JACC Vol. 57, No. 4, 2011
January 25, 2011:427–36

Abbreviations and Acronyms

AF = atrial fibrillation
CI = confidence interval
OR = odds ratio
PAF = paroxysmal atrial fibrillation
RR = relative risk

ship between any degree of alcohol consumption and the risk of AF as well as AF risk in relation to heavy alcohol drinking.

Methods

Search strategy. Electronic literature searches (Medline, January 1966 to December 2009; and Embase, January 1974 to December 2009) to identify studies describing alcohol intake and AF were conducted using medical subject headings related to alcohol (alcohol drinking OR alcohol related disorders OR alcoholism OR alcoholic beverage OR ethanol) and AF (arrhythmias OR atrial fibrillation). Reference lists from the identified articles were manually examined for relevant new articles. This process was repeated until no additional articles could be identified. No language restriction was imposed.

For inclusion, a study had to fulfill the following criteria: 1) have a cohort or case-control design; 2) identify AF as an outcome variable of interest separate from other arrhythmias; and 3) provide or allow calculation of the effect measure (i.e., relative risk [RR] in a prospective study or odds ratio [OR] in a retrospective study) with its corresponding confidence interval (CI). As an exception, because of the overlap between AF and atrial flutter, studies in which AF and atrial flutter were combined as a study outcome were also included. However, when data on risk of both AF only and the combination of AF and atrial flutter were provided simultaneously in 1 study, we used data on the risk of AF only. We excluded studies wherein alcohol consumption was classified as "yes" or "no" because the degree of daily alcohol consumption could not be ascertained through such a response.

Data extraction. Two of our investigators (S.K. and H.S.) independently reviewed all relevant articles and identified eligible studies. Discrepancies were resolved by group discussion. We extracted the following data from each publication: first author's name, year of publication, geographic region, design of the observational study (i.e., cohort or case-control), selection of study population (i.e., hospital-based or population-based), participants' characteristics (i.e., age [mean or range], proportion of men, and whether participants with heart disease that influenced AF risk were excluded), characteristics of outcome (i.e., onset or recurrence, dominantly paroxysmal atrial fibrillation [PAF] or dominantly chronic [persistent] AF, and whether atrial flutter was included in the study outcome), methods of assessment of alcohol consumption (i.e., questionnaire, interview, or reviews of medical records and registries), methods for ascertainment of AF (i.e., electrocardiogram screening, registries, or participant's report), category of alcohol intake, number of participants and cases, and study-specific controlled variables.

The effect measure in each study was extracted or, if the effect measure for AF was not provided, it was calculated based on data on the number of cases and noncases in referent and exposed groups. In principle, we defined the lowest alcohol intake category or no drinking as the referent group and the other category as the exposed group. When a study classified >2 alcohol intake categories, we extracted or calculated all available effect measures for AF. If a study provided several effect measures, such as unadjusted and adjusted effect measures, the most completely adjusted effect measure was used.

The effect measures were transformed to their natural logarithm (log OR/RR). Fundamentally, the standard error (SE) was calculated from the corresponding CI. In some studies (9–11), the SE corresponding to the log OR/RR was not provided. Then we directly calculated the SE corresponding to the log OR/RR using data on the number of cases and noncases in the exposed and referent groups in each comparison as follows:

$$SE^2 = \frac{1}{C_1} + \frac{1}{N_1} + \frac{1}{C_0} + \frac{1}{N_0}$$

(in case of log OR) (9,11) or:

$$SE^2 = \frac{1}{C_1} - \frac{1}{C_1 + N_1} + \frac{1}{C_0} - \frac{1}{C_0 + N_0}$$

(in case of log RR) (10), where C_1 and N_1 indicate the number of cases and noncases in the exposed group, respectively, and C_0 and N_0 indicate the number of cases and noncases in the referent group, respectively. If necessary, the effect measure and its corresponding SE were approximated from figures in the manuscripts using an image scanner (CanoScan LiDE 500F [resolution 600 dpi], Canon, Inc., Tokyo, Japan).

To standardize alcohol intake, we used a common scale (grams per day) for ethanol consumption. When a study used the number of drinks per day as a unit of alcohol intake, the unit was transformed into grams of ethanol according to the study-specific methods for estimating the amount of ethanol per drink. If the amount of ethanol per drink was not specified, the unit was considered equivalent to 12 g ethanol (12).

For each study, data on the mean level of daily alcohol intake for each category were extracted or calculated as point estimates of ethanol consumption. When this information was not provided, we assigned the mid-point of the upper and lower boundaries in each category as the average intake. If the highest category had an open upper boundary, mean alcohol intake was estimated to be 1.2 times the lower boundary (13).

Data synthesis. To summarize the association of habitual heavy alcohol consumption with the risk of AF, the effect measures were pooled for the highest versus lowest alcohol intake category. Based on the definition of heavy alcohol

drinking by the National Institute on Alcohol Abuse and Alcoholism (12), we limited this analysis to studies in which the highest alcohol intake category was defined as consumption of 2 or more drinks per day for men, 1 or more drinks per day for women, and 1.5 or more drinks per day for the combination of men and women. Subjects described as "alcohol abusers" and "alcoholics" were also considered to be heavy drinkers. The pooled estimate was calculated by averaging the log OR/RRs weighted by the inverse of variance based on a fixed- or random-effects model. We used the results from the random-effects model if between-study heterogeneity, which was assessed by Q statistics and I-squared (14), was significant (15). Because daily alcohol consumption in heavy alcohol drinkers varied from study to study, we conducted stratified analysis according to the degree of "heavy" drinking. Analyses were also stratified by the pre-specified study characteristics. We also conducted meta-regression analyses to assess the influence of study characteristics on study results.

The possibility of publication bias was assessed primarily by visual inspection of a funnel plot in which the effect measure in the individual study was plotted against its corresponding SE. The funnel plot is expected to be symmetrical with respect to the overall estimate if publication bias is absent. We secondarily assessed the possibility of publication bias by 2 formal tests: the Begg's adjusted rank correlation test (16) and the Egger's regression asymmetry test (17). If publication bias was statistically suspected, we also followed the Duval and Tweedie "trim and fill" procedure (18) for further estimation of the possible effect of the publication bias. This method considers the possibility of hypothetical unpublished studies that would have allowed a funnel plot to be symmetrical and recalculates a pooled estimate after imputation of the effect measures of the hypothetical studies as though they actually existed.

We primarily used weighted, least-squared regression models (19) to explore the dose-response relationship between alcohol intake and the risk of AF by regressing the log OR/RR of AF on the alcohol dose. To further investigate the shape of the relationship between the level of alcohol consumption and the risk of AF, we used restricted cubic splines with knots at the 25th, 50th, and 75th centiles of the distribution of alcohol consumption. These analyses were limited to data from studies with a referent category whose mean alcohol consumption was reported or estimated to be less than 1 drink per day, so that overlapping of alcohol intake of exposed and referent groups could be avoided as much as possible. Two-sided p values of ≤ 0.05 were considered statistically significant except for tests of publication bias for which the recommended level is p value ≤ 0.10 (20). Data were analyzed using STATA software version 10 (STATA Corp., College Station, Texas).

Results

Study characteristics. Figure 1 shows details of the literature search. Our electronic literature search resulted in retrieval of 1,754 citations (591 from Medline and 1,163 from Embase). Of these, 1,673 citations were excluded after the first screening. Eighty-one papers as well as 20 additional papers identified by manual search were left for full-text review. After this review, of the 101 papers, 87 were excluded for the reasons shown in Figure 1. Finally, 14 studies (9–11,21–31), which comprised 130,820 participants and 7,558 cases, were included in this meta-analysis.

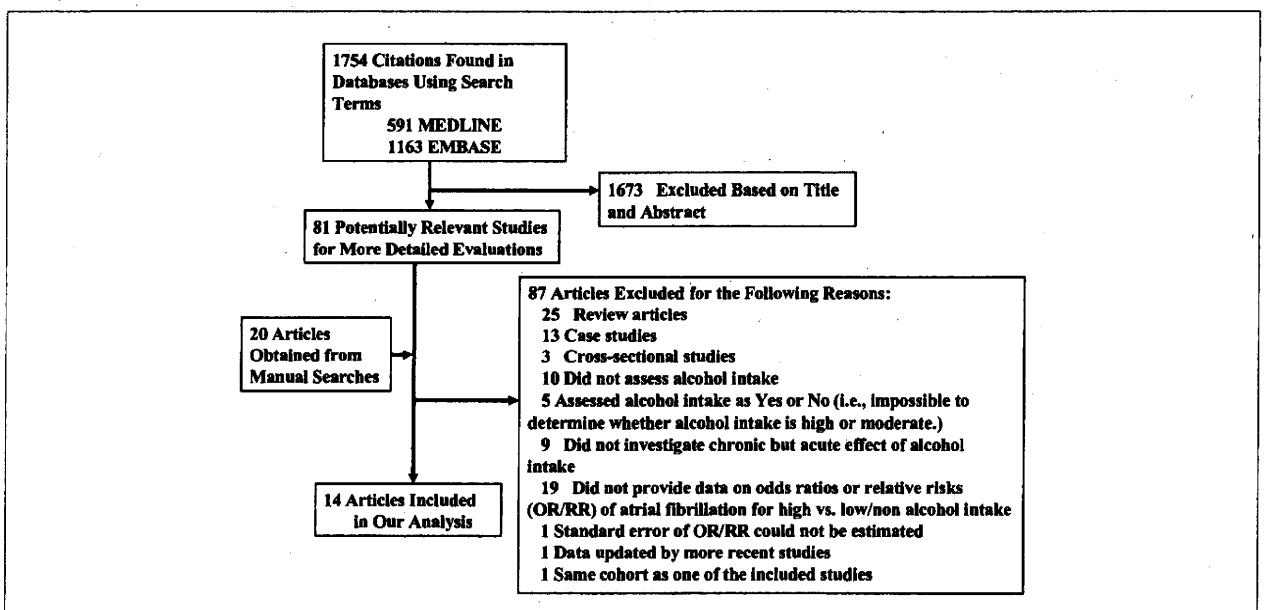

Figure 1 Study Flow Chart of Meta-Analysis

Table 1 Characteristics of Studies Included in the Meta-Analysis

Authors (Ref. #)		Year of Publication	Design	Selection of Population	Country	Age (yrs), Range (Mean)	% Men
Rich et al. (9)		1985	Case-control	Hospital-based	U.S.	18–70	76
Cohen et al. (10)		1988	Cohort	Population-based	U.S.	NA	NA
Krahn et al. (21)		1995	Cohort	Population-based	Canada	18–62 (31)	100
Wilhelmsen et al. (22)		2001	Cohort	Population-based	Sweden	47–55	100
Ruigomez et al. (23)		2002	Case-control	Hospital-based	Sweden	40–89	46
Djousse et al. (24)	Men	2004	Cohort	Population-based	U.S.	28–62	100
	Women						0
Frost and Vestergaard (25)	Men	2004	Cohort	Hospital-based	Denmark	50–64	100
	Women						0
Mattioli et al. (11)		2005	Case-control	Population-based	Italy	54	74
Mukamal et al. (26)	Men	2005	Cohort	Population-based	U.S.	26–75 (51)	100
	Women					26–73 (52)	0
Ruigomez et al. (27)	PAF	2005	Case-control	Hospital-based	Sweden	40–89	47
	Chronic		Cohort	Hospital-based		40–89	49
Planas et al. (28)		2006	Cohort	Hospital-based	Spain	53	64
Mukamal et al. (29)		2007	Cohort	Population-based	U.S.	≥65	42
Conen et al. (30)		2008	Cohort	Population-based	Switzerland	≥45 (53)	0
Marcus et al. (31)		2008	Case-control	Both	U.S.	53	75

		Were Patients With Heart Disease Excluded?	PAF Dominant or Chronic-AF Dominant	Was Method to Distinguish PAF From Chronic AF Described?	Onset or Recurrent AF	Was Atrial Flutter Among AF Events Included?	Method of Ascertaining AF	Method for Assessment of Exposure
Rich et al. (9)		Yes	PAF	Yes	Onset	No	Medical records	Medical records
Cohen et al. (10)		No	Chronic	No	Onset	No	Medical records	Questionnaires
Krahn et al. (21)		No	Chronic	No	Onset	No	ECG screening or physicians' report	Medical records
Wilhelmsen et al. (22)		No	Chronic	No	Onset	No	Registries	Questionnaires
Ruigomez et al. (23)		No	Chronic	Yes	Onset	No	Registries	Medical records
Djousse et al. (24)		No	Chronic	No	Onset	Yes	ECG screening	Questionnaires
Frost and Vestergaard (25)		Yes	Chronic	No	Onset	Yes	Registries	Questionnaires
Mattioli et al. (11)		Yes	PAF	Yes	Onset	No	Medical records	Questionnaires
Mukamal et al. (26)		Yes	Chronic	No	Onset	Yes	ECG screening, medical records, Registries	Interviews
Ruigomez et al. (27)	PAF	No	PAF	Yes	Onset	No	Registries	Medical records
	Chronic	No	Chronic		Recurrent	No	Registries	
Planas et al. (28)		Yes	PAF	Yes	Recurrent	No	ECG screening	Medical records
Mukamal et al. (29)		No	Chronic	No	Onset	Yes	ECG screening, medical records, Registries	Questionnaires
Conen et al. (30)		Yes	Chronic	No	Onset	No	Participants' reports	Questionnaires
Marcus et al. (31)		No	PAF	No	Onset	No	Medical records	Interviews

Continued on next page

Characteristics of the 14 included studies are shown in Table 1. Nine studies used a cohort design, 4 studies used a case-control design, and 1 study reported data from both case-control and cohort designs. All studies were conducted in Western countries (7 in Europe and 7 in North America).

For assessing daily alcohol consumption, only 4 of the 14 studies (25,26,29,31) validated methods to assess alcohol intake. All effect measures were controlled for age and sex, and most of the included reports (10 studies) made adjustments for heart disease, which potentially elevates the risk of AF, or excluded participants with heart disease. Only 3 studies (10,29,31) considered racial differences among the participants.

Risk of AF through heavy alcohol consumption. The lowest amount of alcohol consumed in the highest category in each study ranged from 1.5 to 6 drinks per day. Consequently, in all 14 studies, the criteria for heavy alcohol drinking as previously defined were met (12). Three studies reported separate results according to sex. One study analyzed 2 populations separately according to age (≤60 years or >60 years), and 1 study indicated 2 risk measures (1 for PAF and another for the progression from an AF episode to permanent AF). Finally, 19 effect measures were analyzed to

Table 1 Continued

		Category of Alcohol Intake (Ethanol Consumption)	No. of Cases	No. of Participants	Duration (yrs)*
Rich et al. (9)		>70 ml/day or not	58	116	—
Cohen et al. (10)		6 or more drinks/day or <1 drink/day	28	3,966	—
Krahn et al. (21)		Self- and physician-reported alcoholism or not	299	3,983	44
Wilhelmsen et al. (22)		Alcohol abuse or not	754	7,495	25.2
Ruigomez et al. (23)		None, 1–5, 6–15, 16–42, >42 U/week (1 U = 10 ml)	1,035	6,035	—
Djousse et al. (24)	Men	None, 0.1–12, 12.1–24, 24.1–36, >36 g/day	544	2,921	≥24
	Women		511	2,806	
Frost and Vestergaard (25)	Men	Quintile (4.1, 12.1, 20.0, 36.1, 68.7 g/day)	374	22,528	5.7
	Women	Quintile (1.1, 4.6, 9.4, 15.6, 38.8 g/day)	182	25,421	5.8
Mattioli et al. (11)		0, 1–20, 21–50, >50 ml/day	116	232	—
Mukamal et al. (26)	Men	<1, 1–6, 7–13, 14–20, 21–27, 28–34, >34 drinks/week (1 drink = 12 g)	548	7,588	16.3
	Women	<1, 1–6, 7–13, 14–20, >20 drinks/week (1 drink = 12 g)	523	8,827	18.8
Ruigomez et al. (27)	PAF	None, 1–7, 8–21, 21 U/week (1 U = 10 ml)	525	5,525	—
	Chronic		70	418	2.7
Planas et al. (28)		>40 g/day or not (men); >20 g/day or not (women)	32	115	2.5
Mukamal et al. (29)		None, former, <1, 1–6, 7–13, >13 Drinks/week (1 drink = 13.3 g)	1,232	5,609	9.1
Conen et al. (30)		None, <1, 1–2, >2 drinks/day (1 drink = 15 g)	653	34,175	12.4
Marcus et al. (31)		>1.5 drinks/day or not	74	260	—

		Age/Sex	Smoking	Study BMI or WC	Confounders SBP or HT	Heart Disease†	Other Control Variables	Total No. of Control Variables
Rich et al. (9)		✓				✓		2
Cohen et al. (10)		✓	✓				Race	3
Krahn et al. (21)		✓						1
Wilhelmsen et al. (22)		✓						1
Ruigomez et al. (23)		✓				✓		2
Djousse et al. (24)		✓			✓	✓		3
Frost and Vestergaard (25)		✓	✓	✓	✓	✓		5
Mattioli et al. (11)		✓				✓		2
Mukamal et al. (26)		✓	✓	✓	✓	✓	Education, income, diabetes, physical activity, respiratory function	10
Ruigomez et al. (27)	PAF	✓						1
	Chronic							
Planas et al. (28)		✓			✓	✓		3
Mukamal et al. (29)		✓		✓	✓	✓	Race, income, diabetes, use of psychoactive medication	8
Conen et al. (30)		✓	✓	✓	✓	✓	Diabetes	6
Marcus et al. (31)		✓		✓	✓	✓	Race	5

*Duration is the duration between the time point of alcohol consumption and subsequent observation. †Cardiac function (e.g., left ventricular end-systolic volume, left atrial size) was involved in heart disease.

AF = atrial fibrillation; BMI = body mass index; ECG = electrocardiogram; HT = hypertension; NA = not available; PAF = paroxysmal atrial fibrillation; SBP = systolic blood pressure; WC = waist circumference.

summarize the risk of AF in relation to heavy alcohol intake. The pooled estimate of OR/RR for the highest category of alcohol consumption compared with the lowest category in individual studies was 1.51 (95% CI: 1.31 to 1.74) (Fig. 2).

There was significant between-study heterogeneity in the effect measures (Q-squared, 33.2; I-squared, 45.8%; p = 0.02). Table 2 shows results of stratified and meta-regression analyses across a number of key study characteristics to explore causes of the study heterogeneity. On the whole, a positive association between AF risk and heavy alcohol consumption was consistently found in all stratified analyses.

In the stratified analysis by mean alcohol intake in the highest intake group, the pooled estimates of AF for <4 drinks per day, or 48 g per day, and ≥4 drinks per day was 1.32 (95% CI: 1.15 to 1.50) and 1.74 (95% CI: 1.35 to 2.24), respectively. However, the difference was not significant (p = 0.17). When analyses were limited to the 6 studies that regarded nondrinkers as the referent group, the

JACC Vol. 57, No. 4, 2011
January 25, 2011:427–36

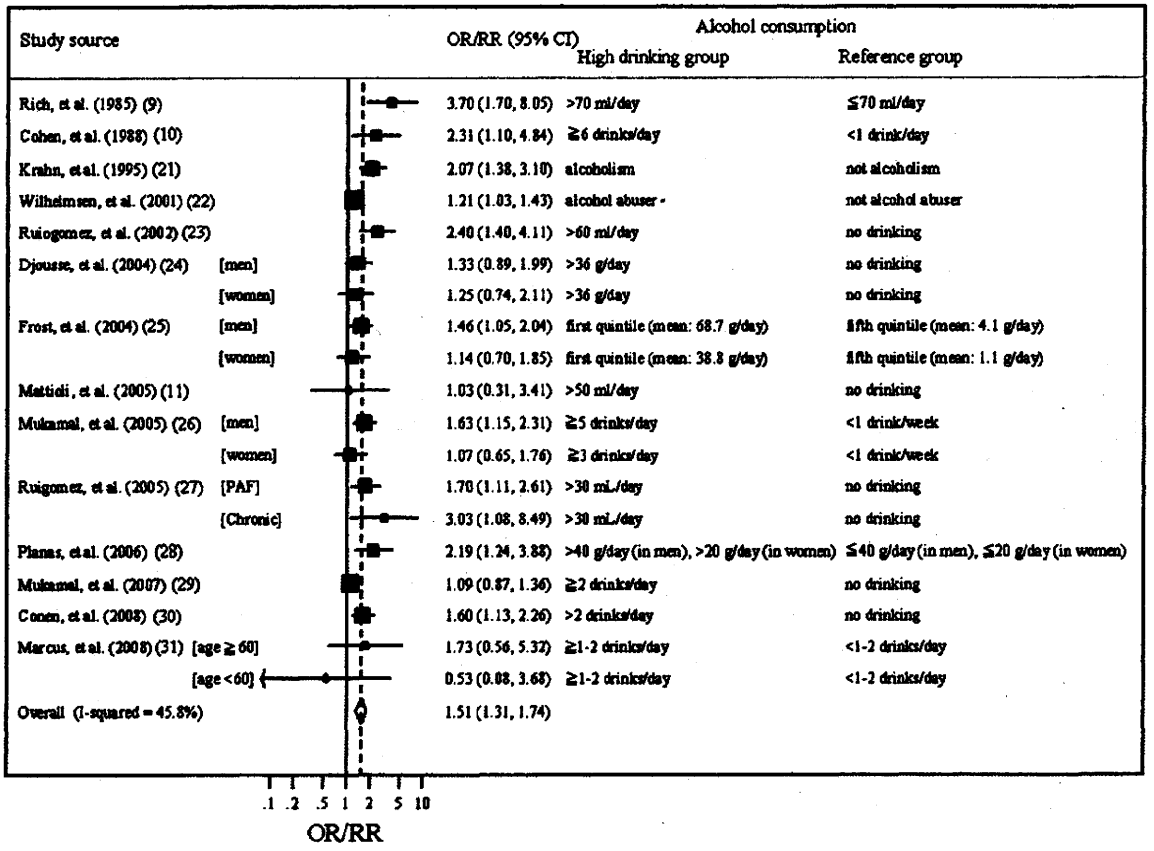

Figure 2 **OR/RR and 95% CI of AF by Alcohol Intake Group**

Odds ratio (OR) or relative risk (RR) and 95% confidence interval (CI) of atrial fibrillation (AF) for highest versus lowest alcohol intake groups. **Size of squares** reflects the statistical weight of each study. Overall estimate is indicated by **unshaded diamond**. PAF = paroxysmal atrial fibrillation.

pooled estimate for the highest category was 1.36 (95% CI: 1.18 to 1.57).

Stratified analysis by geographic region, participants' sex, or whether persons with heart disease were included did not show any significant difference in pooled estimates between strata. Using a case-control design seemed to produce a substantially larger AF risk (pooled estimates 1.98 [95% CI: 1.49 to 1.63]) compared with the use of other designs (pooled estimate 1.34 [95% CI: 1.22 to 1.47]). However, these differences were not borderline significant ($p = 0.06$). Selecting hospital-based participants produced a borderline significantly larger pooled estimate in comparison with population-based participants (pooled estimate 1.75 [95% CI: 1.45 to 2.11] vs. 1.30 [95% CI: 1.18 to 1.44]; $p = 0.049$).

Strong associations were observed when the type of AF end point in the study was PAF-dominant (pooled estimate 1.92 [95% CI: 1.44 to 2.56]) or AF recurrence (pooled estimate 2.37 [95% CI: 1.44 to 3.90]) whereas a significantly weaker association was observed in studies that included atrial flutter as a study end point (pooled estimate 1.25 [95% CI: 1.10 to 1.43]) than in those that did not (pooled estimate 1.83 [95% CI: 1.45 to 2.30]; $p = 0.02$).

The method for ascertainment of AF did not significantly affect the magnitude of the association between high alcohol consumption and AF risk. However, a significantly stronger association was observed when reviewed data on alcohol intake were based on medical records or registries (pooled estimate 2.17 [95% CI: 1.74 to 2.70]) compared with other methods of determining alcohol consumption, such as questionnaires or interviews (pooled estimate 1.28 [95% CI: 1.16 to 1.41]; $p < 0.001$). The influence of study adjustments for possible confounders was not significant, although AF effect measures were attenuated with adjustment for hypertension or blood pressure (pooled estimate 1.33 [95% CI: 1.17 to 1.50]).

Publication bias was visually suggested by the asymmetrical funnel plot of the reported results (Fig. 3), which was also statistically supported by Egger's test ($p = 0.03$) but not Begg's test ($p = 0.31$). We attempted to adjust for this publication bias using the trim and fill method (18). After 4

Table 2 Stratified Analyses of Pooled Relative Risk of Atrial Fibrillation for Highest Alcohol Intake Versus Lowest Alcohol Intake

Variable	No. of Data Units	Risk Estimates (95% CI)	Q Statistics	I-Squared	p Value of Heterogeneity	Meta-Regression*
Mean estimated alcohol intake of exposed group						
<4 drinks (48 g) per day	11	1.32 (1.15–1.50)	13.1	23.6%	0.22	Referent
≥4 drinks (48 g) per day or alcohol abuser	8	1.74 (1.35–2.24)	18.8	62.8%	0.009	0.16
Drinking status of referent group						
Nondrinkers	8	1.36 (1.18–1.57)	12.6	44.6%	0.08	Referent
Light drinkers	11	1.56 (1.27–1.91)	20.5	51.1%	0.03	0.73
Design						
Cohort	13	1.34 (1.22–1.47)	20.4	41.1%	0.06	Referent
Case-control	6	1.98 (1.49–2.63)	6.5	22.5%	0.26	0.06
Selection of study population						
Population-based	10	1.30 (1.18–1.44)	14.3	37.0%	0.11	Referent
Hospital-based	7	1.75 (1.45–2.11)	10.8	44.2%	0.10	0.06
Both population- and hospital-based	2	1.28 (0.49–3.39)	1.1	6.7%	0.30	0.88
Geographic region						
North America	10	1.50 (1.19–1.90)	19.5	53.7%	0.02	Referent
Europe	9	1.40 (1.25–1.58)	13.7	41.8%	0.09	0.76
Sex						
Men	4	1.32 (1.06–1.64)	2.3	—	0.51	Referent
Women	5	1.37 (1.21–1.55)	7.3	45.0%	0.12	0.47
Men/women	10	1.85 (1.33–2.56)	22.8	60.5%	0.007	0.32
Excluding participants with heart disease						
No	11	1.50 (1.24–1.82)	20.5	51.3%	0.03	Referent
Yes	8	1.53 (1.31–1.80)	10.6	34.1%	0.16	0.79
Was atrial flutter included as AF outcome?						
No	12	1.83 (1.45–2.30)	23.8	53.9%	0.01	Referent
Yes	7	1.25 (1.10–1.43)	5.1	—	0.52	0.02
Type of AF outcome						
Chronic AF dominant	14	1.43 (1.24–1.66)	22.0	45.4%	0.04	Referent
PAF dominant	6	1.92 (1.44–2.56)	6.0	17.2%	0.30	0.11
Was AF outcome the first episode or recurrence?						
First episode	17	1.46 (1.27–1.69)	28.5	43.8%	0.03	Referent
Recurrence	2	2.37 (1.44–3.90)	0.3	—	0.59	0.10
Methods for assessment of alcohol intake						
Questionnaires	9	1.26 (1.13–1.40)	7.4	—	0.50	Referent
Interviews	4	1.41 (1.07–1.85)	3.0	—	0.39	0.45
Historical data reviews	6	2.17 (1.74–2.70)	3.7	—	0.60	<0.001
Methods for ascertainment of AF						
ECG screening	3	1.47 (1.12–1.95)	2.5	20.0%	0.29	Referent
Medical records	5	2.14 (1.38–3.31)	5.5	27.6%	0.24	0.28
Registries	6	1.35 (1.19–1.54)	10.2	51.1%	0.07	1.00
Others†	5	1.43 (1.11–1.85)	10.8	62.9%	0.03	0.83
Study adjustment						
Smoking						
No	13	1.58 (1.29–1.95)	28.0	57.2%	0.005	Referent
Yes	6	1.47 (1.24–1.73)	4.6	—	0.46	0.68
Obesity (BMI or waist circumference)						
No	11	1.76 (1.40–2.21)	23.3	57.1%	0.01	Referent
Yes	8	1.29 (1.13–1.48)	7.9	10.9%	0.34	0.07
SBP or HT						
No	9	1.93 (1.40–2.64)	20.8	66.3%	0.004	Referent
Yes	10	1.33 (1.17–1.50)	11.0	9.4%	0.35	0.06
Heart disease						
No	5	1.74 (1.24–2.43)	11.3	64.4%	0.02	Referent
Yes	14	1.39 (1.24–1.56)	22.0	40.8%	0.06	0.44

*Represents test for significance of the study modification across strata. †Participants' report or using combination of registry with electrocardiographic screening.
CI = confidence interval; other abbreviations as in Table 1.

JACC Vol. 57, No. 4, 2011
January 25, 2011:427–36

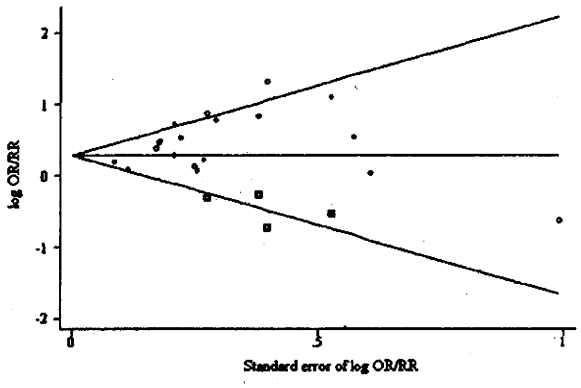

Figure 3 Funnel Plot of High Alcohol Consumption and AF

Funnel plot of high alcohol consumption and atrial fibrillation (AF) risk before and after adjustment for publication bias using trim and fill procedures. Hypothetical dummy studies indicated by **circles within squares** are added to the genuine studies indicated by **circles**. OR = odds ratio; RR = relative risk.

negative unpublished results were incorporated to produce a hypothetically symmetrical funnel plot, the pooled estimate of AF for heavy alcohol consumption was modestly attenuated to be 1.39 (95% CI: 1.19 to 1.62) but remained statistically significant ($p < 0.001$).

Dose-response relationship between alcohol intake and AF risk. Nine studies (10,11,23–27,29,30) involving 126,051 participants and 6,341 cases were eligible for analysis of the dose-response relationship between different categories of alcohol intake and AF risk. The alcohol dose in these studies ranged from 4.0 to 86.4 g per day. Figure 4 illustrates the linear and spline regression curves for AF risk related to daily alcohol intake. The linear dose-response curve showed a significant relationship between alcohol intake and AF risk ($R^2 = 0.43$, $p < 0.001$). The coefficient for the linear term was $7.4 \pm 1.3 \times 10^{-3}$, meaning that the incremental increase in relative risk of AF per 10 g alcohol consumption per day was $e^{10\times7.4\pm1.3\times10^{-3}} = 1.08$ (95% CI: 1.05 to 1.10). Also, in the spline regression model, AF risk significantly increased with larger daily alcohol consumption levels ($R^2 = 0.44$, $p < 0.001$). However, the fit of this model was not significantly different from that of the linear regression model ($p = 0.77$).

Discussion

Our study is the first to systematically review the literature on the association between alcohol consumption and the risk of AF. In this review, high alcohol intake was shown to be associated with a significant elevation in AF risk, both by overall analysis and across a number of stratified analyses based on key characteristics of study methods, although there was substantial study heterogeneity in the magnitude of AF risk, partly due to variability in study design and methodology. Even though, in principle, observational studies do not allow for proof of causality, there are several theoretically plausible speculations for the cause-effect relationship between high alcohol intake and the development of AF.

One speculation is based on biological findings that suggest a harmful effect of high alcohol intake on maintenance of normal heart rhythm, including the achievement of a hyperadrenergic state (32), impairment of vagal tone (33), direct effect on myocardial structure (34), and various electrophysiological changes in atrial cells (e.g., increase in intra-atrial conduction time represented by a length of the P-wave, reduction in the refractory period, negative inotropic effect through calcium-channel inhibition in ventricular cells) (31,35,36).

Another speculation is based on reports suggesting that the development of chronic heart failure accompanied by long-term excessive alcohol consumption may result in elevated AF risk (2). Particularly, dilated cardiomyopathy is typical of alcohol abusers with chronic heart failure. The average total lifetime alcohol consumption was reported to be significantly greater in patients with dilated cardiomyopathy than in a population-based control group (37). Moreover, alcoholics were found to have progressive dilated cardiomyopathy in proportion to the duration of heavy drinking even before the clinical appearance of chronic heart failure (38).

It remains to be established whether the dose-response relationship between daily alcohol consumption and AF risk is interpreted as linear or not (e.g., J-shaped curve, threshold curve). While we identified a linear association of daily alcohol consumption with the risk of AF, a J-shaped relation or threshold value was not observed from the

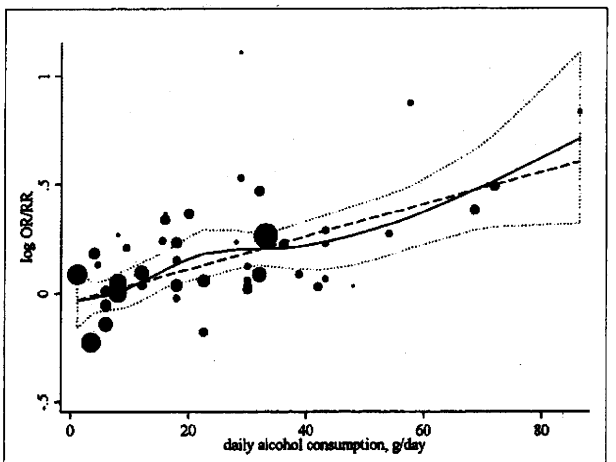

Figure 4 Regression of Natural Log OR/RR for Atrial Fibrillation on Daily Alcohol Consumption

The **solid curve** and its accompanying area indicate the log odds ratio or relative risk (OR/RR) and its corresponding 95% confidence interval based on a restricted cubic spline regression model with knots at 8, 22.5, and 33.1 g/day of alcohol consumption. This model did not significantly improve the fit compared with the linear regression model expressed by the **dotted line**. The area of each data point is proportional to its statistical weight.

current spline regression curve. These regression analyses suggest no evidence that moderate alcohol consumption is beneficial in ameliorating the risk of AF, unlike that of cardiovascular disease (7). If anything, moderate alcohol drinkers may have a greater risk of AF than nondrinkers, although the AF risk is not as large as that for heavy drinkers.

Study limitations. First, the definition of heavy drinking is heterogeneous across studies. Second, the majority of the included studies did not state whether the method used for assessment of alcohol intake was validated. Third, few studies considered racial differences among participants. Differences in ethnicity or proportion of whites and nonwhites among different studies might have affected AF risk estimates. Fourth, asymptomatic PAF could have been missed in any of these studies. Given that heavy alcohol drinkers are likely to have experienced PAF, the risk of AF in relation to high alcohol intake would be underestimated in any of the examined studies. Fifth, no studies have investigated the effect of different types of alcoholic beverages on AF risk, although it has been reported that wine has a better effect on cardiovascular disease (39). Sixth, it could not be ruled out whether a particular drinking pattern, such as whether alcohol was consumed with a specific food or at meals, could have contributed to the AF risk irrespective of alcohol dosage. For example, alcohol is usually consumed during meals, as in Mediterranean countries (39), which might explain a beneficial effect.

A meta-analysis cannot completely solve problems with confounders that vary from study to study. Lack of adjustment for possible confounders could also produce a superficially strong association between high alcohol consumption and AF risk. For example, a weaker association between high alcohol consumption and AF risk was observed when studies included blood pressure values or the presence of hypertension among study confounders. In fact, hypertension was reported to be an independent risk factor for AF (2), and the risk of hypertension increases linearly with alcohol consumption (40). Possibly, the AF risk associated with high alcohol consumption is partly explained by an alcohol-related development of hypertension. In addition, other factors that could not be specified by this meta-analysis or were not specified in the individual included studies might contribute to residual confounding (e.g., objective sleep apnea [41], diabetes mellitus [42]).

Lastly, results that indicated risk of AF could be biased by study design and other methodological features. For example, larger AF risk estimates were observed in studies having a case-control design compared with studies having a cohort design or in hospital-based studies compared with population-based studies. The AF risk could have been overestimated by exaggeration of alcohol intake in patients with AF in studies with a case-control design (i.e., recall bias) or an unavoidable reduction in alcohol intake as a result of a control subject having an illness in a hospital-based setting (i.e., selection bias). However, underestimation of AF risk is possible if patients did not truthfully report the full extent of alcohol intake when completing questionnaires.

Conclusions

Habitual heavy alcohol drinking is associated with an increased risk of AF, although several study limitations exist and must be recognized. The relationship between daily alcohol consumption and the risk of AF was explained by a linear dose-response model, suggesting that not consuming alcohol at all is the most favorable behavior for avoiding AF rather than moderate alcohol consumption. Further investigation is needed to establish the extent to which this association is explained by a causal relationship.

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Key Words: alcohol ■ atrial fibrillation ■ meta-analysis.

Atherosclerosis 210 (2010) 674–677

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Short communication

Are serum cholesterol levels associated with silent brain infarcts? The Seiryo Clinic Study

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ARTICLE INFO

Article history:
Received 16 August 2009
Received in revised form
25 December 2009
Accepted 1 January 2010
Available online 22 January 2010

Keywords:
Cerebral infarction
Magnetic resonance imaging
Cholesterol
Multivariate analysis

ABSTRACT

Objective: High levels of serum cholesterol are associated with the risk of stroke. However, the association of serum cholesterol with silent brain infarcts (SBIs) is unclear. We investigated the association between SBI and various clinical factors.
Methods: We conducted a cross-sectional study that included 324 apparently healthy Japanese men (mean age 53.8 ± 9.2 years). Combinations of three types of scan (T1-weighted, T2-weighted and FLAIR images) were used to detect and discriminate SBI.
Results: Serum cholesterol was significantly associated with SBI [total cholesterol, odds ratio (OR) 3.75 (95% confidence interval (CI) 1.45–9.68); LDL-cholesterol, OR 2.54 (95% CI 1.03–6.27), and non-HDL-cholesterol, OR 2.54 (95% CI 1.03–6.27)] after adjustment for age, smoking status, serum triglycerides, maximal-intima-media thickness, obesity, hypertension, diabetes mellitus, hyperuricemia, coronary heart disease and lipid-lowering agent use.
Conclusion: Our cross-sectional data suggest that serum cholesterol levels are associated with SBI independently of known confounders.

1. Introduction

The significance of detecting silent brain infarction (SBI) and its associated factors is increasing in the primary prevention of stroke and dementia, which threaten quality of life [1]. Although increased total cholesterol (TC) levels are known to be associated with a higher risk of ischemic stroke [2], it remains unclear whether SBI is associated with serum cholesterol [1]. The aim of this study was to investigate factors associated with the development of SBI.

2. Methods

Subjects of the Seiryo Clinic Study were a cohort of apparently healthy Japanese men, aged 30–80 years who consecutively had undergone MRI of the brain as part of a health check-up between May 2000 and April 2008 in northern Japan. From the 483 persons who had MRI, measurements of maximal-intima-media thickness (maximal-IMT) and blood pressure, as well as blood tests, we excluded women ($n = 137$) and all men who had symptomatic stroke ($n = 2$), epilepsia ($n = 1$), a space-occupying lesion shown on brain MRI due to brain tumor ($n = 2$), pituitary adenomas ($n = 2$), vascular malformations ($n = 2$), venous angioma ($n = 1$), meningioma ($n = 1$), microbleeding ($n = 1$), brain contusion ($n = 1$), brain injury ($n = 1$) or serum triglyceride (TG) ≥4.52 mmol/L ($n = 8$). Ultimately, 324 men were analyzed.

Blood pressure was measured in a resting state while sitting, and body mass index (BMI) was calculated by weight [kg]/(height [m])^2. The blood test was performed under fasting conditions. Serum TC and HDL-cholesterol, trygricerides, glucose and uric acid were measured by an autoanalyzer. Serum LDL-cholesterol was calculated by the Friedewald equation [3], and non-HDL-cholesterol was determined by subtracting HDL-cholesterol from TC.

Carotid arteries were evaluated independently by two trained technicians using high-resolution B-mode ultrasonography SSA-550A (Toshiba, Tokyo, Japan). The technicians were also masked to clinical information. Four segments of the common carotid artery and of the internal carotid artery at the near and far wall on both left and right sides were examined [4] and maximal-intima-media thickness (maximal-IMT) was obtained.

The following data were obtained by a questionnaire completed by the participant: smoking habit, medication and medical history.

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doi:10.1016/j.atherosclerosis.2010.01.008

Table 1
Clinical characteristics of the 324 study men according to the presence of silent brain infarcts.

	All (n=324)	With SBI (n=17)	Without SBI (n=307)	P
Age, year	53.8±9.2	63.1±7.2	53.3±9.0	<0.001
Body mass index, kg/m^2	24.6±3.1	25.4±3.4	24.5±3.1	0.28
Obesity (body mass index ≥25)	130 (40%)	8 (47%)	122 (40%)	0.55
Systolic blood pressure, mmHg	121±15	132±11	120±15	0.001
Diastolic blood pressure, mmHg	75.4±9.9	79.6±7.7	75.1±10.0	0.07
Hypertension	102 (31%)	12 (71%)	90 (29%)	<0.001
Fasting blood sugar, mmol/L	5.5±1.2	5.6±0.6	5.5±1.2	0.89
Diabetes mellitus	38 (12%)	2 (12%)	36 (12%)	1.00
Total cholesterol, mmol/L	5.19±0.80	5.32±0.81	5.18±0.80	0.50
LDL-cholesterol, mmol/L	3.48±0.77	3.57±0.73	3.47±0.78	0.59
Non-HDL-cholesterol, mmol/L	3.77±0.83	3.84±0.74	3.76±0.83	0.71
HDL-cholesterol, mmol/L	1.43±0.37	1.48±0.37	1.42±0.36	0.54
Triglycerides, mmol/L	1.45±0.75	1.33±0.56	1.45±0.76	0.50
Use of lipid-lowering agents	26 (8%)	1 (6%)	25 (8%)	0.73
Uric acid, μmol/L	357±70	378±62	356±71	0.20
Hyperuricemia	82 (25%)	7 (41%)	75 (24%)	0.14
Maximal-IMT, mm	1.44±0.75	2.03±0.63	1.41±0.74	<0.001
Maximal-IMT, top tertile (≥1.6 mm)	112 (35%)	14 (82%)	98 (32%)	<0.001
Current smoker	100 (31%)	6 (35%)	94 (31%)	0.69
Coronary heart disease	8 (2%)	2 (12%)	6 (2%)	0.06

Values are means ± S.D. or numbers (percentages). P values were obtained using the analysis of variance for continuous variables and chi-square test for categorical variables.

Table 2
Logistic regression model to investigate the clinical factors associated with the presence of silent brain infarcts according to each cholesterol levels in Japanese men.

Variables	Odd ratio (95% confidence interval)			
	Model 1	Model 2	Model 3	Model 4
Total cholesterol, per mmol/L	3.75 (1.45–9.68)**			
LDL-cholesterol, per mmol/L		2.54 (1.03–6.27)*		
Non-HDL-cholesterol, per mmol/L			2.54 (1.03–6.27)*	
HDL-cholesterol, per mmol/L				4.52 (0.97–21.0)
Triglycerides, per mmol/L	0.58 (0.19–1.73)	0.67 (0.23–1.97)	0.56 (0.18–1.76)	1.36 (0.48–3.84)
Use of lipid-lowering agent (Yes vs No)	0.54 (0.05–6.27)	0.42 (0.04–4.38)	0.42 (0.04–4.38)	0.52 (0.04–5.77)
Age, per year	1.15 (1.05–1.25)**	1.12 (1.04–1.21)**	1.12 (1.04–1.21)**	1.12 (1.03–1.21)**
Obesity (body mass index ≥25 kg/m^2 vs <25 kg/m^2)	0.98 (0.29–3.29)	0.81 (0.25–2.70)	0.81 (0.25–2.70)	1.12 (0.32–3.91)
Maximal-IMT (≥1.6 mm vs <1.6 mm) (highest tertile vs middle or lowest tertiles)	6.48 (1.33–31.6)*	5.03 (1.14–22.3)*	5.03 (1.14–22.3)*	5.51 (1.31–23.1)*
Hypertension (Yes vs No)	7.03 (1.77–28.0)**	5.46 (1.51–19.8)**	5.46 (1.51–19.8)**	3.98 (1.19–13.3)*
Diabetes mellitus (Yes vs No)	0.46 (0.07–2.83)	0.55 (0.09–3.30)	0.55 (0.09–3.30)	0.38 (0.05–2.60)
Hyperuricemia (Yes vs No)	3.46 (0.97–12.3)	3.01 (0.87–10.4)	3.01 (0.87–10.4)	2.66 (0.80–8.88)
Coronary heart disease (Yes vs No)	3.40 (0.38–30.5)	3.33 (0.42–26.2)	3.33 (0.42–26.2)	3.46 (0.38–31.5)
Current smoker (vs never and former smoker)	4.13 (1.07–15.9)*	3.15 (0.87–11.4)	3.15 (0.87–11.4)	2.60 (0.76–8.91)

Odds ratios were adjusted for all of the other covariates listed in Table 2.
* P<0.05.
** P<0.01.

No subject had a previous transient ischemic attack nor was atrial fibrillation (AF) shown by electrocardiogram.

Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg or diastolic blood pressure (DBP) ≥90 mmHg, a previous diagnosis of hypertension, or treatment with antihypertensive medications agents. Diabetes mellitus was defined as fasting blood sugar ≥126 mg/dL (7 mmol/L), a previous diagnosis of diabetes mellitus or the use of antidiabetic agents. Hyperuricemia was defined as uric acid ≥7 mg/dL (416 μmol/L), use of medication for hyperuricemia or a history of hyperuricemia. Smoking status was defined as current smoker and past or never smoker. Coronary heart disease was defined as a history or the presence of myocardial infarction or angina pectoris.

SBI and other brain lesions were assessed by a SIGNA 1.5T MRI scanner (GE Healthcare, Waukesha, WI) with T1-weighted, T2-weighted and FLAIR (fluid attenuated inversion recovery) scans with an axial AC-PC line, 5-mm slice thickness and a 2-mm interslice gap. We defined SBI according to the criteria of the Japanese Society for the Detection of Asymptomatic Brain Disease [5]: namely, a focal hyperintensity area ≥2 mm in diameter on T2-weighted images and low intensity on T1-weighted images. We also used a combination of the three types of scan to discriminate SBI from white matter lesions [6] or dilated perivascular spaces. Two board-certified radiologists who were masked to clinical data independently assessed the images. Final decisions were made by including two or more radiologists for consensus.

To examine the potential risk factors associated with SBI, logistic regression analysis was performed by entering all possible confounders determined by biological measurements and results of the questionnaire as explanatory variables. A two-sided P value of less than 0.05 was statistically significant. All analyses were performed with SPSS (Ver.15.0J for Windows, Chicago, IL) software. The institutional review board of this institution approved this study.

3. Results

Seventeen participants (5.2%) had SBI. A total of 31 infarcts were detected, with locations in the basal ganglia (77%,) subcortical area (17%), and cerebellum (6%). No infarcts were noted in the cortical area.

Table 1 shows baseline characteristics of participants with and without SBI. Participants with SBI were significantly older and had higher blood pressure and maximal-IMT values than those without SBI. Odds ratios (OR) of increased risk of SBI per unit IMT, SBP

and DBP were 2.09 (95% confidence interval (CI) 1.30–3.35, *P* value 0.002), 1.05 (95% CI 1.02–1.09, *P* value 0.002), and 1.07 (95% CI 0.99–1.09, *P* value 0.074), respectively, by univariate analysis.

Results of logistic regression analyses to examine the possible factors associated with SBI are shown in Table 2. Serum cholesterol (TC, LDL and Non-HDL) levels were significantly associated with SBI (*P* value, 0.006 for TC; 0.042 for LDL-cholesterol; 0.042 for non-HDL-cholesterol) after adjustment for age, smoking status, triglycerides, obesity, highest tertile of maximal-IMT (≥1.6 mm) and history of hypertension, diabetes mellitus, hyperuricemia, coronary heart disease and use of lipid-lowering agents in the multivariate analysis (Models 1, 2 and 3). There was a tendency for a positive association of HDL-cholesterol with SBI (*P* value, 0.06) (Model 4).

4. Discussion

Our results showed an independent association between serum cholesterol levels and SBI after controlling for classical risk factors and IMT. This supports results of a recent study [7]; however, most previous studies have not indicated a possible association between cholesterol values and the presence of SBI [1,8,9].

Hypertension is known to be strongly associated with SBI [1]. We also found significant association between hypertension and SBI. However, we simultaneously found significant association between serum cholesterol levels and SBI. Results of a meta-analysis [10] indicated that TC was significantly associated with ischemic stroke in subjects with normal blood pressure (SBP ≤125 mmHg). Consequently, this might account for our findings, because the average SBP of our subjects was 121 mmHg. In contrast, the association between SBI and IMT has been established [4,8,9].

In population studies in the United States [8] and Europe [9], SBI included not only non-cortical but also cortical lesions. In addition, those study participants were older than those in the present study and a few percent had AF. In contrast, our subjects, who had undergone health screening, had neither cortical infarcts nor AF. Most of the infarcts detected were in the basal ganglia. We speculate that the inconsistency between our results and those of others was due to differences in the brain infarction site, as well as in age and race, which could influence the association of serum cholesterol with SBI.

Uehara [11] suggested dyslipidemia as a potential associated factor for SBI in the basal ganglia, not in white matter. In subjects who use antihypertensive agents, LDL-3 levels are associated with the prevalence of silent lacunar infarcts in basal ganglia not in white matter [12]. Therefore, cholesterol is possibly that serum cholesterol is associated more with non-cortical infarcts, especially in basal ganglia.

In this study, HDL-cholesterol tended to be positively associated with SBI. However, the TC-to-HDL-cholesterol ratio (TC/HDL-C) was not significantly associated with SBI [OR 0.92 (95% CI 0.42–2.03), *P* value 0.84]. Although a recent study recommended the usefulness of TC/HDL-C for predicting stroke [2], SBI was not associated with TC/HDL-C.

Our study has one strong point: SBI was evaluated by a combination of three images involving FLAIR to ensure a higher reliability of discrimination [6]. In most previous studies, a lack of proton density or FLAIR images for diagnosis of SBI might have reduced the specificity [13].

We should address several limitations. First, the relatively small number of participants resulted in a small number of cases with SBI. Second, our results cannot be applied to women, as women were excluded because too few attended the health screening for an investigation by multivariate analysis. Third, our subjects may not have been representative of the general population because of selection bias. These participants might have been more concerned about their health than the general male population, thus allowing more early detection of hypertension and diabetes mellitus. Fourth, although obstructive sleep apnoea is a risk factor for SBI, we did not investigate its association with SBI. Fifth, we could not elucidate risk factors because of the cross-sectional study design.

Additionally, we unfortunately did not evaluate serum lipoprotein (a) [Lp (a)], although Lp (a), which has a structure like LDL-cholesterol, has been reported to be associated with lacunar infarcts defined by MRI [14]. Therefore, we cannot predict the extent to which Lp (a) confounded the relationship between cholesterol and SBI and it is possible that the observed significant association between cholesterol and SBI could be partially explained by Lp (a).

In conclusion, our cross-sectional data suggest that serum cholesterol is a key factor in the development of SBI. From these findings, evaluating serum cholesterol levels together with brain MRI might be recommended in the primary prevention of SBI as well as prevention of symptomatic infarction. Further studies are necessary to clarify what characteristics in a population and what infarction sites would indicate that serum cholesterol is strongly associated with SBI.

Acknowledgements

We thank Dr. Akira Kushi and Dr. Jutaro Takahashi for contribution on the estimation of infarcts on MRI. Hiroko Uzurahashi and Yutaka Furukawa are acknowledged for their efforts in collecting data.

Contributors: Dr. Sone had full access to all of the data in the study and takes responsibility for the accuracy of the data analysis. Study concept and design is by Asumi, Yamaguchi, Saito, Kodama, Fukuda, Sone. Acquisition of data is by Asumi, Yamaguchi, Miyazawa, Matsui. Analysis and interpretation of data is by Asumi, Yamaguchi, Saito, Kodama, Miyazawa, Matsui, Suzuki, Fukuda, Sone. Drafting of the manuscript is done by Asumi, Yamaguchi, Sone. Critical revision of the manuscript for important intellectual content is by Asumi, Yamaguchi, Saito, Kodama, Miyazawa, Matsui, Suzuki, Fukuda, Sone. Statistical analysis is by Asumi, Yamaguchi, Saito, Kodama, Sone. Administrative, technical, or material support is by Asumi, Yamaguchi, Saito, Kodama, Miyazawa, Matsui, Fukuda, Sone and study supervision is by Fukuda, Sone.

Sources of funding: Dr. Sone and Dr. Kodama are recipients of a Grant-in-Aid for Scientific Research (#20300227) and Postdoctoral Research Fellowship respectively, both from the Japan Society for the Promotion of Science (JSPS). This study is also supported by the Ministry of Health, Labor and Welfare, Japan.

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Diabetologia
DOI 10.1007/s00125-010-2025-0

ARTICLE

Low transition rate from normo- and low microalbuminuria to proteinuria in Japanese type 2 diabetic individuals: the Japan Diabetes Complications Study (JDCS)

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Received: 30 July 2010 / Accepted: 30 November 2010

Abstract
Aims/hypothesis The aim of the study was to determine the transition rate and factors associated with the progression of normo- and low microalbuminuria to diabetic nephropathy (overt proteinuria).
Methods For 8 years we prospectively observed 1,558 Japanese patients with type 2 diabetes mellitus whose basal urinary albumin:creatinine ratio (UACR) had been measured as <17.0 mg/mmol at entry. The incidence of nephropathy (UACR >33.9 mg/mmol) was determined by measuring UACR twice a year.
Results Progression to nephropathy occurred in 74 patients. The annual transition rate was 0.67%, and was substantially higher for the low-microalbuminuric group than for the normoalbuminuric group (1.85% and 0.23%, respectively; hazard ratio for the low-microalbuminuric group 8.45, $p<0.01$). The hazard ratio for an HbA_{1c} of 7–9% or ≥9% was 2.72 ($p<0.01$) or 5.81 ($p<0.01$) relative to HbA_{1c} <7.0%, respectively. In comparison with individuals with a systolic blood pressure (SBP) of <120 mmHg, the hazard ratios for patients with an SBP of 120–140 mmHg or ≥140 mmHg were 2.31 ($p=0.06$) and 3.54 ($p<0.01$), respectively. Smoking also affected progression to proteinuria (hazard ratio 1.99, $p<0.01$). In contrast, 30.3% of the low-microalbuminuric group returned to normoalbuminuria (i.e. were in remission).
Conclusions/interpretation These results suggest that if patients with type 2 diabetes mellitus are receiving

Electronic supplementary material The online version of this article (doi:10.1007/s00125-010-2025-0) contains supplementary material, which is available to authorised users.

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Published online: 01 February 2011

treatment from diabetologists for hyperglycaemia and hypertension when they are in the early stages of nephropathy (i.e. normo- or low microalbuminuria), their rate of transition to proteinuria is considerably lowered, and that differentiating patients with low microalbuminuria from those with high microalbuminuria might be clinically useful.

Trial registration UMIN Clinical Trials Registry C000000222
Funding The study was funded by the Ministry of Health, Labour and Welfare, Japan.

Keywords Blood pressure · Diabetic nephropathy · Glycaemic control · Progression · Remission · Smoking

Abbreviations

ACE	Angiotensin-converting enzyme
ARB	Angiotensin receptor blocker
eGFR	Estimated glomerular filtration rate
ESRD	End-stage renal disease
INNOVATION	Incipient to Overt: Angiotensin II Receptor Blocker, Telmisartan, Investigation on Type 2 Diabetic Nephropathy
JDCS	Japan Diabetes Complications Study
SBP	Systolic blood pressure
UACR	Urinary albumin/creatinine ratio
UKPDS	UK Prospective Diabetes Study

Introduction

Diabetic nephropathy is the most common cause of end-stage renal disease (ESRD) in many countries, including Japan [1–3]. In the UK Prospective Diabetes Study (UKPDS), 24.9% of patients developed microalbuminuria within 10 years of diagnosis of type 2 diabetes, but only 0.8% developed ESRD, as assessed by an elevated plasma creatinine level (>250 μmol/l) or the need for renal replacement therapy [4]. Annual rates of transition between successive stages within the classic paradigm of normoalbuminuria to microalbuminuria to macroalbuminuria to ESRD were 2–3% per year [4].

In Japan, the number of patients requiring renal replacement therapy has increased threefold in less than 15 years [3]. Among 36,017 patients who started haemodialysis in 2007, the number of diabetic patients has reached 15,663 (43.5%) [3]. In Hong Kong, the overall number of people receiving renal replacement therapy increased by 50% between 1995 and 1999, and in the diabetic group, a 100% increase was observed [5]. Thus, Asians have a predisposition to diabetic nephropathy and ESRD. In fact, the recent Japanese Incipient to Overt: Angiotensin II Receptor Blocker, Telmisartan, Investigation on Type 2 Diabetic Nephropathy (INNOVATION) trial revealed that about 50% of diabetic individuals with high microalbuminuria (urinary albumin/creatinine ratio [UACR] between 11.3 and 33.9 mg/mmol [100–300 mg/g]) progressed to proteinuria within 2 years [6], indicating that progression is very rapid once high microalbuminuria develops. On the other hand, intervention using angiotensin receptor blockers (ARBs) such as losartan or telmisartan seems to be very effective in Asians in comparison with Europeans [6, 7]. The Japan Diabetes Complications Study (JDCS) is a nationwide randomised controlled study of type 2 diabetic patients focusing on lifestyle modification [8, 9]. Although the status of control of most classic cardiovascular risk factors, including body weight, glycaemia, serum lipids and blood pressure, did not differ between the two groups during the study period, the incidence of stroke in the intensive lifestyle intervention group (0.55/100 patient-years) was significantly lower than in the control group (0.95/100 patient-years) by Kaplan–Meier analysis, while the incidence of nephropathy did not differ significantly between the groups [9]. Here, we report the rate of transition and factors associated with the development and/or progression of normo- and low microalbuminuria to diabetic nephropathy (overt proteinuria) in this JDCS cohort.

Methods

In 1996, 2,205 patients aged 40–70 years with previously diagnosed type 2 diabetes and HbA_{1c} levels of >6.5% were recruited and registered from 59 hospitals specialising in diabetes care. The protocol for the study, which was in accordance with the Declaration of Helsinki and the Ethical Guidelines for Clinical/Epidemiological Studies of the Japanese Ministry of Health, Labour and Welfare, received ethics approval from the institutional review boards of all the participating institutions. Written informed consent was obtained from all the patients enrolled. The inclusion criteria for participating patients have been described previously by Sone et al. [8]. A final total of 2,033 patients aged 58.5±6.9 years (mean ± SD) were included in the study, and their diabetes duration was 10.9±7.2 years.

The recruited patients were randomly allocated to either an intensive lifestyle intervention group or a conventional treatment group. Details of the intervention have been described previously by Sone et al. [8, 9]. We selected a cohort of 1,558 patients in whom the mean value of the two-spot UACR was <17.0 mg/mmol (150 mg/g) without microscopic haematuria or other clinical findings indicating other renal diseases. We followed this cohort for 8 years, and measured their body weight, waist/hip circumference

and blood pressure at least twice a year. Fasting plasma glucose, HbA_{1c}, serum lipids and serum creatinine levels were also determined twice a year. Spot UACR was also determined at least twice a year using the turbidimetric immunoassay to measure the urinary albumin concentration. We defined normoalbuminuria as a UACR of <3.4 mg/mmol (30 mg/g), and low microalbuminuria as a UACR of 3.4 to 17.0 mg/mmol (30 to 150 mg/g). Estimated glomerular filtration rate (eGFR) was calculated using serum creatinine levels according to the modification of diet in renal disease (MDRD) formula modified for Japanese populations [10].

Statistical analyses The primary endpoint for the nephropathy analysis was transition from normo- or low microalbuminuria to proteinuria (>33.9 mg/mmol [300 mg/g]) in two consecutive urine samples. Transition to proteinuria was summarised by the annual rate of transition to proteinuria and the remission proportion was defined as those patients whose mean UACR at the final two visits was <3.4 mg/mmol. Risk factors for proteinuria were explored by the following survival analysis methods. Univariate analyses were performed by the Kaplan–Meier method, logrank test, and univariate Cox regression with a 95% CI. Multivariate Cox regression was also used. The SAS software package (version 9.2, SAS Institute, Cary, NC, USA) was used for all analyses, with the level of significance set at $p<0.05$.

Results

Tables 1 and 2 give the baseline characteristics and glycaemic and blood pressure control at baseline, and at 4 and 8 years after the start of observation. As shown in Table 2, the proportion of patients who were receiving insulin injections increased from 20.7% to 41.9% over 8 years. The use of antihypertensive agents also increased over this period from 28.2% to 42.0%. In particular, usage of renin–angiotensin system inhibitors such as angiotensin-converting enzyme (ACE) inhibitors and/or ARBs increased from 12.3% to 28.4% over 8 years. The use of statins also increased from 20.5% to 31.1%. Over a median follow-up period of 7.98 years, 74 patients developed proteinuria. The annual transition rate was 0.67 per 100 person-years (95% CI 0.53–0.84). For the low-microalbuminuric group, the annual transition rate per 100 person-years was substantially higher than for the normoalbuminuric patients (1.85 [95% CI 1.43–2.41] and 0.23 [95% CI 0.14–0.36]), respectively. On the other hand, remission (i.e. normalisation) occurred in 137 (30.3%) of the 452 individuals with low microalbuminuria (Table 3).

Table 1 Baseline characteristics of 1,558 patients included in the nephropathy analysis

Variable	Mean ± SD[a]
n (men/women)	1,558 (813/745)
Age (years)	58.5±6.9
BMI (kg/m^2)	23.0±2.9
Waist (cm)	79.4±9.2
SBP (mmHg)	132.4±15.8
DBP (mmHg)	76.6±9.5
Fasting plasma glucose (mmol/l)	8.9±2.4
HbA_{1c} (%)	7.8±1.3
Duration of diabetes (years)	10.7±7.1
Serum total cholesterol (mmol/l)	5.19±0.89
Serum triacylglycerols (mmol/l)[b]	1.15±0.82
Serum HDL-cholesterol (mmol/l)	1.41±0.43
UACR (mg/mmol)[b]	1.8±3.0
eGFR (ml min^{-1} 1.73 m^{-2})[b]	81.3±32.1
Current/past/never smoker (%)	27/24/49
Ethanol intake: 0/1–38/≧38 g/day (%)	62/31/7

DBP, diastolic blood pressure

[a] Unless otherwise stated

[b] Median±interquartile range

Figure 1 shows the Kaplan–Meier curves for progression to overt nephropathy on the basis of UACR (Fig. 1a), HbA_{1c} level (Fig. 1b), systolic blood pressure (SBP, Fig. 1c) and smoking status (Fig. 1d). As can be seen, patients with higher UACR, higher HbA_{1c}, higher SBP or current smokers had a higher risk for progression to proteinuria. The hazard ratio for the low-microalbuminuric group was 8.45 ($p<0.01$) relative to the normoalbuminuric group. Stratification of eGFR to >90, 60–90 and <60 ml min^{-1} 1.73 m^{-2} did not predict progression to proteinuria. The hazard ratio of HbA_{1c} for a range of 7–9% or for ≥9% was 2.72 ($p<0.01$) or 5.81 ($p<0.01$) relative to an HbA_{1c} of <7%, respectively. In comparison with individuals with an SBP of <120 mmHg, the hazard ratio for patients with an SBP of 120–140 mmHg or ≥140 mmHg was 2.31 ($p=0.06$) and 3.54 ($p<0.01$), respectively. Smoking also affected progression to proteinuria, with a hazard ratio of 1.99 ($p<0.01$).

Table 4 shows risk factors for the development of proteinuria based on multivariate Cox regression analysis. All the factors shown to be significant by univariate analysis—UACR, HbA_{1c} level, SBP level and smoking status—were significantly associated with the development of proteinuria after adjustment for other clinical factors. Multivariate Cox regression analysis showed that the hazard ratio for use of ACE inhibitors and/or ARBs was 1.49 (95% CI 0.83–2.69, $p=0.19$) and that the hazard ratio for use of statins was 0.73 (95% CI 0.38–1.41, $p=0.35$) in relation to the progression to proteinuria.

Table 2 Measures of glycaemic and blood pressure control at the baseline and at 4 and 8 years after the start of intervention

Variable	Baseline	4 years after start of intervention	8 years after start of intervention
BMI (kg/m^2)	23.0±2.9	23.0±3.0	23.0±3.1
SBP (mmHg)	132.4±15.8	132.5±15.4	132.5±15.9
DBP (mmHg)	76.6±9.5	75.9±9.1	74.0±10.0
Fasting plasma glucose (mmol/l)	8.9±2.4	8.9±2.6	8.6±2.5
HbA_{1c} (%)	7.8±1.3	7.7±1.2	7.7±2.0
Hypoglycaemic agent (%)			
Any use	84.4	89.3	86.6
Insulin	20.7	30.1	41.9
Sulfonylurea	62.3	63.3	59.7
Alpha-glucosidase inhibitor	25.9	29.9	28.8
Biguanide	7.5	16.1	32.8
Insulin sensitiser	1.2	8.0	9.1
Antihypertensive agent (%)			
Any use	28.2	33.3	42.0
ACE inhibitor/ARB	12.3	16.6	28.4
Calcium-channel blocker	20.7	24.4	27.2
Diuretic	1.2	1.1	2.9
Other	6.0	7.1	8.6
Statin (%)	20.5	23.7	31.1

Each value is expressed as mean ± SD or percentage

DBP, diastolic blood pressure

Discussion

Based on the main result of the JDCS study, which was reported previously by Sone et al., the incidence of stroke in the intensive lifestyle intervention group was significantly lower, by 38%, than in the control group, while the incidence of nephropathy did not differ significantly between the groups [9]. Lifestyle intervention resulted in a small but significant temporary improvement of glycaemic control and only minimal changes in other known risk factors for diabetic complications, including blood pressure, indicating the difficulty of changing the lifestyle of patients with long-term diabetes. In this sense, patients who participated in this study could be considered as representative of the general population of patients with type 2 diabetes. This might explain why there was no difference in the incidence of diabetic nephropathy. The main finding of interest in this study was that the annual incidence of proteinuria was as low as 0.67% (0.67/100 person-years), in marked contrast to previous reports. In the UKPDS, the annual rates of transition from normoalbuminuria to microalbuminuria and from microalbuminuria to macroalbuminuria in newly diagnosed patients with type 2 diabetes were 2% and 2.8% per year, respectively [4]. Ravid et al. [11] reported higher progression rates in type 2 diabetic patients in Israel, i.e. 35% from normoalbuminuria to microalbuminuria and 16% from normoalbuminuria to macroalbuminuria during 7.8 years. In Pima Indians with normotensive type 2 diabetes, Nelson et al. [12] also reported that the rates of progression from normoalbuminuria to microalbuminuria and to macroalbuminuria during 4.7 years was 37.8% and 4.3%, respectively. In Japan, a clinic-based observational 6.8 year longitudinal study of 426 patients who developed diabetes before the age of 30 years revealed that the incidence of proteinuria developing from normoalbuminuria or microalbuminuria was 1.41/100 person-years [13]. In another Japanese clinic-based observational longitudinal study conducted for 6 years, 28% of 216 patients enrolled from 1996 to 1998 showed progression from microalbuminuria to proteinuria [14]. It is difficult to compare the annual incidence of proteinuria with that found in other studies because the stages of nephropathy

Table 3 Mean UACR measured at the final two visits stratified by the basal value

Basal UACR (mg/mmol)	Final UACR (mg/mmol)			
	<3.4	3.4–17.0	17.0–33.9	≥33.9
<3.4	817 (73.9)	244 (22.1)	27 (2.4)	18 (1.6)
3.4–17.0	137 (30.3)	203 (44.9)	56 (12.4)	56 (12.4)
Total	954 (61.2)	447 (28.7)	83 (5.3)	74 (4.8)

Data shown are *n* (%)

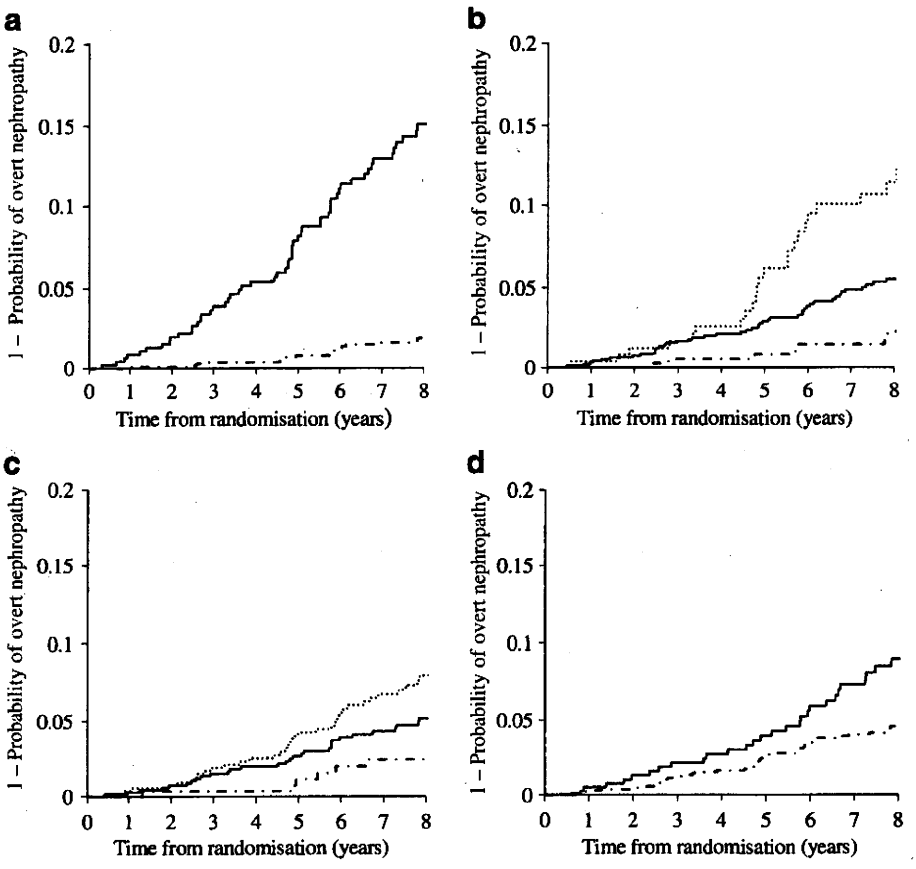

Fig. 1 Kaplan–Meier curves for progression to overt nephropathy according to: UACR (**a**), HbA_{1c} levels (**b**), SBP (**c**) and smoking status (**d**). **a** The hazard ratio for the low-microalbuminuric group (solid line) was 8.45 (95% CI 4.97–14.38, $p<0.01$) relative to the normoalbuminuric group (dashed–dotted line). **b** The hazard ratio of HbA_{1c} for a range of 7–9% (solid line) and for ≥9% (dotted line) was 2.72 (95% CI 1.22–6.03, $p<0.01$) and 5.81 (95% CI 2.49–13.55, $p<0.01$), respectively, relative to an HbA_{1c} of <7% (dashed–dotted line). **c** The hazard ratio for an SBP of 120–140 mmHg (solid line) or ≥140 mmHg (dotted line) was 2.31 (95% CI 0.96–5.54, $p<0.06$) and 3.54 (95% CI 1.50–8.40, $p<0.01$), respectively, relative to an SBP of <120 mmHg (dashed–dotted line). **d** The hazard ratio for current smoking (solid line) was 1.99 (95% CI 1.24–3.18, $p<0.01$) relative to past smoking or never smoked (dashed–dotted line)

differ from one study to another. However, the rate of transition to proteinuria in the JDCS seems to be very low. Of course, one of the reasons for this low incidence might be that two-thirds of the enrolled patients had normoalbuminuria and one-third had low microalbuminuria. In contrast, the placebo group in the INNOVATION trial showed a considerably higher transition rate, amounting to 50%, from high microalbuminuria to proteinuria within 2 years, with a UACR between 11.3 and 33.9 mg/mmol [6], although the UACR was determined using the first-voided

Table 4 Risk factors for progression to proteinuria demonstrated by multivariate Cox regression analysis

Risk factor	Hazard ratio	95% CI	*p* value
Conventional/intervention	1.01	0.63–1.61	0.98
Age, +10 years	1.03	0.71–1.49	0.87
Sex, woman/man	0.74	0.41–1.34	0.32
Duration, +10 years	1.16	0.80–1.68	0.44
BMI, +1 kg/m^2	1.01	0.93–1.10	0.73
SBP, 120–140/<120 mmHg	1.90	0.73–4.95	0.19
SBP, ≥140/<120 mmHg	2.55	0.98–6.63	0.05
HbA_{1c}, 7–9/<7%	2.22	1.00–4.96	0.05
HbA_{1c}, ≥9/<7%	4.16	1.73–10.04	<0.01
LDL-cholesterol, ≥4.0/<4.0 mmol/l	0.85	0.48–1.49	0.57
Triacylglycerol, ≥2.3/<2.3 mmol/l	1.60	0.88–2.89	0.12
HDL-cholesterol, ≥1.0/<1.0 mmol/l	1.43	0.79–2.61	0.24
UACR, ≥3.4/<3.4 mg/mmol	6.98	4.02–12.10	<0.01
Current smoker/past or never smoker	1.87	1.07–3.25	0.03
Ethanol intake, ≥38 g/<38 g/day	0.99	0.98–1.01	0.38

Missing values meant 126 patients were excluded

morning urine. Taken together with these studies, the data suggest that the current treatment by diabetologists along with administration of the usual hypoglycaemic and hypotensive drugs from the stage of normoalbuminuria or low microalbuminuria reduced the annual incidence of proteinuria to a level as low as 0.67/100 person-years. Ideally, however, the inclusion of a control group receiving placebo and matched to the drug-treated diabetic patients would be desirable in order to allow a firm conclusion to be drawn, although admittedly this would be ethically problematic. As the baseline UACR profoundly affected the cumulative incidence of proteinuria, it might be clinically useful to divide patients with microalbuminuria into low- and high-risk groups, i.e. those with low and high microalbuminuria, although the cut-off value remains to be determined.

In the present study, progression to proteinuria was independently associated with higher baseline HbA_{1c} and SBP levels in addition to an elevated baseline UACR. Furthermore, smoking was also a significant predictor of proteinuria. These results are consistent with previous studies [11, 15]. In the UKPDS, the risk factors most highly associated with proteinuria were reported to be urinary albumin, plasma creatinine, waist circumference, SBP, glycaemic control, LDL-cholesterol, and plasma triacylglycerol [15]. Indian-Asian ethnicity was also an independent risk factor for microalbuminuria and/or proteinuria [12, 15]. Smoking and male sex were reported to be independent predictors of proteinuria in addition to plasma cholesterol, mean blood pressure and HbA_{1c} [11]. Based on these epidemiological studies, tight glycaemic control has been reported to be effective for preventing the onset and/or progression of nephropathy in clinical trials such as the Diabetes Control and Complications Trial (DCCT), the Kumamoto study and the UKPDS [16–18]. Strict blood pressure control, especially with ACE inhibitors or ARBs, has also been demonstrated to be effective for delaying the progression of diabetic nephropathy [6, 7, 19–21]. However, in the present study, the initial usage of an ACE inhibitor and/or ARB, or statin was not significantly associated with the prevention of proteinuria. As this study was designed to clarify the effects of lifestyle intervention on subsequent occurrence of diabetic complications, it might have been difficult to recognise the effects of such drugs on the progression of diabetic nephropathy. In some studies, normalisation of microalbuminuria, i.e. remission/regression, has also been reported [6, 12]. In fact, in our study, 30.3% of 452 individuals with low microalbuminuria demonstrated normalisation.

However, following the advent of modern therapeutics, especially hypoglycaemic and antihypertensive agents, diabetic nephropathy is the most common cause of ESRD, and the number of patients being started on haemodialysis is still increasing dramatically in many countries, particularly in Asia. Our data have major clinical relevance because we have demonstrated that the initiation of hypoglycaemic and antihypertensive treatment from the early stage of nephropathy might lower the rate of transition to proteinuria even in the Japanese, who are highly susceptible to diabetic nephropathy. To reduce the number of patients who require haemodialysis, it is very important to measure UACR, make a diagnosis of diabetic nephropathy, define the stage of nephropathy and initiate strict glycaemic and blood pressure control as early as at the normo- or low-microalbuminuria stage.

Acknowledgements We thank the many diabetologists (the members of the JDCS Group are listed in the Electronic supplementary material [ESM]) and patients at the 59 participating institutions throughout Japan. Part of this study has been reported previously in abstract form ([2008] *Diabetes* 57 (Suppl 1):A210). This study was financially supported by the Ministry of Health, Labour and Welfare, Japan. The sponsor had no role in the design or conduct of the study.

Duality of interest The authors declare that there is no conflict of interest associated with the manuscript.

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