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Retrospective exploratory analyses on gender differences in determinants for incidence and progression of diabetic retinopathy in Japanese patients with type 2 diabetes mellitus

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Abstract. Gender differences in risks for macrovascular complications in type 2 diabetes mellitus (T2DM) have been well established. However, the impact of gender differences on diabetic retinopathy (DR) has not been fully elucidated. We therefore retrospectively explored gender-specific determinants for DR in patients with T2DM in a small sized Japanese cohort in Okinawa. There were 214 patients who were diagnosed as no DR (n = 142) and non-proliferative DR (n = 72) in 2009. During the follow-up of median 7 years, 41/142 of incidence, 26/72 of progression, and 67/214 of incidence and progression were observed, respectively. DR was assessed using the modified international clinical DR severity scales. The risks for incidence, progression as well as incidence and progression of DR were comparable between men and women, respectively. Cox proportional hazard models in multivariate analyses demonstrated that the only common determinant in both men and women for DR was the duration of T2DM. Regarding gender-specific determinants, lower level of serum albumin in men as well as higher HbA1c, lower level of estimated glomerular filtration rate, and lower level of serum uric acid in women were extracted, respectively. Although precise mechanisms for such gender-specific determinants of DR still remain unsolved, the present study would highlight a couple of factors associated with gender-specific determinants for DR in a limited numbers of Japanese cohort. Prospective observational studies on gender-specific determinants of DR in a large scale cohort are warranted to further clarify underlying mechanisms.

Key words: Diabetic retinopathy, Gender difference, Gender-specific determinant, Retrospective study

RESEARCH in gender difference regarding risk of type 2 diabetes mellitus (T2DM) and related cardiovascular disease (CVD) have attracted considerable attention in both clinics and academy [1-4]. Of note, the prevalence of T2DM is higher in young women than young men due to more severe insulin resistance during puberty, whilst systemic insulin resistance is greater in middle-aged men than middle-aged women [5, 6]. In accordance with this notion, here in Japan, age-standardized diabetes prevalence estimates based on the Japanese population over 20 years of age was 6.1% among women (95% confidence interval [CI] 5.5–6.7) and 9.9% (95% CI 9.2–10.6) among men at 2010 [7]. Moreover, it is important to note that the risk of CVD is relatively higher in women with T2DM than in men with T2DM, as evidenced by a systematic review evaluating 85,000 patients [8]. Potential

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mechanisms for gender differences of T2DM on CVD risk include biological and physiological factors as well as disparities in disease management between genders [4]. It is generally accepted that women develop T2DM at a higher body mass index (BMI) than men [9]. Therefore, women may experience more prolonged exposure to overweight prior to the diagnosis of T2DM compared with men [9]. Furthermore, it is recognized that cardiorespiratory fitness and physical activity levels are lower in women than men [10-12].

While gender differences in risks for macrovascular complications in T2DM have been well established [8, 13], impact of gender differences on microvascular complications [14], especially diabetic retinopathy (DR), have been poorly elucidated [15]. Although there would be a possible gender differences among determinants for incidence and progression of DR, such differences between men and women have been poorly examined [16]. If gender-specific determinants for DR are identified, it may be possible to more effectively predict and intervene at early incidence of DR. In this context, we retrospectively explored gender-specific determinants for DR in patients with T2DM utilizing a Japanese cohort.

Materials and Methods

Study design and subjects

The present study was approved by the institutional ethical committee (approved number 765, institutional ethical committee of University of the Ryukyus, and Tomishiro Central Hospital approved on February 27, 2015) and was conducted in accordance with the Declaration of Helsinki. We enrolled 975 patients (578 men and 397 women) with T2DM at clinics either in University of the Ryukyus or Department of Diabetes and Life-style related Disease Center, Tomishiro Central Hospital, from January to December in 2009 (Fig. 1. Dataset 1). We excluded a total of 480 subjects (282 men and 198 women) from analyses because of missing data [*i.e.* 122 subjects (67 men and 55 women) with no recorded BMI values and 358 subjects (215 men and 143 women) with no recorded ALB values, respectively], with 495 subjects (296 men and 199 women) analyzed (Fig. 1. Dataset 2). Because incidence and progression of DR has been focused in the present study, among those 495 patients, 92 subjects (56 men and 36 women) with proliferative DR were excluded as well as 189 subjects (121 men and 68 women) who had failed to attend ophthalmologist appointments or followed up for less than 4 years were also excluded. Consequently, 214 subjects (119 men and 95 women) were analyzed in the present study (Fig. 1. Full analyses set). Patterns in missing data were visually assessed by taking the combinations of missing variables of 975 enrolled patients into account (Supplemental Fig. 1).

Measurements

Data were obtained from electric medical records in University of the Ryukyus and Tomishiro Central Hospital. Results of the first fundus examination in 2009 as well as the first blood sampling examination in 2009 were analyzed. In most cases, both fundus examination and blood sampling were done at the same day, but in a few cases, there was a time point difference ranging from one to three months. Serum biochemical variables were measured with conventional automated analyzers. Dyslipidemia was identified by the current use of anti-dyslipidemia drugs. Overt proteinuria was defined as positive if ones showed ±, 1+, 2+, 3+, 4+ by semi-qualitative urinary protein stick test at two consecutive follow-up months.

Assessment of DR

The severity of DR was determined by qualified ophthalmologists in either University of the Ryukyus Hospital or Tomishiro Central Hospital. Following the modified international clinical DR severity scales [17], we divided subjects into three groups (no diabetic retinopathy (NDR) as stage 1, non-proliferative diabetic retinopathy (NPDR) as stage 2, proliferative diabetic retinopathy (PDR) as stage 3). In case the severity of the right and left eyes were different, severer eye condition was taken for the staging.

Assessment of incidence and progression of DR

Incidence and progression of DR were assessed from the series of fundus examination from 2009 to 2013 or later. Incidence of DR was assessed by worsening in stage (from stage 1 to stage 2 or stage 3), and progression of DR was defined as a worsening in stage (from stage 2 to stage 3) [18, 19].

Statistical analyses

The continuous variables of normal distribution are represented by mean (standard deviation SD), the continuous variables of non-normal distribution are represented by median (25%, 75%), and the categorical variables are represented by *n* (%), respectively. For comparison between the groups, two-tailed unpaired *t* test was used for continuous variables of the normal distribution, and Kruskal-Wallis test was used for continuous variables of the non-normal distribution. Categorical variables were analyzed by Fisher's exact tests.

Univariate Cox proportional hazard models were used to identify determinants for the incidence and progression of DR from 2009 to 2013 or later. Age, gender,

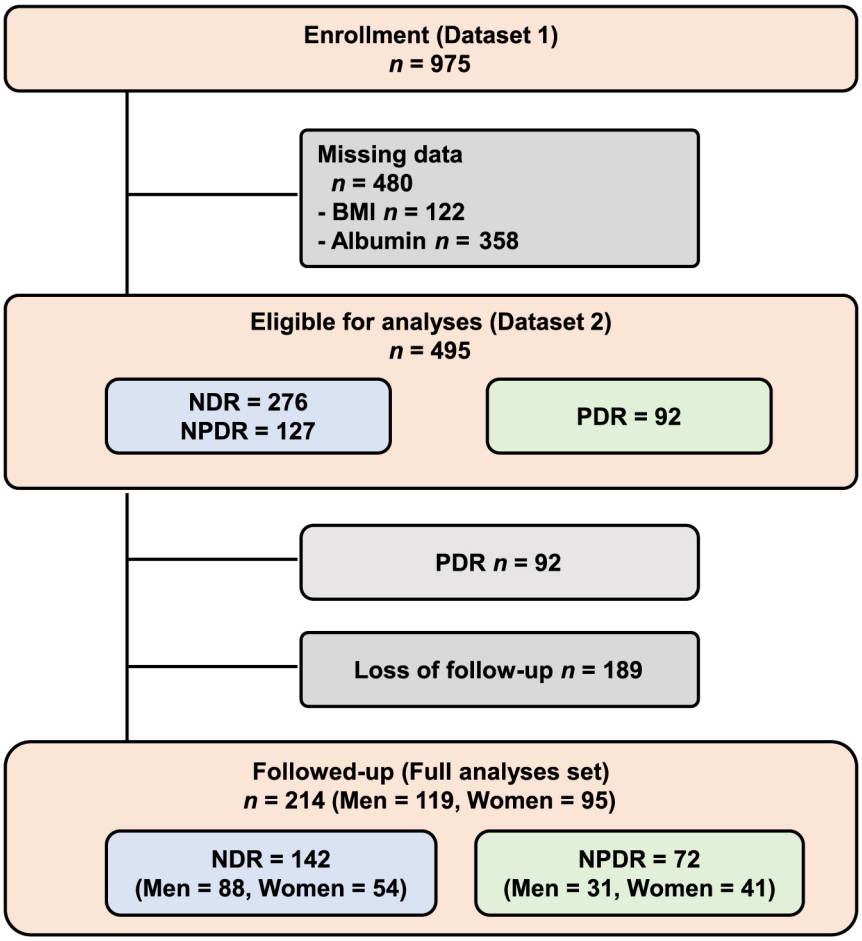

Fig. 1 Research design

We enrolled 975 patients (578 men and 397 women) with T2DM from January to December in 2009 (Dataset 1). After 480 (282 men and 198 women) excluded due to missing data [*i.e.* 122 patients (67 men and 55 women) with no recorded values BMI and 358 patients (215 men and 143 women) with no recorded ALB values, respectively], 495 patients (296 men and 199 women) either with NDR & NPDR or PDR were included (Dataset 2). Full analyses set included 214 patients (119 men and 95 women) after excluded with PDR (n = 92, men 56 and women 36) and due to lack of follow-up (n = 189, men 121 and 68 women). *N*, number; BMI, body mass index; NDR, no diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

BMI, use of anti-hypertensive drugs, duration of T2DM, dyslipidemia, overt proteinuria, history of smoking, diabetic family history, each value for HbA1c, albumin (ALB), estimated glomerular filtration rate (eGFR), uric acid (UA), hypertriglyceridemia, low-dencity lipoprotein (LDL-C), high-density lipoprotein cholesterol (HDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltransferase (γ-GT) in serum were employed as dependent variables. Parameters with highly skewed distribution including HbA1c, triglyceride, eGFR, AST, ALT, and γ-GT were logged for analyses. Multivariate Cox proportional hazard models were adjusted by age and BMI or by age, BMI, duration of T2DM, use of anti-hypertensive drugs, overt proteinuria, HbA1c, ALB, eGFR and UA with a specific focus on a gender differences. The deviance residual plots were used to confirm the Cox proportional assumption in each model.

Kaplan-Meier free-survival analyses for the incidence, progression as well as incidence and progression of DR were performed as follows. Time zero was defined as the day of the first retinopathy assessment, and observations were censored at the primary outcome (incidence and progression of DR) with the event or the last assessment of retinopathy during the follow-up period. Non-informative censoring was used.

For sensitivity analyses, we assessed patterns in missing data as follows (Supplemental Fig. 1). We first identified combinations of missing variables and showed the observed numbers as intersection size. The most frequent missing combinations, sole Fundus examination (FE) (n = 66) and sole LDL (n = 57) were less than 10% of all

samples (n = 975) and the combinations containing ALB (ALB + FE, ALB + LDL, sole ALB, ALB + LDL + FE) were less than 5% of all samples. We therefore considered the patterns in missing data as completely at random.

Statistical analyses were performed using standard software package (R 3.4.3, JMP version 12; SAS Institute Inc., Cary, NC) unless otherwise indicated. The missing values in the dataset were analyzed by using R package "naniar" to visually confirm the missing pattern [20]. Kaplan-Meier Plots were made by using the statistical package, Jskm: Kaplan-Meier Plot with 'ggplot2'.R package version 0.4.1 (Zarathu Co.,Ltd). P value was two-sided test and significance was accepted at $p < 0.05$.

Results

General characteristics

Clinical profile of the cohort (n = 214, 56% men) is shown in Table 1. Mean value of age was 63 ± 12 (men 62 ± 11, women 65 ± 12) years, and mean BMI was 25.4 ± 4.3 (men 25.0 ± 4.3, women 25.9 ± 4.3) kg/m^2. HbA1c level at baseline was 7.8 ± 1.8 (men 7.7 ± 1.7, women 8.0 ± 2.0) %. Median duration of T2DM was 10 (5, 16) [men 10 (5, 15), women 10 (6, 20)] years.

Cox proportional hazard model for exploring determinants associated with incidence, progression as well as incidence and progression of DR

As shown in Fig. 2, during the follow-up of median 7 years (range 4–8 years), incidence was 29% (41/142), progression was 36% (26/72), and incidence and progression were 31% (67/214), respectively. In men, incidence was 25% (22/88), progression was 42% (13/31), and incidence and progression were 29% (35/119), respectively. In women, incidence was 35% (19/54), progression was 32% (13/41), and incidence and progression were 34% (32/95), respectively. Cox proportional hazard model analyses for exploring determinants associated with incidence, progression as well as incidence and progression of DR were shown in Table 2. In multivariate analyses adjusted by age, BMI, duration of T2DM, use of anti-hypertensive drugs, overt proteinuria, HbA1c, ALB, eGFR and UA, determinants associated with incidence included higher log HbA1c level (p = 0.002) and lower level of UA (p = 0.018) in all, no factors in men, and lower level of log eGFR (p = 0.029) and lower level of UA (p = 0.012) in women, respectively. Furthermore, determinants associated with progression included longer duration of T2DM ($p < 0.001$), no use of anti-hypertensive drugs (p = 0.018) and lower level of serum ALB (p = 0.014) in all, longer duration of T2DM (p = 0.027) and lower level of serum ALB (p = 0.009) in men, and longer duration of T2DM (p = 0.034) and higher log HbA1c level (p = 0.038) in women, respectively. On the other hand, determinants associated with both incidence and progression included higher log HbA1c level (p = 0.001), lower level of log eGFR (p = 0.043) and lower level of UA (p = 0.004) in all, no factors in men, and higher log HbA1c level (p = 0.006), lower level of log eGFR (p = 0.007) and lower level of UA (p = 0.006) in women, respectively.

Kaplan-Meier free-survival analyses for the incidence, progression as well as incidence and progression of DR

There were no significant differences in free-survival for incidence, progression as well as incidence and progression of DR between men and women (p = 0.71, p = 0.42 and p = 0.41, respectively) (Fig. 3). When stratified by the value of HbA1c, incidence, progression as well as incidence and progression of DR were increased with HbA1c ≥8% in all and in women (all; p = 0.027, p = 0.009, $p < 0.001$, women; p = 0.018, p = 0.01, $p < 0.001$, respectively), but not in men (Fig. 4). When stratified by the value of UA, incidence of DR was increased in UA <7.0 mg/dL in all (p = 0.034) (Fig. 5). When stratified by the duration of T2DM, progression in all ($p < 0.001$), men ($p < 0.001$), women ($p < 0.001$) and incidence and progression in all (p = 0.002) and men (p = 0.014) were increased in the duration of T2DM ≥10 years (Fig. 6). When stratified by the value of ALB, progression of DR was increased with ALB <4.0 g/dL in men (p = 0.012) (Fig. 7). When stratified by the value of eGFR <60 mL/min/1.73 m^2, there were no significant differences among groups (Fig. 8). When stratified by the value of HbA1c, incidence in all (p = 0.029) and women (p = 0.017) and incidence and progression in women (p = 0.003) were increased with HbA1c ≥7% (Supplemental Fig. 2). When stratified by the use of anti-hypertensive drugs, incidence and progression of DR were increased with no use of anti-hypertensive drugs in women (p = 0.028) (Supplemental Fig. 3).

Discussion

The major findings in the present study are that lower level of serum albumin was identified as a male-specific determinant for DR, whilst higher HbA1c, lower level of eGFR and lower level of serum uric acid for DR were deemed as female-specific determinants. Although precise mechanisms for such gender-specific determinants of DR still remain unsolved, the present study would highlight that there is an underreporting of gender differences regarding determinants of DR. In the present study, the risks for incidence, progression as well as incidence and progression of DR were comparable between men

Table 1 General characteristcs of the studied patients

	All				Men				Women			
	Total	NDR	NPDR	*p* value		NDR	NPDR	*p* value		NDR	NPDR	*p* value
	n = 214	*n* = 142	*n* = 72		*n* = 119	*n* = 88	*n* = 31		*n* = 95	*n* = 54	*n* = 41	
Men, *n* (%)	119/214 (56)	88/142 (62)	31/72 (43)	0.02								
Age (year)	63 ± 12	64 ± 13	63 ± 10	0.74	62 ± 11	63 ± 13	62 ± 9	0.56	65 ± 12	66 ± 14	63 ± 11	0.55
BMI (kg/m^2)	25.4 ± 4.3	25.6 ± 4.5	25.6 ± 4.2	0.15	25.0 ± 4.3	25.6 ± 4.8	24.7 ± 3.6	0.19	25.9 ± 4.3	25.8 ± 4.1	26.4 ± 4.6	0.36
Duration of T2DM (years)	10 (5, 16)	6 (3, 10)	10 (7, 17)	<0.0001	10 (5, 15)	6 (3, 10)	10 (5, 18)	<0.0001	10 (6, 20)	6 (3, 11)	10 (7, 17)	<0.0001
Anti-hypertensive drugs, *n* (%)	158/214 (74)	100/142 (70)	58/72 (81)	0.64	90/119 (76)	65/88 (74)	25/31 (81)	0.72	68/95 (72)	35/54 (65)	33/41 (80)	0.62
Dyslipidemia, *n* (%)	96/148 (65)	55/69 (80)	41/62 (66)	0.30	47/77 (61)	31/45 (69)	16/25 (64)	0.80	49/71 (69)	24/54 (44)	25/37 (68)	0.39
Overt proteinuria, *n* (%)	66/194 (34)	25/91 (27)	41/76 (54)	<0.001	38/106 (36)	19/62 (31)	19/31 (61)	0.001	28/88 (32)	6/29 (7)	22/40 (55)	0.0001
History of smoking, *n* (%)	89/214 (42)	57/142 (40)	32/72 (44)	0.23	75/119 (63)	52/88 (59)	23/31 (74)	0.54	14/95 (15)	5/54 (9)	9/41 (22)	0.05
Diabetic family history of diabetes, *n* (%)	67/214 (31)	41/142 (29)	26/72 (36)	0.29	34/119 (29)	23/88 (26)	11/31 (29)	0.55	33/95 (35)	18/54 (33)	15/41 (37)	0.59
Cessation of treatment, *n* (%)	21/214 (10)	12/142 (9)	9/72 (13)	0.02	15/119 (13)	10/88 (11)	5/31 (16)	0.01	6/95 (6)	2/54 (4)	4/41 (9)	0.12
HbA1c (%)	7.8 ± 1.8	7.4 ± 1.6	8.1 ± 1.8	0.001	7.7 ± 1.7	7.4 ± 1.6	8.1 ± 2.1	0.17	8.0 ± 2.0	7.2 ± 1.6	8.1 ± 1.7	0.001
ALB (mg/dL)	4.0 ± 0.5	4.2 ± 0.3	3.9 ± 0.5	0.001	4.1 ± 0.5	4.2 ± 0.4	4.0 ± 0.4	0.00	4.0 ± 0.4	4.1 ± 0.3	3.9 ± 0.5	0.05
eGFR (mL/min/1.73 m^2)	60 (23, 82)	64 (32, 83)	58 (18, 75)	0.45	63 (25, 83)	67 (32, 85)	61 (40, 81)	0.46	54 (19, 79)	56 (33, 79)	49 (17, 74)	0.20
UA (mg/dL)	5.6 ± 1.5	5.8 ± 1.5	5.6 ± 1.5	0.31	5.8 ± 1.5	6.0 ± 1.4	5.7 ± 1.5	0.39	5.3 ± 1.5	5.2 ± 1.6	5.5 ± 1.4	0.63
Hypertriglyceridemia, *n* (%)	70/180 (39)	51/84 (61)	19/68 (30)	0.41	40/101 (40)	34/56 (61)	6/30 (20)	0.13	30/79 (38)	17/28 (61)	13/38 (34)	0.95
LDL-C (mg/dL)	99 ± 42	93 ± 41	105 ± 45	0.41	99 ± 42	100 ± 41	98 ± 53	0.99	98 ± 42	80 ± 39	110 ± 39	0.07
HDL-C (mg/dL)	50 ± 13	49 ± 12	51 ± 14	0.55	49 ± 13	47 ± 12	48 ± 13	0.13	53 ± 13	54 ± 10	53 ± 15	0.56
AST (IU/L)	19 (16, 26)	21 (17, 26)	18 (15, 26)	0.23	19 (16, 26)	24 (17, 28)	16 (14, 20)	0.02	21 (16, 26)	19 (16, 24)	22 (16, 28)	0.18
ALT (IU/L)	19 (15, 32)	21 (16, 34)	18 (14, 29)	0.21	24 (16, 34)	27 (18, 41)	20 (15, 26)	0.03	18 (13, 26)	18 (14, 19)	18 (13, 32)	0.29
γ-GT (IU/L)	26 (17, 46)	26 (19, 46)	26 (16, 47)	0.64	32 (21, 51)	30 (22, 53)	37 (18, 55)	0.21	21 (15, 33)	22 (17, 33)	21 (15, 36)	0.35

Date are expressed as mean (SD), median (IQR), and *n* (%). *P* values were calculated by two-tailed unpaired *t* test. NDR, no diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; BMI, body mass index; HbA1c, glycated hemoglobin; ALB, albumin; eGFR, estimated glomerular filtration; UA, uric acid; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GT, γ-glutamyltransferase. Dyslipidemia means use of anti-dyslipidemia drugs; Overt proteinuria means positive of urinary qualitative at two consecutive follow-up months; Hypertriglyceridemia means >150 mg/dL.

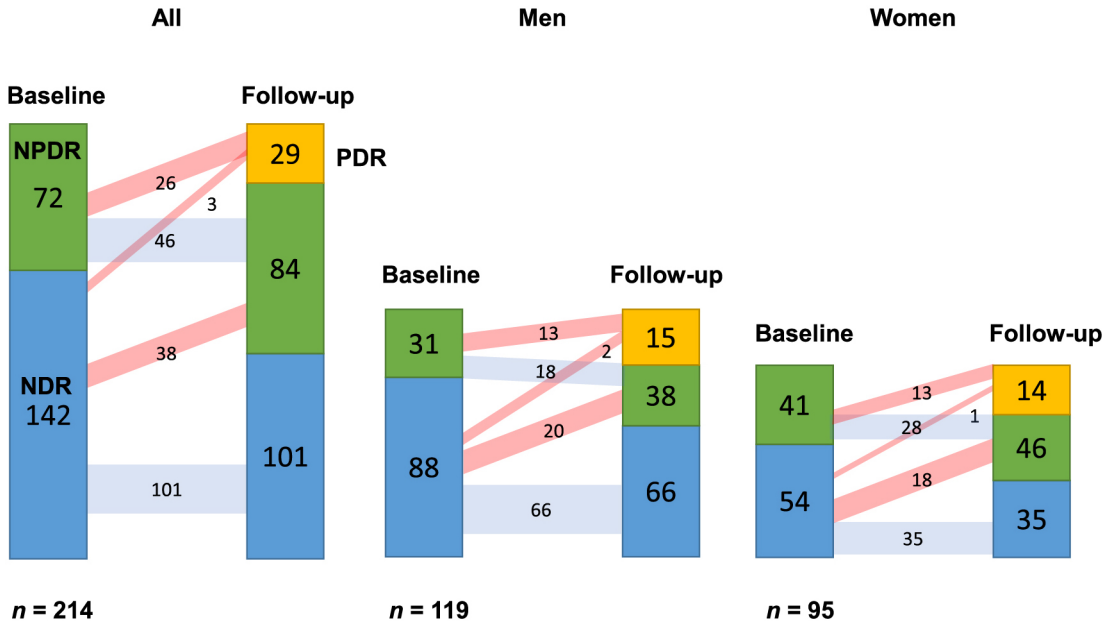

Fig. 2 Distribution of patients with T2DM on the stage of DR at baseline and during the follow-up period
Numbers of incidence or progression are shown in light red band and those of no changes are shown in light blue band. NDR, no diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy.

and women (n = 41; men 25%, women 35%, p = 0.25, n = 26; men 42%, women 32%, p = 0.46, n = 67; men 29%, women 34%, p = 0.55, respectively). According to previous reports, DR risk was greater in men [21-24]. On the other hand, some reports demonstrated that DR risk was greater in women [25, 26]. Collectively, there is still a longstanding controversy as to whether gender is an independent risk factor for DR. Cox proportional hazards models in the present study showed that the only common determinant in incidence, progression as well as incidence and progression of DR for both men and women was the duration of T2DM in progression of DR. This finding is in agreement with many previous reports [26-31].

Lower level of serum albumin as a male-specific determinant for the progression of DR

In previous reports, impact of serum ALB level on DR was evaluated in combined population of both genders. A report from Japan was 130 population size (men 80,women 50) and another report from China was 104 (men 82, women 22) [32, 33]. In contrast, the present study demonstrated that lower level of serum ALB was a male-specific determinant for incidence and progression of DR. Lower level of serum ALB reflects at least partly, decomposition of ALB under poorly controlled status as well as a urinary loss by proteinuria [32]. Relatively larger mass of skeletal muscle and accelerated protein catabolism associated with impaired insulin secretion in men may account for such a difference observed between men and women [6]. Gender difference in urinary loss of ALB has also been reported [34-36]. Both estrogens and androgens play crucial roles in the pathophysiology of diabetic kidney disease *via* the suppression or activation of renin-angiotensin-aldosterone system (RAAS), respectively. In this context, imbalance of sex hormones may also influence severity of proteinuria by diabetic nephropathy through RAAS, possibly causing gender difference in DR risks [36, 37]. Further studies are warranted to test this hypothesis by evaluating circulating level of a variety of sex hormones in a prospective setting.

Higher level of HbA1c as a female-specific determinant for the progression, incidence and progression of DR

In the present study, higher HbA1c level was significantly associated with progression, incidence and progression of DR only in women. Because it is well-known that higher HbA1c level is a strong risk in incidence and progression of DR for both men and women [16, 21], the lack of a significant association in men would be extremely unexpected. Relatively small size of population may fail to reproduce the finding that higher HbA1c level is associated with incidence and progression of DR in men. We think it important to reexamine whether higher HbA1c level is a men-specific determinant associated with incidence and progression of DR in a larger cohort in a future. It is also another reason that men may

Table 2 Cox proportional hazard model for identifying determinants associated with incidence, progression, incidence and progression of diabetic retinopathy

A. Incidence

	All							Men							Women						
	Events/ Cases	Univariate		Adjusted by age and BMI		Adjusted by age, BMI, duration of T2DM, use of anti-hypertensive drugs, overt proteinuria, HbA1c, ALB, eGFR and UA		Events/ Cases	Univariate		Adjusted by age and BMI		Adjusted by age, BMI, duration of T2DM, use of anti-hypertensive drugs, overt proteinuria, HbA1c, ALB, eGFR and UA		Events/ Cases	Univariate		Adjusted by age and BMI		Adjusted by age, BMI, duration of T2DM, use of anti-hypertensive drugs, overt proteinuria, HbA1c, ALB, eGFR and UA	
		Estimate	*p* value	Estimate	*p* value	Estimate	*p* value		Estimate	*p* value	Estimate	*p* value	Estimate	*p* value		Estimate	*p* value	Estimate	*p* value	Estimate	*p* value
Men, *n* (%)	41/214	–0.11	0.71	–0.17	0.6																
Age (year)	41/214	–0.01	0.5			0.01	0.43	22/119	–0.005	0.79			0.03	0.33	19/95	–0.017	0.35			0	0.96
BMI (kg/m^2)	41/214	–0.01	0.8			0.03	0.42	22/119	–0.014	0.77			0.05	0.48	19/95	0.005	0.93			0.01	0.91
Duration of T2DM (years)	41/212	–0.03	0.15	–0.03	0.14	–0.05	0.079	22/119	–0.028	0.37	–0.03	0.35	–0.03	0.57	19/93	–0.038	0.24	–0.04	0.25	–0.06	0.2
Anti-hypertensive drugs, *n* (%)	41/214	–0.33	0.32	–0.27	0.45	–0.42	0.33	22/119	0.124	0.81	0.23	0.66	–0.16	0.83	19/95	–0.818	0.079	–0.78	0.12	–0.85	0.17
Dyslipidemia, *n* (%)	23/148	0.14	0.76	0.21	0.65			11/77	0.019	0.98	0.13	0.85			12/71	0.286	0.67	0.3	0.66		
Overt proteinuria, *n* (%)	38/194	–0.57	0.14	–0.58	0.13	0.13	0.79	19/106	–0.541	0.3	–0.56	0.29	0.04	0.96	19/88	–0.663	0.24	–0.67	0.24	0.36	0.63
History of smoking, *n* (%)	41/214	–0.23	0.48	–0.29	0.38			22/119	–0.196	0.65	–0.21	0.63			19/95	–0.253	0.74	–0.4	0.6		
Diabetic family history, *n* (%)	41/214	–0.02	0.96	–0.04	0.91			22/119	–0.225	0.64	–0.23	0.64			19/95	0.266	0.57	0.23	0.63		
Log HbA1c (%)	33/197	1.75	0.017	1.78	0.018	0.26	0.002	15/108	1.646	0.14	1.69	0.14	0.25	0.06	18/89	1.594	0.1	1.51	0.12	0.24	0.077
ALB (mg/dL)	41/214	0.14	0.71	0.14	0.7	0.57	0.27	22/119	–0.044	0.93	–0.01	0.98	0.62	0.49	19/95	0.523	0.38	0.58	0.35	1.18	0.14
Log eGFR (mL/min/ 1.73 m^2)	38/208	0	0.55	0	0.64	–0.01	0.064	19/113	0.01	0.19	0.01	0.2	0	0.8	19/95	–0.003	0.72	0	0.62	–0.02	0.029
UA (mg/dL)	39/195	–0.3	0.007	–0.3	0.009	–0.36	0.018	21/111	–0.209	0.15	–0.21	0.15	–0.01	0.96	18/84	–0.489	0.012	–0.49	0.014	–0.62	0.012
Log Hypertriglyceridemia, *n* (%)	32/180	0.19	0.6	0.16	0.66			16/101	0.048	0.92	0.03	0.96			16/79	0.294	0.56	0.25	0.63		
LDL-C (mg/dL)	18/111	0	0.42	0	0.65			9/61	0.001	0.9	0	0.98			9/50	0.008	0.34	0.01	0.5		
HDL-C (mg/dL)	34/176	0	0.84	0	0.84			17/101	0.003	0.86	0	0.86			17/75	–0.019	0.4	–0.02	0.44		
Log AST (IU/L)	38/201	–0.32	0.39	–0.32	0.39			21/115	–1.037	0.035	–1.03	0.038			17/86	1.022	0.081	1	0.085		
Log ALT (IU/L)	41/199	–0.05	0.84	–0.09	0.76			22/114	–0.311	0.39	–0.34	0.39			19/85	0.547	0.18	0.51	0.22		
Log γ-GT (IU/L)	36/187	0.12	0.55	0.11	0.62			20/104	0.27	0.3	0.32	0.23			16/83	–0.057	0.87	–0.23	0.51		

Univariate logistic regression analysis and multivariate logistic regression analysis for adjusted age, BMI, duration of T2DM, anti-hypertensive drugs, overt proteinuria, HbA1c, ALB, eGFR and UA with the incidence of diabetic retinopathy as a dependent variable. Abbreviations are the same in Table 1.

Table 2 Cont.

B. Progression

	All							Men							Women						
	Events/ Cases	Univariate		Adjusted by age and BMI		Adjusted by age, BMI, duration of T2DM, use of anti-hypertensive drugs, overt proteinuria, HbA1c, ALB, eGFR and UA		Events/ Cases	Univariate		Adjusted by age and BMI		Adjusted by age, BMI, duration of T2DM, use of anti-hypertensive drugs, overt proteinuria, HbA1c, ALB, eGFR and UA		Events/ Cases	Univariate		Adjusted by age and BMI		Adjusted by age, BMI, duration of T2DM, use of anti-hypertensive drugs, overt proteinuria, HbA1c, ALB, eGFR and UA	
		Estimate	*p* value	Estimate	*p* value	Estimate	*p* value		Estimate	*p* value	Estimate	*p* value	Estimate	*p* value		Estimate	*p* value	Estimate	*p* value	Estimate	*p* value
Men, *n* (%)	26/214	–0.31	0.43	–0.53	0.2																
Age (year)	26/214	0.01	0.41			0.03	0.24	13/119	0.004	0.85			0.04	0.39	13/95	0.025	0.36			0.04	0.34
BMI (kg/m^2)	26/214	–0.11	0.027			0.01	0.89	13/119	–0.093	0.19			0.05	0.56	13/95	–0.154	0.051			–0.07	0.49
Duration of T2DM (years)	26/212	0.1	<0.001	0.1	<0.001	0.12	<0.001	13/119	0.116	<0.001	0.11	<0.001	0.14	0.027	13/95	0.092	<0.001	0.08	0.001	0.07	0.034
Anti-hypertensive drugs, *n* (%)	26/214	–0.3	0.48	–0.24	0.6	–1.45	0.018	13/119	0.079	0.9	0.33	0.64	–2.38	0.055	13/93	–0.632	0.27	–0.63	0.29	–0.67	0.43
Dyslipidemia, *n* (%)	15/148	0.67	0.3	0.64	0.33			6/77	0.206	0.81	0.12	0.89			9/71	1.039	0.33	1.29	0.23		
Overt proteinuria, *n* (%)	24/194	0.7	0.09	0.63	0.13	0.23	0.65	11/106	2.04	0.009	2	0.011	1.14	0.24	13/88	–0.42	0.53	–0.65	0.35	–0.06	0.94
History of smoking, *n* (%)	26/214	0.3	0.45	0.37	0.37			13/119	0.215	0.72	0.4	0.54			13/95	1.014	0.094	1.17	0.072		
Diabetic family history, *n* (%)	26/214	–0.08	0.85	0.03	0.94			13/119	–0.168	0.78	–0.09	0.88			13/95	–0.041	0.94	0.03	0.95		
Log HbA1c (%)	26/197	2.18	0.006	2.39	0.004	0.11	0.36	13/108	0.378	0.77	0.44	0.73	–0.22	0.48	13/89	3.622	<0.001	4.61	<0.001	0.34	0.038
ALB (mg/dL)	26/214	–1.05	0.004	–0.99	0.01	–1.42	0.014	13/119	–1.53	<0.001	–1.51	0.001	–2.29	0.009	13/95	–0.078	0.91	–0.08	0.91	–0.54	0.53
Log eGFR (mL/min/ 1.73 m^2)	26/208	–0.01	0.23	–0.01	0.44	0	0.59	13/113	–0.016	0.074	–0.01	0.12	0.01	0.7	13/95	0.002	0.81	0.01	0.52	–0.01	0.38
UA (mg/dL)	26/195	–0.16	0.22	–0.2	0.14	–0.15	0.33	13/111	–0.188	0.31	–0.21	0.28	–0.14	0.56	13/84	–0.108	0.59	–0.12	0.54	–0.23	0.47
Log Hypertriglyceridemia, *n* (%)	25/180	–0.39	0.36	–0.25	0.56			13/101	–0.626	0.3	–0.44	0.48			12/79	–0.146	0.81	0.07	0.92		
LDL-C (mg/dL)	11/111	0	0.61	0	0.66			5/61	–0.005	0.64	–0.01	0.64			6/50	–0.003	0.76	0	0.97		
HDL-C (mg/dL)	19/176	0.02	0.38	0.01	0.5			10/101	0.031	0.16	0.03	0.26			9/75	–0.016	0.61	–0.01	0.7		
Log AST (IU/L)	23/201	–0.83	0.1	–0.79	0.13			13/115	–0.471	0.44	–0.43	0.49			10/86	–1.429	0.12	–1.63	0.098		
Log ALT (IU/L)	25/199	–0.74	0.051	–0.65	0.099			13/114	–0.613	0.2	–0.56	0.26			12/85	–0.889	0.2	–0.77	0.29		
Log γ-GT (IU/L)	22/187	–0.42	0.15	–0.32	0.3			10/104	–0.204	0.62	–0.11	0.81			12/83	–0.535	0.21	–0.36	0.43		

Univariate logistic regression analysis and multivariate logistic regression analysis for adjusted age, BMI, duration of T2DM, anti-hypertensive drugs, overt proteinuria, HbA1c, ALB, eGFR and UA with the progression of diabetic retinopathy as a dependent variable. Abbreviations are the same in Table 1.

Table 2 Cont.

C. Incidence and progression

	All							Men							Women						
	Events/ Cases	Univariate		Adjusted by age and BMI		Adjusted by age, BMI, duration of T2DM, use of anti-hypertensive drugs, overt proteinuria, HbA1c, ALB, eGFR and UA		Events/ Cases	Univariate		Adjusted by age and BMI		Adjusted by age, BMI, duration of T2DM, use of anti-hypertensive drugs, overt proteinuria, HbA1c, ALB, eGFR and UA		Events/ Cases	Univariate		Adjusted by age and BMI		Adjusted by age, BMI, duration of T2DM, use of anti-hypertensive drugs, overt proteinuria, HbA1c, ALB, eGFR and UA	
		Estimate	*p* value	Estimate	*p* value	Estimate	*p* value		Estimate	*p* value	Estimate	*p* value	Estimate	*p* value		Estimate	*p* value	Estimate	*p* value	Estimate	*p* value
Men, *n* (%)	67/214	–0.19	0.44	–0.19	0.25																
Age (year)	67/214	0	0.96			0.02	0.26	35/119	–0.003	0.83			0.02	0.470	32/95	–0.002	0.9			0.01	0.58
BMI (kg/m^2)	67/214	–0.05	0.1			0.02	0.52	35/119	–0.041	0.31			0.06	0.270	32/95	–0.058	0.21			–0.02	0.71
Duration of T2DM (years)	67/212	0.04	0.003	0.04	0.007	0.03	0.093	35/119	0.039	0.042	0.04	0.054	0.03	0.270	32/93	0.035	0.046	0.03	0.093	0.02	0.4
Anti-hypertensive drugs, *n* (%)	67/214	–0.33	0.22	–0.25	0.37	–0.52	0.11	35/119	0.112	0.78	0.29	0.50	–0.55	0.340	32/95	–0.765	0.034	–0.77	0.045	–0.74	0.13
Dyslipidemia, *n* (%)	38/148	0.33	0.38	0.34	0.36			17/77	0.071	0.89	0.11	0.83			21/71	0.552	0.32	0.62	0.27		
Overt proteinuria, *n* (%)	62/194	–0.03	0.91	–0.05	0.85	0.11	0.76	30/106	0.4	0.27	0.39	0.30	0.39	0.480	32/88	–0.596	0.17	–0.66	0.13	–0.04	0.93
History of smoking, *n* (%)	67/214	–0.01	0.98	–0.04	0.87			35/119	–0.025	0.94	–0.01	0.98			32/95	0.388	0.39	0.37	0.44		
Diabetic family history, *n* (%)	67/214	–0.04	0.88	–0.01	0.97			35/119	–0.209	0.58	–0.19	0.63			32/95	0.153	0.67	0.13	0.72		
Log HbA1c (%)	59/197	2.11	<0.001	2.21	<0.001	0.22	0.001	28/108	1.231	0.15	1.26	0.14	0.12	0.360	31/89	2.637	<0.001	2.82	<0.001	0.26	0.006
ALB (mg/dL)	67/214	–0.41	0.12	–0.37	0.17	–0.23	0.5	35/119	–0.784	0.016	–0.73	0.029	–0.6	0.240	32/95	0.281	0.53	0.3	0.52	0.4	0.48
Log eGFR (mL/min/ 1.73 m^2)	64/208	0	0.77	0	0.91	–0.01	0.043	32/113	0	0.94	0	0.99	0	0.560	32/95	–0.001	0.93	0	0.95	–0.02	0.007
UA (mg/dL)	65/195	–0.26	0.003	–0.26	0.002	–0.31	0.004	34/111	–0.224	0.051	–0.23	0.051	–0.13	0.390	31/84	–0.332	0.017	–0.32	0.023	–0.52	0.006
Log Hypertriglyceridemia, *n* (%)	57/180	–0.05	0.84	0	0.99			29/101	–0.23	0.55	–0.14	0.72			28/79	0.107	0.78	0.16	0.70		
LDL-C (mg/dL)	29/111	0	0.76	0	0.94			14/61	–0.001	0.84	0	0.78			15/50	0.003	0.59	0	0.62		
HDL-C (mg/dL)	53/176	0	0.76	0	0.86			27/101	0.013	0.35	0.01	0.43			26/75	–0.018	0.31	–0.02	0.38		
Log AST (IU/L)	61/201	–0.52	0.086	–0.49	0.1			34/115	–0.833	0.033	–0.8	0.04			27/86	0.21	0.67	0.22	0.66		
Log ALT (IU/L)	66/199	–0.29	0.18	–0.28	0.22			35/114	–0.398	0.17	–0.39	0.2			31/85	0.082	0.82	0.1	0.77		
Log γ-GT (IU/L)	58/187	–0.07	0.69	–0.04	0.83			30/104	0.141	0.52	0.22	0.35			28/83	–0.265	0.33	–0.28	0.32		

Univariate logistic regression analysis and multivariate logistic regression analysis for adjusted age, BMI, duration of T2DM, anti-hypertensive drugs, overt proteinuria, HbA1c, ALB, eGFR and UA with the incidence and progression of diabetic retinopathy as a dependent variable. Abbreviations are the same in Table 1.

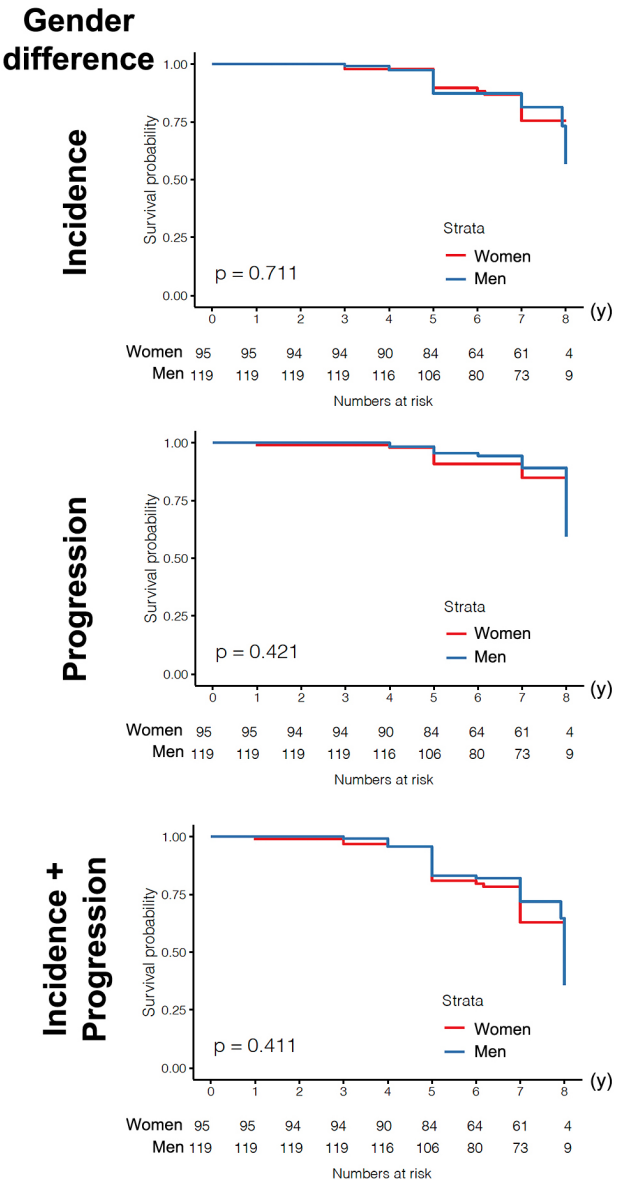

Fig. 3 Kaplan-Meier free-survival analyses for the incidence, progression as well as incidence and progression of DR in men and women with T2DM

Time zero was defined as the day of the first retinopathy assessment, and observations were censored at the primary outcome (incidence and progression of DR) with the event or the last assessment of retinopathy during the follow-up period. *P* values were obtained by log-rank test.

realize better control of HbA1c than women due to (1) medical disparities (social and economic advantages, *i.e.*, higher education, higher participation in paid work, and higher incomes), (2) gender-related prescribing biases (earlier intensive treatments without avoidance of teratogenic medications), and (3) gender-related differences in attitudes and beliefs about their health status and requirement for medications [38-40]. The finding in the present study that higher HbA1c level in men was not correlated with determinant for DR could be ascribed to relatively better control of HbA1c in men than women. In any cases, prospective studies are warranted to test this speculation.

Lower level of estimated glomerular filtration rate as a female-specific determinant for incidence, incidence and progression of DR

In the present study, lower level of eGFR was a female-specific determinant for incidence, incidence and progression of DR. It is widely accepted that diabetic nephropathy (DN) is associated with DR risk [41], additionally, lower level of eGFR is also significant correlation with incidence of DR [42]. However, little is known about gender difference in correlation between reduction in eGFR and DR risk. On the other hand, a recent study demonstrated that epidemiological cross-sectional data pointed to a higher risk of renal disease in women than men among T2DM [43]. The angiotensin-converting enzyme (ACE) insertion (I)/deletion (D) polymorphism could influence predisposition for DN by vascular modulation in the kidney, and futhermore, sex-specific differences in gene polymorphism were suggested by one study showing that women with T2DM carrying the ACE D allele had a higher risk for development of DN, while no such difference was observed in men with T2DM [44]. However, further studies are warranted to evaluate eGFR for risk factor of DR with stratification by gender in a prospective study.

Lower level of serum uric acid as a female-specific determinant for the incidence, incidence and progression of DR

In the present study, lower level of serum UA was a female-specific determinant for incidence, incidence and progression of DR. It is known that serum UA level in premenopausal women is clearly lower than in men, partly due to estrogen-induced degradation of urate reabsorptive transporter 1 (URAT1) [45]. On the other hand, circulating level of UA has been proposed to play a pivotal role in the antioxidant defense systems [46-48]. In a cross-sectional study examining the balance of oxidative stress in an early stage of type 1 diabetes mellitus without diabetic complications, Marra et al. reported that both reduced antioxidant activity and increased oxidative stress preferentially ocuured in women [49]. In this context, it may be possible that reduced antioxidant activity through lower level of serum UA would be involved, at least in part, in the augmentation of DR determinant in women with T2DM. However, further studies are warranted to evaluate a line of markers for oxidative stress in a prospective study.

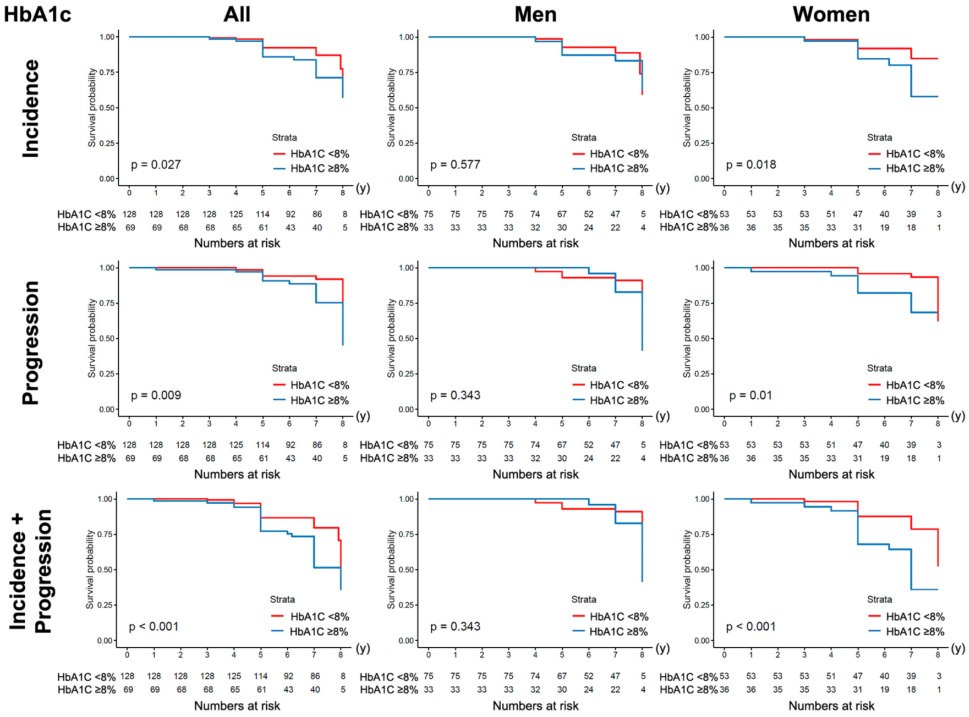

Fig. 4 Kaplan-Meier free-survival analyses for the incidence, progression as well as incidence and progression of DR in patients with T2DM according to the value of baseline HbA1c with <8% or with ≥8%

Time zero was defined as the day of the first retinopathy assessment, and observations were censored at the primary outcome (incidence and progression of DR) with the event or the last assessment of retinopathy during the follow-up period. *P* values were obtained by log-rank test.

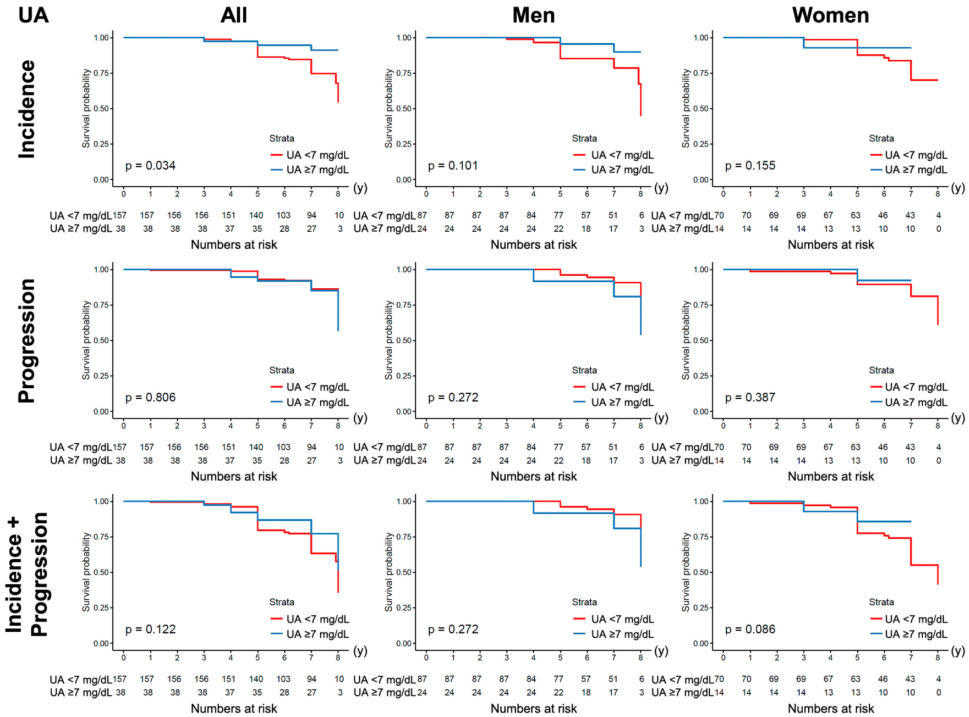

Fig. 5 Kaplan-Meier free-survival analyses for the incidence, progression as well as incidence and progression of DR in patients with T2DM according to the value of baseline uric acid with <7.0 mg/dL or with ≥7.0 mg/dL

Time zero was defined as the day of the first retinopathy assessment, and observations were censored at the primary outcome (incidence and progression of DR) with the event or the last assessment of retinopathy during the follow-up period. *P* values were obtained by log-rank test.

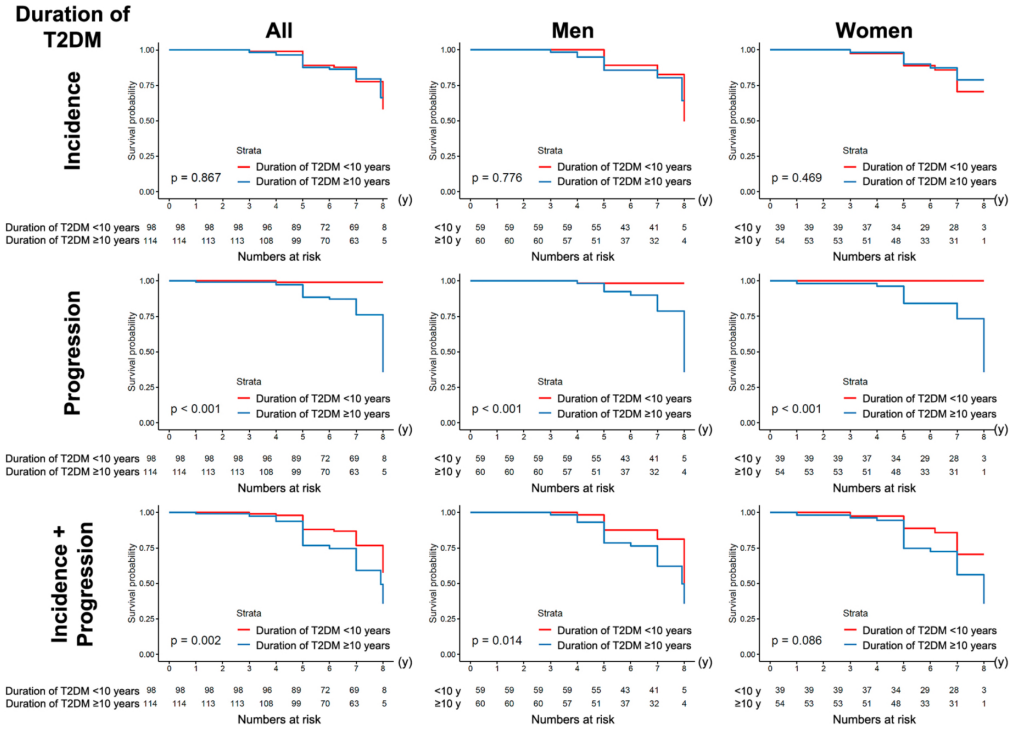

Fig. 6 Kaplan-Meier free-survival analyses for the incidence, progression as well as incidence and progression of DR in patients with T2DM according to baseline duration of diabetes with <10 years or with ≥10 years

Time zero was defined as the day of the first retinopathy assessment, and observations were censored at the primary outcome (incidence and progression of DR) with the event or the last assessment of retinopathy during the follow-up period. *P* values were obtained by log-rank test.

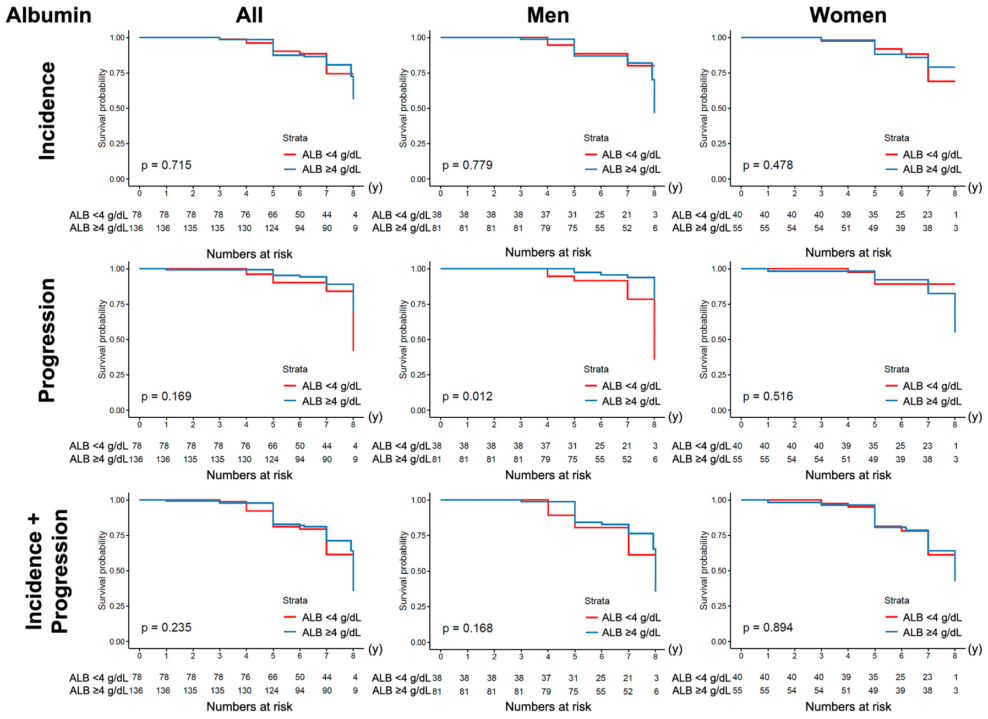

Fig. 7 Kaplan-Meier free-survival analyses for the incidence, progression as well as incidence and progression of DR in patients with T2DM according to the value of baseline serum albumin with <4 g/dL or with ≥4 g/dL

Time zero was defined as the day of the first retinopathy assessment, and observations were censored at the primary outcome (incidence and progression of DR) with the event or the last assessment of retinopathy during the follow-up period. *P* values were obtained by log-rank test.

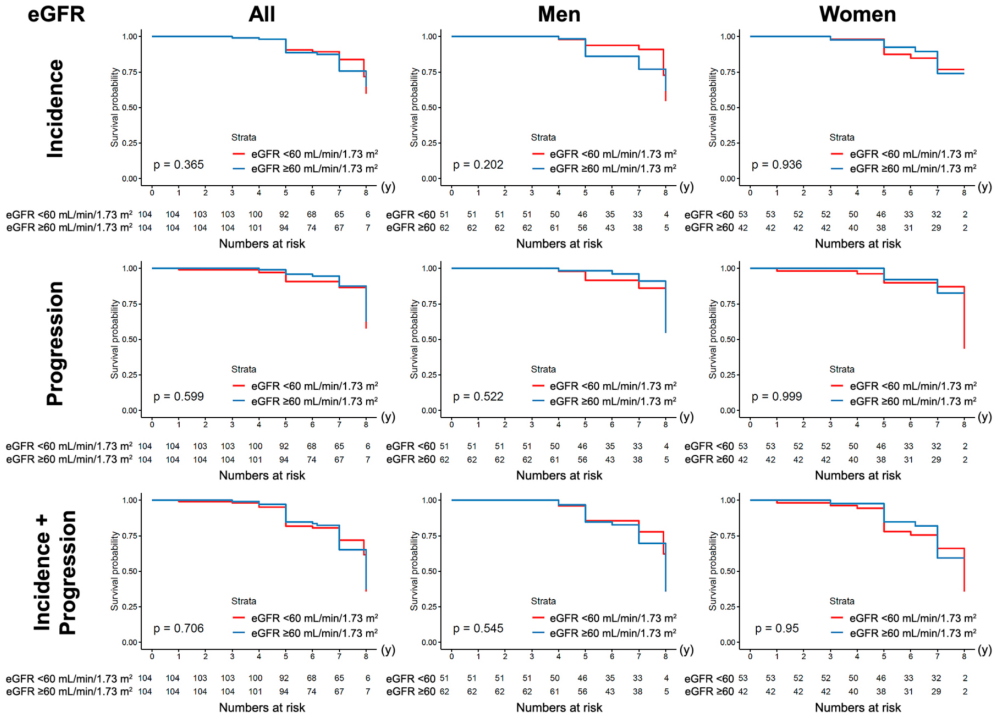

Fig. 8 Kaplan-Meier free-survival analyses for the incidence, progression as well as incidence and progression of DR in patients with T2DM according to the value of baseline estimated glomerular filtration rate with <60 mL/min/1.73 m^2 or with ≥60 mL/min/1.73 m^2

Time zero was defined as the day of the first retinopathy assessment, and observations were censored at the primary outcome (incidence and progression of DR) with the event or the last assessment of retinopathy during the follow-up period. *P* values were obtained by log-rank test.

Study limitations

We do acknowledge that there are a couple of limitations in the present study. First, because of a retrospective design, the present study failed to obtain considerable amount of critical data as well as to evaluate the fundus in patients. Second, although higher HbA1c level was an unwavering strong risk factor for DR, it was a determinant for DR somehow for only women but not men in the present study. Third, the present study did not assess directly blood pressure, but alternatively defined the presence of hypertension by medications. This may cause underdiagnosis of hypertension. Fourth, data was derived only from Japanese patients, thereby raising concerns regarding generalizations in multiethnic populations. Fifth, the present study was a retrospective design and fundus of patients was not regularly evaluated. Therefore, subtle changes for DR might be overlooked during the early phase, and therefore, it would be difficult to accurately determine the incidence or progression of DR. Sixth, the observational design cannot clarify causal relationships. Seventh, because of the small size of population, DR determinants for gender difference could be underestimated. In the present study, apparently-different prevalence rate of smoking history (men 63% *vs.* women 15%) could be also underrated. Since deleterious effects of smoking on DR in patients with type 2 diabetes remain unclarified [50], future studies are warranted to explore possible gender difference in smoking determinant on DR.

Conclusions

Although precise mechanisms for gender-specific determinants of DR still remain unsolved, the present study in a Japanese cohort extracts a couple of possibilities of gender differences in determinants for DR. We believe it valuable to highlight an underreporting of gender differences regarding determinants of DR. Prospective observational studies on gender-specific determinants of DR are warranted to further clarify underlying mechanisms.

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Disclosure

None of the authors has a potential conflict of interest to disclose.

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