Genetic-Biochemical Studies of Alloalbuminemia, I.
A Family of Fast Type Alloalbuminemia
(Albumin Kyoto)*

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Introduction
During the past decade, several genetically determined protein variants have been discovered as a result of the introduction of new electrophoretic techniques. The albumin variants, so-called bis- or double albuminemia was first described by Knedel (1957).1) Most of the albumin variants were reported as slow mobility type and only about ten families of the fast type have been reported.

The present study showed the abnormal electrophoretic pattern of serum albumin, so-called fast type, in a patient with recurrent stomatitis and her families. The first case of fast type alloalbuminemia in Japan was reported by Kawai et al.(1967)2) and the present case will be the second in our country. Short notes of this case were presented at the 24th General Meeting of the Society of Electrophoresis (Yamamoto et al.).3) In the present paper, Alb\textsuperscript{n} (normal), Alb\textsuperscript{f} (fast) and Alb\textsuperscript{s} (slow) were adopted as abbreviations.

Materials, Experiments and Results
The index case was of a 48 year-old female with recurrent stomatitis during the past two years and was admitted to the Fukuchiyama National Hospital (Kyoto). Her laboratory findings were almost normal. Although some abnormal findings were observed, none of these could be related to the presence of the abnormal albumin. Examination of the liver functions showed slight abnormality in T. T. T. and B. S. P. tests. Serum total cholesterol, triglyceride and phospholipid were also slightly elevated. No hypertensive blood pressure was observed (130/80 mmHg). The serum protein concentration was 7.5 g/dl, in which 64.7% was albumin. A bone marrow specimen showed increased number of megalocytes, despite of a normal platelet count in the peripheral blood. The chromosomal analysis was also carried out in the direct method of a bone marrow, indicating the normal karyotype. The chromosomal deletion, breakage or excess in number was not detected.

The cellulose acetate electrophoretic pattern showed two albumin bands, one with normal and the other with fast mobility. The two bands are present in similar amount of albumin, with 0.90 in the ratio of Alb\textsuperscript{f} to Alb\textsuperscript{n} (Fig. 1).

The pedigree of this family is shown in Fig. 2. This family was originated from the Kumamoto Prefecture, and named as albumin Kyoto according to the present address of the propositus. Thirteen cases through 3 generations were found to be of alloalbuminemia. The family segregation of variant gene indicates autosomal codominant inheritance. The liver functions of these alloalbuminemics were
normal except the index case (II-11). The examination of lipid metabolism including cholesterol, triglyceride and phospholipid were carried out for all of the 13 positive cases, indicating slight abnormality in some cases. Among the cases which showed hypercholesteremia, I-5 was 85 years old, III-11 was pregnant, and in II-7, II-12 and III-8 blood was drawn just after meal. III-11 showed elevated serum phospholipid. The serum total albumin level was 4.5 g/dl in the average of 13 positive cases. The albumins are consisted of two fractions: Albf 26.1–35.4% (mean: 30.8%) and Albh 31.9–39.0% (mean: 34.5%) by means of Ponceau 3R staining after electrophoresis. Therefore, the ratio of Albf to Albh would be less than 1.0 in every case (mean: 0.89), if the binding capacity of both fractions with Ponceau dye was assumed to be the same. Although some abnormal findings were recorded when the 13 persons having variant were examined, none of these could be related to the presence of the variant albumin (Table 1).

1. Electrophoresis

Separation of variant albumins was excellent in cellulose acetate electrophoresis using veronal buffer

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Total protein (g/dl)</th>
<th>A/G</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>Phospholipid (mg/dl)</th>
<th>Total albumin (g/dl)</th>
<th>Albf (%)</th>
<th>Albh (%)</th>
<th>Albf/Albh</th>
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<tr>
<td>I — 5</td>
<td>83</td>
<td>F</td>
<td>6.7</td>
<td>1.38</td>
<td>110</td>
<td>174</td>
<td>135</td>
<td>3.9</td>
<td>26.1</td>
<td>31.9</td>
<td>0.82</td>
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<tr>
<td>I — 7</td>
<td>55</td>
<td>M</td>
<td>7.3</td>
<td>2.38</td>
<td>167</td>
<td>152</td>
<td>182</td>
<td>5.1</td>
<td>33.3</td>
<td>37.0</td>
<td>0.90</td>
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<tr>
<td>II — 11</td>
<td>48</td>
<td>F</td>
<td>7.5</td>
<td>1.82</td>
<td>240</td>
<td>172</td>
<td>237</td>
<td>4.3</td>
<td>30.4</td>
<td>34.2</td>
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<td>II — 12</td>
<td>46</td>
<td>M</td>
<td>6.8</td>
<td>2.04</td>
<td>162</td>
<td>167</td>
<td>196</td>
<td>4.6</td>
<td>31.4</td>
<td>35.7</td>
<td>0.88</td>
</tr>
<tr>
<td>II — 13</td>
<td>43</td>
<td>M</td>
<td>6.6</td>
<td>2.90</td>
<td>149</td>
<td>89</td>
<td>193</td>
<td>4.9</td>
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<td>7.6</td>
<td>2.13</td>
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<td>108</td>
<td>209</td>
<td>5.2</td>
<td>32.0</td>
<td>36.0</td>
<td>0.89</td>
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<td>F</td>
<td>6.4</td>
<td>1.13</td>
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<td>116</td>
<td>158</td>
<td>3.4</td>
<td>20.0</td>
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<tr>
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<td>132</td>
<td>154</td>
<td>3.9</td>
<td>29.0</td>
<td>33.0</td>
<td>0.88</td>
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<td>2.00</td>
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<td>236</td>
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<td>1.79</td>
<td>139</td>
<td>97</td>
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<td>30.8</td>
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<tr>
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<td>2.35</td>
<td>265</td>
<td>125</td>
<td>202</td>
<td>4.8</td>
<td>35.1</td>
<td>35.1</td>
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<tr>
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<td>1.89</td>
<td>256</td>
<td>203</td>
<td>282</td>
<td>4.0</td>
<td>32.7</td>
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<tr>
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<td>7</td>
<td>M</td>
<td>6.9</td>
<td>1.86</td>
<td>190</td>
<td>76</td>
<td>202</td>
<td>4.5</td>
<td>32.0</td>
<td>33.0</td>
<td>0.97</td>
</tr>
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</table>

Mean 0.89
Clear separation was obtained at pH 8.6. No separation was observed below pH 4.0 or above pH 11.0. The storage of the samples in the freezer for many days did not influence their separation. Clear separation of the two albumins was also observed in starch gel, starch block, agar gel and disc electrophoresis.

2. Column chromatography

The supernatant obtained from the patient serum at 50% saturation of ammonium sulfate was applied to the DEAE cellulose column equilibrated with 0.005 M phosphate buffer at pH 6.0 and eluted with linear salt gradient. Two major peaks were obtained corresponding to normal and fast albumins. Calcium phosphate chromatography as well as Sephadex G-200 gel filtration failed to distinguish them.

3. Ultracentrifugation

Ultracentrifugation of the albumin fractions from both normal and variant albumins showed the peak at 4.25 S, indicating that the variant albumin from the patient had the same molecular weight as normal albumin as shown in Fig. 3.

4. Functional studies

Bromphenol blue (BPB) binding test was carried out by two different methods.

i) Variable amount of BPB were incubated with the serum, and cellulose acetate electrophoresis was carried out. The dye binding capacity was greater in Albf fraction when a small amount of dye solution (0.05%) was added (dye/serum = 1/3 in volume). When the larger amount was added, the dye was bound to both bands with equal density.

ii) Three aliquots of BPB were incubated, with purified Alb0 and Albf at the same protein concentration and with saline as a control. Each sample was passed through the same Sephadex G-200 gel filtration column, and OD595 of the effluent was recorded. The control sample showed a single peak of free BPB, while those incubated with either Alb0 or Albf showed another peak in front of the control BPB peak. Elution patterns shown in Fig. 4 indicated that equal amount of BPB was bound to Alb0 and Albf. Excess free dye peak was observed at the same position as the control BPB peak.

5. Thyroxine binding

Thyroxine-131I (Specific activity 28.4 mc/mg) was added to the serum at the final concentrations of 0.25, 0.50, 0.75, 1.00 and 1.50 μg/ml, and cellulose acetate electrophoresis of each sample was carried out. After the run, the paper strips were stained and one part of each sample was exposed to the X ray film to obtain autoradiogram, and the other to calculate the radioactivity by a scintillation counter. The ratios of Albf to Alb0 in radio-
activity were counted as 1.07, 1.08, 1.51, 1.81 and 2.06 at each concentration, respectively. The thyroxine binding experiment revealed greater capacity in Albf than in Albn and, moreover, the difference in capacity was greater at higher protein concentration.

6. Immunological study

Immunoelectrophoretic pattern against anti-human albumin rabbit immune serum (home made immune serum) showed a biphasic precipitin line in the patients with alloalbuminemia. But by Ouchterlony’s technique, both albumins showed the same antigenicity against the anti-human albumin rabbit immune serum. With cross antigen-antibody electrophoresis according to Laurell (1965), the fast type albumin showed a rather broad rocket, while the slow type albumin (Albumin Otsu) showed a narrower rocket (Fig. 5).

![Image of agarose electrophoresis pattern](image)

Agarose electrophoresis

Fig. 5. Antigen-antibody crossed electrophoretic patterns of fast (top) and slow (middle) type alloalbuminemia compared with that of normal serum (bottom).

Antibody: anti-human albumin rabbit immune serum.

Discussion

Alloalbuminemia occurs rarely having usually a genetic basis, and sometimes accompanies certain diseases coincidentally. Weitkamp et al. (1967) compared the electrophoretic mobility of many of available variants on starch gel (pH 8.6) and classified them to six groups. The present fast-moving variant might be classified to a “faster” group by the electrophoretic mobility which is similar to the albumin Naskapi occurring very common in the North American Indian (Melartin and Blumberg, 1966). Two samples, fast (Albumin Kyoto) and slow (Albumin Otsu) found by the authors were sent to Dr. L.R. Weitkamp (University of Rochester) to be compared with their reference samples. A comparative studies of the electrophoretic mobility among these cases were presented at the 24th General Meeting of the Society of Electrophoresis (Yamamoto et al.).

The present case (Albumin Kyoto) showed a different mobility from Albumin Naskapi and Albumin Gent.

The molecular size of both (Albf and Albn) albumins was proved almost to be the same by Sephadex gel filtration and ultracentrifugation in our study. Peptide analysis might reveal definite structural difference between the two albumins as in the case of albumin Oliphant and albumin Ann Arbor in which only one glutamic acid in the former was shown by Winter et al. (1972) to be substituted by a lysine in the latter.

Seventeen slow type and three fast type families with genetically determined alloalbuminemia have been published in Japan since the first report of Sofue and Ohbayashi (1967). The first case of fast moving type alloalbuminemia in Japan was reported by Kawai et al. (1967) and the present case is the second. More recently another case of the fast type was reported in a patient with chronic myeloid leukemia (Sezaki et al.).

In our study, the fast type (found in Fukuchiyama National Hospital) and the slow type (in Otsu City Hospital) are both detected by the central laboratory in the hospitals during routine protein fractionation by cellulose acetate electrophoresis. Both hospitals have already studied about 20,000 cases for serum protein fractionation since cellulose
acetate electrophoresis was introduced to these laboratories. However, the incidence of alloalbuminemia in our country could not be estimated as one in twenty thousands, because only one buffer system was usually used for electrophoretic protein fractionation in the routine laboratory work. Albumin variants having a slight electrophoretic difference from normal might be missed, unless different buffer systems are used for the separation. Alloalbuminemia seems to exist more often than expected in our country.

Although some abnormal clinical findings including the liver function and lipid metabolism in the present families were recorded, these were very slight and could not be observed as the common abnormalities through 13 alloalbuminemias. The ratios of Albf/Albn were between 0.61 and 1.00 by Ponceau 3R staining, with the average 0.89 in 13 cases. It is an interesting finding that all of the positive cases had the ratios less than 1.00. It should be noted that the ratio of the amount of variant albumin to normal albumin were found to be near 1.0 for the families that have been reported.

Serum albumin is the major plasma protein which binds to transport many substances in the blood. In our study, bromphenol blue (BPB) binds more readily to Albf at low concentrations of the dye. Thyroxine binding was also greater in Albf, though no preferential binding was reported in albumin Naskapi (Melartin, 1967).12 Sarcione and Aungst (1962)12 and Tarnosky and Lestas (1964)13 showed that only the variant band of the albumin in heterozygous sera (albumin B and albumin Reading, respectively) binds thyroxine at low concentrations. These differences could be due to the different quantities of thyroxine used. Bromsulphalein (BSP) retention as one of the liver function tests was slightly prolonged to 12.3% at 30 minutes and 10.0% at 45 minutes, though BSP test was performed only for proband. The binding capacity of the variant albumin to BSP dye suggested some difference from normal albumin. However, the binding test to BSP dye in vitro failed.

Antigenicity of both albumins could not be differenciated by Ouchterlony's technique against the anti-human albumin rabbit serum. Using the new technique of cross antigen-antibody electrophoresis, the patterns of our fast type showed a rather broad rocket, being different from that of the slow type, and suggesting the further possibility of getting new type of information by analyses of various different types of alloalbuminemia from different families. Variant albumin genes (such as albumin Naskapi and albumin Mexico) are widespread in American Indians. They must be of some selective value, and the polymorphism would have been generated as a result of differences in the selective values for the different genotypes, such as a system of sickle cell hemoglobin. It is very difficult to detect the selective forces which bear on the polymorphism. The possible approach on this problem is to study pharmacogenetic behavior of albumin variants, because barbiturates, many antibiotics, digitalis glucosides, salicylates etc. are bound to albumin. More extensive studies should be desired also in our country, from the point of genetics, anthropology and also therapeutic importance.

**Summary**

In a family of the fast type alloalbuminemia, 13 heterozygous cases were found through 3 generations, showing the autosomal codominant inheritance. The presence of the variant albumin was not directly associated with any disease. Binding studies seem to indicate that Albf has greater ability to bind BPB dye and thyroxine, when small amounts of those were added. No immunological difference between Albf and Albn was observed.

**Acknowledgement**

We thank Dr. N. Fujiki (Department of Genetics, Institute for Developmental Research. Aichi Prefectural Colony) for his criticism of the manuscript, and Mr. J. Shiomi (Fukuchiyama National Hospital) for his technical assistance.

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最近の電気泳動法の進歩により，遺伝的に異型を示す
血清蛋白が数多く発見されるようになった。Alb においては、Knedel (1957) が遺伝性の血清蛋白異常として報告したのが最初で Doppel-Albuminämie として発表して以来、世界各地での報告がみられる。本邦では祖父江ら (1967) の 1 家系 9 名が最初で、現在まで 20 家系の報告がある。

私達は fast type 1 家系、slow type 2 家系を発見し、第 24 回電気泳動学会総会において発表した。本文論文は fast type の 1 家系について遺伝学的な検討を行なったものである。

Fast type としては、河合ら(1967)の報告が最初で、本例は 2 例目であり、最近、顕崎ら(1974)が 3 例目の報告をしている。

本家系は頭面的な口内炎を主訴とする 48 歳の女性を発端者とし、3世代に及ぶ 13 名に陽性者を認め、出身地は鳥取県である。発端者の病所より Albumin Kyoto と命名した。遺伝形式は共通性で、いずれもヘテロ接合者であり、発端者を含め 25 名に各種臨床検査を施行したが、陽性者の有無の所見は認めなかった。

Ge との遺伝的連鎖を調べるため、型判定を行なったが、すべて 1 型で有効な情報は得られなかった。発端者には骨髄穿刺、末梢血での染色体分析も施行したが異常所見は認めなかった。

電気泳動では、セルロースアセテート膜、寒天ゲル、酸塩素ゲル、ディスク泳動、酸塩素ブロックを試みたが、pH 4.0 ～ 11.0 にて、いずれも明確に異常バンドを分離した。本血清と私達が大阪市で発見した slow type の Albumin Otsu の血清を Dr. Weitkamp (Univ. of Rochester) と Dr. Neel (Univ. of Michigan) に送り、reference samples との比較泳動を依頼したが、そのいずれも異なることが判明した。

両 Alb 分画の Ponceau 3R との結合能が等しいと仮定すれば、Alb と Alb との濃度比は 0.61 ～ 1.00 の間にあり、13 名の平均値は 0.89 で、いずれも Alb の方が増量していた。

脂質代謝異常の報告例があるが、本家系にても数名の異常者が存在したが、本症に特有といえない。肝機能検査で発端者に BSP の遅延がみられたので、in vitro で alb との結合を試みたが、うまく結合しなかった。DEAE セルロースカムと酸塩素ブロック泳動により alb 両分画を分離し、濃度を一定にし、Sephadex G-200 により BPB 色素との結合能（色素遊離の状態で）を調べたが、両者に差を認めなかった。またセルロースアセテート泳動により、BPB 色素、131I-thyroxine との結合能をみると、これらを少くすると、fast 分画により多く結合するという成績をえた。両分画でそれぞれ家系を免疫して抗原性的差異をみたが、両者に差はなかった。超遠沈、アミノ酸分析でも差を認めず、最近 Winter ら(1972)が新たに、ペプタイド分析を行ない、異常分画のアミノ酸配列を調べ、そのアミノ酸が置換されて電気泳動上 fast type を示すのか解明してゆきたい。

References


