European Heart Journal Supplements (2020) **22** (Supplement L), L105–L109
The Heart of the Matter
doi:10.1093/eurheartj/suaa146

ESC
European Society
of Cardiology

The omics of channelopathies and cardiomyopathies: what we know and how they are useful

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KEYWORDS
Sudden cardiac death;
Brugada syndrome;
Channelopathies;
Cardiomyopathy;
Genetics

Sudden cardiac death results from arrhythmias commonly caused by channelopathies and cardiomyopathies, often due to several genetic factors. An emerging concept is that these disease states may in fact overlap, with variants in traditionally classified 'cardiomyopathy genes' resulting in 'channelopathies phenotypes'. Another important concept is the influence of both genetic and non-genetic factors in disease expression, leading to the utilization of systems biology approaches, such as genomics/epigenomics, transcriptomics, proteomics, metabolomics, lipidomics, and glycomics, to understand the disease severity and progression and to determine the prognosis and the best course of treatment. In fact, our group has discovered significant differences in metabolites, proteins, and lipids between controls and Brugada syndrome patients. Omics approaches are useful in overcoming the dogma that both channelopathies and cardiomyopathies exist as Mendelian disorders (caused by a mutation in a single gene). This shift in understanding could lead to new diagnostic and therapeutic approaches.

Introduction

Sudden cardiac death (SCD) in young, otherwise healthy individuals is often due to ventricular tachyarrhythmias, resulting from a variety of diseases, traditionally grouped into the categories of channelopathies and cardiomyopathies. Channelopathies are primarily electrical disorders affecting ion channels, while cardiomyopathies affect sarcomeric proteins, desmosomes, the cytoskeleton, and the nuclear envelope. Examples of diseases traditionally classified as channelopathies include Brugada syndrome (BrS), long-QT syndrome (LQTS), short-QT syndrome (SQTS), and catecholaminergic polymorphic ventricular tachycardia. Examples of genetic cardiomyopathies are hypertrophic cardiomyopathy (HCM), dilated cardiomyopathy (DCM), left ventricular non-compaction (LVNC), arrhythmogenic right ventricular cardiomyopathy (ARVC), and restrictive cardiomyopathy.

Identifying patients at risk of arrhythmic events is challenging, as SCD may be the first symptom of such conditions. However, risk stratification is not completely reliable yet in most of these conditions, making the clinical management quite problematic. Often the implantable cardioverter-defibrillator (ICD) is the only reliable prophylactic measure. However, ICD implantation comes with many pitfalls, including inappropriate shocks, the need for battery replacement, potential infection or broken leads, and psychological consequences. Besides, ICD aims to treat, rather than prevent, the potential fatal arrhythmias.

An emerging concept is that channelopathies and cardiomyopathies may overlap, as patients harbouring variants in genes associated with channelopathies may develop 'cardiomyopathy phenotypes', and patients with genetic cardiomyopathies often develop 'arrhythmic phenotypes' resembling channelopathies. While several reports have already been published in this field,[1–3] this line of research is still in its infancy. Another emerging concept is the interaction between both genetic and non-genetic factors in the disease expression, relative to arrhythmogenesis and myocardial dysfunction. In order to assess the mechanisms

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Table 1 Definitions of omics

Genomics	Application of molecular biology techniques to the complete DNA sequencing in a given organism.
Epigenomics	Study of the functional interaction among coding and non-coding regions and regulatory genes.
Transcriptomics	Application of molecular biology techniques to study RNA produced from DNA.
Metabolomics	Large-scale study of small molecules produced by living cells.
Proteomics	Application of biochemical techniques to study the complete set of proteins produced by an organism.
Lipidomics	Full characterization of lipid molecular species compared to proteins.
Glycomics	Comprehensive study of glycans that a cell or tissue produces under specified conditions of time, location, and environment.
Glycoproteomics	The systems-level analysis of glycoproteins, including their protein identities, sites of glycosylation, and glycan structures.

behind overlap and interaction of these two disease types, a new approach is required, encompassing whole genome sequencing (genomic), the RNA landscape (transcriptomic), the protein compounds derived from RNA (proteomic), and the characterization of lipid molecules (lipidomics). Such systems biology approach is defined as 'omics' (*Table 1*).

Omics are providing the instruments to shed a new light onto the source of the wide phenotypic variability, even in the presence of similar genotypes. In cardio-genetics, omics may assist to overcome the dogma that both channelopathies and cardiomyopathies behave as Mendelian disorders, and may help to better understand the disease course and prognosis, improving the diagnostic approach and therapeutic management.

The interaction between genetic background and environmental factors is complex, and often it is difficult to discern the relative contribution of genetic and environmental factors that may result in a certain disease condition. Many examples exist of environmental factors (including pharmaceutical treatments) that may influence the clinical phenotype of either channelopathies or cardiomyopathies. For example, LQTS or BrS may become overt only after being exposed to certain drugs or stressful conditions, or post-chemotherapy dilative cardiomyopathy may be triggered especially in the presence of a genetic background that predisposes a patient to this effect of the therapy. In this view, omics can contribute to understand the mechanisms, to discover the links, and to identify new biomarkers that may lead to innovative diagnostic and therapeutic strategies.

Genomics/epigenomics

Early genetic studies by Sanger were sequencing screened patients for variants in candidate genes thought to be causative for the clinical phenotypes (*Figure 1*). Variants in several genes, including *KCNQ1*, *SCN5A*, *KCNH2*, *RYR2*, *PKP2*, *DSP*, and *MYBPC3*[4] were discovered over time in patients with personal and familial history of SCD, but, nonetheless, channelopathies and cardiomyopathies were still considered two completely separate conditions.

With the development of next-generation sequencing, moving towards the study of the whole DNA sequence, this paradigm became obsolete, since genomic studies provided increasing evidence of cardiomyopathy gene mutations in patients bearing a channelopathy phenotype, and vice versa. At our centre, out of 200 BrS patients who tested positive during genetic testing, 95 did not harbour variants in the *SCN5A* gene, but rather in an array of other genes (*Figure 2*). After *SCN5A*, variants were most commonly found in the *AKAP9* gene (13%), followed by *SCN10A* (12%), *MYBPC3* (9%), *CACNA1C* (7%), *TRPM4* (7%), *DSG2* (4%), *PKP2* (4%), *ABCC9* (2%), *LMNA* (2%), and *CBL* (2%). Regardless of the genotype, these patients exhibited similar arrhythmogenic substrates, demonstrating that variants in an array of genes are associated with BrS. This data demonstrates that the BrS phenotype cannot currently be explained by variants in a single gene, but rather, BrS is associated with variants in genes encoding for a wide variety of proteins, including signalling, channel, sarcomeric, and desmosomal proteins.

The complex functional interaction among coding and non-coding regions and regulatory genes is defined as epigenomics. Recent evidence suggests that mutations in regulatory genes may influence the clinical expression of gene mutations associated with channelopathies or cardiomyopathies. For example, experimental evidence suggested that *MYBPC3* pathogenic mutations, known to be associated with DCM, can be modulated by epigenetic factors.[5] Similarly, a recent study suggests a role for the differential methylation and imprinting of *KCNQ1* (a gene involved in type 1 LQTS) in the risk for symptomatic prolonged QT interval.[6] Moreover, the reversible modifications occurring in DNA or histones modulates gene expression even without altering the DNA sequence. The ways by which these complex processes occur are yet incompletely understood but can be considered part of epigenomics. However, epigenomics alone cannot entirely explain the complex link between channelopathies and cardiomyopathies. Therefore, the latest studies employ the whole genomic approach to clarify the contribution of additional coding and non-coding regions to these disease conditions. For example, HCM or DCM are thought to be inherited only in an autosomal dominant manner,[7] but about 5% of affected patients have been reported to harbour more than one mutation, consistent with the hypothesis of an oligogenic inheritance. Additionally, some recent exome studies provided evidence of a possible high false positive rate in genetic testing for both HCM and DCM.[8]

Along these lines, it is likely that channelopathies and cardiomyopathies are not monogenic disorders and that

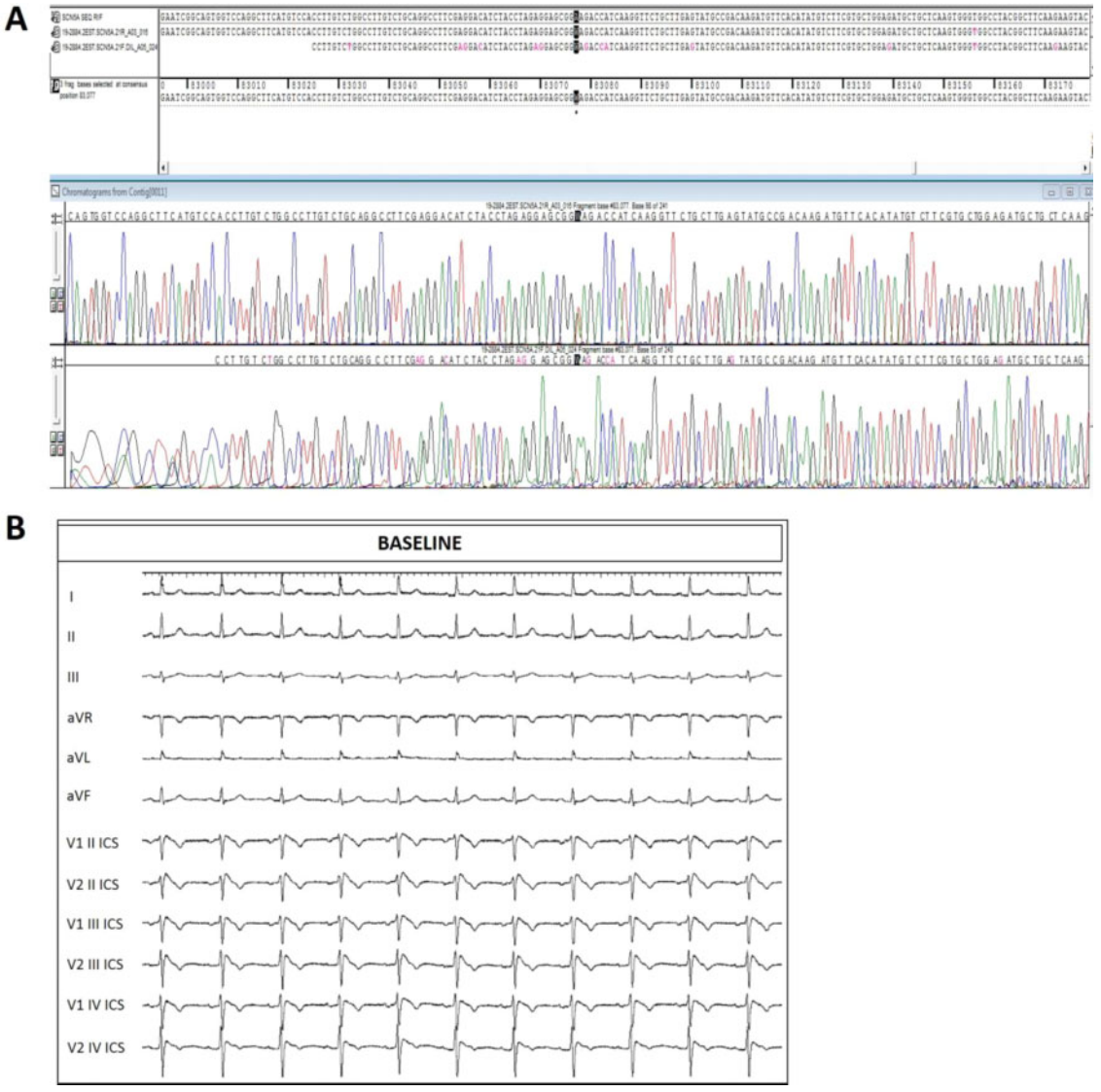

Figure 1 (*A*) Representative Sanger sequencing for the confirmation of the *SCN5A* heterozygous mutation (NM_198056.2):c.3697A>T found in a BrS patient. (*B*) Spontaneous 12-lead ECG demonstrating the type 1 BrS pattern in the patient described in *Figure 1*.

they are possibly influenced, or even caused by, an interaction between genomic factors, including modifier genes, and environmental factors. This would also explain the pleomorphic phenotypes that can emerge even within a single patient, for example, a spontaneous type 1 BrS electrocardiogram pattern that occurs only transiently, or the fact that patients can live for decades completely asymptomatic, then suddenly experience cardiac arrest, often under very specific conditions, such as while sleeping or during a febrile state, something that appears to also be influenced by age and gender.[2]

There have been numerous studies linking several genes to SCD, ranging from sodium channel, calcium channel, potassium channel, desmosomal, and sarcomeric genetic variants. In spite of this, the genetics of such conditions still remains elusive, without any known disease-causing mutation found in approximately 20% of families meeting clinical diagnostic criteria for LQTS or in approximately 60% of BrS patients.[9,10]

Most genetic studies on SCD have focused on the *SCN5A* gene, which encodes for the alpha subunit of the cardiac sodium channel. *SCN5A* heterozygous mutations are considered causative for a variety of both channelopathies and cardiomyopathy phenotypes, including LQTS, BrS, ARVC, LVNC, DCM, idiopathic ventricular fibrillation, sick sinus syndrome, and progressive heart block.[2]

In BrS, due to the low prevalence of molecular confirmation, many clinical genomic studies have been performed in search of candidate genes, many of those suggesting that other sodium channel genes, for example, *SCN1B* and *SCN10A*, might play a role in the pathogenesis of BrS.[2,11] However, a recent report underlined that, so far, only the *SCN5A* gene can be considered as causative for BrS, since it is the only BrS-associated gene that has withstood a systematic, evidence-based evaluation supporting the genotype–phenotype correlation.[12]

Other ionic channels besides sodium channels are implicated in the genesis of SCD. The function of calcium

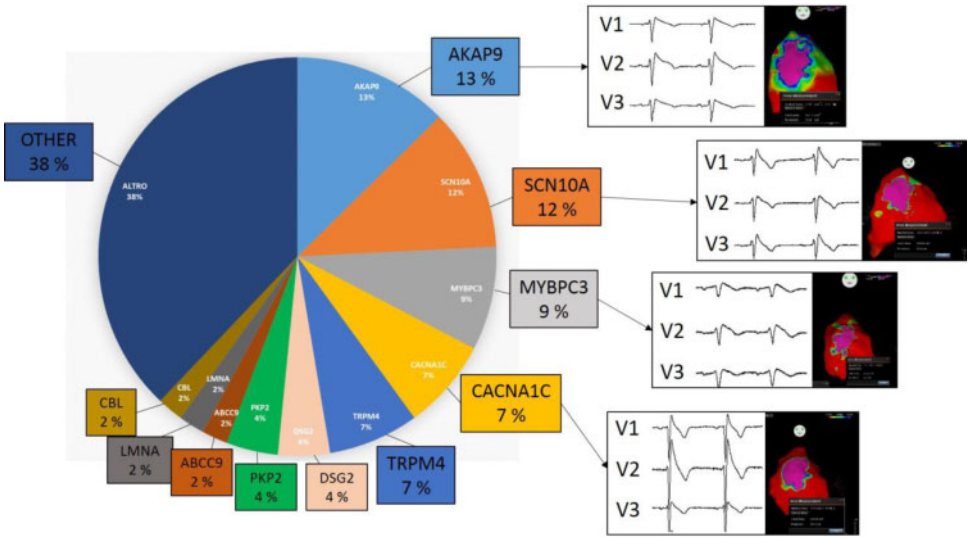

Figure 2 At our centre, out of 200 BrS patients who tested positive during genetic testing, 95 did not harbour variants in the *SCN5A* gene, but rather in an array of other genes. After *SCN5A*, variants were most commonly found in the *AKAP9* gene (13%), followed by *SCN10A* (12%), *MYBPC3* (9%), *CACNA1C* (7%), *TRPM4* (7%), *DSG2* (4%), *PKP2* (4%), *ABCC9* (2%), *LMNA* (2%), and *CBL* (2%).

channels, including L-type calcium channels, ryanodine receptors, the sodium/calcium exchanger, the sarco/endoplasmic reticulum Ca^{2+}-ATPase, and phospholamban, is central to excitation–contraction coupling, and the dysregulation of any of these proteins can lead to fatal arrhythmias. The most relevant genes implicated in these pathways are *CACNA1C*, *CACNB2*, *CACNA2D1*, *RYR2*[2] (associated with LQTS), and *CACNA1C*[9] (associated with BrS).

Other genes involved in the genesis of SCD are those encoding potassium channels, such as *KCNQ1* (associated with type 1 LQTS) and *KCNH2* (associated with many different arrhythmic phenotypes, including type 2 LQTS, SQTS, and BrS).

Of note, heterozygous mutations in *SCN5A*, the major gene associated with cardiac channelopathies, were observed in patients with cardiomyopathies. Indeed, the finding of right ventricular dysfunction is not rare in BrS patients with *SCN5A* mutations. Therefore, it is now a well-established concept that *SCN5A* is a pleiotropic gene, causative of both electrical and structural phenotypes. However, the mechanisms behind such *SCN5A* pleiotropism are not yet understood, and, so far, genomic approaches have provided limited clues relating to this issue.

Several channelopathy-associated ion channel genes have been associated also with cardiomyopathies, including *SCN5A* and *KCNQ1* with DCM, the *KCNQ1*, *RYR2*, and *HCN4* genes with LVNC, and *RYR2* with ARVC.[15] However, these findings are less common than those observed with *SCN5A*. Therefore, it is possible that other omics approaches, beyond genomics and epigenomics, will explain the mechanisms of gene pleiotropism and overlapping syndromes between channelopathies and cardiomyopathies.

From genomic/epigenomic to other omics approaches

The complex scenario of cardio-genetic conditions can be studied with other omics approaches, such as proteomics, transcriptomics, lipidomics, metabolomics, and glycomics. So far, such methods are still underutilized in cardiogenetics, even though the role of environmental and metabolic variations in determining the phenotype of channelopathies and cardiomyopathies has long been recognized.

Omics approaches could also be utilized to better differentiate BrS from Brugada phenocopies that can result from a number of non-genetic factors, including acute myocardial ischaemia, pulmonary embolism, electrolyte abnormalities, or adverse drug reactions, such as beta-receptor blocker or cocaine or marijuana use, in the absence of an identifiable genetic background.

As examples of proteomic changes, in the plasma of BrS patients, levels of apolipoprotein E, prothrombin, vitronectin, complement-factor H, vitamin-D-binding protein, voltage-dependent anion-selective channel protein 3, and clusterin have been reportedly increased, while levels of alpha-1-antitrypsin, fibrinogen, and angiotensinogen were decreased when compared to control subjects.[13] Additionally, our group recently discovered significant differences in circulating metabolites, proteins, and lipids between controls and BrS patients with a type 1 pattern expressed either spontaneously or after ajmaline administration, providing evidence that BrS may be a metabolomic disease (unpublished data). Such changes might affect the ionic channel traffic, explaining dynamic variation in BrS phenotypes.

The protein encoded by *SCN5A* (called the $Na_V1.5$ protein) undergoes several post-translational modifications, such as phosphorylation and sialylation. Both these processes have been implicated in BrS pathogenesis.[14] Following these findings, proteomic changes may be interpreted as a ‘fingerprint’ of the disease process. For example, we recently observed that BrS patients displayed post-translational modification of Nav1.5, compared to controls. This may provide a new diagnostic method and may be a new prognostic tool for the management of BrS patients.

Other omics, such as transcriptomic, metabolomics, lipidomics, and glycomics are still in an early phase due to methodological issues. Our group is pioneering their application both to BrS and to other related conditions.

Conclusions

Cardio-genetic diseases were always considered pure Mendelian disorders, in spite of much contradicting evidence. The technical improvements in biosciences have enabled the omics approach to illuminate that cardiogenetic diseases follow a more complex pathogenesis than a 'one mutation in a single gene' mechanism. This is true, especially for conditions without a well-established genetic background, such as BrS and some forms of cardiomyopathies. Omics approaches can be used in these conditions to understand disease severity, natural progression, prognosis, and overlap between seemingly distinct phenotypes. Moreover, omics approaches can impact the clinical management and treatment plan with positive consequences for affected patients.

Conflict of interest: none declared.

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