Case Report

Impaired Insulin Secretion in Four Tangier Disease Patients with ABCA1 Mutations

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Aim: Tangier disease (TD), caused by deficiency of ATP-binding cassette transporter A1, is characterized by the absence of high density lipoprotein and the accumulation of cholesteryl esters in many tissues. Recently, it has been reported that ABCA1 is expressed in pancreatic β cells and mice with specific inactivation of ABCA1 in β cells showed markedly impaired insulin secretion, suggesting that ABCA1 deficiency may be involved in diabetes. The aim of the current study was to confirm these findings by the oral glucose tolerance test (OGTT) in human subjects with ABCA1 deficiency.

Methods and Results: Four Japanese patients with TD were investigated by OGTT with 75 g glucose. In all TD patients, the plasma glucose concentration after 30 min progressively increased, indicating a type 2 diabetic pattern; however the plasma insulin concentration did not respond well to glucose increase. The calculated insulinogenic index was significantly lower in TD patients than in non-diabetic controls (0.055 ± 0.034 vs 0.775 ± 0.538, mean ± SD, p < 0.05, respectively).

Conclusions: Although the number of TD patients was very small in the current study, these observations indicated a possible mechanism that glucose-stimulated insulin secretion might be impaired in human TD patients with ABCA1 mutations. Taken together, ABCA1 may be involved in insulin secretion from pancreatic β-cells.


Key words: ABCA1, OGTT, Insulinogenic index, Tangier disease

Introduction

Tangier disease (TD) is a rare autosomal recessive disease caused by mutations in the ATP-binding cassette transporter A1 (ABCA1) gene. Patients with TD are accompanied by high density lipoprotein (HDL) deficiency, resulting in the accumulation of cholesteryl ester in many tissues, such as the tonsils, liver, spleen, lymph nodes, gastrointestinal mucosa, and peripheral nerves. We previously investigated patients with TD, and found abnormal lipid rafts and the reduction of cdc42 in ABCA1-deficient cells. Furthermore,
ABCA1 was reported to be expressed in pancreatic β-cells\(^4\), suggesting that ABCA1 deficiency along with abnormal lipid rafts and cdc42 might lead to the dysfunction of pancreatic β cells in TD patients; however, little is known about the effect of ABCA1 deficiency on the endocrine function of the pancreas. Recently, Brunham LR et al. analyzed mice with specific inactivation of Abca1 in β cells and revealed markedly impaired glucose tolerance, defective insulin secretion, but normal insulin sensitivity\(^5\). In the present study, we focused on patients with TD and performed the oral glucose tolerance test (OGTT) with 75 g glucose.

**Case Presentation**

Four Japanese patients with TD were subjected to the current study (Table 1).

Case 1 was a 48-year-old man, who was first referred to our clinic in January 1989 because of marked hypocholesterolemia, very low HDL cholesterol, anemia, and hyperbilirubinemia. He had orange tonsils, corneal opacity, hepatosplenomegaly, and thrombocytopenia. OGTT was performed in 1995. At this time, fasting plasma glucose (FPG) was 163 mg/dL, insulin concentration was 4.0 µU/mL, and HbA1c was 5.8%. In 1997, this patient began to have exertional chest pain and coronary angiography revealed massive and longitudinal diffuse calcifications in the 3 coronary arteries, including the left main trunk\(^3,6,7\). He was found to be homozygous for a mutation at G1158A (Ala255Thr) of the ABCA1 gene\(^7\).

Case 2 was a 71-year-old woman\(^6,7\) who showed a typical TD phenotype, such as hepatosplenomegaly and orange tonsils. She had coronary artery disease (CAD) and suddenly died of acute myocardial infarction at the age of 73. This patient was homozygous for a mutation at C5946T (Arg1851Stop)\(^7\).

Case 3 was diagnosed with homozygous TD in 1985\(^8\). He was a 44-year-old man, who also had orange tonsils, foamy macrophages in the gastric mucosa, and hepatosplenomegaly. OGTT was performed. FPG was 180 mg/dL and insulin concentration was 3.8 µU/mL. At first he did not have CAD; however, he had a CABG operation for the 3 coronary arteries in 2005. This patient was heterozygous for an Asn935His mutation; however, the other mutation in the ABCA1 gene has not been identified yet.

Case 4 was diagnosed with homozygous TD in 2002. He had also orange tonsils and foamy macrophages in the gastric mucosa\(^9\). He had not only hepatosplenomegaly but also chronic viral hepatitis type C. OGTT was performed. FPG was 176 mg/dL and insulin concentration was 4.3 µU/mL. At this time, he did not have CAD. This patient was homozygous for a mutation at A3198C (Asn935His)\(^9\).

To show the normal range, 297 subjects without diabetes, hyperlipidemia and hypertension were selected from subjects undergoing an annual health examination in the institutions that participated in the Japanese Visceral Fat Syndrome (J-VFS) Study Committee of the Ministry of Health and Welfare of Japan\(^10\). After selecting the subjects, who were matched for age and BMI, we analyzed 123 control

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**Table 1. Clinical characteristics of Japanese patients with Tangier disease and normal subjects**

<table>
<thead>
<tr>
<th></th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Normal (n=123)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABCA1 mutations</td>
<td>Ala255Thr</td>
<td>Arg1851Stop</td>
<td>Asn935His/N.D.</td>
<td>Asn935His</td>
<td></td>
</tr>
<tr>
<td>Age (years)/Sex (M/F)</td>
<td>54M</td>
<td>71F</td>
<td>44M</td>
<td>74M</td>
<td>55.3 ± 6.9 (M94/F29)</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
<td>24.6</td>
<td>–</td>
<td>23.5</td>
<td>22.4</td>
<td>23.4 ± 0.8</td>
</tr>
<tr>
<td>Fasting plasma glucose (mg/dL)</td>
<td>163</td>
<td>180</td>
<td>180</td>
<td>176</td>
<td>93.8 ± 6.9</td>
</tr>
<tr>
<td>Fasting plasma insulin (µU/mL)</td>
<td>4.0</td>
<td>4.0</td>
<td>3.0</td>
<td>4.26</td>
<td>5.1 ± 3.0</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>5.8</td>
<td>7.9</td>
<td>–</td>
<td>6.1</td>
<td>4.7 ± 0.3</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>35</td>
<td>59</td>
<td>64</td>
<td>69</td>
<td>198.3 ± 31.1</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>0**</td>
<td>6.0</td>
<td>2.5</td>
<td>3.5</td>
<td>52.2 ± 14.1</td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>395</td>
<td>162</td>
<td>272</td>
<td>42</td>
<td>127.0 ± 92.1</td>
</tr>
<tr>
<td>Coronary artery disease</td>
<td>(+)</td>
<td>(+)</td>
<td>(+)</td>
<td>(−)</td>
<td></td>
</tr>
<tr>
<td>Sudden death</td>
<td>3VD, CABG</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

3VD: triple vessel disease, CABG: coronary artery bypass graft surgery
Data are the means ± SD.
*N.D.: The other mutation has not been identified so far.
**Less than sensitivity
subjects. The subjects ranged from 40 to 76 years of age and from 22 to 25 BMI, respectively. Written informed consent was obtained from all subjects. The experimental protocol was approved by the ethics committee of Osaka University.

**Results**

**Clinical and Biochemical Characteristics of Patients with TD Disease**

The body mass index (BMI) of TD Case 1, 3 and 4 was 24.6, 23.5 and 22.4, respectively. They were not obese. Although detailed data of Case 2 were not available, we had information that she was not obese. FPG level was elevated 163, 180, 180 and 176, indicating the presence of diabetes. Fasting plasma insulin level was within the normal range and relatively low. Serum total cholesterol was decreased in all TD patients, whereas triglycerides were increased in two TD patients. Three patients with TD are suffering from CAD.

**Oral Glucose Tolerance Test**

In order to know the characteristics of the pattern of insulin secretion in patients with TD and control subjects, 75 g-OGTT was performed at the first medical examination without any treatment. Plasma samples were collected and analyzed before and 30, 60, and 120 min after OGTT. Plasma glucose (PG) and insulin concentrations were measured (Fig. 1A, B).

We calculated a homeostasis model assessment for insulin resistance (HOMA-IR) and insulinogenic index \( \Delta I_{30-0}/\Delta G_{30-0} \) as a measure of early-phase insulin secretory response to an oral glucose load\(^{11, 12} \). The formulas used to calculate the HOMA-IR and insulinogenic index were as follows: HOMA-IR: \[ \text{fasting glucose (mg/dL)} \times \text{fasting insulin (μU/mL)} / 405 \]; insulinogenic index \( \Delta I_{30-0}/\Delta G_{30-0} \): \[ \text{insulin (30 min - 0 min)} / \text{glucose (30 min - 0 min)} \]. Results are expressed as the means ± SE.

As shown in Fig. 1B, the insulin response in TD patients was markedly disrupted compared to that of normal subjects. Especially in case 1 and 4, the responses of insulin were poor at 30 min, while prominent at 60 and 120 min. Insulin secretion measured by the insulinogenic index in TD patients was markedly decreased compared with normal subjects while there was no significant difference in insulin resistance, measured by HOMA-IR between TD patients and normal subjects (Table 2). These results demonstrated an impaired insulin secretion but normal insulin sensitivity in patients with TD.

**Discussion**

In the present study, we reported the results of glucose and insulin measurements by OGTT in 4 patients with TD. We emphasized and documented that all 4 subjects had diabetes mellitus accompanied by relatively low fasting insulin levels, severely disrupted insulin responses to glucose and an absence of insulin resistance. Cases 2, 3 and 4 particularly showed...
impaired early-phase insulin secretion, which was observed in a mouse model; however, in Cases 2 and 3, insulin secretion was completely disrupted. These patterns were not specific to TD patients, but are common in advanced type 2 diabetes. Furthermore, we cannot exclude the possibility that HOMA-IR was underestimated in our cases, since fasting PG levels were more than 160 mg/dL. In the mouse model of ABCA1 deficiency, those mice did not show the phenotype of fasting hyperglycemia, while TD patients in the current study showed fasting hyperglycemia; therefore, we hypothesized that impaired insulin secretion due to ABCA1 deficiency in β cells caused hyperglycemia, and thus sugar toxicity may further attenuate insulin secretion. Furthermore, although Tangier disease is an extremely rare disorder, all 4 TD patients suffered from diabetes mellitus. These results might be very difficult to explain by coincidence and might reflect the deleterious effect of cholesterol accumulation in β cells as it has been demonstrated in a mouse model of Tangier disease. Asian and Japanese populations have been reported to have a background of reduced insulin reserve in the pancreas compared with Western populations. Thus, impaired insulin secretion might have been accelerated further in these Japanese TD patients according to age. To demonstrate an association between ABCA1 defect and an impairment of insulin secretion in humans, we should find more patients and conduct further prospective studies.

A relationship between leukocyte ABCA1 gene expression and fasting glucose in healthy men was demonstrated by Albrecht et al.13, who argued the effect of glucose on leukocyte ABCA1 expression. In contrast, we focused on the effect of the defective condition of HDL on β cells, which leads to impaired cholesterol efflux from β cells. Since the mechanism proposed in the current study might be a novel pathophysiological pathway toward diabetes, it would be worth trying to expand the study to investigate type 2 diabetic patients, especially those accompanied by low HDL-cholesterol levels. Recently, some variants of ABCA1 causing low HDL were reported and one of these variants was associated not only with decreased HDL cholesterol and apolipoprotein A-I levels but also type 2 diabetes14.

It is widely believed that ABCA1 plays a key role in the regulation of cholesterol homeostasis and HDL production; therefore, it would be of interest to investigate whether ABCA1 defects in β cells are dominantly involved in β cell dysfunction in type 2 diabetes or whether cholesterol deposition in β cells by impaired apoA-1-mediated cholesterol efflux causes β cell dysfunction.

In conclusion, all patients with TD suffered from type 2 diabetes. A characteristic of diabetes in TD patients might be the extremely low value of insulinogenic index in OGTT. The present study strongly demonstrates the possibility that ABCA1 plays a critical functional role in insulin secretion from β cells.

Acknowledgements

This work was supported by grants-in-aid (No. 11557055 and No. 10671070) from the Japanese Ministry of Education, Science, Sports, and Culture, an International HDL Research Awards Program grant from Pfizer, a grant from Takeda Medical Research Foundation, and a grant from the ONO Medical Research Foundation and the Novartis Foundation for Gerontological Research to S. Yamashita.

We thank H. Iwahashi, E. Fukuda-Akita and A. Fukuda-Tokunaga for their valuable help with this study.

References