Genetics

Genetic diversity of the *KCNE1* gene and susceptibility to postoperative atrial fibrillation

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Background The human *KCNE1* protein forms the β-subunit of the IKs potassium channel and is important in the regulation of the atrial action potential duration. The purpose of this study was to investigate the association between the nonsynonymous 112G>A mutation of the *KCNE1* gene and postcardiac surgery atrial fibrillation (AF).

Methods and results A cohort of patients scheduled for cardiac surgery was prospectively recruited. The genotype of 112G>A polymorphism was determined using polymerase chain reaction/restriction fragment analysis and confirmed with direct sequencing of the polymerase chain reaction product. In total, 509 patients were recruited in the study, of whom 203 (39.9%) had at least 1 qualifying episode of postoperative AF. An increased frequency of the G allele was observed in the postoperative AF group compared with the group without postoperative AF (0.628 vs 0.552, respectively, $P = .016$). The individual's relative risk of postoperative AF increased as the number of G alleles increased from 1.36 (95% CI 0.89-2.08) for G allele heterozygotes to 1.62 (95% CI 1.08-2.43) for G allele homozygotes ($P = .04$ for trend). The multivariate analysis revealed the abnormal ejection fraction (odds ratio [OR] 1.585, 95% CI 1.076-2.331, $P = .020$), age (OR 1.043, 95% CI 1.022-1.064, $P < .001$), type of surgery (aortic valve replacement) (OR 1.869, 95% CI 1.094-3.194, $P = .022$), and the 112G>A genotype (OR 1.401 [in additive model], 95% CI 1.052-1.865, $P = .021$) to be independent predictors of postoperative AF.

Conclusion This study confirmed the association of the 112G>A polymorphism and postoperative AF in a cohort of patients undergoing cardiac surgery. (Am Heart J 2014;167:274-280.e1.)

Atrial arrhythmias and atrial fibrillation (AF), in particular, commonly occur postoperatively.[1,2] The reported incidence of postcardiac surgery AF ranges from 5% to 60%.[1,2] Lower incidence has been reported following noncardiac surgery ranging from 0.3% to 29%.[1,3] This variation in incidence reflects differences in patient demographics, techniques for rhythm monitoring, and inconsistency in criteria for diagnosis. Postoperative AF is associated with significant morbidity, mortality, and a considerable increase of inhospital stay and treatment costs.[4,5]

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The epidemiology, clinical consequences, and preventive strategies of postoperative AF have been previously assessed. Nevertheless, the exact pathophysiologic mechanisms responsible for the onset and perpetuation of postoperative atrial arrhythmias are not completely understood.[4,5] Postoperative AF appears to be associated with inflammation, sympathetic activations, and oxidative stress.[1]

The identification of extended families with AF and the mapping of discrete genetic loci in such families have raised the possibility that genetic factors might affect susceptibility to AF.[6] A variety of genome-wide association studies have identified genomic regions associated with AF. Among these, the 4q25, 16q22, and 1q21 seem to be of particular interest,[7-11] providing a compelling argument that inherited gene defects potentially predispose individuals to AF per se or AF as part of other arrhythmic and/or cardiomyopathic syndromes. Thus, AF appears to be the result of a complex interaction between genetics and environmental factors.[6,12-14]

The *KCNE1* gene forms the β-subunit of the potassium channel carrying the cardiac Iks current.[15] Cardiac Iks channels contribute to atrial repolarization in the late phase of the action potential.[16] A nonsynonymous, single-

nucleotide polymorphism (SNP) in the *KCNE1* gene (A to G substitution at position 112, rs1805127), leading to a glycine substitution for serine at amino-acid position 38 (S38G), was previously associated with increased AF incidence.[17,18] Of note, the S38G variant in the *KCNE1* gene reduces Iks current density and prolongs the atrial action potential duration, whereas familial AF-causing mutations in Iks channel genes have all had gain-of-function effects.[8,16]

In the present study, we assessed the impact of the KNCE1 S38G polymorphism on the incidence of postoperative AF in a prospectively recruited cohort of patients subjected to cardiac surgery. To the best of our knowledge, no data are available on the effect of the *KCNE1* polymorphisms to postoperative AF.

Methods

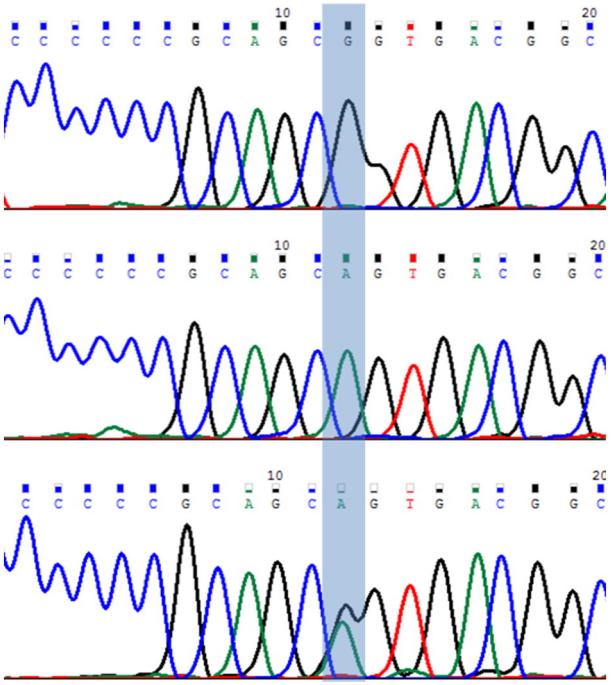
Study population

A prospective observational study was designed to assess the impact of polymorphisms of the *KCNE1* gene on susceptibility to AF associated with cardiac surgery. Patients undergoing cardiac surgery were prospectively recruited from the Onassis Cardiac Surgery Center of Athens, between January 2011 and May 2011. The study aimed for the consecutive enrollment of patients. Exclusion criteria included age <18 years, history of AF, or anatomic valvular heart disease predisposing to AF (atrial diameter on echocardiography >50 mm, or mitral stenosis). This study further excluded patients with mitral valve disease as the primary indication for cardiac surgery. However, we included cases necessitating intervention to the mitral valve if coronary artery disease was the primary indication for cardiac surgery. Each patient underwent a standardized interview and physical examination to identify past medical conditions, medication intake, and possible triggers for AF. All the patients received standard postsurgical care, including intensive care treatment for 24 to 72 hours postsurgery. Approval for the present study was obtained from the ethics committee of the Onassis Cardiac Surgery Centre, and all the study participants provided informed consent.

Clinical definitions and end points

New-onset AF occurring postoperatively and before hospital discharge was the study's end point. Qualifying episodes of AF were considered to be those episodes lasting >60 minutes requiring antiarrhythmic treatment or direct current cardioversion. All episodes were documented on telemetry and confirmed with 12-lead electrocardiogram. *Hypertension* was defined as systolic and/or diastolic blood pressure above the recommended upper limits by the European Society of Cardiology guidelines on the management of hypertension[19] or treatment with an antihypertensive agent at the time of recruitment. *Diabetes mellitus* was defined as fasting glucose ≥126 mg/dL or oral glucose challenge ≥200 mg/dL after 2 hours or diabetes treated with oral antidiabetic agent, insulin, or both agents. *Hypercholesterolemia* was defined as a concentration of the serum cholesterol levels (≥200 mg/dL) or the use of lipid-lowering agents. Left ventricular ejection fraction was calculated using 2-dimensional echocardiography or left ventriculography during cardiac catheterization. *Chronic renal failure* was defined as serum creatinine levels >2.0 mg/dL or treatment with dialysis.

Figure 1



Representative sequencing electrophoretograms depicting the SNP rs1805127 of the gene *KCNE1* (nucleic acid change, G112A; amino acid change, S38G).

Genotyping

Ten milliliters of blood was drawn from all the study participants. DNA was extracted following standard procedures.[20] Polymerase chain reaction (PCR) was performed to isolate a 508-fragment of the *KCNE1* gene framing the 112G>A locus and 6 previously described SNPs.[21] In total, 192 PCR products were purified and subjected to direct sequencing at GATC Biotech (GATC Biotech, Konstanz, Germany) (Figure 1). For the remainder of the study, genotyping of the participant was performed by restriction fragment length polymorphism analysis. To the best of our knowledge, there is no known linkage disequilibrium between the polymorphism investigated in this study and other polymorphisms that can introduce bias in the reported associations.

Statistical analysis

Means and SDs were calculated for the continuous variables. Frequencies and percentages were calculated for the categorical variables. Continuous variables were analyzed using 1-way analysis of variance or independent-sample *t* test, whereas categorical variables were analyzed using the χ^2 or Fisher exact test as appropriate. The χ^2 test was used to test the deviation of genotype distribution from the predicted genotype frequencies based on the Hardy-Weinberg equilibrium. The

Table I. Demographic and clinical characteristics of the population studied

		No AF (n = 306)	AF (n = 203)	*P*
Age (mean ± SD)		64 (9)	68 (10)	<.0001
Age	<65	153 (50)	68 (33.5)	<.0001
	65-75	103 (33.7)	82 (40.4)	.133
	>75	50 (16.3)	53 (26.1)	.009
Female gender		42 (13.7)	34 (16.7)	.375
Risk factors				
Hypertension		180 (88.7)	259 (84.6)	.237
Diabetes		99 (32.4)	79 (38.9)	.130
Hyperlipidemia		267 (87.3)	172 (84.7)	.433
Current smoker		88 (43.3)	165 (46.1)	.585
Family history of CAD		131 (42.8)	79 (38.9)	.409
Myocardial infarction (history)		107 (35)	73 (36)	.850
Chronic renal failure		9 (2.9)	9 (4.4)	.463
Ejection fraction (mean ± SD)		52.4 (7.8)	51.5 (8)	.228
Ejection fraction	<35	2 (0.7)	2 (1)	.653
	35-44	44 (14.4)	33 (16.3)	.614
	45-54	84 (27.5)	69 (34)	.139
	>55	176 (57.5)	99 (48.8)	.06
Procedure characteristics				
CABG (isolated)		255 (83.3)	159 (78.3)	.165
CABG plus valve/aorta intervention		23 (7.5)	20 (9.8)	.416
Valve/aorta intervention (isolated)		28 (9.2)	24 (11.8)	.371
Off-pump CPB		20 (6.5)	8 (3.9)	.238
No. of grafts	1	32 (10.5)	18 (8.9)	.649
	2	104 (34)	66 (32.5)	.774
	>2	142 (46.4)	95 (46.8)	1.000
No. of arterial grafts	0	37 (12.1)	31 (15.3)	.184
	1	177 (57.8)	130 (64)	.167
	>1	92 (30.1)	42 (20.7)	.024
Concurrent valve repair		36 (11.8)	41 (20.2)	.011
Aortic valve replacement		32 (10.5)	40 (19.7)	.004
Mitral valve replacement/repair		6 (2)	3 (1.5)	1.000
Ascending aortic aneurysm		24 (7.8)	15 (7.4)	1.000
Pump duration (mean ± SD)		103 (44)	108 (37)	.263
Aortic clamp duration (mean ± SD)		74 (38)	76 (33)	.247
Perioperative medication				
β-Blockers preoperative		230 (75.2)	154 (75.9)	.916
Statins preoperative		257 (84)	156 (76.8)	.049
ACE inhibitors preoperative		205 (67)	144 (70.9)	.381
Amiodarone preoperative		–	–	–
β-Blockers postoperative		270 (88.2)	169 (83.3)	.116
Statins postoperative		224 (73.2)	143 (70.4)	.545
ACE inhibitors postoperative		104 (34)	59 (29.1)	.286
Amiodarone postoperative*		25 (8.2)	166 (81.8)	<.001
β-Blocker interruption		18 (5.9)	19 (9.4)	.163
Statin interruption		49 (16)	28 (13.8)	.529
ACE inhibitors interruption		128 (41.8)	98 (48.3)	.172
POAF risk index[22] (mean ± SD)		17 (11)	21 (12)	<.001

Values represent number or patients (percentages) unless otherwise indicated. Statistical significances are derived by the χ^2/Fisher exact test analysis for categorical/dichotomous variables or independent-sample *t* test for continuous variables. *CAD*, Coronary artery disease; *CABG*, coronary artery bypass grafting; *CPB*, cardiopulmonary bypass; *POAF*, postoperative AF.

* Amiodarone postoperatively was given to patients after AF as antiarrhythmic treatment.

stepwise binary logistic regression analysis was used to assess factors independently associated with the study's outcome. The association between the 112G>A polymorphism of the *KCNE1* gene and postoperative AF was assessed by binary logistic regression analysis under a dominant, recessive, and additive genetic model.

Assuming a 30% event rate in the rare allele group and 0.45 rare allele frequency, with the given sample size, we will be able to detect true odds ratios (ORs) for disease of 0.52 or 1.78 in the rare allele group relative to the common allele group with a probability (power) of 0.8. The Type I error probability associated with this test of the null hypothesis that this OR equals 1 is 0.05.

$P < .05$ was considered statistically significant. Analysis was performed with the SPSS version 17.0 (SPSS, Inc, Chicago, IL) and the R statistical package (www.r-project.org).

Table II. Genotype and allele frequencies in the cohort

Genotype	Total cohort	No AF	AF	P*
GG	164 (32.2)	88 (28.8)	76 (37.4)	.042
AG	265 (52.1)	162 (52.9)	103 (50.7)	.651
AA	80 (15.7)	56 (18.3)	24 (11.8)	.062
G allele frequency	0.583	0.552	0.628	.016
Total	509	306 (60.1)	203 (39.9)	

* Statistical significances are derived by χ^2/Fisher exact test.

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Results

Clinical characteristics

In total, 509 patients were recruited in the study, with a mean age of 65 (SD 10), and 85.1% of these patients were male. Of the 509 patients, 203 (39.9%) presented at least 1 qualifying episode of AF postcardiac surgery. Clinical and demographic characteristics of the study population are summarized in Table I. Results of the univariate analysis showed that clinical factors associated with postoperative AF included age and type of surgery (aortic valve replacement and use of >1 arterial graft) (Table I).

Allele, genotype frequencies, and Hardy-Weinberg equilibrium

A 508-bp fragment of the coding sequence of *KCNE1* gene was screened for changes by direct sequencing, and 1 polymorphism was identified (112G>A). The allele frequency of the remaining 5 SNPs in our pilot cohort of 192 patients who underwent sequencing was <0.02, and they were not included in further analysis. Genotype and allele frequencies in the study's population are summarized in Table II. Observed allele frequencies were in accordance with expected frequencies by the Hardy-Weinberg equilibrium in the total cohort and the end point–positive and end point–negative subgroups (Table III).

The 112G>A polymorphism and incidence of postoperative AF

A higher frequency of G allele was observed in the postoperative AF group compared with the group without postoperative AF (0.628 vs 0.552, respectively, $P = .016$). Accordingly, the individual's relative risk of postoperative AF increased as the number of G alleles increased from 1.36 (95% CI 0.89-2.08) for G allele heterozygotes to 1.62 (95% CI 1.08-2.43) for G allele homozygotes ($P = .04$ for trend) (Figure 2).

Table III. Observed and expected genotype frequencies according to the Hardy-Weinberg equilibrium

	Observed			Expected				
	GG	AG	AA	GG	AG	AA	χ^2	P
Total cohort	164	265	80	172.72	247.57	88.72	2.52	.11
AF	76	103	24	80.08	94.84	28.08	1.5	.22
No AF	88	162	56	93.34	151.33	61.34	1.52	.22

The results of the multivariate stepwise binary regression analysis revealed that the abnormal ejection fraction, age, and type of surgery (aortic valve replacement) as well as the 112G>A genotype assessed under the dominant (GG or AG vs AA) and additive (risk of disease per no. of G alleles) effects were independent predictors of postoperative AF (Table IV). A model composing of these 4 factors (age, genotype, aortic valve replacement, and abnormal ejection fraction) could explain 10% of the variation in the binary outcome.

Two models including clinical factors and clinical and genotype factors were developed. A comparison of these models is summarized in Table V.

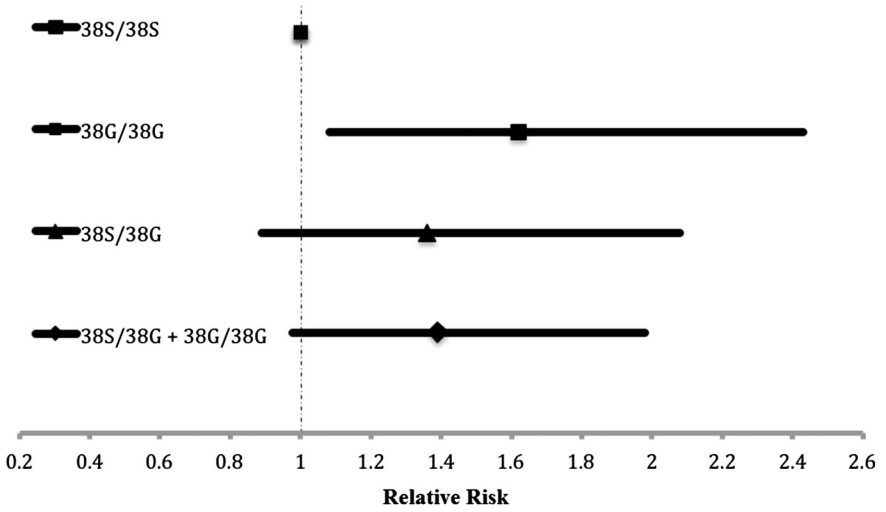
No association was observed between the 112G>A polymorphism and demographic or clinical factors, including severity of coronary artery disease and left ventricular systolic dysfunction.

Discussion

This is the first study assessing the *KCNE1* gene polymorphisms as potential predisposing factors for postoperative AF in white subjects. Our results showed an association between the number of G alleles and the risk of postoperative AF. Patients with 2 G alleles have an approximately 60% higher risk for postoperative AF, using the AA genotype as the baseline risk. Moreover, the multiple logistic regression analysis demonstrated that this effect was independent of patient- and operation-related factors, including periprocedural medication (β-blockers, angiotensin-converting enzyme inhibitors, statins), and type of procedure. Our findings are in agreement with previous epidemiological observations that suggested an association between the 112G>A polymorphism and AF in general population[17,18] and functional studies that demonstrated an important impact of the 112G>A polymorphism on the regulation of the Iks current.[16]

Postoperative AF is the most common complication of cardiac surgery and an ideal setting for assessing genetic predisposition to AF.[1,2] It provides an opportunity for prospective recruitment of an AF-naive population, possibly to present AF at a given time point and with an extremely high event rate. The reasons for some patients developing postoperative AF, whereas others do not, remain unclear. It has been hypothesized that

Figure 2



Relative risk for AF. Ratios were calculated compared with patients with minK 38S/38S genotype. Bars, 95% CIs.

Table IV. Clinical and genetic factors independently associated with postsurgery AF in stepwise binary logistic regression analysis

		95% CI		
Recessive model	**OR**	**Lower**	**Upper**	***P***
Genotype				
GG	1.477	0.978	2.233	.064
Age (y)	1.041	1.041	1.062	<.001
Aortic valve replacement	1.880	1.105	3.195	.020
Abnormal ejection fraction*	1.605	1.093	2.353	.016
Dominant model				
Genotype				
GG or AG	1.751	1.007	3.045	.047
Age (y)	1.042	1.021	1.063	<.001
Aortic valve replacement	1.845	1.082	3.144	.024
Abnormal ejection fraction*	1.600	1.088	2.353	.017
Additive model				
Genotype				
No. of G alleles	1.401	1.052	1.865	.021
Age (y)	1.043	1.022	1.064	<.001
Aortic valve replacement	1.869	1.094	3.194	.022
Abnormal ejection fraction*	1.585	1.076	2.331	.020

Dominant, recessive, and additive genetic models were assessed. Variables entered in step 1 model: age, gender, diabetes mellitus, hypertension, smoking, hyperlipidemia, family history of coronary artery disease, chronic renal failure, type of surgery, and number of arterial grafts.
*Defined as ejection fraction <0.55.

Table V. Comparison of prediction models with and without genotype data

	Clinical model*	**Clinical/genotype model***
Log likelihood	655.9	649.5
HL χ^2 (*df*)	2.111 (8)	7.596 (8)
Nagelkerke's R^2	0.074	0.090
Area under the curve	0.638 (0.590-0.687)	0.655 (0.606-0.703)

*The clinical model includes age, abnormal ejection fraction, and aortic valve replacement. The clinical/genotype model includes the clinical factors plus number of G alleles. *HL*, Hosmer and Lemeshow.

inflammation, oxidative stress, and sympathetic activation can potentially trigger AF in the fraction of postcardiac surgery patients predisposed to AF.[1] Several factors have been shown to confer susceptibility to AF post surgery, including clinical and genetic factors. Notably, some of the most potent clinical risk factors for postoperative AF (age and structural heart disease) also confer susceptibility to AF in the general population.[5,22-24] In our analysis, as in most previous studies, age, type of surgery (aortic valve replacement), and impaired left ventricular systolic emerged as independent predictors of postsurgical AF.[22-24]

Genetic variability of the *KCNE1* gene has been previously associated with AF.[17,18] *KCNE1* encodes the β-subunit of cardiac Iks channel, and its expression in atrial tissue is documented both at the messenger RNA and protein level.[18] The *KCNE1* 38G variant has been associated with a higher risk of AF in a number of different ethnic populations.[17,18] Cardiac Iks channels are capable of modulating the action potential duration and restitution properties of the atrial tissue as part of their contribution in the slowly activating delayed rectifier potassium current.

Currently, the functional role of *KCNE1* in the pathogenesis of postoperative AF is unclear. Ehrlich et al[16] have demonstrated that the *KCNE1* 38G isoform is associated with a reduced Iks current and increased action potential duration, possibly due to a decreased

Iks channel membrane expression. Furthermore, Heerd et al[25] showed that there is a strong correlation between atrial *KCNE1* down-regulation and postoperative AF in Sinclair swine.

For the present analysis, a prospective study design with a sufficient sample size was used to detect clinically relevant phenotype-genotype interactions, taking into consideration all possible clinical and demographic characteristics. Targeted SNP analysis was carried out, selecting the 112G>A *KCNE1* gene polymorphism due to the existing convincing evidence of its role in the pathophysiology of AF derived from both electrophysiological and genetic-epidemiological studies. Direct sequencing of a large part of the gene's coding sequence confirmed the existence of one polymorphism (112G>A) with clinically relevant allele frequency, in accordance with previously published data.[21]

The pathophysiologic background underlying this association is not clear. The role of *KCNE1* gene is not well defined, and the functional consequences of the S38G polymorphism are poorly understood. It is possible that the prolongation of the action potential duration associated with reduced Iks current provides a substrate for atrial arrhythmias that can become clinically evident in the presence of inflammatory, chemical, mechanical, or electrical triggers. However, the exact mechanism linking causative genetic variants with clinical outcome and susceptibility to AF remains to be elucidated.

Limitations

This is a single-center study consisting only of South European whites limited by the lack of external validations. Thus, the results obtained in this study cannot be extrapolated in different ethnic groups and should be further validated. Despite that, in our cohort, the S38G genotype appears to improve the predictive performance of clinical data, safe conclusions cannot be made without an external validation. Nevertheless, our findings are supported by previous epidemiological observations and functional studies.[16-18]

Every effort has been made to exclude history of AF from the recruited population. However, the presence of asymptomatic AF in our sample cannot be excluded, although an accurate interview weighted to symptoms associated with dysrhythmias has been performed. We used a short follow-up period (postoperative hospitalization), following the study design of previous studies in the field. Thus, AF episodes that occurred after hospital discharge were missed. There are likely factors that could predispose to postoperative AF that were not validated in this analysis including inflammatory and oxidative stress markers. Finally, results from the setting of postoperative AF, although suggestive of possible genotype-phenotype interactions, cannot be extrapolated in the general population.

Conclusion

In the present study, we confirmed the association of the 112G>A polymorphism of the *KCNE1* gene and postoperative AF in a cohort of patients undergoing cardiac surgery. In addition, we demonstrated that a number of patient- and surgery-related factors, including age, abnormal left ventricular ejection fraction, type of surgery, and the 112G>A genotype accounted for a significant part of the risk for postoperative AF. Prospective interventional studies using preoperative risk profile (clinical and genetic) to apply preventive strategies are, therefore, required to determine the potential clinical implications of this association.

See the online Appendix Supplementary Figure.

Disclosures

All authors have no conflicts of interest or relationship with the industry to declare related to this work.

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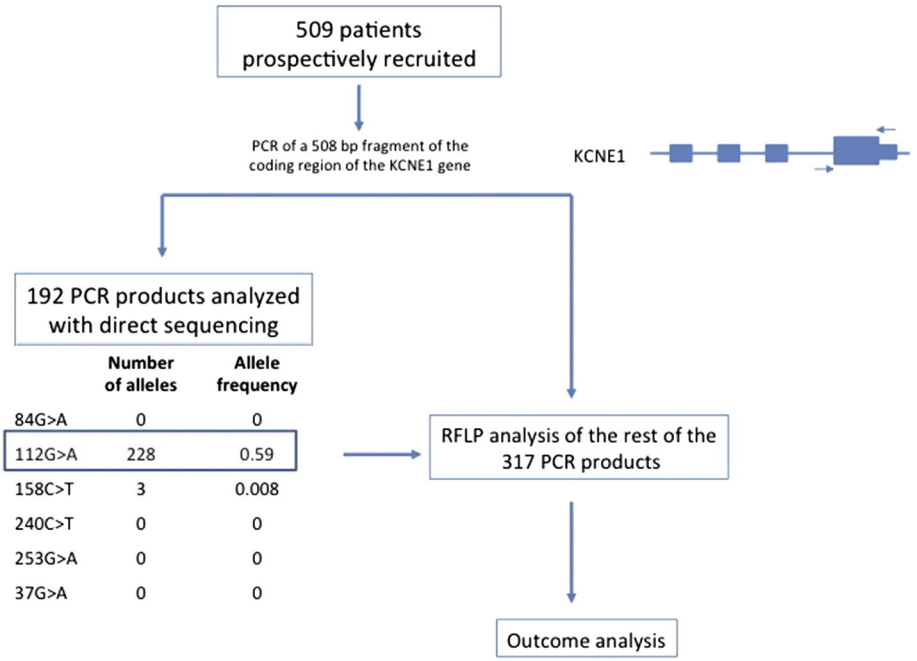
Appendix

Supplementary Figure



Outline of the study design and the methods that have been used. Six polymorphism have been previously identified in the 508-bp gene fragment (84G>A, 112G>A, 158C>T, 240C>T, 253G>A, 37G>A).[21] Two were detected in our population. The 158C>T is a synonymous polymorphism with allele frequency in our sample 0.008. Thus, further analysis by restriction fragment length polymorphism was carried out only for the 112G>A polymorphism. The structure of the KCNE1 gene is also illustrated. Thin lines represent introns, whereas exons are depicted as boxes. Small boxes are noncoding regions, and large boxes are coding regions. Arrows indicate the location of PCR primers (gene size 65,585 bp).