T2238C ANP gene variant and risk of recurrent acute coronary syndromes in an Italian cohort of ischemic heart disease patients

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Abstract

Background. The role of C2238/ANP minor allele (MA), at the T2238C ANP gene variant, as predisposing risk factor for acute cardiovascular events has been previously reported. We aimed at evaluating, by a retrospective approach, the long-term impact of C2238/ANP-MA carrier status towards the risk of recurrent acute coronary syndromes (re-ACS) in an Italian cohort of ischemic heart disease patients.

Methods. 379 patients (males=80.5%; mean age=62.5±9.2 years) presenting with ACS were retrospectively analyzed. Mean follow up was 5.1±3.5 years (range between 1-26 years). Occurrence of new episodes of unstable angina, NSTE myocardial infarction, STE myocardial infarction over the years was recorded and compared between subjects not carrying and subjects carrying C2238/ANP-MA.

Results. At univariate analysis, C2238/ANP-MA carrier status and treatment with beta-blocker, aspirin and statin were associated with risk of re-ACS. Multivariate analysis confirmed that hypercholesterolemia ($p<0.0001$) and C2238/ANP-MA carrier status ($p<0.05$) were both significantly and independently associated with increased risk of re-ACS. Both treatments with beta-blocker and with statin were significantly associated with reduced risk of re-ACS ($p=0.01$ and $p<0.01$, respectively). Age above 55 years was associated with recurrence of ACS in C2238/ANP-MA carriers (HR 1.427, 95% CI 1.066-1.911, $p=0.017$).

Kaplan Meyer curves confirmed highest risk of new events occurrence in C2238/ANP-MA carriers ($p=0.035$).

Conclusions. The present results demonstrate that C2238/ANP-MA carrier status is an independent risk factor for ACS recurrence in an Italian cohort of ischemic heart disease patients over the long term, and they support the role of C2238/ANP-MA as a negative prognostic factor in coronary artery disease patients.

Key words: atrial natriuretic peptide, T2238C variant, acute coronary syndrome, prognosis, cardiovascular risk

Introduction

Atrial natriuretic peptide (ANP) is a cardiac hormone which exerts beneficial effects on the cardiovascular system through its natriuretic, diuretic and vasodilator properties (1). Depending upon either abnormal circulating concentrations or peptide structural alterations, ANP may contribute to the risk of cardiovascular events (2, 3). A common molecular variant of pre-proANP gene, depending on the substitution of thymidine (T) with cytosine (C) in position 2238, with a frequency ranging from 13 to 23% in the general population, leads to the synthesis of a 30 amino acid long αANP. This molecular ANP variant has raised attention in both scientific and medical communities. In fact, C2238 minor allele (C2238/ANP-MA) has been shown to affect the incidence of cardiovascular events [stroke and myocardial infarction (MI)] in different human populations (4-8). We previously reported that C2238/ANP-MA negatively affects vascular function and we observed a significant increase in the occurrence of acute coronary syndromes (ACS) and of cardiovascular mortality in subjects carrying the C2238/ANP-MA from two European cohorts of stable angina patients, thus revealing for the first time a negative prognostic role in stable coronary artery disease (CAD) (7).

However, contrasting evidence on the role of C2238/ANP-MA in CVD predisposition has also been reported (4, 9, 10).

Therefore, additional studies are needed to fully assess the role of C2238/ANP-MA carrier status as a novel genetic factor associated with increased cardiovascular risk and with worse prognosis in patients affected by CAD, particularly after a prolonged follow-up.

Thus, the present study was performed to evaluate the long-term prognostic impact of C2238/ANP-MA on the incidence of recurrent ACS episodes in a cohort of Italian patients affected by CAD.

Methods

Sample study population

This was a single center retrospective study that included a cohort of 379 patients affected by CAD admitted to the UOC Cardiologia, S.Andrea Hospital in Rome, Italy, from March 2001 till

December 2012. Out of 379 patients, 345 individuals had their hospitalization for a first ACS episode at our Institution and were subsequently followed. The remaining 34 patients had already had a first ACS episode before admission at our hospital. Follow-up data of all patients were obtained through review of ambulatory visits, phone calls and careful revision of medical records. Thus, a detailed and complete cardiac history was available for all patients included into the study.

Criteria for inclusion of all patients were: 1) ACS episode (unstable angina, non-ST-segment elevation MI, ST-segment elevation MI) as first manifestation of CAD, diagnosed on the basis of standard criteria (11); 2) evidence of critical coronary artery stenosis in at least one vessel (≥70%), as documented by coronary angiography; 3) availability of a well documented clinical follow-up after the first ACS episode. Exclusion criteria were: 1) lack of coronary angiography; 2) lack of a well documented clinical follow-up; 3) lack of availability of most of the parameters chosen for statistical analysis; 4) patients with neoplasia and short life expectancy (< 6 months).

The following parameters were recorded for each patient: demographic data, presence/absence of hypertension, hypercholesterolemia, diabetes, tobacco use, prescribed medications, data on cardiac geometry and function, type of coronary revascularization following the first ACS episode. Hypertension was diagnosed on the basis of WHO/ISH criteria (12) and if subjects were routinely receiving antihypertensive therapy. Hypercholesterolemia was defined by a total cholesterol blood level >220 mg/dl. Type 1 and 2 diabetes mellitus were diagnosed according to the American Diabetes Association (ADA) guidelines (13) and/or if subjects were receiving anti-diabetic therapy. Smoking habit was also recorded (i.e. smokers were considered former only if they had stopped smoking > 2 months before entering into the analysis). Presence of metabolic syndrome was assessed according to the Adult Treatment Panel III (ATP III) report (14).

At the time of recruitment for the analysis, each patient was recalled at our hospital center and, after providing informed written consent, underwent a venous blood sample drawing for assessment of T2238C allele carrier status. The study was approved by the ethic committee of S.Andrea Hospital.

Follow up and definition of end points

Following the first ACS episode, clinical follow-up of each patient was well documented with available information regarding re-hospitalizations, new revascularization procedures and medical treatments administered over the years. The mean follow up time was 5.1±3.5 years (range between 1-26 years). Patients performed periodic check-up visits with a frequency of 1-2 per year.

The end-point of the study was a new acute coronary event (unstable angina, non-ST-segment elevation MI, ST-segment elevation MI).

Genetic analysis

Genomic DNA was extracted by a commercially available kit (Qiagen). Characterization of allele frequency and genotype distribution of the T2238C ANP variant was performed by a previously reported procedure (5, 7).

Statistical analysis

Data analysis regarding sample characteristics was performed with SPSS software package (version 20.0, SPSS Inc., Chicago, Illinois). Normal data distribution was assessed through Kolmogorov-Smirnov test. Continous variables are expressed as mean ± SD; categorical variables are expressed with the corresponding frequencies and percentages.

Differences between C2238/ANP-MA carriers and non carriers were tested using two-sample t-test or Mann Whitney test, when appropriate, for quantitative variables. Chi-square test was used for categorical variables. Bonferroni correction was used when appropriate.

Genotype frequencies were evaluated and Hardy-Weinberg equilibrium (HWE) was tested using Pearson's Chi-square test. The assumption of a dominant model for genotype analysis was considered (score of 0 for wild type, 1 for combined heterozygote and double mutant individuals). Due to the low number of C2238/ANP-MA homozygotes, no recessive model of inheritance was included into the analysis.

At univariate analysis the following predictors were considered: C2238/ANP-MA carrier status, sex, age at first event, diabetes, hypercholesterolemia, hypertension, metabolic syndrome, multiple risk factors (2 or more), treatment with beta-blocker, anti-aldosteronic drug, hydrochlorothiazide,

acetylsalicylic acid, nitrate, calcium channel blocker, angiotensin converting enzyme inhibitor, AT1 receptor blocker, other anti-platelet drugs, statin.

Multivariate models were selected using a forward stepwise regression method based on optimization of the Akaike Information Criterion (AIC), finally selecting the following covariates: C2238/ANP-MA carrier status, hypercholesterolemia, treatments with beta blocker, acetylsalicylic acid, statin.

All possible bivariate interactions based on the final model were considered.

The risk associated with C2238/ANP-MA carrier status for occurrence of multiple events per patient was analyzed by means of Cox regression models with Gamma distributed frailty at both univariate and multivariate analyses. Inclusion of a frailty term in Cox regression represents a suitable approach to take into account the dependence arising from repeated measures of time-to-event in the same patient, as previously reported (15-17). Cox regression with Gamma distributed frailty was performed using R software version 2.14.

Survival curves for ACS recurrence were constructed using the Kaplan-Meyer method.

A p value <0.05 was considered as statistically significant in all analyses.

Results

Clinical characteristics of the patients at the time of enrolment and clinical follow up.

Table I shows the main clinical characteristics of our study cohort and the risk factors distribution, based on the ANP allele carrier status, at the time of their first ACS episode. ST-segment elevation MI represented the most frequent type of ACS, followed by unstable angina and non-ST-segment elevation MI. Males were 80.5%; mean age was 62.5±9.2 years. A large percentage of patients were hypertensives (73.6%), with diabetes and hypercholesterolemia also occurring at higher frequencies (30% and 62.5%, respectively) as compared to the Italian general population (18, 19). Knowledge about hypertensive status was not known for 15 patients. Knowledge about metabolic syndrome carrier status was not known for 30 patients.

Of note, percentage of hypertensive subjects did not differ between wild type and mutant ANP allele carriers.

No difference was observed with regard to cardiac systolic function between subjects not carrying and subjects carrying C2238/ANP-MA (p=0.434).

Severity of CAD (number of diseased vessels and degree of stenosis) was comparable between the two groups (p=0.944 for number of diseased vessels; p=0.609 for degree of stenosis). In addition, no significant difference was detected between the two groups with regard to the type of coronary revascularization performed at the first ACS episode, p=0.748 between the two groups; number of bar metal stent and of drug eluting stent, p=0.495 between the two groups (Table 2).

Allele and genotype frequencies for the T2238C ANP gene variant are shown in Table 3. They were in HWE (p=0.821). Percentage of allele frequencies for the T2238C ANP variant was similar to that previously reported in other CAD cohorts (5, 7).

Table 4 reports the medical treatments administered to patients during the follow up time (up to 20 years), based on the allele carrier status for T2238C/ANP-MA. Knowledge of medical treatments administered at beginning of cardiac disease history was not available only for the 10 patients with the longest follow up (26 years). There was no statistically significant difference in the treatment strategies between patients not carrying and those carrying the C2238/ANP-MA.

Evaluation of risk factors for re-ACS

A careful screening of clinical documentation of all patients throughout the follow-up time was performed by trained medical personnel. Fig. 1 shows results of univariate Cox analysis, based on the events registered over the follow-up time in the two groups of patients (STEMI=36% in wild type, 38% in mutant; NSTEMI=22% in wild type, 24% in mutant; UA=38% in wild type, 41% in mutant). In particular, new ACS occurred at an earlier time in patients carrying the C2238/ANP-MA.

We then evaluated what variables were significantly associated with recurrence of ACS during follow-up time. Table 5 and Table 6 report the results obtained by both univariate and multivariate analyses. At univariate analysis, C2238-MA carrier status and treatments with beta-blocker, statin

and aspirin showed a significant impact on re-ACS (Table 5). At multivariate Cox analysis C2238/ANP-MA remained a significant predictor of re-ACS, along with hypercholesterolemia (Table 6). On the other hand, both treatments with beta-blocker and with statin were confirmed as significant protective factors towards re-ACS occurrence over the follow up time (Table 6). None of the possible bivariate interactions, considered on the basis of the final model, was significant.

We then wanted to evaluate whether the concomitant presence of other risk factors could potentiate the detrimental effect of C2238/ANP-MA on re-ACS. Therefore, we stratified our population by gender, by age at first event, by absence/presence of diabetes, of metabolic syndrome, of hypertension, of hypercholesterolemia. As a result, we found that age above 55 years (HR 1.427, 95% CI 1.066-1.911, p=0.017) was significantly associated with recurrence of ACS in C2238/ANP-MA carriers.

Of note, both gender and left ventricular function did not exert any significant impact on ACS recurrence in C2238/ANP-MA carriers, although a trend towards protection was observed in subjects with maintained left ventricular function.

Kaplan Meyer curves based on re-ACS occurrence clearly showed that subjects carrying the C2238/ANP-MA were those with the highest risk of new events occurrence (p=0.035, Fig.2).

Discussion

Our study demonstrates that carrier status of the C2238/ANP-MA at the T2238C ANP gene variant is significantly associated with a long-term increased incidence of recurrent ACS in ischemic heart disease patients from an Italian population.

The C2238/ANP-MA has been previously shown to associate with increased risk of acute cardiovascular events in different human cohorts (4-8), although with some controversies (4, 9, 10). We recently reported for the first time its negative prognostic role in two distinct populations of stable angina patients, leaving unaddressed, at that time, the impact of this ANP gene variant on cardiovascular outcome of patients who already experienced an ACS (7). In this regard, our current study extends previous evidence on the role of C2238/ANP-MA as a genetic risk factor for CAD,

and it demonstrates that after a long follow-up the C2238/ANP-MA is still significantly associated with recurrent ACS in patients who already had an ACS as first manifestation of CAD.

The clinical characteristics of our population were comparable to those of previously reported ischemic heart disease patients cohorts, with higher incidence of common risk factors for myocardial infarction, i.e. hypertension, diabetes, hypercholesterolemia. The latter turned out to be, by multivariate analysis, a significant risk factor for recurrence of ACS in our study sample, whereas both hypertension and diabetes were not (likely as a result of the ongoing medical treatments).

With regard to the impact of therapy, use of both beta-blocker and statin was confirmed as a highly protective strategy for secondary prevention of ACS in our patients. The latter finding is consistent with previously reported observations in other cohorts of CAD patients (20-23).

Importantly, despite the protective role of medical treatments, carrier status for the C2238/ANP-MA was revealed as an independent risk factor for recurrence of ACS. Not surprisingly, its dangerous effect was favoured by age above 55 years, that is a well known cardiovascular risk factor on its own (24, 25). The majority of patients enrolled in the study were males. In fact, it is known that male sex is a not modifiable risk factor for AMI (26).

The molecular mechanisms underpinning the deleterious vascular effects and the negative role of this ANP variant on the risk of acute cardiovascular events have been recently dissected out (27, 28). In fact, we documented, by both in vitro and ex vivo experiments, that CC2238 αANP promotes vascular damage through a deregulated activation of the natriuretic peptide type C receptor signalling that is associated with inhibition of Akt pathway by inhibition of cAMP/PKA axis. In particular, we were able to demonstrate that healthy subjects free of cardiovascular risk factors and carrying the C2238/ANP-MA had a significantly impaired endothelial-dependent vasorelaxation. Selective inhibition of NPR-C completely restored endothelial viability and function in rat vessels exposed to mutant ANP (28). Thus, our previous data provide clear evidence of a tight pathophysiological link between mutant ANP and increased vascular damage with consequent vascular events.

Recently, the C2238/ANP-MA turned out to be a significant risk factor for acute cardiovascular events in a large general population, followed up to nine years, from Minnesota, USA (29).

Our present data further suggest that a partial inhibition of NPR-C would be recommended in subjects carrying C2238/ANP-MA in order to reduce their cardiovascular risk, particularly if affected by ischemic heart disease.

In addition, our new results strengthen the need to introduce into clinical practice the characterization of T2238C/ANP allele carrier status in CAD patients. Knowledge of the C2238/ANP-MA carrier status, before a NPR-C targeted therapy becomes available, contributes to identify a more complete cardiovascular risk profile of affected patients and it provides a straightforward indication for more aggressive treatment strategies as well as for more frequent clinical monitoring. It is also likely that knowledge of the C2238/ANP-MA carrier status may contribute to improve the management of all cardiovascular disease patients. Notably, diuretic treatment led to optimal blood pressure control and to a significant reduction of cardiovascular risk in hypertensive patients carrying C2238/ANP-MA from the ALLHAT population (30). The latter evidence suggests an impaired diuretic effect of the ANP variant and that diuretics should be considered as first-choice therapeutic agent for patients carrying the C2238/ANP-MA. Despite the lack of appropriate suitability of our study sample to verify this issue (since therapies had been randomly rather than genotype-based assigned over the years), we observed, among hypertensives, a trend towards higher percentage of C2238 allele versus wild type allele carriers assuming diuretics (18% vs 12%).

Study limitations. Our study was a retrospective, observational investigation limited to the information available from the electronic health records of our center and to the patients medical documentation. Moreover, due to the retrospective approach of our analysis, all deaths were missed and they could not be considered as an end point of the current study. As a consequence, we focused our interest on recurrence of acute coronary events, as the major end point of the analysis, in order to evaluate the cardiovascular outcome of patients following an ACS episode.

Conclusions. Subjects experiencing ACS are more prone to develop new coronary events over a long-term follow up if carriers of the C2238/ANP-MA. Our new findings further support the role of this ANP variant as a novel cardiovascular genetic risk factor. Furthermore, they highlight the need to screen the presence of this variant in CAD patients in order to improve both clinical and therapeutic managements.

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Figures legends

Figure 1

Results of univariate Cox analysis, based on the events registered over the follow-up time in the two groups of patients: C2238/ANP-MA carriers (red dots) and wild type ANP allele carriers (blue dots).

Figure 2

Kaplan Meyer curves for ACS recurrence in patients carrying the C2238/ANP-MA (red line) vs patients carrying the wild type ANP allele (blue line).

Table 1: Characteristics of the study sample at the time of enrolment.

Genotype	Gender	Mean age at first event (years ± SD)	Hypertension (n = 364)	Diabetes (n, %)	Hypercholesterolemia (n, %)	Metabolic Syndrome (n = 349)	Diagnosis at first event	Mean ejection fraction at enrollment (%± SD)
C2238 carriers (n = 103)	males: 89 (85,6%) females: 15 (14,4%)	62,3 ± 8,9	Yes: 75 (72.8%) No: 23 (22.3%) Unknown=3	Yes:32 (30,1%) No: 72 (69,9%)	Yes: 61 (58,7%) No: 43 (41,3%)	Yes: 49 (47,6%) No: 45 (43,7%) Unknown=10	STEMI: 42 (40,8%) NSTEMI: 23 (22,3%) UA: 38 (36,9%)	53 ± 9
TT2238 carriers (n = 276)	males: 219 (79,6%) females: 56 (20,4%)	62,1 ± 9,6	Yes: 194 (70.3%) No: 76 (27.5%) Unknown=12	Yes: 82 (29,7%) No: 194 (70,3%)	Yes: 176 (64%) No: 99 (36%)	Yes: 123 (44.6%) No: 132 (47,8%) Unknown=20	STEMI: 126 (46,6%) NSTEMI: 67 (24,2%) UA: 83 (30,0%)	51 ± 10

Legend: STEMI=ST-segment elevation myocardial infarction; NSTEMI=non-ST-segment elevation myocardial infarction; UA=unstable angina.

Table 2. Types of coronary revascularization at first ACS episode

Genotype	PCI+stent PCI+POBA	BMS/DES	CABG	No revascularization
T2238 allele carrier	310	177/97	42	30
C2238 MA carrier	116	57/31	19	11

Legend:
PCI= percutaneous coronary intervention; POBA= percutaneous balloon angioplasty; BMS=bar metal stent; DES= drug eluting stent; CABG=coronary artery by pass grafting
MA=minor allele

Table 3: Alleles and genotypes frequencies.

Genotypes	Frequency	Percentage
TT2238	276	72,8%
T2238C	97	25,6%
CC2238	6	1,6%
Alleles		
T		85,6%
C		14,4%

Table 4: Pharmacological therapy during follow-up in C2238/ANP-MA and wild-type individuals.

T2238C allele carrier status	Pharmacological Therapy		Years of follow up (n)			
			0-5 (185)	0-10 (145)	0-20 (39)	p-value
Wild type	Beta-Blocker	No	65	37	16	0,718
		Yes	73	65	9	
Minor		No	21	20	6	
		Yes	26	23	7	
Wild type	Anti-Aldosteronic	No	133	100	22	0,898
		Yes	5	2	2	
Minor		No	45	39	13	
		Yes	2	4	0	
Wild type	Hydrochlorothiazide	No	123	96	24	0,06
		Yes	15	6	1	
Minor		No	40	37	11	
		Yes	7	6	2	
Wild type	Acetylsalycilic Acid	No	32	38	14	0,291
		Yes	106	64	11	
Minor		No	9	14	3	
		Yes	38	29	10	
Wild type	Nytroglicerin	No	97	69	15	0,267
		Yes	41	33	10	
Minor		No	30	28	6	
		Yes	16	15	7	
Wild type	Calcium channel Blocker	No	110	76	22	0,275
		Yes	28	26	3	
Minor		No	42	34	9	
		Yes	4	9	4	
Wild type	ACE-Inhibitor	No	77	67	17	0,908
		Yes	61	35	8	
Minor		No	29	27	6	
		Yes	17	16	7	
Wild type	Angiotensin Receptor Blocker	No	109	77	21	0,999
		Yes	29	25	4	
Minor		No	32	37	13	
		Yes	14	6	0	
Wild type	Other Anti-Platelet Drugs	No	84	84	17	0,991
		Yes	54	18	8	
Minor		No	28	34	10	
		Yes	18	9	3	
Wild type	Statin	No	35	22	7	0,191
		Yes	103	80	18	
Minor		No	14	13	4	
		Yes	32	30	9	

Table 5: Predictors of ACS recurrence at follow-up by univariate analysis.

	HR (95% CI)	p-value
C2238/ANP-MA	1,321 (1,000 – 1,730)	**0,035**
Sex	1,066 (0,771 – 1,474)	0,699
Age	1,022 (0,989 – 1,015)	0,751
Diabetes	1,122 (0,879 – 1,432)	0,355
Hypercholesterolemia	1,240 (0,956 – 1,607)	0,105
Hypertension	0,818 (0,620 – 1,079)	0,155
Metabolic Sindrome	0,927 (0,550 – 1,562)	0,775
EF	0,340 (0,107 – 1,080)	0,070
Beta-Blocker	0,711 (0,551 – 0,918)	**0,009**
Antialdosteronic drug	0,766 (0,361 – 1,624)	0,487
Hydrochlorothiazide	0,600 (0,336 – 1,072)	0,085
Acetylsalicilic acid	0,711 (0,553 – 0,915)	**0,008**
Nitrates	1,233 (0,949 – 1,602)	0,116
Calcium channel blocker	1,006 (0,732 – 1,384)	0,969
ACE-Inhibitor	0,881 (0,672 – 1,156)	0,362
Angiotensin Receptor Blocker	0,813 (0,577 – 1,145)	0,235
Statin	0,726 (0,562 – 0,938)	**0,014**

Legend: ACE=angiotensin converting enzyme; EF=ejection fraction

Table 6: Predictors of ACS recurrence at follow-up by multivariate analysis.

	HR (95% CI)	p-value
C2238/ANP MA	1,321 (1,010 – 1,730)	**0,042**
Hypercholesterolemia	1,471 (1,104 – 1,959)	**< 0,001**
Beta-Blocker	0,691 (0,519 – 0,920)	**0,011**
Acetylsalicilic acid	0,798 (0,614 – 1,037)	0,091
Statin	0,619 (0,453 – 0,847)	**0,002**

Figure 1

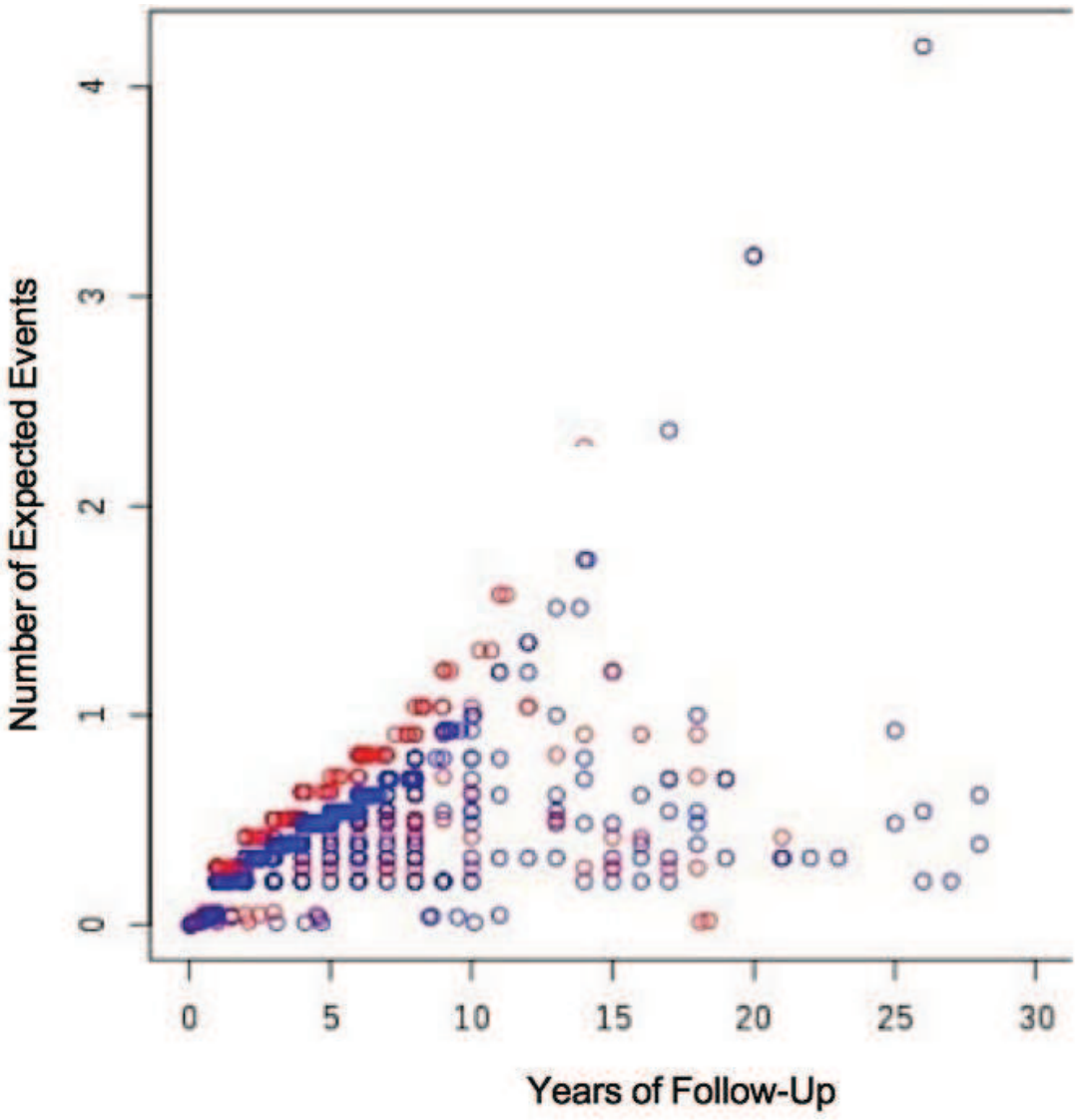
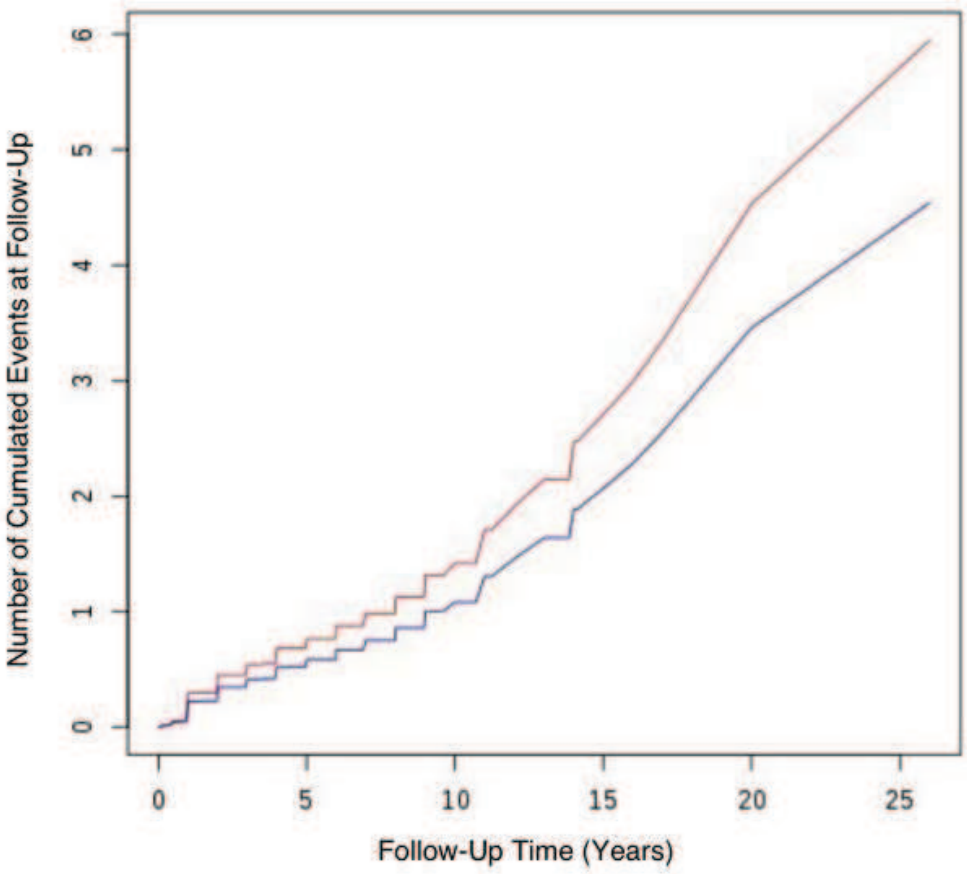

Number of Expected Events
0
1
2
3
4
0
5
10
15
20
25
30
Years of Follow-Up

Figure 2


Number of Cumulated Events at Follow-Up
0
1
2
3
4
5
6
0
5
10
15
20
25
Follow-Up Time (Years)