Refinement of hotspots in Long-QT Syndrome associated genes

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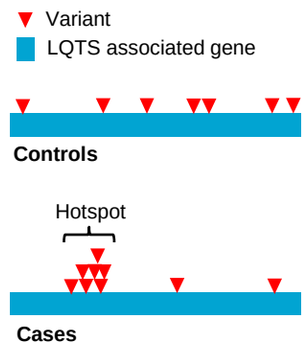
1) Background

- Long-QT syndrome (LQTS) is an inherited arrythmia syndrome.
- There is a high risk of sudden cardiac death if untreated.
- LQTS is caused by variants in genes encoding cardiac ion channel subunits or channel associated proteins.
- Genetic testing plays a vital role in patient management and in identifying at risk family members.
- However, it can be difficult to differentiate between benign and pathogenic variants.
- Regional enrichment of pathogenic variants (hotspots) in LQTS cases can help variant interpretation[1-4].
- **Aim**: compare variant frequency and distribution in LQTS cases and controls to identify 'hotspots' in LQTS associated genes.



2) Methods

- **Collate variants from 21 genes from our LQTS cohort**: 2598 probands referred to the Oxford Regional Genetics Laboratory (ORGL) for LQTS genetic testing.
- **Filter for rare missense variants**: rare defined as variant having a filtering allele frequency (FAF) of ≤5x10-5 in the Genome Aggregation Database exome cohort (gnomAD v2.1.1)[5].
- **Gene burden analysis**: case-control analyses of rare missense variants to calculate odds ratio (OR) and confidence interval (CI) for each gene.
- **Regional burden analysis**: we used the GAM framework[6] which combines gene burden and positional information to calculate an OR and CI for each codon.
- **Reclassification of variants**: we defined hotspots by lower 95% CI (strong >100, moderate 20-100, supporting 10-20) to use as evidence for variant classification.

Case data: probands referred to the ORGL for LQTS genetic testing

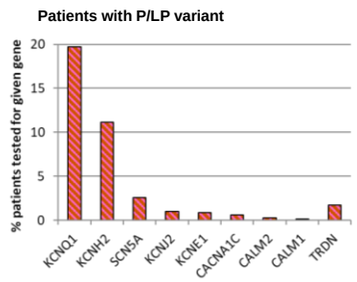
vs

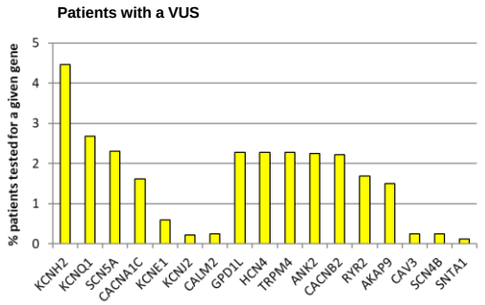
Control data: individuals from gnomAD v.3.1 genome cohort.

3) Results

A. Most pathogenic (P) and likely pathogenic (LP) variants are in *KCNQ1, KCNH2* and *SCN5A*

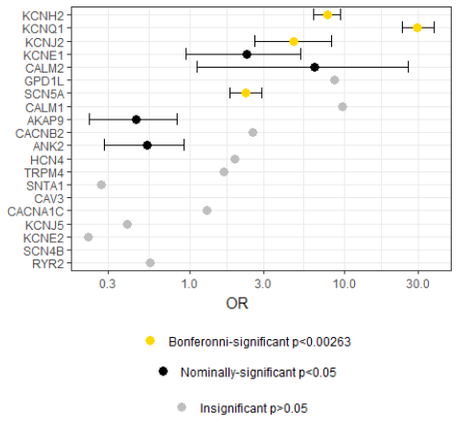
- 656 variants in 21 genes.
- Some genes have only had variants of unknown significance (VUS) reported.



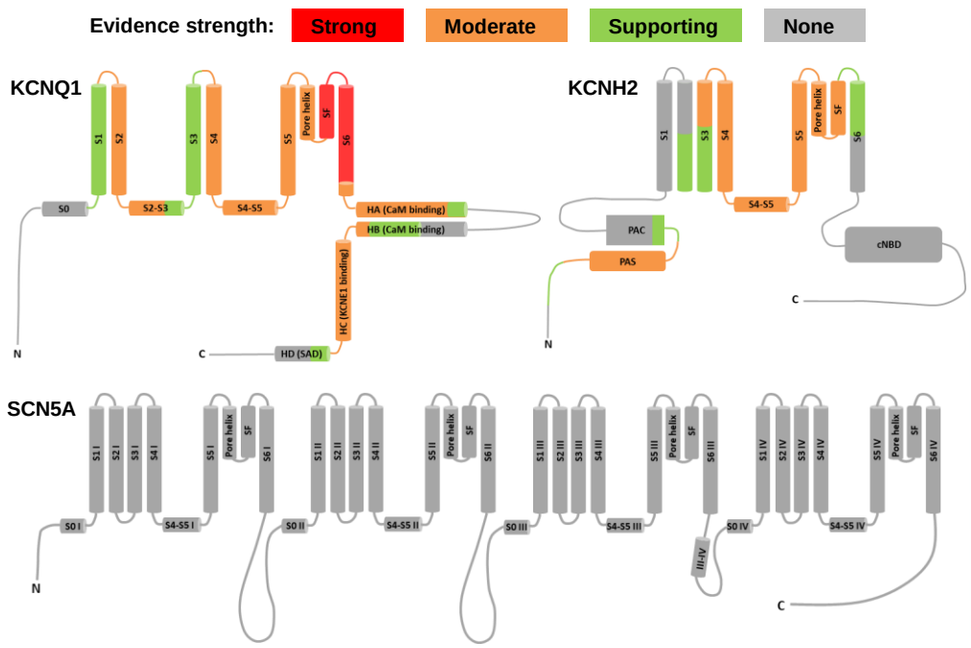


B. Rare missense variants are enriched in *KCNQ1, KCNH2* and *SCN5A*

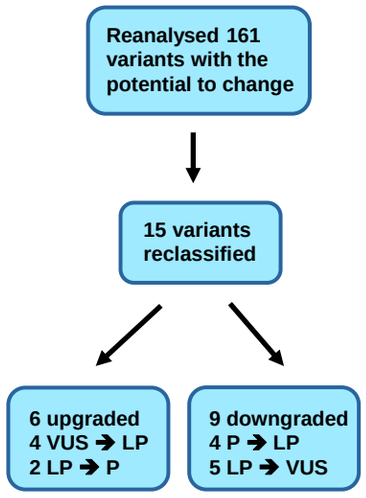
KCNJ2, KCNE1, and *CALM2* also show enrichment for rare missense variants in the LQTS cohort.



C. Rare missense variants show regional enrichment (hotspots) in KCNQ1 and KCNH2 but not SCN5A



D. Applying information about hotspots changes the classification of variants in our cohort



6) Conclusions

- Rare missense variants are enriched in *KCNH2, KCNQ1, KCNJ2, KCNE1, CALM2* and *SCN5A.*
- Regional burden analysis refined previously described hotspots[2-4] in KCNQ1 and KCNH2 but found no hotspot in SCN5A.
- We have demonstrated the clinical utility of these refined hotspots by reclassifying 15 variants in our cohort.

7) Future work

- Incorporate more data from other NHS laboratories and/or published datasets.
- This may allow us to do hotspot analysis of other LQTS associated genes such as *KCNJ2* and *KCNE1*.
- Use the GAM framework to identify hotspots in SCN5A in a Bragada syndrome cohort

References: 1) Richards S, et al. Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015. 2) Kapa S, et al. Genetic testing for long-qt syndrome: Distinguishing pathogenic mutations from benign variants. Circulation. 2009. 3) Kapplinger JD, et al. Spectrum and prevalence of mutations from the first 2,500 consecutive unrelated patients referred for the FAMILION® long QT syndrome genetic test. Hear Rhythm. 2009. 4) Walsh R, et al. Enhancing rare variant interpretation in inherited arrhythmias through quantitative analysis of consortium disease cohorts and population controls. Genet Med. 2020. 5) The genome Aggregation Database (gnomAD) | MacArthur Lab [Internet]. [cited 2021 Mar 4]. Available from: https://macarthurlab.org/2017/02/27/the-genome-aggregation-database-gnomad/ 6) Waring A, et al. Data-driven modelling of mutational hotspots and in silico predictors in hypertrophic cardiomyopathy. J Med Genet. 2020.