

**Status and physiological significance of circulating adiponectin in the very old and centenarians: an observational study**

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24   **Abstract**

25   **Background:** High levels of circulating adiponectin are associated with increased insulin sensitivity,  
26   low prevalence of diabetes, and low body mass index (BMI); however, high levels of circulating  
27   adiponectin are also associated with increased mortality in the 60–70 age group. In this study, we  
28   aimed to clarify factors associated with circulating high-molecular-weight (cHMW) adiponectin  
29   levels and their association with mortality in the very old (85–89 years old) and centenarians.

30   **Methods:** The study included 812 (women: 84.4%) for centenarians and 1,498 (women: 51.7%)  
31   for the very old. The genomic DNA sequence data were obtained by whole genome sequencing  
32   or DNA microarray-imputation methods. LASSO and multivariate regression analyses were  
33   used to evaluate cHMW adiponectin characteristics and associated factors. All-cause mortality  
34   was analyzed in three quantile groups of cHMW adiponectin levels using Cox regression.

35   **Results:** The cHMW adiponectin levels were increased significantly beyond 100 years of age,  
36   were negatively associated with diabetes prevalence, and were associated with SNVs in *CDH13*  
37   ( $p = 2.21 \times 10^{-22}$ ) and *ADIPOQ* ( $p = 5.72 \times 10^{-7}$ ). Multivariate regression analysis revealed that  
38   genetic variants, BMI, and high-density lipoprotein cholesterol (HDL) were the main factors  
39   associated with cHMW adiponectin levels in the very old, whereas the BMI showed no  
40   association in centenarians. The hazard ratios for all-cause mortality in the intermediate and  
41   high cHMW adiponectin groups in very old men were significantly higher rather than those for  
42   all-cause mortality in the low level cHMW adiponectin group, even after adjustment with BMI.  
43   In contrast, the hazard ratios for all-cause mortality were significantly higher for high cHMW  
44   adiponectin groups in very old women, but were not significant after adjustment with BMI.

45   **Conclusions:** cHMW adiponectin levels increased with age until centenarians, and the  
46   contribution of known major factors associated with cHMW adiponectin levels, including BMI  
47   and HDL, varies with age, suggesting that its physiological significance also varies with age in  
48   the oldest old.

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61

## Introduction

Adiponectin is an adipocyte-derived hormone that plays a vital role in metabolism, including lipid and glucose metabolism, and occurs in circulation at concentrations of up to 0.05% of total plasma protein<sup>1,2</sup>. Circulating adiponectin forms three major multimer complexes, including a trimer, hexamer, and high-molecular-weight form. Among these forms, circulating high-molecular-weight (cHMW) adiponectin shows more potent biological activity than that of the other two forms<sup>3</sup>. Previous studies in the mouse model studies have shown that cHMW adiponectin enhances insulin sensitivity and plasma lipid clearance; high levels of cHMW adiponectin improve the stability of lipid homeostasis and provided systemic tolerance to obesity under normal physiological conditions<sup>4,5,6</sup>. Adiponectin knock-out mice showed mild or moderate insulin resistance, which is exacerbated by a high-fat diet<sup>7,8</sup>. However, adiponectin knock-out mice are viable under regular physiological conditions, indicating that adiponectin is not essential for survival under regular dietary conditions<sup>7,8</sup>. Therefore, adiponectin function is considered inconspicuous under normal conditions and should become prominent under physiological stress such as hyperglycemia.

In humans, adiponectin shows strong negative associations with body mass index (BMI), the prevalence of type 2 diabetes (T2DM), and hypertension<sup>9,10,11</sup>. However, high levels of adiponectin have also been associated with an increased risk of cardiovascular disease (CVD) in adults in their 60s and 70s<sup>12,13,14,15</sup>. These contradictory findings indicate that environmental and related physiological changes could alter the level and function of adiponectin; therefore, the analysis of adiponectin in adults aged 80 years and older would be essential to elucidate the significance of adiponectin in aging.

Centenarians are individuals aged 100 years and older and characterized by a low incidence of life-threatening diseases, such as CVD and T2DM. They serve as potential models for successful aging<sup>16,17</sup>. Previous studies have reported that cHMW adiponectin levels increase with age and, specifically, that centenarians show comparatively high levels<sup>18,19</sup>. Low BMI may contribute to high adiponectin levels and insulin resistance in older adults ages above 60 years, and transgenic mouse models have shown prolonged health span and median lifespan. However, the physiological significance of high cHMW adiponectin levels in adults aged above 80 years is still unclear<sup>20,21,22</sup>. To provide evidence for understanding the physiological function and significance of adiponectin in the oldest old, this study aimed to determine the status and factors associated with cHMW adiponectin levels in 2,310 adults aged  $\geq 85$  years, including 812 centenarians.



96 **Methods**

97 **Study populations**

98 We used data from four prospective cohort studies of the oldest old in Japan: the TCS and  
99 JSS for centenarians and the TOOTH and KAWP for the very old (aged 85–99 years).  
100 Recruitment was conducted as previously described<sup>17, 23, 24, 25, 26, 27, 28</sup>. From the TCS and JSS,  
101 155 participants were excluded due to a lack of cHMW adiponectin level data; thus, 812  
102 centenarians were enrolled (127 men and 685 women with a median age of 105.3 [interquartile  
103 range (IQR): 100.9–106.8] and 106.0 years [IQR: 103.9–107.2], respectively). The TOOTH and  
104 KAWP surveys are community-based prospective cohort studies of individuals between 85 and  
105 102 years (TOOTH) and 85 and 90 years (KAWP), respectively. Data for 542 (236 men and 306  
106 women) and 1,026 (513 men and 513 women) individual medical examinations are included in  
107 the TOOTH and KAWP studies, respectively. Of these, 63 individuals from the TOOTH study  
108 were excluded because they were older than 90, and 7 individuals from the KAWP study were  
109 excluded due to a lack of cHMW adiponectin level data; thus, 1,498 individuals were enrolled  
110 as the very old (724 men and 774 women with a median age of 86.9 [IQR: 85.9–88.2] and 87.0  
111 years [IQR: 86.0–88.4], respectively, Figure 1–figure supplements 1 and 2, Supplementary File  
112 1).

113 All of the KAWP, TOOTH, TCS, and JSS have been managed by the Center for  
114 Supercentenarian Medical Research, Keio University School of Medicine. Written informed  
115 consent was obtained either from the participant or from a proxy if the participant lacked the  
116 capacity to provide consent. The ethics committee approved all cohort studies of the Keio  
117 University School of Medicine (ID: 20021020, 20022020, 20070047, and 20160297). The  
118 TOOTH and KAWP studies are registered in the University Hospital Medical Information  
119 Network Clinical Trial Registry (ID: UMIN000001842 and UMIN000026053).

120

121 **Baseline examination**

122 All participants were examined by experienced geriatricians at the time of enrolment,  
123 following previously described protocols<sup>17, 23, 24, 25, 26</sup>. Our assessment considered medical  
124 histories, lifestyle factors, and physical and cognitive functions. A mini-mental state  
125 examination (MMSE; 0–30 points) was used to assess cognitive function. The five-item world  
126 health organization well-being index (WHO5; 0–5 points) was used to assess current mental  
127 well-being. Instrumental activities of daily living (IADLs) were assessed using the Lawton scale  
128 (0–5 points) and independent IADL was defined as 5 points on the Lawton scale. The  
129 concentration of blood biomarkers, including cHMW adiponectin, NTproBNP, cystatin C, and  
130 interleukin-6 (IL-6), was measured according to previously described protocols<sup>29</sup>. Blood test  
131 results for HDLC, LDLC, TCHO, TG, choline esterase (CHE), aspartate aminotransferase

(AST),  $\gamma$ -glutamyl transpeptidase ( $\gamma$ GTP), lactate dehydrogenase (LDH), uric acid (UA), albumin (ALB), and HbA1c content were also obtained using previously described protocols<sup>29</sup>. A person with DM was defined as follows: individuals with glycated hemoglobin (HbA1c)  $\geq$  6.5%, those receiving antidiabetic drug therapy, or those receiving insulin injections (Supplementary File 1).

### Measurement of cHMW adiponectin levels

The plasma cHMW adiponectin levels were measured using the Human HMW Adiponectin/Acrp30 Immunoassay Quantikine ELISA Kit (R&D Systems, Inc., Minneapolis, MN, USA) according to manufacture protocol.

### Whole-genome DNA sequencing

Total genomic DNA was extracted from whole blood using a FlexGene DNA Kit (Qiagen, Hilden, Germany). The whole-genome DNA sequence of 440 centenarians was determined using whole-genome DNA sequencing with previously described protocols<sup>30</sup>.

### Genotyping using DNA microarray and imputation

The genotypes of 0.65 M SNVs of 367 centenarians were determined using an Axiom Japonica Array NEO according to the manufacturer's protocol. The genotypes of 0.65 M SNVs of 1,015 individuals in the KAWP study were determined using an Infinium Asian Screening Array-24 v1.0 BeadChip kit according to the manufacturer's protocol. All DNA microarray scan images were analyzed using previously described protocols<sup>30</sup>.

### Meta quantitative trait association analysis for cHMW adiponectin level

To identify cHMW adiponectin level-associated SNVs in the very old and centenarians, we analyzed the association among cHMW adiponectin level and genetic variants using quantitative trait association analysis with the PLINK program (version 1.90) adjusted for sex and age at entry against 440 WGS and DNA microarray-imputed data for 367 centenarians and 1,015 very old, respectively<sup>31</sup>. These quantitative trait association analyses were meta-analyzed using Metal (released on 2020-05-05)<sup>32</sup>. Finally, we obtained meta-quantitative trait association data between 5.75 M SNVs and cHMW adiponectin levels for 1,822 individuals. A Manhattan plot was created using the qqman package (version 0.1.8) in program R<sup>33</sup>. An enlarged view of a Manhattan plot with recombination rate information was generated using LocusZoom (version 1.3)<sup>34</sup>.

### Genotyping and minor allele frequency of rs4783244 (*CDH13*) and rs11711353 (*ADIPOQ*)

168 To determine the rs4783244 and rs11711353 genotypes and minor allele frequency in the  
169 very old and centenarians, we genotyped these SNVs using the TaqMan SNP Genotyping Assay  
170 system according to the manufacturer's protocols.

171 Minor allele frequency of rs4783244 and rs11711353 for Japanese controls (ToMMo  
172 38KJPN) was used in the jMorp database (<https://jmorp.megabank.tohoku.ac.jp>).

173

#### 174 **LASSO and multivariate analysis**

175 For LASSO and further multivariate analysis, cHMW adiponectin level was used as the  
176 outcome, and LASSO was used to evaluated 32 factors by LASSO including age at entry, BMI,  
177 systolic blood pressure (SBP), years of education, smoking history, IADL score, hand grip,  
178 cognitive impairment (MMSE:  $\leq 23$ ), WHO5 score, self-reported disease histories (heart disease,  
179 diabetes, cancers, renal disease, fracture), biomarkers in blood (HDLc, TCHO, LDLc, TG,  
180 CHE, AST, ALT,  $\gamma$ GTP, LDH, UA, ALB, CstC, NTproBNP, HbA1c, IL6), and genetic factors  
181 (sex, *CDH13* rs4783244, *ADIPOQ* rs11711353) for the very old, and 26 factors including age at  
182 entry, BMI, SBP, five educational category, smoking history, activities of daily living (ADL)  
183 score, self-reported disease histories (heart disease, diabetes, cancers, renal disease, fracture),  
184 biomarkers in blood (HDLc, TCHO, LDLc, TG, CHE,  $\gamma$ GTP, UA, ALB, CstC, NTproBNP,  
185 HbA1c, IL6), and genetic factors (sex, *CDH13* rs4783244, *ADIPOQ* rs11711353) for  
186 centenarians (Supplementary File 1). After excluding samples with any missing values in the  
187 selected factors, 1,314 very old and 352 centenarians were selected.

188

#### 189 **Survival analysis**

190 For survival analysis, all-cause, cancer-case, CVD-cause, and pneumonia-cause mortalities  
191 were used as outcome, and BMI, cHMW adiponectin level, disease history (DM), number of  
192 allele for *CDH13* rs4783244 and *ADIPOQ* rs11711353, age at entry, HDLc, and years of  
193 education were used as potential confounder and effect modifiers based on the results of  
194 multivariate analysis. The very old and centenarians were grouped into three quantile cHMW  
195 adiponectin level groups (high, intermediate, and low) against 1,425 very old (678 men and 747  
196 women) and 545 centenarians (90 men and 455 women) for whom survival time information  
197 was available.

198

#### 199 **Statistical analyses**

200 Baseline characteristics, medical history, plasma biomarkers, and genotype data are expressed  
201 as a median or number with a percentage or interquartile range (IQR). The difference in baseline  
202 data was evaluated using Wilcoxon rank-sum, chi-square, and Fisher's Exact tests  
203 (Supplementary File 1). Multivariate logistic regression analyses were performed using a

204 generalized linear model with factors selected by LASSO. All statistical analyses were  
205 performed using program R (version 4.0.3) with exactRankTests (wilcox.exact, Wilcoxon  
206 rank-sum test [version 0.8-31]), glmnet (LASSO and multivariate analyses [version 4.1]),  
207 survival (survival analysis (survfit, coxph, and cox.zph) [version 3.2-13]), powerSurvEpi  
208 (statistic power calculation [ver. 0.1.3]) and default packages.

209

## 210 **Results**

### 211 **Baseline characteristics of the very old and centenarian cohorts**

212 This study used data collected from prospective cohort studies, including the Tokyo Oldest  
213 Old Survey on Total Health (TOOTH) and Kawasaki Aging Wellbeing Project (KAWP) for the  
214 very old (aged 85–89 years) as well as the Tokyo Centenarian Study (TCS) and Japanese  
215 Semi-supercentenarian Study (JSS) for centenarians (aged 100 years and older, Figure 1a)<sup>17, 23,</sup>  
216 <sup>24, 25, 26, 27, 28</sup>. The data for cHMW adiponectin levels were available for 812 centenarians  
217 (woman: 84.4%, 87.7% in Japanese census data in 2020) and 1,498 very old (woman: 51.7%,  
218 64.4% in Japanese census data in 2020, Figure 1–figure supplement 1). Participant  
219 characteristics at enrollment are presented in Supplementary File 1. The flow chart for the  
220 analysis is shown in Figure 1–figure supplement 2.

221 cHMW adiponectin levels increased with age from 30–70 years old and are higher in women  
222 than those in men<sup>19</sup>. Our findings in this study were consistent in that cHMW adiponectin levels  
223 gradually increased with age (Figure 1b; also observed in the longitudinal data of the TOOTH  
224 study, Figure 1–figure supplement 3), with a similar difference observed between sexes of the  
225 very old and centenarians (Figure 1c,d).

226

### 227 **Single nucleotide variations in the promoter regions of *CDH13* and *ADIPOQ* were** 228 **associated with cHMW adiponectin levels in the very old and centenarians**

229 A previous genome-wide association study (GWAS) has revealed that cHMW adiponectin  
230 levels are associated with two major loci, including *CDH13* (also called T-cadherin) and  
231 *ADIPOQ* (gene corresponding to adiponectin), both in European and multi-ethnic cohorts<sup>35</sup>. To  
232 confirm this association in the very old and centenarians, we quantitatively assessed 5.75 M  
233 single nucleotide variants (SNVs) adjusted for age at entry and sex from the genome data for  
234 1,822 individuals, including whole-genome DNA sequences for 440 centenarians, imputed  
235 microarray analysis data for 367 centenarians, and imputed microarray analysis data for 1,015  
236 very old (Figure 2a, Figure 2–figure supplement 1). We found that rs12051213 T>C SNV,  
237 located near exon 1 of *CDH13*, was the locus most significantly associated with cHMW  
238 adiponectin levels ( $p = 2.21 \times 10^{-22}$ , Z score = -9.73), and rs11711353 A>G SNV, located near  
239 exon 1 of *ADIPOQ*, was the second-most significant locus ( $p = 5.72 \times 10^{-7}$ , Z score = 5.00). The

GWAS results also revealed that rs12051213, rs11711353, and other associated SNVs were mainly located around exon 1, indicating that these variants would be associated with the expression of *CDH13* and *ADIPOQ* genes (Figure 2b,c). For the *CDH13* locus, rs4783244 ( $p = 5.39 \times 10^{-22}$ ,  $Z$  score = -9.64) was located near rs12051213, another SNV commonly used as cHMW adiponectin level-associated SNV; therefore, we selected rs4783244 as a representative SNV among *CDH13*-associated SNVs. To confirm the association between these SNVs and cHMW adiponectin levels, we determined the genotype of these two SNVs against the very old and centenarians using a TaqMan assay. As a result, no significant difference in minor allele frequency was found between Japanese control (ToMMo 38KJPN), the very old, and centenarian men and women using Fisher's exact test and multiple testing (Figure 2–figure supplement 2). We compared the genotype-based distribution of cHMW adiponectin levels by genotype (Figure 2d, Figure 2–figure supplement 2). cHMW adiponectin levels were found to vary significantly between the rs4783244 reference allele homozygote and rs4783244 alternative allele heterozygote both in the very old and centenarians. However, except for very old men, no significant difference was observed between the rs4783244 alternative allele heterozygote and rs4783244 alternative allele homozygote in the very old or centenarians. Additionally, cHMW adiponectin levels varied significantly among several allele combinations of rs11711353 in very old or centenarian women but not in very old or centenarian men (Figure 2–figure supplement 3). These data indicated that both major loci (rs4783244 of *CDH13* and rs11711353 of *ADIPOQ*) were associated with cHMW adiponectin levels in the very old and centenarians. However, the effects depended on age and sex.

### **The characteristics of cHMW adiponectin levels in the very old and centenarians**

A previous study reported a negative association between cHMW adiponectin levels and T2DM prevalence and BMI, as well as a positive association with insulin sensitivity index, triglyceride (TG) content, and high-density lipoprotein cholesterol (HDL) levels<sup>9, 19</sup>. To evaluate these associations in the oldest old, we analyzed the association between cHMW adiponectin levels and diabetes mellitus (DM), HDL, and BMI (Figure 3). A person with DM was defined as follows: individuals with glycated hemoglobin (HbA1c)  $\geq 6.5\%$ , those receiving antidiabetic drug therapy, or those receiving insulin injections (Figure 3a,b). We found that cHMW adiponectin levels in the DM group were significantly lower than those in the non-DM group in both the very old and centenarians, indicating that adiponectin is associated with the DM pathway, regardless of age.

Although blood-lipid contents, including total cholesterol (TCHO), HDL, low-density lipoprotein cholesterol (LDL), and TG gradually decreased with age from the very old to centenarians (Supplementary File 1), a positive association was observed between cHMW

adiponectin and HDLC levels (Figure 3c,d). A negative association between cHMW adiponectin levels and BMI was observed in the very old, though this association was less prominent in centenarians (Figure 3e,f). These findings suggested that the physiological factors associated with adiponectin may vary from the very old to centenarians.

## **The factors associated with cHMW adiponectin levels vary between the very old and centenarians**

In our multivariate regression analysis of cHMW adiponectin levels, we initially selected 32 factors for the very old, including cHMW adiponectin level-associated genetic factors (genotypes of rs4783244 in *CDH13* and rs11711353 in *ADIPOQ*), and 26 factors for centenarians based on a previous report<sup>19,20</sup>. To reduce the effects of multicollinearity, we used a Least Absolute Shrinkage and Selection Operator (LASSO) method with five-fold cross-validation and identified 19 factors for the very old and 7 factors for centenarians (Figure 4-figure supplement 1). According to the multivariate regression analysis for the very old, 14 significant factors for men and 10 significant factors for women were identified (Figure 4a, Supplementary Files 2–4); among centenarians, three significant factors for men and four significant factors for women were identified (Figure 4b, Supplementary Files 5–7). An analysis of deviance revealed that the total variance of known cHMW adiponectin level-associated factors was 36.8–42.0% in the very old and centenarian men and 18.4% in centenarian women (Figure 4c,d, Supplementary Files 8–11). These results suggest that the genotypes of rs4783244 in *CDH13*, HDLC, BMI, and lipid metabolism-associated factors, including HDLC and TG, are major factors associated with cHMW adiponectin levels in both sexes of the very old. Furthermore, the genotypes of rs4783244 in *CDH13* and HDLC were also associated with cHMW adiponectin levels in centenarians. Significantly, the current known factors associated with cHMW adiponectin levels were expected to correspond to 18.4% of the total variance in centenarian women, indicating a reduced contribution of known factors associated with cHMW adiponectin levels in centenarians. Thus, major cHMW adiponectin-associated factors found in the very old would not be responsible for the age-dependent increment of cHMW adiponectin levels.

## **Higher cHMW adiponectin levels in very old men was positively associated with high all-cause mortality rates, independent of BMI**

High cHMW adiponectin levels are associated with increased all-cause mortality and CVD risk in adults in their 60s and 70s<sup>12,13,14,15</sup>. To evaluate the effects of cHMW adiponectin levels on mortality in the very old and centenarians, hazard ratios of all-cause mortality were analyzed using Cox promotional hazards models for three quantiles of cHMW adiponectin levels (i.e.,

high, intermediate, and low) in 1,425 very old (678 men and 747 women) and 545 centenarians (90 men and 455 women) for whom both survival time information and a number of covariates were available. Prior to the analysis, the availability of sufficient samples and events for all-cause mortality were ensured for the survival analysis of the very old and centenarian women and there was no significant difference in the proportional hazards assumption of the cHMW adiponectin level and each of the covariates (Figure 5-figure supplements 1 and 2). However, the statistical power analysis indicated that there were not sufficient events, and samples were ensured for the centenarian men even if they were divided into two groups. Within the follow-up periods, 145 (21.3%) men and 101 (13.5%) women died in the very old, whereas 89 (98.9%) men and 542 (99.4%) women died in the centenarians (Figure 5 and Supplementary File 1). As a result, the hazard ratios of all-cause mortality for intermediate and high levels of cHMW adiponectin groups in very old men were significantly higher (HR: 1.67 and 2.32) rather than those of the all-cause mortality of the low cHMW adiponectin level group (reference), even after adjustment for BMI (HR: 1.60 and 2.12). In contrast, the hazard ratio for all-cause mortality was significantly higher for the high cHMW adiponectin levels group in very old women was significantly higher (HR: 1.89), but was not significant after adjustment for BMI (HR: 1.41, (Figure 5)). This trend was also observed in the centenarian women.

To further elucidate the factors associated with mortality, we also analyzed cause-specific mortality associated with cancer, CVD, and pneumonia in the very old (Figure 5-figure supplements 3-5). The total number of events for each cause-specific mortality was 59 (cancer), 53 (CVD), and 40 (pneumonia), indicating that the analysis lacked sufficient statistical power. Testing populations with a 5% difference in event frequency would require approximately 440 samples for each group.

## Discussion

The results of this study showed that cHMW adiponectin levels increased with age up to centenarians, although the associated factors varied with sex. Therefore, we are further elucidating whether the increment of cHMW adiponectin level with age extends into very old and exceptionally old age. Meta-GWAS with cHMW adiponectin levels revealed that the SNVs of two loci containing the promoter regions of *CDH13* and *ADIPOQ* genes were associated with cHMW adiponectin levels. The levels of HDLC were associated with those of cHMW adiponectin both in the very old and centenarians, though the association with BMI was relatively weaker in centenarians. The multivariate regression analysis with factor selection using the LASSO method revealed that genetic variants, BMI, and lipids were major factors associated with cHMW adiponectin level in the very old; here, BMI was not selected as an associated factor in centenarians. The analysis of deviance revealed that the contribution of

known factors to cHMW adiponectin levels decreased in centenarian women, suggesting that the major factors in the very old would not be responsible for the age-dependent increase in cHMW adiponectin levels. The high cHMW adiponectin levels in very old men were associated with all-cause mortality independently of BMI; however, no association was observed between the cHMW adiponectin levels and all-cause mortality in very old and centenarian women. Therefore, the contribution of known major factors associated with cHMW adiponectin levels, including BMI and lipid content, varies with age, suggesting that its physiological significance also varies with age in the oldest old.

The salutary effects of adiponectin on glucose homeostasis, insulin sensitivity, and chronic low-grade inflammation, and the inverse association between the incidence of T2DM and cHMW adiponectin levels are known<sup>36, 37, 38</sup>. We have previously reported that a low incidence of T2DM is a characteristic of centenarians; therefore, we deduced that the high cHMW adiponectin levels in centenarians would be partially influenced by a low incidence of T2DM. In the present study, the T2DM group showed significantly lower levels of cHMW adiponectin, regardless of the cohort, suggesting the physiological significance of cHMW adiponectin levels in the context of insulin sensitivity and T2DM incidence is consistent across ages.

We revealed that very old men with high cHMW adiponectin levels show high rates of all-cause mortality, consistent with previous reports for adults in their 60s and 70s<sup>12, 13, 14, 15</sup>. Moreover, cHMW adiponectin levels were associated with all-cause mortality independently of BMI. Excess weight loss can cause frailty in the oldest old, exacerbating mortality rates and death due to pneumonia<sup>39</sup>. Based on these results, we deduced that a combination of high cHMW adiponectin levels and low BMI may exert synergistic effects in the mortality among very old men. We also revealed that high cHMW adiponectin levels were not associated with mortality both in very old and centenarian women. Surprisingly, strength of the association between BMI and cHMW adiponectin level decreased in centenarians. Although the major factors associated with cHMW adiponectin level in centenarians were unknown, these results suggest that the factors associated with cHMW adiponectin levels vary with age, which would also alter the physiological significance of cHMW adiponectin level as it relates to mortality.

Frailty is an important concept in health maintenance and the process of functional decline in the oldest old. Recently, plasma adiponectin levels have been positively associated with frailty in the oldest old<sup>40, 41</sup>. In our cohort, most centenarians were classified as frail according to the current frailty criteria, so it is difficult to assess frailty in centenarians. For the very old, only the KAWP, one of the cohorts that included the very old, collected sufficient data to assess frailty. Using these limited data for the very old, we analyzed the distribution of cHMW adiponectin levels in each frailty category and analyzed their association with the revised J-CHS frailty index criteria using multiple regression analysis<sup>42</sup>. As a result, we found that cHMW



adiponectin levels were significantly associated with frailty, both in very old men and women (Figure 5-figure supplement 6). The cHMW adiponectin level was also significantly associated with frailty in very old women even after adjustment for BMI; however, no significant association was observed in very old men after adjustment by BMI. Thus, cHMW adiponectin levels would be associated with frailty in the very old, especially in women.

Although cHMW adiponectin levels increased with age, their association with BMI was comparatively lower in centenarians than that in the very old. This raises the question of which cells are responsible for the increased expression of adiponectin with aging. One hypothesis is that the clearance mechanism of adiponectin from the blood may be impaired by reduced kidney function, resulting in an accumulation of cHMW adiponectin. However, we did not observe a significant association between the levels of cHMW adiponectin and plasma cystatin C, one of the kidney function markers. Another hypothesis is that aging would cause ectopic *ADIPOQ* gene expression, increasing cHMW adiponectin levels. Re-analysis of *in silico* mouse single-cell transcriptomic data revealed that a small number of cells derived from subcutaneous adipose tissue expressed high levels of *ADIPOQ*, including brown, gonadal, mesenteric, and subcutaneous adipose tissues (Figure 5-figure supplement 7)<sup>43</sup>. Furthermore, a re-analysis of mouse whole-body single-cell transcriptomic data from 24 tissues during 1–30 months of age revealed that *ADIPOQ* mRNA was rarely expressed in tissues other than the fat tissue, even at advanced ages of 24, 27, and 30 months (Figure 5-figure supplement 8)<sup>43</sup>. These findings indicate that no universal mechanism between humans and mice would exist to induce cHMW adiponectin through ectopic expression of the *ADIPOQ* gene by aging.

The study had the following limitations: 1) Surveys of centenarian surveys tend to have many missing values due to their limited physical and cognitive function; therefore, multivariate analysis using a series of covariates tends to reduce the number of samples to be analyzed. 2) Although the short survival time of centenarian in this showed no association between cHMW adiponectin level and all-cause mortality in this study, strong factors associated with survival, such as N-terminal pro-brain natriuretic peptide (NTproBNP) and albumin (ALB), tend to be detectable, while weaker factors are more difficult to detect. 3) Cox regression for all-cause mortality in centenarian men and cause-specific mortality in the very old men was statistically underpowered due to the insufficient size of samples and/or events. CVD mortality in very old men showed a trend to be associated with cHMW adiponectin levels, but statistically, twice the number of events or twice the number of total samples are needed to assess this. 4) Analysis of cHMW adiponectin levels and frailty in centenarians is difficult because most centenarians would be classified as frail according to the current frailty criteria. Of the two cohort studies of very old participants, the TOOTH study did not have sufficient data adjusted for the evaluation of J-CHS frailty criteria. Therefore, the association between cHMW adiponectin levels and

420 frailty was analyzed in selected samples derived only from the KAWP study only. This was only  
421 a cross-sectional analysis, and further analysis would be needed to prove causality. Therefore,  
422 these are described only in the Discussion section.

423 In this study, we verified the association among cHMW adiponectin level, BMI, and all-cause  
424 mortality in the very old and centenarians. Due to changes in the physiological significance of  
425 BMI between young and old ages, the appropriate BMI value is expected to vary with age.  
426 While a low BMI is recommended at a young age due to the risk of diabetes and metabolic  
427 syndrome, a high (though not excessively high) BMI is recommended at a later stage of life to  
428 decrease the risk of frailty and mortality. Therefore, the biological significance of cHMW  
429 adiponectin levels would be also changed depending on the biological significance of BMI in  
430 the aging process. The reasons for the high cHMW adiponectin levels and loss of association  
431 with BMI in centenarians remain unknown; however, future research should focus on  
432 identifying cells that expressing adiponectin, which should clarify its physiological significance  
433 in the oldest old.

434

435

#### 436 **Data availability**

437 The cHMW adiponectin levels and covariates data were deposited with this manuscript as  
438 source data files. The data with age for the very old and centenarians have ethical and legal  
439 restrictions to public deposition due to avoid personal identification, and will be available upon  
440 request with an appropriate research arrangement with approval of the Research Ethics  
441 Committee of Keio University School of Medicine for Clinical Research. To request, please  
442 contact Takashi Sasaki (corresponding author) via e-mail: sasasa@z5.keio.jp.

443

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591 **Figure legends**

592 **Figure 1 | Analysis workflow and distribution of circulating high-molecular-weight**  
593 **(cHMW) adiponectin levels in the very old and centenarians**

594 (a), Sample summary and analysis workflow of cHMW adiponectin levels. (b), Distribution of  
595 cHMW adiponectin levels in older adults and centenarians. cHMW adiponectin levels gradually  
596 increased with age in the very old to centenarians. (c), Distribution of cHMW adiponectin levels  
597 in older men and women. (d), Distribution of cHMW adiponectin levels in centenarian men and  
598 women. The difference in cHMW adiponectin levels was significant between sexes in both the  
599 very old and centenarians.

600

601 **Figure 2 | Meta- genome wide association study (GWAS) for cHMW adiponectin levels in**  
602 **the very old and centenarians**

603 (a), Meta-GWAS analysis for cHMW adiponectin levels in the very old and centenarians.  
604 Number of samples for the very old and centenarian were 1,015 and 807, respectively. Loci for  
605 *CDH13* (rs12051213, C: reference allele, T: alternative allele,  $p = 2.45 \times 10^{-22}$ ) and *ADIPOQ*  
606 (rs11711353, G: reference allele, A: alternative allele,  $p = 6.68 \times 10^{-7}$ ) were detected using  
607 meta-GWAS for cHMW adiponectin levels in older adults and centenarians. (b), A GWAS  
608 enlarged view of the *CDH13* region. (c), A GWAS enlarged view of the *ADIPOQ* region. (d),  
609 Distribution of cHMW adiponectin levels in rs4783244 (*CDH13*) genotypes of the very old and  
610 centenarians. cHMW adiponectin levels varied significantly between the rs4783244 reference  
611 allele homozygote and rs4783244 alternative allele heterozygote in the very old and  
612 centenarians. Except in very old men, no significant difference was observed between the  
613 rs4783244 alternative allele heterozygote and rs4783244 alternative allele homozygote in the  
614 very old or centenarians.

615

616 **Figure 3 | Association between cHMW adiponectin level, high-density lipoprotein**  
617 **cholesterol (HDLc), body mass index (BMI), and glycated hemoglobin (HbA1c)**

618 (a, b), Distribution of cHMW adiponectin levels in the diabetes mellitus (DM) and non-DM  
619 groups. A person with DM was defined as follows: individuals with glycated hemoglobin  
620 ( $\text{HbA1c} \geq 6.5\%$ ), those receiving antidiabetic drug therapy, or those receiving insulin injections.  
621 cHMW adiponectin levels in the DM group were significantly lower than those in the non-DM  
622 group in the very old and centenarians. (c, d), Association between cHMW adiponectin levels  
623 and HDLC content. A positive association was observed between cHMW adiponectin levels  
624 and HDLC content in the very old and centenarians. (e, f), Association between cHMW  
625 adiponectin levels and BMI. A strong negative association was observed between cHMW  
626 adiponectin levels and BMI in the very old, though this association was rarely observed in



centenarians.

**Figure 4 | Multivariate analysis for cHMW adiponectin levels in the very old and centenarians**

(a), Multivariate analysis for cHMW adiponectin levels in very old men and women; 14 significant factors for very old men and 10 significant factors for very old women were identified. (b), Multivariate analysis for cHMW adiponectin levels in centenarian men and women; 3 significant factors for centenarian men and 4 significant factors for centenarian women were identified. (c), The contribution rate for each factor in very old men and women was estimated by analysis of variance. (d), The contribution rate for each factor in centenarian men and women was estimated using analysis of variance. The total variance of known cHMW adiponectin level associated factors corresponded to 36.8–42.0% in very old and centenarian men and 18.4% in centenarian women.

**Figure 5 | Survival analysis using Cox promotional hazards model for three quantile cHMW adiponectin level groups**

(a, b), Survival analysis of very old men and women using the Cox promotional hazards model for three quantile cHMW adiponectin level groups. Seven covariates (model1) and seven covariates with BMI were used for calculating the multiple regression analysis of Cox promotional hazards model. Hazard ratio for low concentration adiponectin group was calculated as the reference. The statistics power analysis using powerSurvEpi (ver. 0.1.3) indicated that survival analyses for both very old men and women have sufficient number of samples and events. (c, d), Survival analysis of the centenarian men and women using Cox promotional hazards model for three quantile cHMW adiponectin level groups. Three covariates (model2) and three covariates with BMI were used for calculation of multiple regression analysis of Cox promotional hazards model. Hazard ratio for low concentration adiponectin group was calculated as the reference. The statistics power analysis using powerSurvEpi (ver. 0.1.3) indicated that survival analysis for centenarian women has sufficient number of samples and events, however, survival analysis for centenarian men was underpowered due to insufficient number of events.

659 **Figure 1-figure supplement 1 | Description of the cohorts in this study**

660 (a) Description of the cohorts for centenarian studies. (b) Description of the cohorts for the very old  
661 studies.

662

663 **Figure 1-figure supplement 2 | Flow chart for analysis in this study**

664 The numbers on the flowchart indicate the number of samples used in each analysis.

665

666 **Figure 1-figure supplement 3 | Transition of cHMW adiponectin level in the longitudinal data  
667 of TOOTH study**

668 (a) Transition of cHMW adiponectin level at baseline and 3-year follow-up studies. (b), Difference  
669 of cHMW adiponectin level between baseline and 3-year follow-up studies. The cHMW adiponectin  
670 level is gradually increasing during very old.

671

672 **Figure 2-figure supplement 1 | GWAS for cHMW adiponectin level**

673 (a) GWAS for cHMW adiponectin level in centenarians (n=440) determined by whole genome  
674 sequencing (WGS). (b) GWAS for cHMW adiponectin level in centenarians (n=367)  
675 determined by genotyping by DNA microarray (Japonica V3 array) and DNA sequence imputation.  
676 (c) GWAS result for cHMW adiponectin level in the very old (n=1,015) determined by genotyping  
677 using DNA microarray (Asian screening array, Illumina) and DNA sequence imputation.

678

679 **Figure 2-figure supplement 2 | Minor allele frequency comparison of rs4783244 (CDH13) and  
680 rs11711353 (ADIPOQ)**

681 Allele frequency differences were statistically tested using the Fisher Exact test (fisher.test in R  
682 stats package (version4.2.2)). (a) rs4783244 men (CDH13). (b) rs4783244 women (CDH13). (c)  
683 rs11711353 men (ADIPOQ). (d) rs11711353 women (ADIPOQ).

684

685 **Figure 2-figure supplement 3 | Distribution of cHMW adiponectin level in rs11711353  
686 (ADIPOQ) genotypes of the very old and centenarians**

687

688 **Figure 4-figure supplement 1 | LASSO with five-fold cross-validation against 1,326 very old  
689 and 352 centenarians**

690 (a) LASSO with five-fold validation analysis against 1,326 very old. As a result of five-fold  
691 cross-validation, 19 factors shown in black in the bottom column were selected for further  
692 multivariate regression analysis. (b) LASSO with five-fold validation analysis against 352  
693 centenarians. As a result of five-fold cross-validation, 7 factors shown in black in the bottom column  
694 were selected for further multivariate regression analysis.

695

696 **Figure 5-figure supplement 1 | The proportional hazards assumption test for a Cox regression**  
697 **model fit**

698 (a) Very old men. (b) Very old women. For calculation of proportional hazards assumption test,  
699 cox.zph in survival package [version 3.2-13] was used.

700

701 **Figure 5-figure supplement 2 | The proportional hazards assumption test for a Cox regression**  
702 **model fit**

703 (a) Centenarian men. (b) Centenarian women. For calculation of proportional hazards assumption  
704 test, cox.zph in survival package [version 3.2-13] was used.

705

706 **Figure 5-figure supplement 3 | Survival time analysis for cancer-cause mortality against the**  
707 **three quantile groups of cHMW adiponectin levels in very old men and women**

708 Survival analysis for cancer-cause mortality of very old men and women grouped by cHMW  
709 adiponectin level by cox regression analysis. The statistics power analysis by powerSurvEpi (ver.  
710 0.1.3) indicated that this survival analysis was underpowered due to insufficient number of events.

711

712 **Figure 5-figure supplement 4 | Survival time analysis for cardiovascular disease-cause**  
713 **mortality against the three quantile groups of cHMW adiponectin levels in very old men and**  
714 **women**

715 Survival analysis for cardiovascular disease-cause mortality of very old men and women grouped by  
716 cHMW adiponectin level by cox regression analysis. The statistics power analysis by powerSurvEpi  
717 (ver. 0.1.3) indicated that this survival analysis was underpowered due to insufficient number of  
718 events.

719

720 **Figure 5-figure supplement 5 | Survival time analysis for pneumonia-cause mortality against**  
721 **the three quantile groups of cHMW adiponectin levels in very old men and women**

722 Survival analysis for pneumonia-cause mortality of very old men and women grouped by cHMW  
723 adiponectin level by cox regression analysis. The statistics power analysis by powerSurvEpi (ver.  
724 0.1.3) indicated that this survival analysis was underpowered due to insufficient number of events.

725

726 **Figure 5-figure supplement 6 | J-CHS frailty index distribution against the three quantile**  
727 **groups of cHMW adiponectin levels and multiple regression analysis in very old men and**  
728 **women (KAWP)**

729 (a) J-CHS frailty index distribution against the three quantile groups of cHMW adiponectin levels in  
730 very old men and women. (b) Multiple regression analysis between cHMW adiponectin level and  
731 J-CHS frailty index.

732

733 **Figure 5-figure supplement 7 | Adiponectin mRNA expression analysis of single-cell RNA-seq**  
734 **for four kinds of mouse adipose tissue**

735 We re-analyzed single-cell RNA-seq results ([https://tabula-muris-senis.ds.czbiohub.org](https://tabula-muris-senis.ds.czbiohub.org/fat/droplet/)  
736 [/fat/droplet/](https://tabula-muris-senis.ds.czbiohub.org/fat/droplet/)) for four kinds of mouse adipose tissues including brown adipose tissue (Bat), gonadal  
737 adipose tissue (Gat), mesenteric adipose tissue (Mat), and subcutaneous adipose tissue (Scat). Dots  
738 with the blue color indicated adiponectin-expressed cells. These single-cell RNA-seq results  
739 suggested that Scat is one of the major adiponectin-expressed cells in adipose tissues.

740

741 **Figure 5-figure supplement 8 | Ectopic expression of adiponectin with aging in mouse**

742 We re-analyzed single-cell RNA-seq results of 24 kinds of tissue in 1, 3, 18, 21, 24, 30 months  
743 (<https://tabula-muris-senis.ds.czbiohub.org/fat/droplet/>). Major adiponectin-expressed cells were  
744 adipose tissue and no obvious ectopic adiponectin expression was observed in the time series of  
745 mouse cells.

746

747 **Supplementary File 1 | Participants' characteristics at enrollment**  
748 1: Wilcoxon ranking test, 2: Fisher's exact test, 3: Chi-square test. Abbreviations: IQR, inter-quartile  
749 range; BMI, body mass index; SBP, systolic blood pressure; IADL, instrumental activities of daily  
750 living; ADL, activities of daily living; MMSE, mini mental state examination; HMW, high molecular  
751 weight; HDLC, high density lipoprotein cholesterol; LDLC, low density lipoprotein cholesterol;  
752 TCHO, total cholesterol; TG, triglyceride; CHE, choline esterase; AST, aspartate aminotransferase;  
753  $\gamma$ GTP, g-glutamyl transpeptidase; LDH, lactate dehydrogenase; UA, uric acid; ALB, albumin; Alt,  
754 alternative; MAF, minor allele frequency.  
755

756 **Supplementary File 2 | Coefficients for generalized linear model analysis of plasma HMW**  
757 **adiponectin level in very old men (n=643)**  
758

759 **Supplementary File 3 | Coefficients for generalized linear model analysis of plasma HMW**  
760 **adiponectin level in very old women (n=683)**  
761

762 **Supplementary File 4 | Coefficients for generalized linear model analysis of plasma HMW**  
763 **adiponectin level in the very old (n=1,326)**  
764

765 **Supplementary File 5 | Coefficients for generalized linear model analysis of plasma HMW**  
766 **adiponectin level in centenarian men (n=63)**  
767

768 **Supplementary File 6 | Coefficients for generalized linear model analysis of plasma HMW**  
769 **adiponectin level in centenarian women (n=289)**  
770

771 **Supplementary File 7 | Coefficients for generalized linear model analysis of plasma HMW**  
772 **adiponectin level in centenarian (n=352)**  
773

774 **Supplementary File 8 | Analysis of variance of plasma HMW adiponectin level by ANOVA in**  
775 **very old men (n=643)**  
776

777 **Supplementary File 9 | Analysis of variance of plasma HMW adiponectin level by ANOVA in**  
778 **very old women (n=683)**  
779

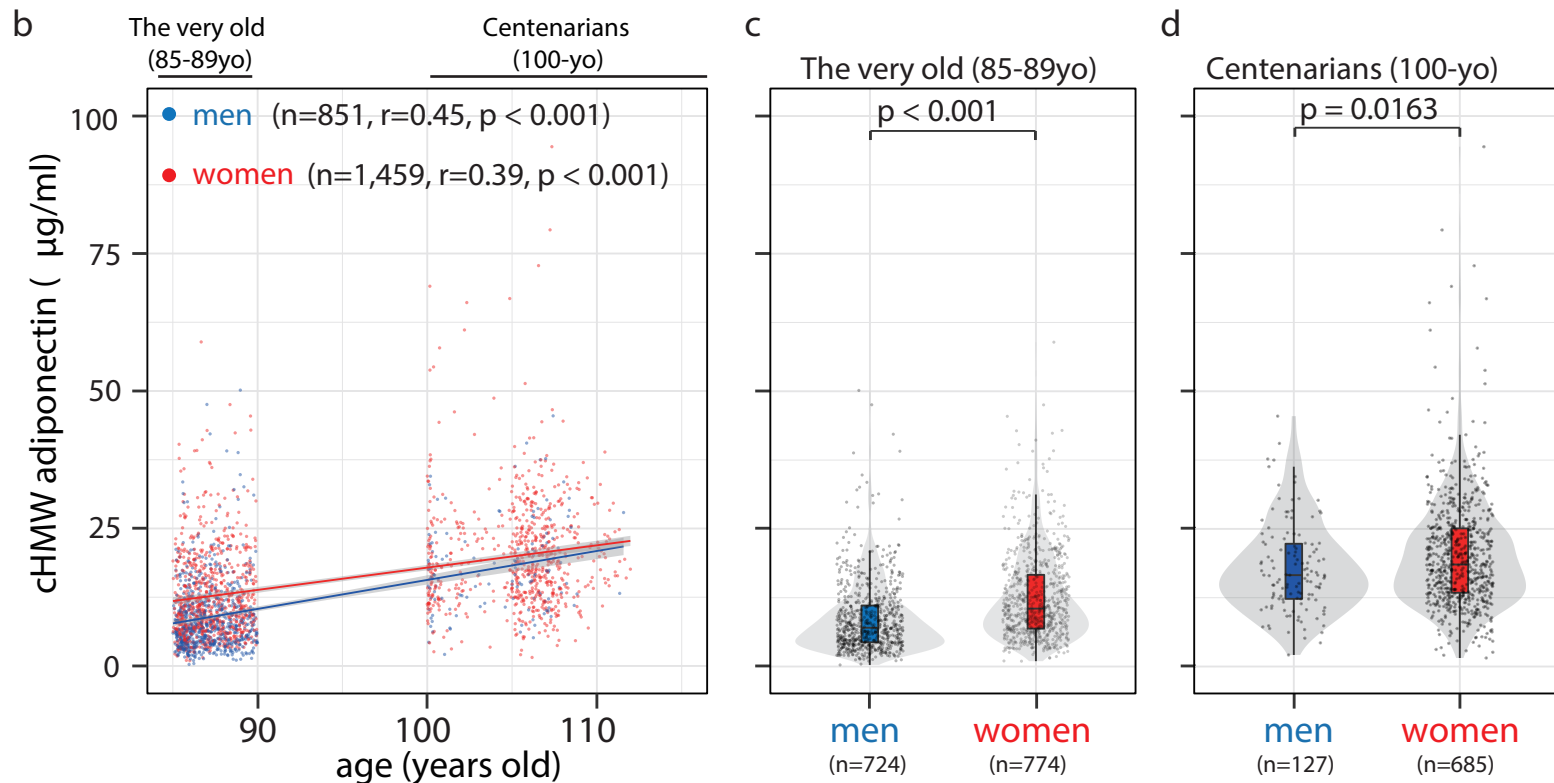
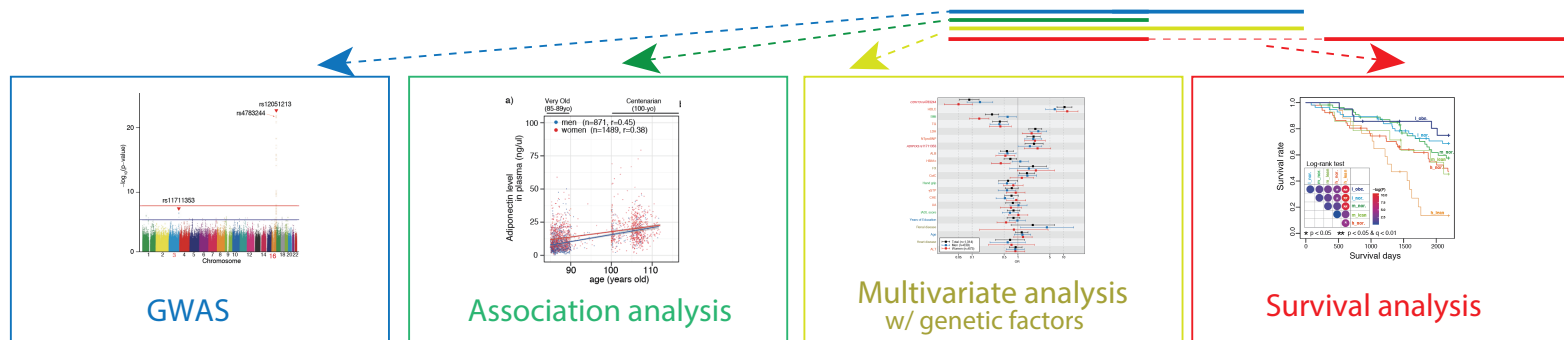
780 **Supplementary File 10 | Analysis of variance of plasma HMW adiponectin level by ANOVA in**  
781 **centenarian men (n=63)**  
782

783 **Supplementary File 11 | Analysis of variance of plasma HMW adiponectin level by ANOVA in**  
784 **centenarian women (n=289)**  
785

786     **Figure 1 source data1**  
787     Source data for figure 1 including 812 centenarians and 1,498 very old data.  
788  
789     **Figure 2 source data1**  
790     Source data for figure 1 including 812 centenarians and 1,498 very old data.  
791  
792     **Figure 3 source data1**  
793     Source data for figure 1 including 812 centenarians and 1,498 very old data.  
794  
795     **Source Code File 1**  
796     R script code file for Figure 1c.  
797  
798     **Source Code File 2**  
799     R script code file for Figure 1d.  
800  
801     **Source Code File 3**  
802     R script code file for Figure 2d.  
803  
804     **Source Code File 4**  
805     R script code file for Figures 3ab.  
806  
807     **Source Code File 5**  
808     R script code file for Figures 3c-f.  
809

a

Group	Cohort	Age at entry	n (women%)	data		
				Survey	Genetic	Survival
Centenarians n = 812		100- yo	812 (84.4%)	Baseline	6M SNVs	~ 10 years later
The very old n = 1498	KAWP	85-89 yo	1019 (49.7%)	Baseline	6M SNVs	~ 3.5 years later
	TOOTH	85-89 yo	479 (55.7%)	Baseline 3 years later	2 SNVs	~ 6 years later

Adiponectin  
AnalysisFigure 1  
Sasaki et al.



a

## Centenarians (n=967)

Study: Tokyo Centenarian Study (TCS) ref: Gondo Y. *et al* (2006)  
 Target age:  $\geq 100$ yo.  
 Entry period: Sept. 2000 ~ Oct 2003  
 Follow up period: ~ July 2018  
 Number of Entry: 238

Study: Japan Semi-supercentenarian Study (JSS) ref:  
 Target age:  $\geq 105$ yo. ref: Arai Y. *et al* (2014)  
 Entry period: Sept. 2002 ~ March 2019  
 Follow up period: ~ March. 2019  
 Number of Entry: 729



no cHMW Adiponectin data: 155

**812** (127 men (median age: 105.3 yo [IQR: 100.9–106.8]),  
 685 women (median age: 106.0 yo [IQR: 103.9–107.2]))

ref:

Gondo Y, *et al*. Functional status of centenarians in Tokyo, Japan: developing better phenotypes of exceptional longevity. *J Gerontol A Biol Sci Med Sci* **61**, 305-310 (2006).

Arai Y, *et al*. Physical independence and mortality at the extreme limit of life span: supercentenarians study in Japan. *J Gerontol A Biol Sci Med Sci* **69**, 486-494 (2014).

Arai Y, *et al*. The Tokyo Oldest Old survey on Total Health (TOOTH): a longitudinal cohort study of multidimensional components of health and well-being. *BMC Geriatr* **10**, 35 (2010).

Arai Y, *et al*. Behavioral changes and hygiene practices of older adults in Japan during the first wave of COVID-19 emergency. *BMC Geriatr* **21**, 137 (2021).

b

## The very old (n=1,568)

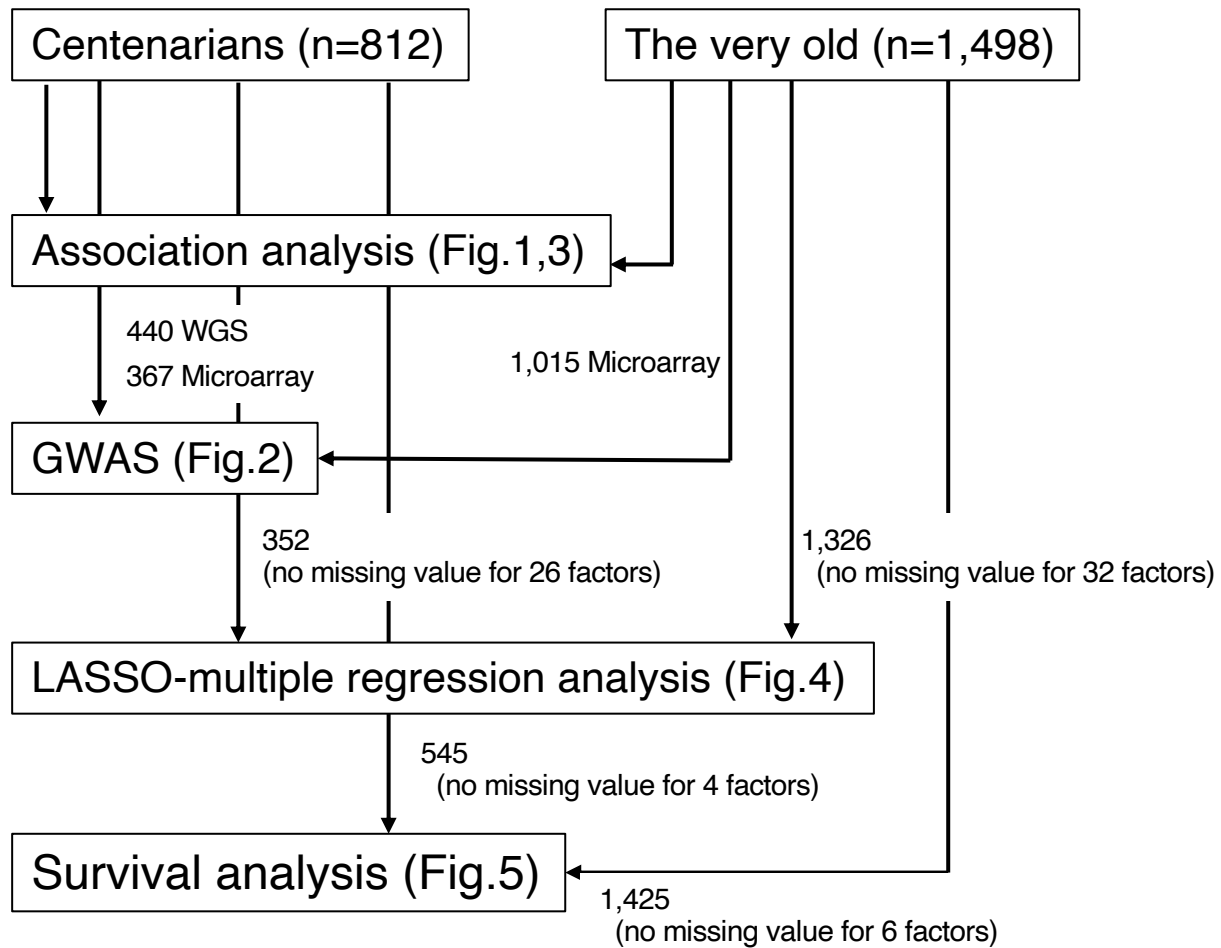
Study: The Tokyo Oldest Old Survey on Total Health (TOOTH)  
 Target age:  $\geq 85$ yo. ref: Arai Y *et al*. (2010)  
 Entry period: March 2008 ~ Dec. 2009  
 Follow up period: ~ Jan. 2016  
 Number of Entry: 542

Study: Kawasaki Aging and Wellbeing Project (KAWP)  
 Target age: 85-89yo. ref: Arai Y *et al*. (2021)  
 Entry period: March, 2017 ~ Dec. 2019  
 Follow up period: ~ Sep 2022  
 Number of Entry: 1026

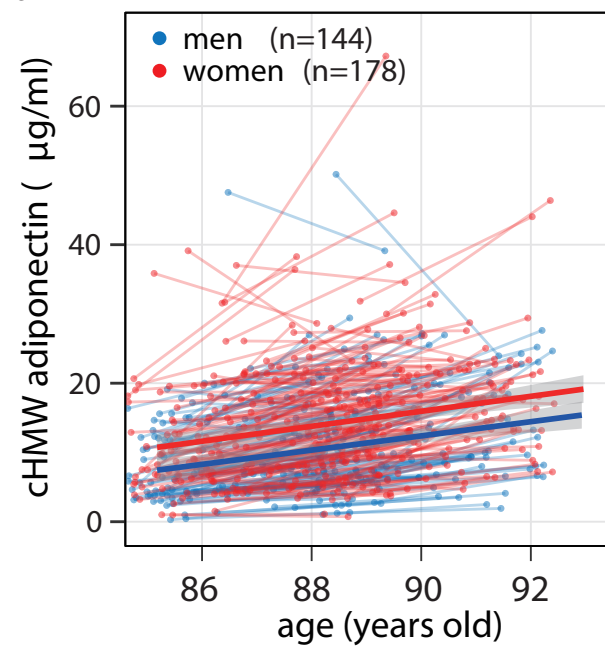


no cHMW Adiponectin data: 7  
 out of age ( $\geq 90$  yo): 63

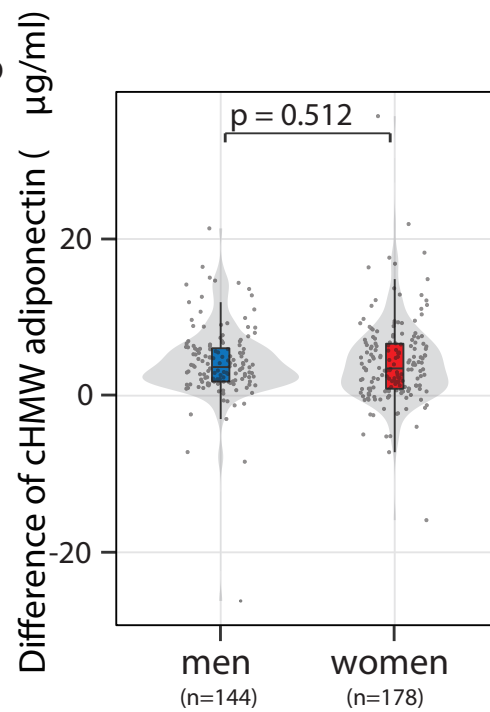
**1,498** (724 men (median age: 86.9 [IQR: 85.9–88.2]),  
 774 women (median age: 87.0 years [IQR: 86.0–88.4]))



a



b



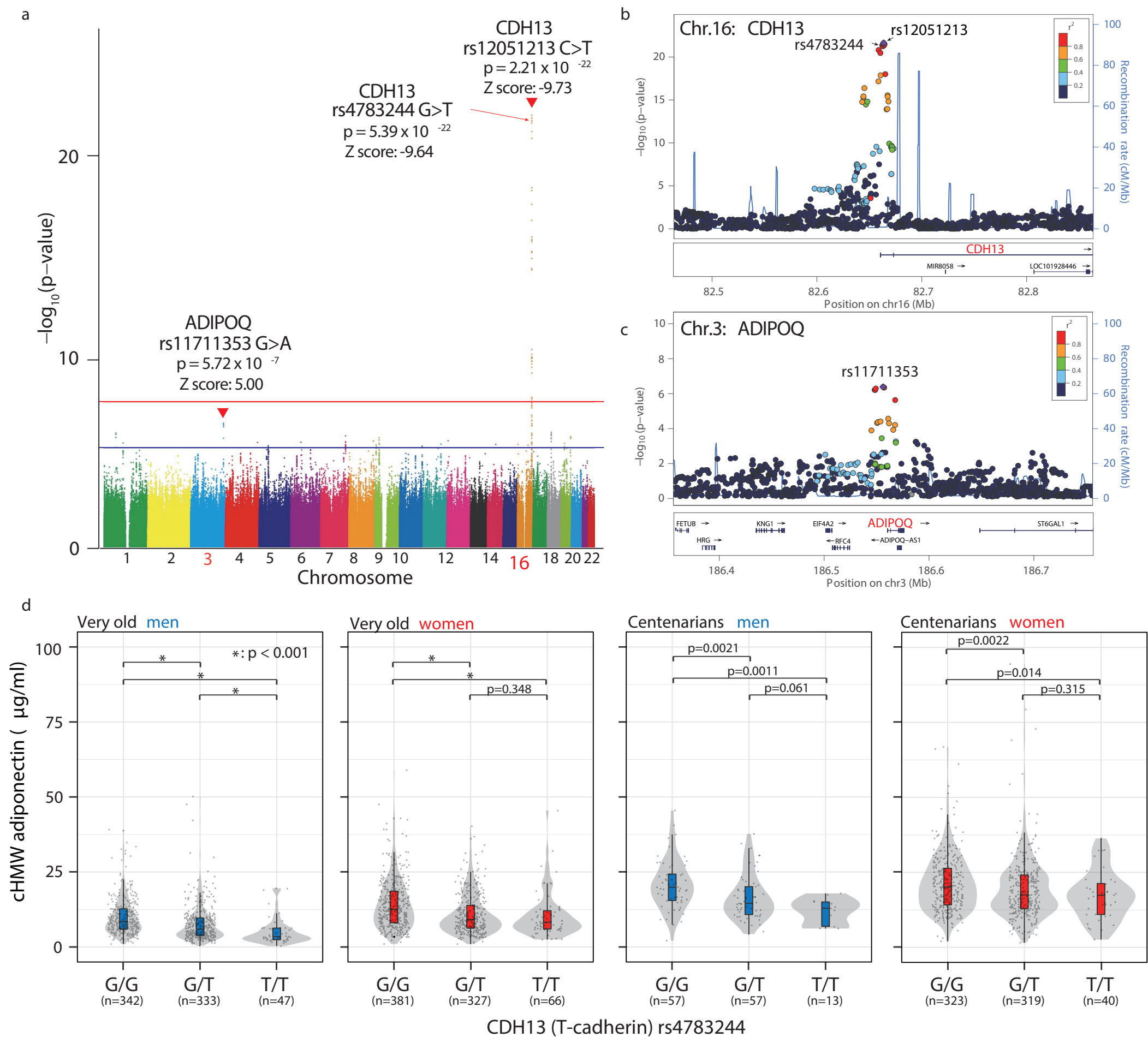
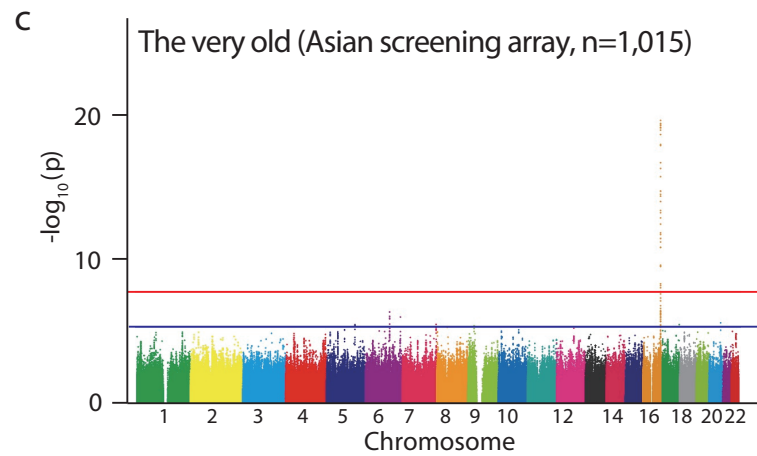
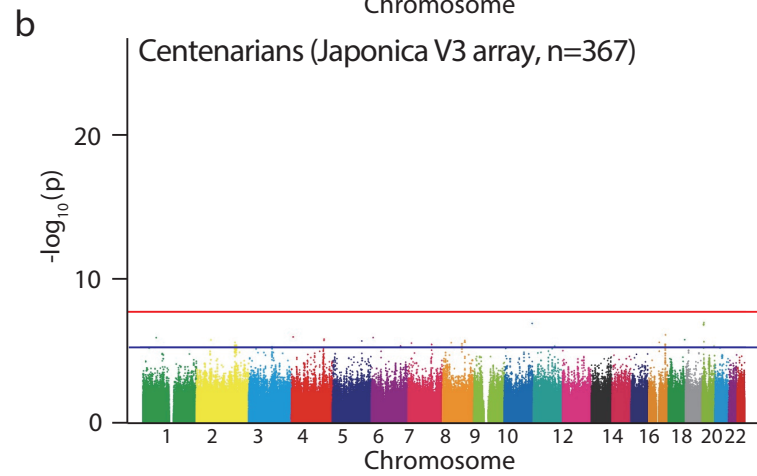
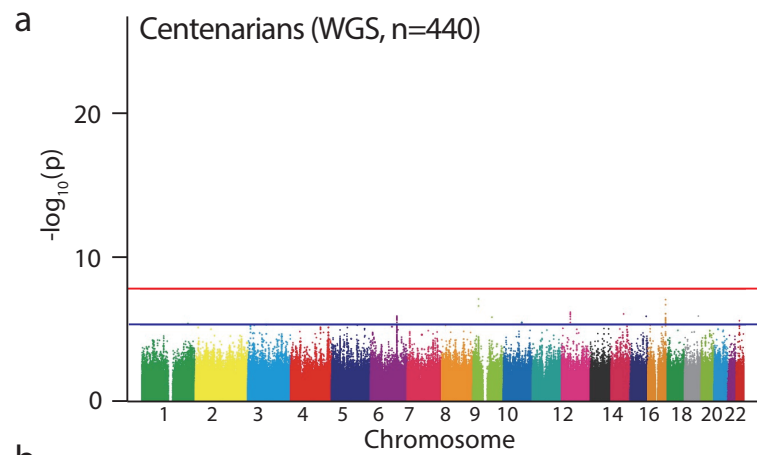
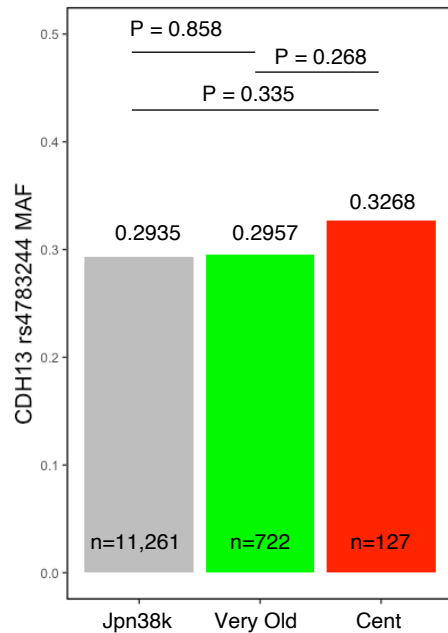


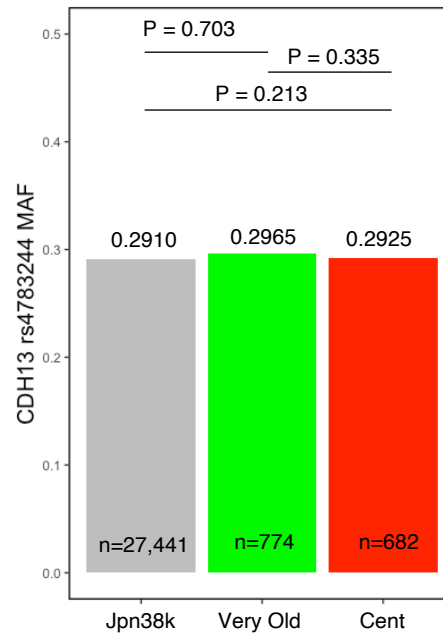
Fig. 2 Sasaki et al.



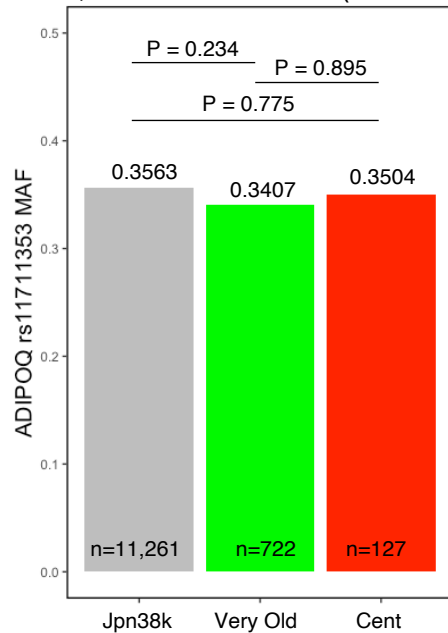
a, rs4783244 men (CDH13)



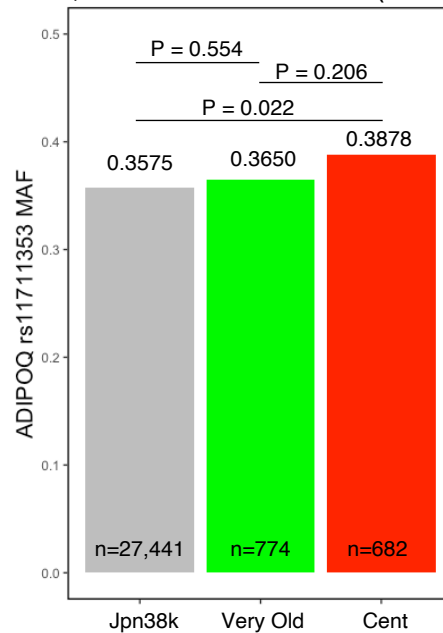
b, rs4783244 women (CDH13)

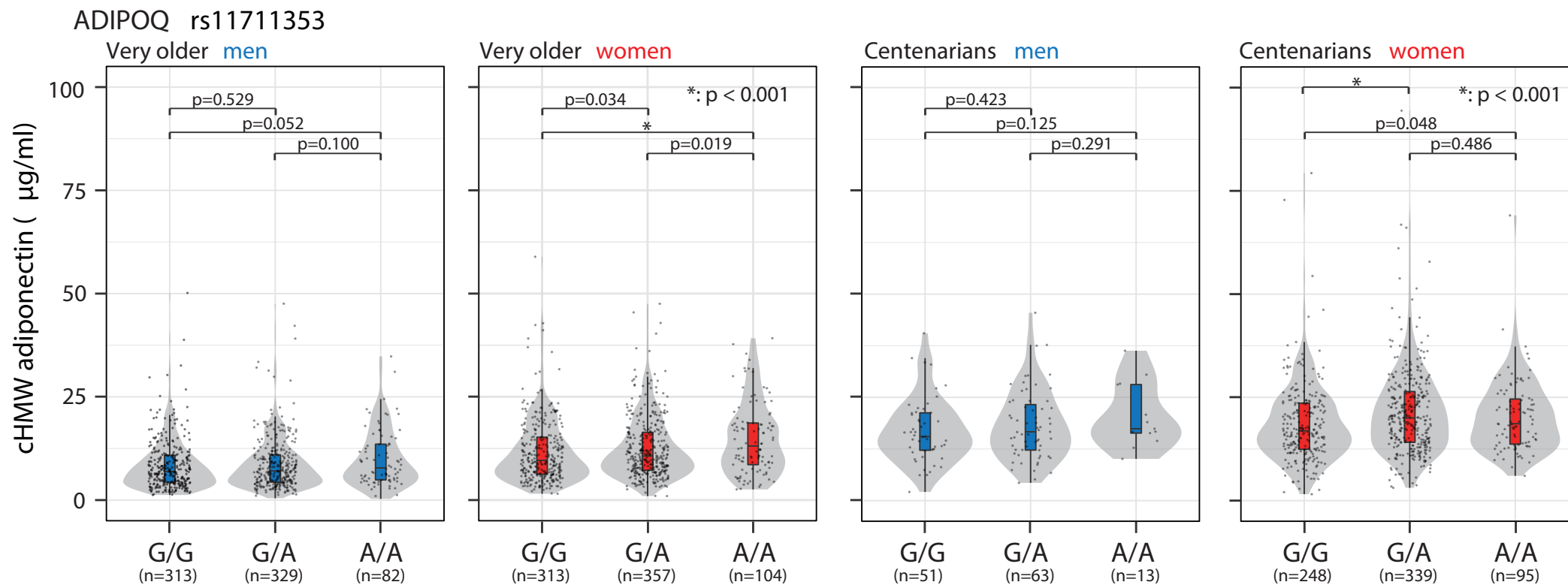


c, rs11711353 men (ADIPOQ)



c, rs11711353 women (ADIPOQ)





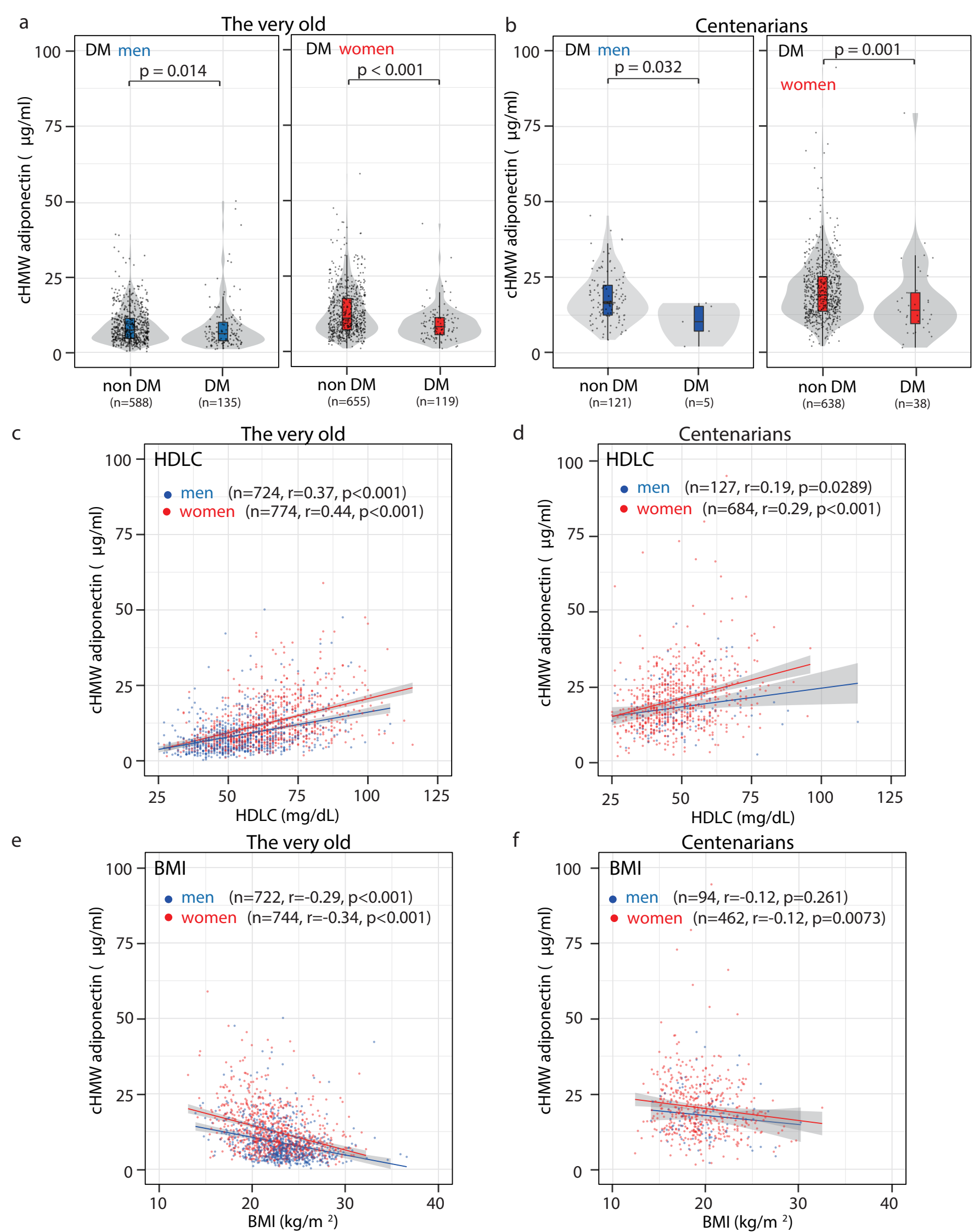
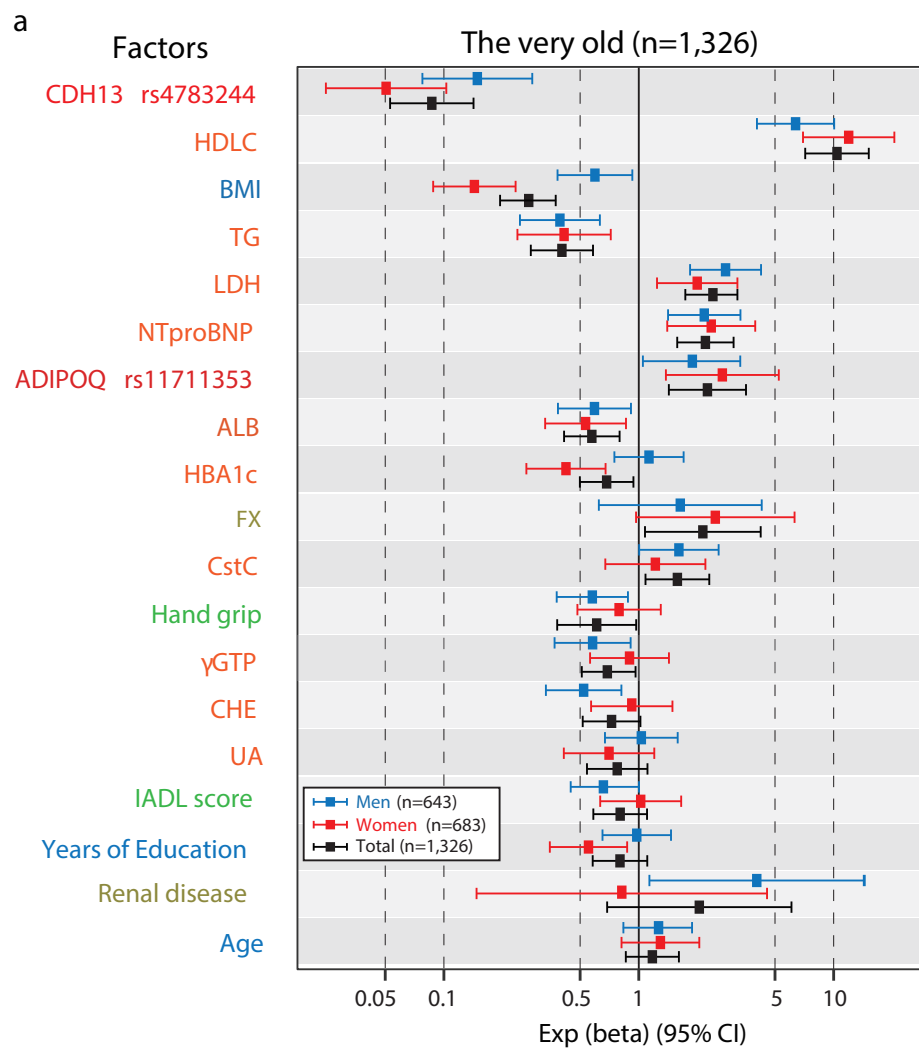


Figure 3 Sasaki et al.





#### Basic information (3)

Age  
BMI  
Years of education

#### Physical and Cognitive function (2)

IADL score  
Hand grip

#### Disease histories (2)

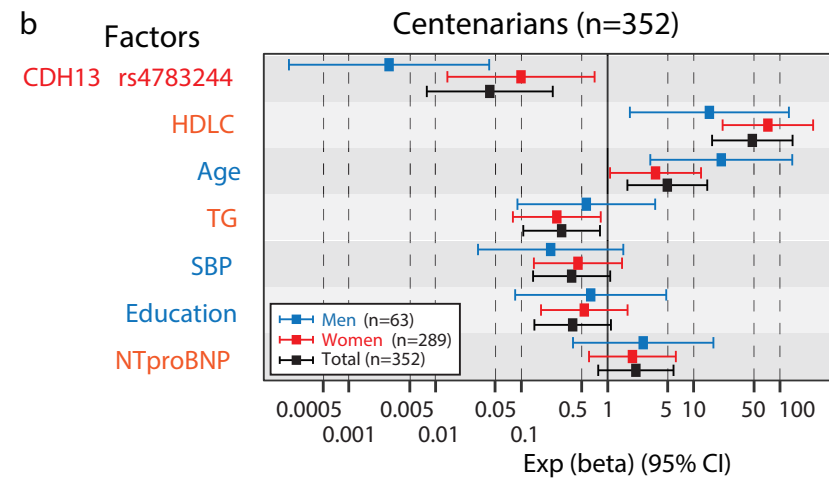
Renal disease  
Fracture

#### Genetic factors (2)

CDH13 rs4783244  
ADIPOQ rs11711353

#### Biomarkers in blood (10)

HDLC  
TG  
CHE  
yGTP  
LDH  
UA  
ALB  
CstC  
NTproBNP  
HBA1c



#### Basic information (3)

Age  
SBP  
Education

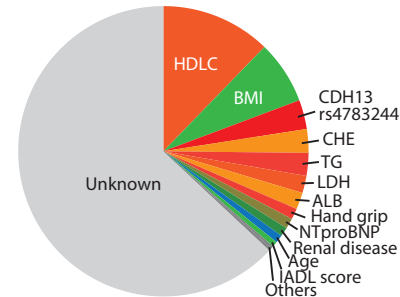
#### Biomarkers in blood (3)

HDLC  
TG  
NTproBNP

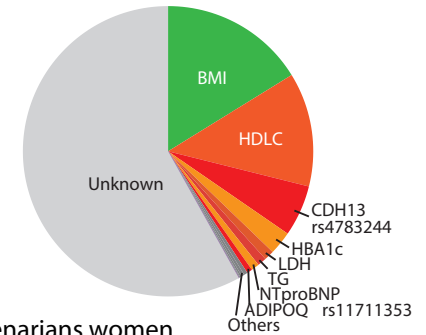
#### Genetic factors (1)

CDH13 rs4783244

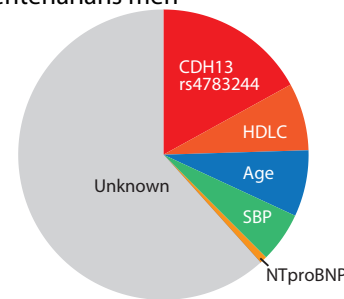
#### c Very older men



#### Very older women



#### d Centenarians men



#### Centenarians women

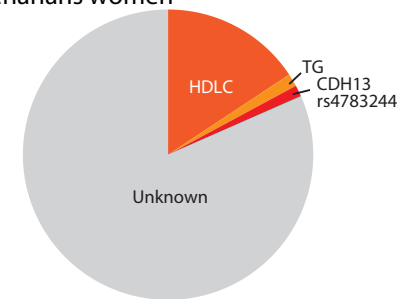
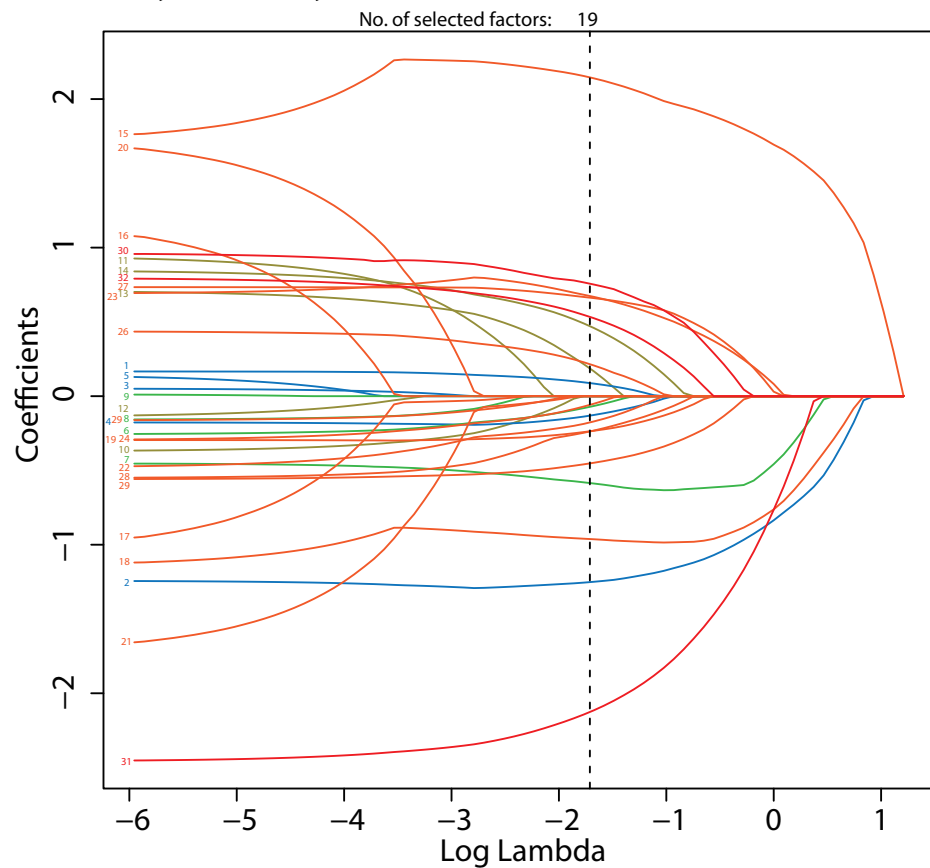


Figure 4 Sasaki et al.

(a) The very old (85-89yo, n=1,326)



#### Basic information (5)

- 1 Age
- 2 BMI
- 3 SBP
- 4 Years of education
- 5 Smoking history

#### Physical and Cognitive function (4)

- 6 IADL score
- 7 Hand grip
- 8 Cognitive impairment
- 9 WHO5 score

#### Disease histories (5)

- 10 Heart disease
- 11 Diabetes
- 12 Cancers
- 13 Renal disease
- 14 Fracture

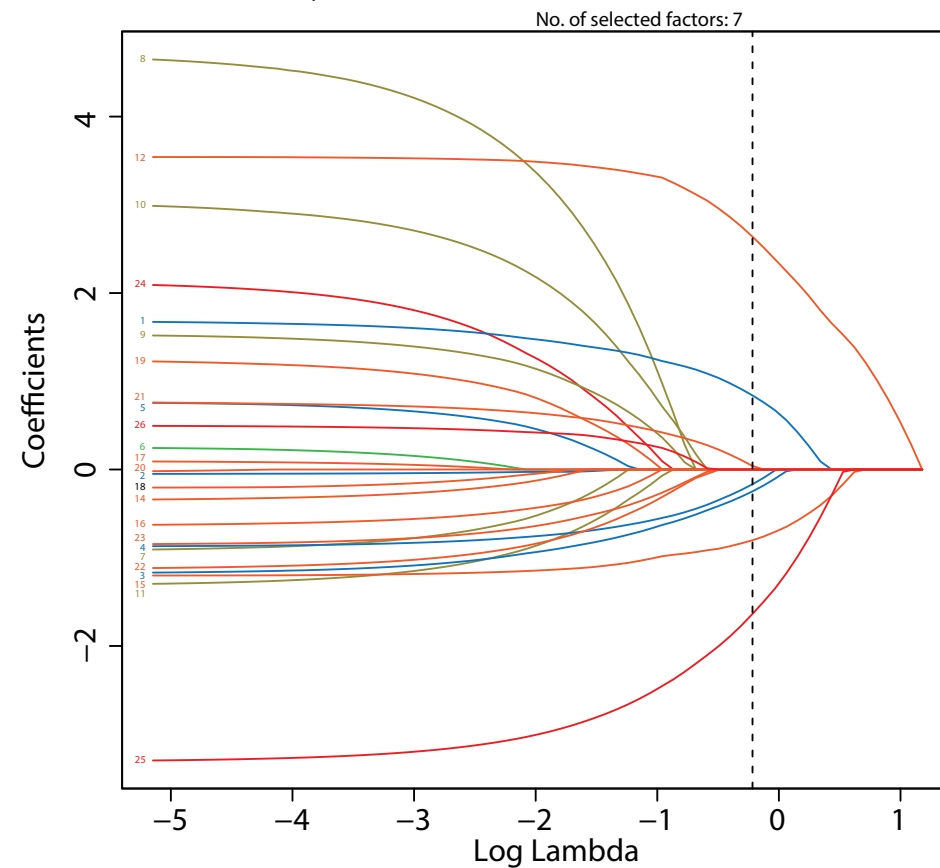
#### Biomarkers in blood (15)

- 15 HDLC
- 16 TCHO
- 17 LDLC
- 18 TG
- 19 CHE
- 20 AST
- 21 ALT
- 22  $\gamma$ GTP
- 23 LDH
- 24 UA
- 25 ALB
- 26 CstC
- 27 NTproBNP
- 28 HBA1c
- 29 IL6

#### Genetic factors (3)

- 30 SEX
- 31 CDH13 rs4783244
- 32 ADIPOQ rs11711353

(b) Centenarians (100-yo, n=352)



#### Basic information (5)

- 1 Age
- 2 BMI
- 3 SBP
- 4 Education category
- 5 Smoking history

#### Physical and Cognitive function (1)

- 6 ADL score

#### Disease histories (5)

- 7 Heart disease
- 8 Diabetes
- 9 Cancers
- 10 Renal disease
- 11 Fracture

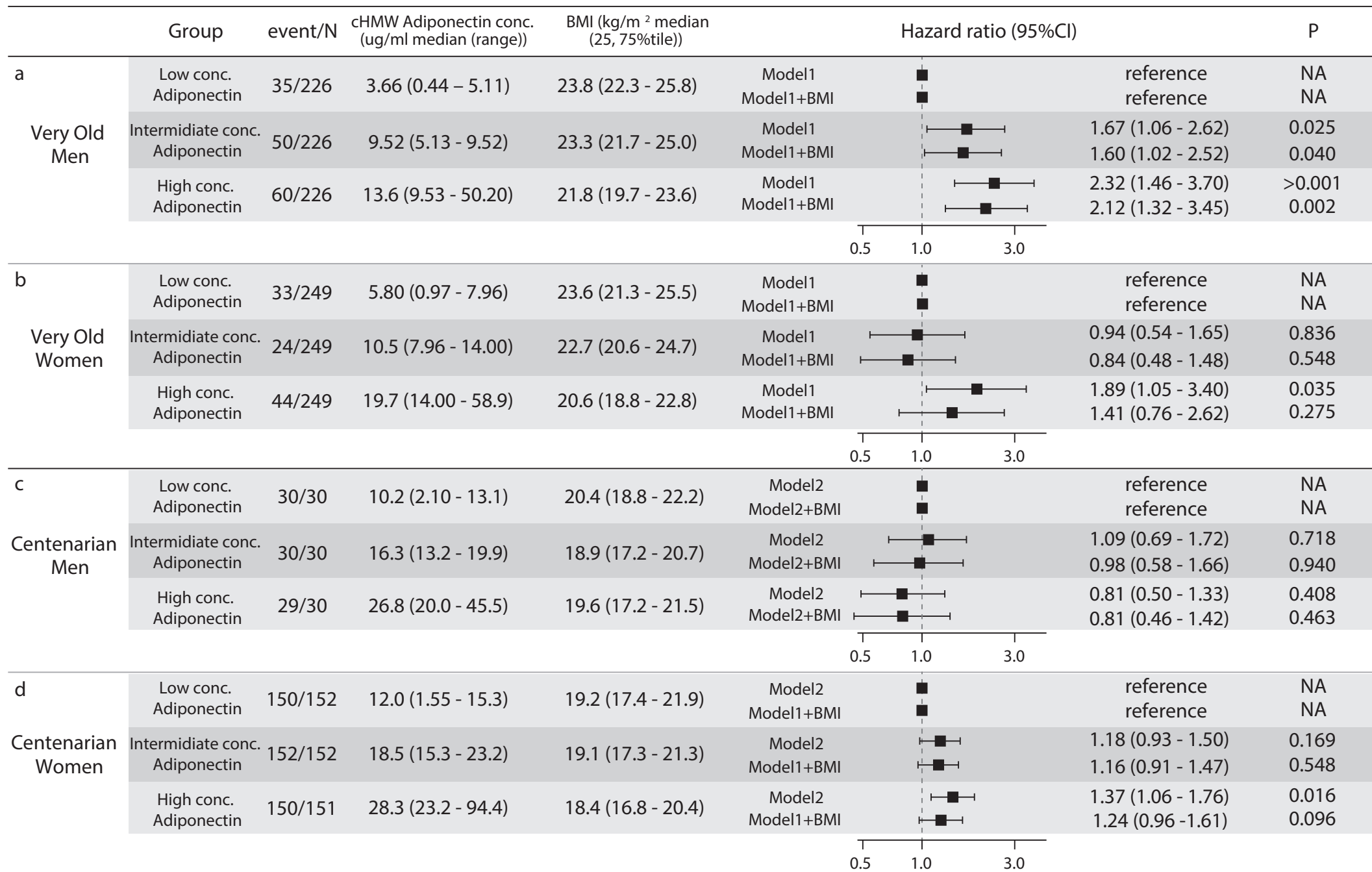
#### Biomarkers in blood (12)

- 12 HDLC
- 13 TCHO
- 14 LDLC
- 15 TG
- 16 CHE
- 17  $\gamma$ GTP
- 18 UA
- 19 ALB
- 20 CstC
- 21 NTproBNP
- 22 HBA1c
- 23 IL6

#### Genetic factors (3)

- 24 SEX
- 25 CDH13 rs4783244
- 26 ADIPOQ rs11711353

## All-Cause mortality

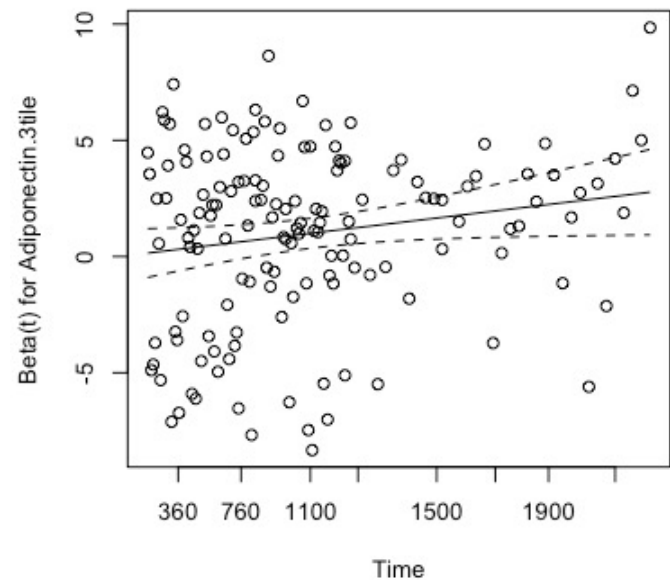


Model1 covariates: Age, cohort, HDLC, disease history (Diabetes),

CDH13 rs11711353, ADIPOQ rs4783244, Years of education

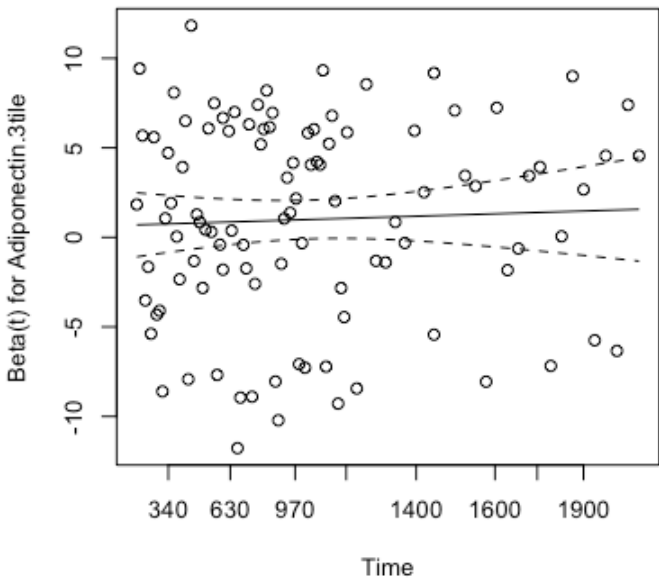
Model2 covariates: Age, HDLC, CDH13 rs11711353

a. Very old men



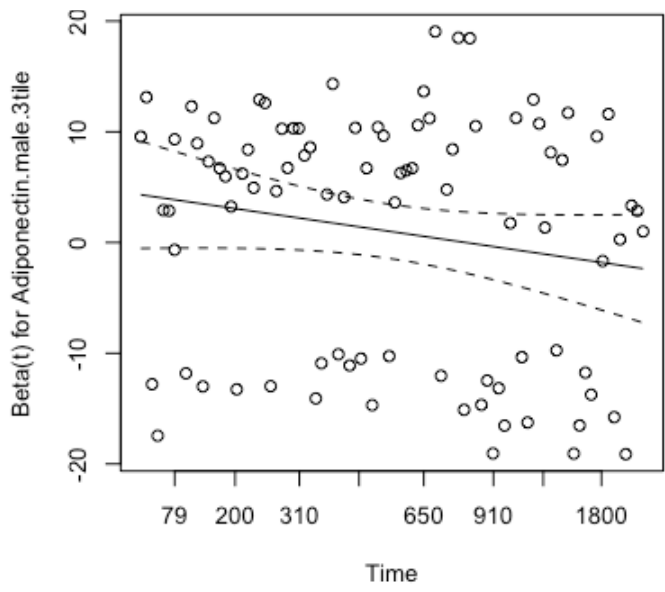
	chisq	df	p
Adiponectin.3tile	5.6258	2	0.060
Age	0.4014	1	0.526
cohort	3.3586	1	0.067
HDLC	0.2860	1	0.593
DM	0.0184	1	0.892
rs11711353	0.1363	1	0.712
rs4783244	0.5392	1	0.463
years of education	2.4766	1	0.116
BMI	0.9791	1	0.322
GLOBAL	14.6985	10	0.143

b. Very old women



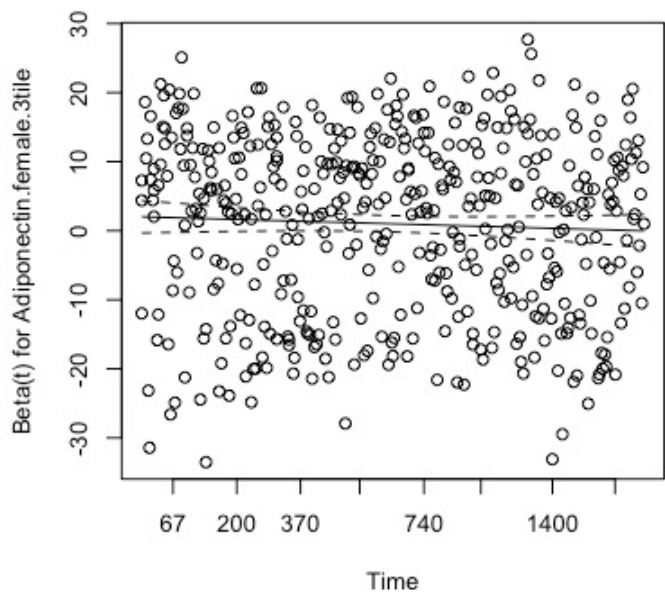
	chisq	df	p
Adiponectin.3tile	0.21036	2	0.90
Age	0.33001	1	0.57
cohort	1.96893	1	0.16
HDLC	0.00235	1	0.96
DM_adipo	0.80508	1	0.37
rs11711353_num.taqman	1.51935	1	0.22
rs4783244_num.taqman	0.01977	1	0.89
years_of_education	0.06784	1	0.79
BMI	0.74597	1	0.39
GLOBAL	6.93027	10	0.73

a. Centenarian men



	chisq	df	p
Adiponectin.male.3tile	3.513	2	0.173
Age	3.243	1	0.072
HDL	0.957	1	0.328
rs4783244	0.152	1	0.697
BMI	0.711	1	0.399
GLOBAL	12.810	6	0.046

b. Centenarian women



	chisq	df	p
Adiponectin.female.3tile	1.35e+00	2	0.51
Age	4.25e-01	1	0.51
HDL	5.03e-02	1	0.82
rs4783244	1.63e-08	1	1.00
BMI	5.93e-01	1	0.44
GLOBAL	2.19e+00	6	0.90

## Cancer-cause mortality

	Group	event/N	cHMW Adiponectin conc. (ug/ml median (range))	BMI (kg/m <sup>2</sup> median (25, 75%tile))		Hazard ratio		P
Very Old Men	Low conc. Adiponectin	13/226	3.66 (0.44 - 3.64)	23.8 (22.4 - 25.8)	Model1	■	reference	NA
					Model1+BMI	■	reference	NA
	Intermediate conc. Adiponectin	13/226	9.52 (5.11 - 9.52)	23.3 (21.8 - 25.0)	Model1	■	1.14 (0.51 - 2.53)	0.753
					Model1+BMI	■	1.18 (0.53 - 2.65)	0.686
	High conc. Adiponectin	13/226	13.5 (9.52 - 50.2)	21.8 (19.7 - 23.6)	Model1	■	1.25 (0.53 - 2.99)	0.610
					Model1+BMI	■	1.36 (0.56 - 3.31)	0.501
						0.3 1.0 3.0		
Very Old Women	Low conc. Adiponectin	3/249	5.80 (0.97 - 7.96)	23.6 (21.3 - 25.5)	Model1	■	reference	NA
					Model1+BMI	■	reference	NA
	Intermediate conc. Adiponectin	8/249	10.5 (7.96 - 14.00)	22.7 (20.6 - 24.7)	Model1	■	3.16 (0.79 - 12.74)	0.105
					Model1+BMI	■	2.98 (0.73 - 12.12)	0.128
	High conc. Adiponectin	9/249	19.7 (14.00 - 58.9)	20.6 (18.8 - 22.8)	Model1	■	3.39 (0.73 - 15.77)	0.119
					Model1+BMI	■	2.94 (0.60 - 14.36)	0.184
						0.3 1.0 3.0		

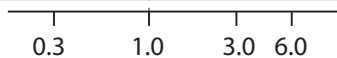
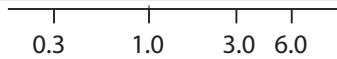
Model1 covariates: Age, HDLC, CDH13 rs11711353

## Cardiovascular disease-cause mortality

	Group	event/N	cHMW Adiponectin conc. (ug/ml median (range))	BMI (kg/m <sup>2</sup> median (25, 75%tile))	Hazard ratio		P
Very Old Men	Low conc. Adiponectin	6/226	3.66 (0.44 - 3.64)	23.8 (22.4 - 25.8)	Model1	reference	NA
					Model1+BMI	reference	NA
	Intermediate conc. Adiponectin	8/226	9.52 (5.11 - 9.52)	23.3 (21.8 - 25.0)	Model1	1.58 (0.52 - 4.79)	0.418
					Model1+BMI	1.50 (0.49 - 4.55)	0.475
	High conc. Adiponectin	12/226	13.5 (9.52 - 50.2)	21.8 (19.7 - 23.6)	Model1	3.16 (1.06 - 9.41)	0.039
					Model1+BMI	2.80 (0.91 - 8.61)	0.072
					0.3 1.0 3.0		
Very Old Women	Low conc. Adiponectin	12/249	5.80 (0.97 - 7.96)	23.6 (21.3 - 25.5)	Model1	reference	NA
					Model1+BMI	reference	NA
	Intermediate conc. Adiponectin	3/249	10.5 (7.96 - 14.00)	22.7 (20.6 - 24.7)	Model1	0.30 (0.08 - 1.11)	0.105
					Model1+BMI	0.27 (0.07 - 1.03)	0.056
	High conc. Adiponectin	12/249	19.7 (14.00 - 58.9)	20.6 (18.8 - 22.8)	Model1	1.04 (0.35 - 3.09)	0.946
					Model1+BMI	0.86 (0.27 - 2.72)	0.794
					0.3 1.0 3.0		

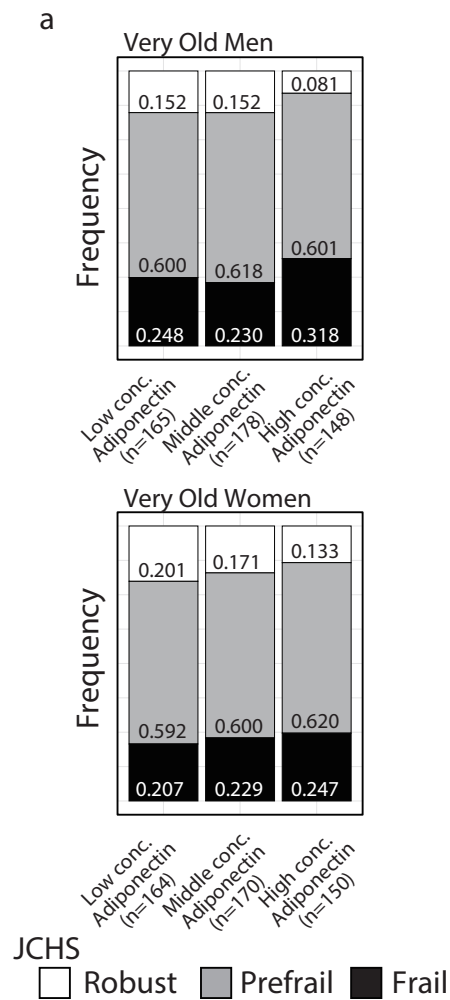
Model1 covariates: Age, HDLC, CDH13 rs11711353

## Pneumonia disease-cuase mortality

	Group	event/N	cHMW Adiponectin conc. (ug/ml median (range))	BMI (kg/m <sup>2</sup> median (25, 75%tile))	Hazard ratio		P
Very Old Men	Low conc. Adiponectin	6/226	3.66 (0.44 - 3.64)	23.8 (22.4 - 25.8)	Model1	reference	NA
					Model1+BMI	reference	NA
	Intermediate conc. Adiponectin	11/226	9.52 (5.11 - 9.52)	23.3 (21.8 - 25.0)	Model1	2.26 (0.80 - 6.39)	0.124
					Model1+BMI	1.83 (0.64 - 5.21)	0.257
	High conc. Adiponectin	11/226	13.5 (9.52 - 50.2)	21.8 (19.7 - 23.6)	Model1	2.22 (0.74 - 6.69)	0.157
				Model1+BMI	1.47 (0.46 - 4.68)	0.516	
							
					0.3 1.0 3.0 6.0		
Very Old Women	Low conc. Adiponectin	4/249	5.80 (0.97 - 7.96)	23.6 (21.3 - 25.5)	Model1	reference	NA
					Model1+BMI	reference	NA
	Intermediate conc. Adiponectin	2/249	10.5 (7.96 - 14.00)	22.7 (20.6 - 24.7)	Model1	1.31 (0.21 - 8.18)	0.770
					Model1+BMI	1.13 (0.18 - 7.23)	0.895
	High conc. Adiponectin	6/249	19.7 (14.00 - 58.9)	20.6 (18.8 - 22.8)	Model1	5.87 (1.01 - 34.14)	0.049
				Model1+BMI	3.80 (0.59 - 24.69)	0.161	
							
					0.3 1.0 3.0 6.0		

Model1 covariates: Age, HDLC, CDH13 rs11711353



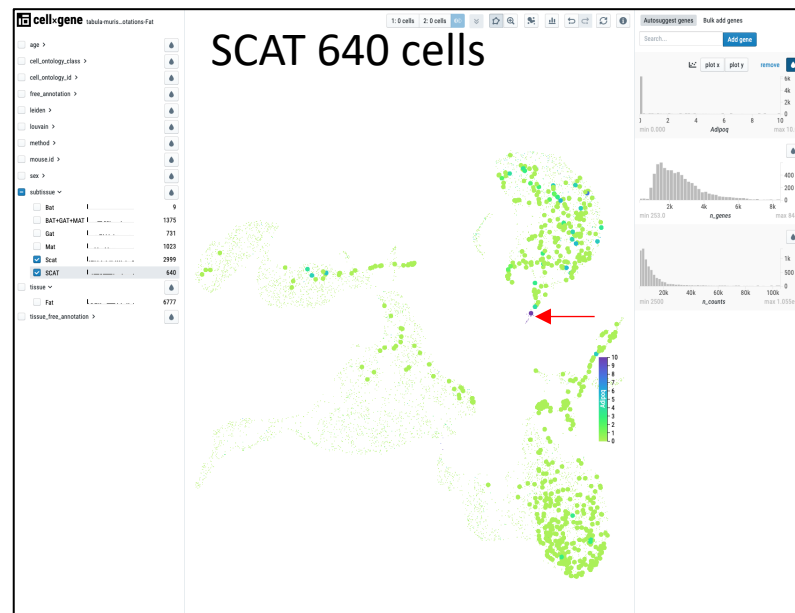
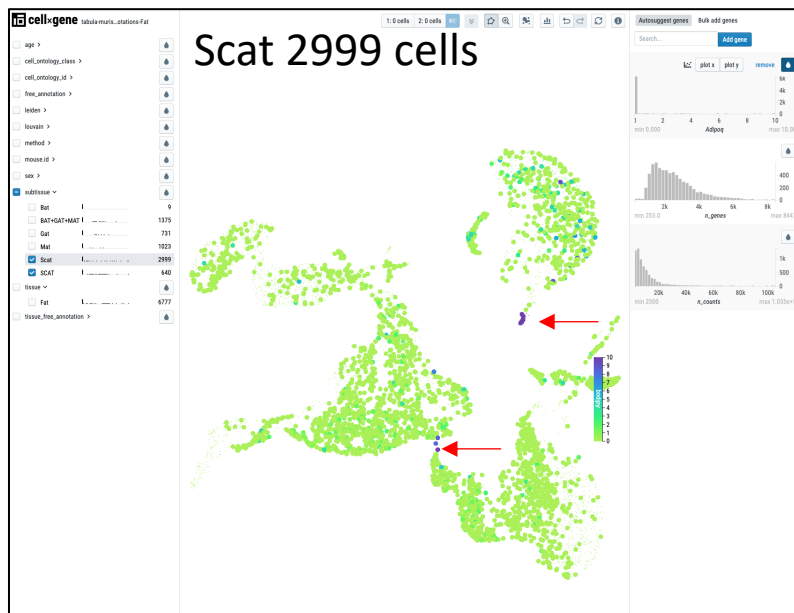
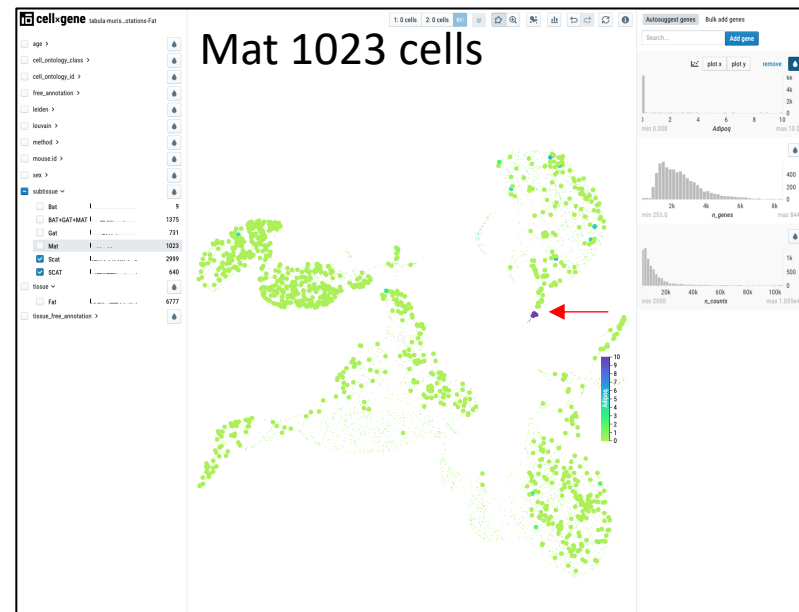
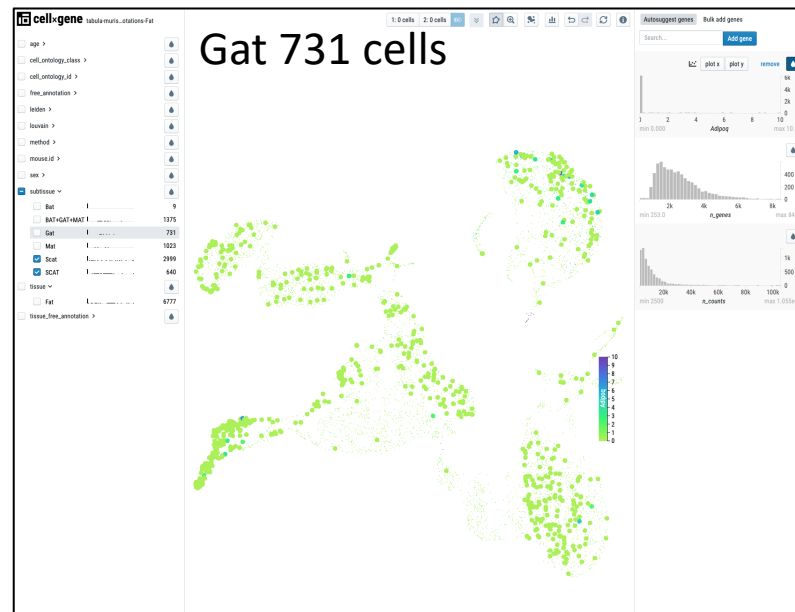
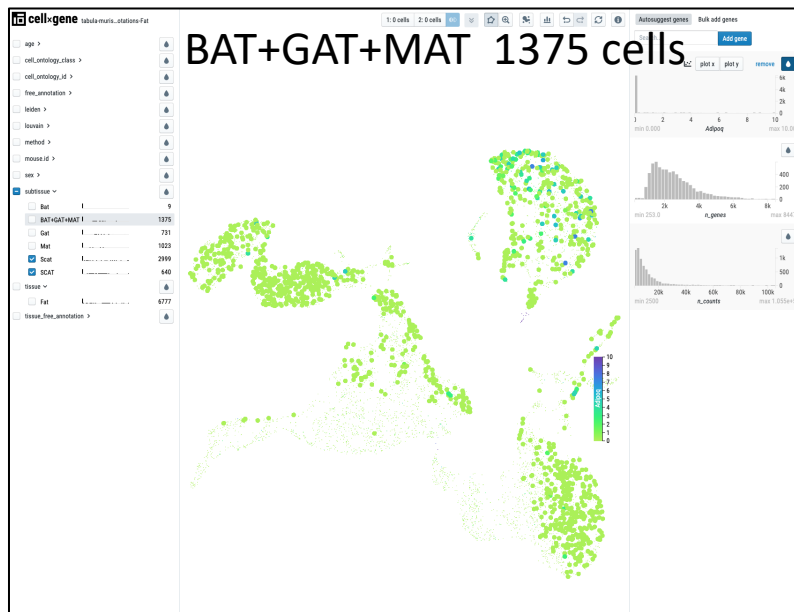


b

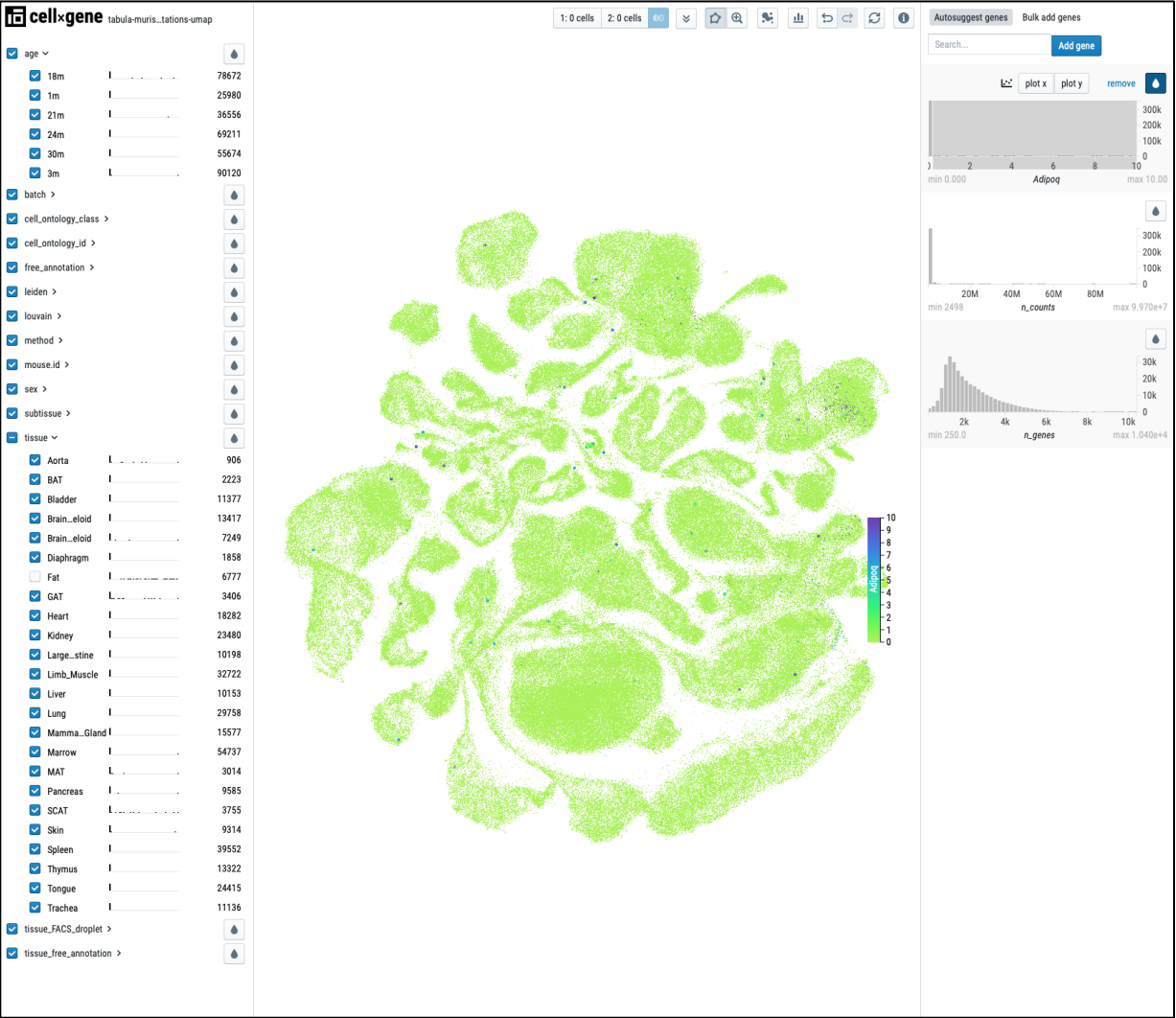
## Mutiple regression analysis between cHMW adiponectin level and Frailty

	Group	N	cHMW Adiponectin level (ug/ml median (range))		OR (95%CI)	P
Very Old Men	Low conc. Adiponectin	165	3.58 (1.23 - 5.11)	Model1	reference	NA
				Model1+BMI	reference	NA
	Intermediate conc. Adiponectin	178	6.98 (5.13 - 9.52)	Model1	0.99 (0.79 - 1.24)	0.929
				Model1+BMI	0.98 (0.79 - 1.22)	0.868
	High conc. Adiponectin	148	13.2 (9.58 - 42.20)	Model1	1.28 (1.00 - 1.63)	0.054
				Model1+BMI	1.22 (0.95 - 1.56)	0.126
Very Old Women	Low conc. Adiponectin	164	6.08 (1.60 - 7.92)	Model1	reference	NA
				Model1+BMI	reference	NA
	Intermediate conc. Adiponectin	170	10.5 (7.96 - 13.90)	Model1	1.12 (0.90 - 1.39)	0.329
				Model1+BMI	1.12 (0.90 - 1.39)	0.319
	High conc. Adiponectin	150	19.4 (14.0 - 58.9)	Model1	1.35 (1.04 - 1.74)	0.023
				Model1+BMI	1.36 (1.04 - 1.78)	0.023

Model1 covariates: Age, cohort, HDLC, disease history (Diabetes), CDH13 rs11711353, ADIPOQ rs4783244, Years of education



non fat tissue



adipose tissue

