【S-11】

Genetic Variations and Functional Analysis in HERG (KCNH2) Potassium Channel

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For the last 10 years, pharmacogenetic and pathogenetic studies have been performed to discover and characterize the many polymorphic variations and disease-causing mutations in the genes encoding the cardiac ion channels. As such efforts long QT syndrome (LQTS), an arrhythmogenic disorder characterized by prolongation of the QT interval on eletrocardiograms (ECGs), often causes syncope or sudden cardiac death as a result of recurrent and lethal arrhythmias. Chromosome 7-linked inherited LQTS (LQT2) is caused by mutations in human *ether-a-go-go*-related gene (HERG;KCNH2), whereas drug-induced LQTS is caused primarily by HERG channel block. We screened single nucleotide polymorphisms (SNPs) in HERG (LQT2) to understand a comprehensive association of frequent SNPs with QT-interval in the healthy Korean population. We found 17 SNPs in promoter, exon and exon/intron boundary regions. Six of the 17 SNPs were in the coding range. The remaining 11 SNPs were located in introns (9 SNPs) or in promoter regions (2 SNPs). All SNPs except K897T have not been previously described. In addition, we tested LQTS mutations in HERG polymorphic channels to characterize their functional properties using biochemical and voltage-clamp techniques. Conclusionally, we suggest that analysis of these SNPs in a much larger populations would enable establishment of common polymorphisms that are associated with QT interval. These could facilitate prediction of arrhythmia risk in the healthy population as well as LQTS patients.

GENETIC VARIATIONS AND FUNCTIONAL ANALYSIS IN *HERG* (*KCNH2*) POTASSIUM CHANNEL

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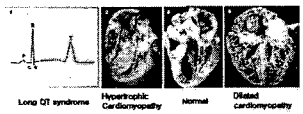
Common Disease with Ion Channel Targets

- Alzheimer's
- Parkinsonism
- Anethesia
- Asthma
- Cystic fibrosis
- Diabetes
- Epilepsy
- Gastro-esophageal reflex
- Glaucoma
- Systemic hypertension
- Pulmonary hypertension
- Immune suppression
- Irritable bowl syndrome
- Stroke
- Urinary incontinence
- Deafness

[redacted]

Condition and Genes causative for cardiac arrhythmia and sudden death

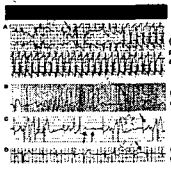


Long QT syndrome	Hypertrophic Cardiomyopathy	Normal	Dilated cardiomyopathy
KCNQ1	MYH7		
KCNH2			
KCNE1	MYBP3C		
KCNE2			
SCN5A	TNNT2		

Definition of Long QT Syndrome (LQTS)

Prolongation of the QT interval corrected for heart rate (QTc) on the surface electrocardiogram associated with T-wave abnormalities, relative bradycardia, and ventricular tachyarrhythmias

[redacted]



(A) The abnormal QT interval is followed by 7 beats of polymorphic ventricular tachycardia (torsade de pointes) after dofetilide treatment.

(B)Torsade de pointes in a patient treated with the antipsychotic haloperidol.

(C) Torsade de pointes in a patient with complete heart block

(D) Markedly abnormal postpause repolarization in a patient with advanced heart failure

Diagnosis of LQT syndrome

-electrocardiographic findings

-QTc greater than 0.44 s,

-presence of symptoms (syncope, seizures, and sudden death)

-family history

Causes of Long QT interval

Congenital (at least six genetic mutations identified)

- Romano-Ward syndrome(autosomal dominant)
- Jervell and Lange-Nielsen syndrome (cardiac abnormality-autosomal dominant & associated deafness-autosomal recessive)
- Brugada syndrome

Acquired

- Drugs
- Cardiac pathology(heart failure, ischemia, myocarditis)
- Electrolyte abnormality(hypokalemia, hypomagnesemia)
- Cerebrovascular disease(subarachnoid haemmorhage, ischemic stroke
- Severe bradycardia (especially complete heart block)
- Hyperthyroidism/hypothyroidism

Drugs That May Provoke Life-threatening Arrhythmias in LQTS

Antiarrhythmics
Amiodarone, Disopyramide, Dofetilide, Ibutilide, Procainamide, Quinidine, Sotalol

Antimicrobial &Antifungals
Amantadine (Symmetrel), Azithromycin, Chloroquine, Clarithromycin (Biaxin), Clindamycin (Cleocin)**, Erythromycin, Gatifloxacin, Halofantrine, Itraconazole, Moxifloxacin (Avelox), Pentamidine (NebuPent), Sparfloxacin (Zagam), Sulfamethoxazole-Trimethoprim (Bactrim, Septra)

Psychotropics
Dolasetron (Anzemet), Doxepin (Sinequan), Haloperidol (Haldol), Levacetylmethadol (Orlaam), Mesoridazine (Serentil), Phenothiazines,
Risperidone (Risperdal), Thiothixene (Navane), Thioridazine (Mellaril), Thorazine, Tricyclics, Ziprasidone (Geodon)

Others (Antihistamine)
Albuterol (Proventil), Bepridil (Vascor), Diuretics (water pills), Epinephrine (Adrenaline), Felbamate (Felbatrol), Ketanserin, Methadone, Pimozide (Orap)

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Drugs withdrawn by QT prolongation from major markets since 1990

Astemizole (Hismanal)	Allergies	Withdrawn voluntarily by manufacturer 1999(US)	QT interval prolongation and TdP	NA	*KCNH2, KCNQ1, KCNE1, KCNE2, KCNE3, CYP2J2*
Cisapride (Propulsid)	Nocturnal heartburn due to gastroesophageal reflux	Withdrawn by manufacturer/health authorities 2000 (most major markets)	QT interval prolongation and TdP	$950 million in 1999	*KCNH2, KCNQ1, KCNE1, KCNE2, KCNE3,SCN5A*
Droperidol (Inapsine)	Premedication for anesthesia	Withdrawn 2001 (UK)	QT interval prolongation and TdP	NA	*KCNH2, KCNQ1, KCNE1, KCNE2, KCNE3*
Grepafloxacin (Raxar, Vaxar)	Bacterial infections	Withdrawn by manufacturers 1999 (US and elsewhere)	QT interval prolongation and TdP	$23.5million in 24 months on market	*KCNH2, KCNQ1, KCNE1, KCNE2, KCNE3*
Sertindole (Serdolect)	Schizophrenia	Withdrawn 1998 (worldwide)	QT interval prolongation and TdP	$15.6 million in 1998	*KCNH2, KCNQ1, KCNE1, KCNE2, KCNE3*
Terfenadine (Seldane, Triludan)	Allergies	Withdrawn by manufacturer/health authorities(1997-1999)(5 comunities);restricted in others	Drug interaction, QT interval prolongation and TdP	$600 million per year before drug-interaction warnings	*KCNH2, KCNQ1, KCNJ11, KCNA5,*
Terodiline	Urinary incontinence	Withdrawn 1992 (worldwide)	TdP	NA	*KCNH2, KCNQ1, KCNE1, KCNE2, KCNE3, CYP2C19*

Nature Genetics 37(7):671-681, 2005

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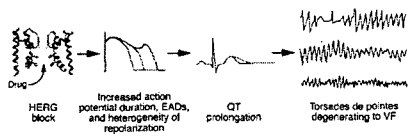
Mechanisms of sudden death with HERG blockade



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OUTLINE

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What are SNPs ?

ACGTTTGGATAC
TGCAAACTTATG

ACGTTTGTATAC
TGCAAACCTATG

- Single nucleotide polymorphisms consist of a single change in the DNA code.
- SNPs occur with various allele frequencies. Those in the 20-40% range are useful for genetic mapping.
- Those at frequencies between 1% and 20% may be used with candidate gene approaches.
- Changes at <1% are called variants.

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SNPs vs. Mutation?

SNP	Mutation
1 base pair change	1+base pair changes(deletions)
No necessary phenotype	Phenotype manifestation
Affect any region of genome	Affect genes
Present in populations > 1%	Present in population < 1%
Occur naturally	Occur naturally or in the lab
Germline inheritance	Germline or somatic inheritance

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What are the effects of SNPs?

Where	Result		Effect
In coding region	May be silent, e.g.,UUG→CUG, leu in both cases	sSNP	Usually no change in phenotype
In coding region	May change amino acid sequence, e.g., UUC→UUA, phe to leu, Some characterize these as the least common and most valuable SNPs, Many being patented	nSNP	Phenotype change (may be subtle depending on amino acid replacement and position)
In coding region	May create a "Stop"codon, e. g., UCA→UGA, ser to stop		Phenotype change
In coding region	May affect the rate of transcription (up-or down-regulate)	nSNP	Possible phenotype Change
Other regions	No affect on gene products(?). May act as genetic markers for multi-component diseases. These are sometimes called anonymous SNPs and are the most common.	rSNP	

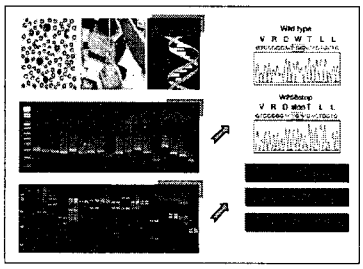
How many SNPs are there ?

- It is estimated that the human genome contains between 3 million and 6 million SNPs spaced irregularly at intervals of 500 to 1,000 bases.
- The SNP Consortium estimates that as many as 300,000 SNPs may be needed to fuel studies.
- 100,000 or more SNPs may be required for complex disease gene discovery

LQT Candidate Genes

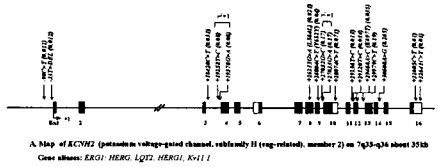
1. KCNQ1(Type 1 LQTs)-16 exons
2. HERG(Type 2 LQTs)-15 exons
3. SCN5A(Type 3 LQTs)-28 exons
4. KCNE1(Type 4 LQTs)-1 exon
5. KCNE2(Type 6 LQTs)-1 exon
6. 기타(LQTs 관련유전자)

SSCP & Direct Sequencing



HERG Channel Allelic Variants Identified in normal Korean (n=54)

num	PROMER	REGION	POS.	CONSENS	RARE	Freq.	Name
1	1F	promoter	-98	C	81	T	1	0.012	-98C>T (0.012)
2	1F	promoter	-33	T	81	DEL	1	0.012	-33 T>DEL (0.012)
3	5R	INT1	16428	C	81	T	3	0.036	16428C>T (0.036)
4	6F	INT3	19254	T	92	C	8	0.08	19254 T>C (0.08)
5	6F	INT3	19378	G	92	A	8	0.08	19378 G>A (0.08)
6	10F	EX8	26213	G	91	A	8	0.023	26213 G>A (0.9648,) (0.023)
7	11F	EX9	26884	C	96	T	4	0.04	26884 C>T (V652V) (0.04)
8	11F	INT9	27032	G	83	C	17	0.17	27032 G>C (0.17)
9	11F	INT9	27033	G	83	A	17	0.17	27033 G>A (0.17)
10	13F	EX10	28074	C	93	T	7	0.073	28074 C>T (0.073)
11	15F	INT11	29236	T	89	C	1	0.011	29236 T>C (0.011)
12	15F	INT11	29320	T	83	C	3	0.034	29320 T>C (0.034)
13	15F	EX12	29668	A	80	C	8	0.085	29668 A>C (K897T) (0.085)
14	16F	INT12	29979	C	81	T	10	0.10	29979 C>T (0.10)
15	17F	INT14	30604	A	72	G	26	0.265	30604 A>G (0.265)
16	19F	EX16	32005	C	97	T	1	0.01	32005 C>T (0.01)
17	19F	EX16	32641	C	97	T	1	0.01	32641 C>T (0.01)



A Map of *KCNH2* (potassium voltage-gated channel, subfamily H (eag-related), member 2) on 7q35-q36 about 35kb

Gene aliases: *ERG1, HERG, LQT2, HERG1, Kv11.1*

HERG Channel Allelic Variants Identified in normal Subjects

	Amino acid change	Amino acid change	Amino acid change	Amino acid change	Region	H.F
542	R181Q(G>A)	N33T(A>C)			N-terminus	1.3
558	G187S(G>A)	R176W(C>T)			N-terminus	0.3
Del559~567	GAG187~189del	V2G1S(T>G)			N-terminus	1.0
569	A190T(G>A)	H254Q(C>G)			N-terminus	0.3
607	A203T(G>A)	P347S(C>T)		P215A(C>G)	N-terminus	0.3
769	R257H(A>C)				N-terminus	0.3
1099	T367S(A>T)				N-terminus	0.3
2617	G873S(G>A)	C723R(T>C)			N-terminus	0.3
2650	K897T(A>C)	K897T(A>C)	K897T(A>C)	K897T(A>C)	C-terminus	8.2
2729	P910L(C>T)	P911L(C>T)	A915V(C>T)		C-terminus	0.3
2900	P967L(C>T)	L1023del3067~3069			C-terminus	0.3
3103	R1033W(C>T)				C-terminus	0.3
3140	R1047L(G>T)	R1047L(G>T)			C-terminus	0.3
3173	A1058E(C>A)				C-terminus	0.3
3203	Q1068R(A>G)				C-terminus	0.3

Mayo Clin Proc. 2003;78:1479-1487

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Comparison of HERG Mutation in between American, Japanese and Danish Patients

and Coding					Expression Studies
Pore Region					
	7	L552S	R534C	R534C	
	7	A561V	A561V	A561V	dominant negative
	7	A561T			
	7	P568L		G572R	
	7	583stop			
	7	G604S			
	7	D609N	Y611H		
	7	T613M			dominant negative
	7	A614V	A614V		dominant negative
	7	F627L		S621N	
	7	G628S			dominant negative
	7	M629I		M629I	
	7	Y630A	Y630L		
	7	K639E			

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Comparison of HERG Mutation in between American, Japanese and Danish Patient

and Coding					Expression Studies
N-terminus region					
	2	S2R			
	2	F29L	F29L		↑Rate of deactivation
	2	K33T			↑Rate of deactivation
	2	C44stop			
	2	G47V			
	2	R56Q			↑Rate of deactivation
	2	D56G			↑Rate of deactivation
	2	H70R			↑Rate of deactivation
	2	P72Q			
	2	A78P			↑Rate of deactivation
	2	fsA83/37ins	82-84in		
	2	L86R			↑Rate of deactivation
	3	M124R	I36T		↑Rate of deactivation
	4	fsV95/63ins	K101E		

(continued)

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Comparison of HERG Mutation in between American, Japanese and Danish Patient

and Coding						Expression Studies
	6	G27Gsplice		R366X		
	6	S429stop		1400M		
	6	S429L	T474I			
	6	T436M	Y493X			
C-terminus region						
	9	R744stop	Q725X			
	9	R752W	frameshift			Trafficking abnormality
	9	L793splice				
	10	F805C	frameshift			Trafficking abnormality
	10	V822M				Trafficking abnormality
	10	R823W				
	10	A865T				
	12	P917L				
	12	R920Is				
	12	fsGly925				
	12	fsP968/4ins				
	13	W1001stop				Assembly abnormality
Subtotal		104	68	60	0	

(continued)

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Nucleotide substitutions(missense/nonsense)	106
Nucleotide substitutions(splicing)	4
Nucleotide substitutions (regulatory)	0
Small deletions	17
Small insertions	10
Small indels	0
Gross deletions	2
Gross insertions & duplications	2
Complex rearrangements	1
Repeat variations	0
TOTAL	142

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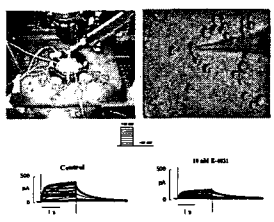

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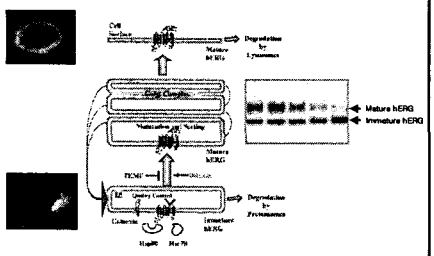
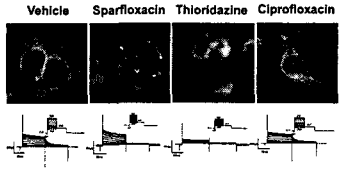
A Model for Biosynthesis of hERG



Drugs with hERG trafficking defects in HERG-GFP



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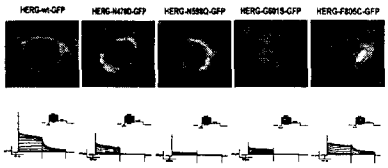
Transient Transfection Assay



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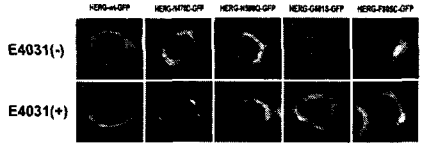
OBJECT : 1. Wild-type(WT) cell line – hERG trafficking inhibitors
2. Trafficking defective mutant hERG – hERG channel blockers



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CONCLUSION

1. First comprehensive determination of the frequency of cardiac potassium channel variants (HERG) in apparently Korean healthy individuals
2. Facilitate functional studies of HERG potassium channel variants
3. Prediction of QT-prolongation in clinically important drugs, and drug discovery
4. Facilitate predictive testing for individual arrhythmia risk
5. Enable safer prescription of drugs associated with induced QT prolongation
6. Fine establishment of common polymorphism in a much larger population

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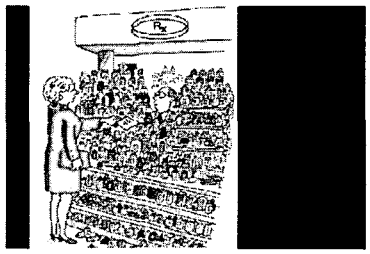

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