Circulating Apolipoprotein L1 is associated with insulin resistance-induced abnormal lipid metabolism

Kenji Nishimura1, Taichi Murakami1*, Toshihiro Sakurai2, Masashi Miyoshi3, Kiyoe Kurahashi4, Seiji Kishi1, Masanori Tamaki1, Tatsuya Tominaga5, Sumiko Yoshida4, Kojiro Nagai1, Hideharu Abe1, Shu-Ping Hui2, Kazuhiko Kotani6 & Toshio Doi1

Circulating Apolipoprotein L1 (ApoL1) is a component of pre-β1-high-density lipoprotein (HDL), however little is known about the relationship of ApoL1 with cardiometabolic factors. Considering previous studies reporting the correlation of ApoL1 to triglyceride, we have hypothesized that ApoL1 associates with insulin-related metabolism. The current study examined their associations in 126 non-diabetic subjects and 36 patients with type 2 diabetes (T2DM). Non-diabetic subjects demonstrated triglyceride (standardized coefficients [s.c.] = 0.204, \( p < 0.05 \)), body mass index (s.c. = 0.232, \( p < 0.05 \)) and HDL cholesterol (s.c. = −0.203, \( p < 0.05 \)) as independent determinant of ApoL1 levels, and the significant elevation of ApoL1 in metabolic syndrome. Lipoprotein fractionation analysis revealed the predominant distribution of ApoL1 in large HDL fraction, and the significant increase of ApoL1 in large LDL fraction in high ApoL1 samples with insulin resistance. In T2DM, ApoL1 was higher in T2DM with metabolic syndrome, however ApoL1 was lower with \( \beta \) cell dysfunction. Insulin significantly promotes ApoL1 synthesis and secretion in HepG2 cells. In conclusion, circulating ApoL1 may be associated with abnormal HDL metabolism in insulin resistant status. This may suggest a regulation of insulin signal on the ApoL1 level, leading to offer a novel insight to the ApoL1 biology.

Apolipoprotein L1 (ApoL1) was originally recognized as a component of human high-density lipoprotein (HDL) particles. ApoL1 possesses amphipathic helices, which confer a high level of lipophilicity; therefore, ApoL1 is entirely bound to HDL during the circulation of normolipidemic population. ApoL1 gene on chromosome 22 comprises putative sterol response elements in the promoter region, through which the transcription of genes involved in lipid metabolism is presumed to be activated. Circulating ApoL1 levels correlate with the plasma triglyceride (TG) levels, and increases in subjects with a deficiency of cholesteryl ester transfer protein (CETP) that exchanges cholesterol and TG between the TG-rich lipoprotein and HDL. Recent research reported that ApoL1-containing HDL subsets that comprise ApoA-I and ApoE are huge pre-β1-HDL particles. The evidence indicates that the HDL particles carrying ApoL1 are the characteristic subspecies in terms of HDL metabolism or bioactivity.

In innate immunity, HDL ApoL1 is the trypanolytic factor in human serum, which forms anion channels on the lysosomal membrane of Trypanosoma brucei that causes sleeping sickness. Additionally, the association between plasma ApoL1 and the clinical characteristics including lipid-related factors in normolipidemic, dyslipidemic, or diabetic subjects, has also been explored. Duchateau et al. reported the independent positive correlation of ApoL1 with total cholesterol (Total-C) and TG in normolipidemia, and with TG in dyslipidemia. Moreover, it was observed that ApoL1 was elevated in the non-insulin dependent diabetic patients with hypertriglyceridemia. Albert et al. reported that TG and hyperglycemia are the strongest predictors of plasma ApoL1 in dyslipidemic coronary artery disease subjects with low HDL cholesterol (HDL-C). Intriguingly, proteomic...
The comparison between non-obesity, abdominal obesity, and pre-Mets/Mets. We classified 126 facility volunteers into 3 groups, namely, non-obese, abdominally obese, and preliminary metabolic syndrome (pre-Mets)/Mets; hence, we combined both groups as pre-Mets/Mets. All data are listed in Table 2. The median ages of the non-obese, abdominally obese, and preliminary metabolic syndrome (pre-Mets)/Mets participants were 32.0 (26.0–38.0), 31.0 (27.3.0–41.0), and 41.5 (35.0–44.8) years, respectively, wherein the pre-Mets/Mets group is significantly older than the non-obese group (p < 0.01). The male percentage in the pre-Mets/Mets group was 81.8%, which was significantly higher than that of the non-obese group. In the non-obese group, the median WC and the mean BMI were 74.0 (68.0–80.8) cm and 20.7 ± 2.2 kg/m² and all other characteristics were within normal limit. In the abdominally obese group defined as having central obesity without metabolic syndrome, the median WC and the mean BMI were 81.8%, which was significantly higher than that of the non-obese group. In the non-obese group, all other characteristics revealed similar levels with the non-obese group. Between the abdominally obese group and the pre-Mets/Mets group, the mean BMI was 22.0 [19.0–27.8] µg/mL, p < 0.001) or abdominally obese group (25.0 [22.5–26.8] µg/mL, p < 0.05). No significant difference was observed in ApoL1 between the non-obese and abdominal obese groups (Fig. 1a). Insulin resistance, as a consequence of accumulating visceral fat, is the basic pathophysiology in Mets; therefore, we evaluated the relationship with Homeostasis model assessment insulin resistance (HOMA-IR) as insulin resistance index. As assumed, ApoL1 demonstrated the significant positive correlation with HOMA-IR (p < 0.01, r = 0.240; Fig. 1b). These data suggest that serum ApoL1 is associated with dyslipidemia in the Mets.

### Results

Univariable linear regression analysis of characteristics relative to ApoL1 in non-diabetic volunteers. Initially, we examined the correlation of serum ApoL1 levels with clinical variables in all non-diabetic volunteers. In this study, 126 institute volunteers (male: 76 [60.3%], female: 50 [39.7%]) were enrolled, and their median age was 34.0 (26.3–41.0) years. Their median waist circumference (WC) and body mass index (BMI) were 79.0 (70.0–87.0) cm and 21.9 (19.5–24.9) kg/m², and the mean systolic and diastolic blood pressure (BP) values were 113.8 ± 11.0 mmHg, respectively. The percentage values of the habitual drinker and present smoker were 28.6% and 16.7%, respectively. The median value of serum ApoL1 was 24.0 (20.0–29.0) µg/mL. All characteristics in the serum and urine samples were within normal limits. Univariable linear regression analysis revealed the significant correlation of log ApoL1 with sex (standardized coefficients [s.c.] = 0.269, p < 0.001), WC (s.c. = 0.412, p < 0.001), BMI (s.c. = 0.430, p < 0.001), systolic BP (s.c. = 0.233, p < 0.01), diastolic BP (s.c. = 0.268, p < 0.01), low-density lipoprotein (LDL-C) (s.c. = 0.236, p < 0.01), log HDL-C (s.c. = 0.421, p < 0.001), log TG (s.c. = 0.416, p < 0.001), log γ-glutamyltransferase (γ-GTP) (s.c. = 0.342, p < 0.001), uric acid (s.c. = 0.343, p < 0.001), fasting blood sugar (FBS) (s.c. = 0.299, p < 0.001), log insulin (s.c. = 0.205, p < 0.05), and log adiponectin (s.c. = −0.362, p < 0.001) (Supplementary Table S1).

Multivariable stepwise linear regression analysis of characteristics relative to ApoL1 in non-diabetic volunteers. Furthermore, we determined the independently correlated factors among these 13 characteristics found by univariable linear regression analysis. Multivariable stepwise linear regression analysis identified log BMI (s.c. = 0.232, p < 0.05), log TG (s.c. = 0.204, p < 0.05), and log HDL-C (s.c. = −0.203, p < 0.05) as the independent determinant of serum ApoL1 levels (Table 1). The positive correlation of log TG and log BMI and the inverse correlation of log HDL-C with log ApoL1 indicate the association of serum ApoL1 with typical characteristics in the serum and urine samples were within normal limits. Univariable linear regression analysis demonstrated the positive correlation of the in vitro antioxidative capacity and ApoL1 peptide count on purified HDL determined by mass spectrometry.

These data suggest that ApoL1 may be associated with lipid and glucose metabolisms or may exert an antioxidative effect as a distinct HDL subspecies in certain dyslipidemic subjects. We postulate that serum ApoL1 levels may elevate and exert certain biological activity in subjects with insulin resistance that frequently presents dyslipidemia marked by hypertriglyceridemia; however, the association between ApoL1 and insulin resistance has not been reported. The present study aimed to examine this association in the nondiabetic volunteers and patients with type 2 diabetes (T2DM) characterized by insulin resistance.

### Table 1. Multivariable stepwise linear regression analysis of characteristics relative to log ApoL1 in non-diabetic volunteers. BMI, TG and HDL-C were independent determinants of serum ApoL1 levels. BMI, body mass index; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; ApoL1, apolipoproteinL1.

| characteristic | B     | standard deviation error | standardized coefficients | p value |
|---------------|-------|-------------------------|--------------------------|---------|
| log BMI (kg/m²) | 0.396 | 0.165 | 0.232 | < 0.05 |
| log TG (mg/dL) | 0.127 | 0.058 | 0.204 | < 0.05 |
| log HDL-C (mg/dL) | −0.265 | 0.127 | −0.203 | < 0.05 |
Table 2. Baseline characteristics of non-obesity, abdominal obesity and pre-Mets/Mets. Data are presented as mean ± standard deviation, as number (%) or as median (interquartile range) if skewed. aCalculated with one way ANOVA in parametric characteristics or with Kruskal-Wallis in non-parametric characteristics. bCalculated with Tukey in parametric characteristics or with Steel-Dwass in non-parametric characteristics between non-obese participants, abdominal obese participants and preMets/Mets. Mets, metabolic syndrome; BMI, body mass index; BP, blood pressure; Total-C, total cholesterol; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; TG, triglycerides; γ-GTP, γ-glutamyltransferase; eGFR, estimated glomerular filtration rate; FBS, fasting blood sugar; U-Alb/Cr, urine albumin to creatinine ratio; U-8OHdG/Cr, urine 8-Hydroxy-2′-Deoxyguanosine to creatinine ratio; ApoL1, apolipoproteinL1.

### Lipoprotein fractionation analysis in low and high ApoL1 subjects.

Next, we compared ApoL1 distribution among lipoprotein fractions between low ApoL1 subjects (N = 5) and high ApoL1 subjects (N = 5). Serum ApoL1 levels were 24.2 ± 3.3 and 42.6 ± 9.9 μg/mL in low and high ApoL1 subjects, respectively. In high ApoL1 group, the WC (p < 0.05), BMI (p < 0.01), TG (p < 0.05), and insulin level (p < 0.05) were higher, whereas HDL-C (p < 0.05) and adiponectin (p > 0.01) levels were lower (Supplementary Table S2). Western blotting of serum in dematured condition identified two different signals of ApoL1, 42 kDa and 39 kDa (Fig. 2a), wherein not 42 kDa, but 39 kDa ApoL1 significantly increased in the high ApoL1 group (Fig. 2b).

Furthermore, we quantified cholesterol, TG, and ApoL1 in lipoprotein fractions separated by gel filtration chromatography. The lipid profile is shown in Fig. 3 and Supplementary Table S3. Western blotting of ApoL1 in lipoprotein fractions revealed that ApoL1 was predominantly distributed in the large HDL fractions irrespective of the serum ApoL1 levels (Fig. 4a). ApoL1 is known to express in two different sizes of HDL complexes in large LDL and large HDL fractions in the normolipemic subjects. In addition, ApoL1 expression levels are significantly enriched in the smaller complex. Our data of western blotting is consistent with the results of previous studies. Interestingly, ApoL1 appeared to a lesser extent in LDL fractions of high ApoL1 sample. Quantification of ApoL1 by ELISA revealed the significant increase in the LDL fractions. ApoL1 in HDL fractions also indicated the trend to increase (Fig. 4b); however, it was non-significant. These findings indicate that two different size ApoL1-containing complexes may be differentially metabolized in abnormal HDL metabolism.

### Evaluation of circulating ApoL1 in T2DM.

We examined ApoL1 expression in 36 patients with T2DM characterized by insulin resistance (Table 3). The mean age was 47.0 ± 9.9 years, and male frequency was 47.2%. The average of the abdominal circumference was 99.8 ± 18.2 cm, and BMI was 29.6 ± 7.9 kg/m². The median FBS was 138.0 (123.5–186.0) mg/dl and the mean HbA1c was 9.7 ± 2.5%, thus indicating poor glycemic control. The median insulin-to-creatinine ratio was 29.1 (7.2–284.3) μg/mgCr, in which overt albuminuria is 25.0%, microalbuminuria is 22.2%. ApoL1 revealed positive correlation with WC (p < 0.05, r = 0.38) and log TG (p < 0.05, r = 0.37), and negative correlation with log adiponectin (p < 0.05, r = 0.41) in overall patients with T2DM (Supplementary Fig. S1). However, unexpectedly, no significant difference of serum ApoL1 levels was
observed between all T2DM patients and 90 nondiabetic non-obese volunteers. Therefore, we compared T2DM without Mets and with Mets (Table 3). ApoL1 was significantly lower in T2DM without Mets, wherein c-peptide also presented the significant decrease despite of hyperglycemia, thus indicating β cell dysfunction in this group.

In addition, Interestingly, U-8OHdG/Cr was significantly lower in T2DM with Mets. Furthermore, ApoL1 is inversely correlated with log U-8OHdG/Cr ($p < 0.05$, $r = 0.40$).

**In vitro** examination on the insulin signaling-mediated ApoL1 expression and secretion in HepG2 cells. We hypothesized that insulin signal may regulate the ApoL1 expression in hepatic cells based on our clinical data, thus we examined the effect of insulin on ApoL1 expression in HepG2 cells and secretion in media. Previous studies reported HepG2 cells expresses insulin receptor and present insulin-dependent apo-protein secretion. We incubated HepG2 cells in the absence of insulin or in the presence of 100 nM insulin. Incubation with 100 nM insulin for 6h results in the significant upregulation of ApoL1 mRNA ($p < 0.05$) (Fig. 5a). ApoL1 protein levels in HepG2 cells were significantly increased after 12h insulin stimulation ($p < 0.05$) (Fig. 5b). In addition, ApoL1 protein secretion in media showed the significant increase in the presence of 100 nM insulin for 24h ($p < 0.05$) (Fig. 5c). ApoL1 protein in cells or media was detected as a single band of 42 kDa by western blot, which may attribute to the some difference of proteolytic regulation between *in vivo* and *in vitro*. These data suggest that insulin signaling may regulate ApoL1 synthesis and secretion in hepatic cells

**Discussion**

This observational study revealed several novel findings regarding insulin resistance-mediated ApoL1 biology in Mets and T2DM, including the elevation of serum ApoL1 in Mets. In nondiabetic volunteers, high BMI, hypertriglyceridemia, and low HDL-C were identified as independent characteristics that determined serum ApoL1 levels and are typical features associated with Mets. To the best of our knowledge, this is the first study to report a
correlation between ApoL1 and HDL-C or BMI\textsuperscript{17,11}. Specifically, serum ApoL1 in Mets was significantly higher than in non-obesity or abdominal obesity without insulin resistance. The abnormal lipid metabolism in Mets is characterized by elevated VLDL, hypertriglyceridemia, high levels of LDL-C, increased CETP activity, and low levels of HDL-C, in which insulin resistance and increased circulating free fatty acid (FFA) resulting from adipose tissue hyperplasia and hypertrophy are considered as fundamental pathogenesis\textsuperscript{12-15}. This study also found a significant correlation between serum ApoL1 and HOMA-IR in all non-diabetic volunteers. These findings suggest that serum ApoL1 may not increase in obesity alone, but it may be elevated in non-diabetic Mets marked by insulin resistance-mediated dyslipidemia.

Figure 2. Immunoblot analysis of serum ApoL1 in low and high ApoL1 groups. (a) Western blotting detected 42 kDa and 39 kDa ApoL1 in serum. (b) Signal intensity of 39 kDa ApoL1 was significantly increased in high ApoL1 group. ApoL1, apolipoproteinL1.

Figure 3. Lipid profile in lipoprotein fractionation analysis by gel filtration chromatography in low and high ApoL1 groups. VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein.
Figure 4. Alteration of ApoL1 distribution among lipoprotein fractions in low and high ApoL1 groups. (a) ApoL1 expression in lipoprotein fractions was determined by Western blotting. A representative data of low ApoL1 group and high ApoL1 group are presented. (b) Quantitative evaluation of ApoL1 in lipoprotein fractions. P value was calculated with student’s t-test. VLDL, very low-density lipoprotein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; ApoL1, apolipoproteinL1.

Table 3. Baseline characteristics of patients with T2DM. Data are presented as mean ± standard deviation, as number (%) or as median (interquartile range) if skewed. P value was calculated with student’s t-test in parametric variables or Mann–Whitney’s U test was used in non-parametric variables for comparison between T2DM without Mets and T2DM with Mets. T2DM, type2 diabetes mellitus; Mets, metabolic syndrome; BMI, body mass index; BP, blood pressure; Total-C, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; γ-GTP, γ-glutamyltransferase; eGFR, estimated glomerular filtration rate; FBS, fasting blood sugar; U-Alb/Cr, urine albumin to creatinine ratio; U-8OHdG/Cr, urine 8-Hydroxy-2’-Deoxyguanosine to creatinine ratio; ApoL1, apolipoproteinL1.
The ApoL1 increase in T2DM is consistent with the results of this study because insulin resistance is the common basal pathophysiology of Mets and T2DM. However, patients with T2DM unexpectedly demonstrated no significant increase in serum ApoL1 compared with the non-obese volunteers in this study. In contrast, those with T2DM exhibited a significant increase in WC, BMI, TG, and adiponectin compared with non-obese volunteers, thus suggesting a difference in ApoL1 regulation between Mets and T2DM. Considering that insulin secretory capacity in T2DM varies, we examined the presence of Mets in T2DM. T2DM without Mets had significantly lower c-peptide and ApoL1 levels, despite high FBS levels similar to T2DM with Mets, thereby indicating that absolute insulin deficiency due to β cell dysfunction is associated with low ApoL1 levels in T2DM without Mets. ApoL1 protein levels in the circulation are primarily determined by liver secretion. We postulated that insulin, not hyperglycemia, promoted ApoL1 secretion in the Mets liver. We finally showed that insulin regulated ApoL1 synthesis and secretion in HepG2 cells. Hence, we conclude that ApoL1 synthesis and secretion in the liver attribute, in part, to insulin signaling in Mets or T2DM.

Alteration of the lipid and lipoprotein profile induced by human African trypanosomiasis (HAT), wherein ApoL1-containing particles function as lytic factors, has been reported. Huet et al. reported that patients with HAT presented with an altered lipid and lipoprotein profile, including hypertriglyceridemia, high LDL-C, low HDL-C, and reduced ApoA-1. Lamour et al. found hypertriglyceridemia and lowered plasma levels of phospholipid and sphingolipid as metabolic markers in HAT. Vervet monkeys infected with Trypanosoma brucei presented with hypoglycemia, hypertriglyceridemia, and low HDL-C. Further, Trypanosoma infection-induced

Figure 5. in vitro examination on insulin-mediated ApoL1 expression and secretion in HepG2 cells. (a) Expression of ApoL1 mRNA in HepG2 cells is determined by quantitative PCR. Arbitrary unit of ApoL1/ GAPDH mRNA expression is shown. (b) ApoL1 protein expression in HepG2 cells is evaluated by western blotting. A representative data of ApoL1 in the absence of insulin or in the presence of 100 nM insulin are presented. Arbitrary unit of ApoL1/GAPDH protein expression is shown. (c) Secreted ApoL1 in culture media is evaluated by western blotting. A representative data of ApoL1 in the absence of insulin or in the presence of 100 nM insulin are presented. Arbitrary unit of ApoL1 protein expression corrected by protein concentration in media is shown. P value was calculated with student's t-test. Data is shown as mean SD of three to five independent experiments. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; ApoL1, apolipoproteinL1.
TNF has been shown to inhibit lipoprotein lipase activity in a rodent model; therefore, increased lipolysis from adipose tissue may result in lipid abnormalities. These findings indicate some similarities in the pathophysiology of dyslipidemia between Mets and HAT. In addition, circulating ApoL1 expression was reported to be increased by *Trypanosoma brucei* infection. The interaction of the altered lipid profile and increased ApoL1 expression remains unclear; however, some metabolic factors or pro-inflammatory cytokines induced by trypanosoma infection may alter lipid and lipoprotein metabolism in HAT.

A second major finding was the distinct expression and distribution pattern of ApoL1 in subjects with increased ApoL1 levels resulting from insulin resistance. Circulating ApoL1 was reported to show a bimodal distribution among lipoprotein fractions. Duchateau et al. reported that ApoL1 existed in the large HDL and VLDL fractions under non-denatured conditions, and that ApoL1-containing lipoproteins exhibited pre-β mobility. Weckerle et al. also found ApoL1 in the LDL and large HDL fractions by FPLC separation, in which the ApoL1 protein was highly enriched in the large HDL particle size. They also found that these ApoL1-carrying HDL subspecies expressed ApoA-I and ApoE and migrated in the pre-β position. Interestingly, plasma levels of pre-β HDL have been reported to increase in parallel with TG and BMI in Mets. Lipoprotein fractionation analysis in this study indicated the predominant expression of ApoL1 in the large HDL fraction, independent of ApoL1 levels. However, ApoL1 in the LDL fraction, which corresponds with the larger ApoL1-containing complex, presented a significant increase (1.7-fold) in the high ApoL1 subjects, thus suggesting that two different size ApoL1-enriched complexes may be differentially metabolized in the abnormal HDL metabolic cycle of Mets.

Moreover, we detected serum ApoL1 at two different sizes of 42 and 39 kDa by western blotting, which are regarded as a precursor and a signal peptide-deleted activated form, respectively. Compared with low ApoL1 subjects, high ApoL1 subjects exhibited a significant increase in total ApoL1 and 39 kDa ApoL1, thus indicating that circulating ApoL1 in Mets may be transcriptionally increased and proteolytically activated. The alteration in HDL apoprotein expression levels or distribution contributes to HDL metabolism or bioactivity in pathological conditions, including Mets. These data suggest the possibility that the change in distribution or proteolytic activation of ApoL1 may be associated with ApoL1-containing HDL activity or metabolism in Mets.

The function of circulating ApoL1 is another important point to consider. Therefore, we explored the association of ApoL1 levels with arterial stiffness and oxidative stress in T2DM. ApoL1 did not correlate with arterial stiffness, as evaluated by pulse wave velocity in this study, which may be consistent with the previous observation that ApoL1 levels did not correlate with the progression of coronary artery disease. These data support that ApoL1 is not a strong pro-atherogenic molecule, although serum ApoL1 levels have been shown to be associated with known pro-atherogenic markers such as hypertriglyceridemia or low HDL-C.

Additionally, we measured urinary 8-OHDG to evaluate oxidative DNA damage throughout the body. U-8-OHDG/Cr in T2DM without Mets was significantly higher than in T2DM with Mets, and log U-8-OHDG/Cr was inversely correlated with serum ApoL1 levels. One of the possible explanations for this result is the antioxidative activity of ApoL1-enriched HDL. Little is known about the anti-oxidative capacity of ApoL1. Davidson et al. reported a positive correlation of ApoL1 peptide count on HDL by mass spectrometry and the anti-oxidative capacity of HDL. In that study, the correlation analysis found a strong relationship between ApoL1, paraoxonase1, paraoxonase2, and ApoF, thus suggesting potent anti-oxidative activity of these apoproteins. Another possibility is that the oxidative stress-mediated β cell dysfunction may result in reduced insulin secretion and ApoL1 production in T2DM without Mets. A third possible explanation is the association between urinary 8-OHDG and ApoL1 in kidney cells. ApoL1 protein is expressed in glomerular podocytes or proximal tubular cells, and the expression levels have been shown to decrease in diseased kidney. Conversely, urinary 8-OHDG excretion increases in human and rodent kidney diseases. ApoL1 in kidney cells is assumed to be derived from both endogenous synthesis and extracellular resource, and to serve a functional role. ApoL1 localized in kidney cells may affect urinary 8-OHDG excretion. Our data suggest several hypotheses on the ApoL1 effect; however, further investigation is required to clarify the causal association and mechanism between ApoL1 and oxidative stress.

This study had certain limitations. First, this study was conducted with our institute volunteers, in which the job types were slightly biased; therefore, the baseline data may differ from the general population. However, the pre-Mets/Mets ratio in our subjects (total 17.4%, 11.1% aged 20–40 years, 33.3% aged 20–40 years) does not differ from recent data of the Japan National Health and Nutrition Survey. Thus, the correlation between ApoL1 and clinical factors in this study is similar to that in the Japanese population with insulin resistance. Another limitation is that we did not examine gene mutations related to kidney disease in black Americans. The gene variant is known to determine trypanolytic activity or prognosis of kidney diseases. Proteomic analysis revealed variant-related quantitative changes in apoproteins on ApoL1-containing HDL subspecies; however, it remains unclear if these variants alter lipid metabolism, HDL function, or cardiovascular disease susceptibility. Furthermore, the gene variants have not been examined in the Japanese population. Therefore, our study may provide insight into variant-independent ApoL1 biology.

In summary, the current observational study and in vitro examination demonstrated insulin resistance-mediated ApoL1 synthesis and secretion in the liver and alteration of ApoL1 distribution between lipoprotein fractions in Mets. These data may provide new insights into ApoL1 biology in insulin resistance-mediated abnormal lipid metabolism.

**Material and Method**

**Design and subjects.** This study enrolled 126 non-diabetic volunteers from age 20 to age 60. These non-diabetic volunteers who consisted of doctor, nurse, clinical laboratory technician, medical representative and medical student were recruited from our institute with their consent. We measured BP, height, body weight and WC, and collected serum and urine after an overnight fasting. According to the criteria of International Diabetes
Federation, Mets was defined as having more than any two of the four factors including raised TG (≥150 mg/dL), reduced HDL-C (<40 mg/dL in males or <50 mg/dL in females), raised BP (systolic BP ≥ 130 or diastolic BP ≥ 85 mmHg), or raised FBS (≥100 mg/dL), in addition to central obesity (WC: male ≥ 90 cm and female ≥ 80 cm). Subject with abdominal obesity and one of the four factors was classified as pre-Mets. Subject with central obesity and without metabolic abnormality was defined as abdominal obesity. In addition, 36 patients with T2DM from age 20 to age 60 who were hospitalized in Tokushima University Hospital for the purpose of glycemic control were also registered as diabetic participants with their consent. For the analysis of ApoL1 distribution among lipoprotein fractions in low ApoL1 subjects and high ApoL1 subjects, we collected additional serum samples from 5 males in the top 25th percentile and the bottom 25th percentile of serum ApoL1 levels, respectively.

**Blood collection, biochemical analysis, and sample.** Venous blood and urine were collected after an overnight fasting. Serum was then isolated by centrifugation (2,500 g for 10 min) and stored at −80 °C until analysis. Serum levels of Total-C, LDL-C, HDL-C, TG, γ-GTP, albumin, uric acid, creatinine, blood sugar, insulin, HbA1c and adiponectin, and urine levels of albumin, creatinine and 8OHdG were measured. 8OHdG was evaluated by ICR-001 (Techno Medica, Japan) and U-8OHdG/Cr was used as a marker of oxidative DNA damage. Estimated glomerular filtration rate of each participant was calculated from serum creatinine and age by using the 3-variable Japanese equation as follows; estimated glomerular filtration rate (ml/min/1.73m²) = 194 X Age−0.287 X Creatinine (mg/dL)−1.094 (X 0.739 if female). BMI was calculated as weight in kilograms divided by height in meters squared. HOMA-IR, insulin resistance index, was calculated from FBS and insulin as follows; HOMA-IR = fasting insulin (μU/mL) X FBS (mg/dL)/405.

**Gel filtration chromatography.** Serum samples (0.3 mL) were fractionated by gel filtration chromatography on a Superose 6 column (GE Healthcare, Little Chalfont, UK) in a liquid chromatography apparatus (Shimadzu, Kyoto, Japan) using a procedure modified from a previous report41. The column was eluted with 50 mmol/L phosphate buffer (pH7.4) containing 150 mmol/L NaCl and 1 mmol/L EDTA at a flow rate of 0.5 mL/min. Eluted fractions (0.5 mL each) were subjected to lipid analysis and immunoassays. Lipoprotein fractions were defined by eluted fraction volume as follows; very low-density lipoprotein (VLDL) 7.5–9 mL, large LDL 10.5–12 mL, small LDL 12–13.5 mL, large HDL 16–17.5 mL, small HDL, and very small HDL 17.5–19.0 mL.

**ApoL1 ELISA examination.** ApoL1 concentration in serum and in lipoprotein fraction was measured by a human specific sandwich ELISA (KE00047) (proteintech, IL, USA). Briefly, serum was diluted 1:10,000 and assayed in duplicate according to the manufacturer's instructions. For the examination of lipoprotein fractions, samples were diluted 1:250.

**Cell culture.** HepG2 cells were cultivated in DMEM containing 10% fetal calf serum, 5%CO2 at 37 °C. For RNA isolation or protein extraction, HepG2 cells were seeded on 6-well plates at a density of 1 × 10⁶ cells/well and cultivated for 24 h. The culture medium was replaced with a fresh one without fetal calf serum, and cells were additionally incubated for 12 h before insulin administration. Cells were incubated in the absence of insulin or in the presence of 100 nM insulin (Wako, Tokyo, Japan) RNA or whole cell extract was collected after 6 h or after 12 h incubation with insulin, respectively. Culture media for 24 h insulin incubation was collected and concentrated by gel filtration column (Sartorius, Gottingen, Germany) for the evaluation of ApoL1 secretion.

**Western blotting.** One μL of serum, 15 μg of whole cell lysate, 16 μL of concentrated culture media of HepG2 or 10 μL of lipoprotein solution were heated to 70 °C for 15 minutes in SDS gel-loading buffer, and applied to SDS gel electrophoresis, thereafter proteins were transferred to nitrocellulose filters (GE Healthcare, Little Chalfont, UK). The blots were incubated with rabbit anti-ApoL1 antibody (SIGMA, MO, USA) or rabbit anti-human GAPDH antibody (Abcam, Cambridge, UK), followed by incubation with horseradish peroxidase-conjugated goat anti-rabbit IgG (IBL, Gunma, Japan). Signal intensity of ApoL1 was measured by Image J.

**RNA isolation and quantitative PCR.** The total RNA was extracted from cultured HepG2 cells by RNAiso Plus (Takara, Ootsu, Japan). Quantitative PCR was performed on BIO-RAD MJ Mini Personal Thermal Cycler (BIO-RAD, CA, USA) using SYBR Green PCR Master Mix (Applied Biosystems). The following PCR primers were used; human ApoL1: sense 5'-ggatctgaaccaacactg-3', antisense 5'-caaggggctatcggagga-3' and human GAPDH: sense 5'-tttggctacagcaacagg-3', antisense 5'-ggatctcttcctctcctg-3'.

**Ethical approval.** This study was approved by the Ethics Committee of Tokushima University Hospital (No.2128, No.3306) and Hokkaido University (No.17–53–1), and was performed in compliance with the Helsinki Declaration. Written informed consent was obtained from all participants.

**Statistics.** The results are shown as mean ± standard deviation, as number (%) or as median (interquartile range) if skewed. A test for normality was performed using the Kolmogorov-Smirnov test. Student's t-test was used in parametric variables and Mann -Whitney's U test was used in non-parametric variables for comparison between two groups. For the regression analysis, correlations were determined by univariate linear regression analysis, and multiple stepwise regression was used to confirm significant independent correlation. When data were non-normal distribution, log transformation was applied to correct skewness. For the assessment of potential differences in mean value, one-factor ANOVA was performed. Subsequently, as for the characteristic that presents significant difference between three groups, multiple comparison analysis was performed. Those that
were normally distributed were subjected to multiple comparison by Tukey's test, and those that were non-normal distribution were subjected to multiple comparison by Kruskal-Wallis test. Statistical analyses were performed with IBM SPSS statistics. A p value < 0.05 was considered significant.

Received: 23 November 2018; Accepted: 23 September 2019; Published online: 16 October 2019

References
1. Duchateau, P. N. et al. Apolipoprotein L, a new human high density lipoprotein apolipoprotein expressed by the pancreas. Identification, cloning, characterization, and plasma distribution of apolipoprotein L. J. Biol Chem 272, 25576–25582 (1997)
2. Duchateau, P. N., Pullinger, C. R., Cho, M. H., Eng, C. & Kane, J. P. Apolipoprotein L gene family: tissue-specific expression, splicing, promoter regions; discovery of a new gene. J Lipid Res 42, 620–630 (2001)
3. Duchateau, P. N. et al. Plasma apolipoprotein L1 concentrations correlate with plasma triglycerides and cholesterol levels in normolipidemic, hyperlipidemic, and diabetic subjects. J Lipid Res 41, 1231–1236 (2000)
4. Weckerle, A. et al. Characterization of circulating APOL1 protein complexes in African Americans. J Lipid Res 57, 120–130 (2016)
5. Vanhamme, L. et al. Apolipoprotein L-I is the trypanosome lytic factor of human serum. Nature 422, 83–87 (2003)
6. Genovese, G. et al. Association of trypanolytic ApoL1 variants with kidney disease in African Americans. Science (New York, N.Y.) 329, 841–845 (2010)
7. Albert, T. S. et al. Apolipoprotein L1 is positively associated with hyperglycemia and plasma triglycerides in CAD patients with low HDL. J Lipid Res 46, 469–474 (2005)
8. Davidson, W. S. et al. Proteomic analysis of defined HDL subpopulations reveals particle-specific protein clusters: relevance to antioxidative function. Arteriosclerost. thrombos, and vascular biology 29, 870–876 (2009)
9. Yuasa, T. et al. Sequential cleavage of insulin receptor by complement 2 and gamma-secretase impairs insulin signalling. Diabetologia 59, 2711–2721 (2016)
10. Shavva, V. S. et al. Insulin-Mediated Downregulation of Apolipoprotein A-I Gene in Human Hepatoma Cell Line HepG2: The Role of Interaction Between FOXO1 and LXRbeta Transcription Factors. Journal of cellular biochemistry 118, 382–396 (2017)
11. Bruggeman, L. A. et al. Plasma apolipoprotein L1 levels do not correlate with CKD. Journal of the American Society of Nephrology: JASN 23, 634–644 (2014)
12. Kaur, J. A comprehensive review on metabolic syndrome. Cardioiology research and practice 2014, 943162 (2014)
13. Halberg, N., Wernstedt-Asterholm, I. & Scherer, P. E. The adipocyte as an endocrine cell. Endocrinology and metabolism clinics of North America 37, 753–768, x–xi (2008)
14. Lamarche, B. et al. Triglyceride enrichment of HDL enhances in vivo metabolic clearance of HDL apo A-I in healthy men. The Journal of clinical investigation 103 (1999)
15. Deeb, S. S., Zambon, A., Carr, M. C., Ayyobi, A. F. & Brunzell, J. D. Hepatic lipase and dyslipidemia: interactions among genetic variants, obesity, gender, and diet. J Lipid Res 44, 1279–1286 (2003)
16. Shukla, K. et al. Most ApoL1 Is Secreted by the Liver. Journal of the American Society of Nephrology: JASN 28, 1079–1083 (2017)
17. Huet, G. et al. Serum lipid and lipoprotein abnormalities in human african trypanosomiasis. Transactions of the Royal Society of Tropical Medicine and Hygiene 84, 792–794 (1990)
18. Lamour, S. D. et al. Discovery of Infection Associated Metabolic Markers in Human African Trypanosomiasis. PLoS neglected tropical diseases 9, e0004200 (2015)
19. Gaithuma, A. K. et al. Lipid metabolism and other metabolic changes in vervet monkeys experimentally infected with Trypanosoma brucei rhodesiense. Journal of medical primatology 41, 75–81 (2012)
20. Rouzer, C. A. & Cerami, A. Hypertriglyceridemia associated with Trypanosoma brucei brucei infection in rabbits: role of defective triglyceride removal. Molecular and biochemical parasitology 2, 31–38 (1980)
21. Iboudo, H. et al. APO1 expression is induced by Trypanosoma brucei gambiense infection but is not associated with differential susceptibility to sleeping sickness. Infection, genetics and evolution: journal of molecular epidemiology and evolutionary genetics in infectious diseases 12, 1519–1523 (2012)
22. Lee, J. Y. & Parks, J. S. ATP-binding cassette transporter A1 and its role in HDL formation. Current opinion in lipidology 16, 19–25 (2005)
23. O'Connor, P. M. et al. Prebeta-1 HDL in plasma of normolipidemic individuals: influences of plasma lipoproteins, age, and gender. J Lipid Res 39, 670–678 (1998)
24. Tsimihodimos, V. et al. Plasma triglyceride levels and body mass index values are the most important determinants of prebeta-1 HDL concentrations in patients with various types of primary dyslipidemia. Atherosclerosis 208, 506–511 (2010)
25. Patsch, J., Karlin, J. B., Scott, L. W., Smith, L. C. & Gatto, A. M. Jr. Inverse relationship between blood levels of high density lipoprotein subfraction 2 and magnitude of postprandial lipemia. Proceedings of the National Academy of Sciences of the United States of America 80, 1449–1453 (1983)
26. Dullaart, R. P., Plomgaard, P., de Vries, R., Dahlback, B. & Nielsen, L. B. Plasma apolipoprotein M is reduced in metabolic syndrome but does not predict intima media thickness. Atherosclerosis 222, 151–157 (2012)
27. Gordon, S. M. et al. The effects of type 2 diabetes on lipoprotein composition and arterial stiffness in male youth. Diabetes 62, 2958–2967 (2013)
28. Maraninchi, M. et al. Impact of bariatric surgery on apolipoprotein C-III levels and lipoprotein distribution in obese human subjects. Journal of clinical lipidology 11, 495–506.e493 (2017)
29. Cubedo, J., Pedro, T., Alonso, R., Mata, P. & Badimon, L. Apolipoprotein L1 in human postprandial lipoprotein and cardiovascular event presentation in patients with familial hypercholesterolemia. J Lipid Res 57, 1039–1073 (2016)
30. Loft, S., Fischer-Nielsen, A., Jeding, I. B., Vistisen, K. & Poulsen, H. E. 8-Hydroxydeoxyguanosine as a urinary biomarker of oxidative DNA damage. Journal of toxicology and environmental health 40, 391–404 (1993)
31. Krauss, S. et al. Supernova-mediated activation of uncoupling protein 2 causes pancreatic beta cell dysfunction. The Journal of clinical investigation 112, 1831–1842 (2003)
32. Zhou, H. et al. Urinary marker for oxidative stress in kidneys in cisplatin-induced acute renal failure in rats. Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association 21, 616–623 (2006)
33. Boona, C., Wunsawan, R., Tungsanga, K. & Tosukhowong, P. Urinary 8-hydroxydeoxyguanosine is elevated in patients with nephrolithiasis. Urological research 35, 185–191 (2007)
34. Muraya, Y. & Bo, O. Effect of clofibrate on fatty acid metabolism in the kidney of puromycin-induced nephrotic rats. Clinical and experimental nephrology 20, 862–870 (2016)
35. Madhavan, S. M. et al. APO1 localization in normal kidney and nondiabetic kidney disease. Journal of the American Society of Nephrology: JASN 22, 2119–2128 (2011)
36. Ma, L. et al. Localization of APO1 protein and mRNA in the human kidney: nondiseased tissue, primary cells, and immortalized cell lines. Journal of the American Society of Nephrology: JASN 26, 339–348 (2015).
37. Kopp, J. B. et al. Clinical Features and Histology of Apolipoprotein L1-Associated Nephropathy in the FSGS Clinical Trial. *Journal of the American Society of Nephrology: JASN* **26**, 1443–1448 (2015).

38. McLean, N. O., Robinson, T. W. & Freedman, B. I. APOL1 Gene Kidney Risk Variants and Cardiovascular Disease: Getting to the Heart of the Matter. *American journal of kidney diseases: the official journal of the National Kidney Foundation* **70**, 281–289 (2017).

39. Kozlitina, J. et al. Plasma Levels of Risk-Variant APOL1 Do Not Associate with Renal Disease in a Population-Based Cohort. *Journal of the American Society of Nephrology: JASN* **27**, 3204–3219 (2016).

40. Gutierrez, O. M. et al. APOL1 nephropathy risk variants are associated with altered high-density lipoprotein profiles in African Americans. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association* **31**, 602–608 (2016).

41. Sakurai, T. et al. Novel monoclonal antibody recognizing triglyceride-rich oxidized LDLs associated with severe liver disease and small oxidized LDLs in normal subjects. *Annals of clinical biochemistry* **49**, 456–462 (2012).

**Acknowledgements**

This study is supported by Grant-in-Aid for Scientific Research (C) (15K09264). We thank Arisa Ochi, Hisae Nakagawa, Sanae Hayashi, and Ryoko Uemoto for their technical support. We appreciate Yoshihiro Okayama for his support about statistical analysis and editage for English proofreading. We are grateful to Dr. Jeffrey Kopp, Dr. Munehide Matsuhashi, Dr. Ken-ichi Aihara and Dr. Tomoyuki Yuasa for their technical advice.

**Author contributions**

K.N. collected samples and information of volunteer and analyzed the data. T.M. organized this study, analyzed the data and made the manuscript. T.S. and S.H. performed lipoprotein fractionation analysis. M.M. measured serum and urine samples including ApoL1. K.K. and S.Y. collected samples and information of T2DM and analyzed the data. K.K. contributed the statistical analysis and edited the manuscript. M.T., S.K. and T.T. analyzed the data. K.N., H.A. and T.D. analyzed data and edited the manuscript.

**Competing interests**

The authors declare no competing interests.

**Additional information**

**Supplementary information** is available for this paper at https://doi.org/10.1038/s41598-019-51367-7.

**Correspondence** and requests for materials should be addressed to T.M.

**Reprints and permissions information** is available at www.nature.com/reprints.

**Publisher’s note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

**Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The images or other third party material in this article are included in the article’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the article’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this license, visit http://creativecommons.org/licenses/by/4.0/.

© The Author(s) 2019