Genetic polymorphisms in *KCNQ1*, *HERG*, *KCNE1* and *KCNE2* genes in the Chinese, Malay and Indian populations of Singapore

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Aims

To determine the genetic variability of long QT syndrome (LQTS)-associated genes (*KCNQ1*, *HERG*, *KCNE1* and *KCNE2*) among three distinct ethnic groups in the Singapore population.

Methods

Genomic DNA samples from up to 265 normal healthy Chinese, 118 Malay and 139 Indian volunteer subjects were screened for genetic variations in the coding region of the LQTS-associated genes using denaturing high-performance liquid chromatography and sequencing analyses.

Results

In total, 37 single nucleotide polymorphisms (SNPs) were identified in the coding exons of the LQTS-associated potassium ion channel genes, seven of which were novel nonsynonymous polymorphisms. SNPs 356G→A (exon 1 of *KCNQ1*), 2624C→T and 2893G→A (exon 11 of *HERG*), 3164G→A, 3322C→G and 3460G→A (exon 14 of *HERG*), and 79C→T (exon 3 of *KCNE2*) resulted in Gly119Asp, Thr875Met, Gly965Arg, Arg1055Gln, Leu1108Val, Gly1154Ser and Arg27Cys amino acid substitutions, respectively. In addition, 16 intronic variants were detected. The functional consequence of these variants has not been studied and their association with risk of LQTS is unclear.

Conclusions

There exist multiple genetic polymorphisms of the LQTS-associated genes in the three distinct Asian populations. Though the functional significance of many of these SNPs is unknown, this interindividual and interethnic genetic variability may underlie the different susceptibilities of individuals to developing LQTS.

Introduction

Long QT syndrome (LQTS) is a disorder of ventricular repolarization characterized by electrocardiographic abnormalities, predominantly a prolongation of the QT interval and ventricular tachyarrhythmia termed *torsades de pointes*, often leading to syncope, seizures and sudden death [1]. There are two forms of congenital LQTS: the Romano–Ward syndrome (RWS) [2] and the

Jervell and Lange–Nielsen syndrome (JLNS) [3]. Acquired LQTS is often a complication from therapy or trigger with precipitating factors such as psychological and physical stress [4].

Congenital LQTS is a genetically heterogeneous disorder associated with mutations in various cardiac ion channel genes. To date, at least six gene loci have been identified for the disorder, of which four encode for

potassium channels (KCNQ1, HERG, KCNE1 and KCNE2), and one encodes for the sodium channel (SCN5A). *KCNQ1*, the gene responsible for causing LQT1, was mapped to chromosome 11p15.5 [5], *HERG* to LQT2 locus on chromosome 7q35-36 [6], *SCN5A* to LQT3 locus on chromosome 3p21-24 [7], *KCNE1* to LQT5 locus on chromosome 21q22 [8] and *KCNE2* to LQT6 locus on chromosome 21q22 [9]. LQT4 has been attributed to ankyrin B mutation [10] and its locus was mapped to chromosome 4q25-27 [11].

Drug-induced LQTS is a serious adverse effect for a wide spectrum of drugs, including antiarrhythmic drugs [12] and noncardiac drugs such as antihistamines [13], prokinetic agents [14], antipsychotics [15] and antimicrobials [16]. De Ponti *et al.* have published a comprehensive review on the various noncardiac drugs known to cause QT prolongation [17]. Genetic polymorphisms in the LQTS-associated genes have been reported to modulate the response to drugs and predispose individuals to acquired LQTS [9, 14, 18–22]. It was estimated that as many as 10–15% of drug-induced QT prolongation cases can be associated with mutations in ion channel genes [20].

To date, numerous mutations have been identified across the coding region of the ion channel genes in LQTS patients. One obvious deficiency of the current knowledge is the lack of data on the population frequencies of the single nucleotide polymorphisms (SNPs) in the general population, particularly those of Asian origin. At present, the exact association of many SNPs with cardiac pathology is still unclear; few mutations have been functionally characterized to ascertain their potential severity and those identified in LQTS probands were usually deemed to be pathological and causative of the disorder. It is often assumed that the identification of genetic variants in either individuals or families indicates a causative role of these variants. These variants may, however, be occurring in high frequency among healthy individuals in the population and may in fact represent common benign polymorphisms.

In this study, we report the polymorphism pattern of the two genes accountable for the majority of LQTS cases: *KCNQ1* and *HERG* [23], as well as their respective auxiliary subunits, *KCNE1* and *KCNE2*, in three distinct Asian populations, i.e. Chinese, Malay and Indian. The other known LQTS-associated genes, *SCN5A* and ankyrin B, were not screened in this population study due to their minor contribution to the overall incidence of the disorder. This report provides a useful database for researchers to identify potential LQTS-causing mutations for functional studies or make comparisons among different ethnic groups. This may have

important clinical implications in tailoring drug therapy for specific population groups.

Methods

Sample collection and DNA isolation

A total of 265, 118, and 139 unrelated normal, healthy Chinese, Malay and Indian individuals, respectively, aged 18–35 years old were screened in this study. Of the Chinese subjects, 90 were archived samples from previous studies. All volunteer subjects were recruited in accordance with ethics requirements (Institutional Review Board, National University Hospital, Singapore) and written informed consent. The volunteer subjects were required to declare a medical history free of cardiac conditions and of the respective ethnic descent for two previous generations as inclusion criteria for participation in the study. Whole blood (10 ml) was obtained from them using standard blood sampling method. The genomic DNA extracted from peripheral leucocytes by standard methods was used as templates in amplification of the exons in the entire coding region of *KCNQ1*, *HERG*, *KCNE1* and *KCNE2* genes. The archived Chinese DNA samples were insufficient for analysis of all fragments, hence the sample size for some fragments is 175 instead of 265 (Tables 1 and 2).

Polymerase chain reaction amplification

DNA fragments were generated using specially designed primers based on flanking intronic sequences (Lasergene DNASTAR software) and combinations of primers as described in previous studies, with modifications of the amplification conditions [9, 24–27]. The primer pairs and amplification conditions of the polymerase chain reaction (PCR) fragments are available at the NUS Pharmacogenetics Laboratory website (<http://www.med.nus.edu.sg/medphc/PGLab/research/lqts.htm>). The exons are numbered according to the GenBank sequences in the National Center for Biotechnology Information (NCBI). This numbering nomenclature differs from those reported by Splawski *et al.* [24] and Jongbloed *et al.* [25]. The amplifications were performed in a total volume of 50 µl containing 1 × Master Mix (Promega, Madison, WI, USA), 0.2 µM of each primer (Research Biolabs, Singapore) and 100 ng of DNA. Due to the rich GC content of Exon 1.1 of *KCNQ1*, FastStart Taq DNA Polymerase (Roche Diagnostics GmbH, Mannheim, Germany) was necessary for amplification of the fragment. GC-clamped primers ('cgg gcg ggg gcg gcg gga cgg gcg cgg ggc gcg ggcg' incorporated at the 5' end of reverse primers) were designed using DCode WinMelt Version 2.0 software for subsequent denaturing gradient gel electro-

Table 1

Population-specific allele frequencies of SNPs in KCNQ1 and KCNE1 genes

Gene	Region	Nucleotide change	Amino acid change	Allele frequency			Previously reported AF/[HF] (ethnicity; n)	Reference
				Chinese (n = 265)	Malay (n = 118)	Indian (n = 139)		
KCNQ1	Exon 1	C354T	T118T	0	0	0.004	NA	This study
	Exon 1	G356A	G119D	0	0	0.004	NA	This study
	Intron 1	386 + C9A	–	0.002	0	0	NA	This study
	Exon 1b	C435T	I145I	0.055	0.025	0.014	0.06 (Japanese; 50)	[45]
	Exon 2	C483T	I161I	0.002	0	0	NA	This study
	Intron 2	605 – C31T	–	0.002	0	0	NA	This study
	Intron 5	921 + C13T	–	0	0.004	0	NA	This study
	Exon 6	C972T	V324V	0	0	0.004	NA	This study
	Exon 6	C1008T	A336A	0.004	0	0	NA	This study
	Intron 8	1251 + C9A	–	0	0.004	0	NA	This study
	Exon 9	C1343G	P448R*	0.086†	0.064	0	0.14‡ (Chinese), ~0.20‡ (Asians)	[28] [30]
	Intron 10	1514 + G7T	–	0	0.025	0.004	NA	This study
	Intron 10	1514 + C18T	–	0.025	0.030	0.036	NA	This study
	Intron 10	1515 – A32T	–	0.002	0	0	NA	This study
	Intron 11	1590 + T14C	–	0	0	0.004	NA	This study
	Intron 11	1590 + A31T	–	0.004	0.008	0	NA	This study
	Intron 11	1590 + G50C	–	0	0	0.004	NA	This study
	Exon 12	G1638A	S546S	0.243	0.174	0.194	0.19 (Caucasians; 32), [0.12] (Dutch/Belgian; 32)	[22] [25]
	Intron 12	1685 + G36A	–	0.243	0.174	0.194	0.33 (Caucasians; 32)	[22]
	Exon 14	T1746C	D582D	0	0	0.004	NA	This study
	Exon 15	C1860T	H620H	0.002	0.004	0	NA	This study
	Exon 15	G1927A	G643S*	0.008	0.008	0	0.09 (Japanese; 50), [0.11] (Japanese; 110)	[31] [32]
	Exon 15	C1944T	V648V	0.002	0	0	NA	This study
	Exon 15	C1986T	Y662Y	0	0	0.054	0.33 (Caucasians; 32), [0.35] (Dutch/Belgian; 32)	[22] [25]
KCNE1	Exon 3	G30A	T10T	0.002	0.004	0	NA	This study
	Exon 3	G112A	G38S	0.306	0.246	0.255	0.33 (Caucasians; 32), [0.31] (Dutch/Belgian; 32), 0.22 (Caucasians; 16)	[22] [25] [33]
	Exon 3	C162T	F54F	0.006	0	0	NA	This study
	Exon 3	G253A	D85N*	0.004	0	0	0 (Caucasians; 32), [0.03] (Dutch/Belgian; 32)	[22] [25]

The nucleotide numbering starts from the ATG start codon. NCBI GenBank Accession Numbers: AF000571 (KCNQ1) and NM_000219 (KCNE1). *Functional characterization has been published. †Sample size equals 175. ‡Type of frequency not stated. AF, Allele frequency; HF, heterozygous frequency; n, number of subjects; NA, not available.

phoresis (DGGE) application. For denaturing high-performance liquid chromatography (DHPLC) application, appropriate primer pairs were designed using the WAVEMAKER 4.0 software.

Following an initial predenaturation step at 94 °C for 3 min, the reactions were cycled 35 times through denaturation at 94 °C for 1 min, variable annealing temperatures for 1 min and extension at 72 °C for 1 min. The

reactions were terminated by an additional extension step at 72 °C for 10 min. The PCR reactions were run on the Peltier Thermal Cycler (DNA Engine Dyad; MJ Research Inc, Waltham, MA, USA).

DGGE

DGGE (DCode Universal Mutation Detection System; Bio-Rad Laboratories, Life Science Research Group,

Table 2

Population-specific allele frequencies of SNPs in HERG and KCNE2 genes

Gene	Region	Nucleotide change	Amino acid change	Allele frequency			Previously reported AF/[HF] (ethnicity; n)	Reference
				Chinese (n = 265)	Malay (n = 118)	Indian (n = 139)		
HERG	Intron 5	1128 + G7A	–	0	0.008	0	NA	This study
	Exon 6	T1467C	I489I	0.351†	0.441	0.327	0.19 (Caucasians; 32), [0.45] (Dutch/Belgian; 32), 0.82 (Danish; 46)	[22] [41&[25] rsqb;
	Exon 6	T1539C	F513F	0.351†	0.441	0.327	0.19 (Caucasians; 32), [0.43] (Dutch/Belgian; 32), 0.82 (Danish; 46)	[22] [25] [41]
	Exon 7	C1635T	Y545Y	0.002	0	0	NA	This study
	Exon 7	G1692A	L564L	0.143†	0.233	0.511	0.36 (Caucasians; 32), [0.50] (Dutch/Belgian; 32), 0.58 (Danish; 39)	[22] [25] [41]
	Exon 7	C1887T	N629N	0.002	0	0	NA	This study
	Exon 8	C1956T	Y652Y	0.085	0.144	0.331	0.41 (Caucasians; 32), [0.65] (Dutch/Belgian; 32), 0.38 (Danish; 47)	[22] [25] [41]
	Exon 8	C2127T	N709N	0	0	0.007	NA	This study
	Exon 9	C2331T	T777T	0.004	0	0	NA	This study
	Exon 11	G2617A	G873S	0.002	0	0	[0.003] (Black; 305) [33]	[35]
	Exon 11	C2624T	T875M	0	0	0.004	NA	This study
	Exon 11	A2690C	K897T*	0.047	0.119	0.158	0.14 (Caucasians; 92), 0.23 (Caucasians; 32), [0.41] (Dutch/Belgian; 32), 0.16 (Finnish; 201)	[20] [22] [25] [36]
	Exon 12	G2893A	G965R	0	0	0.004	NA	This study
	Intron 12	2965 + C11T	–	0.002	0	0	NA	This study
	Intron 12	2965 + G38A	–	0	0.004	0	NA	This study
	Exon 13	C3063T	S1021S	0	0.004	0	NA	This study
	Exon 13	G3140T	R1047L*	0	0.004	0	0.04 (Danish; 40)	[41]
	Intron 13	3152 + G22A	–	0.645†	0.508	0.356	[0.48] (Dutch/Belgian; 32), 0.10 (Danish; 40)	[25] [41]
	Intron 13	3152 + G27C	–	0	0.008	0	NA	This study
	Exon 14	G3164A	R1055Q	0	0.004	0	NA	This study
	Exon 14	C3297G	P1099P	0.002	0	0	NA	This study
	Exon 14	C3322G	L1108V	0	0	0.004	NA	This study
	Exon 15	G3460A	G1154S	0	0.004	0	NA	This study
KCNE2	Exon 3	C79T	R27C	0.003†	0	0	NA	This study
	Exon 3	G354A	G118G	0.006†	0	0	NA	This study

The nucleotide numbering starts from the ATG start codon. NCBI GenBank Accession Numbers: AF363636 (HERG) and NM_172201 (KCNE2). *Functional characterization has been published. †Sample size equals 175. AF, Allele frequency; HF, heterozygous frequency; n, number of subjects; NA, not available.

Hercules, CA, USA) was initially employed as the mutational screening method. An 8% polyacrylamide gel with 40–70% denaturant was casted with the aid of the Model 475 gradient delivery system by dispensing the high- and low-density solutions. This method was used to analyse the 90 archived Chinese samples for the *KCNQ1* gene.

Prior to DGGE, the PCR products were subjected to a heteroduplex formation cycle involving denaturation at 95 °C for 5 min, followed by 65 °C for 1 h and 37 °C for 1 h. Ten microlitres of PCR product was mixed with an equal volume of 2 × loading dye and loaded into the wells of the polyacrylamide gel placed in the tank with 7 l of 1 × TBE buffer preheated to 60 °C. Wild-type,

heterozygous and mutant samples were each loaded into the gel to serve as controls. The gel was run at 180 mV for 7 h. The PCR products which produced heteroduplex bands or suspected mutants with a shift in band position on the gel were processed for DNA sequencing to determine the underlying mutation.

DHPLC

Mutational screening was subsequently accomplished using DHPLC (Transgenomic WAVE Nucleic Acid Fragment Analysis System; Omaha, NE, USA). Prior to replacing the DGGE technique with the DHPLC method, a validation study was performed to ensure concordance in the results obtained using the two systems. This involved analysing the genetic profile of the 90 archived samples in the polymorphic fragment *KCNQ1* exon 12 previously screened using DGGE, and 100% concordance was achieved.

Prior to DHPLC, the PCR products were subjected to a heteroduplex formation cycle. The PCR product was spiked with an equal volume of known wild-type product for the same fragment. The contents were then denatured at 95 °C for 5 min, followed by a decrement of 1 °C per s until 25 °C to allow heteroduplex formation. Ten microlitres of the mixture was then injected into the DNASEp cartridge maintained at the predicted oven temperature. Elution of the samples from the stationary phase was performed at a constant flow rate of 0.9 ml min⁻¹ using a linear acetonitrile gradient. The temperature and elution gradient were predicted by the WAVEMAKER 4.0 software. Heterozygous profiles were identified by visual inspection of the chromatograms displaying additional earlier elution peaks due to the less stable heteroduplex populations. Homozygous profiles displayed only a single elution peak. The PCR products with heterozygous profiles were then sequenced to determine the underlying mutation.

DNA sequencing

Prior to sequencing, unincorporated deoxynucleotide triphosphates and excess primers were removed from the 12 µl of PCR products using 2 units of Exonuclease 1 (New England Biolabs, Beverly, MA, USA) and 1 unit of Shrimp Alkaline Phosphatase (Promega) by incubating at 37 °C for 15 min, followed by enzyme deactivation at 80 °C for 20 min. The sequencing reactions were carried out using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The sequences of genomic fragments were analysed on the automated ABI Prism Model 3100 Avant Genetic Analyser (Applied Biosystems). The

results were blasted against the published sequences (NCBI GenBank Accession no. AF000571:*KCNQ1*, AF363636:*HERG*, NM_000219:*KCNE1*, NM_172201:*KCNE2*) using the NCBI bl2seq Similarity Alignment Tool Program.

Results

A systematic survey of the four LQTS-associated genes revealed a total of seven novel nonsynonymous SNPs (G119D-*KCNQ1*, T875M-, G965R-, R1055Q-, L1108V- and G1154S-*HERG*, and R27C-*KCNE2*). In addition, two reported nonsynonymous SNPs and 10 synonymous SNPs were uncovered in *KCNQ1*, and three reported nonsynonymous SNPs and 10 synonymous SNPs in *HERG*. Apart from coding variants, 11 and five intronic variants were uncovered in *KCNQ1* and *HERG*, respectively. Analysis of the auxiliary subunits revealed two each of reported nonsynonymous SNPs and synonymous SNPs in *KCNE1*, and one synonymous SNP in *KCNE2*. The population-specific allele frequencies of the SNPs identified are depicted in Tables 1 and 2.

Discussion

From this study, we have determined the pattern of genetic variability of the cardiac ion channel genes (*KCNQ1*, *HERG*, *KCNE1* and *KCNE2*) associated with LQTS in the three distinct Asian populations (Chinese, Malay and Indian). In total, we identified 37 SNPs across the coding region of the four genes. Of these SNPs, seven were novel nonsynonymous variants: one each in *KCNQ1* and *KCNE2*, and five in *HERG*. The published population frequencies for the reported SNPs are shown in Tables 1 and 2. To our knowledge, this is the first comprehensive study on the allele frequencies of the spectrum of genetic variants across the coding region of *KCNQ1*, *HERG*, *KCNE1* and *KCNE2* genes in apparently healthy individuals of the three main Asian populations (Tables 1 and 2).

G119D-*KCNQ1* is a novel SNP present in an Indian subject. P448R-*KCNQ1* was once thought to be causative of LQTS [23] but was later discovered to be an ethnic-specific polymorphism present in 14% to approximately 20% of the Asian population [20, 28, 29]. In our population, this variant is present in higher frequency among Chinese (8.6%) than Malays (6.4%), and is totally absent in the Indians. This variant was found to increase current by twofold when expressed alone, but no apparent difference was observed when coexpressed with the auxiliary subunit *KCNE1* [30]. G643S-*KCNQ1* is a relatively rare variant in our population (<1%) compared with the Japanese population (~10%) [31, 32]

and found to predispose individuals to arrhythmias [32]. Functional studies demonstrated that this variant resulted in a decrease in outward K^+ current density and an accelerated deactivation process. It was postulated that G643S-KCNQ1 is associated with a mild phenotype which is clinically manifested during adverse conditions such as states of hypokalaemia or bradyarrhythmias [32]. The variant G38S-KCNE1 was reported to have an allele frequency of 22–33% among the caucasian population [22, 33], a figure reasonably consistent with our data. There is no statistically significant difference in the allele frequencies among the three ethnic groups ($P > 0.05$). D85N-KCNE1 was a former mutation identified in acquired LQTS patients [22]. It was present in higher frequency among acquired LQTS patients (7%) than normal controls (2–4%) [34], and reduced current by about half [30]. It was detected in two of our Chinese subjects.

G873S-HERG has a heterozygous frequency of 0.3% among black subjects [35] and was found in a single Chinese subject. K897T-HERG has an allele frequency of 14–23% in the caucasian population [20, 22] and 16% in the Finnish population [36]. It occurs at highest frequency among Indians (15.8%), followed by Malays (11.9%) and Chinese (4.7%). The allele frequency differs significantly between Chinese and Malays ($\chi^2 = 13.839$, $P = 0.0002$) as well as between Chinese and Indians ($\chi^2 = 27.955$, $P < 0.0001$), but not between Malays and Indians ($\chi^2 = 1.626$, $P = 0.2022$). The debate over its phenotypic consequence [37, 38] and electrophysiological effects [39, 40] remains inconclusive. R1047L-HERG has an allele frequency of 4% in the Danish population [41] and reported findings on its electrophysiology properties were inconsistent [40, 42]. This variant was present only in a Malay subject in this study. T875M-, G965R-, and L1108V-HERG are novel SNPs detected in separate Indian subjects, while R1055Q- and G1154S-HERG are novel SNPs found in individual Malay subjects. R27C-KCNE2 is a novel SNP detected in a Chinese subject.

Among the amino acid variants identified, the nonpolar glycine of G119D-KCNQ1, G965R- and G1154S-HERG is replaced by the negatively charged polar aspartic acid, positively charged polar arginine and uncharged polar serine, respectively. T875M- and R1055Q-HERG result from the substitution of polar threonine with nonpolar methionine and arginine with uncharged polar glutamine, respectively. The arginine of R27C-KCNE2 is replaced by uncharged cysteine. L1108V-HERG is the variant with the most minor change, with both amino acid residues leucine and valine having nonpolar side chains, and thus likely to

produce the least effect. However, the prediction of the effect on the final protein based on the degree of structural modification is largely speculative. The majority of the nonsynonymous SNPs of the *HERG* gene identified in our apparently normal subjects reside in the C-terminal region. This is consistent with the findings published by Ackerman *et al.*, who speculated that the N- and C-termini regions are more tolerant of amino acid substitutions [35]. This hypothesis is substantiated by the fact that most LQTS-causing mutations reside in the core regions of the channels [23]. However, mutations in the C-terminus of HERG have been associated with altered activation–deactivation properties [43] and defective trafficking of the protein [44]. Regardless of the considerable effort devoted to finding genotype–phenotype relationships, the potential severity of these mutations is difficult to evaluate and their exact electrophysiological properties and prediction of clinical outcome demand more specific experiments. It should therefore be emphasized that the phenotypic consequence of the variants identified in this study is still unclear as no functional study has yet been done. Moreover, a genotype–phenotype correlation is not possible as the study subjects have not undergone cardiac evaluation.

Despite the direct relevance of genetic polymorphisms of these ion channel genes in clinical cardiology and therapeutics, large-scale population screening is not routine practice due to the continuously expanding list of disease-causing genes. Moreover, due to the low prevalence of the disorder and the large number of exons in the disease-causing genes, mutational analysis of the population at large is not economically worthwhile. Furthermore, the task of screening for mutations using the current state of the art technology is still labourious and time-consuming. Therefore, molecular diagnosis is usually limited to family members of clinically diagnosed LQTS patients. Presymptomatic diagnosis serves to identify asymptomatic gene carriers of pathological mutations and initiate prophylactic therapy and education on proper lifestyle management for these high-risk individuals. Since drug-induced LQTS has been reported to be a serious adverse effect for a wide spectrum of drugs, genotyping the individual prior to the administration of known QT-prolonging drugs allows the clinician to assess the risk–benefit ratio and optimize the drug dosage regimen. Genetic polymorphisms of ion channel genes thus play a major role in interindividual variability in drug response and susceptibility to LQTS. This has significant clinical relevance in the management of high-risk individuals.

The identification of pathological mutations and the profiling of the polymorphism pattern in the population

at large will have a major impact on therapeutics and the clinical development of new chemical entities. The findings of this study provide information on the profile of genetic variants in the three major ethnic groups of the Singapore population. This lays the foundation for future work involving clinical samples in distinguishing potential disease-causing mutations from common benign polymorphisms. The identification of amino acid variants paves the way to characterizing their functional consequences and their response to candidate drugs. The availability of the bank of cumulative genetic information will aid molecular diagnosis and predict the susceptibility of individuals to drug-induced LQTS. This increasing awareness of drug-gene interactions will inevitably lead to the design of safer and more effective drugs. This justifies the integration of pharmacogenetics in the drug development programme and clinical prescription of medications.

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References

- Vincent GM. The molecular genetics of the long QT syndrome: genes causing fainting and sudden death. *Annu Rev Med* 1998; 49: 263–74.
- Ward OC. A new familial cardiac syndrome in children. *J Ir Med Assoc* 1964; 54: 103–6.
- Jervell A, Lange-Nielsen F. Congenital deaf-mutism, function heart disease with prolongation of the QT interval and sudden death. *Am Heart J* 1957; 54: 59–68.
- Schwartz PJ, Zaza A, Locati E, Moss AJ. Stress and sudden death. The case of the long QT syndrome. *Circulation* 1991; 83 (Suppl. II): 1171–80.
- Keating M, Atkinson D, Dunn C, Timothy K, Vincent GM, Leppert M. Linkage of a cardiac arrhythmia, the long QT syndrome, and the Harvey ras-1 gene. *Science* 1991; 252: 704–6.
- Curran ME, Splawski I, Timothy KW, Vincent GM, Green ED, Keating MT. A molecular basis for cardiac arrhythmia: HERG mutations cause long QT syndrome. *Cell* 1995; 80: 795–803.
- Jiang C, Atkinson D, Towbin JA, Splawski I, Lehmann MH, Li H, Timothy K, Taggart RT, Schwartz PJ, Vincent GM, Moss AJ, Keating MT. Two long QT syndrome loci map to chromosomes 3 and 7 with evidence for further heterogeneity. *Nat Genet* 1994; 8: 141–7.
- Barhanin J, Lesage F, Guillemare E, Fink M, Lazdunski M, Romey G. KvLQT1 and Isk (mink) proteins associate to form the I_{Ks} cardiac potassium current. *Nature* 1996; 384: 78–80.
- Abbott GW, Sesti F, Splawski I, Buck ME, Lehmann MH, Timothy KW, Keating MT, Goldstein SA. MiRP1 forms I_{Kr} potassium channels with HERG and is associated with cardiac arrhythmia. *Cell* 1999; 97: 175–87.
- Mohler PJ, Schott JJ, Gramolini AO, Dilly KW, Guatimosim S, duBell WH, Song LS, Haurogne K, Kyndt F, Ali ME, Rogers TB, Lederer WJ, Escande D, Le Marec H, Bennett V. Ankyrin-B mutation causes type 4 long-QT cardiac arrhythmia and sudden cardiac death. *Nature* 2003; 421: 634–9.
- Schott JJ, Charpentier F, Peltier S, Foley P, Drouin E, Bouhour JB, Donnelly P, Vergnaud G, Bachner L, Moisan JP, Marec HL, Pascal O. Mapping of a gene for long QT syndrome to chromosome 4q25–27. *Am J Hum Genet* 1995; 57: 1114–22.
- Viskin S. Long QT syndromes and torsade de pointes. *Lancet* 1999; 354: 1625–33.
- Woosley RL, Chen Y, Freiman JP, Gillis RA. Mechanism of the cardiotoxic actions of terfenadine. *JAMA* 1993; 269: 1532–6.
- Napolitano C, Schwartz PJ, Brown AM, Ronchetti E, Bianchi L, Pinnavaia A, Acquaro G, Priori SG. Evidence for a cardiac ion channel mutation underlying drug-induced QT prolongation and life-threatening arrhythmias. *J Cardiovasc Electrophysiol* 2000; 11: 691–6.
- Reilly JG, Ayis SA, Ferrier IN, Jones SJ, Thomas SH. QTc-interval abnormalities and psychotropic drug therapy in psychiatric patients. *Lancet* 2000; 355: 1048–52.
- Brandriss MW, Richardson WS, Barold SS. Erythromycin-induced QT prolongation and polymorphic ventricular tachycardia (torsades de pointes): case report and review. *Clin Infect Dis* 1994; 18: 995–8.
- De Ponti F, Poluzzi E, Montanaro N. QT-interval prolongation by non-cardiac drugs: lessons to be learned from recent experience. *Eur J Clin Pharmacol* 2000; 56: 1–18 (Review).
- Donger C, Denjoy I, Berthet M, Neyroud N, Cruaud C, Bennaceur M, Chivoret G, Schwartz K, Coumel P, Guicheney P. KVLQT1 C-terminal missense mutation causes a forme fruste long-QT syndrome. *Circulation* 1997; 96: 2778–81.
- Piippo K, Holmstrom S, Swan H, Viitasalo M, Raatikka M, Toivonen L, Kontula K. Effect of the antimalarial drug halofantrine in the long QT syndrome due to a mutation of the cardiac sodium channel gene SCN5A. *Am J Cardiol* 2001; 87: 909–11.
- Yang P, Kanki H, Drolet B, Yang T, Wei J, Viswanathan PC, Hohnloser SH, Shimizu W, Schwartz PJ, Stanton M, Murray KT, Norris K, George AL Jr, Roden DM. Allelic variants in long-QT disease genes in patients with drug-associated torsades de pointes. *Circulation* 2002; 105: 1943–8.
- Splawski I, Timothy KW, Tateyama M, Clancy CE, Malhotra A, Beggs AH, Cappuccino FP, Sagnella GA, Kass RS, Keating MT. Variant of SCN5A sodium channel implicated in risk of cardiac arrhythmia. *Science* 2002; 297: 1333–6.
- Paulussen AD, Gilissen RA, Armstrong M, Doevendans PA, Verhasselt P, Smeets HJ, Schulze-Bahr E, Haverkamp W, Breithardt G, Cohen N, Aerssens J. Genetic variations of KCNQ1, KCNH2, SCN5A, KCNE1, and KCNE2 in drug-induced long QT syndrome patients. *J Mol Med* 2004; 82: 182–8.
- Splawski I, Shen J, Timothy KW, Lehmann MH, Priori S, Robinson JL, Moss AJ, Schwartz PJ, Towbin JA, Vincent GM, Keating MT.

- Spectrum of mutations in long-QT syndrome genes. KVLQT1, HERG, SCN5a, KCNE1, and KCNE2. *Circulation* 2000; 102: 1178–85.
- 24 Splawski I, Shen J, Timothy KW, Vincent GM, Lehmann MH, Keating MT. Genomic structure of three long QT syndrome genes: KVLQT1, HERG, and KCNE1. *Genomics* 1998; 51: 86–97.
 - 25 Jongbloed R, Marcelis C, Velter C, Doevendans P, Geraedts J, Smeets H. DHPLC analysis of potassium ion channel genes in congenital long QT syndrome. *Hum Mutat* 2002; 20: 382–91.
 - 26 Syrris P, Murray A, Carter ND, McKenna WM, Jeffery S. Mutation detection in long QT syndrome: a comprehensive set of primers and PCR conditions. *J Med Genet* 2001; 38: 705–10.
 - 27 Itoh T, Tanaka T, Nagai R, Kamiya T, Sawayama T, Nakayama T, Tomoike H, Sakurada H, Yazaki Y, Nakamura Y. Genomic organization and mutational analysis of HERG, a gene responsible for familial long QT syndrome. *Hum Genet* 1998; 102: 435–9.
 - 28 Liang LZD, Cai LL, Wu JX, Zheng T, Qi TX. A novel KCNQ1 mutation in Chinese with congenital long QT syndrome. *Zhonghua Er Ke Za Zhi* 2003; 41: 724–7.
 - 29 Sharma D, Glatter KA, Timofeyev V, Tuteja D, Zhang Z, Rodriguez J, Tester DJ, Low R, Scheinman MM, Ackerman MJ, Chiamvimonvat N. Characterization of a KCNQ1/KVLQT1 polymorphism in Asian families with LQT2: implications for genetic testing. *J Mol Cell Cardiol* 2004; 37: 79–89.
 - 30 Westenskow P, Splawski I, Timothy KW, Keating MT, Sanguinetti MC. Compound mutations: a common cause of severe long-QT syndrome. *Circulation* 2004; 109: 1834–41.
 - 31 Iwasa H, Itoh T, Nagai R, Nakamura Y, Tanaka T. Twenty single nucleotide polymorphisms (SNPs) and their allelic frequencies in four genes that are responsible for familial long QT syndrome in the Japanese population. *J Hum Genet* 2000; 45: 182–3.
 - 32 Kubota T, Horie M, Takano M, Yoshida H, Takenaka K, Watanabe E, Tsuchiya T, Otani H, Sasayama S. Evidence for a single nucleotide polymorphism in the KCNQ1 potassium channel that underlies susceptibility to life-threatening arrhythmias. *J Cardiovasc Electrophysiol* 2001; 12: 1223–9.
 - 33 Lai LP, Deng CL, Moss AJ, Kass RS, Liang CS. Polymorphism of the gene encoding a human minimal potassium ion channel (Mink). *Gene* 1994; 151: 339–40.
 - 34 Wei J, Yang ICH, Tapper AR, Murray KT, Viswanathan P, Rudy Y, Bennett PB, Balser JR, Roden DM, George AL. KCNQ1 polymorphism confers risk of drug-induced long QT syndrome by altering kinetic properties of I_{Ks} potassium channels. *Circulation* 1999; 100: 1495.
 - 35 Ackerman MJ, Tester DJ, Jones GS, Will ML, Burrow CR, Curran ME. Ethnic differences in cardiac potassium channel variants: implications for genetic susceptibility to sudden cardiac death and genetic testing for congenital long QT syndrome. *Mayo Clin Proc* 2003; 78: 1479–87.
 - 36 Laitinen P, Fodstad H, Piippo K, Swan H, Toivonen L, Viitasalo M, Kaprio J, Kontula K. Survey of the coding region of the HERG gene in long QT syndrome reveals six novel mutations and an amino acid polymorphism with possible phenotypic effects. *Hum Mutat* 2000; 15: 580–1.
 - 37 Pietila E, Fodstad H, Niskasaari E, Laitinen PJ, Swan H, Savolainen M, Kesaniemi YA, Kontula K, Huikuri HV. Association between HERG K897T polymorphism and QT interval in middle-aged Finnish women. *J Am Coll Cardiol* 2002; 40: 511–4.
 - 38 Bezzina CR, Verkerk AO, Busjahn A, Jeron A, Erdmann J, Koopmann TT, Bhuiyan ZA, Wilders R, Mannens MM, Tan HL, Luft FC, Schunkert H, Wilde AA. A common polymorphism in KCNH2 (HERG) hastens cardiac repolarization. *Cardiovasc Res* 2003; 59: 27–36.
 - 39 Paavonen KJ, Chapman H, Laitinen PJ, Fodstad H, Piippo K, Swan H, Toivonen L, Viitasalo M, Kontula K, Pasternack M. Functional characterization of the common amino acid 897 polymorphism of the cardiac potassium channel KCNH2 (HERG). *Cardiovasc Res* 2003; 59: 603–11.
 - 40 Anson BD, Ackerman MJ, Tester DJ, Will ML, Delisle BP, Anderson CL, January CT. Molecular and functional characterization of common polymorphisms in HERG (KCNH2) potassium channels. *Am J Physiol Heart Circ Physiol* 2004; 286: H2434–41.
 - 41 Larsen LA, Andersen PS, Kanters J, Svendsen IH, Jacobsen JR, Vuust J, Wettrell G, Tranebjaerg L, Bathen J, Christiansen M. Screening for mutations and polymorphisms in the genes KCNH2 and KCNE2 encoding the cardiac HERG/MiRP1 ion channel: implications for acquired and congenital long Q–T syndrome. *Clin Chem* 2001; 47: 1390–5.
 - 42 Sun Z, Milos PM, Thompson JF, Lloyd DB, Mank-Seymour A, Richmond J, Cordes JS, Zhou J. Role of a KCNH2 polymorphism (R1047 L) in dofetilide-induced Torsades de Pointes. *J Mol Cell Cardiol* 2004; 37: 1031–9.
 - 43 Nakajima T, Kurabayashi M, Ohyama Y, Kaneko Y, Furukawa T, Itoh T, Taniguchi Y, Tanaka T, Nakamura Y, Hiraoka M, Nagai R. Characterization of S818L mutation in HERG C-terminus in LQT2. Modification of activation–deactivation gating properties. *FEBS Lett* 2000; 481: 197–203.
 - 44 Gong Q, Keeney DR, Robinson JC, Zhou Z. Defective assembly and trafficking of mutant HERG channels with C-terminal truncations in long QT syndrome. *J Mol Cell Cardiol* 2004; 37: 1225–33.
 - 45 Itoh T, Tanaka T, Nagai R, Kikuchi K, Ogawa S, Okada S, Yamagata S, Yano K, Yazaki Y, Nakamura Y. Genomic organization and mutational analysis of KVLQT1, a gene responsible for familial long QT syndrome. *Hum Genet* 1998; 103: 290–4.