

# Clinical Spectrum Of *CACNA1C* Variants, Revisited

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## Research

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# Abstract

## Background

CACNA1C is a gene encoding the CaV1.2 calcium channel and several cardiac conditions are potentially associated with pathogenic variants in this gene. The aim of this study is to explore genotype-phenotype correlations related to *CACNA1C* ever described variants and vast phenotypic spectrum.

## Methods

We analyzed 102 patients with *CACNA1C* variants (*CACNA1Cv*) (9 our cohort and 93 from literature). We studied the association between *CACNA1Cv* and clinical parameters: arrhythmias, structural heart defects, cardiomyopathy and survival. We followed the American College Medical Genetics (ACMG) scoring system to grade variants' pathogenicity and their domains.

## Results *CACNA1Cv*

with high ACMG scores were associated with higher mortality than variants with lower scores ( $p < 0.001$ ). *CACNA1Cv* in Cytoplasmic and Transmembrane domains were associated with higher mortality than other domains ( $p = 0.005$ ). Multivariate analysis for higher ACMG scores, indicates cardiomyopathy and a lesser extent domain, as independent risk factor for mortality ( $p = 0.031$  and  $p = 0.04$ ). Cytoplasmic domain variants were frequently associated with long-QT syndrome; C-terminal variants were often linked to Brugada syndrome. Parental mosaicism was relatively high (4–5%) and must not be overlooked in parents' phenotypic analysis and in calculation of disease recurrence risk

## Conclusion

To the best of our knowledge, this is the first study trying to create genotype-phenotype correlation and better risk stratification in *CACNA1Cv* in relation to survival and long-term results.

# Background

*CACNA1C* is a gene encoding the CaV1.2 calcium channel, which is critical for the plateau phase of the cardiac action potential, cellular excitability, and excitation–contraction coupling<sup>1, 2, 3, 4, 5</sup>. Several conditions are potentially associated with pathogenic variants in this gene including electrical, myocardial and structural defects. Moreover, extracardiac involvement is described, as well as a multisystemic disorder (Timothy Syndrome) characterized by different degrees of cardiac involvement (arrhythmic and structural), fingers syndactyly, developmental delay and recurrent infections.

Abnormalities related to *CACNA1C* variants vary greatly and may include any of the followings: i. electrical defects such as long QT syndrome (LQTS), Brugada syndrome (BrS), short QT syndrome (SQTS) and sudden cardiac death (SCD); ii. myocardial abnormalities, including hypertrophic

cardiomyopathy (HCM); iii. structural heart defects such as tetralogy of Fallot and ventricular septal defects<sup>6, 7, 8, 9</sup>. For some of these disease entities a causal association has recently been questioned<sup>10</sup>.

It is difficult to estimate the phenotypic spectrum of *CACNA1C* variants due to its wide variability. Historically, two major variants were described (p.Gly406Arg and p.Gly402Ser); however, with the progressive use of next generation sequencing (NGS) more variants are described and genetic heterogeneity is increasing. Moreover, genotype-phenotype correlation is still undone. Previous studies have only discussed fragmented aspects of the phenotypic spectrum.

## Methods

The aim of this study is to explore this relationship, based on our cohort and review of current evidence on known *CACNA1C* variants.

### Bambino Gesù Children Hospital cohort (OPBG):

We reviewed medical records of patients referred to our tertiary care centre for molecular analysis of *CACNA1C*. All data, including the molecular and cardiac diagnosis, were extracted from our database, which includes records on electrocardiograms (ECG), Holter ECG monitoring, echocardiography, stress test results and angiography procedures. The study protocol conforms to ethical guidelines of the 1975 Declaration of Helsinki, as reflected in a priori approval by the Institution's Human Research Committee.

We performed, on peripheral blood DNA extracted from patients, molecular analysis of *CACNA1C* (mRNA: NM\_000719) coding regions and flanking splicing regions through high-throughput targeted resequencing using an Arrhythmia custom panel and successively analyzed with NextSeq550 platform (Illumina, San Diego, CA). This panel includes causative genes for the following syndromes: LQTS, BrS, Catecholaminergic Polymorphic Ventricular Tachycardia (CPVT) and SQTS. Variant analysis was performed with Illumina Variant Studio Software and Integrative Genome Viewer (IGV). Sanger sequencing confirmed all genetic variants detected in the index cases on re-extracted DNA, using standard protocols.

### Literature Review

We searched PubMed for any published studies describing *CACNA1C* variant(s). Search strategy included "*CACNA1C*" and any of the following terms: "Timothy", "Brugada", "Long QT", "LQTS", "Cardiomyopathy" and "Rhythm". Inclusion criteria were: any article reporting data on *CACNA1C* and clinical presentation of the patient(s) involved; exclusion criteria were: duplicated cohorts and/or articles, molecular studies and articles written in language other than English. Full papers were carefully reviewed and considered according to the previous criteria. All articles were independently analyzed by three authors (BC, MG, FC). In case of dispute, an agreement was negotiated among the three authors. References of selected papers were cross-checked with the same inclusion and exclusion criteria. Papers included at the end of the

screening process were fully reviewed, and study characteristics were extracted and tabulated in an MS Excel for Windows (Microsoft Corporation, Redmond, Washington)- *available upon request*.

## **Variants classification**

For each variant, we considered the ACMG (American College of Medical Genetics) classification to establish the pathogenicity of the molecular variants<sup>10</sup>. This classification allowed us to classify variants using specific terminology identified in Mendelian disorders ('pathogenic' (ACMG score 5), 'likely pathogenic' (ACMG score 4), 'uncertain significance - VUS' (ACMG score 3), 'likely benign' (ACMG score 2), and 'benign' (ACMG score 1)). We excluded from the analysis patients with variants identified by the ACMG score as "benign" or "likely benign", distributed uniformly across all regions. We used this classification and stipulated it in further statistical analysis and in supplementary table 1A and 1B.

## **Clinical parameters**

We analyzed different parameters for each patient (literature and OPBG cohort): family history, prenatal findings, birth weight, gender, median age at diagnosis, and survival.

Electrocardiographic features have been investigated specifically for: abnormalities of QT corrected interval (QTc), PR, QRS, ST, J wave and T wave, Brugada pattern, presence and type of atrioventricular block (AVB), sinus node dysfunction, atrial tachyarrhythmias, ventricular tachycardia/ fibrillation and torsade de pointes.

Standard Echocardiogram data were collected with a focus on findings suggestive for hypertrophic cardiomyopathy (HCM) and ventricular septal defects.

We have also considered the presence of cyanosis, history of syncope or aborted SCD, hypotension, congenital heart defects and type of management (surgical, conservative or hemodynamic studies) including post-operative complications. The presence of dysmorphic features and syndactyly were evaluated. The analysis included any abnormal growth pattern, developmental delay, history of seizures, hypoglycemia, sepsis/recurrent infections and finally genetic investigation, with peculiar attention to *CACNA1C* variants.

## **Statistical Analysis**

All statistical analysis was performed using SPSS Statistics 21 package (IBM Corporation, Armonk, NY, USA). Continuous variables are presented as mean values (standard deviation (SD) and range) or median values (interquartile range, IQR), as appropriate. Categorical variables are expressed as absolute numbers or percentages. Fisher's exact test was used to compare groups < 5. Survival data were analyzed and graphically reported by the Kaplan-Meier method. Log rank test was used for comparison between groups. Patients who did not experience an event (cardiac arrest, aborted cardiac arrest) were censored at the time of the last follow-up. Univariate analysis was performed by logistic regression analysis for each variable (Arrhythmic event, LQT, BrS, ICD/PMK, HCM, CHD and mortality). Variables with P values less than 0.2 in the univariate analysis were considered eligible for entering multivariable analysis. Multiple

regression analysis was also performed. Results are expressed as odds ratios (OR) with 95% confidence intervals (CI). P-value was considered significant when  $\leq 0.05$ .

## Results

### OPBG Cohort

We identified 19 patients from our database (10 females and 9 males). Cardiac diagnosis varied from: LQTS (n = 13), BrS (n = 2), CPVT (n = 3), and asymptomatic (n = 1) teenager who is a first-degree relative of an individual with SCD. The age at first observation was < 18-years-old in 76% of patients. Eight (42%) patients showed a positive family history of LQTS or SCD. Supplementary material Table 1A includes list of *CACNA1C* variants and domain localization in our OPBG Cohort.

Although rare, the following variants (p.Val1707Ile, p.Ala1717Gly, p.Thr1870Met, p.Arg1973Gln) were previously reported with conflicting interpretations of pathogenicity and potential benign effect: ClinVar ID 190622, 93411, 191567 and 93419 respectively<sup>12, 13, 14</sup>. For these reasons we decided to exclude them from statistical analysis in order to avoid bias in terms of correlation to morbidity and mortality. Thus, we excluded 10 patients with any of these variants from our analysis. This reduced our cohort to 9 patients.

#### Literature Review analysis

We identified 235 articles from the literature (Fig. 1). 185 articles were excluded according to the aforementioned criteria (1 article not in English language; 118 reporting basic science studies; 66 duplicated cohorts). Fourteen patients of these remaining 50 articles were further excluded because *CACNA1C* variant was mentioned but without any specific molecular details: no nucleotide nor amino acid references (Table 1B in supplementary materials include data from this group of patients). Thus, 50 articles were included in our literature review (Fig. 1), reporting data of 97 patients.

Some specific issues are relevant to point out. The variant described by Delphine M. Beziau in 2014<sup>15</sup> in a family with combination of *CACNA1C* (p.Asn300Asp) and *SCN5A* (p.Gln1695Ter) with a rather complex phenotype of Brugada Syndrome (5 individuals), cardiac conduction defects (CCD, 3 individuals) and a reported short QT interval (4 individuals, < 350msec, i.e. longer than warranted for the diagnosis SQTs). The other characteristics match with loss of function *SCN5A*. Family analysis did not show complete segregation of the different arrhythmias with specific variants and it was complicated in one individual with rare *ABCC9* variant. The study was inconclusive and reflects great difficulties in the NGS era of interpretation of molecular results with multiple variants with intersecting pathways.

### Molecular characterization

We catalogued 97 *CACNA1C* variants from literature and 9 from OPBG cohort according to one of the 6 groups of the protein localization/domain [UniProtKB - Q13936 (*CAC1C\_HUMAN*)]. These domains are:

1. **N-terminal, cytoplasmic;**

2. **Transmembrane;**
3. **Extracellular;**
4. **Cytoplasmic;**
5. **Intramembrane, pore-forming;**
6. **C-terminal, cytoplasmic.**

In supplementary materials table 2 we annotated aminoacidic division for each proteic domain.

For each domain group, we reported the main findings including gender, family history, median age at diagnosis, QTc, survival, SCD, ICD, tachyarrhythmia, Brugada pattern, HCM, and CHD.

We summarized relevant clinical data related to *CACNA1C* variants in a bar graph included in Fig. 2.

Moreover a detailed analysis including full description of domain to domain analysis is included in supplementary file 1. The file includes relative references for this specific section.

## Outcomes

We performed univariate analysis for each outcome (Arrhythmic event, LQT, BrS, ICD/PMK, HCM, CHD and mortality), with N-terminal domain as reference (table 1).

Extracellular (OR: 13.33 [95%CI 1.06–166.3]) and C-terminal (OR: 6.78 [95%CI 1.516–30.39]) domains were significantly associated with arrhythmic events; variants in cytoplasmic domain was significantly associated with LQTS (OR: 0.057 [95%CI 0.05–0.627]), whereas variants in C-Terminal domain were associated (OR: 0.098 [95%CI 0.22–0.44]) with Brugada pattern at surface ECG. No significant association was observed for HCM, CHD and Death.

We performed Kaplan Meier analysis for survival related to *CACNA1C* localization and depending on ACMG pathogenicity scoring (Fig. 3a and 3b).

In order to explore potential domains at higher risk for mortality at follow-up, we performed another analysis (Fig. 3c) considering patients with variants in cytoplasmic and transmembrane domains versus others.

To explore the relationship between variants ACMG score, localization and HCM, we performed a Cox-regression analysis. Domains were categorized in two main groups: Cytoplasmic and Transmembrane vs other domains. Results

HCM and high score ACMG resulted as a predictor of mortality independently from variants domain localization.

## Discussion

CACNA1C pore-forming, alpha-1C subunit of the voltage-gated calcium channel gives rise to L-type calcium currents mediates influx of calcium ions into the cytoplasm, and thereby triggers calcium release from the sarcoplasm<sup>1,2</sup>. CaV1.2—the predominant L-type calcium channel in the cardio-vascular system and in the brain has an intermediate voltage-dependence of activation<sup>3</sup>. It is composed of four homologous but non-identical domains (repeats I, II, III, IV), each consisting of six membrane-spanning helices (S1-S6). Helices S1 through S4 of each repeat form voltage-sensing domains (VSD); helices S5, S6 and the connecting P-loop of all four repeats together form the channel pore<sup>16</sup>.

*CACNA1C* variants are relatively rare and maybe related to high clinical heterogeneity, not only in arrhythmic conditions but also in structural progressive myocardial disease and CHD. Previous studies are mainly divided into two groups: those discussing basic science studies and animal models (which were not the topic of this study) and those describing heterogeneous cohorts of patients. To the best of our knowledge, this is the first comprehensive study which includes previously described variants and a cohort of 9 patients from our centre. Variants were classified according to their localization, in order to identify genotype-phenotype correlation and potential risk stratification in relation to survival and long-term results in each category.

Different survival rates are identified according to ACMG variants pathogenicity score and variants localization, as explained by Kaplan-Meier curves. In fact, Fig. 3a considers the different variant localisation domains (N-terminal, Extracellular, Transmembrane, Cytoplasmic and C-terminal) with a p-value of 0.028.

While Fig. 3b includes Kaplan Meier analysis for survival expressed in years depending on the degree of variant pathogenicity according to ACMG score (Log rank = 0.000). Pathogenic and likely pathogenic variants are shown in a single plot (ACMG score = 4–5), the second plot represents Variant of Uncertain Significance (VUS) with ACMG score = 3, and the last plot represents Not Applicable (NA) variants for the lack of data (ACMG score < 3). There is significance difference between the three plots with a log rank of 0.000. This means that validity of the ACMG score is supported by the present analysis, with the score of pathogenicity (ACMG score 4–5) that result associated to high mortality.

This study might help in determining the prognosis especially in terms of active management for early therapeutic intervention, close follow-up and potential benefit from early implantation of ICD/PMK.

The last Kaplan Meier curve (Fig. 3c) compared survival between combined Cytoplasmic domains and Transmembrane variants versus other domains (N-Terminal, Extracellular and C-terminal). There was significance between the two curves in terms of mortality with the first showing highest mortality compared to the latter domains (p-value = 0.005).

Univariate analysis considering localisation domain (table 1) showed, using N-terminal domain as reference, that arrhythmic event was significantly related to C-terminal and Extracellular domain (p = 0–012 and p = 0.044, respectively), LQTS was correlated to Cytoplasmic domain (p = 0.019). Cytoplasmic domain is important for Calcium ion selectivity and permeability and is performed by four internal repeats

that contains five hydrophobic transmembrane segments (S1, S2, S3, S5, and S6) and one positively charged transmembrane segment (S4). S4 segments represent the voltage-sensor and are characterized by a series of positively charged amino acids at every third position. LQT electrophysiological phenotype is characterized by loss of current density and gain-of-function shift in activation leading to increased steady-state current<sup>4</sup>.

Brugada syndrome is significantly related to C-terminal domain ( $p = 0-002$ ). Calcium binding region is localized in C-terminal domain. Variants might cause loss of a low-affinity interaction with CALM1 or loss of channel inactivation by Ca (2+) and calmodulin<sup>17</sup>.

Multivariate analysis has shown that HCM and ACMG variants score pathogenicity (4–5) are independent predictors of mortality. The p-value is 0.031 for HCM and 0.04 for variants pathogenicity (supplementary Table 3). There is no significance considering variants localisation domain. This suggests that the differences among different regions in Figs. 3 and 5 is possibly due to a higher incidence of HCM in *CACNA1C* variants located in the transmembrane and cytosolic domains, as showed in Fig. 2. In order to exclude other known genetic factors, we analysed with exome sequencing our patient with the classic *CACNA1C* variant without identifying any pathogenic variants of genes related to HCM. This data strengthens the potential role of this gene on structural myocardial changes. A similar finding has been reported previously in literature<sup>8</sup> where authors have demonstrated that exome sequencing identified only one variant that was related to a complex phenotype in a family where different members showed LQTS, HCM and CHDs.

In order to give a comprehensive vision of genotype phenotype correlation, we summarized in Fig. 4 the topographic representation of different variants domains and associated major clinical features and outcome.

Another aspect that need to be considered is the mosaicism. Mosaicism refers to the co-existence, in an individual, of cells with different genotypes, although derived from a single zygote, so that some cells may present with the gene mutation (and the resulting loss/gain of function), and others do not<sup>18</sup>. The incidence of mosaicism in the human is underestimated, especially in the low-grade mosaicism. While somatic mosaicism has been implicated in over 30, monogenic disorders, mosaicism is rarely reported in LQTS<sup>19</sup>. In this review we identified five reports<sup>3, 20, 21, 22, 23</sup> with parental mosaicism. It makes a relatively high percentage for such a small cohort, reaching 4–5% of the total analysed series. These observations have important consequences for genetic counselling, as previously identified de novo mutations may represent parental mosaicism. A shared partial phenotype should not be dismissed as a benign or insignificant finding but should be evaluated further to rule out the possibility of parental mosaicism concealing a potentially fatal heritable disease.

Another previously and relatively widely reported aspect related to *CACNA1C* variants is the association with multisystemic disorder, well recognized as Timothy syndrome. It is characterized by cardiac involvement (LQTS, HCM, CHD), hand/foot variable syndactyly, facial dysmorphic features (depressed

nasal bridge, low-set ears, thin vermilion border of the upper lip, and round face), and neurodevelopmental features including global developmental delays and autism spectrum disorders. Another less investigated aspect is immunodeficiency and recurrent infections<sup>24-27</sup>.

## Conclusions

In conclusion, our study did not identify any sex predominance in any domain. The domain most frequent associated with LQTS is the cytoplasmic domain (table 1). The domain potentially linked to Brugada syndrome is N-terminal. Mortality is statistically significant considering ACMG score  $p = 0.000$  (Fig. 3a) and localisation domain  $p = 0.028$  (Fig. 3b), even more significant when we considered Cytoplasmic and Transmembrane vs other groups  $p = 0.005$  (Fig. 3c). HCM and pathogenicity are considered independent risk factor for mortality from localisation of domains ( $p = 0.031$  and  $0.04$ ). To the best of our knowledge this is the first comprehensive study trying to create phenotype genotype correlation and better risk stratification for patients carrying variants with *CACNA1C*, even if we have to consider the limitations of the study.

## Study Limitation

Due to the rarity of the condition, the main limitation of the study is the number of patients. Such a small number may prevent to find any conclusive findings regarding localization domain as an independent predictor of death. Another limitation regards the attempt of using published data to draw meaningful conclusions. There were some difficulties in retrieving data for patients from literature review and some genetic tests (fourteen patients) were unfortunately unavailable. We used in our review analysis information that we have found. Moreover, for younger ones, instead, there is a brief follow-up, which may lead to underestimation of long-term morbidity and mortality. The retrospective study is also a limitation, but this is necessary since this is a rare cardiac condition.

## Abbreviations

ACMG

American College Medical Genetics;

AVB

Atrioventricular block;

CI

confidence intervals;

CPVT

Catecholaminergic Polymorphic Ventricular Tachycardia;

ECG

Electrocardiograms;

HCM

hypertrophic cardiomyopathy;

IQR  
Interquartile range;  
IGV  
Integrative Genome Viewer;  
LQTS  
Long-QT syndrome;  
NA  
Not Applicable;  
NGS  
Next generation sequencing;  
OR  
odds ratios;  
QTc  
QT corrected interval;  
SQTS  
Short QT syndrome;  
SCD  
sudden cardiac death;  
VUS  
Variant of Uncertain Significance.

## **Declarations**

### **Ethics approval**

The study protocol conforms to ethical guidelines of the 1975 Declaration of Helsinki, as reflected in a priori approval by the Institution's Human Research Committee.

### **Consent for publication**

Not applicable

### **Availability of data and materials**

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### **Competing interests**

The authors declare that they have no competing interests

### **Funding**

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## Authors' contributions

AB critically reviewed and interpreted patient data, literature review and write the manuscript. BC collected data and participate in writing manuscript. MG and NC analysed and interpreted patient data and contributed in review the manuscript. FC contributed in collecting patients' data. BC, MG, FC independently performed the literature review. DR, CDM an MSS contributed in interpreting arrhythmic data and reviewed the manuscript. BD, AN, ASA, FB reviewed the manuscript and gave the final approval. All authors read and approved the final manuscript.

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There are no conflicts of interest

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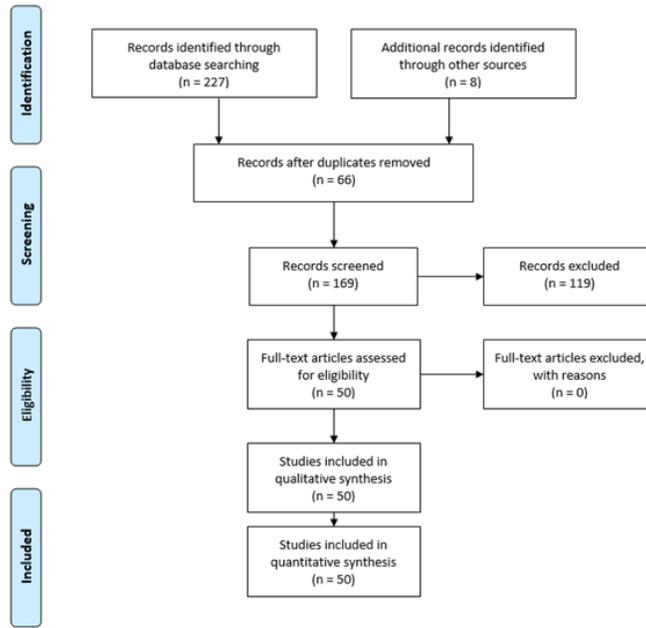
## Table

**Table 1. Results of univariate logistic regression for outcomes among the different variants for each domain.**

Variables	OR	95% CI		P-value
		Lower	Upper	
<b>Arrhythmic event</b>				
N-terminal (ref)	-			.097
Transmembrane	2.667	.123	57.620	.532
Extracellular	13.333	1.069	166.374	.044
Cytoplasmic	10.667	.823	138.222	.070
C-terminal	6.788	1.516	30.392	.012
<b>LQTS</b>				
N-terminal (ref)	-			.001
Transmembrane	.100	.006	1.712	.112
Extracellular	.700	.037	13.179	.812
Cytoplasmic	.057	.005	.627	.019
C-terminal	1.733	.163	18.398	.648
<b>Brugada</b>				
N-terminal (ref)	-			.003
Transmembrane	1.286	.158	10.450	.814
Extracellular	.286	.041	1.981	.205
Cytoplasmic	1.500	.290	7.753	.629
C-terminal	.098	.022	.440	.002
<b>ICD/PMK</b>				
N-terminal (ref)	-			.167
Transmembrane	.000	.000	.	.999
Extracellular	1.000	.091	11.028	1.000
Cytoplasmic	.000	.000	.	1.000
C-terminal	6.000	.963	37.381	.055

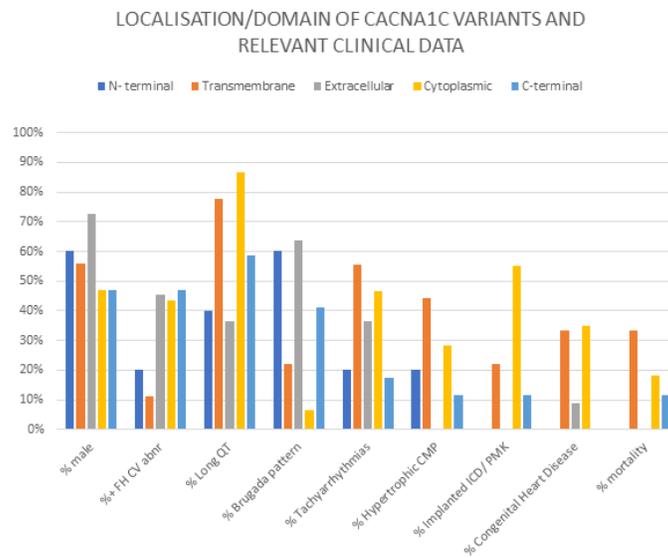
<b>Table 1. Results of univariate logistic regression for outcomes among the different variants for each domain.</b>				
<b>HCM</b>				
N-terminal (ref)	-			.925
Transmembrane	1.000	.053	18.915	1.000
Extracellular	2.667	.277	25.636	.396
Cytoplasmic	.000	.000	.	.999
C-terminal	1.478	.242	9.028	.672
<b>CHD</b>				
N-terminal (ref)	-			.917
Transmembrane	807.737	.000	.	.999
Extracellular	969.284	.000	.	.999
Cytoplasmic	538.491	.000	.	.999
C-terminal	1.413.540	.000	.	.999
<b>Death</b>				
N-terminal (ref)	-			.721
Transmembrane	.000	.000	.	.999
Extracellular	4.200	.535	32.955	.172
Cytoplasmic	.000	.000	.	.999
C-terminal	1.571	.311	7.937	.584

## Figures



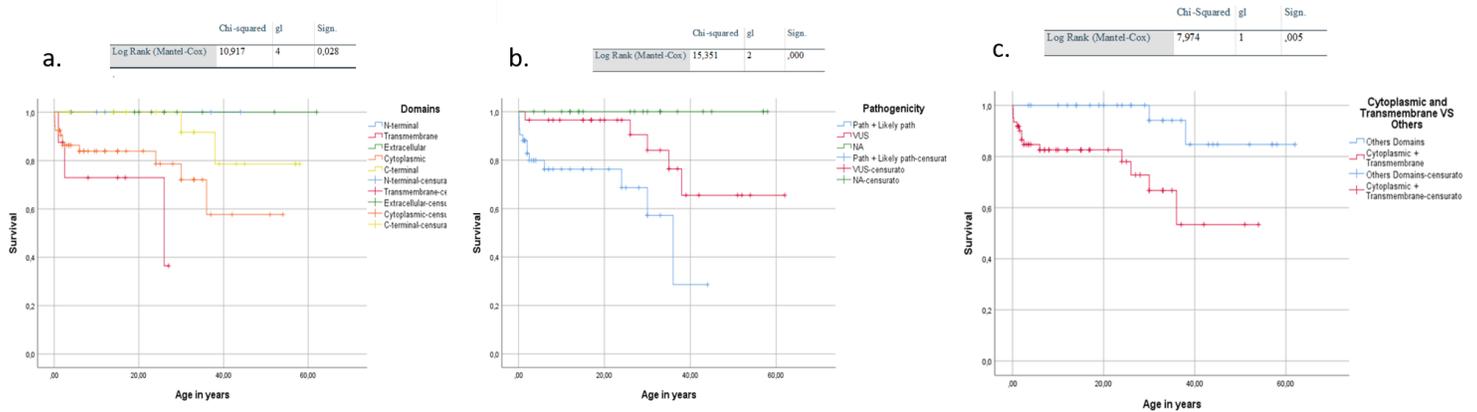
**Figure 1**

Flow chart explaining the methodology undertaken for systematic review of literature.



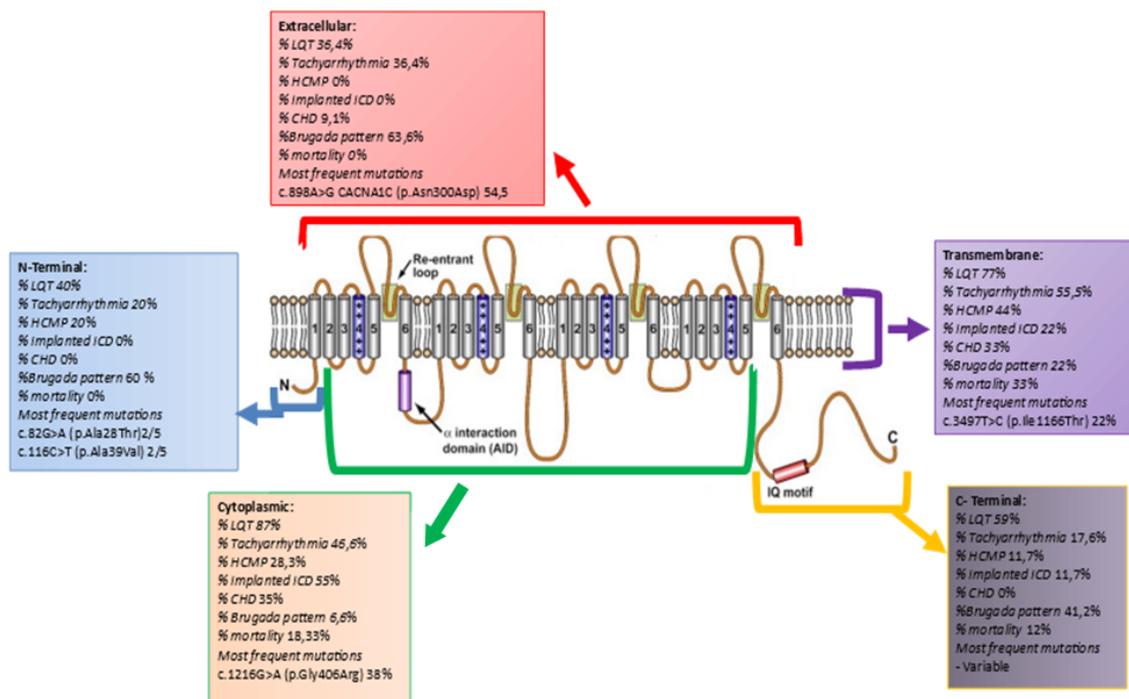
**Figure 2**

Bar graph of Localisation Domain of CACNA1C variants and relevant clinical data (domains-to-domains analysis).



**Figure 3**

3a: Legend: Kaplan Meier analysis shows the survival expressed in years depending on the type of variants (Log rank=0.028). 3b. Kaplan Meier analysis shows the survival expressed in years depending on the pathogenicity of variants according to ACMG score. Log rank 0.000. Legend: VUS = Variants Uncertain Significance; NA = not applicable. 3c. Kaplan Meier analysis shows survival expressed in years depending variants localisation domains. The two plots represent Cytoplasmic and Transmembrane domains and Other domains (Log rank 0.005)



## Figure 4

Topographic representation of variants from different domains and associated major clinical features and outcome.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [BabanSupplementaryTable1A.docx](#)
- [BabanSupplementaryTable2.docx](#)
- [BabanSupplementaryTable1B.docx](#)
- [BabanSupplementaryFile1.docx](#)
- [BabanSupplementaryTable3.docx](#)