Recent studies have demonstrated that changes in the distribution pattern of Cx43, such as Cx43 lateralization, play important roles in impulse conduction slowing, even if the expression levels of Cx43 protein are not decreased.[20,21] Moreover, Gard *et al.*[10] indicated that increased amounts of Cx43 may be located within non-junctional pools in mice because confocal analysis showed a marked reduction in the amount of Cx43 present in gap junctions, whereas immunoblotting demonstrated roughly normal levels of Cx43 protein in the ventricular myocardium, suggesting that diminished Cx43 in gap junctions in the mice was not due to limited Cx43 expression but rather to an inability of Cx43 to localize normally at cell-to-cell junctions. In the present study, changes in the distribution pattern of Cx43 protein, such as Cx43 lateralization, were observed in ventricles from R120G TG mice. Moreover, although we could not quantitatively measure changes in Cx43 distribution, the amount of linear staining of Cx43 at the lateral cell margins was decreased in ventricles from R120G TG mice following nicorandil. Therefore, the present results suggest that changes in the distribution pattern of Cx43 protein, such as lateralization, play a key role in the impulse conduction slowing in ventricles from R120G TG mice, even when expression levels of Cx43 protein have increased, and that nicorandil may improve impulse conduction slowing by normalizing increased Cx43 expression and its distribution in R120G TG mice.

It is known that monophasic action potential upstroke reflects tissue excitability, whereas the optical action potential upstroke reflects both tissue excitability and intercellular coupling. In the present study, the upstroke ratio of Phase 0 depolarization from the monophasic action potential was decreased, suggesting that tissue excitability decreases in ventricles from R120G TG mice. The cardiac Na^+ channel (Nav1.5) plays key roles in cardiac conduction through tissue excitability, and impulse conduction slowing is associated with decreased protein expression levels of Nav1.5 in hearts.[22] Although the cause of the discrepancy between the increased Nav1.5 expression and impulse conduction slowing is unclear, a recent study demonstrated that loss of expression of Cx43 led to a reduction in the abundance of the Nav1.5 signal at the intercalated discs.[23] These results suggest that Cx43 modulates the expression of Nav1.5 protein. In the present study, both Cx43 and Nav1.5 protein expression was increased, and heterogeneous distribution of Cx43, such as lateralization, was observed. Therefore, although we did not examine the distribution pattern of Nav1.5, it is heterogeneous in R120G TG mouse hearts, which may induce impulse conduction slowing. However, in the present study chronic nicorandil treatment improved increased Cx43 protein expression levels, whereas it did not improve increased Nav1.5 expression, suggesting that Cx43 does not modulate the expression of Nav1.5 protein in the TG mice. Nevertheless, increased Nav1.5 may participate in ventricular conduction slowing because the action potential upstroke calculated from the monophasic action potential is reduced in ventricles from TG mice (i.e. decreases in tissue excitability). Moreover, nicorandil did not improve the decreased upstroke ratio of Phase 0 depolarization calculated from the monophasic action potential, whereas it improved the decreased upstroke ratio of Phase 0 depolarization calculated from the optical action potential, suggesting that nicorandil improves intercellular coupling, leading to improvements in impulse conduction slowing.

In the present study, nicorandil improved impulse conduction slowing and inhibited increases in total and p-Cx43 protein expression, leading to inhibition of electrically induced VT. In a previous study, we demonstrated that cell viability was dose-dependently recovered in myocytes overexpressing mutant *HSPB5* (i.e. R120G TG) following nicorandil treatment.[9] Nicorandil treatment also inhibited the increase in BAX, the decrease in BCL2, activation of caspase 3 and apoptotic cell death induced by mutant *HSPB5*. Moreover, nicorandil treatment prolonged the survival of R120G TG mice. Therefore, we concluded in the previous study that nicorandil prolonged the survival of R120G TG mice by protecting against mitochondrial impairment.[9] Recently, Zhang *et al.*[24] showed that the opening of mitoK_{ATP} channels in astrocytes can reverse rotenone-induced dysfunction of astrocytic Cx43 and therefore protect against the toxicity of rotenone in astrocytes. Several studies suggest that the cardioprotective effects of nicorandil are mediated by activation of mitoK_{ATP} channels in myocytes.[2,4] Therefore, nicorandil may improve dysfunction of cardiac Cx43 by opening of mitoK_{ATP} channels.

In conclusion, we found that overexpression of the R120G missense mutation in *HSPB5* in the heart slowed ventricular impulse conduction and caused electrically induced VT. In addition, R120G TG mice exhibited increased Cx43 and Nav1.5 protein expression, as well as heterogeneous Cx43 distribution in the ventricles. Moreover, nicorandil improved impulse conduction slowing, normalized increases in Cx43 protein expression and reduced the generation of electrically induced VT. These results suggest that the electrical and structural remodelling, especially in terms of the expression levels and heterogeneous distribution of Cx43, contributes to the generation of VT and that nicorandil prevents VT induction by normalizing Cx43 expression in this mouse model of desmin-related cardiomyopathy.

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DISCLOSURE

The authors declare no conflicts of interest.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Movie S1. Spiral re-entry.

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ORIGINAL ARTICLE
Renal Disease

Comparison of Predictability of Future Cardiovascular Events Between Chronic Kidney Disease (CKD) Stage Based on CKD Epidemiology Collaboration Equation and That Based on Modification of Diet in Renal Disease Equation in the Japanese General Population

– Iwate KENCO Study –

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Background: Whether estimated glomerular filtration rate (eGFR) calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Study equation ($eGFR_{CKDEPI}$) improves risk prediction compared to that calculated using the Modification of Diet in Renal Disease (MDRD) study equation ($eGFR_{MDRD}$) has not been examined in a prospective study in Japanese people.

Methods and Results: Participants (n=24,560) were divided into 4 stages (1, ≥90; 2, 60–89 (reference); 3a, 45–59; 3b+ <45 $ml \cdot min^{-1} \cdot 1.73 m^{-2}$) according to $eGFR_{CKDEPI}$ or $eGFR_{MDRD}$. Endpoints were all-cause death, myocardial infarction (MI) and stroke. Area under the receiver operating characteristic curves (95% confidence intervals) for predicting all-cause death, MI and stroke by $eGFR_{CKDEPI}$ vs. $eGFR_{MDRD}$ were 0.680 (0.662–0.697) vs. 0.582 (0.562–0.602); 0.718 (0.665–0.771) vs. 0.642 (0.581–0.703); and 0.656 (0.636–0.676) vs. 0.576 (0.553–0.599), respectively. Multivariate-adjusted Cox regression and Poisson regression analysis results were similar for adjusted incidence rates and adjusted hazard ratios in each corresponding stage between the 2 models and no differences were found in model assessment parameters. Net reclassification improvement (NRI) for predicting all-cause death, MI and stroke were estimated to be 6.7% (P<0.001), –1.89% (P=0.029) and –0.20% (P=0.421), respectively.

Conclusions: Better discrimination was achieved using $eGFR_{CKDEPI}$ than $eGFR_{MDRD}$ on univariate analysis. NRI analysis indicated that the use of $eGFR_{CKDEPI}$ instead of $eGFR_{MDRD}$ offered a significant improvement in reclassification of death risk. (*Circ J* 2013; **77:** 1315–1325)

Key Words: Chronic kidney disease; CKD-EPI equation; Estimated glomerular filtration rate; MDRD equation; Model assessment

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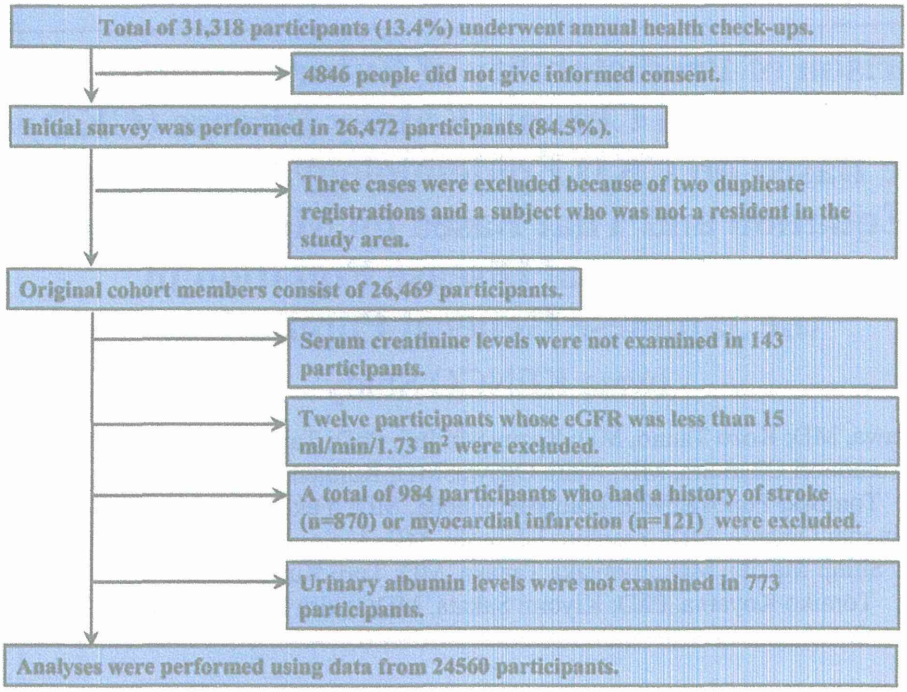

Figure 1. Flow chart of the procedure used to select participants for the Iwate KENCO study. The original cohort consisted of 26,469 participants. We excluded a total of 1,909 participants who lacked data for serum creatinine (n=143), 984 participants with a history of myocardial infarction or stroke (n=984), and 785 participants who lacked data used for analysis. eGFR, estimated glomerular filtration rate.

Chronic kidney disease (CKD) contributes not only to the risk for development of end-stage renal disease but also to the risk for cardiovascular morbidity and mortality.[1] CKD also increases risks for cardiovascular morbidity and mortality in Japanese people.[2–6] The National Kidney Foundation and the American Heart Association have proposed using CKD in cardiovascular risk stratification and treatment guidelines.[1,7] Defining and staging of kidney disease requires combining information on kidney damage, usually detected using albuminuria, and decreased renal function, usually based on glomerular filtration rate (GFR).[7]

The Modification of Diet in Renal Disease (MDRD) Study equation is the most widely used equation.[8] Recently, the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) developed a new equation.[9] The CKD-EPI Study equation was shown to be more accurate than the MDRD Study equation for the calculation of estimated GFR (eGFR).[9] Both the MDRD equation modified by a Japanese coefficient[10] and CKD-EPI equation modified by a Japanese coefficient[11] have recently been developed. The CKD-EPI equation modified by a Japanese coefficient was also shown to be more accurate than the MDRD equation modified by a Japanese coefficient using inulin clearance as a gold standard.[11]

The development of a more accurate equation for eGFR requires reclassification of CKD stage using the new equation instead of the old equation and confirmation of the concordance of CKD stage between the 2 models. Reclassification of CKD stage and correlations between the 2 equations were examined for US subjects.[9,12] Horio et al also examined proportions in each CKD stage separately for the 2 equations.[11]

The development of a more accurate equation for eGFR also requires reassessment of the predictability of CKD in prospective longitudinal studies using the new equation for eGFR. Comparisons of risk predictabilities have been widely used in the cardiovascular field, and new statistical methods have also been developed.[13–20] Recently, Matsushita et al used a new statistical method for comparing risk predictabilities between the 2 types of eGFR.[12] They showed that improved reclassification of CKD stage was observed using $eGFR_{CKDEPI}$ instead of $eGFR_{MDRD}$, with statistical significance in all 4 endpoints (death, myocardial infarction [MI], stroke and end-stage renal disease).

They noted, however, that considerable racial difference existed in correlations between the 2 types of eGFR and in risk predictabilities. They also noted that similar analyses should be performed for ethnicities other than white and African-American people.[12] It is necessary to compare correlations between the 2 types of eGFR and compare risk predictability for CKD stage between the 2 CKD stage models based on $eGFR_{CKDEPI}$ and $eGFR_{MDRD}$ in other ethnicities including Japanese people. Therefore, we compared correlations between the 2 types of eGFR and compared the risk predictability of models based on $eGFR_{CKDEPI}$ and on $eGFR_{MDRD}$ using traditional and newly developed techniques in statistics to examine model accuracy in univariate- and multivariate-adjusted analyses based on a prospective study in Japanese people.

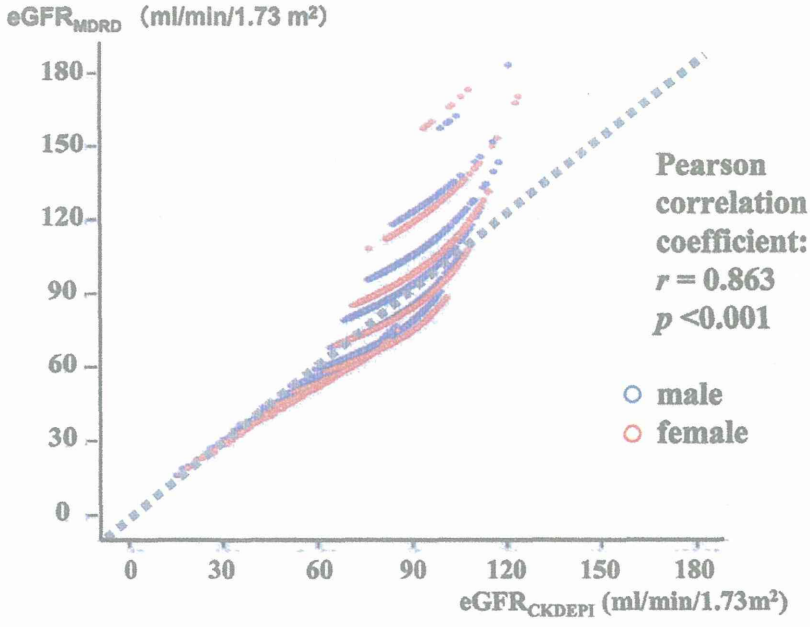

Figure 2. Relationship between eGFRCKDEPI and eGFRMDRD. Points along the diagonal dotted line, accordance between eGFRCKDEPI and eGFRMDRD; points below the diagonal dotted line, underestimation by eGFRMDRD; points over the diagonal dotted line, overestimation by eGFRMDRD. eGFRCKDEPI, estimated glomerular filtration rate (eGFR) calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Study equation; eGFRMDRD, eGFR calculated using the Modification of Diet in Renal Disease (MDRD) study equation.

Methods

Subjects

The subjects were participants of the Iwate-Kenpoku cohort (Iwate-KENCO) study. The Iwate KENCO Study was designed to determine the effects of traditional risk factors and new biomarkers on cardiovascular morbidity and mortality in the Japanese general population. The study area is a typical rural area of Japan (**Figure S1**) with a low move-out/move-in population and a high proportion of elderly people. The methodology of the Iwate-KENCO study has been described elsewhere.[21,22] The initial surveys were carried out from 2002 to 2004. The original cohort study members consisted of 26,469 participants. We excluded participants who lacked data for serum creatinine (n=143), participants with a history of MI or stroke (n=984), and participants who lacked data for at least 1 factor that was used for analysis as an explanatory variable (n=785). Finally, we analyzed data from 24,560 participants (**Figure 1**). The study was approved by the Medical Ethics Committee of Iwate Medical University and conducted in accordance with the guidelines of the Declaration of Helsinki.

Measurements

The initial examinations consisted of a questionnaire, measurements of blood pressure and anthropometric data, and blood tests. Serum creatinine level was measured using an enzymatic assay on an automated analyzer (HITACHI 7700). The methods for measuring serum lipid profile, plasma glucose level, and plasma glycosylated hemoglobin (HbA_{1c}) have been previously described.[21] In this analysis, HbA_{1c} level (National Glycohemoglobin Standardization Program [NGSP] equivalent value) was modified by adding 0.4% to the estimated value (the Japan Diabetes Society [JDS] value) according to the Guidelines of the JDS.[23] Urine albumin was assessed quantitatively using an immunonephelometric method (N-antiserum albumin, Dade Behring) and urine creatinine was measured quantitatively on enzymatic colorimetric test.[24] The urine albumin-creatinine ratio (UACR) was used because the accuracy of the ratio in comparison to 24-h urine sample has been demonstrated in previous studies.[25,26] The data-gathering methodology has been previously described.[21]

Classification and Definition

eGFR was calculated using both MDRD and CKD-EPI equations modified by a Japanese coefficient (eGFRCKDEPI and eGFRMDRD) as shown in **Table S1**.[10,11] Participants were divided into 4 categories (1, $\geq 90\,ml \cdot min^{-1} \cdot 1.73\,m^{-2}$; 2, 60–$89\,ml \cdot min^{-1} \cdot 1.73\,m^{-2}$; 3a, 45–$59\,ml \cdot min^{-1} \cdot 1.73\,m^{-2}$; 3b+, $<45\,ml \cdot min^{-1} \cdot 1.73\,m^{-2}$) according to both GFR based on eGFRCKDEPI and on eGFRMDRD. Albuminuria was defined as the presence of microalbuminuria or macroalbuminuria. Microalbuminuria was defined as a UACR of 30–299 mg/g and macroalbuminuria was defined as a UACR ≥300 mg/g.

Hypertension was defined as systolic blood pressure (SBP) ≥140 mmHg, diastolic blood pressure ≥90 mmHg, use of antihypertensive agents or a combination of these. Diabetes was defined as plasma glucose ≥200 mg/dl, plasma HbA_{1c} (NSGP equivalent value) ≥6.5%, use of anti-diabetes agents or a combination of these. Dyslipidemia was defined as serum total cholesterol (TC) ≥220 mg/dl, serum high-density-lipoprotein cholesterol (HDLC) <40 mg/dl, use of anti-hyperlipidemia agents or a combination of these. Regular alcohol drinking was defined as drinking ≥5 days/week. Exercise habit was defined as doing exercise for at least 60 min 8 days per month.

Outcome Measures

In this cohort study, the endpoints were all-cause death, incident MI and incident stroke. To ascertain subjects' vital status, follow-up surveys were performed in 2006 and 2009. The investigators visited each municipality and reviewed the Basic Resident Register sheets in each local government to confirm the dates of death and move-out of participants. Persons who were known to be alive at the end of follow-up and those who had moved away from the study area were treated as censored

Table 1. Subject Characteristics vs. CKD Stage

CKD stage (ml·min^{-1}·1.73 m^{-2})	eGFR ≥90	60≤eGFR<90	45≤eGFR<60	eGFR <45
CKD-EPI equation (no. subjects)	2,504	20,607	1,259	190
Age (years)	43.0±9.0	63.9±9.3	73.0±7.6	75.2±6.8
BMI (kg/m^2)	23.3±3.6	24.1±3.2	24.3±3.3	24.6±3.4
SBP (mmHg)	116±17.7	128±19.9	133±19.8	134±21.5
TC (mg/dl)	189±33.7	202±32.6	202±33.2	200±38.6
HDLC (mg/dl)	61.4±14.5	59.6±14.9	55.6±14.4	53.3±14.4
HbA_{1c} (mg/dl)	5.33±0.77	5.53±0.66	5.59±0.65	5.64±0.68
SCr (mg/dl)	0.6±0.1	0.7±0.1	1.0±0.1	1.4±0.3
UACR† (mg/g)	10.7 (6.6–19.1)	14.9 (8.5–29.3)	18.8 (9.5–47.2)	56.2 (20.0–317)
Comorbid conditions and habits (%)				
Microalbuminuria	332 (13.3)	4,668 (22.7)	374 (29.7)	77 (40.5)
Macroalbuminuria	21 (0.8)	351 (1.7)	66 (5.2)	49 (25.8)
Hypertension	352 (14.1)	8,653 (42.0)	752 (59.7)	123 (64.7)
Diabetes mellitus	76 (3.0)	1,070 (5.2)	87 (6.9)	19 (10.0)
Dyslipidemia	540 (21.6)	7,600 (36.9)	534 (42.4)	92 (48.4)
Current smoker	577 (23.0)	2,395 (11.6)	120 (9.5)	16 (8.4)
Past smoker	230 (9.2)	2,295 (11.1)	230 (18.3)	34 (17.9)
Regular drinker	579 (23.1)	3748 (18.2)	184 (14.6)	25 (13.2)
MDRD equation (no. subjects)	4,449	17,128	2,711	272
Age (years)	56.2±12.2	62.7±10.9	68.7±8.5	73.3±8.2
BMI (kg/m^2)	23.8±3.5	24.0±3.2	24.4±3.3	24.6±3.4
SBP (mmHg)	124±20.1	127±20.0	131±19.6	134±20.9
TC (mg/dl)	197±33.5	201±32.8	204±32.9	201±38.3
HDLC (mg/dl)	60.7±14.8	59.7±14.9	57.1±14.7	54.2±13.8
HbA_{1c} (mg/dl)	5.54±0.90	5.50±0.61	5.54±0.58	5.62±0.71
SCr (mg/dl)	0.5±0.1	0.7±0.1	0.9±0.1	1.3±0.3
UACR (mg/g)†	15.3 (8.9–30.8)	14.0 (8.1–27.4)	15.4 (8.2–35.5)	38.7 (14.8–180)
Comorbid conditions and habits (%)				
Microalbuminuria	1,076 (24.2)	3,594 (21.0)	677 (25.0)	104 (38.2)
Macroalbuminuria	66 (1.5)	263 (1.5)	103 (3.8)	55 (20.2)
Hypertension	1,452 (32.6)	6,805 (39.7)	1,451 (53.5)	172 (63.2)
Diabetes mellitus	273 (6.1)	815 (4.8)	137 (5.1)	27 (9.9)
Dyslipidemia	1,396 (31.4)	6,090 (35.6)	1,151 (42.5)	129 (47.4)
Current smoker	743 (16.7)	2,110 (12.3)	237 (8.7)	18 (6.6)
Past smoker	402 (9.0)	1,952 (11.4)	398 (14.7)	37 (13.6)
Regular drinker	944 (21.2)	3,175 (18.5)	387 (14.3)	30 (11.0)

Data given as mean±SD, n (%) or †median (interquartile range).
AMI, acute myocardial infarction; BMI, body mass index; CKD, chronic kidney disease; CKD-EPI, Chronic Kidney Disease Epidemiology Collaboration; eGFR, estimated glomerular filtration rate; HbA_{1c}, glycosylated hemoglobin; HDLC, high-density lipoprotein cholesterol; MDRD, Modification of Diet in Renal Disease; SBP, systolic blood pressure; SCr, serum creatinine; TC, total cholesterol; UACR, urinary albumin to creatinine ratio.

cases.

Stroke events were identified by assessing the Iwate Prefecture Stroke Registration program, which included the entire area where the subjects lived. Details of this registry have been described previously.[22,27] The medical records of all medical facilities within the survey area were verified every year to ensure complete capture of all data from 2006 to 2009 by the physicians and trained research nurses. Incidents of acute MI (AMI) were identified by accessing data from Northern Iwate Heart Disease Registry Consortium, which has been collecting data since 2002. The registration of AMI was based on the criteria of the MONICA study. Details of this registry have been described previously.[22,28] To verify the accuracy of the data, physicians and trained research nurses also checked the medical records of the referral hospitals.

Statistical Analysis

The relationship between $eGFR_{CKDEPI}$ and $eGFR_{MDRD}$ was illustrated using a scatter diagram. Pearson correlation coefficient (r) between $eGFR_{CKDEPI}$ and $eGFR_{MDRD}$ was calculated. Concordance between each corresponding CKD stage according to $eGFR_{CKDEPI}$ and $eGFR_{MDRD}$, was examined on a cross-table for the 4 CKD categories of $eGFR_{CKDEPI}$ and $eGFR_{MDRD}$. Baseline characteristics are listed according to CKD stage based on both $eGFR_{CKDEPI}$ and $eGFR_{MDRD}$ and according to endpoint category. Risk factor-related variables are expressed as mean±SD, proportions (expressed as percentages) or median (interquartile range). Proportion of each CKD stage was compared between GFR_{CKDEPI} and $eGFR_{MDRD}$ by chi-square test.

We defined follow-up time as the period from the initial survey to the first outcome or end of observation. Individuals

Table 2. Baseline Subject Characteristics vs. Endpoint

	All subjects	No events survivor	Died	Incident AMI	Incident stroke
Subjects (n)	24,560	22,246	851	78	605
Age (years)	62.3±11.4	61.3±11.3	71.3±9.0	70.3±7.6	69.9±8.1
Male	8,368 (34.1)	7,334 (33.0)	320 (37.6)	55 (70.5)	96 (36.0)
BMI (kg/m^2)	24.0±3.3	24.0±3.3	23.5±3.5	24.3±3.2	24.3±3.5
SBP (mmHg)	127±20.1	126±20.0	133±20.5	134±19.8	138±20.6
TC (mg/dl)	201±33.0	201±33.0	192±33.9	206±32.6	198±32.7
HDLC (mg/dl)	59.5±14.9	59.7±14.8	56.4±15.4	51.3±14.2	57.5±14.9
HbA$_{1c}$ (%)	5.52±0.67	5.50±0.65	5.63±0.91	5.77±0.72	5.67±0.83
SCr (mg/dl)	0.7±0.2	0.7±0.2	0.8±0.2	0.8±0.2	0.7±0.2
eGFR$_{CKDEPI}$ (ml·min^{-1}·1.73m^{-2})	77.6±10.6	78.4±10.3	71.0±12.0	69.3±10.8	72.2±10.3
eGFR$_{MDRD}$ (ml·min^{-1}·1.73m^{-2})	75.9±15.4	76.5±15.2	71.8±16.8	68.3±14.1	72.1±15.1
UACR (mg/g)†	14.5 (8.2–29.1)	14.0 (8.0–27.4)	19.5 (10.4–49.6)	20.4 (9.3–51.9)	22.8 (12.6–51.8)
Comorbid conditions and habits					
Microalbuminuria	5,453 (22.6)	4,664 (21.3)	273 (34.0)	24 (32.4)	218 (37.8)
Macroalbuminuria	487 (2.0)	373 (1.7)	47 (5.5)	4 (5.1)	29 (4.8)
Hypertension	9,881 (40.2)	8,529 (38.3)	447 (52.5)	51 (65.4)	412 (68.1)
Diabetes mellitus	1,252 (5.1)	1,028 (4.6)	88 (10.3)	8 (10.3)	60 (9.9)
Dyslipidemia	8,770 (35.7)	7,945 (35.7)	274 (32.2)	35 (44.9)	215 (35.5)
Current smoker	3,110 (12.7)	2,761 (12.4)	184 (21.6)	19 (24.4)	108 (17.9)
Past smoker	2,789 (11.4)	2,451 (11.0)	176 (20.7)	16 (20.5)	97 (16.0)
Regular drinker	4,537 (18.5)	4,085 (18.4)	217 (25.5)	15 (19.2)	155 (25.6)
CKD stage based on eGFR$_{CKDEPI}$ (ml·min^{-1}·1.73m^{-2})					
GFR ≥90	2,508 (10.2)	2,470 (11.1)	26 (3.1)	1 (1.3)	11 (1.8)
60≤GFR<90	20,612 (83.9)	18,716 (84.1)	695 (81.7)	62 (79.5)	521 (86.1)
45≤GFR<60	1,259 (5.1)	951 (4.3)	96 (11.3)	14 (17.9)	61 (10.1)
GFR <45	190 (0.8)	118 (0.5)	34 (4.0)	1 (1.3)	12 (2.0)
CKD stage based on eGFR$_{MDRD}$ (ml·min^{-1}·1.73m^{-2})					
GFR ≥90	4,453 (18.1)	4,209 (18.9)	112 (13.2)	5 (6.4)	69 (11.4)
60≤GFR<90	17,133 (69.7)	15,582 (70.0)	568 (66.7)	48 (61.5)	428 (70.7)
45≤GFR<60	2,711 (11.0)	2,280 (10.2)	132 (15.5)	24 (30.8)	94 (15.5)
GFR <45	272 (1.1)	184 (0.8)	39 (4.6)	1 (1.3)	14 (2.3)

Data given as mean±SD, n (%) or †median (interquartile range).
Abbreviations as in Table 1.

who were free of outcomes by 5-year follow-up were subjected to censoring. Receiver operating characteristic (ROC) curves were drawn and the areas under the curves (AUROC) were calculated for each equation to compare the discrimination abilities of the 2 models. Crude, sex- and age-adjusted, and multivariate-adjusted mortality and incidence rates of AMI and stroke (/1,000 person-years) were determined in the 4 groups according to eGFR (CKD stages 1, 2, 3a and 3b+) on Poisson regression analysis. Multivariate-adjusted mortality and incidence rates were estimated after adjusting for age, sex, SBP, body mass index, TC, HDLC, HbA$_{1c}$, existence of albuminuria, smoking habit, regular drinking habit and exercise habit.

Relative risks for all-cause death, incident AMI, and incident stroke were estimated in each category and compared with the reference group (60≤eGFR<90 ml·min^{-1}·1.73 m^{-2}) in a Cox regression model for the same explanatory variables as those used in Poisson regression analysis separately for the 2 models of eGFR$_{CKDEPI}$ and eGFR$_{MDRD}$ CKD stage. The performances of the multivariate models were quantified using Harrell's concordance statistics (Harrell's C).[14] The Akaike information criterion (AIC)[29] and Bayesian information criterion (BIC)[30] were also estimated for these models. We also quantified the degree of correct reclassification by estimating net reclassification improvement (NRI) using cross-categories of eGFR for both equations.[20,31]

All P-values were 2-tailed, and P<0.05 was considered to be statistically significant. Statistical analysis was performed using PASW version 18.0 (IBM Japan, Tokyo, Japan) and STATA version STATA/SE 11 (STATA, College Station, TX, USA).

Results

Figure 2 shows a scatter graph of eGFR$_{CKDEPI}$ and eGFR$_{MDRD}$. The overall correlation between eGFR$_{CKDEPI}$ and eGFR$_{MDRD}$ was relatively good (r=0.863, P<0.001.). Using eGFR$_{CKDEPI}$ as the gold standard, eGFR$_{MDRD}$ mildly to moderately underestimated GFR in persons with eGFR$_{CKDEPI}$ 45–90 ml·min^{-1}·1.73 m^{-2}. Because most of the present participants belonged to this category, eGFR$_{MDRD}$ was likely to underestimate GFR in the total subjects. In contrast, eGFR$_{MDRD}$ moderately to greatly overestimated GFR in persons with eGFR$_{CKDEPI}$ 90–120 ml·min^{-1}·1.73 m^{-2}, especially in female participants. Table S2 is a cross-table of the 4 CKD categories of eGFR$_{CKDEPI}$ and eGFR$_{MDRD}$. Underestimation of eGFR$_{MDRD}$ was observed in 30%

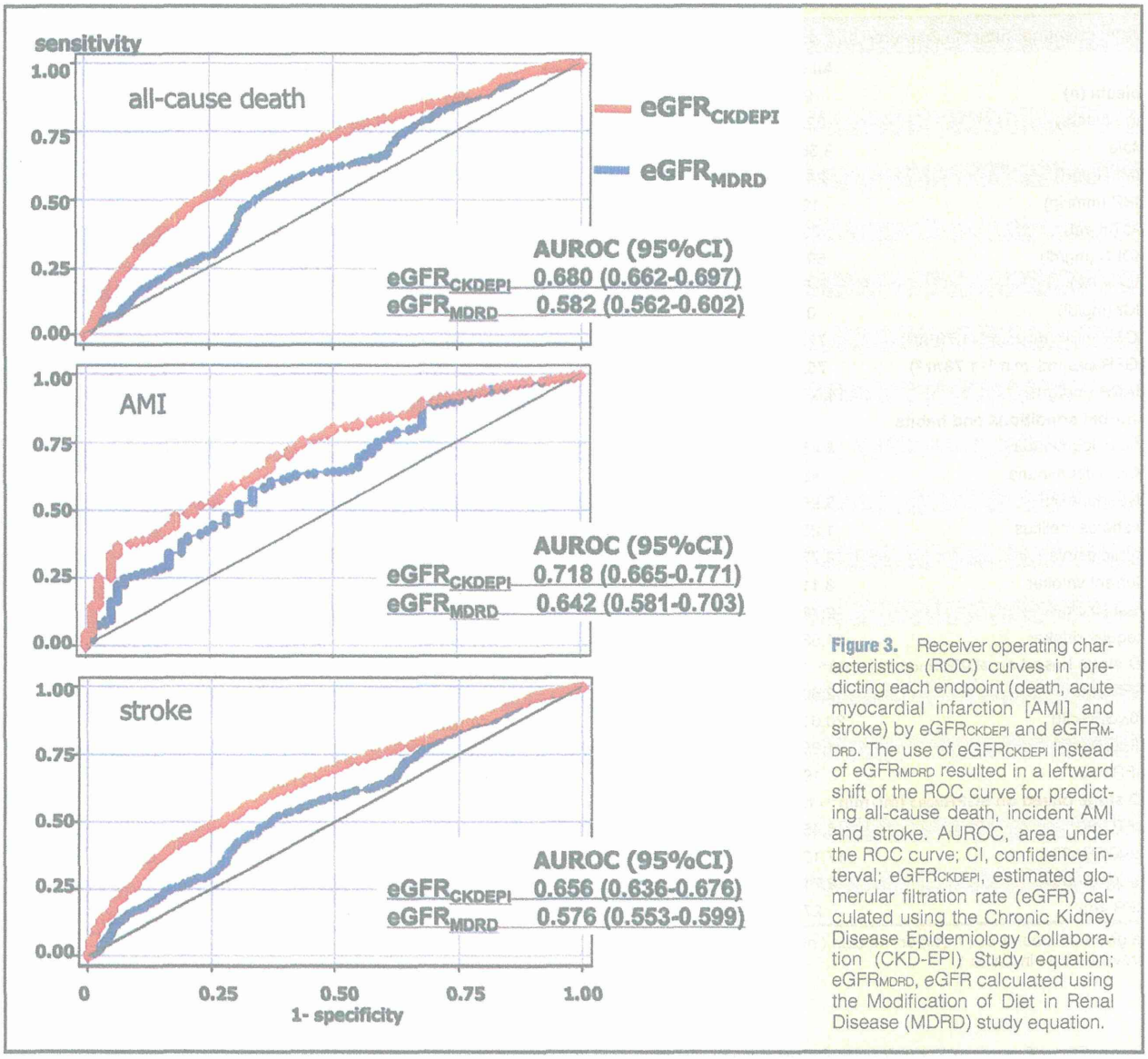

Figure 3. Receiver operating characteristics (ROC) curves in predicting each endpoint (death, acute myocardial infarction [AMI] and stroke) by eGFR$_{CKDEPI}$ and eGFR$_{MDRD}$. The use of eGFR$_{CKDEPI}$ instead of eGFR$_{MDRD}$ resulted in a leftward shift of the ROC curve for predicting all-cause death, incident AMI and stroke. AUROC, area under the ROC curve; CI, confidence interval; eGFR$_{CKDEPI}$, estimated glomerular filtration rate (eGFR) calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Study equation; eGFR$_{MDRD}$, eGFR calculated using the Modification of Diet in Renal Disease (MDRD) study equation.

of subjects in stage 1 (eGFR ≥90 ml·min^{-1}·1.73 m^{-2}) and in 7% of subjects in stage 2 (69–89 ml·min^{-1}·1.73 m^{-2}) using eGFR$_{CKDEPI}$ as the gold standard, while eGFR$_{MDRD}$ was overestimated in 13% of subjects in stage 2. Subclassification and age-stratification of CKD stage showed that significantly different age distributions between corresponding CKD stage based on eGFR$_{CKDEPI}$ and eGFR$_{MDRD}$ contributed to over/underestimations. We have already listed the concordance/discordant results of CKD stage in detail elsewhere.[32]

Table 1 lists baseline characteristics vs. CKD stage based on eGFR$_{CKDEPI}$ and on eGFR$_{MDRD}$, and also the differences in baseline characteristics in each CKD stage between the models using eGFR$_{CKDEPI}$ and eGFR$_{MDRD}$. Age distributions were very different between the 2 models. In stage 1 (eGFR ≥90 ml·min^{-1}·1.73 m^{-2}), mean age was 43.0 years and had a narrow distribution in the eGFR$_{CKDEPI}$ model, while it was 56.2 years with a wide distribution in the eGFR$_{MDRD}$ model. The difference in mean age in CKD stage 1 was >13 years. Higher age with a narrower age distribution was observed in CKD stage 3a and stage 3b+ in the eGFR$_{CKDEPI}$ model compared to the eGFR$_{MDRD}$ model.

After completion of 5-year follow-up, the observed patient-years were 136,961. The mean follow-up period was 5.6 years. There were 851 deaths, 78 cases of AMI and 605 cases of stroke during the observation period. Table 2 lists baseline characteristics of participants stratified by endpoint. The mean age of subjects who died during the observation period was 71.3 years, which was 10 years older than that of subjects who survived without events. The mean age of subjects in whom AMI or stroke occurred was approximately 70 years, which was 9 years older than that of survivors with no events. For the total subjects, differences in prevalence of CKD stage between eGFR$_{CKDEPI}$ and eGFR$_{MDRD}$ were large in stage 1 (10.2% vs. 18.1%, χ^2=633, P<0.001) and stage 2 (83.9% vs. 69.7%, χ^2=1,382, P<0.001). The proportion of subjects with eGFR <60 ml·min^{-1}·1.73 m^{-2} was 5.9% for eGFR$_{CKDEPI}$ and 12.1% for eGFR$_{MDRD}$ (χ^2=584, P<0.001).

Figure 3 shows ROC curves for predicting each endpoint

Table 3. Events (Death or Incidence) and Mortality/Incidence Rates vs. eGFR

CKD stage (ml·min^{-1}·1.73 m^{-2})	eGFR ≥90	60≤eGFR<90	45≤eGFR<60	eGFR <45
CKD-EPI equation (n)	2,504	20,607	1,259	190
Death				
No. events (crude)	26 (1.86)	695 (6.04)	96 (14.0)	34 (33.4)
Sex- and age-adjusted (95% CI)	6.68 (4.05–9.30)	3.03 (2.66–3.41)	3.26 (2.43–4.08)	6.65 (4.14–9.16)
Multivariate-adjusted (95% CI)	7.21 (4.32–10.1)	3.70 (3.17–4.23)	4.09 (3.01–5.16)	7.50 (4.62–10.4)
AMI				
No. events (crude)	1 (0.07)	62 (0.54)	14 (2.06)	1 (0.98)
Sex- and age-adjusted (95% CI)	0.18 (0.00–0.53)	0.34 (0.22–0.47)	0.72 (0.22–1.23)	0.31 (0.00–0.95)
Multivariate-adjusted (95% CI)	0.15 (0.00–0.45)	0.27 (0.14–0.40)	0.50 (0.11–0.89)	0.19 (0.00–0.58)
Stroke				
No. events (crude)	11 (0.79)	521 (4.58)	61 (9.14)	12 (12.1)
Sex- and age-adjusted (95% CI)	2.27 (0.91–3.64)	2.92 (2.54–3.30)	3.19 (2.23–4.15)	3.71 (1.50–5.92)
Multivariate-adjusted (95% CI)	2.61 (1.03–4.20)	3.56 (2.99–4.12)	4.01 (2.75–5.27)	4.17 (1.66–6.69)
MDRD equation (n)	4,449	17,128	2,711	272
Death				
No. events (crude)	112 (4.47)	568 (5.95)	132 (8.88)	39 (26.6)
Sex- and age-adjusted (95% CI)	4.21 (3.40–5.02)	3.04 (2.65–3.43)	3.02 (2.38–3.66)	6.46 (4.19–8.73)
Multivariate-adjusted (95% CI)	4.80 (3.82–5.78)	3.73 (3.18–4.27)	3.79 (2.94–4.64)	7.36 (4.72–10.0)
AMI				
No. events (crude)	5 (0.20)	48 (0.50)	24 (1.62)	1 (0.68)
Sex- and age-adjusted (95% CI)	0.21 (0.02–0.39)	0.32 (0.20–0.45)	0.77 (0.35–1.19)	0.26 (0.00–0.80)
Multivariate-adjusted (95% CI)	0.17 (0.01–0.33)	0.26 (0.13–0.39)	0.56 (0.20–0.92)	0.17 (0.00–0.52)
Stroke				
No. events (crude)	69 (2.77)	428 (4.53)	94 (6.43)	14 (9.76)
Sex- and age-adjusted (95% CI)	2.81 (2.13–3.48)	2.88 (2.49–3.26)	2.89 (2.19–3.59)	3.25 (1.46–5.04)
Multivariate-adjusted (95% CI)	3.19 (2.37–4.00)	3.56 (2.98–4.14)	3.64 (2.70–4.59)	3.68 (1.63–5.72)

Crude, crude mortality/incidence rates; multivariate-adjusted, multivariate-adjusted mortality/incidence rates (/1,000 person-years); sex- and age-adjusted, sex- and age-adjusted mortality/incidence rates (/1,000 person-years). Multivariate adjustment for risk factors: age, SBP, BMI, TC, HDLC, HbA_{1c}, smoking habit and regular drinking habit.
CI, confidence interval. Other abbreviations as in Table 1.

Table 4. Adjusted HR (95% CIs) for Endpoint According to eGFR Type

CKD stage (ml·min^{-1}·1.73 m^{-2})	eGFR ≥90	60≤eGFR<90	45≤eGFR<60	eGFR <45	Model parameters in multivariate-adjusted Cox regression analysis		
Estimated using CKD-EPI equation							
Subjects	2,504	20,607	1,259	190	AIC	BIC	Harrell's C
Death	1.93 (1.25–2.98)	Ref	1.12 (0.90–1.40)	2.05 (1.43–2.92)	15,858	15,947	0.739
AMI	0.55 (0.07–4.34)	Ref	1.86 (1.01–3.44)	0.71 (0.10–5.24)	1,449	1,538	0.790
Stroke	0.72 (0.38–1.36)	Ref	1.13 (0.86–1.48)	1.17 (0.66–2.10)	11,441	11,530	0.729
Estimated using MDRD equation							
Subjects (n)	4,449	17,128	2,711	272	AIC	BIC	Harrell's C
Death	1.28 (1.04–1.58)	Ref	1.03 (0.85–1.25)	1.99 (1.43–2.78)	15,863	15,952	0.737
AMI	0.65 (0.25–1.67)	Ref	2.14 (1.29–3.55)	0.65 (0.09–4.78)	1,446	1,536	0.797
Stroke	0.89 (0.68–1.15)	Ref	1.02 (0.82–1.28)	1.03 (0.00–0.45)	11,442	11,531	0.729

Data given as multivariate-adjusted HR (95% CI). Adjustment for risk factors: age, sex, SBP, BMI, TC, HDLC, HbA_{1c}, existence of albuminuria, smoking habit, regular drinking habit and exercise habit.
AIC, Akaike's information criterion; BIC, Baysian information criterion; Harrell's C, Harrell's concordance statistics; HR, hazard ratio. Other abbreviations as in Tables 1,2.

according to eGFRCKDEPI and eGFRMDRD. The use of eGFRCKDEPI instead of eGFRMDRD results in a leftward shift of the ROC curve in prediction of all-cause death, incident AMI and stroke. AUROCs (95% confidence interval [95% CI]) for eGFRCKDEPI vs. eGFRMDRD were 0.680 (0.662–0.697) vs. 0.582 (0.562–0.602) in predicting all-cause death, 0.718 (0.665–0.771) vs. 0.642 (0.581–0.703) in predicting AMI and 0.656 (0.636–0.676) vs. 0.576 (0.553–0.599) in predicting stroke.

Table 3 lists number of events (death, AMI and stroke) and crude mortality and incidence rates, sex- and age-adjusted and multivariate-adjusted mortality and incidence rates, and their 95% CI (expressed as /1,000 person-years) in the 4 categories separately for the 2 models based on eGFRCKDEPI and on eGFRMDRD. A clear steep linear relationship between eGFR

Table 5. Reclassification of eGFR Categories According to Endpoint

CKD stage	eGFR$_{CKDEPI}$				Total
	eGFR ≥90	60≤eGFR<90	45≤eGFR<60	eGFR <45	
eGFR$_{MDRD}$					
Participants who died					
GFR ≥90	25	87*	0	0	112
60≤GFR<90	1**	567	0	0	568
45≤GFR<60	0	41**	91	0	132
GFR <45	0	0	5**	34	39
Total	26	695	96	34	851
Participants who did not die					
GFR ≥90	1,741	2,596**	0	0	4,337
60≤GFR<90	737*	15,823	0	0	16,560
45≤GFR<60	0	1,493*	1,086	0	2,579
GFR <45	0	0	77*	156	233
Total	2,478	19,912	1,163	156	23,709
Participants who developed AMI					
GFR ≥90	1	4*	0	0	5
60≤GFR<90	0**	48	0	0	48
45≤GFR<60	0	10**	14	0	24
GFR <45	0	0	0**	1	1
Total	1	62	14	1	78
Participants who did not develop AMI					
GFR ≥90	1,765	2,679**	0	0	4,444
60≤GFR<90	738*	16,342	0	0	17,080
45≤GFR<60	0	1,524*	1,163	0	2,687
GFR <45	0	0	82*	189	271
Total	2,503	20,545	1,245	189	24,482
Participants who developed stroke					
GFR ≥90	10	59*	0	0	69
60≤GFR<90	1**	427	0	0	428
45≤GFR<60	0	35**	59	0	94
GFR <45	0	0	2**	12	14
Total	11	521	61	12	605
Participants who did not develop stroke					
GFR ≥90	1,756	2,624**	0	0	4,380
60≤GFR<90	737*	15,963	0	0	16,700
45≤GFR<60	0	1,499*	1,118	0	2,617
GFR <45	0	0	80*	178	258
Total	2,493	20,086	1,198	178	23,955

*Improved reclassification; **worse reclassification using the CKD-EPI equation instead of the MDRD equation. Units of GFR, $ml \cdot min^{-1} \cdot 1.73 m^{-2}$.
Abbreviations as in Table 1.

and crude mortality was observed for the eGFR$_{CKDEPI}$ model, while that for eGFR and crude mortality in the GFR$_{MDRD}$ model was more of a gradual slope.

The relationship between CKD stage and death risk was U-shaped in the sex- and age-adjusted models and multivariate-adjusted models, and the lowest mortality rate was observed in stage 2 ($60–89 ml \cdot min^{-1} \cdot 1.73 m^{-2}$) for both the eGFR$_{CKDEPI}$ and eGFR$_{MDRD}$ models. The adjusted mortality rate in stage 3a was almost identical to that in stage 2 for the eGFR$_{MDRD}$ model, while a slightly higher mortality rate in stage 3a than in stage 2 was observed for the eGFR$_{CKDEPI}$ model. Adjusted mortality rate was significantly higher in stage 1 than in stage 2 for the eGFR$_{CKDEPI}$ model, while it was not significantly higher in the eGFR$_{MDRD}$ model. Adjusted incidence rates of AMI and stroke were similar between the 2 models for each stage.

Table 4 lists relative risks for death, AMI and stroke according to CKD stage for both the eGFR$_{CKDEPI}$ and eGFR$_{MDRD}$ models. Also listed are the results of model assessment using post-estimation analysis. A typical U-shaped relationship between mortality risk and CKD stage was observed for eGFR$_{CKDEPI}$, and a J-shaped relationship between mortality risk and CKD stage was observed for eGFR$_{MDRD}$. Two-fold higher risk for death was observed in stage 1 and stage 3b+ (eGFR $<45 ml \cdot min^{-1} \cdot 1.73 m^{-2}$) in the eGFR$_{CKDEPI}$ model, while mildly elevated risk for death was observed in stage 1 in the eGFR$_{MDRD}$ model. Risks for AMI and stroke were similar in each stage regardless of eGFR type. Although AUROC indicated that eGFR$_{CKDEPI}$ accurately discriminated subjects into persons with events or not, all model parameters (AIC,