP, CP and total porphyrin (UP+CP) concentration as compared to the reference 2.5-97.5th percentile limit values (0-20.8, 11.7-93.1, and 15.9-102.9 μg/g creatinine for UP, CP and UP+CP, respectively) reported by Alves et al [12]. The difference of these porphyrin concentrations between porphyria types was not significant. The proportion of urinary CP/UP >6.0 in VP subjects (3/5, 60.0%) was significantly higher than that in AIP subjects (0/6, 0%, Chi-square test, p=0.026).

As shown in Table 3, difference of PBG-D ac-

### Table 1 Urinary hemprecousor concentration (μg/g creatinine) of AIP subjects

<table>
<thead>
<tr>
<th>Subject Age, sex</th>
<th>Uroporphyrins (UP)</th>
<th>Coproporphyrins (CP)</th>
<th>Total porphyrin</th>
<th>CP/UP ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female; age: 50</td>
<td>154.4</td>
<td>794.3</td>
<td>948.7</td>
<td>5.14</td>
</tr>
<tr>
<td>Male; age: 48</td>
<td>172.1</td>
<td>332.0</td>
<td>504.1</td>
<td>1.93</td>
</tr>
<tr>
<td>Female; age: 45</td>
<td>25.4</td>
<td>133.5</td>
<td>158.9</td>
<td>5.26</td>
</tr>
<tr>
<td>Male; age: 18</td>
<td>17.3</td>
<td>78.0</td>
<td>95.3</td>
<td>4.51</td>
</tr>
<tr>
<td>Male; age: 17</td>
<td>21.0</td>
<td>88.6</td>
<td>110.6</td>
<td>4.27</td>
</tr>
<tr>
<td>Female; age: 20</td>
<td>35.6</td>
<td>165.4</td>
<td>201.0</td>
<td>4.65</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>71.0 ± 72.0</td>
<td>265.4 ± 274.8</td>
<td>336.4 ± 335.2</td>
<td>4.29 ± 1.21</td>
</tr>
</tbody>
</table>

### Table 2 Urinary hemprecousor concentration (μg/g creatinine) of VP subjects

<table>
<thead>
<tr>
<th>Subject Age, sex</th>
<th>Uroporphyrins (UP)</th>
<th>Coproporphyrins (CP)</th>
<th>Total porphyrin</th>
<th>CP/UP ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female; age: 73</td>
<td>37.3</td>
<td>105.6</td>
<td>142.9</td>
<td>2.83</td>
</tr>
<tr>
<td>Female; age: 42</td>
<td>50.0</td>
<td>478.6</td>
<td>528.6</td>
<td>9.57</td>
</tr>
<tr>
<td>Female; age: 55</td>
<td>58.0</td>
<td>356.6</td>
<td>414.6</td>
<td>6.15</td>
</tr>
<tr>
<td>Male; age: 8</td>
<td>27.4</td>
<td>130.3</td>
<td>157.6</td>
<td>4.76</td>
</tr>
<tr>
<td>Male; age: 5</td>
<td>25.1</td>
<td>221.1</td>
<td>246.2</td>
<td>8.81</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>39.6 ± 14.2</td>
<td>258.4 ± 157.5</td>
<td>297.9 ± 168.2</td>
<td>6.42 ± 2.80</td>
</tr>
</tbody>
</table>

### Table 3 PBG-D activity (μmol UP/ml RBC/hr) of AIP and VP subjects

<table>
<thead>
<tr>
<th>Type of porphyr ia</th>
<th>Subject Age, sex</th>
<th>PBG-D</th>
</tr>
</thead>
<tbody>
<tr>
<td>AIP</td>
<td>Female; age: 50</td>
<td>15.1**</td>
</tr>
<tr>
<td></td>
<td>Male; age: 48</td>
<td>19.2'</td>
</tr>
<tr>
<td></td>
<td>Female; age: 45</td>
<td>17.6'</td>
</tr>
<tr>
<td></td>
<td>Male; age: 18</td>
<td>38.8^a</td>
</tr>
<tr>
<td></td>
<td>Male; age: 17</td>
<td>39.4^a</td>
</tr>
<tr>
<td></td>
<td>Female; age: 20</td>
<td>18.9'</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>25.9 ± 12.1</td>
</tr>
<tr>
<td>VP</td>
<td>Female; age: 73</td>
<td>44.5^a</td>
</tr>
<tr>
<td></td>
<td>Female; age: 42</td>
<td>27.8^a</td>
</tr>
<tr>
<td></td>
<td>Female; age: 55</td>
<td>37.8^a</td>
</tr>
<tr>
<td></td>
<td>Male; age: 8</td>
<td>27.8^a</td>
</tr>
<tr>
<td></td>
<td>Male; age: 5</td>
<td>33.1^a</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>31.6 ± 4.8</td>
</tr>
</tbody>
</table>

*Value below the mean-1SD of 16.1 in AIP patient reported by Tanaka et al [13]
'Value below the low end reference range of 20.9 reported by Santos et al [14]
^Value over the mean+2SD of 23.0 in AIP patient reported by Tanaka et al [13]
tivity between porphyria types was not significant. All VP subjects showed PBG-D activity over the mean +2SD of 23.0 reported by Tanaka et al [13] in AIP patient. The proportion of PBG-D activity below the low end reference range of 20.9 reported by Santos et al [14] or mean-1SD of 16.1 reported by Tanaka et al [13] in AIP patient (4/6, 66.7%) was significantly higher than that in VP subjects (0/5, 0%, Chi-square test, p=0.022).

DISCUSSION

Urinary porphyrins determined in this study are byproducts of intermediates in the heme biosynthetic pathway and these are important for screening and diagnosis of various hereditary porphyria (Fig.1), metabolic disorders and xenobiotic exposures. AIP is characterized by a deficiency of the PBG-D activity in an early step in heme synthesis [15, 16], and the porphyrin precursor metabolites of PBG and 5-aminolevulinic acid accumulate in the cytoplasm [17]. UP and CP are also increased in urine of AIP due to chemical oxidized conversion from excessive PBG [18].

No significant difference of PBG-D activity between AIP and VP in this study suggests that AIP shows no significant decrease of PBG-D activity in a latent state. In this study, significantly more AIP subjects showed PBG-D activity below the low end reference value reported by Tanaka et al [13] or Santos et al [14]. As our previous study revealed the negative spike of PBG-D activity associated to AIP attacks [13], these subjects of low PBG-D activity might be in a sub-acute or pre-acute state.

VP shows deficient activity of protoporphyrinogen oxidase (PPOX) that catalyzes the removal of 6 hydrogen atoms from the protoporphyrinogen IX (PP) to protoporphyrin IX by oxidation in the penultimate step (seventh step) of heme biosynthetic pathway (Fig.1) [19]. The defect of PPOX results in the accumulation of lipophilic PP associated with the excessive production of other hydrophilic CP, UP, ALA and PBG under the condition of stressed heme synthetic pathway [20]. Thus the observed subjects of AIP and VP in this study showed abnormally high urinary profile of UP and CP.

Water solubility of porphyrins is related to the number of free carboxyl side-chains binding to tet-
rapyrole ring. The most hydrophilic porphyrin UP has a total of eight carboxylate residues around the periphery and is excreted rapidly via urine because of its high water solubility. Also HEPA with 7 carboxyl side-chains, HEXA with 6 carboxyl side-chains and PENTA with 5 carboxyl side-chains show high hydrophilic nature and these are also eliminated through the body via urine. CP with 5 carboxyl side-chains shows slight lipophilic nature. PP bearing only two carboxyl groups is not eliminated via urine because of its high lipophilic nature and detectable only in blood and stool. Although most part of CP eliminated via urine, partially enters enterohepatic circulation by the liver uptake and finally excreted in feces via bile. CP and PP may accumulate in bone marrow or liver, which then accumulate throughout the body [21, 22]. Accumulated CP and PP absorb UV light energy intensively, resulting in transitions to excited electronic states to produce free radicals and singlet oxygen, which in turn cause oxidative damage of surrounding tissues cells and DNA [23]. Thus, porphyrins are considered to be solar induced carcinogenicity.

In the present study, the risk of cancer death showed 4-fold significant increase in VP family and no significant increase in AIP family when compared to Japanese national data. Although UP, CP and total porphyrin determined in this study did not show significant difference between AIP and VP family, the proportion of urinary CP/UP > 6.0 in VP subjects was significantly higher than that in AIP subjects. As urinary screening is an effective tool to investigate porphyrina [24] and fraction mismatch of urinary CP/UP has been used to explore porphyrin metabolism [25-28]. Urinary CP/UP ratio increase may reflect the accumulation of CP and PP under the circumstance of excess porphyrin production, there seemed to be a relationship between the risk of cancer death and urinary CP/UP ratio. The result of this study suggests that high urinary CP/UP > 6.0 can be a future risk of cancers.

Lipophilic part of CP and PP is filtered out by the liver, secreted in the bile to the small intestine, and enters enterohepatic circulation recycle from the liver to the small intestine. Thus, stool containing CP and PP passes out through the body via intestinal tract in VP [29]. Present study showed that 7 cases of 17 cancer death (41.1%) in VP family related to the organs above mentioned enterohepatic circulation route (liver, gall bladder and lower intestinal tract) and 1.58 times higher than Japanese national data of proportional mortality rates from malignant neoplasms by site in 2007. It can be assumed that the more excretion and longer retention of CP and PP in the bile or stool, the higher the cancer risk around enterohepatic circulation route in VP, because of CP and PP carcinogenicity.

It is well discussed that lifestyle modification to avoid aggravating factors (drugs, alcohol, organic solvent, heavy metals, diets, stress, illness and sun exposure) is the most effective way to prevent acute attack of porphyria and a glucose 10% infusion, hematin and heme arginate (Normosang®; domestically unapproved drug in Japan) are established for treatment of crisis [30-32], however there has been no commonly accepted standard clinical benchmark to predict porphyria related complications. The present study revealed that urinary porphyrin index of CP/UP > 6.0 can be a good prospective approach to assessment for future cancer risk especially for the enterohepatic circulation route in VP. We propose that the periodical examination of these indices will be helpful for the long-term convalescence management of porphyria.

REFERENCES


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