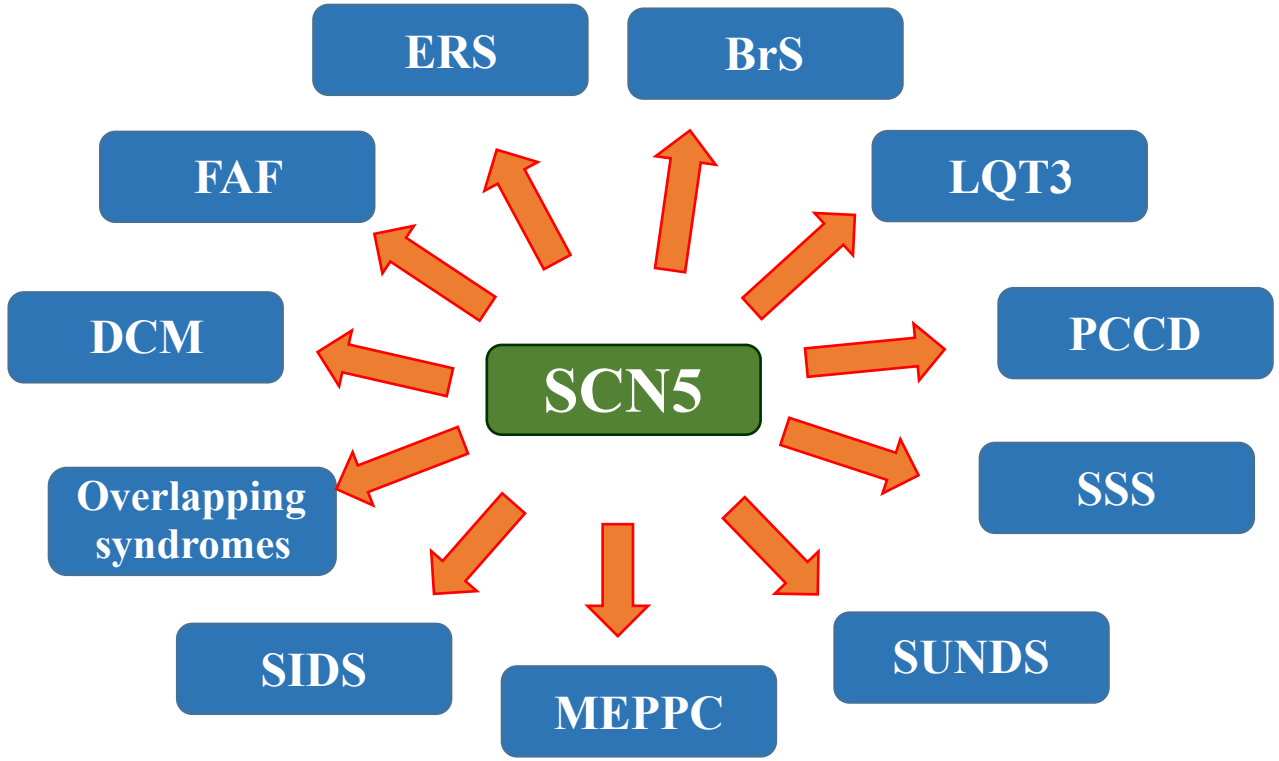
Brugada syndrome and its allelic disorders: the “Na^+ channel syndrome"

Genes associated with Brugada syndrome and allelic disorders with their mutations (Brugada R 201; Bezzina 20013)

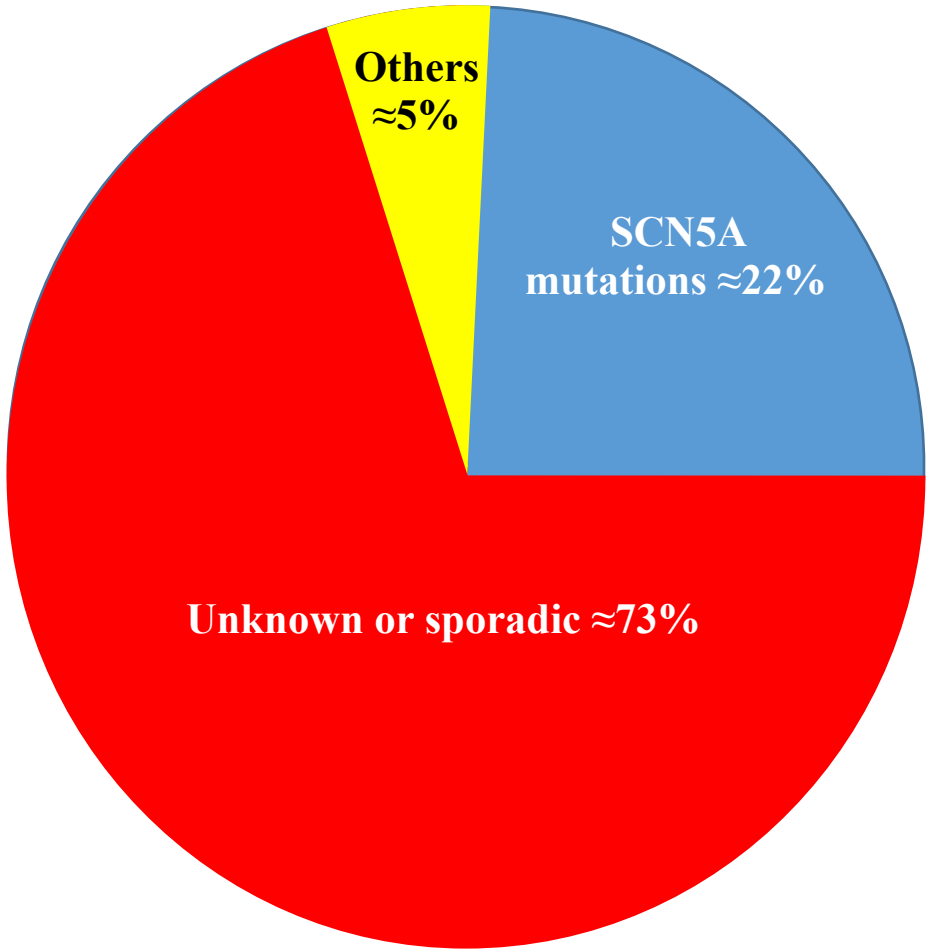
Although BrS is inherited as an autosomal dominant trait, more than half of BrS may be sporadic. Approximately 20% to 30% of BrS is caused by loss-of-function mutations in the *SCN5A*-encoded cardiac sodium channel, classified as Brugada syndrome type 1 (BrS1). Currently, more than 180 mutations in at lest 21 genes have been associated with BrS : ***SCN5A,*** *SCN1B, SCN2B, SCN3B, SCN10A, CACNA1C*, *CACNB2*, *CACNA2D1, GPD1L, KCND3, KCNE3, KCNE1L, (KCNE5), KCNJ8, HCN4, ABCC9, RANGRF, PKP2*, *FGF12, SLMAP, TRPM4 (***Stallmeyer 2012: Liu 2013***) and HEY2* (**Bezzina 2013**). ≈ 25%-30% of BrS is accounted for by pathogenic variants in this 18 genes mentioned. BrS is a rare cardiac rhythm disorder associated with SCD. Mutations in the Na^+ channel gene SCN5A are found in ~20% (**Kapplinger 2010**) of cases while mutations in other genes collectively account for <5%. Although BrS is considered a mendelian disorder with autosomal dominant transmission, studies in families harboring SCN5A mutations have demonstrated low disease penetrance (**Priori 2000; Probst 2009**) and, in some instances, absence of the familial SCN5A mutation in some affected family members. Also, many cases are sporadic (**Schulze-Bahr 2003; Hermida 2010**), and familial linkage analyses have largely been unsuccessful in uncovering new disease-causing genes. These observations suggest a more complex inheritance model. Identifying new genetic risk factors could assist in further diagnosis, provide new insights into underlying molecular mechanisms and yield new information relevant to the broader problem of SCD.

Multiple cardiac phenotypes that are associated with SCN5A mutations (Veltmann 2016)



Scheme showing the multiple cardiac phenotypes that are associated with *SCN5A* mutations (allelic disease). BrS, Brugada syndrome; DCM, dilated cardiomyopathy; ERS, early repolarization syndrome; FAF, familial atrial fibrillation; LQTS, long QT syndrome (LQT3); MEPPC, multifocal ectopic Purkinje-related premature contractions; PCCD, progressive cardiac conduction defects; SIDS, sudden infant death syndrome; SSS, sick sinus syndrome; sudden unexplained nocturnal death syndrome (SUNDS); Overlapping syndromes (Phenotypic overlap of LQT3 BrS), PCCD, and SND). It is appropriate to consider the "Na^+ channel syndrome" (mutations in sodium channel α-subunit gene SCN5A) as a unique clinical entity that may manifest itself with a broad spectrum of possible phenotypes**.** Mutations in the sodium channel gene SCN5A are found in ≈20% of cases while mutations in other genes collectively account for <5%. t ion channel dysfunction, in particular in the cardiac sodium channel, may not be a prerequisite for BrS (**Veerman 20016**). The cardiac Na^+ channel (Nav1.5) conducts a depolarizing inward Na^+ current that is responsible for the the upstroke Phase 0 of the AP in fast fibers. Changes in Na^+ currents can affect conduction velocity and impulse propagation. The cardiac Nav1.5 is also involved in determination of the AP duration, since some channels may reopen during the plateau phase, generating a persistent or late inward current in phase 2.

Genes Implicated in Brugada Syndrome



Mutations in multiple genes have been identified in BrS SCN%A mutations are reported in 15-30% of patients with BrS. Others include SCN1B, SCN2B, SCN3B, SCN10A, CACNA1C, CACNB2, CACNA2D1, GPD1L, KCND3, KCNE3, KCNE1L, (KCNE5), KCNJ8, HCN4, ABCC9, RANGRF, PKP2, FGF12, SLMAP, and TRPM4 . The remaining are unknown or sporadic.

Allele: One of alternative forms at a genetic locus on a single chromosome. For loci in most of the genome, a human has two chromosomes, which may carry the same or two different alleles. Genetically induced ventricular arrhythmias can be divided in two subgroups: the primary electrical disorders or channelopathies, and the secondary arrhythmogenic cardiomyopathies (**Boussy 2008**).

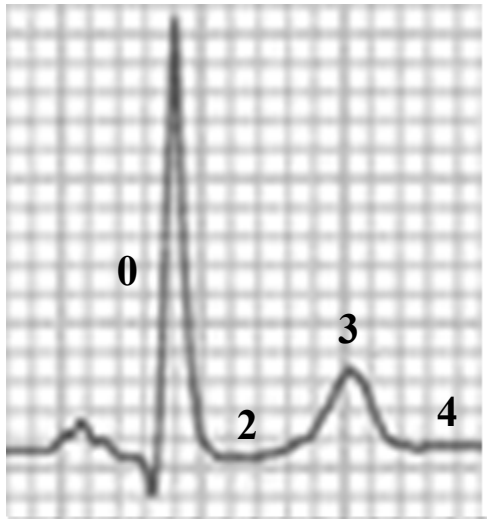
It is now appropriate to consider the "Na^+ channel syndrome" (mutations in sodium channel alpha-subunit gene (SCN5A) as a unique clinical entity that may manifest itself with a broad spectrum of possible phenotypes (**Napolitano 2003**).

Allelic entities associated with SCN5A mutations

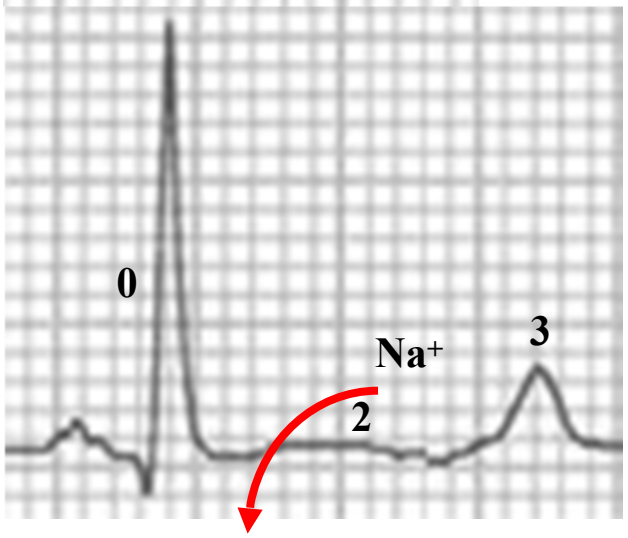
a) *LQT3 variant of long QT syndrome* Type 3 long-QT syndrome (LQT-3) is caused by gain-of-function mutations in the SCN5A encoding the cardiac sodium channel. In addition to the characteristic QT prolongation, LQT3 carriers regularly present with bradycardia and sinus pauses. Na+ channel mutations displaying a persistent inward current or a negative shift in inactivation may account for the bradycardia seen in LQT3 patients, whereas SA node pauses or arrest may result from failure of SA node cells to repolarize under conditions of extra net inward current *(***Veldkamp 2003**).

Characteristics of LQT3 variant, SCN5A mutation

Long QT interval by ST segment prolongation.
Delayed appearance of T wave, significant dependence on heart rate of QT interval, affected gene: SCN5A, p21-24 mutation in chromosome 3, TAP phase: plateau, dome or phase 2 by persistent sodium inflow.



Normal



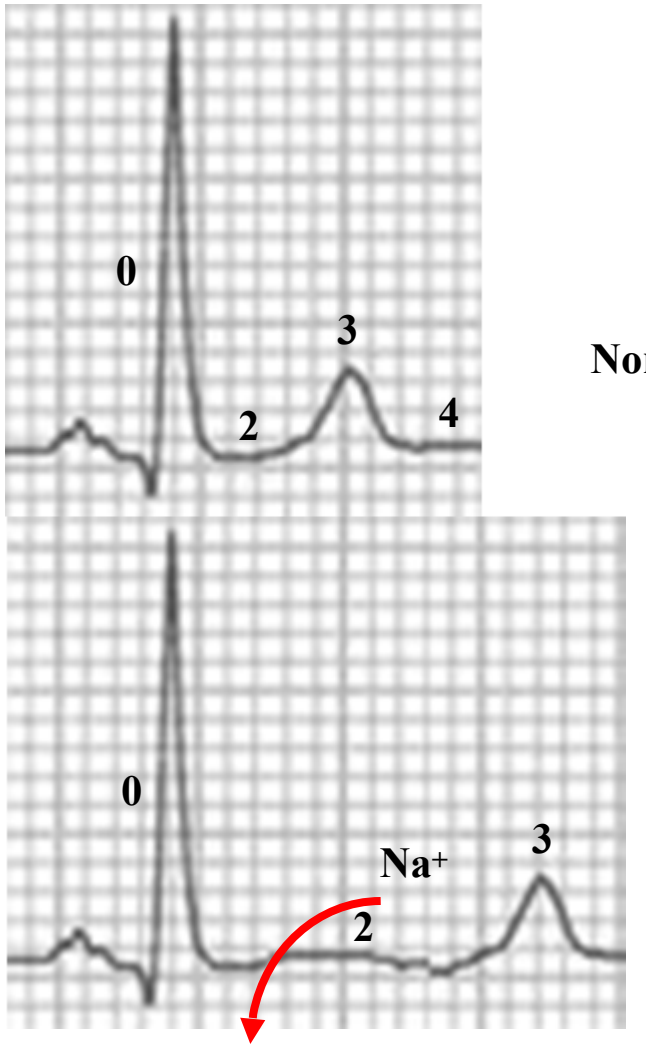
≈ 1% of total

Delayed appearance of T wave

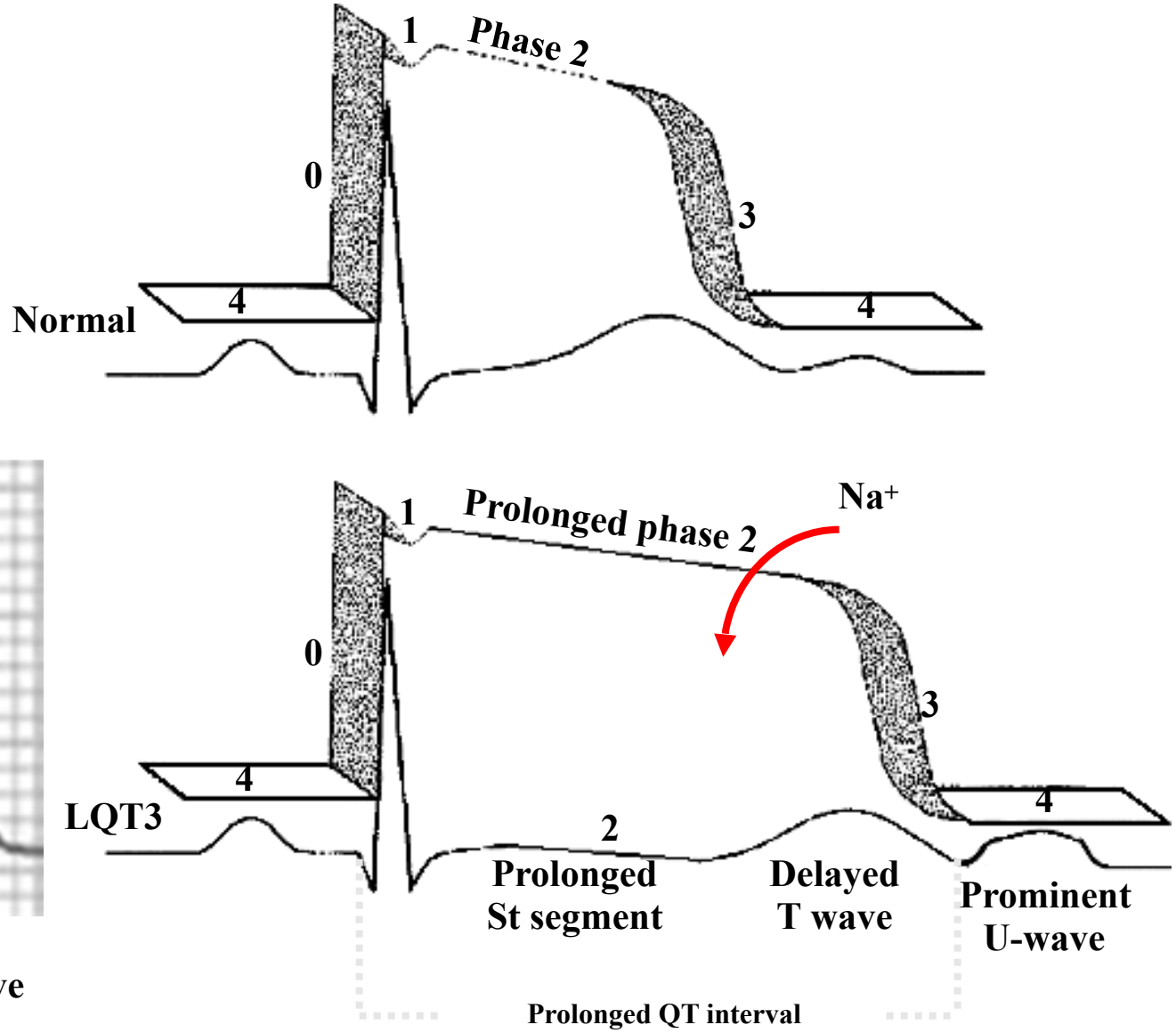
≈ 80% of events during sleeping or at rest

Male sex has higher risk This is the mirror image of Brugada syndrome

Normal ECG and action potential versus LQT3 ECG and action potential

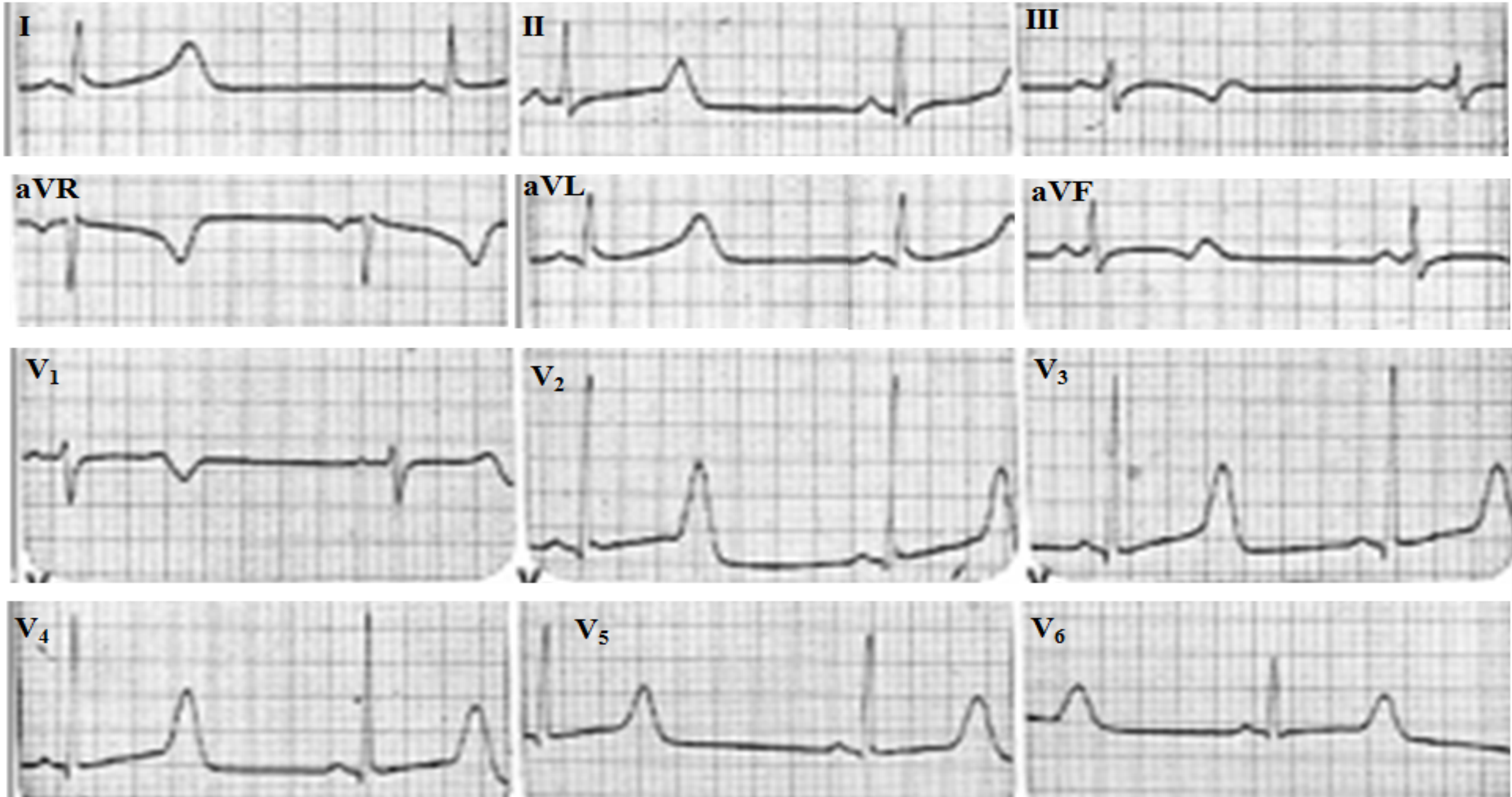


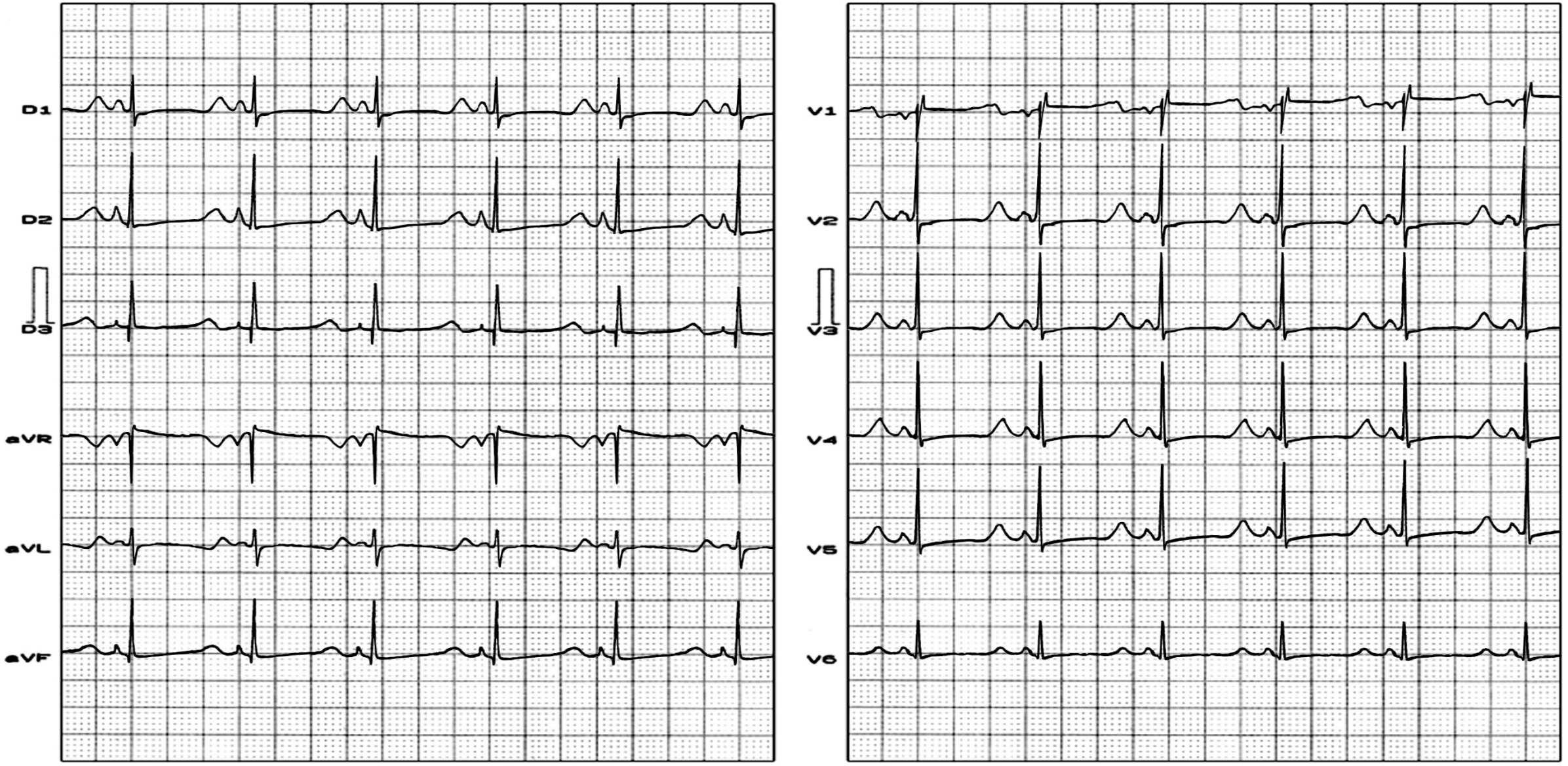
Delayed appearance of T wave



Characteristics of LQT3 variant, SCN5A mutation

LQT3 ECG





This ECG belong a new born with LQT3 variant. Clear ST segment prolongation and delayed appearance of T wave. affected gene: SCN5A, p21-24 mutation in chromosome 3, AP phase: plateau, dome or phase 2 by persistent sodium inflow.

b) *Brugada Syndrome type 1*

c) ***Isolated Progressive Cardiac Conduction (Defect PCCD) (Lenègre disease)*** it is characterized by progressive alteration of cardiac conduction through the atrioventricular node, His-Purkinje system, with right or left bundle branch block, fascicular blocks and QRS widening. In some instances, the disorder may progress to third degree AV block or complete atrioventricular block, with syncope and even death. While the role of genetic factors in conduction disease has been suggested as early as the 1970s, it was only recently that specific genetic loci have been reported. Multiple mutations in the gene encoding for the cardiac voltage-gated sodium channel (SCN5A), which plays a fundamental role in the initiation, propagation, and maintenance of normal cardiac rhythm **(Viswanathan 2006)**.

d) *Idiopathic ventricular fibrillation (IVF) or Genuine idiopathic ventricular fibrillation* J-point elevation has been considered an innocent finding among healthy young individuals (the "early repolarization" pattern). However, this ECG finding is increasingly being associated with idiopathic ventricular fibrillation (IVF). J-point elevation is more common among patients with IVF than among matched control subjects. This is true for J-point elevation in the inferior leads and for J-point elevation in leads I and VL. J-point elevation from V4 to V6 occurs with equal frequency among patients and matched control subjects. Male subjects had J-point elevation more often than female subjects and young athletes had J-point elevation more often than healthy adults but less often than patients with IVF. The presence of ST-segment elevation or

e. QRS slurring did not add diagnostic value to the presence of J-point elevation. J-point elevation is found more frequently among patients with IVF than among healthy control subjects. The frequency of J-point elevation among young athletes is intermediate (higher than among healthy adults but lower than among patients with IVF) (**Rosso 2008**).

f. *Sudden infant death syndrome (SIDS) or sudden unexpected infant death* (**Van Norstrand 2007**) is characterized by the sudden death of an infant that occurs during sleep and remains unexplained despite thorough examination. In addition to clinical associations such as prone sleeping and exposure to cigarette smoke, several genetic factors have been identified with regard to SIDS, including autonomic disorders, immunologic polymorphisms and metabolic disorders. In the past decade, postmortem genetic analysis ('molecular autopsy') of SIDS cases has revealed a number of cardiac ion channel mutations that are associated with arrhythmia syndromes, including the long QT syndrome, Brugada syndrome and short QT syndrome. Mutations have been found in genes encoding (subunits of) cardiac potassium, sodium and calcium channels, as well as in genes involved in the trafficking or regulation of these channels.At least one out of five SIDS victims carries a mutation in a cardiac ion channel-related gene and that the majority of these mutations are of a known malignant phenotype. Genetic analysis is therefore recommended in cases of SIDS. More research is required to further elucidate the pathophysiology of SIDS and to determine whether genetic or electrocardiographic screening of apparently healthy infants should be pursued (**Klaver 2011**).

g. *Sudden Unexpected Nocturnal Death Syndrome (SUNDS) Atrial standstill*.

h. *Cardiac sodium channel (SCN5A) variants associated with atrial fibrillation* (**Darbar 2008**).

i. *Arrhythmogenic right ventricular cardiomyopathy and SCN5A* mutation (**Erkapic 2008**).

Mixed forms, **Cardiac sodium channel *"overlap syndromes of cardiac sodium channelopathy."* different faces of SCN5A mutations *(Remme 2008)***

a) ***Phenotypic overlap of*** long QT syndrome (LQT3) ***with Brugada syndrome*** (BrS). The cardiac sodium channel gene, SCN5A, is involved in both the BrS and the LQT3. BrS mutations cause a reduced sodium current, while LQT3 mutations are associated with a gain of function. The effects of class I antiarrhythmic drugs have been used to differentiate these diseases. Intravenous flecainide is used as a highly specific test to unmask the ECG phenotype 1 of the Brugada syndrome. The same drugs act as a gene-specific therapy in this disorder by contrasting the effect of mutations in LQT3 has been explored. phenotypic overlap may exist between the Brugada syndrome and LQT3. Has been reported a large family with a SCN5A mutation and a "mixed" ECG pattern (prolonged QT interval and ST-segment elevation). Flecainide challenge may elicit ST-segment elevation in some LQT3 patients. The presence of "intermediate" phenotypes highlights a remarkable heterogeneity suggesting that clinical features may depend upon the single mutation. Only deepened understanding of the genotype-phenotype correlation

a. will allow the definition of the individual patient's risk and the development of guidelines for clinical management.**(Cerrone 2001**). Flecainide may induce ST segment elevation in LQT3 patients, raising concerns about the safety of flecainide therapy and demonstrating the existence of an intriguing overlap between LQT3 and BrS (**Priori 2000**).

b. To investigate the basis for this overlap, Makita et al (**Makita 2008**) genotyped a cohort of 44 LQT3 families of multiple ethnicities from 7 referral centers and found a high prevalence of the E1784K mutation in SCN5A. Of 41 E1784K carriers, 93% had LQT3, 22% had BrS, and 39% had sinus node dysfunction. Heterologously expressed E1784K channels showed a 15.0-mV negative shift in the voltage dependence of Na channel inactivation and a 7.5-fold increase in flecainide affinity for resting-state channels, properties also seen with other LQT3 mutations associated with a mixed clinical phenotype. Furthermore, these properties were absent in Na channels harboring the T1304M mutation, which is associated with LQT3 without a mixed clinical phenotype. A negative shift of steady-state Na channel inactivation and enhanced tonic block by class IC drugs represent common biophysical mechanisms underlying the phenotypic overlap of LQT3 and BrS and further indicate that class IC drugs should be avoided in patients with Na channels displaying these behaviors.

c. BrS and progressive dromotropic disorders of The His Purkinje system: "Lenègre disease" or BrS with marked conduction disease (**Vorobiof 2008**). The most common phenotype of gene carriers of a BrS-type SCN5A mutation is progressive cardiac conduction defects similar to the Lenègre disease phenotype. In consequence, carriers of a SCN5A mutation need a clinical and ECG follow-up because of the risk associated with severe conduction defects (**Probst 2006; Shimizu 2006**). The occurrence of BrS and PCCD in the same family could be due to a single SCN5A novel mutation, the P1438L SCN5A mutation (**Six 2008**). Vorobiof et al (**Vorobiof 2008**) studied a case of 51-year-old woman with an overlap syndrome: BrS and distal atrioventricular conduction disease secondary to a novel SCN5A mutation. At electrophysiological testing she was found to have marked infrahisian conduction disease and had easily inducible polymorphic VT. She underwent implantation of a dual-chamber implantable ICD and family screening was recommended. Genetic analysis revealed a novel nonsense mutation in the gene encoding for the sodium channel (SCN5A). Five months after ICD implantation the patient had an episode of VF documented on ICD interrogation.

d. Brugada syndrome with sick sinus syndrome (**Nakazato 2004**).

e. Benito et al (**Benito 2008**) presented the first report showing an association of familial atrial fibrillation and LQT-3 due to a mutation in SCN5A. Additionally, fecainide is usefull in this particular complex phenotype, both as a diagnostic tool for LQT-3 and as an acute treatment for AF. Sick sinus syndrome, intraventricular conduction disease, and monomorphic sustained ventricular tachycardia.

f. Sinus dysfunction and BrS: computational analysis of a novel sodium channel mutation, E161K, suggests that a loss of sodium channel function is not only associated with Brugada syndrome and conduction disease, but may also cause sinus node dysfunction in carriers of this mutation (**Smits 2005**).

g. Coexistent manifestations of the Andersen-Tawil and BrSs (**Shandling 2008**).

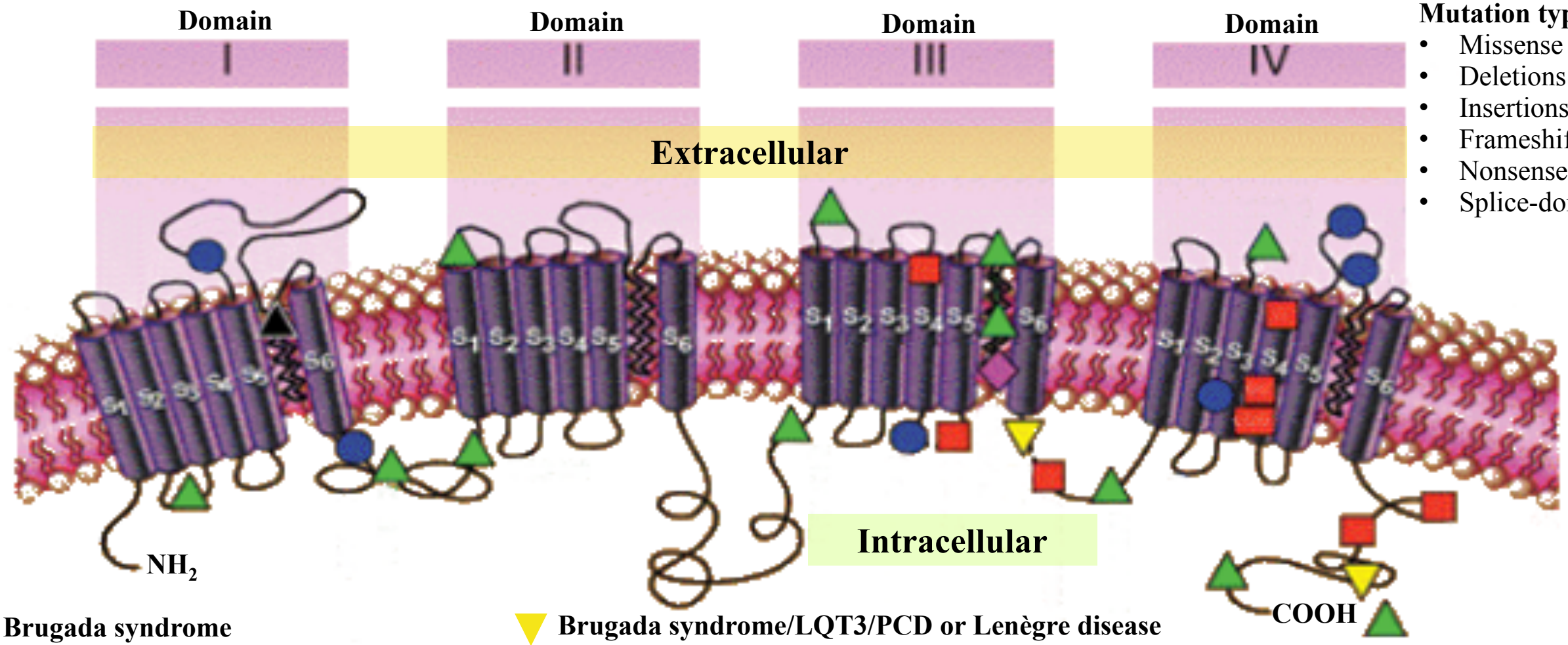
h. The delQKP-1507-1509 cardiac sodium channel mutation has in association the following features: of LQT3, cardiac conduction defects, including second-degree AV block, intermittent third-degree AV block, left anterior fascicular block (LAFB), incomplete right bundle-branch

i. block (IRBBB), and dilated cardiomyopathy (DCM). The cardiac sodium channel mutation delQKP 1507-1509 is associated with the expanding phenotypic spectrum of LQT3, conduction disorder, dilated cardiomyopathy, and high incidence of youth sudden death.

j. SCN5A Mutation autosomal dominant Associated with dilated cardiomyopathy (right and occasionally left ventricular dilatation and dysfunction) cardiac conduction disorder, sinus node dysfunction, and arrhythmia (**McNair 2004**). This is a heterozygous G-to-A mutation at position 3823 that changed an aspartic acid to asparagine (D1275N) in a highly conserved residue of exon 21. This mutation was present in all affected family members, was absent in 300 control chromosomes, and predicted a change of charge within the S3 segment of domain III.

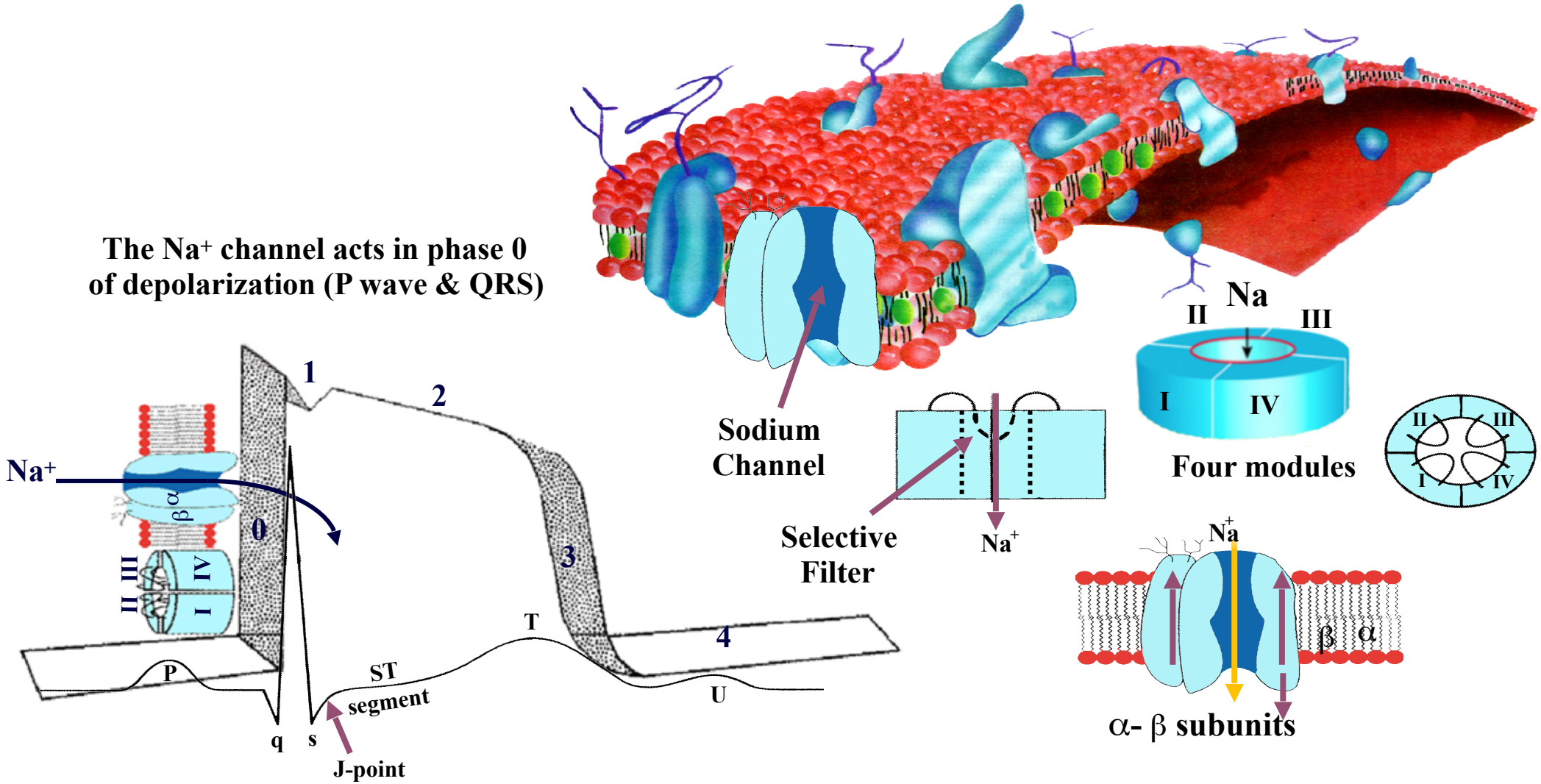
k) Congenital sick sinus disease, a Brugada-like electrocardiogram and recurrent aborted sudden death. This region of the SCN5A gene is not commonly involved in the pathogenesis of the BrS and associated disorders (**London 2007**). They constitute a spectrum of disease entities termed Na+ channelopathies. These diseases are allelic disorders, if not the same disease with variable penetrance and variable modifiers worldwide. Interestingly, death occurs during sleep in all of these disorders, suggesting a common mechanism. To date, mutational analyses have revealed about 103 distinct mutations in SCN5A, of which at least more than 30 mutations are associated with LQT3, whereas the rest of the mutations are affiliated with the remaining sodium channel disorders. The majority of these mutations are missense. However, other types such as deletions, insertions, frameshifts, nonsense and splice-donor errors have also been reported.

Sodium channel mutation in SCN5A gene and its phenotypes

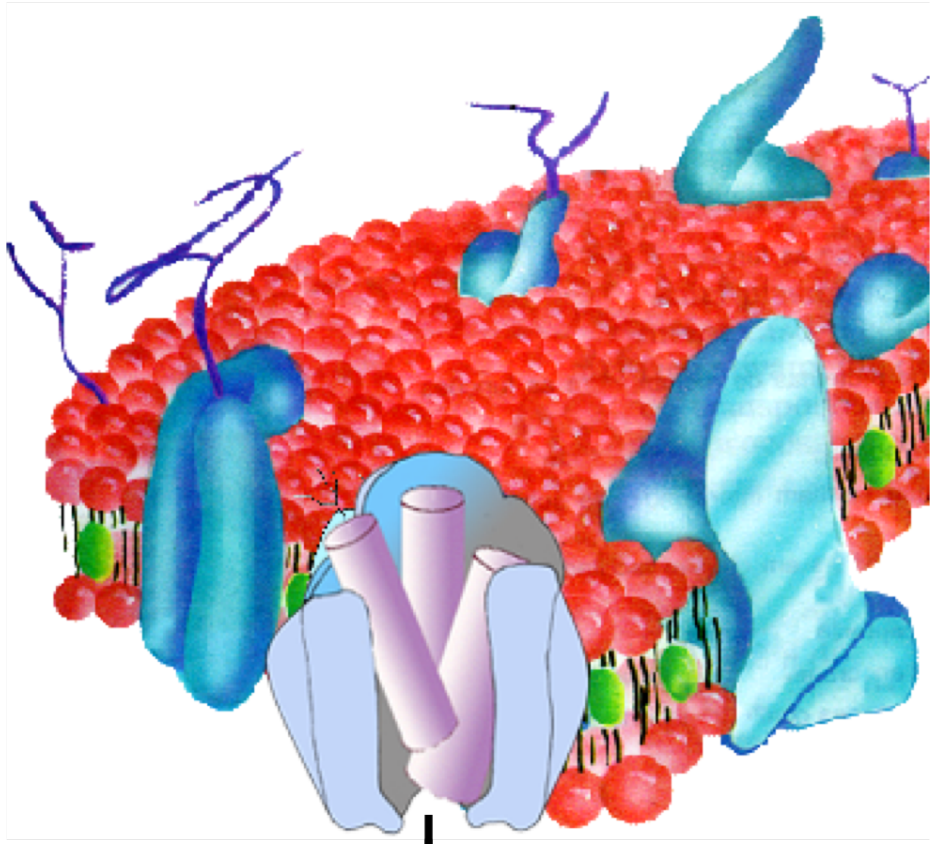


The predicted secondary structure of the cardiac sodium channel and locations of mutations causing the BrS, LQT3, Lenègre disease, overpaying syndromes and atrial standing . The channel consists of four putative transmembrane domains (I–IV), with each domain containing six transmembrane segments (S1–S6 BrS mutations green triangles, LQT3 red square, Lenègre disease blue circles, overlapying BrS and Lenègre disease trapezoid pink and black triangle atrial standing.

Structure of the sodium channel



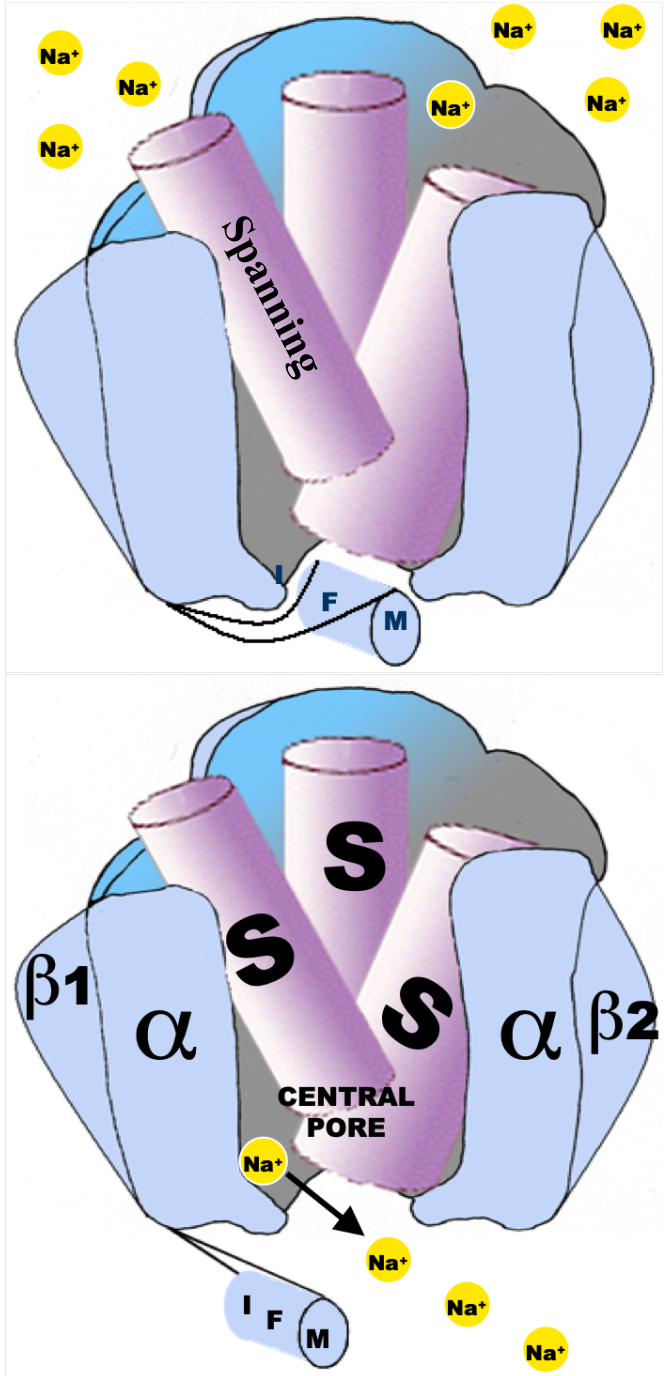
Characteristics of the Na^+ channel. This is a protein structure, formed by four modules that surround a central pore. This channel has a main structure, called α and other surrounding accessory ones called β1 and β-2. This channel is very important in stimulus conduction and cell activation. Inherited mutations in SCN5A, the gene encoding the cardiac Na^+ channel, provoke life-threatening cardiac arrhythmias, often by modifying these voltage-dependent conformational changes. $Na_v1.5$ consists of four domains (DI–DIV), each containing six transmembrane segments (S1–S6); S4 segments are positively charged and act as voltage sensors.



STATES OF THE SODIUM CHANNEL

CLOSED

OPEN

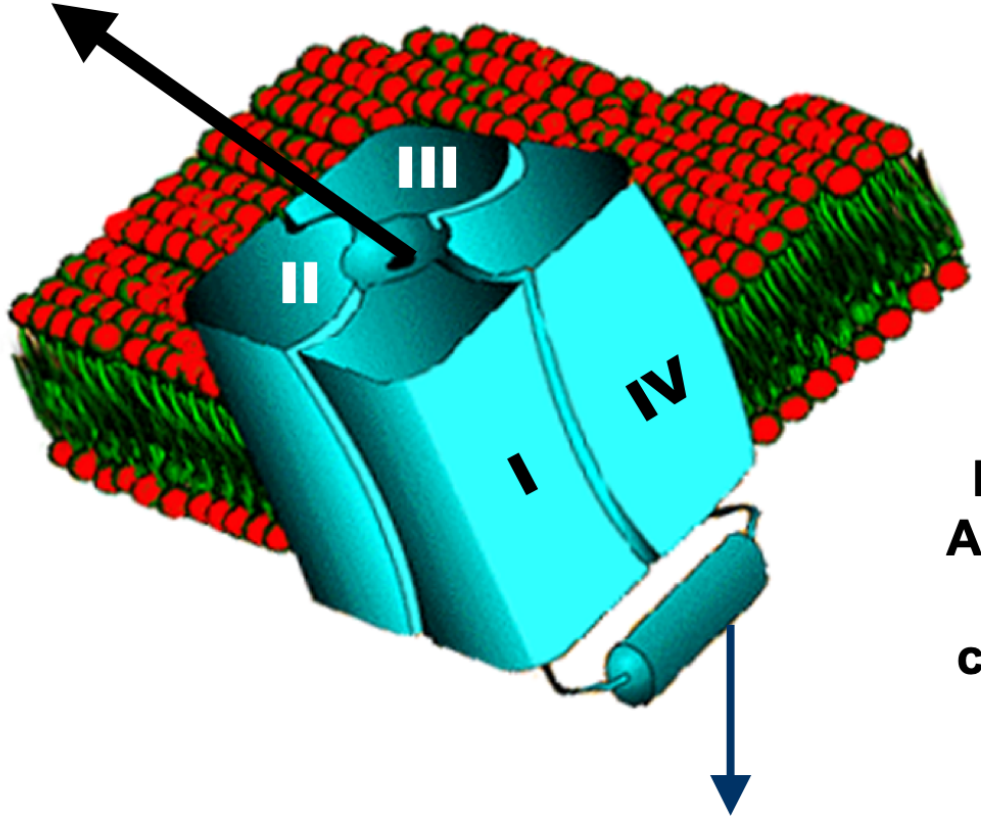


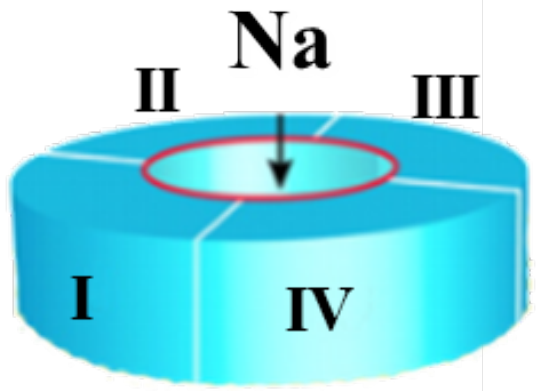
Membrane Spanning subunits.

The critical residue (Phe1489F) is shown as occluding the intracellular mouth of the pore.

The architecture of the Na^+ channel is modular around a central pore.

Extracellular mouth of the Na^+ channel pore

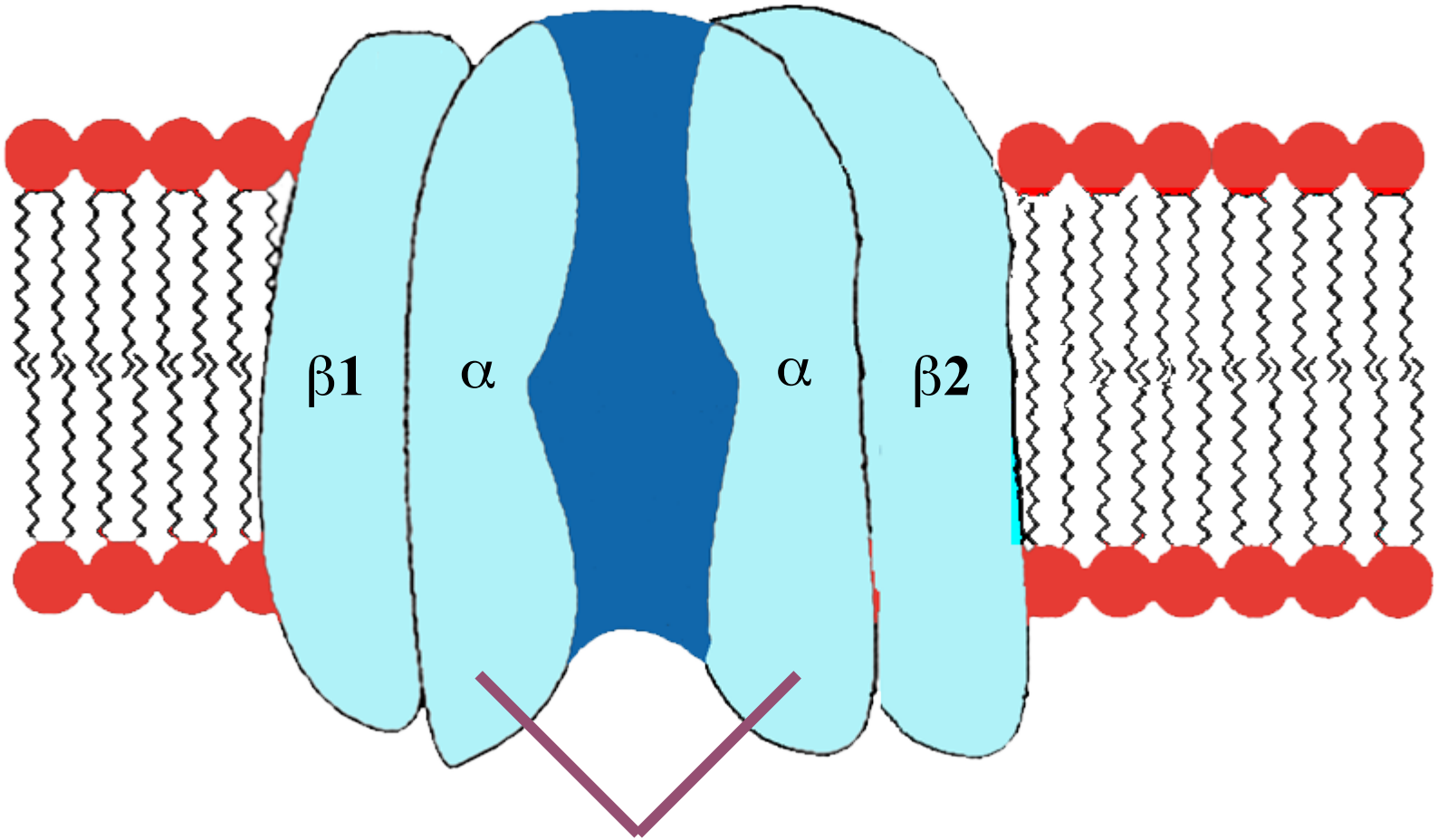




Domains I, II, III and IV At the center of the four Domains(pore) is the channel through which the Na+ ions move.

The intracellular loop connecting domains III and IV of the Na+ channel is depicted as forming A hinged lid. The critical residue (Phe1489F) is shown as occluding the intracellular mouth of the pore (1)

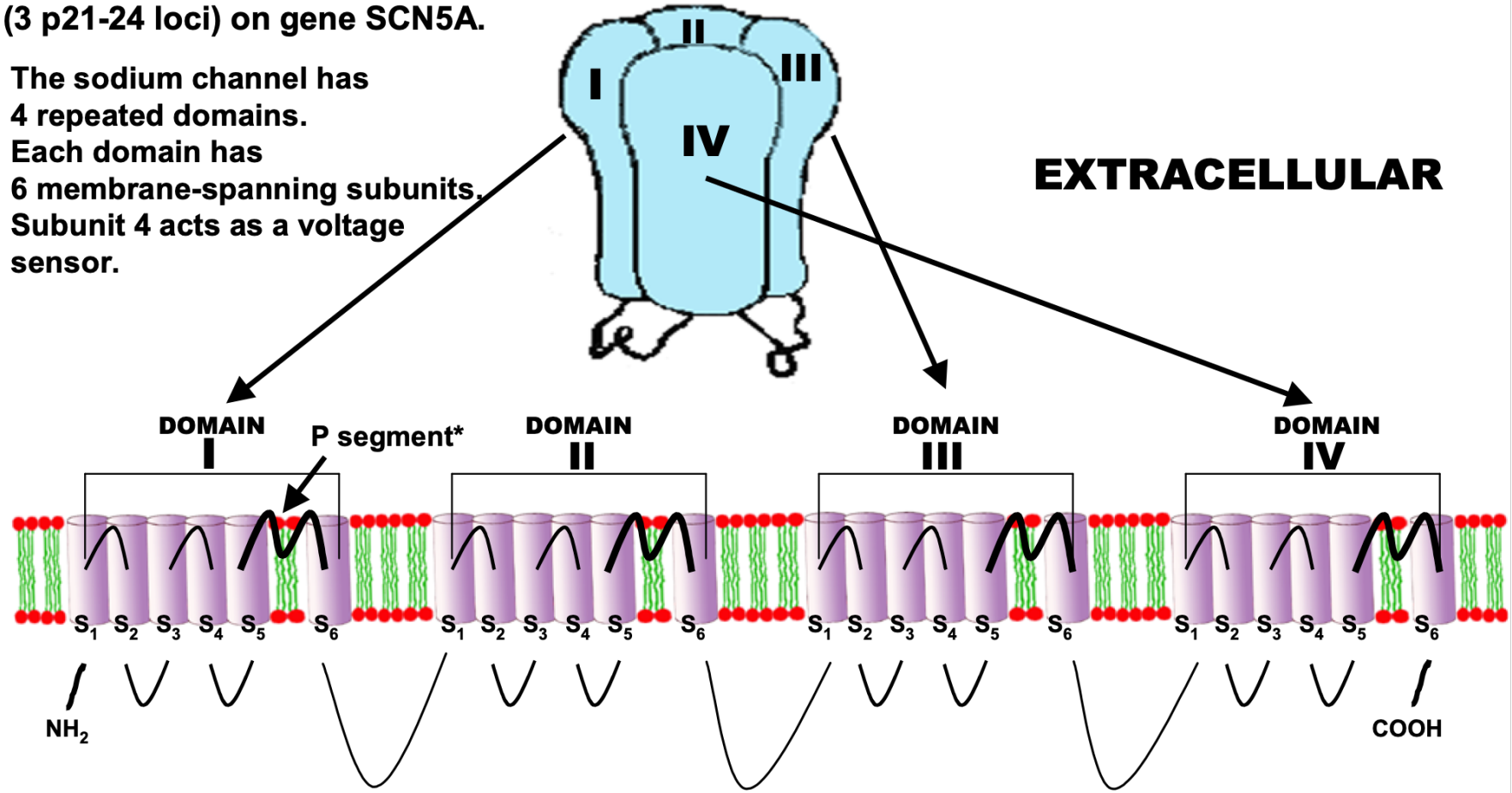
Sodium channel subunits



Molecular structure of the cardiac sodium channel: main α-subunit ($Na_v1.5$) and the -β1- β2subunits of the cardiac Na^+ channel. The β-subunit consists of one single transmembrane segment. The four domains(I-IV) with 6 transmembrane segments (1-6). of $Na_v1.5$ fold around an ion-conducting pore, The expression and function of $Na_v1.5$ is regulated by β-subunits and several directly or indirectly interacting regulatory proteinsα and β subunits of sodium channels.

The human cardiac isoform (hH1) has been mapped to the short arm of chromosome 3 (3 p21-24 loci) on gene SCN5A.

The sodium channel has
4 repeated domains.
Each domain has
6 membrane-spanning subunits.
Subunit 4 acts as a voltage sensor.



Membrane Spanning subunits.

S_4 Voltage sensor

* The S5-S6 linkers or P segment of each domain come together to form the lining of the pore.