Down's Syndrome and Acute Leukemia in Children: An Analysis of Phenotype by Use of Monoclonal Antibodies and Electron Microscopic Platelet Peroxidase Reaction

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The clinical, hematologic, and immunophenotypic features in 20 patients with Down's syndrome (DS) and acute leukemia were analyzed. Of the 20 patients, all 14 patients who were 3 years old and less were diagnosed as having acute megakaryoblastic leukemia (AMKL) by use of platelet-specific monoclonal antibodies and platelet peroxidase (PPO) reaction in electron microscopy. They were characterized by the presence of bone marrow fibrosis, having a history of myelodysplastic syndrome (MDS) and a poor response to chemotherapy. Only one patient has remained in continuous complete remission for more than 1 year. Acute leukemia in six patients who were older than 4 years was classified as common acute lymphoblastic leukemia without Down's syndrome.

It is well known that the frequency of acute leukemia is much higher in patients with than in those without Down's syndrome (DS).1,2 Large-scale surveys on acute leukemia in patients with DS conducted by several groups3-6 have suggested that the distribution of types of leukemia in patients with DS is similar to that in normal children. However, it is noteworthy that in these reports the classification of acute leukemia was based on morphologic study alone. Lewis5 reported on a child with DS who developed acute megakaryoblastic leukemia (AMKL) and suggested that trisomy 21 could be linked with AMKL. Since then there have been additional reports on AMKL-DS.4,8 AMKL was first described in 1931,9 but due to insufficient specific morphologic and cytochemical characteristics it was paid little attention. In recent years, the development of platelet peroxidase (PPO) reaction in electron microscopy and monoclonal platelet-specific antibodies has made it possible to identify AMKL.

The present study was designed to analyze the clinical, hematologic, and immunophenotypic characteristics of acute leukemia in patients with DS. To determine the cell lineage of acute leukemia, we used a panel of monoclonal antibodies (MoAbs) and ultrastructural PPO in 20 patients with acute leukemia-DS.

MATERIALS AND METHODS

Patients. All participating institutions in the Japanese society for aggressive therapy-oriented pediatric hematologists and oncologists were asked to provide information on patients with acute leukemia-DS that they had treated over a 5-year period. Twenty patients with acute leukemia-DS were diagnosed between September 1983 and August 1988. Patients 6, 7, 11, and 13 have been reported previously.9-10 The patients were classified according to the French-American-British (FAB) Co-operative Group criteria,11 which defined acute leukemia as the presence of over 30% blasts in the bone marrow.

Bone marrow trephine biopsies were performed in the patients with inaspirable marrow. The presence and degree of fibrosis was determined by reticulin staining and graded using a scoring system.12

Morphology and cytochemistry. Bone marrow and peripheral blood samples were stained with Wright-Giemsa and cytochemical methods for peroxidase, periodic acid-Schiff (PAS), and a-naphthyl butyrate esterase with and without sodium fluoride inhibition.

Electron microscopic studies. For ultrastructural morphology, theuffy coat layer of the peripheral blood was fixed in a mixture of paraformaldehyde, glutaraldehyde, and tannic acid for detection of PPO activity as described by Breton-Gorius and Guichard.13 All specimens were postfixed with osmium tetroxide, dehydrated, and processed in epoxy resin for transmission electron microscopy.

Immunologic study. Mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation from peripheral blood and/or bone marrow. In patients with bone marrow fibrosis precluding an adequate aspiration, immunologic studies were performed on peripheral blood mononuclear cells. The cells were analyzed by indirect immunofluorescence with a panel of MoAbs. The specifici...
ties of MoAbs used were as follows: (1) granulocyte and/or monocyte: My7 (CD13; Coulter Immunology, Hialeah, FL), My4 (CD14; Coulter), and My9 (CD33; Coulter); (2) platelets: Tp80I4 or HPL1, both against glycoprotein IIb/IIIa (CDw41), and AN51I6 or HPL14, both against glycoprotein Ib (CDw42); (3) T lymphocyte: OKT3 (CD3; Ortho Pharmaceutical Corp, Raritan, NJ), and OKTl 1 (CD2; Ortho); (4) B lymphocyte: B1 (CD20; Coulter) and B4 (CD19; Coulter).

Common acute lymphoblastic leukemia antigens (CALLA) were detected with J5 (CD10; Coulter) and HLA DR antigen with I2 (Coulter) or OK DR (Ortho). For assessment of megakaryocytic lineage, a minimum of 10% blast cells positive for PPO reaction or one or more of the platelet-specific MoAbs was required. The reason for using the 10% cut-off was that none of the patients with acute nonlymphoblastic leukemia other than AMKL had more than 5% positive cells for the platelet-specific antibodies for PPO reaction in electron microscopy in our laboratories.

RESULTS

Clinical and laboratory data. The clinical and laboratory data of the 20 patients are summarized in Table 1. In 14 of the 20 patients, the involvement of the megakaryocytic lineage was assessed, and the remaining 6 patients were classified as having CALLA-positive ALL.

The age of the patients with AMKL ranged from 10 months to 3 years. Ten patients were male and four were female. All patients with ALL were older than 4 years, and they ranged in age from 4 1/2 to 9 years (Fig 1). White blood cell (WBC) counts for patients with AMKL ranged from 4.6 to 40.6 x 10^9/L (median, 10.1 x 10^9/L), platelet counts from 0.2 to 108 x 10^9/L (median, 25.0 x 10^9/L), and hemoglobin levels from 2.9 to 12.6 g/dL (median, 8.1 g/dL). The bone marrow was difficult to aspirate and trephine biopsies were performed in seven patients; they showed a marked increase of marrow reticulin and were assessed as having grade IV fibrosis (Fig 2).

Seven of these 14 patients had a history of myelodysplastic syndrome (MDS) before AMKL developed. The initial FAB diagnosis was refractory anemia (RA) in two patients and RA with excess of blasts (RAEB) in five. Most of the patients presented with easy bruising and purpura. Laboratory examination showed thrombocytopenia with or without a low percentage of blasts in the peripheral blood (Table 2). Although there was some variety in the manifestations in different patients, the bone marrow smears were characterized by abnormalities in the three cell lines. The median observation time between initial diagnosis and moment of

Table 1. Clinical and Laboratory Data of Acute Leukemia in DS

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age</th>
<th>Sex</th>
<th>Hb (g/dL)</th>
<th>Leukocytes (x 10^9/L)</th>
<th>Platelets (x 10^9/L)</th>
<th>Peripheral Blasts (%)</th>
<th>Bone Marrow Fibrosis</th>
<th>History of MDS</th>
<th>Treatment</th>
<th>Response</th>
<th>Duration of Remission (mo)</th>
<th>Survival (mo)</th>
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<tbody>
<tr>
<td>1</td>
<td>1 y 10 mo</td>
<td>M</td>
<td>6.6</td>
<td>27.9</td>
<td>36.0</td>
<td>30</td>
<td>1</td>
<td>+</td>
<td>ADR, AraC, 6MP, Pred</td>
<td>CR</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>3 y</td>
<td>M</td>
<td>2.9</td>
<td>4.4</td>
<td>10.0</td>
<td>40</td>
<td>1</td>
<td>+</td>
<td>DNR, AraC, VP-16</td>
<td>CR</td>
<td>6+</td>
<td>7+</td>
</tr>
<tr>
<td>3</td>
<td>1 y 8 mo</td>
<td>M</td>
<td>6.8</td>
<td>4.6</td>
<td>7.0</td>
<td>65</td>
<td>1</td>
<td>+</td>
<td>DNR, AraC, VP-16</td>
<td>CR</td>
<td>6+</td>
<td>10+</td>
</tr>
<tr>
<td>4</td>
<td>1 y 5 mo</td>
<td>M</td>
<td>9.2</td>
<td>18.2</td>
<td>10.0</td>
<td>40</td>
<td>1</td>
<td>+</td>
<td>DNR, 6MP, AraC, Pred</td>
<td>NR</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>1 y 11 mo</td>
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<td>3.6</td>
<td>27.9</td>
<td>108.0</td>
<td>57</td>
<td>1</td>
<td>+</td>
<td>ADR, 6MP, AraC, Pred</td>
<td>NR</td>
<td>0</td>
<td>20+</td>
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<td>1 y 8 mo</td>
<td>F</td>
<td>8.1</td>
<td>40.6</td>
<td>47.0</td>
<td>32</td>
<td>1</td>
<td>-</td>
<td>VCR, CY, ADR, Pred</td>
<td>CR</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>2 y</td>
<td>F</td>
<td>12.0</td>
<td>10.1</td>
<td>17.0</td>
<td>30</td>
<td>1</td>
<td>-</td>
<td>DNR, AraC</td>
<td>CR</td>
<td>18+</td>
<td>19+</td>
</tr>
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<td>F</td>
<td>7.7</td>
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<td>0.2</td>
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<td>+</td>
<td>DNR, AraC</td>
<td>CR</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>1 y 9 mo</td>
<td>M</td>
<td>10.4</td>
<td>7.2</td>
<td>33.0</td>
<td>24</td>
<td>1</td>
<td>+</td>
<td>DNR, AraC, VP-16</td>
<td>CR</td>
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<td>1</td>
</tr>
<tr>
<td>10</td>
<td>2 y 3 mo</td>
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<td>10.5</td>
<td>0.9</td>
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<td>1</td>
<td>+</td>
<td>DNR, AraC</td>
<td>CR</td>
<td>5+</td>
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<tr>
<td>11</td>
<td>1 y 6 mo</td>
<td>M</td>
<td>12.6</td>
<td>6.6</td>
<td>30.0</td>
<td>3</td>
<td>+</td>
<td>Low-dose BH-AC</td>
<td>NR</td>
<td>0</td>
<td>18+</td>
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<tr>
<td>12</td>
<td>1 y 7 mo</td>
<td>M</td>
<td>5.4</td>
<td>5.1</td>
<td>33.0</td>
<td>20</td>
<td>-</td>
<td>Low-dose AraC</td>
<td>CR</td>
<td>6+</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>2 y 4 mo</td>
<td>M</td>
<td>9.5</td>
<td>6.5</td>
<td>11.0</td>
<td>9</td>
<td>-</td>
<td>+</td>
<td>ACM, BH-AC</td>
<td>CR</td>
<td>2+</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
<td>10 mo</td>
<td>M</td>
<td>8.2</td>
<td>10.9</td>
<td>25.0</td>
<td>30</td>
<td>-</td>
<td>Low-dose AraC</td>
<td>CR</td>
<td>4</td>
<td>22+</td>
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</tr>
<tr>
<td>15</td>
<td>7 y 8 mo</td>
<td>M</td>
<td>8.0</td>
<td>5.4</td>
<td>25.0</td>
<td>38</td>
<td>-</td>
<td>VCR, CY, DNR</td>
<td>Pred</td>
<td>10+</td>
<td>11+</td>
<td></td>
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<tr>
<td>16</td>
<td>9 y 9 mo</td>
<td>M</td>
<td>6.8</td>
<td>4.1</td>
<td>71.0</td>
<td>16</td>
<td>-</td>
<td>VCR, L-ASP, Pred</td>
<td>CR</td>
<td>22+</td>
<td>23+</td>
<td></td>
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<tr>
<td>17</td>
<td>5 y 7 mo</td>
<td>F</td>
<td>12.5</td>
<td>9.3</td>
<td>62.0</td>
<td>0</td>
<td>-</td>
<td>VCR, L-ASP, Pred</td>
<td>CR</td>
<td>2+</td>
<td>3+</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>6 y 9 mo</td>
<td>M</td>
<td>4.4</td>
<td>9.1</td>
<td>11.0</td>
<td>93</td>
<td>-</td>
<td>VCR, L-ASP, Pred</td>
<td>CR</td>
<td>14+</td>
<td>15+</td>
<td></td>
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<tr>
<td>19</td>
<td>5 y 3 mo</td>
<td>M</td>
<td>12.0</td>
<td>12.1</td>
<td>92.0</td>
<td>21</td>
<td>-</td>
<td>VCR, L-ASP, Pred</td>
<td>CR</td>
<td>11+</td>
<td>12+</td>
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<td>4 y 6 mo</td>
<td>F</td>
<td>11.6</td>
<td>3.7</td>
<td>26.6</td>
<td>0.5</td>
<td>-</td>
<td>VCR, L-ASP, Pred</td>
<td>CR</td>
<td>52+</td>
<td>53+</td>
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</table>

Abbreviations: CR, complete remission; NR, no response; CALLA, common acute lymphoblastic leukemia antigen; MDS, myelodysplastic syndrome; ADR, adriamycin; AraC, cytarabine; 6MP, 6 mercaptopurine; Pred, prednisone; DNR, daunorubicin; VP-16, etoposide; CY, cyclophosphamide; BH-AC, 4N-behenoyl-1-β-arabinofuranosylcytosine; ACM, aclacinomycin; VCR, vincristine; L-ASP, L-asparaginase.
overt leukemia for these seven patients was 6 months, with a range of 3 to 9 months.

Eleven of the 14 patients achieved complete remissions of short duration. Only one patient has remained in continuous complete remission for more than 1 year.

In patients with ALL, WBC counts ranged from 3.7 to 12.0 x 10^9/L (median, 9.1 x 10^9/L), platelet counts from 11.0 to 92.0 (median, 62.0 x 10^9/L), and hemoglobin levels from 4.4 to 12.5 g/dL (median, 11.6 g/dL). Bone marrow fibrosis and history of MDS were not noted in any of the patients. All six patients achieved a complete remission and have remained in continuous complete remission from 10 to 52 months from the initial diagnosis.

Morphology and cytochemistry. The blasts in the patients with AMKL were generally undifferentiated with a lymphoid-looking appearance. The undifferentiated blasts had high nuclear-cytoplasmic ratios with basophilic cytoplasm. Some of differentiated blasts showed cytoplasmic budding or cytoplasmic granules (Fig 3). Blasts were uniformly negative for myeloperoxidase, a-naphthyl butyrate esterase, and PAS staining. The blasts were classified as L1 according to FAB criteria in all of the patients with ALL.

Electron microscopic morphology and cytochemistry. The blasts of AMKL were characterized by occasionally indented round nuclei with sparse nuclear chromatin and a large nucleolus. The cytoplasm contained numerous small mitochondria. Demarcation membranes and specific granules could not be identified (Fig 4).

PPO reactions were studied in patients 1 through 13 and patient 18. In 10 of the 14 patients with AMKL, PPO reactivity was observed in the nuclear envelope and rough endoplasmic reticulum (Fig 5). PPO reaction was absent in the blasts of a patient with ALL. The percentages of PPO positivity in blasts are shown in Table 3.

Immunologic findings and PPO activity. The results of the immunologic studies are shown in Table 3. Immunologic markers were studied with MoAbs against platelet glycoproteins in 16 patients. In 14 of the 16 patients studied, the blast cells were positive for one or two anti-platelet MoAbs. The blast cells from patients 4, 6, and 8, which did not show PPO activity, reacted with one or two anti-platelet MoAbs. Based on these findings, patients 1 through 14 were diagnosed as having AMKL. The blast cells from patients 15 through 20 reacted with HLA DR, B4 (CD19), and CALLA (CD10) and did not react with the two MoAbs against platelets. They were classified as CALLA-positive ALL. In patient 16, the blast cells also expressed myeloid-associated surface antigens.

DISCUSSION

The present study has clearly shown that the distribution of acute leukemia types is quite different from that in

Table 2. Initial Clinical and Hematologic Findings of Seven Patients With MDS-DS

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age (mo)</th>
<th>Duration From Initial Presentation to Overt Leukemia (mo)</th>
<th>Leukocytes (x 10^9/L)</th>
<th>Blasts (%)</th>
<th>Hb (g/dL)</th>
<th>Platelets (x 10^9/L)</th>
<th>Cellularity</th>
<th>Blasts (%)</th>
<th>FAB Classification at Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>6</td>
<td>8.0</td>
<td>2.0</td>
<td>10.1</td>
<td>90</td>
<td>Increased</td>
<td>8.0</td>
<td>RAEB</td>
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<tr>
<td>7</td>
<td>18</td>
<td>6</td>
<td>4.6</td>
<td>0</td>
<td>9.8</td>
<td>77</td>
<td>Increased</td>
<td>2.0</td>
<td>RA</td>
</tr>
<tr>
<td>8</td>
<td>24</td>
<td>8</td>
<td>6.4</td>
<td>2.0</td>
<td>11.3</td>
<td>11</td>
<td>Increased</td>
<td>7.5</td>
<td>RAEB</td>
</tr>
<tr>
<td>9</td>
<td>18</td>
<td>3</td>
<td>7.2</td>
<td>3.0</td>
<td>9.5</td>
<td>30</td>
<td>Increased</td>
<td>11.0</td>
<td>RAEB</td>
</tr>
<tr>
<td>10</td>
<td>18</td>
<td>9</td>
<td>8.9</td>
<td>1.0</td>
<td>13.7</td>
<td>62</td>
<td>Increased</td>
<td>3.0</td>
<td>RA</td>
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<tr>
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<td>15</td>
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<td>0</td>
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<td>64</td>
<td>Increased</td>
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<td>RAEB</td>
</tr>
<tr>
<td>13</td>
<td>22</td>
<td>6</td>
<td>3.7</td>
<td>4.0</td>
<td>9.9</td>
<td>46</td>
<td>Increased</td>
<td>13.0</td>
<td>RAEB</td>
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</tbody>
</table>

Abbreviations: FAB, French-American-British; RA, refractory anemia; RAEB, refractory anemia with excess of blasts.
children without DS. Of the 20 patients with acute leukemia-DS, all of the 14 patients who were 3 years old and less were diagnosed as having AMKL, whereas the acute leukemia of six patients who were older than 4 years was classified as common-ALL. Although the blast cells from these six patients were not thoroughly studied with platelet-specific MoAbs and PPO reaction, the cytochemical and immunologic features were typical of common ALL. In a survey from the United Kingdom and the Irish Republic between 1971 and 1986, the immunologic phenotype of the blasts was studied in 34 children with ALL-DS, of whom 31 had common-ALL. There was a significantly higher proportion of common-ALL in the patients with DS compared with the proportion in normal children.18

Because of the difficulty in recognizing the early stage of megakaryocytic lineage by light microscopic features alone, AMKL was considered an exceptional variant of acute myeloid leukemia. In recent years, the development of the PPO reaction in electron microscopy13 and monoclonal and polyclonal platelet-specific antibodies14-17 has made it possible to diagnose more cases. Because the classification of acute leukemia was based on morphologic study alone in previous reports, AMKL was probably not recognized and thus was classified among other types of leukemia. Zipursky et al18 reviewed 24 patients with acute leukemia-DS diagnosed between 1972 and 1982, when platelet-specific antibodies and PPO reaction in electron microscopy were not available. Four of the 24 patients were retrospectively classified as AMKL on the basis of characteristic morphology.

The blast cells from patient 16 simultaneously expressed lymphoid- and myeloid-associated antigens. Mirro et al19 showed that the leukemic blasts from 18 of 95 children with a diagnosis of ALL by standard diagnostic criteria expressed myeloid-associated cell surface antigen and proposed the term “acute mixed lineage leukemia” to describe such cases. Although the blast cells from patient 16 probably belonged to this type of leukemia, the clinical features were not different from those of the remaining five patients.

The patients with AMKL-DS were different from those with ALL-DS, not only in terms of age but also in some of the other clinical features. The patients with AMKL-DS were characterized by the presence of bone marrow fibrosis, history of MDS, and poor response to chemotherapy. Prominent bone marrow fibrosis is one of the characteristics in patients with AMKL.20 In 20 children reported with AMKL, bone marrow fibrosis was present in all children who had trephine biopsy performed.21

Interestingly, there was a history of MDS in seven of the 14 patients with AMKL-DS, but in none of those with
ALL-DS. MDS has also been observed elsewhere in patients with acute leukemia-DS.\(^2\)\(^3\)\(^4\) Transformations to overt leukemia occurred within 9 months from the initial presentation in all of the patients. MDS is rare in childhood and has been described mainly in elderly patients. Aged patients who undergo acute transformation usually show a poor response to chemotherapy and rarely can achieve a complete remission.\(^5\)\(^6\) Likewise, few responses to chemotherapy have been noted in adults with AMKL.\(^2\)\(^5\)\(^6\) In our series, seven of the eight patients who transformed to AMKL from MDS achieved a complete remission with chemotherapy, which lasted for more than 1 year. Only one patient has remained in continuous complete remission for more than 1 year.

In contrast to patients with AMKL-DS, patients with ALL-DS have a favorable prognosis. All six patients with ALL-DS achieved complete remissions and remained in continuous complete remission from 10 to 52 months from the initial diagnosis. All the patients had CALLA-positive blasts and belonged to a standard-risk group based on the prognostic factors of age and WBC count. In previous studies, patients with ALL-DS had a poorer 5-year survival rate than those without DS.\(^4\)\(^9\) In those studies, a substantial proportion of the patients (30% to 40%) were 3 years old or less at diagnosis. It is possible that some of the DS patients had AMKL and that the overall outcome of the patients with ALL-DS was worsened by this.

We conclude that AMKL accounts for a high percentage of leukemia in children with DS who are less than 3 years old. The children with AMKL-DS are different from those with ALL-DS not only in terms of age, but also because they present with bone marrow fibrosis, have a history of MDS, and show a poor response to chemotherapy.

### REFERENCES

expression in cells from acute megakaryoblastic leukemia with Down’s syndrome. Blood 70:368, 1987


Down's syndrome and acute leukemia in children: an analysis of phenotype by use of monoclonal antibodies and electron microscopic platelet peroxidase reaction [see comments]

S Kojima, T Matsuyama, T Sato, K Horibe, S Konishi, M Tsuchida, Y Hayashi, H Kigasawa, Y Akiyama and J Okamura

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