# RARE VARIATION IN DRUG METABOLISM AND LONG QT GENES AND THE GENETIC SUSCEPTIBILITY TO ACQUIRED LONG QT SYNDROME

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**Abbreviations**.

ADR: adverse drug reaction;

aLQTS: acquired LQTS;

cLQTS: congenital LQTS;

ECG: Electrocardiogram;

ExAC: Exome Aggregation Consortium

gnomAD: Genome Aggregation Database

LQTS: long QT syndrome;

MAF: minor allele frequency;

QTc: corrected QT interval;

SCD: sudden cardiac death;

SIDS: Sudden Infant Death Syndrome;

TdP: torsades de pointes;

VF: ventricular fibrillation;

VT: ventricular tachycardia

**ABSTRACT**

**Background**: Acquired long QT syndrome (aLQTS) is a serious unpredictable adverse drug reaction. Pharmacogenomic markers may predict risk.

**Methods and Results**: Computational methods identified proteins interacting most significantly with 216 QT-prolonging drugs deemed culpable for aLQTS in 153 patients [58 years (range:14-88), 98.7% Caucasian, 85.6% symptomatic]. All cases underwent sequencing of 31 candidate genes arising from this analysis and/or associating with LQTS. Variants were filtered using a minor allele frequency (MAF) <0.01 and classified for susceptibility for aLQTS. In 25.5% cases, 46 aLQTS variants were identified: 22.2% cases carried at least one LQTS variant; 7.8% cases carried cytochrome-P450 (CYP) variants. Of 12 identified CYP susceptibility variants, 11 (92%) affected the enzyme that metabolised at least one culprit drug to which the subject had been exposed. Drug-drug interactions that affected culprit drug metabolism were found in 19% of cases. More than one LQTS variant, CYP variant and/or drug interaction was present in 7.8% of cases. Gene-burden analyses of the primary cohort compared to control exomes (n=452), and an independent replication aLQTS exome sequencing cohort (n=67) and control (n=148) demonstrated an increased burden of rare (MAF<0.01) variants in CYP genes but not LQTS genes.

**Conclusion**: Rare susceptibility variants in CYP enzyme genes are potentially important pharmacogenomic risk markers for aLQTS and should form part of personalised medicine. Together with drug interactions they emerge as an important pharmacokinetic pathway. Furthermore, pathogenic/likely pathogenic and rare susceptibility LQTS variants predispose to aLQTS although their importance in Caucasians may be less than previously suspected. (*250 words*)

**Keywords**: Torsades de Pointes; long QT syndrome; acquired long QT syndrome; adverse drug reaction; arrhythmia; pharmacogenomics.

## INTRODUCTION

Acquired long QT syndrome (aLQTS) is associated with prolongation of the QT interval on the electrocardiogram (ECG) and Torsades de Pointes (TdP) in the setting of triggering factors.[1,2] It is rare, often unpredictable and can be due to a serious adverse drug reaction (ADR) during treatment with cardiovascular and non-cardiovascular medications[1] and as such is a cause for relabelling and withdrawal. It is an important issue for the pharmaceutical industry and public health and thorough QT studies have become a standard component of new drug evaluation.[1,3]

A number of clinical factors have been identified that suggest an individual may be at increased risk for aLQTS including: female gender; acute and chronic metabolic abnormalities such as hypokalaemia and liver disease; heart disease, including bradycardia and recent conversion from atrial fibrillation to normal rhythm; and drug-specific factors such as dose, drug pharmacokinetics and pharmacodynamics and route of administration.[2,4] Inhibition of the cardiac repolarizing potassium current IKr, and to a lesser extent IKs, is the most common mechanism across multiple drugs.[15] In addition, inhibition of drug metabolism or elimination leading to high plasma concentrations has been implicated.[6] The sporadic nature of aLQTS and the similarity with the congenital condition has however suggested that they may share some common genetic background.[4,7] Indeed studies have identified putative pathogenic LQTS associated rare variants in 10% to 28% of aLQTS (aLQTS) cases, often with normal QT intervals after drug withdrawal.[8–11] This has led to the concept of the repolarization reserve, the physiological capacity for cardiac repolarisation which is in part genetically predetermined and upon which additional insults such as hypokalaemia and/or a QT prolonging drug may act and precipitate aLQTS.[1,4]

The ARITMO consortium aimed to identify rare genetic predisposition to aLQTS in known and novel candidate genes and their association with other risk factors to identify

personalised risk.

## METHODS

A multicentre international cohort of likely aLQTS cases was collected from four groups: two population series: the Drug-induced Arrhythmia Risk Evaluation (DARE) study in UK (n=97)[12,13] and the Berlin Case-Control Surveillance Study (FAKOS) in Germany (n=41)[14]; and case series from Denmark (n=9) and Italy (n=6). Cases were included if they were exposed to one or more medications deemed culpable for an event that met criteria for case definition, following review by an expert panel: QTc prolongation with documented TdP and/or ventricular fibrillation (VF); cardiac syncope without vagal features but with QTc prolongation >450 ms for males and >470 ms for females; or QTc prolongation >500 ms alone.[2] Where possible, the QTc interval was measured in the presence of the culprit drug at initial presentation and after drug removal.

Culprit drugs were categorized by their potential to cause QT prolongation and/or TdP according to CredibleMeds (https://crediblemeds.org): known risk of TdP (KR), possible risk of TdP (PR); and conditional risk of TdP (CR). Drug to drug interactions were analysed according to the Drug Interaction Checker from Medscape (http://reference.medscape.com/drug-interactionchecker). In CYP gene variant carriers, CYP450 to drug interactions were analysed according to the Flockhart Table™ from Indiana University (http://medicine.iupui.edu/clinpharm/ddis/main-table/).

**Selection of candidate genes:** In order to identify all the drugs consistently associated with TdP, we curated and integrated drug information from open repositories of package inserts (DailyMed, SAEPI, CTD, DPD). The chemical structures of TdP-associated drugs were used to identify all the proteins that are known to interact with these

drugs in public pharmacological databases (see online methods).[15] Next, we used chemoinformatic methods implemented in the CLARITY software to predict additional proteins that could also be interacting with culprit drugs (see online methods).[16] A list of 62 targets was collated for the aforementioned culprit drugs linked to TdP and sorted by the minimum affinity at which they become statistically significant (Table S1). The top 15 targets included three LQTS genes (*KCNH2*, *KCNQ1* and *SCN5A*) as well as five CYP450 genes and five non-cardiac ion channels. We also added the seven proteins significantly associated with the two specific drugs most commonly implicated in our cases; namely sotalol and amiodarone (Table S2). We finally included 9 additional genes associated with LQTS at the time of panel design (such as *KCNE1*, *KCNE2*) for which very limited *in vitro* affinity data for drugs were available and thus, were not prioritised by the statistical analysis. The final list contains 31 candidate genes (Table S2).

**Genetic methods:** The genetic methodology is shown in Figure 1. Genetic analyses were approved by each Institutional Ethics Committee. Genomic DNA was isolated from peripheral leucocytes using conventional methods for all samples. Primers were custom designed to capture the exons and their intronic borders for the 31 genes of interest. Libraries were constructed using 10 ng of DNA sample per primer pool per case. The prepared library was quantified and sequenced on the Ion PGM Sequencer. BAM files were transferred to Ion Reporter Software for variant calling. The variants were annotated using the Ingenuity Variant Analysis Software. All variants identified were filtered for a call quality of >20 and read depth of >5 (see online detailed methods). Any variant found in the Genome Aggregation Database (gnomAD) (http://gnomad.broadinstitute.org) with a minor allele frequency (MAF) >1% was excluded and all remaining variants were considered rare and were confirmed using Sanger sequencing.

**Control and replication cohorts**: 452 randomly selected exomes from the 1958 birth cohort were employed as a general population control series for the primary cohort (n=153).[17] All data were analysed with a bespoke pipeline (https://github.com/sgul-genetics-centre-bioinformatics/SGUL-Exome-Pipeline-on-HPC). Genomic VCF files were created for the samples in each cohort, merged and jointly called according to GATK best practices (https://gatk.broadinstitute.org/hc/en-us/sections/360007226651-Best-Practices-Workflows). Variants were subjected to hard filtering according to low depth (<6 supporting reads) and genotype quality (<30) using GATK. The control exome data were harmonised with the IonTorrent data and filtered using gnomAD (novel variants or MAF<1%).

A replication cohort consisting of 67 cases not associated with the primary analysis and 148 controls from Vanderbilt University, USA, was identified. This cohort had previously undergone targeted panel sequencing using the Illumina HiSeq2500 platform and Agilent SureSelect capture kit as described for a prior analysis of LQTS genes.[18] The cases were known aLQTS cases with documented TdP after exposure to a QT prolonging medication. The control cohort were all exposed to sotalol and did not have any evidence of LQTS or TdP after exposure. Genomic VCF files were filtered similarly. Both datasets were filtered to only include novel variants or MAF<1% in gnomAD.

**Rare variant burden analyses:** The primary cohort case-control and replication case-control datasets underwent rare variant burden analyses. Two main genes lists were explored (table S2): CYP genes and 'main LQTS' genes (*KCNH2*, *KCNQ1*, *SCN5A*)[19]. We employed optimal sequence kernel association test (SKAT-O) as well as collapsing and combine rare variants (CMC) test.[20,21] The burden tests were implemented in RVtests software.[22] Thresholds of 0.0000001 (lower threshold) and 0.001 (upper threshold) were used. A comparison of synonymous rare variants in each gene was made for both primary and replication analyses.

**Case based analysis:** aLQTS was considered a complex trait and a potentially polygenic disorder rather than a monogenic disease. Therefore, we assessed genetic predisposition and susceptibility for aLQTS using gnomAD and ClinVar databases (https://www.ncbi.nlm.nih.gov/clinvar/), *in silico* tools (SIFT, PolyPhen2, GERP, Mutation Taster, UMD Predictor and Align GVGD), and published functional data. Susceptibility classification of CYP gene variants was also based upon the The Human Cytochrome P450 Allele Nomenclature Database (http://www.cypalleles.ki.se/) and the Pharmacogenomics Knowledge Base (PharmGKB; http://www.pharmgkb.org/). The variants were classified for susceptibility according to recently proposed aLQTS criteria[23]:

1. **pathogenic/likely pathogenic variants** were ultra-rare (MAF<0.001% in gnomAD) or novel and classified as pathogenic/likely pathogenic according to American College of Genetics and Genomics (ACMG) criteria[24] i.e. potentially causative of LQTS in their own right. Only variants in established LQTS genes fell into this this category.[19]
2. **risk allele variants** were rare (MAF<1% in gnomAD) with functional and/or statistical data supportive of a biologically plausible association with aLQTS but with insufficient evidence to support pathogenicity as defined above.
3. **variants of uncertain significance** were rare (MAF <1% in gnomAD) with no further supportive data or conflicting data.

**Statistical analysis**: Relevant variables were expressed either as counts and percentages or as mean value ± standard deviation. To assess the variables independency, Chi-square and Fisher's exact tests were performed using the PASWw Statistics 18 software (SPSS Inc., Chicago, IL, USA). A two-sided P-value <0.05 was considered statistically significant.

## RESULTS

**Clinical characteristics**. Baseline clinical characteristics for the cohort are shown in Table 1. A total of 153 unrelated aLQTS cases (94 females, 61.4%) were included, with a mean age of 58 years (range: 14-88). The vast majority of cases were Caucasian (148/150, 98.7%).

Clinical presentation: One-hundred-and-thirty-one cases (85.6%) presented with either TdP, VF or cardiac arrest (n=122, 79.7%), or with syncope (n=9, 5.9%). Twenty-two cases (14.4%) were asymptomatic. The QTc interval, where available at presentation (n=105, 68.7%), was prolonged in all patients. The average QTc on admission was 574 +/- 80ms which reduced to an average of 452 +/- 42 ms on removal of the culprit drug(s). Hypokalaemia was documented in 28.1%.

Culprit drugs: A total of 216 QT-prolonging drugs were observed: 1.4 ± 0.7 drugs per patient (median: 1; range 1-4). CredibleMeds categorized 163 drugs as KR, 13 as PR, 47 as CR and 2 to be avoided by LQTS patients. Drug interactions that were likely to increase culprit drug bioavailability and/or pharmacodynamic synergy were found in 29 patients (19.0%), 18 (11.8%) of whom did not carry a genetic variant (Table 2 and Figure 2).

### Rare Variant Burden Analyses.

Primary cohort: Rare variant association studies found a significantly higher burden of nonsynonymous rare variants (MAF<1%) in cases compared to controls for CYP and 'main LQTS' gene sets with CMC (p=0.004 and p=0.002 respectively) but not with SKAT-O.

Replication Cohort: Rare variant association studies confirmed an increased burden of nonsynonymous rare variants (MAF<1%) in the CYP genes in cases compared with

controls (SKAT-O p=0.02, CMC p=0.01). When analysing for 'main LQTS' genes, there was no significant burden identified (CMC p=0.2, SKAT-O p=0.16).

Synonymous rare variants: In both datasets no gene showed significant differences in burden of rare synonymous variants with CMC or SKAT-O.

**Case characteristics**. After annotation, filtering for MAF<1.0% and Sanger confirmation, 46 variants from 39 cases (25.5%) were included (Figure 1). These variants were from two groups: LQTS genes (34 variants, 22.2% patients) and CYP genes (12 variants, 7.8% patients). Six patients (3.9%) carried more than one variant (Table S4). There were no significant baseline clinical differences between carriers and non-carriers (Table 1). In 11 of the 12 (92%) patients who carried CYP rare variants, at least one culprit drug was metabolised by the CYP450 enzyme in which the variant was identified (Table 3, Figure 2). Three patients were found to harbour both a LQTS variant and a CYP variant (Supplementary Appendix Results and Table S3).

There were two ultra-rare/novel variants identified in two non-cardiac ion channel genes (*GRIN3A* and *SCN2A*). These genes are largely expressed in brain tissue with very low expression in heart tissue, and neither gene has prior functional or clinical data associated with aLQTS. There were no statistical, functional and/or clinical data associated with these variants. They were therefore considered VUS (table S4).

**Variant Susceptibility**: Of the 46 rare variants identified within the cohort, 6 (3.9%) were classified as pathogenic/likely pathogenic; 25 were classified as risk allele variants (13 [8.4%] in LQTS genes and 12 [7.8%] in CYP genes) and 15 (9.8%) were classified as variants of uncertain significance (VUS) (Figure 1). The four pathogenic and two likely pathogenic variants were all found in *KCNQ1*. Six distinct CYP variants were classified as risk alleles as they have either previously been associated with decreased enzyme

activity (n=2) or decreased enzyme expression *in vivo* (n=1) or were very rare or novel (MAF <0.001%) variants with supportive *in silico* data (Table S4). There were 13 (9 distinct) rare (MAF<1%) risk alleles in LQTS genes with either functional and/or statistical data, as well as 15 (6 distinct) variants of uncertain significance, all of which were in LQTS genes. The classification of nine rare variants with conflicting interpretations was determined by consensus (Supplementary Appendix Results).

## DISCUSSION

The unpredictable and serious nature of an ADR such as TdP has important implications. By employing a novel approach to unbiased *in silico* target profiling in a large cohort of aLQTS cases, we demonstrate the important and novel finding of rare variation affecting CYP genes that causes loss of function and likely increased bioavailability of drugs culpable for aLQTS. We hypothesised that this would lead to further reduction of repolarisation reserve and increase the susceptibility to aLQTS. This was partially supported by a subsequent unbiased case-control burden analysis for rare variation. We then tested this hypothesis and replicated our findings in an independent case control dataset showing a statistically robust excess of rare CYP variants in cases. In addition, non-genetic pharmacokinetic susceptibility was postulated in the form of impairment of culprit drug metabolism due to drug-drug interactions. We confirmed previous findings of a small but significant yield of pathogenic or likely pathogenic, rare or ultra-rare variation in LQTS genes.[2,4,7] Burden testing of rare variation in LQTS genes was, however, inconsistent across both cohorts.

Thus, over one third of cases had at least one pharmacodynamic or pharmacokinetic susceptibility, or risk factor, identified with a small number having polygenic and/or multi-factorial risk. The role of other recognised intrinsic and extrinsic risk factors especially hypokalaemia as predisposing and/or triggering factors also appeared important.

<u>CYP variation</u>: Common variants in drug metabolism genes including the CYP genes, accounts for variability in pharmacokinetics of liver metabolised drugs, including drugs tht cause QT prolongation.[25] However, the most interesting and novel finding from our data was the identification of rare or ultra-rare variants with known functional effects, i.e. risk allele variants, that may have altered metabolism of the medication responsible for the

event.[26-28] In 11 of the 12 (92%) patients who carried CYP risk alleles, at least one culprit drug was metabolised by the CYP450 enzyme in which the variant was identified. *CYP2B6*-c.499C>G, found in one case, was associated with decreased enzyme activity *in vivo*;[26-27] whereas two other rare CYP variants found in five cases, *CYP2B6*-c.1172T>A and *CYP2B6*-c.415A>G, were associated with decreased enzyme expression *in vivo*.[28] The other CYP risk alleles had not been functionally characterized but *in silico* tools supported likely dysfunction. We then found an excess of CYP rare variation in aLQTS cases compared to healthy controls on statistical burden testing that was more robust in an independent case-control cohort. This supported our hypothesis that rare CYP variants play an important role in a subset of aLQTS.

In this respect, case reports of drug-drug interactions may serve as an indirect validation of this conclusion. For example, a drug-drug interaction between methadone and voriconazole has been attributed to CYP2B6 inhibition by voriconazole causing increased concentrations of methadone leading to TdP.[29] Therefore, voriconazole administration could be regarded as a 'chemical knockout' of CYP2B6, similar to the putative effect of the novel variant *CYP2B6*-c.445G>A identified in a patient in our cohort presenting with TdP whilst receiving methadone.

In a study of the Ontario Drug Benefit Claims Database, strong or moderately strong CYP3A4 inhibitors were co-prescribed in up to 10.7% of 122,233 patients receiving domperidone, a KR drug. Further KR or PR medications were co-prescribed to 18.3% and 18.8% of this cohort respectively, leading to an increase in the proarrhythmic potential of domperidone.[30] Our findings complement these data by emphasising that pharmacogenetic risk may also be involved in the pharmacokinetic interactions leading to drug-induced ADRs. Indeed, a risk allele *CYP3A4*-c.1000G>T was found in a 58-year-old male in our series who presented with TdP whilst receiving Amiodarone, a KR drug metabolised by CYP3A4. And among the six patients being treated with domperidone at

presentation, four carried at least one rare CYP risk allele, three in a CYP gene whose product is known to metabolise domperidone. This was, however, not a consistent observation. Fluoroquinolones are liver metabolised yet none of our aLQTS cases due to fluoroquinolones had an associated CYP risk allele. This may be a chance finding or other proarrhythmic factors may have been at play in these cases. Sotalol is renally excreted in general and as expected, no CYP risk alleles were associated.

Marked variation has been detected in the CYP genes including novel variants and variation with presumed functional effects (nonsense, frameshift truncations, and splice site variants). This variability is over three times more pronounced in African American populations compared with Caucasians. Up to 7.6% of Caucasian individuals studies carried at least one potentially deleterious allele in a major drug-metabolising CYP gene. compared to 11.7% of African American individuals.[31] One strength of our study is that 99% of the cohort was of Caucasian ethnicity, permitting a more consistent case-control analysis.

Understanding the significance of this variability in CYP genes is a challenge for the personalised medicine initiative. For example, there are reported cases of severe neuropsychiatric disturbances in individuals homozygous for loss-of-function alleles in *CYP2B6* when treated with efavirenz which resolve completely upon cessation of the drug.[32]

LQTS variation: The proportion of cases carrying pathogenic or likely pathogenic LQTS variants (3.9%) likely to have a severe impact on repolarization reserve and able to cause monogenic LQTS in their own right, is lower than yields from previous Sanger sequencing studies (average 10%).[2] These early studies tended to focus on the five major LQTS genes (*KCNQ1*, *KCNH2*, *KCNE1*, *KCNE2* and *SCN5A*). The additional yield of rare variants from including other 'minor' genes was, however, only minimal (3.3%) as

*SCN4B*, *AKAP9* and *CAV3* have not been associated with aLQTS previously and are now considered to be uncertain in causing monogenic LQTS.[19] Thus, all were classed as VUS's. Our pathogenic or likely pathogenic yield was also lower than a recent study of 188 aLQTS cases by Itoh et al, where 28% of patients harbored pathogenic variants in the five principal LQTS genes.[9] In our cohort, strict ACMG criteria for pathogenicity were applied and the remainder were classified as risk allele variants (8.4%) or variants of uncertain significance. Combining pathogenic, likely pathogenic variants and risk alleles gave a more comparable yield (12.3%). In addition, there are important ethnic differences between the two cohorts, with Itoh et al having a majority (78%) of Japanese individuals in their cohort, whereas our population was overwhelmingly (98%) Caucasian. Otherwise the other characteristics of the two cohorts (age, gender, QTc interval post-drug) were all similar. It is highly plausible that in Caucasian individuals there is a more polygenic basis to aLQTS disease compared with Japanese. Other reasons for the differences in yield may be due to different genotyping platforms, the slightly more symptomatic nature of their population and the inherent differences in potency of effect on cardiac repolarisation of the drugs to which the individual is exposed (e.g. cardiac drugs have more potent effects than antiemetics). Ramirez et al. had also reported a substantially higher yield of 36% of variants carriers whilst screening 79 candidate cardiac genes in 31 aLQTS patients.[11] However their population was composed only of patients with TdP and pathogenicity was based upon only 2 in silico tools (Polyphen-2 and SIFT) which would now only score a single "supporting (PP3)" evidence criterion in the ACMG guidelines. Other features would be required before pathogenicity can be attributed.[24] Interestingly, only 10 rare variants were identified in 132 cases sequenced for the main LQTS genes[33] and included in a large study of Caucasian cases submitted for GWAS.

Other important risk alleles were identified; though not expected to cause monogenic disease but still demonstrating increased susceptibility to aLQTS by modifying

repolarization reserve. For example, the *KCNE1*-D85N variant was present in four cases (3% of Caucasian subjects) and therefore seems to be overrepresented in our aLQTS population. This variant has previously been associated with an increased risk of both LQTS[34] and aLQTS[9,18], as well as with drug-induced TdP.[35] A minority of the population reported here (n=57) was included in the latter publication.[35]

The contribution to the aLQTS phenotype by some variants, however, remained unclear, and these were classified as variants of uncertain significance. For instance, the *CAV3* variants c.233C>T and c.216C>G were found in five of our cases and have been previously reported in LQTS[36] and SIDS cohorts.[37-38] Their role may be questionable however as both have been identified with a significant prevalence in exome data from population studies[39] and functional data are inconsistent.[40]

When the burden of rare variation in LQTS genes was tested in the primary and replication datasets a statistically significant association was not confirmed. This does not exclude LQTS rare variation as a risk factor but suggests that the importance of rare LQTS variants in predisposing to aLQTS may be less than previously anticipated in Caucasians. Larger series from different ethnic groups with both GWAS and rare variant data will be required to determine the genomic architecture of aLQTS from the perspective of repolarisation reserve.

Drug-Drug Interactions: The high prevalence of drug interactions caused by polypharmacy serves as a further warning over the importance of simple pharmacokinetic considerations when prescribing for patients. Prevention will require more robust systems for flagging up interactions, changing prescriptions or otherwise monitoring patients with an elevated risk profile.

Implications for personalised medicine: It has already been proposed that

pharmacogenomic testing for rare variation in the LQTS genes may help prevent aLQTS.[2] Whilst further functional analyses are required to reinforce our novel findings, these suggest that rare variation in CYP genes is also an important new avenue for identifying individualised risk to prevent the risk of proarrhythmia due to drugs that are liver metabolised. This will require sequencing of a panel of candidate pharmacogenomic genes to identify rare variation prior to drug prescription. Focusing on common variation alone will miss this potential risk. The timing and strategy for delivering this personalised approach remains to be determined.

Limitations: The primary cohort and control were sequenced using different capture systems and platforms due to unavoidable logistical issues. Burden of nonsynomyous variants was however similar for all genes. To overcome this limitation further we also sought a replication cohort where cases and controls had been sequenced with the same capture systems and platform. Obtaining a large enough replication cohort for this rare phenotype was challenging but ultimately successful. Although we were able to replicate increased burden of rare CYP-gene variation in aLQTS cases compared with controls, we were not able to identify specific functionally relevant variants. Rare variants reported previously in public databases but without any functional data were present. These may have functional effects that are important contributors to the risk of TdP but assessing these was beyond the scope of our current project.

## CONCLUSIONS

Our findings support the role for rare genetic susceptibility in both pharmacodynamic and pharmacokinetic risk for aLQTS and illustrate the potential of unbiased computational methods at uncovering new genetic biomarkers of ADRs. Although rare variants in LQTS genes predispose to risk by reducing repolarisation reserve, rare variation in the CYP450 enzyme genes is an important new pathophysiological pathway. Indeed, burden testing suggests a more statistically significant role for susceptibility variants in CYP genes than LQTS genes amongst Caucasians. Together with drug interactions, this finding will need to be included in the personalised medicine initiative if we are to reduce the risk of this important ADR.

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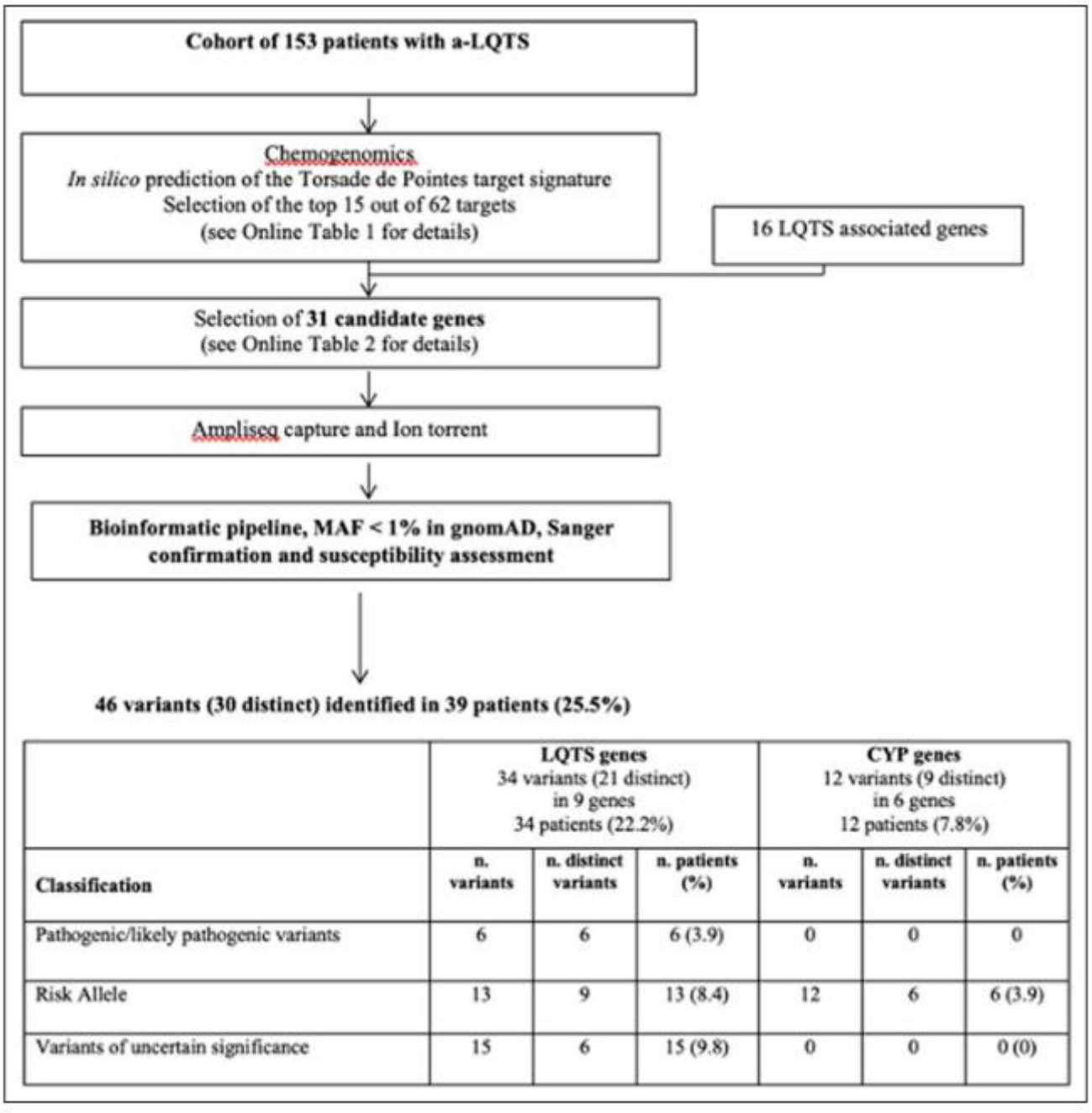
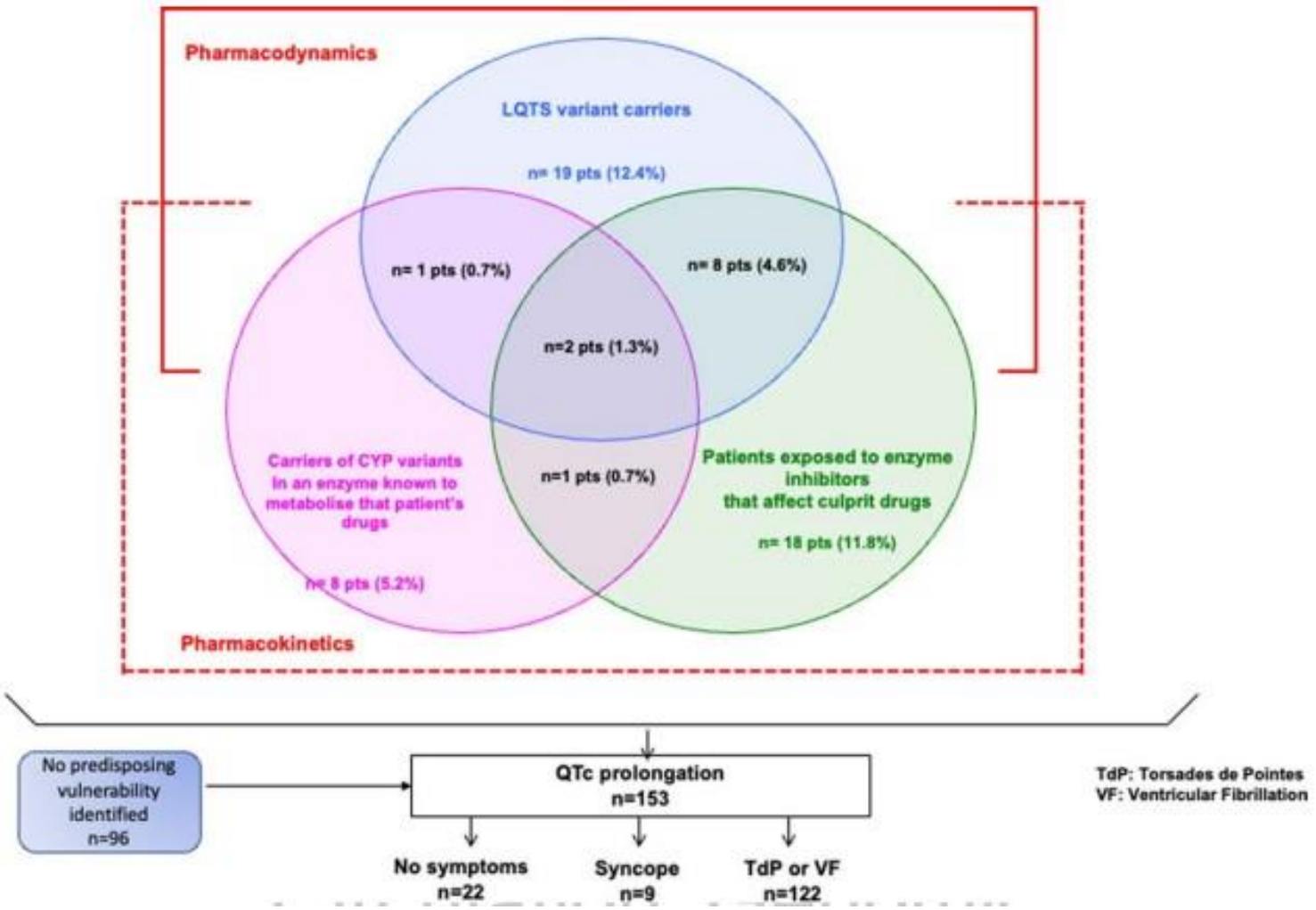
**FIGURES LEGEND**

**Figure 1**: Flow diagram of study




| | **LQTS genes** 34 variants (21 distinct) in 9 genes 34 patients (22.2%) | | | **CYP genes** 12 variants (9 distinct) in 6 genes 12 patients (7.8%) | | |
|---|---|---|---|---|---|---|
| **Classification** | **n. variants** | **n. distinct variants** | **n. patients (%)** | **n. variants** | **n. distinct variants** | **n. patients (%)** |
| Pathogenic/likely pathogenic variants | 6 | 6 | 6 (3.9) | 0 | 0 | 0 |
| Risk Allele | 13 | 9 | 13 (8.4) | 12 | 6 | 6 (3.9) |
| Variants of uncertain significance | 15 | 6 | 15 (9.8) | 0 | 0 | 0 (0) |

**Figure 2**: Complex genetic and environmental background leading to drug-induced cardiac event



**TABLES**

Table 1: Clinical characteristics of all cases and variant carriers compared to non-carriers

| | Overall (n=153) | Carriers (n=39) | Non-carriers (n=114) | P value* |
|---|---|---|---|---|
| Age, mean ± SD<br>median (range) | 58 ± 17<br>58 (14-88) | 57 ± 18<br>59 (14-84) | 59 ± 16<br>63 (17-86) | 0.57 |
| Caucasian, n (%) | 148/150 (98.7) | 38 (97.4) | 110/111 (99.1) | 0.44 |
| QTc on admission, ms [mean ± SD, median (min-max)] | 574.1 ± 80.0<br>560 (460-827) [n=105] | 592.1 ± 100.5<br>560 (475-827) [n=27] | 567.9 ± 71.2<br>564 (460-743) [n=78] | 0.18 |
| QTc after drug removal, ms [mean ± SD, median (min-max)] | 452 ± 42<br>442 (374-622) [n=87] | 452.5 ± 29.1<br>452 (410-520) [n=22] | 451.9 ± 46.6<br>437 (374-622) [n=65] | 0.95 |
| Culprit drugs | | | | |
| Number of culprit drugs per patient | 1.4 ± 0.7<br>1 (1-4) | 1.5 ± 0.6<br>1 (1-3) | 1.4 ± 0.7<br>1 (1-4) | 0.26 |
| Intrinsic risk factors | | | | |
| Female sex, n (%) | 94 (61.4) | 29 (74.3) | 65 (57.0) | 0.055 |

| | | | | |
|---|---|---|---|---|
| Hypothyroidism | 26 (16.9) | 8 (20.5) | 18 (15.8) | 0.50 |
| Heart disease | 74 (48.3) | 20 (51.2) | 54 (47.4) | 0.67 |
| Liver dysfunction (a) | 15 (9.8) | 4 (10.3) | 11 (9.6) | 0.91 |
| Renal dysfunction (b) | 11 (7.2) | 3 (7.7) | 8 (7.0) | 0.89 |
| Extrinsic risk factors | | | | |
| Hypokalaemia | 43 (28.1) | 15 (38.5) | 28 (24.6) | 0.08 |
| Hypomagnesemia | 16 (10.5) | 6 (15.4) | 10 (8.8) | 0.24 |
| Hypocalcaemia | 20 (13.1) | 7 (17.9) | 13 (11.4) | 0.28 |
| Extreme bradycardia <40/min | 12 (7.8) | 1 (2.6) | 11 (9.6) | 0.16 |
| Cardiac event | | | | |
| TdP, VF or cardiac arrest | 122 (79.7) | 29 (74.3) | 93 (73.6) | 0.33 |
| Syncope | 9 (5.9) | 4 (10.2) | 5 (4.3) | 0.18 |
| QTc prolongation alone | 22 (14.4) | 6 (15.4) | 16 (14.0) | 0.84 |

* Comparison between carriers and non-carriers.

(a) in case of liver metabolism of the culprit drug

(b) in case of renal elimination of the culprit drug

Table 2: Drug interactions that affect culprit drugs (n=29 patients)

| Patients | Variant | Drug-1 Generic name | Risk | Drug-2 Generic name | Risk | Drug-3 Generic name | Risk | Drug-4 Generic name | Risk | Drug interaction (type) |
|---|---|---|---|---|---|---|---|---|---|---|
| ARITMO-6 | KCNH2-c.3163C>T p. R1055W | flecainide | KR | disopyramide | KR | n/a | n/a | n/a | n/a | disopyramide and flecainide both increase QTc interval. Pharmacodynamic synergy |
| ARITMO-9 | No variant | fluconazole | KR | clarithromycin | KR | n/a | n/a | n/a | n/a | (1) fluconazole will increase the level or effect of clarithromycin by affecting hepatic/intestinal enzyme CYP3A4 metabolism. (2) clarithromycin and fluconazole both increase QTc interval. Pharmacodynamic synergy |
| ARITMO-13 | No variant | methadone | KR | venlafaxine | KR | n/a | n/a | n/a | n/a | methadone and venlafaxine both increase QTc interval. |

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|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Pharmacodynamic synergy |
| ARITMO-16 | CAV3-<br>c.233C>T<br>p. T78M<br>and<br>SCN5A-<br>c.569G>A<br>p. R190Q | amiodarone | KR | sotalol | KR | n/a | n/a | n/a | n/a | amiodarone and sotalol both increase QTc interval.<br>Pharmacodynamic synergy |
| ARITMO-23 | No variant | sotalol | KR | fluoxetine | CR | n/a | n/a | n/a | n/a | fluoxetine and sotalol both increase QTc interval.<br>Pharmacodynamic synergy |
| ARITMO-28 | KCNQ1-<br>c.727C>T<br>p. R243C | amiodarone | KR | amitriptyline | CR | n/a | n/a | n/a | n/a | (1) amiodarone will increase the level or effect of amitriptyline by P-glycoprotein (MDR1) efflux transporter.<br>(2) amitriptyline and amiodarone both increase QTc interval. |

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| | | | | | | | | | | Pharmacodynamic synergy |
| ARITMO-30 | No variant | citalopram | KR | indapamide | CR | n/a | n/a | n/a | n/a | indapamide and citalopram both increase QTc interval. Pharmacodynamic synergy |
| ARITMO-34 | CYP2B6-c.415A>G p. K139E | amiodarone | KR | fluoxetine | CR | n/a | n/a | n/a | n/a | (1) amiodarone will increase the level or effect of fluoxetine by affecting hepatic enzyme CYP2D6 metabolism. (2) amiodarone and fluoxetine both increase QTc interval. Pharmacodynamic synergy |
| ARITMO-38 | No variant | procainamide | KR | citalopram | KR | amitriptyline | CR | quinine | CR | (1) procainamide will increase the level or effect of quinine by basic (cationic) drug competition for renal tubular clearance. (2) procainamide and quinine both increase QTc interval |

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|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | (3) amitriptyline and procainamide both increase QTc interval. Pharmacodynamic synergy<br>(4) procainamide and citalopram both increase QTc interval. Pharmacodynamic synergy<br>(5) quinine and citalopram both increase QTc interval. Pharmacodynamic synergy<br>(6) amitriptyline and quinine both increase QTc interval. |
| ARITMO-50 | No variant | amiodarone | KR | erythromycin | KR | n/a | n/a | n/a | n/a | (1) erythromycin base will increase the level or effect of amiodarone by affecting hepatic/intestinal enzyme CYP3A4 metabolism.<br>(2) amiodarone and erythromycin |

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| | | | | | | | | | | base both increase QTc interval. Pharmacodynamic synergy. |
| ARITMO-61 | No variant | thioridazine | KR | fluoxetine | CR | n/a | n/a | n/a | n/a | (1) thioridazine will increase the level or effect of fluoxetine by affecting hepatic enzyme CYP2D6 metabolism. (2) fluoxetine increases levels of thioridazine by decreasing metabolism. (3) fluoxetine will increase the level or effect of thioridazine by affecting hepatic enzyme CYP2D6 metabolism. (4) thioridazine and fluoxetine both increase QTc interval. Pharmacodynamic synergy |
| ARITMO-73 | No variant | amiodarone | KR | trimethoprim | TA | n/a | n/a | n/a | n/a | (1) amiodarone will increase the |

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| | | | | | | | | | | level or effect of trimethoprim by basic (cationic) drug competition for renal tubular clearance.<br>(2) amiodarone and trimethoprim both increase QTc interval. Pharmacodynamic synergy |
| ARITMO-84 | No variant | amiodarone | KR | flecainide | KR | n/a | n/a | n/a | n/a | (1) amiodarone will increase the level or effect of flecainide by affecting hepatic enzyme CYP2D6 metabolism.<br>(2) amiodarone and flecainide both increase QTc interval. Pharmacodynamic synergy |
| ARITMO-88 | KCNE1-c.253G>A p. D85N and | amiodarone | KR | fluconazole | KR | domperidone | KR | n/a | n/a | (1) fluconazole will increase the level or effect of amiodarone by affecting hepatic/intestinal enzyme CYP3A4 metabolism. |

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|---|---|---|---|---|---|---|---|---|---|---|
| | CYP2D6-<br>c.404C>T<br>p. S135F | | | | | | | | | (2) Amiodarone: CYP2D6 inhibitor<br>(3) amiodarone and fluconazole both increase QTc interval. Pharmacodynamic synergy |
| ARITMO-89 | KCNE1-<br>c.253G>A<br>p. D85N | flecainide | KR | citalopram | KR | n/a | n/a | n/a | n/a | flecainide and citalopram both increase QTc interval. Pharmacodynamic synergy |
| ARITMO-90 | No variant | amiodarone | KR | disopyramide | KR | n/a | n/a | n/a | n/a | amiodarone and disopyramide both increase QTc interval. Pharmacodynamic synergy |
| ARITMO-93 | KCNQ1-<br>c.733G>A<br>p. G245R | cisapride | KR | itraconazole | CR | n/a | n/a | n/a | n/a | (1) itraconazole will increase the level or effect of cisapride by affecting hepatic/intestinal enzyme CYP3A4 metabolism.<br>(2) cisapride and itraconazole both increase QTc interval. Pharmacodynamic synergy |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| ARITMO-98 | ANK2-c.9854T>C p. I3285T and CYP2E1-c.377G>A p. R126Q | haloperidol | KR | pimozide | KR | hydrochlorothiazide | CR | n/a | n/a | haloperidol and pimozide both increase QTc interval. Pharmacodynamic synergy |
| ARITMO-104 | No variant | methadone | KR | pimozide | KR | n/a | n/a | n/a | n/a | methadone and pimozide both increase QTc interval. Pharmacodynamic synergy |
| ARITMO-108 | No variant | ciprofloxacin | KR | haloperidol | KR | n/a | n/a | n/a | n/a | ciprofloxacin and haloperidol both increase QTc interval. Pharmacodynamic synergy |
| ARITMO-110 | No variant | promethazine | PR | trimipramine | PR | fluoxetine | CR | quetiapine | CR | (1) fluoxetine will increase the level or effect of promethazine by affecting hepatic enzyme CYP2D6 metabolism. |

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| | | | | | | | | | | (2) quetiapine, fluoxetine. Either increases toxicity of the other by QTc interval.<br>(3) quetiapine, trimipramine. Either increases toxicity of the other by QTc interval.<br>(4) trimipramine, promethazine. Either increases levels of the other by decreasing metabolism. Minor/Significance Unknown.<br>(5) trimipramine, promethazine. Either increases levels of the other by pharmacodynamic synergy Minor/Significance Unknown.<br>(6) promethazine and trimipramine both increase QTc interval. |

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| | | | | | | | | | | Pharmacodynamic synergy (7) promethazine and fluoxetine both increase QTc interval. Pharmacodynamic synergy (8) trimipramine and fluoxetine both increase QTc interval. Pharmacodynamic synergy |
| ARITMO-113 | SCN5A-c.1715C>Ap. A572D | amiodarone | KR | hydrochlorothiazide | CR | n/a | n/a | n/a | n/a | amiodarone will increase the level or effect of hydrochlorothiazide by basic (cationic) drug competition for renal tubular clearance. |
| ARITMO-115 | No variant | amiodarone | KR | hydrochlorothiazide | CR | n/a | n/a | n/a | n/a | amiodarone will increase the level or effect of hydrochlorothiazide by basic (cationic) drug competition for renal tubular clearance. |
| ARITMO-124 | No variant | amiodarone | KR | ondansetron | KR | furosemide | CR | n/a | n/a | amiodarone and ondansetron both increase QTc interval. |

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| | | | | | | | | | | Pharmacodynamic synergy |
| ARITMO-126 | KCNE1-c.253G>Ap. D85N | amiodarone | KR | imipramine | PR | n/a | n/a | n/a | n/a | (1) amiodarone will increase the level or effect of imipramine by affecting hepatic enzyme CYP2D6 metabolism.<br>(2) imipramine and amiodarone both increase QTc interval. Pharmacodynamic synergy |
| ARITMO-135 | No variant | amiodarone | KR | hydrochlorothiazide | CR | n/a | n/a | n/a | n/a | amiodarone will increase the level or effect of hydrochlorothiazide by basic (cationic) drug competition for renal tubular clearance. |
| ARITMO-136 | No variant | clarithromycin | KR | amiodarone | KR | metoclopramide | CR | n/a | n/a | (1) clarithromycin will increase the level or effect of amiodarone by affecting hepatic/intestinal enzyme CYP3A4 metabolism.<br>(2) amiodarone and clarithromycin |

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|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | both increase QTc interval. Pharmacodynamic synergy |
| ARITMO-138 | No variant | levofloxacin | KR | dronedarone | KR | n/a | n/a | n/a | n/a | dronedarone and levofloxacin both increase QTc interval. Pharmacodynamic synergy |
| ARITMO-153 | KCNE2-c.22A>G p. T8A | hydroxyzine | CR | fluoxetine | CR | n/a | n/a | n/a | n/a | hydroxyzine increases toxicity of fluoxetine by QTc interval. |

DDI: drug-drug interaction; KR: known risk; PR: possible risk; CR: conditional risk; TA: drug to avoid in long QT syndrome; n/a: not applicable.

Drug to drug interactions were analysed according to CredibleMeds (https://crediblemeds.org) and to the Drug Interaction Checker from Medscape (http://reference.medscape.com/drug-interactionchecker). Reference for ARITMO-88: Ohyama 2000.[30]

Table 3: Culprit drugs and CYP450 metabolism in patients carrying CYP allelic variants (n=12 patients)

| **Patients** | **CYP variant** | **Classification** | **MAF (gnomAD)** | **Culprit drug-1 Generic name** | **Risk** | **CYP450 metabolism** | **CYP-drug match** | **Culprit drug-2 Generic name** | **Risk** | **CYP metabolism** | **CYP-drug match** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| ARITMO-15 | CYP2B6 c.1172T>A p. I391N | Risk allele | 0.004 | erythromycin | KR | 1A2, **2B6**, 2J2, 3A4, 3A7 | yes | n/a | n/a | n/a | n/a |
| ARITMO-26 | CYP3A4 c.1000G>T p. E333* | Risk allele | 0 | amiodarone | KR | 1A1, 1A2, 2C8, 2C9, 2C19, 2D6, **3A4**, 3A5, 3A7 | yes | n/a | n/a | n/a | n/a |
| ARITMO-29 | CYP2B6 c.1172T>A p. I391N | Risk allele | 0.004 | erythromycin | KR | 1A2, **2B6**, 2J2, 3A4, 3A7 | yes | n/a | n/a | n/a | n/a |
| ARITMO-31 | CYP2B6 c.1172T>A p. I139N | Risk allele | 0.004 | amitriptyline | CR | 1A2, **2B6**, 2C8, 2C9, 2C19, 2D6, | yes | n/a | n/a | n/a | n/a |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 2E1, 3A4 | | | | | |
| ARITMO-34 | CYP2B6 c.415A>G p. K139E | Risk allele | 0.0018 | fluoxetine | CR | 1A2, **2B6**, 2C8, 2C9, 2C19, 2D6, 2E1, 2J2, 3A4 | yes | amiodarone | KR | 1A1, 1A2, 2C8, 2C9, 2C19, 2D6, 3A4, 3A5, 3A7 | no |
| ARITMO-36 | CYP2B6 c.1172T>A p. I391N | Risk allele | 0.004 | domperidone | KR | 1A2, **2B6**, 2C8, 2D6, 3A4, 3A5, 3A7 | yes | n/a | n/a | n/a | n/a |
| ARITMO-86 | CYP2B6 c.499C>G p. P167A | Risk allele | 0.0002 | Pimozide overdose | KR | 1A2, 2C8, 2C9, 2C19, 2D6, 2E1, 2J2, 3A4, 3A5, 3A7 | no | n/a | n/a | n/a | n/a |
| ARITMO-88 | CYP2D6 c.404C>T p. S135F | Risk allele | 0.00003 | amiodarone | KR | 1A1, 1A2, 2C8, 2C9, 2C19, **2D6**, 3A4, 3A5, | yes | domperidone | KR | 1A2, 2B6, 2C8, **2D6**, 3A4, 3A5, 3A7 | yes |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 3A7 | | | | | |
| ARITMO-98 | CYP2E1 c.377G>A p. R126Q | Risk allele | 0.00006 | pimozide | KR | 1A2, 2C8, 2C9, 2C19, 2D6, **2E1**, 2J2, 3A4, 3A5, 3A7 | yes | haloperidol | KR | 1A1, 1A2, 2C8, 2C9, 2C19, 2D6, 2J2, 3A4, 3A5, 3A7 | no |
| ARITMO-134 | CYP2B6 c.923G>A p. R308H | Risk allele | 0.00003 | domperidone | KR | 1A2, **2B6**, 2C8, 2D6, 3A4, 3A5, 3A7 | yes | haloperidol | KR | 1A1, 1A2, 2C8, 2C9, 2C19, 2D6, 2J2, 3A4, 3A5, 3A7 | no |
| ARITMO-139 | CYP2B6 c.445G>A p. E149K | Risk allele | 0.00003 | methadone | KR | 1A2, **2B6**, 2C8, 2C9, 2C18, 2C19, 2D6, 3A4, 3A5, | yes | n/a | n/a | n/a | n/a |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | 3A7 | | | | | |
| ARITMO-150 | CYP1A2 c.1493C>A p. T498N | Risk allele | 0.00003 | amiodarone | KR | 1A1, **1A2**, 2C8, 2C9, 2C19, 2D6, 3A4, 3A5, 3A7 | yes | n/a | n/a | n/a | n/a |

MAF: minor allele frequency; gnomAD: genome aggregation database; KR: known risk of torsades de pointes; CR: conditional risk of torsades de pointes; n/a: not applicable.

Risk category for culprit drugs was determined by CredibleMeds (www.Crediblemeds.org).